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Isolated Hypoglycemia in Children with Cystic Fibrosis: Role of Pancreatic Insufficiency and Glucagon Response

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What is already known on this topic?

The possible mechanisms of hypoglycemia in cystic fibrosis (CF) are delayed and prolonged insulin secretion and/or impaired counterregulatory hormone function. However, CF patients in these studies had hypoglycemia with abnormal glucose tolerance (Hypo+AGT) and pancreatic insufficiency (PI).

What this study adds?

Isolated hypoglycemia in CF patients with pancreatic insufficiency (IsoHypo, PI+) is associated with delayed insulin secretion and impaired glucagon response. However, IsoHypo without pancreatic insufficiency is associated with early and exaggerated insulin secretion with relatively preserved but still insufficient glucagon response to hypoglycemia. IsoHypo PI(+) may be a predecessor to Hypo+AGT, whereas IsoHypo PI(-) appears to represent a milder impairment of glucose homeostasis in CF.

ABSTRACT

Objective: Hypoglycemia is one of the comorbidities that adversely affects the quality of life in patients with cystic fibrosis (CF). Isolated hypoglycemia (IsoHypo) is poorly described in patients with CF and its etiopathogenic significance is unclear. To investigate the etiopathogenesis of IsoHypo and the role of pancreatic insufficiency (PI) in IsoHypo in children with CF.

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Methods: The blood glucose, insulin, and glucagon responses of patients with CF and healthy controls were evaluated during a 3-hour oral glucose tolerance test. Based on the results, the patients were categorized into 5 groups: 1) normal glucose tolerance (NGT); 2) IsoHypo; 3) hypoglycaemia with abnormal glucose tolerance (Hypo+AGT); 4) AGT; and 5) CF-related diabetes. IsoHypo and NGT were sub-classified according to the presence of PI as PI(+) or PI(-). Hypoglycemia was defined as blood glucose <70 mg/dL.

Results: A total of 44 patients with CF and 9 controls. Hypoglycaemia was observed in 21 of 44 patients (47.7%), predominantly as IsoHypo (29.5%). Hypo+AGT was found in eight patients (18.2%). The IsoHypo group exhibited undelayed and higher insulin secretion than the Hypo+AGT group, with IsoHypo PI(-) being less impaired compared to IsoHypo PI(+). Both IsoHypo and Hypo+AGT groups exhibited a blunted rise in glucagon at 180 minutes, with the deficiency being more pronounced in the Hypo+AGT group. Insulin and glucagon responses to oral glucose load in IsoHypo PI(+) were similar to Hypo+AGT, whereas they were less impaired in IsoHypo PI(-) patients who had early and higher insulin secretion.

Conclusion: IsoHypo is common in children with CF and may precede Hypo+AGT in those with PI(+). The abnormal insulin and glucagon responses to glucose appear to be the most significant contributors to the development of IsoHypo in CF.

Keywords: Cystic fibrosis, hypoglycemia, insulin, glucagon, children

Introduction

In recent years, spontaneous or reactive hypoglycemia has been increasingly recognized in individuals with cystic fibrosis (CF), both during oral glucose tolerance test (OGTT) and in daily life. The reported prevalence varies widely, ranging from 7% to 69%, depending on the definition of hypoglycemia and the duration of OGTT (1,2,3,4,5,6,7). It is more frequently observed in 3-hour OGTTs, affecting approximately 45% to 65% of CF patients (6,7,8,9,10). One of the key pathophysiological features observed during OGTT in CF is a disruption of the biphasic insulin secretion pattern. The early (first phase) insulin response is often delayed, and this is followed by a prolonged and dysregulated insulin release. Recent studies suggest that this delayed and sustained hyperinsulinemia may predispose to postprandial or reactive hypoglycemia (8,9,10,11). In addition to beta-cell dysfunction, impaired suppression and a dysregulated response to glucose loading, rather than an absolute reduction, have been described in CF patients with abnormal glucose tolerance (AGT) and pancreatic insufficiency (PI). Furthermore, an inadequate glucagon increase in response to hypoglycemia (inappropriate response) may also contribute to the development of reactive hypoglycemia in these patients (8,9,11). Most studies investigating hypoglycemia in CF have focused on individuals with PI and have not included healthy control groups. Moreover, participants experiencing hypoglycemia in these studies often exhibited AGT (8,9,10,11).In our previous research, we identified cases of isolated hypoglycemia (IsoHypo) during OGTT in some children with CF who had normal glucose tolerance (4). The aim of the present study was to explore the mechanisms underlying IsoHypo and to assess the impact of PI on hypoglycemia in CF. We examined glucose, insulin, and glucagon responses to a three-hour OGTT in CF patients with and without PI, as well as in healthy controls.

Methods

Participants

Participants with CF aged 10-18 years, who had been regularly followed in the pediatric pulmonology and endocrinology departments, were invited to participate in this study (NCT05700604). Individuals on corticosteroid therapy, those who had experienced an acute exacerbation in the last three months, or those with a prior diagnosis of diabetes were excluded from the study. PI was defined as the need for enzyme replacement therapy due to clinical symptoms or a fecal elastase level below 200 µg/g stool and all patients with PI were receiving enzyme replacement therapy. All patients included in the study were receiving inhaled therapies because of underlying pulmonary involvement. None of the patients were on CFTR modulatory treatments and none had overt liver disease. The data collected from participants with CF included height, weight, body mass index (BMI), forced expiratory volume in one second (FEV1), molecular etiology, and the presence of PI.The control group consisted of age-matched healthy, non-diabetic siblings of children with type 1 diabetes, who were under follow-up at our clinic. All controls tested negative for pancreatic β-cell autoantibodies, including anti-glutamic acid decarboxylase, islet cell antibodies, and insulin antibodies. The study protocol was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 09.2019.933, date: 01.11.2019), and written informed consent was obtained from participants or their parents.

Procedures

A 3-hour OGTT was performed in the morning following overnight fasting of at least 8 hours. All participants received oral glucose solution (1.75 g/kg; max: 75 g). Blood samples were collected at 0, 30, 60, 90, 120, 150 and 180 minutes for glucose and insulin, and at 0, 60, 120, 150 and 180 minutes for glucagon measurement. In addition, hemoglobin A1c (HbA1c) and C-reactive protein

(CRP) levels were measured at baseline to evaluate glucose metabolism and systemic inflammation, respectively. OGTT results were classified based on ISPAD guidelines (12). Participants with indeterminate glucose tolerance (INDET) or impaired glucose tolerance (IGT) without hypoglycemia were categorized as AGT. The terminology of "isolated hypoglycemia" (IsoHypo) was used for those who experienced hypoglycemia with normal glucose tolerance (NGT). The term "Hypo+AGT" was applied to the participants who had both hypoglycemia and AGT. According to the International Hypoglycaemia Study Group (IHSG) position statement, hypoglycemia is defined as any venous glucose level below 70 mg/dL (13). Although, in individuals not receiving glucose-lowering treatments, the threshold for defining hypoglycemia is recommended to be <54 mg/dL, venous glucose level below 70 mg/dL was chosen as the study hypoglycemia threshold, since the physiological counterregulatory glucagon response to falling glucose levels is known to begin at approximately 68 mg/dL (2). Following the initial analysis, participants with IsoHypo and NGT were further classified based on the presence of PI to evaluate the effect of PI on hypoglycemia (Figure 1). The NGT PI(-) group was selected as the reference group for hormonal comparisons, as these patients had both NGT and preserved pancreatic function, making them the relatively healthiest CF subgroup in terms of blood glucose homeostasis. This group was therefore considered the most appropriate baseline for evaluating the pathophysiology of isolated hypoglycemia.

Plasma Glucose, Insulin and Glucagon Analysis

Blood samples were collected in EDTA tubes and immediately centrifuged on-site after collection. Glucose and insulin measurements were performed on the same day in the laboratory. Glucose was analyzed using the glucose hexokinase method on the Cobas c701/702 (Roche, Uniq İstanbul, Türkiye), while insulin was measured by ECLIA on the Cobas e801 (Roche, Uniq İstanbul, Türkiye). For glucagon analysis, plasma was separated after centrifugation and stored at 4 °C until the OGTT was completed (180 minutes). Immediately afterward, all glucagon samples were transferred to -80 °C for long-term storage. Plasma glucagon levels was measured using a direct sandwich ELISA technique (Mercodia Glucagon ELISA, cat. no. 10-1271-01, lot no. 29870, Uppsala, Sweden) following the standard manufacturer's protocol. Insulin and glucagon responses were considered "inappropriate" if, during venous glucose concentrations below 70 mg/dL, insulin levels were not suppressed, and/or an increase in glucagon failed to raise venous glucose above 70 mg/dL.

Statistical Analysis

All analyses were made using SPSS, version 20 (IBM Inc. Armonk, NY, USA) and graphical figures were produced with GraphPad Prism, V5.0 software (GraphPad Software Inc., San Diego, California, USA). For groups with fewer than five patients (CF-related diabetes and AGT), data are summarized using only median and min-max values; and they are not included in the comparisons, although data is shown on Table 1. For all other

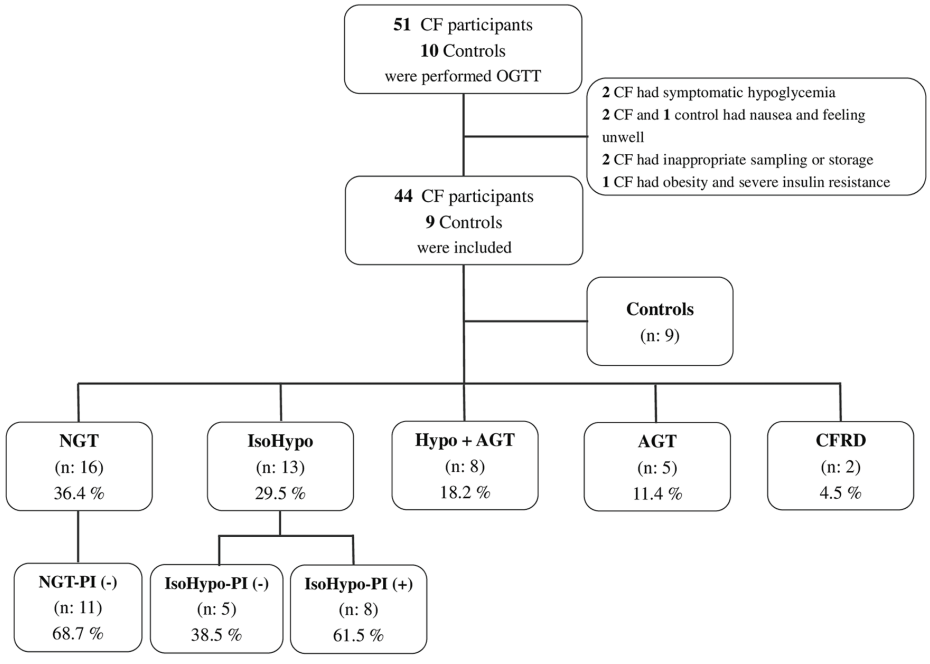

Figure 1. The distribution of the OGTT results
CF: cystic fibrosis, NGT: normal glucose tolerance, OGTT: oral glucose tolerance test, IsoHypo: isolated hypoglycemia

groups, both mean±SD and median (min-max) are reported due to the overall limited sample sizes.Data normality was assessed using Q-Q plot, the Shapiro Wilk test, and the Kolmogorov-Smirnov tests. Depending on normality, group comparisons were performed using either repeated ANOVA or the Kruskal-Wallis test. Post-hoc analysis for ANOVA were conducted using Tukey's or Tamhane's T2 test depending on the homogeneity of variances. For the Kruskal-Wallis test, post-hoc comparisons were performed using Kruskal-Wallis one-way ANOVA (k samples) test. Statistical significance was set at $p<0.05$.

Results

Baseline Characteristics and Prevalence of Hypoglycaemia

A total of 61 participants (51 with CF and 10 healthy controls) were recruited. Two CF participants experienced symptomatic hypoglycemia, necessitating the early termination of their OGTT. In addition, two CF participants and one control were unable to complete the OGTT because of nausea and discomfort. One CF participant with obesity and severe insulin resistance was also excluded. Samples from two CF participants were removed

Table 1. Demographic features and OGTT results of the participants

	Hypoglycemia (+)		**Hypoglycemia (-)**				
	Isolated Hypo (n=13)	**Hypo+AGT (n=8)**	**NGT (n=16)**	**AGT (n=5)**	**CFRD (n=2)**	**Control (n=9)**	**p**
Age, mean±SD	13.5±1.6	13.1±1.8	13.7±2.2	13.3±1.5	15.3 (12.6-17.9)	13.2±1.8	NS
Male, n (%)	10 (77)	4 (50)	9 (56)	3 (60)	1 (50)	4 (44)	NS
BMI SDS	-0.3±1.1 0.0 (-2.1-1.7)	-0.4±1.0 -0.2 (-1.6-1.0)	-0.1±0.8 0.0 (-1.6-1.0)	-0.6±0.5 -0.6 (-1.2-0.2)	-0.0 (-0.2-0.1)	0.0±0.7 -0.4 (-0.8-1.6)	NS
HbA1c, %	5.7±0.3 5.7 (5.1-6.0)	5.7±0.2 5.8 (5.4-6.1)	5.5±0.3 5.6 (4.8-6.0)	5.9±0.3 6.1 (5.6-6.3)	6.7 (5.9-7.5)	5.2±0.2 5.3 (4.9-5.6)	**0.004**$^{§,\&,P}$
CRP (mg/dL)	6.7±9.6 3.2 (3.1-38)	6.9±8.6 3.1 (3.1-27.6)	3.4±0.9 3.1 (3.1-6.8)	6.3±7.1 3.1 (3.1-19.1)	5.0 (3.1-6.9)	0.5±1.0 0.2 (0.1-2.9)	**<0.001**$^{§,\&,P}$
FEV1, %	86±29 94 (34-126)	88±18 87 (53-117)	92±9 94 (68-106)	77±28 77 (58-97)	99 (76-123)		NS
Genotype, n (%)							
ΔF508/ΔF508	1 (8)	2 (25)	1 (6)	1 (20)	1 (50)		NS
ΔF508/nonΔF508	5 (38)	2 (25)	3 (19)	2 (40)			NS
nonΔF508/nonΔF508	7 (54)	4 (50)	12 (75)	2 (40)	1 (50)		NS
PI, n (%)	8 (62)	8 (100)	5 (31)	5 (100)	2 (100)		0.005
OGTT							
Glucose (mg/dL) mean±SD and median (min-max)							
0. min	85±5 86 (75-93)	92±13 91 (80-116)	87±5 84 (80-98)	97±5 97 (91-103)	96 (91-102)	83±4 81 (79-89)	NS
30th min	170±28 173 (93-210)	178±27 174 (153-238)	143±25 138 (83-195)	181±46 195 (116-228)	187 (173-201)	133±16 129 (115-163)	**<0.001**$^{**,§,\#,\&}$
60th min	152±43 162 (50-198)	229±21 230 (204-267)	125±29 118 (85-189)	201±53 202 (119-260)	266 (218-315)	126±26 119 (98-170)	**<0.001**$^{*,\#,\&}$
90th min	120±37 129 (68-167)	177±40 168 (133-250)	105±17 104 (77-137)	173±22 164 (148-202)	292 (221-363)	120±27 112 (93-174)	**0.003**$^{\#,\&}$
120th min	100±23 108 (58-125)	133±35 118 (99-190)	105±12 105 (78-126)	146±27 142 (120-177)	301 (228-375)	101±20 103 (64-130)	NS
150th min	77±20 79 (42-105)	91±23 91 (46-125)	102±17 98 (80-155)	128±30 121 (100-171)	237 (177-297)	90±17 90 (62-110)	**0.025****
180th min	68±16 67 (46-98)	60±10 66 (42-68)	93±17 90 (70-128)	117±26 114 (84-154)	132 (75-190)	87±14 89 (59-102)	**<0.001**$^{**,\#,\&}$

Table 1. Continued

	Hypoglycemia (+)		Hypoglycemia (-)				
Insulin (mIU/mL) mean±SD and median (min-max)							
0. min	8.3±6.1 6.2 (1.7-24.0)	5.9±5.1 4.4 (1.9-18.0)	8.0±4.3 8.6 (1.3-18.4)	8.3±1.5 8.1 (6.4-10.4)	14.3 (4.1-24.5)	10.9±7.5 8.6 (3.0-23.4)	NS
30th min	72.2±75.9 48.0 (8.4-243.0)	20.0±16.1 16.0 (4.4-55.0)	68.3±28.9 68.0 (16.0-113.0)	39.2±19.0 43.0 (8.0-58.0)	16.5 (15.0-18.0)	38.2±30.1 26.0 (3.9-108.0)	**0.002**#
60th min	93.3±91.0 58.0 (14.1-299.0)	63.5±71.1 45.0 (15.5-235.0)	59.5±36.7 46.7 (24.6-140.0)	46.4±28.1 41.9 (15.0-80.6)	22.2 (3.2-41.3)	51.5±25.4 47.3 (18.8-102)	NS
90th min	59.8±55.3 46.0 (2.4-181.0)	72.0±45.1 60.3 (34.2-166.0)	46.1±29.8 37.0 (17.0-103.0)	50.1±19.3 54.0 (28.0-71.0)	24.0 (3.0-45.0)	54.9±49.0 48.0 (3.4-169.0)	NS
120th min	39.5±22.2 38.2 (12.3-89.2)	35.1±13.9 37.4 (6.0-51.1)	41.9±22.4 35.7 (18.6-89.2)	49.5±16.0 41.6 (34.9-73.7)	24.8 (3.0-46.7)	49.2±35.5 48.3 (13.8-135.0)	NS
150th min	16.5±7.1 14.8 (1.3-31.1)	17.5±11.1 18.0 (4.6-31.3)	34.6±17.0 28.1 (12.2-73.6)	37.8±10.7 37.1 (21.9-43.9)	46.4 (11.2-81.6)	31.5±35.3 24.1 (1.1-119.0)	**0.013****
180th min	12.3±8.8 10.0 (0.9-29.0)	8.2±6.1 7.3 (1.5-18.7)	29.2±24.4 20.6 (7.0-105.2)	23.1±11.1 24.5 (4.6-32.1)	40.1 (20.1-59.4)	23.5±18.2 20.4 (6.1-68.4)	**0.004**#
Glucagon (pmol/L) mean±SD and median (min-max)							
0. min	6.1±5.6 4.0 (1.7-22.4)	5.7±2.9 5.7 (2.1-9.7)	4.9±2.2 4.4 (1.3-9.3)	9.7±6.1 10.4 (1.3-16.3)		7.2±3.7 6.4 (3.7-15.8)	NS
60th min	3.4±1.5 3.0 (1.8-6.7)	5.8±5.1 4.2 (0.1-16.7)	2.8±2.3 2.1 (0.4-7.1)	5.5±5.0 4.9 (0.3-11.0)	5.0 (4.2-5.8)	1.7±1.2 1.7 (0.2-3.5)	**0.037**&
120th min	3.8±2.1 3.0 (1.7-8.1)	4.2±3.3 3.2 (1.4-11.6)	3.0±2.0 2.6 (0.1-8.4)	4.2±2.9 4.9 (0.4-6.9)	18.3 (3.2-33.3)	1.3±1.1 1.2 (0.1-3.3)	**0.009**§,&
150th min	4.1±3.0 3.8 (1.4-11.7)	4.0±2.5 3.3 (1.9-9.6)	2.6±1.6 2.0 (0.8-6.0)	4.3±2.9 4.1 (0.6-8.8)	5.0 (3.0-7.0)	2.1±2.3 1.3 (0.5-7.6)	**0.031**&
180th min	6.4±5.9 4.7 (0.6-21.8)	5.1±4.0 4.4 (0.1-11.2)	2.7±1.6 2.2 (0.4-6.6)	2.5±1.4 2.9 (0.3-4.0)	8.7 (4.8-12.6)	5.4±6.6 3.3 (1.0-21.2)	NS

Analyses were performed only for groups with more than five patients; AGT and CFRD groups were excluded. Data are presented as mean±SD and median (min-max). *IsoHypo vs Hypo+AGT, **IsoHypo vs NGT, §IsoHypo vs Controls, #Hypo+AGT vs NGT, &Hypo+AGT vs Controls, PNGT vs Controls

from the analysis because of improper sampling or storage. The final study cohort consisted of 53 participants (44 with CF and 9 controls) and these were included in the final analysis (Figure 1).

Based on the OGTT results, the patients were categorized into 5 groups: 1) normal glucose tolerance (NGT; n=16); 2) isolated hypoglycemia (IsoHypo; n=13); 3) hypoglycemia with abnormal glucose tolerance (Hypo+AGT; n=8); 4) AGT (n=5); and 5) CF-related diabetes (n=2).

The frequency of hypoglycemia in participants with CF was 47.7% (21/44) and 22.2% (2/9) in the healthy controls (p>0.05). The mean HbA1c and CRP levels were higher in all CF groups compared to the controls (p=0.004 and p<0.001, respectively) but no difference was observed between CF groups. There was also no difference in FEV1 and CFTR genotype between the CF groups (Table 1). There was a significant difference in the frequency of PI between OGTT groups in CF participants (p=0.005) (Table 1). Hypoglycemia was significantly worse in CF participants with PI(+) than PI(-) (p=0.006).

Differences in OGTT Characteristics between IsoHypo, Hypo+AGT, NGT in the CF and Healthy Control Groups

While the time of peak glucose was at 30 min in the IsoHypo group, similar to the NGT and control groups, it was delayed in Hypo+AGT to 60 min.Glucose level at 30 minutes was statistically higher in both IsoHypo and Hypo+AGT than in NGT and controls (p<0.001), whereas high glucose levels at 60 and 90 minutes persisted only in Hypo+AGT (p<0.001 and p=0.003, respectively). Glucose at 180 minutes was similar in IsoHypo and Hypo+AGT and lower than NGT and controls (p<0.001). Compared to IsoHypo, Hypo+AGT exhibited a delayed insulin peak, whereas IsoHypo demonstrated the highest peak insulin levels among all groups. Although, the insulin levels at 150 and 180 min in IsoHypo and Hypo+AGT were significantly

lower than NGT (p=0.013 and p=0.004, respectively), they were inappropriately high in the context of lower glucose.In relation to glucagon, both the IsoHypo and Hypo+AGT groups exhibited inadequate suppression in response to glucose increase during the OGTT. This insufficiency was statistically significant in the Hypo+AGT group (p=0.037). In the IsoHypo group, it remained comparable to NGT and controls, even though glucose levels were significantly higher, suggesting an inadequate suppression. Furthermore, despite the presence of hypoglycemia at 180 minutes in the Hypo+AGT and IsoHypo groups, glucagon levels were comparable to those observed in the NGT and control groups (p=0.192) (Figure 2 and Table 1).

3.3. Effect of Pancreatic Insufficiency (PI) in IsoHypo Group

The participants with IsoHypo and NGT were also sub-classified according to the presence of PI as either PI(+) or PI(-). Glucose level was higher at 30 min (p=0.002) and lower at 180 min (p=0.008) in IsoHypo PI(+) compared to NGT PI(-) and controls, but not in IsoHypo PI(-) (Figure 2 and Table 2).

The insulin response to an oral glucose load in the IsoHypo PI(+) group was lower compared to the IsoHypo PI(-) group and was more akin to the response observed in the Hypo+AGT group (Figure 2). Similarly, the glucagon response pattern to the oral glucose load in IsoHypo PI(+) mirrored that of the Hypo+AGT group, demonstrating insufficient suppression and significantly elevated levels despite the glucose increase at 60 minutes during the OGTT (p=0.045). The glucagon response to declining glucose levels at 150 and 180 minutes in IsoHypo PI(+) was weak and inappropriate. In IsoHypo PI(-) group, glucagon levels at 120 and 150 minutes were significantly higher in comparison to the control group (p<0.05), while glucose levels were declining (Figure 2). Nevertheless, the glucagon response in IsoHypo PI(-) was inadequate to prevent mild hypoglycemia.

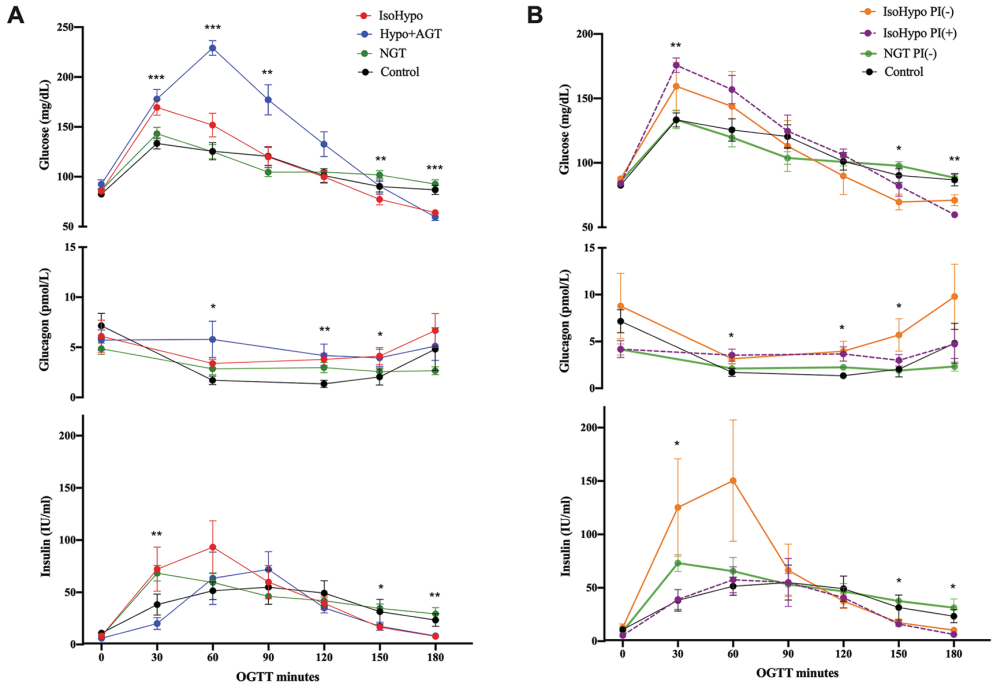

Figure 2. Glucose, insulin, and glucagon changes of the groups during OGTT. In graph A, red line shows the participants with isolated Hypoglycemia (IsoHypo), blue line shows the participants with hypoglycemia with abnormal glucose tolerance (Hypo+AGT), dark green line shows the participants with normal glucose tolerance (NGT), black line shows the healthy controls. Data are presented mean±SE. In graph B, purple line shows the participants with IsoHypo with pancreatic insufficiency [IsoHypo PI(+)], orange line shows the participants with IsoHypo without pancreatic insufficiency [IsoHypo PI(-)], light green line shows the participants with NGT without pancreatic insufficiency [NGT PI(-)], black line shows the healthy controls. Data are presented mean±SE. Statistical significance is indicated as *<0.05, **<0.01, ***<0.001

Table 2. Demographic features and OGTT results of the participants with IsoHypo and NGT according to the presence of pancreatic insufficiency

	IsoHypo PI (-) (n=5)	IsoHypo PI (+) (n=8)	NGT PI (-) (n=11)	Control (n=9)	p
Age (mean)	14.4 ± 1.3	13.0 ± 1.6	14.2 ± 2.4	13.2±1.8	ns
Male, n (%)	5 (100)	5 (63)	5 (45)	4 (44)	0.167
BMI SDS	0.3±1.1 0.4 (-1.4-1.7)	-0.7±0.9 -0.7 (-2.1-0.3)	-0.0±0.7 0.0 (-1.2-1.0)	0.0±0.7 -0.4 (-0.8-1.6)	ns
HbA1c, %	5.6±0.2 5.7 (5.3-5.9)	5.7±0.3 5.8 (5.1-6.0)	5.4±0.3 5.3 (4.8-5.8)	5.2±0.2 5.3 (4.9-5.6)	**0.010**[§,&]
CRP (mg/dL)	3.2±0.1 3.1 (3.1-3.3)	8.9±12 3.6 (3.1-38)	3.5±1.1 3.1 (3.1-6.8)	0.5±1.0 0.2 (0.1-2.9)	**<0.001**[§,&,P]
FEV1, % (mean)	98±16 96 (75-118)	78±35 80 (34-126)	95±6 95 (88-106)		ns
Genotype, n (%)					
ΔF508/ΔF508	-	1 (12.5)	-		ns
ΔF508/nonΔF508	3 (60)	2 (25)	2 (18)		
nonΔF508/nonΔF508	2 (40)	5 (62.5)	9 (82)		
OGTT					
Glucose (mg/dL) mean±SD and median (min-max)					
0. min	88±3 88 (84-91)	84±6 84 (75-93)	85±5 84 (80-97)	83±4 81 (79-89)	ns
30th min	159±41 167 (93-201)	176±16 173 (161-210)	134±22 136 (83-167)	133±16 129 (115-163)	**0.002** [#,&]
60th min	144±61 164 (50-194)	157±31 161 (110-198)	120±24 118 (85-164)	126±26 119 (98-170)	ns
90th min	113±44 109 (68-167)	125±33 142 (77-155)	104±16 106 (77-126)	120±27 112 (93-174)	ns
120th min	90±32 84 (58-125)	106±13 110 (81-122)	101±10 103 (78-112)	101±20 103 (64-130)	ns
150th min	70±14 76 (53-83)	82±23 91 (42-105)	98±10 98 (80-114)	90±17 90 (62-110)	**0.029** [**]
180th min	75±13 81 (57-90)	63±16 59 (46-98)	88±11 86 (70-105)	87±14 89 (59-102)	**0.008** [#,&]
Insulin (mIU/mL) mean±SD and median (min-max)					
0. min	12.9±7.6 14.4 (5.3-24.0)	5.4±2.5 5.7 (1.7-8.6)	9.2±4.5 9.7 (1.3-18.4)	10.9±7.5 8.6 (3.0-23.4)	ns
30th min	125.2±101.9 63.0 (33.1-243.0)	39.0±26.3 34.5 (8.4-89.0)	73.2±25.2 71.0 (35.2-113.1)	38.2±30.1 26.0 (3.9-108.0)	**0.013** [P]
60th min	150.4±126.9 132.6 (14.2-299.0)	57.6±34.4 56.6 (17.4-123)	65.5±42.7 43.9 (24.6-103.0)	51.5±25.4 47.3 (18.8-102)	ns
90th min	66.4±54.8 57.0 (12.3-152.0)	55.1±59.6 36.0 (2.4-181.0)	53.1±32.1 44.5 (18.0-103.0)	54.9±49.0 48.0 (3.4-169.0)	ns
120th min	37.5±15.2 38.3 (12.3-50.6)	40.8±26.7 33.0 (13.2-89.2)	46.7±24.8 39.2 (18.9-89.2)	49.2±35.5 48.3 (13.8-135.0)	ns
150th min	17.3±7.9 14.7 (11.2-31.1)	16.0±7.1 16.6 (1.3-23.8)	37.6±18.5 28.2 (12.2-73.6)	31.5±35.3 24.1 (1.1-119.0)	**0.027** [#]
180th min	17.0±9.3 16.2 (5.2-29.0)	9.3±7.6 7.6 (0.9-26.5)	31.4±27.3 22.5 (9.9-105.0)	23.5±18.2 20.4 (6.1-68.4)	**0.021** [#]

Table 2. Continued

	IsoHypo PI (-) (n=5)	IsoHypo PI (+) (n=8)	NGT PI (-) (n=11)	Control (n=9)	p
Glucagon (pmol/L) mean±SD and median (min-max)					
0. min	8.8±7.8 5.6 (3.5-22.4)	4.2±2.4 3.7 (1.7-8.6)	4.2±1.9 4.1 (1.3-8.9)	7.2±3.7 6.4 (3.7-15.8)	ns
60th min	3.2±1.3 2.9 (1.9-5.0)	3.5±1.7 3.1 (1.8-6.7)	2.1±1.8 1.8 (0.4-6.9)	1.7±1.2 1.7 (0.2-3.5)	**0.045** &
120th min	3.9±2.4 3.1 (2.1-8.1)	3.7±2.0 2.7 (1.7-6.7)	2.2±1.0 2.4 (0.1-3.5)	1.3±1.1 1.2 (0.1-3.3)	**0.011** §
150th min	5.7±3.9 4.7 (1.5-11.7)	3.0±1.6 2.0 (1.4-5.7)	1.9±0.8 1.7 (0.8-4.1)	2.1±2.3 1.3 (0.5-7.6)	**0.047** §
180th min	10.3±7.7 9.6 (1.9-21.8)	4.0±2.9 3.7 (0.6-9.9)	2.3±1.6 2.1 (0.4-6.6)	5.4±6.6 3.3 (1.0-21.2)	ns

Data are presented mean±SD and median (min-max). *IsoHypo PI(-) vs IsoHypo PI(+), **IsoHypo PI(-) vs NGT PI(-), § IsoHypo PI(-) vs Controls, # IsoHypo PI(+) vs NGT PI(-), & IsoHypo PI(+) vs Controls, ᵖNGT PI(-) vs Controls

Discussion

Over the past decade, postprandial or reactive hypoglycemia has been increasingly recognized in patients with CF, although its underlying causes are not fully understood. This study assessed pediatric CF patients with isolated hypoglycemia and explored the role of PI in this condition. The results suggest that isolated hypoglycemia is prevalent among pediatric CF patients and is linked to dysregulated insulin secretion, which involves early and excessive insulin release. Moreover, there was a weakened glucagon response, with insufficient suppression following glucose elevation and an inadequate increase during hypoglycemia. Our findings also suggest that isolated hypoglycemia appears to be an early indicator of hypoglycemia accompanied by abnormal glucose tolerance (Hypo+AGT), but only in individuals with PI. In contrast, isolated hypoglycemia in those with pancreatic sufficiency appears to arise from a distinct mechanism.

The frequency of hypoglycemia observed in this study was comparable to that seen in other 3-hour OGTT studies (6,7,8,10). However, previous research did not differentiate between IsoHypo and Hypo+AGT. In the present study, the frequency of IsoHypo was 61.9%, which is similar to the 66.6% found in our previous pediatric cohort (4). IsoHypo appears to be more common in pediatric CF populations than adult studies, suggesting it may serve as an early indicator of future glucose regulation abnormalities. Delayed and prolonged insulin secretion, along with impaired counterregulatory response, has been proposed as a key factor contributing to reactive hypoglycemia in CF studies (8,9,10,11). In those studies, most hypoglycemic patients had both AGT and PI. In such populations, the delayed and prolonged insulin secretion and/or impaired counterregulatory response are likely causes for hypoglycemia due to the association of AGT with PI. Consistent with this, we also observed β-cell dysfunction in Hypo+AGT group, as reported in other studies on hypoglycemia (8,9,11). However, this mechanism may not apply to CF patients with pancreatic sufficiency (15,16). A critical point in understanding whether other factors, aside from PI, contribute to hypoglycemia is to examine CF patients with isolated hypoglycemia with or without PI. Given that the CFTR mutation distribution in our country differs significantly from that of European and North American cohorts, this genetic variability likely contributes to the higher prevalence of pancreatic sufficiency observed in our cohort. This, in turn, enabled us to evaluate the impact of PI on hypoglycemia more effectively. In the present study, the robust insulin response seen at 30 min during the OGTT in the IsoHypo group indicated a relatively hyperinsulinemic state. When analyzed further, based on the presence of PI, this early and elevated insulin release was found only in the IsoHypo PI(-) subgroup. In contrast, the IsoHypo PI(+) subgroup exhibited a delayed and prolonged insulin secretion, similar to response observed in the Hypo+AGT group.

Glucagon secretion from α-cells has been found to exhibit impaired suppression and a dysregulated response to glucose loading in CF patients with AGT and PI, rather than an absolute reduction (21,22). Recent studies have also reported a diminished glucagon response in individuals with NGT and PI(+), while those with NGT but no PI displayed normal glucagon responses, similar to healthy controls (15). In the present study, both the IsoHypo and Hypo+AGT groups exhibited inadequate glucagon suppression at 60 and 120 min following glucose intake during the OGTT, with the deficiency being more pronounced in the Hypo+AGT group. Subgroup analysis indicated that the IsoHypo PI(+) group had a more pronounced impairment in glucagon suppression, resembling that seen in the Hypo+AGT group, whereas the IsoHypo PI(-) group demonstrated relatively preserved suppression. Furthermore, despite the occurrence of lower glucose at 180 min in both the Hypo+AGT and IsoHypo

groups, glucagon levels at 180 min did not significantly differ from those observed in the NGT and control groups, suggesting an inappropriate glucagon response. When subgroups were compared by the presence or absence of PI, glucagon response to meaningful low glucose at 180 min was weak and insufficient in the IsoHypo PI(+), whereas it remained comparable to the control group in IsoHypo PI(-) (Figure 2B). However, in both groups, the glucagon response was not strong enough to prevent mild hypoglycemia.

Kilberg et al. (8,11) hypothesized that hypoglycemia in PI(-) may reflect a physiological phenomenon similar to that observed in general healthy population, rather than a distinct pathological feature of CF8. In our control group, although the frequency of hypoglycemia was not significantly different from the CF group, the severity was lower than in the IsoHypo PI(-) group. Moreover, two hypoglycemic healthy participants exhibited normal early insulin secretion. Therefore, we believe this effect warrants further investigation in relation to impaired insulin and glucagon secretion in pancreatic-sufficient CF patients. Another possible explanation is the inflammation of islet cells, which has primarily been studied in CF patients with PI (21,22,27). In our cohort, IsoHypo PI(-) and NGT PI(-) groups also had significantly higher CRP levels than healthy controls, suggesting systemic inflammation. This inflammatory condition may contribute to functional abnormalities of islet cells, even in patients with sufficient pancreatic function.

Study Limitations

The main limitations of this study include the lack of frequent sampling for glucagon levels during the early period of the OGTT, which may have resulted in missing important fluctuations. Although our total sample size was relatively large compared with previously published studies in this area, the small number of participants within each subgroup, due to the high number of subgroups analyzed, as well as small sample size of the control group, may limit the generalizability of the findings.

Conclusion

This study provides further evidence suggesting that dysregulated insulin secretion and impaired glucagon response may contribute to hypoglycemia in CF, and that these abnormalities can be observed even in the absence of PI. Isolated hypoglycemia in pediatric CF patients appears to be common and may represent a predecessor Hypo+AGT in pancreatic insufficient CF patients.

Ethics

Ethics Committee Approval: The study protocol was approved by the Marmara University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 09.2019.933, date: 01.11.2019).

Informed Consent: Written informed consent was obtained from participants or their parents.

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Footnotes

Authorship Contributions

Concept: Belma Haliloğlu, Abdullah Bereket, Design: Belma Haliloğlu, Serap Demircioğlu Turan, Abdullah Bereket, Data Collection or Processing: Belma Haliloğlu, Tuba Seven Menevşe, Yasemin Gökdemir, Ela Erdem, Bülent Karadağ, Analysis or Interpretation: Belma Haliloğlu, Seda Güleç Yılmaz, Tuba Akdeniz, Büşra Gürpınar Tosun, Turgay İşbir, Literature Search: Belma Haliloğlu, Tuba Seven Menevşe, Writing: Belma Haliloğlu, Tülay Güran, Abdullah Bereket.

Conflict of Interest: One author of this article, Abdullah Bereket, is a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, he did not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other authors declared no conflict of interest.

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