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Article type : Original Article

**Reducing adverse events associated with the glucagon stimulation test for the assessment of growth hormone deficiency in adults with a high prevalence of pituitary hormone deficiencies**

**Running title: Reducing adverse events with the glucagon stimulation test.**

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/CEN.14464](https://doi.org/10.1111/CEN.14464)

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**Key words:** Glucagon stimulation test, growth hormone, adverse events

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Abstract word count: 250 (250 allowed)

Main text word count: 3,427 (3500 allowed)

References: 34 (35 allowed)

Tables: 4

Figures: 2 (Figures and tables combined, allowed 6)

Supplemental Material: Supplements 1 and 2

**ABSTRACT:**

**Design:** A retrospective review of the adverse events (AEs) in 78 patients during the glucagon stimulation test (GST) for the assessment of growth hormone deficiency (GHD) before and after protocol amendments which aimed to reduce AEs in a group of patients with a high prevalence of pituitary hormone deficiencies.

**Patients:** Based on our observations of frequent AEs during the standard GST protocol in an initial 25 patients (cohort 1), a modified protocol was introduced to include the routine administration of 20mg of hydrocortisone pre-GST in a subsequent 53 patients (cohort 2). Post-hoc analysis of the effect of glucocorticoid dosing pre-GST on AEs was examined in those receiving <20mg hydrocortisone (group A, n=19) versus  $\geq$ 20mg hydrocortisone (group B, n=59).

**Measurements:** AEs including hypotension, hypoglycaemia and nausea/vomiting.

**Results:** Of the 78 patients undergoing the GST, 79% had  $\geq 2$  hormone deficiencies. Rates of AEs were 41% vs 30% for hypotension, 60% vs 28% for hypoglycaemia ( $p < 0.05$ ) and 20% vs 13% for nausea/vomiting in cohort 1 compared with cohort 2, respectively. Post-hoc analysis revealed lower rates of AEs in those receiving  $\geq 20$ mg hydrocortisone (Group B) compared to those receiving  $< 20$ mg due to a reduction in hypoglycaemic events (82% vs 26%,  $p < 0.001$ ) and hypotension (50% vs 27%,  $p = 0.05$ ). Similar numbers of patients in group A and group B met criteria for GHD.

**Conclusions:** In patients with a high prevalence of pituitary deficiencies a modified GST protocol of additional stress dose glucocorticoid attenuated the frequency of AEs without appearing to compromise the performance of the GST.

## INTRODUCTION:

Growth hormone deficiency (GHD) is a distinct clinical syndrome associated with fatigue, reduced quality of life, unfavourable metabolic profile and increased cardiovascular risk.<sup>1,2</sup> Recognition of this syndrome has led to the treatment of adults with severe GHD and the subsequent reporting that recombinant human growth hormone (GH) therapy improves clinical outcomes and quality of life.<sup>3-6</sup>

Recombinant GH has been approved for the treatment of adult GHD since 1996 and subsequently has been reimbursed for use in several countries for many years.<sup>7-11</sup> However, GH was only approved for subsidy in Australia in

December 2018 following advocacy of a professional medical working party to the relevant government agencies.<sup>12</sup> Criteria for subsidised therapy in Australia requires biochemical confirmation of GHD established by one of three dynamic tests: the insulin tolerance test (ITT), the glucagon stimulation test (GST) or the arginine infusion test. Whilst the ITT remains the gold standard for evaluation of adult GHD, there are several disadvantages including patient discomfort from severe hypoglycaemia, contraindication in patients with underlying cardiovascular disease or epilepsy, and the test is labour intensive.<sup>6,13</sup>

Current guidelines support the GST as an acceptable alternative to the ITT,<sup>6,8</sup> as studies have shown that the GST has a similar sensitivity and specificity to the ITT when a peak serum GH level of 3  $\mu\text{g/L}$  is utilised as a cut-off value.<sup>14,15</sup> Furthermore, the GST has been reported to be simple and safe to perform,<sup>6,13-16</sup> and is not influenced by sex or hypothalamic origin of GHD.<sup>6</sup>

The main adverse events (AEs) of the GST that have been reported include nausea, vomiting, sweating, fainting, hypotension and headaches with at least one of these AEs occurring in approximately 10 to 34% of subjects tested.<sup>6,15-17</sup> It is possible that patients with a high burden of pituitary hormone deficiencies and relative secondary adrenal insufficiency account for the majority of AEs reported during the GST.

Here, we relate our experience of a standardised GST protocol that was modified in an attempt to reduce the AEs we observed initially in a group of patients with a high prevalence of hypopituitarism and hence a high likelihood of GHD. We also report the effects of pre-GST glucocorticoid dosing on the rate of AEs categorised on the basis of their pre-GST hydrocortisone-equivalent dose. The hypothesis was that many of the AEs in this patient group could be mitigated by routine additional glucocorticoid dosing.

## Methods

### Study design and setting

This is a retrospective analysis of all GSTs performed between January and December 2019 at St Vincent's Hospital Melbourne, Victoria, Australia; a tertiary referral and university teaching hospital which provides a dedicated multidisciplinary pituitary service. All patients who underwent a GST in the defined time period were included in the analysis. The study consisted of two parts. The first describes the experience of the standard GST which was then modified; the second part relates outcomes for patients based on the dose of glucocorticoid administered prior to the GST (**Figure 1**).

### Part 1: Modification of the GST protocol

#### *Initial testing phase*

Testing was initially performed in 25 patients (cohort 1) between January and May 2019 according to the standard GST protocol (Sd-GST) (**Supplement 1**) using a fixed dose of 1mg glucagon intramuscularly. Serum GH levels were measured by venous sampling at -15 minutes, 0 minutes and then at 30 minute intervals up to 240 minutes in relation to the glucagon injection. GHD was defined as a peak GH level of  $<3.0 \mu\text{g/L}$  as per the Australian Pharmaceutical Benefit Scheme (PBS) criteria.

From March 2019, capillary or venous blood glucose was routinely tested at 30-minute intervals via a point-of-care glucometer and through formal laboratory testing. Prior to this glucose monitoring was not routinely checked. Blood pressure (BP) was measured at the beginning and completion of the test and more frequently if the patients reported symptoms suggestive of hypotension.

Patients were recumbent for at least 15 minutes before baseline BPs were measured.

In this Sd-GST protocol, there were no specific instructions for cortisol deficient patients regarding glucocorticoid administration: dosing was at the discretion of the referring practitioners. Thus, patients in cohort 1 had variable glucocorticoid doses; 4 patients had their dose withheld, 14 had their usual morning dose and 7 patients received stress doses of glucocorticoids. (**Figure 1**).

#### *Subsequent testing phase using a modified protocol*

Following preliminary analysis of safety data obtained from this initial testing described above, procedural amendments were made to the Sd-GST protocol from May 2019 to December 2019 (**supplement 2**) with a further 53 patients tested (cohort 2) using this modified GST (Md-GST). The salient changes were: advice to withhold any antihypertensive medications the night prior and/or morning of the GST, hypocortisolaemic patients administered their usual morning glucocorticoid replacement and all patients received a standardised stress dose of hydrocortisone 20mg orally and metoclopramide 10mg intravenously pre-GST.

BP was routinely measured at 0, 120 and 240 minutes and more frequently if patients were symptomatic of hypotension. Venous glucose was routinely measured at baseline and every 30 minutes via both point-of-care glucometer and formal laboratory testing.

Patients received a weight-adjusted dosing of intramuscular glucagon with patients  $\leq 90$  kilograms receiving 1mg and those  $>90$  kg receiving 1.5mg in keeping with international guidelines.<sup>5</sup>

### **Definition of AEs**

For the purposes of this study, hypoglycaemia, was defined as either a venous or capillary glucose  $\leq 3.5$  mmol/L, hypotension was defined as a systolic BP (SBP)  $< 90$  mmHg or  $\geq 20$  mmHg decrease in SBP compared with baseline, and we recorded patient-reported nausea and/or vomiting. The severity of AEs was not graded.

### **Part 2: Post hoc analysis - effects of pre-GST glucocorticoid dose on AEs**

All 78 patients were divided into two groups: those who received  $< 20$  mg hydrocortisone equivalent (group A,  $n=19$ ); and those who received  $\geq 20$  mg hydrocortisone equivalent (group B,  $n=59$ ), as shown in **Figure 1**. The majority of patients in group B were derived from cohort 2 ( $n=52$ ) but an additional 7 patients from cohort 1 were included in this group. The 19 patients in group A, consisted of 18 patients from cohort 1 with the addition of one patient from cohort 2.

### **Ethical approval**

This study was approved by the Human Research Ethics Committee (HREC) at St Vincent's Health, Australia as a quality and safety audit.

### **Statistical analysis**

Categorical data are reported as number (%) and continuous data as median and interquartile range (IQR). Between group differences were compared by Fisher's Exact Test and Kruskal-Wallis test, as appropriate. Wilcoxon rank sign test was used to compare baseline and nadir glucose and SBP values. Linear regression models were also fitted to examine the relationship between glucose or SBP changes across time, these models included a variable for either cohort or group. Statistical analysis was performed using STATA version 16.1

(STATA LP College Station, TX, USA) and p values <0.05 were considered significant.

## RESULTS

### Part 1: Modification of the GST protocol

#### *Patient characteristics:*

In total, 78 patients were included in this study with 25 patients in cohort 1 and 53 patients in cohort 2. The demographic details of the patients are described in **Table 1**. Patients in cohort 1 were more likely to have  $\geq$  two hormone deficiencies (hypopituitary) (96% vs. 68%,  $p=0.02$ ) and to be on glucocorticoid and GH replacement therapy. This was an expected finding as the preliminary patients undergoing testing for GHD were patients transitioning from self-funded GH therapy onto subsidised GH replacement, rather than case finding in the later time period. There was no significant difference in aetiology of pituitary dysfunction for patients in cohort 1 or cohort 2 (**Figure 2A**).

Overall, of the 78 patients, hypoglycaemia occurred in 35% (24/68), hypotension in 33% (25/75) and nausea/vomiting in 15% (12/78). Ten patients did not have blood glucose measurements and three did not have BP measurements during the test.

In hypopituitary patients who had all three AEs assessed, AEs occurred in 60% (30/50) compared to 66% (12/18) ( $p=0.78$ ) in those without hypopituitarism and in 67% (30/45) compared to 52% (12/23) ( $p=0.3$ ) of patients with and without pre-existing cortisol deficiency.

#### *Outcomes:*

In cohort 1 (n=25), rates of AEs were: 9/15 (60%) for hypoglycaemia, 9/22 (41%) for hypotension and 5/25 (20%) for nausea/vomiting (**Table 2**). Five out of 25 patients (20%) required treatment of symptomatic hypotension. Two patients, who had their normal morning glucocorticoid dose withheld, required intervention from the hospital's medical emergency team but recovered quickly with fluid resuscitation and a stress dose of hydrocortisone and managed to complete their test. Four patients (16%) required treatment for symptomatic hypoglycaemia. One patient prematurely terminated the test due to severe nausea and vomiting which was unresponsive to an antiemetic: this patient had taken their regular dose of glucocorticoid (1mg prednisolone) pre-test.

Furthermore, in cohort 1, 7/25 patients received extra non-standardised doses of glucocorticoids prior to the test. The characteristics and AEs for these seven individual patients are shown in **Table 3**. None of these seven patients experienced hypoglycaemia, one experienced a mild drop in BP and two experienced mild nausea.

In cohort 2, rates of AEs were: 15/53 (28%) for hypoglycaemia, 16/53 (20%) for hypotension and 7/53 (13%) for nausea/vomiting. The rate of hypoglycaemia was significantly lower in cohort 2 compared to cohort 1 (28% vs. 60%), respectively, ( $p=0.03$ ), whilst the rates of hypotension and nausea/vomiting were not statistically different (**Table 2**) between the two cohorts.

In hypopituitary patients who had all three AEs assessed, AEs occurred in 79% (11/14) in cohort 1 compared to 53% (19/36) in cohort 2 ( $p=0.12$ ) and in 85% (11/13) in cohort 1 compared to 59% (19/32) in cohort 2 ( $p=0.17$ ) in patients with pre-existing cortisol deficiency.

Blood glucose decreased in both cohorts (baseline to nadir during the GST), cohort 1 (4.4 (4.1-4.7) versus 3.4 (3.1-4) mmol/L,  $p < 0.01$ ) and cohort 2 (4.5 (4.3-4.8) vs 3.9 (3.5-4.4) mmol/L,  $p < 0.001$ ). However, patients in cohort 2 experienced a relative 0.68 mmol/L (95% CI: 0.14, 1.20) increase in blood glucose compared to those in cohort 1 during the observation period of the study. Whilst one patient in cohort 1 met criteria for Whipple's triad, no patients in cohort 2 met this criteria.

Systolic BP also decreased in both cohorts (baseline to nadir during the GST), cohort 1 (118 (109-126) versus 107 (102-118) mmHg,  $p < 0.01$ ) and cohort 2 (121 (110.5-131) versus 108 (99-119.5) mmHg,  $p < 0.001$ ) but there was no difference in the rate of change in SBP between the two cohorts of patients during the observational period of the study. Similar numbers of patients in cohort 1 and cohort 2 (92% and 93%, respectively) met the GST criteria for GHD.

## **Part 2: Post hoc analysis – effects of pre-GST glucocorticoid dose on AEs**

### *Patient characteristics:*

For this post-hoc analysis, patients were divided into group A (n=19), that received  $< 20$ mg hydrocortisone (equivalent) and group B (n=59), that received  $\geq 20$ mg hydrocortisone (equivalent) prior to the GST. Demographic details of these groups are outlined in **Table 4**. In group A, six patients did not receive any glucocorticoids and 13 patients took their usual hydrocortisone dose (4-16mg) prior to the GST. In group B, 23 patients received 20mg, 31 patients received  $> 20$  to 40mg and 5 received  $> 40$  to 50mg of hydrocortisone before the GST.

The median pre-GST hydrocortisone dose equivalent was 8 mg (range: 0-16) in group A and 24 mg (range: 20-50) in group B ( $p = 0.001$ ). This post hoc

grouping of patients resulted in a matching of all other baseline characteristics and there was no difference in the aetiology of pituitary dysfunction in group A compared to group B (**Figure 2B**).

*Outcomes:*

In total, 18/19 patients in group A and all 59 patients in group B completed the GST. One patient prematurely terminated their GST as outlined above.

In patients who had all three AEs monitored for, AEs occurred in 100% (9/9) in group A and in 51% (21/41) in group B ( $p=0.01$ ) in those patients with hypopituitarism and in 100% (10/10) in group A and in 57% (20/35) ( $p=0.02$ ) in group B in those with pre-existing cortisol deficiency.

Rates of individual AEs are shown in **Table 2**. The rate of hypoglycaemia was significantly less in group B (26%) than in group A (82%),  $p<0.001$ . The reduction in rates of hypotension (29% vs 50%,  $p=0.5$ ) was of borderline significance and those for nausea/vomiting (14% vs 21%) were not statistically significant. When patients taking dexamphetamine were excluded from the analysis, the overall rate of hypotension increased in both groups, but more in group A (67%) than group B (29%),  $p=0.03$ . In two sensitivity analyses we found no difference in AEs for patients given hydrocortisone equivalent doses of  $<10$  mg ( $n=10$ ) versus 10-19 mg ( $n=9$ ), or when given  $\leq 15$ mg ( $n=18$ ), 16-29mg ( $n=48$ ) or  $\geq 30$ mg ( $n=12$ ).

Blood glucose decreased in both groups (baseline to nadir during the GST), Group A (4.3 (4-4.6) vs. 3.3 (3.1-3.5) mmol/L,  $p<0.01$ ) and Group B (4.5 (4.3-4.8) vs. 3.9 (3.5-4.4) mmol/L,  $p<0.001$ ). Patients in group B experienced a relative 0.99 mmol/L (95% CI: 0.56, 1.42) increase in blood glucose compared

to those in Group A. Systolic BP also decreased in both groups (baseline to nadir during the GST), Group A (115 (109-125) vs. 105 (98.5-112) mmHg,  $p < 0.01$ ) and Group B (121 (111-131) vs. 109.5 (101-121) mmHg,  $p < 0.001$ ). However, patients in Group B experienced a 7.1 mmHg (95% CI: 0.7, 13.4) increase in SBP compared to those in Group A. Similar numbers of patients in group A and group B (94% and 92%, respectively), met the GST criteria for GHD.

## Discussion

This single centre experience using the GST for confirming adult GHD highlights several issues. Firstly, this cohort has a high prevalence of hypopituitarism and thus a high pre-test probability of GHD. Secondly, AEs during the GST were more common than reported which may relate to the high prevalence of co-existent hypopituitarism in this patient group. Thirdly, by modification of a Sd-GST protocol, our observations suggest that AEs, specifically hypoglycaemia, are significantly attenuated. Lastly, the routine administration of hydrocortisone in our Md-protocol did not appear to interfere with the performance of the GST, at least within the context of our study design and population.

Our individual rates of hypoglycaemia, hypotension and nausea/vomiting ranged between 20-60% for the Sd-GST. Our AE rate was higher than those previously reported rates of 10-34%.<sup>6,15,16</sup> However, we recognise that the AEs we report were not translated into serious adverse outcomes, but are of clinical importance.

Although AEs occur after administration of glucagon in people without pituitary disease, (for example, in the treatment of diabetes-related hypoglycaemia), it is

likely that rates of glucagon-associated AEs are greater in patients with pituitary disease.<sup>18,19</sup> AEs may be exacerbated by undiagnosed or undertreated secondary adrenal insufficiency, and to our knowledge, no study to date has investigated whether AEs can be mitigated by additional routine pre-GST hydrocortisone in the setting of a high prevalence of hypopituitarism.

Glucagon acts as a vasodilator by reducing peripheral vascular resistance in splanchnic and hepatic vasculature, thereby reducing afterload and BP.<sup>20</sup> However the high rates of hypotension in our study are unlikely to be a result of glucagon alone. Generally, BP decreased in most patients during the GST, presumably as they relaxed over time in the recumbent position. It is noteworthy that all patients in group B who experienced a fall in BP were asymptomatic and that only 4 patients had an SBP below 90 mmHg. Approximately a quarter of patients in each group were taking dexamphetamine prior to the GST for the treatment of hypothalamic obesity. This sympathomimetic medication elevates BP, and potentially confounded the results. When patients taking dexamphetamine were excluded from the analysis, the overall prevalence of hypotension increased in both groups, but more so in group A.

After adoption of the Md-protocol, patients were prophylactically administered 10mg metoclopramide intravenously prior to the GST. Nausea and vomiting are due to glucagon's known effects on the slowing of intestinal motility.<sup>21</sup> Data on the effect of metoclopramide on GH secretion is mixed. Two previous studies have shown no impact on GH levels post metoclopramide,<sup>22,23</sup> whilst two other studies report metoclopramide may stimulate GH secretion including one study of adolescent males of short stature<sup>24</sup> and another in patients with diabetes mellitus on insulin therapy.<sup>25</sup> However, the exact mechanism for this proposed stimulation in both studies was unclear. The effect of metoclopramide on GH

levels appears to have not been a factor in misclassifying GHD in our patient group due to the high prevalence of hypopituitarism in our cohort but requires further investigation. While not statistically significant, the rates of nausea and vomiting were almost halved after the protocol modifications, which is a clinically important outcome.

In view of the high rate of hypoglycaemia identified in our initial cohort, we now routinely measure blood glucose at 30-minute intervals during the GST. The initial blood glucose increasing effects of glucagon are well-described and are routinely applied in clinical practice to treat profound hypoglycaemia.<sup>18</sup> However, this initial increase in glucose stimulates insulin secretion that can result in a subsequent glucose lowering which is usually asymptomatic.<sup>26-28</sup> Our observations suggest that a subsequent decrease in glucose to subnormal values occurs commonly in patients with pituitary disease. Interestingly, hypoglycaemia has not been noted as a prominent AE in previous studies of the GST.<sup>14-16</sup> In our study, the routine administration of at least 20mg of hydrocortisone pre-GST significantly reduced the rate of hypoglycaemia from 82% to 26% of patients.

The decision to administer 20mg of oral hydrocortisone routinely before glucagon was based on previous work carried out by *Jung et al.* which demonstrated that 20mg of oral hydrocortisone achieved supra-physiological levels of cortisol in healthy individuals post dexamethasone suppression.<sup>29</sup> Although, it should be noted that we did not measure blood cortisol levels in the current study, the reduction in the rate of AEs in patients who routinely received 20mg hydrocortisone, regardless of whether a patient was normally taking glucocorticoid replacement, suggests that this dose is adequate in mitigating the AEs of the GST. Unfortunately, our sensitivity analysis failed to help define the optimal hydrocortisone dose to prevent AEs and we accept that lower doses

may have achieved similar results, and this is an area of ongoing research for our group. Importantly, our experience during the Sd-GST suggests that glucocorticoids should not be withheld in those with pre-existing cortisol deficiency.

The effect of glucocorticoids on GH secretion remains contested with previous studies demonstrating that they can both stimulate and inhibit the secretion of GH via a number of mechanisms including the hypothalamus, pituitary and liver.<sup>30-32</sup> The final effect on GH secretion appears to be directly related to the dose and timing of exposure.<sup>31</sup> In human studies, cortisone acetate has been shown to decrease GH secretion acutely one hour after its administration,<sup>30</sup> yet, oral dexamethasone transiently increases GH levels.<sup>33,34</sup> We acknowledge that it still remains unclear how a pre-test dose of glucocorticoid impacts on GH secretion during the GST, but reassuringly, in our study, there was no difference in the rates of detection of GHD in the post hoc analysis groups.

Although the GST is generally considered a safer alternative to the ITT, given the findings of frequent hypotension and hypoglycaemia in our study, and previous reports of severe hypotension in the elderly,<sup>17</sup> it is worth considering whether a GST protocol that does not include modifications to reduce AEs should be considered safe in all patients. Using the Md-GST protocol, we reduced the rate of AEs, and importantly eliminated major AEs.

This study has several limitations. Firstly, all potential confounders cannot be accounted for due to the retrospective nature. Secondly, our sample size was small. Thirdly, evolving protocol changes were made during the time period studied, leading to inconsistency in the way patients were treated and how information on AEs was collected. This may have also resulted in an underestimation of some adverse events, such as hypoglycaemia, as we did not

routinely measure glucose levels in patients initially tested with the Sd-protocol. Furthermore, we did not grade the severity of AEs. Fourthly, the effects of 20mg of hydrocortisone pre-test on the subsequent GH response remain poorly characterised and the results we report may not be applicable to other patient populations. Finally, our study design did not allow for a detailed analysis of the relative impact of all the modifications that we made to our GST protocol, the routine administration of additional doses of hydrocortisone, the withholding of antihypertensive agents and the routine administration of an antiemetic on the reduction in AEs that we observed.

**Conclusion:**

This observational study shows that AEs, particularly hypoglycaemia, occurring with GST can be decreased with modification of GST protocol in a cohort of patients with high prevalence of hypopituitarism and high probability of GHD. It also highlights the importance of not withholding regular glucocorticoids in those with pre-existing cortisol deficiency.

**Conflict of Interest statement:**

RJM has received funding from Pfizer to support dynamic endocrine testing activities in the Department of Endocrinology & Diabetes, St Vincent's Hospital Melbourne. NS received a travel grant from Pfizer to attend a growth hormone workshop.

**Acknowledgement:**

The authors acknowledge the assistance and advice of Associate Professor Warrick Inder in the editing of the manuscript.

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TABLES:

**Table 1:** Baseline characteristics of patients in cohort 1 (Sd-GST protocol) and cohort 2 (Md-GST).

Characteristic	Cohort 1	Cohort 2	P
Number of Patients	25	53	NS
Age (median, IQR)	34 (28 – 45)	33 (24 – 45)	NS
Sex (Female)	15 (60%)	28 (53%)	NS
BMI > 30 kg/m <sup>2</sup>	4/14 (29%) <sup>#</sup>	22/53 (42%)	NS
No pituitary hormone deficiency	0 (0%)	5 (9%)	
1 pituitary hormone deficiency†	1 (4%)	12 (23%)	
2 pituitary hormone deficiencies	5 (20%)	11 (21%)	
3 pituitary hormone deficiencies	5 (20%)	10 (19%)	
4 pituitary hormone deficiencies	11 (44%)	12 (23%)	
5 pituitary hormone deficiencies	3 (12%)	3 (6%)	NS
A total of ≥ 2 pituitary hormone deficiencies	24 (96%)	36 (68%)	P = 0.02
On glucocorticoids pre-GST	22 (88%)	32 (60%)	P = 0.02
Equivalent Hydrocortisone dose taken prior the GST (mg) (median, IQR)	10 (4 – 20)	24 (20 – 30)	P < 0.001

Thyroid hormone replacement	22 (88%)	40 (75%)	NS
Reproductive hormone replacement	17 (68%)	28(53%)	NS
Desmopressin therapy	10 (40%)	17(32%)	NS
Previously on GH replacement	14 (74%)	10 (19%)	P < 0.01
Patients taking anti-hypertensive agents	1 (4%)	10 (19%)	NS
Taking dexamphetamine	7 (28%)	14 (26%)	NS

# BMI only measured in 14 out of the 25 patients in cohort 1

†Pituitary hormone deficiencies included need for desmopressin therapy

BMI: Body Mass Index

GST: Glucagon Stimulation Test

GH: Growth Hormone

**Table 2:** Adverse events associated with the GST.

A) Sd-GST cohort 1 (n=25), and Md-GST cohort 2 (n=53).

B) Post-hoc analysis: Group A (n=19) received < 20mg hydrocortisone equivalent and group B (n=59) received ≥ 20mg hydrocortisone equivalent immediately prior to the GST.

Adverse events rates were not systematically recorded in all patients initially during the Sd-GST

A) Adverse event	Cohort 1 (n=25)	Cohort 2 (n=53)	P
Hypotension	9/22 (41%)	16/53 (30%)	NS

Hypoglycaemia	9/15 (60%)	15/53 (28%)	< 0.05
Nausea/vomiting	5/25 (20%)	7/53 (13%)	NS
<b>B) Adverse event</b>	<b>Group A (n=19)</b>	<b>Group B (n=59)</b>	<b>P</b>
Hypotension	8/16 (50%)	17/59 (29%)	= 0.05
Hypoglycaemia	9/11 (82%)	15/57 (26%)	< 0.001
Nausea/vomiting	4/19 (21%)	8/59 (14%)	NS

GST: Glucagon Simulation Test

**Table 3:** Outcomes for 7 patients in cohort 1 (n=25) that received various doses of glucocorticoids  $\geq 20$ mg equivalent of hydrocortisone as part of the initial Sd-GST protocol.

Patient	Age	Sex	Diagnosis	Pituitary axis deficiencies	Hydrocortisone dose (mg/day)	Hypoglycaemia	Hypotension	Nausea/vomiting
1	66	M	Craniopharyngioma	3	20	No	No	No
2	62	F	Pituitary adenoma	3	20	No	No	No
3	45	M	Congenital panhypopituitarism	4	20	No	No	Yes
4	55	F	Pituitary adenoma	3	50	Not tested	No	No

5	24	M	Medulloblastoma	4	50	Not tested	No	No
6	28	F	Medulloblastoma	1	50	No	No	No
7	30	M	Cranial irradiation	3	50	No	Yes	Yes

GST: glucagon stimulation test

\* Hydrocortisone equivalent dose administered pre-GST

**Table 4:** Baseline characteristics of patients divided into group A (n=19), that received < 20mg hydrocortisone (equivalent) and group B (n=59) that received  $\geq$  20mg hydrocortisone (equivalent) immediately prior to the GST

Characteristic	Group A	Group B	P
Number of Patients	19	59	NS
Age (median, IQR)	34 (24 – 44)	33 (25 – 46)	NS
Sex (Female)	12 (63%)	31 (53%)	NS
BMI > 30 kg/m <sup>2</sup>	4/13 (31) <sup>#</sup>	22/59 (41%)	NS
No pituitary hormonal deficiency	1 (5%)	4 (7%)	NS
1 pituitary hormone deficiency†	1 (5%)	12 (20%)	
2 pituitary hormone deficiencies	2 (11%)	14 (24%)	
3 pituitary hormone deficiencies	5 (26%)	10 (17%)	
4 pituitary hormone deficiencies	7 (37%)	16 (27%)	
5 pituitary hormone deficiencies	3 (16%)	3 (5%)	NS
A total of $\geq$ 2 pituitary hormone deficiencies	17 (89%)	42 (71%)	NS
On glucocorticoids pre-GST	17 (89%)	37 (63%)	P=0.04
Equivalent Hydrocortisone dose taken prior to the GST (mg)	8 (0 – 10)	24 (20 – 30)	P < 0.001

(median, IQR)			
Thyroid hormone replacement	15 (79%)	47 (80%)	NS
Reproductive hormone replacement	14 (74%)	31 (53%)	NS
Desmopressin therapy	8 (42%)	19 (32%)	NS
Previously on GH replacement	9 (47%)	15 (25%)	NS
Patients taking anti-hypertensive medications	1 (5%)	10 (17%)	NS
Taking dexamphetamine	5 (26%)	16 (27%)	NS

# BMI only measured in 13 out of the 19 patients in Group A

†Pituitary hormone deficiencies included need for desmopressin therapy

BMI: Body Mass Index

GST: Glucagon Stimulation Test

GH: Growth Hormone

## FIGURES AND TABLES

**Figure 1:** Study design.

Part 1: the standard and modified GST protocol to mitigate adverse events.

Part 2: the post hoc analysis of the effects of pre-GST hydrocortisone dosing on adverse events

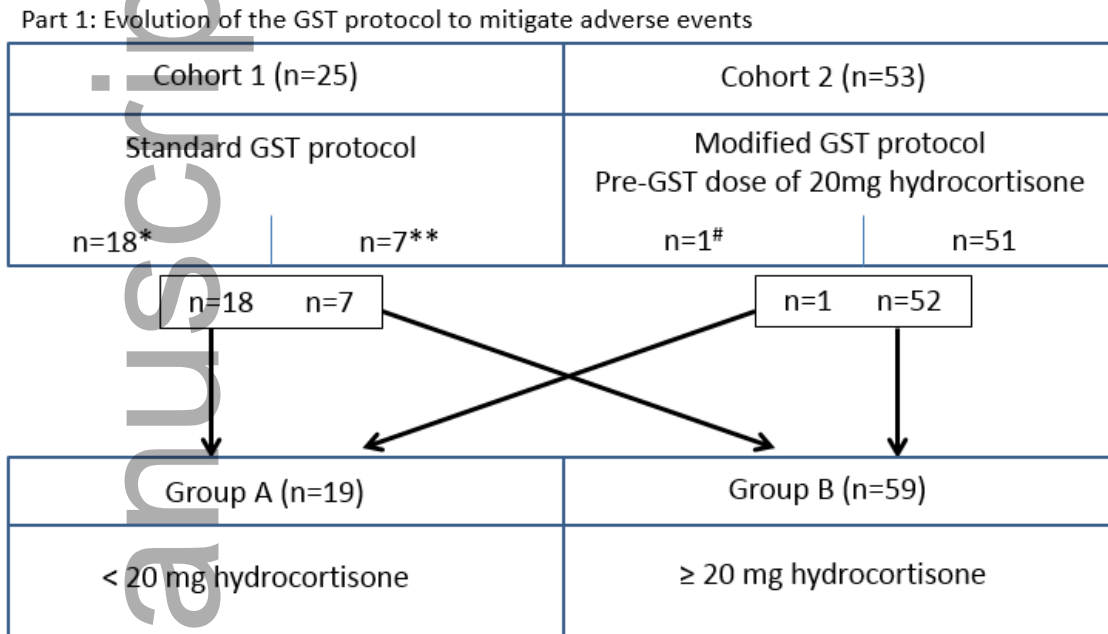
**Figure 2:** Aetiology of pituitary dysfunction. Panel A; cohort 1 (standard protocol) and cohort 2 (modified protocol). Panel B: group A (patients received  $< 20$  mg hydrocortisone equivalent) and group B (patients received  $20$  mg  $\geq$  hydrocortisone equivalent)

SUPPLEMENTARY MATERIAL:

**FIGURE 1: Study design.**

Part 1: the standard and modified GST protocol to mitigate adverse events.

Part 2: the post hoc analysis of the effects of pre-GST hydrocortisone dosing on adverse events



Part 2: Post hoc analysis of the effects of pre GST hydrocortisone dosing on adverse events

GST: glucagon stimulation test

\*No corticosteroid (n=4) or usual corticosteroid dose (n=14) taken prior to the GST

\*\*Stress dosing and/or administration of regular dose of corticosteroid according to the discretion of the referring practitioner.

#One patient failed to receive a pre GST dose of hydrocortisone

**Figure 2:** Aetiology of pituitary dysfunction. Panel A; cohort 1 (original protocol) and cohort 2 (modified protocol). Panel B: group A (patients received < 20 mg hydrocortisone) and group B (patients received 20 mg  $\geq$  hydrocortisone)

