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Research Article

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

Haliloglu B et al. Isolated Hypoglycaemia in Children with CF

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What is already known?
The possible mechanisms of hypoglycaemia in CF are the delayed and prolonged insulin secretion and impaired counterregulatory hormones. However, CF patients in these studies had hypoglycaemia with abnormal glucose tolerance (Hypo+AGT) and pancreatic insufficiency (PI).
What this study adds?
Isolated hypoglycaemia 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 hypoglycaemia. IsoHypo PI(+) might be a predecessor to Hypo+AGT, whereas IsoHypo PI(-) represent a milder impairment in glucose homeostasis in CF.

Abstract
Background: Hypoglycaemia is one of the comorbidities that adversely affects the quality of life in patients with cystic fibrosis (CF). Isolated hypoglycaemia (IsoHypo) is poorly described in patients with CF and its aetiopathogenic significance is unclear.
Aim: To investigate the etiopathogenesis of IsoHypo and the role of pancreatic insufficiency (PI) in IsoHypo in children with CF.
Patients and Methods: The blood glucose, insulin, and glucagon responses of 44 patients with CF and 9 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(-). Hypoglycaemia was defined as <70 mg/dL.
Results: Hypoglycaemia was observed in 21 of 44 patients (47.7%), predominantly as IsoHypo (29.5%). Hypo+AGT was found in 8 patients (18.2%). The IsoHypo group showed undelayed and higher insulin secretion than the Hypo+AGT group, especially in IsoHypo PI(-) compared to IsoHypo PI(+). Both IsoHypo and Hypo+AGT groups exhibited an insufficient increase 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 affected in IsoHypo PI(-) patients who had early and higher insulin secretion.
Conclusion: IsoHypo is common in CF children and might precede Hypo+AGT in those with pancreatic insufficiency. The abnormal insulin and glucagon responses to glucose are the most significant contributors to the development of IsoHypo in CF.
Key words: Cystic fibrosis, hypoglycaemia, insulin, glucagon, children

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1.Introduction

In recent years, spontaneous or reactive hypoglycaemia 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 hypoglycaemia and the duration of OGTT[1-7]. It is more frequently observed in 3-hour OGTTs, affecting approximately 45 to 65% of CF patients[6-10].

One of the key pathophysiological features observed during OGTT in CF is a disruption in 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 hypoglycaemia[8-11]. 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 AGT and PI. Furthermore, an inadequate glucagon increment in response to hypoglycaemia (inappropriate response) may also contribute to the development of reactive hypoglycaemia in these patients [8,9,11]. Most studies investigating hypoglycaemia in CF have focused on individuals with pancreatic insufficiency (PI) and have not included healthy control groups. Moreover, participants experiencing hypoglycaemia in these studies often exhibited abnormal glucose tolerance (AGT)[8-11]. In our previous research, we identified cases of isolated hypoglycaemia (IsoHypo) during OGTT in some CF children who had normal glucose tolerance[4]. This study aims to explore the mechanisms underlying IsoHypo and to assess the impact of PI on hypoglycaemia in CF. We examined glucose, insulin, and glucagon responses to a glucose loading in CF patients with and without PI, as well as in healthy controls.

2. Methods

2.1. 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 3 months, or those with a prior diagnosis of diabetes were excluded from the study. Pancreatic insufficiency (PI) was defined as the need for enzyme replacement therapy due to clinical symptoms or a faecal 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 due to underlying pulmonary involvement. None of the patients were on CFTR modulatory treatments. Additionally, none of the patients had overt liver disease. A total of 61 participants (51 with CF and 10 controls) were recruited. Two CF participants experienced symptomatic hypoglycaemia, necessitating the early termination of their OGTT. Additionally, two CF participants and one control were unable to complete the OGTT due to nausea and discomfort. One CF participant with obesity and severe insulin resistance was also excluded. Samples from two CF participants were removed from the analysis due to improper sampling or storage. As a result, 53 participants (44 with CF and 9 controls) were included in the final analysis (Figure 1). The data collected included height, weight, body mass index (BMI), forced expiratory volume in one second (FEV1), molecular aetiology, 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 Ethics Committee (approval no:09.2019.933), and written informed consent was obtained from participants or their parents.

2.2. Procedures

A 3-h 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. Additionally, HbA1c and CRP levels were measured at baseline to evaluate the glucose metabolism and systemic inflammation, respectively.

OGTT results were classified based on the ISPAD guidelines[12]. Participants with INDET (Indeterminate Glucose Tolerance) or IGT (Impaired Glucose Tolerance) without hypoglycaemia were categorized as Abnormal Glucose Tolerance (AGT). The terminology of “isolated hypoglycaemia” (IsoHypo) was used for those who experienced hypoglycaemia with NGT (Normal Glucose Tolerance). The term “Hypo+AGT” was applied to the participants who had both hypoglycaemia and AGT. According to the International Hypoglycaemia Study Group (IHSG) position statement, hypoglycaemia is defined as any venous glucose level below 70 mg/dL[13]. Although, in individuals not receiving glucose-lowering treatments, the threshold for defining hypoglycaemia is recommended as <54 mg/dL, venous glucose level below 70 mg/dL was chosen as hypoglycaemia 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 hypoglycaemia (Figure 1). The NGT PI(-) group was selected as the reference group for hormonal comparisons, as these patients had normal glucose tolerance and preserved pancreatic function, making them the relatively healthiest CF subgroup. This group was therefore considered the most appropriate baseline for evaluating the pathophysiology of isolated hypoglycaemia.

2.3. 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 analysed using the glucose hexokinase method (Cobas c701/702, Roche), while insulin was measured by ECLIA (Cobas e801 Roche). 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.

2.4. Statistical Analysis

All analyses and graphs were conducted using SPSS (version 20, IBM software) and GraphPad Prism® V5.0 software (GraphPad Software Inc., San Diego, California, USA) respectively. For groups with fewer than five patients (CFRD 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 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 $p < 0.05$.

3. Results

3.1. Baseline characteristics and prevalence of hypoglycaemia

A total of 44 CF patients and 9 healthy controls were included. Based on the results, the patients were categorized into 5 groups: 1) normal glucose tolerance (NGT; n=16), 2) isolated Hypoglycaemia (IsoHypo; n=13), 3) hypoglycaemia with abnormal glucose tolerance (Hypo+AGT; n=8), 4) AGT (n=5), and 5) CF-related diabetes (n=2).

The frequency of hypoglycaemia 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 <0.001, respectively) but no difference was observed among 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). Hypoglycaemia was significantly higher in CF participants with PI(+) than PI(-) ($p=0.006$).

3.2. Differences in OGTT characteristics among IsoHypo, Hypo+AGT, NGT in CF and healthy control groups

While the time of peak glucose was at 30 min in IsoHypo group similar with NGT and controls, it was delayed in Hypo+AGT (at 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 0.004, respectively), they were inappropriately high in the face of lower glucose.

In relation to glucagon, both the IsoHypo and Hypo+AGT groups exhibited inadequate suppression in response to the 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 hypoglycaemia 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 comparison with 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 hypoglycaemia.

4. Discussion

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

The frequency of hypoglycaemia observed in this study was comparable to that seen in other 3-hour OGTT studies[6-8,10]. 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 paediatric cohort[4]. IsoHypo appears to be more common in paediatric 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 hypoglycaemia in CF studies[8-11]. In those studies, most hypoglycaemic patients had both AGT and PI. In such populations, the delayed and prolonged insulin secretion and/or impaired counterregulatory response are likely causes for hypoglycaemia due to the association of AGT with pancreatic insufficiency. In line with that, our study also observed β-cell dysfunction in Hypo+AGT group, as reported in other studies on hypoglycaemia[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 pancreatic insufficiency, contribute to hypoglycaemia is to examine CF patients with isolated hypoglycaemia and/or without pancreatic insufficiency. 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 hypoglycaemia more effectively. In our study, the robust insulin response seen at 30 min ($p=0.002$) during the OGTT in the IsoHypo group indicates a relatively hyperinsulinemic state. When analysed further based on the presence of PI, this early and elevated insulin release was found only in the IsoHypo PI(-) subgroup ($p=0.013$). 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 and 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 ($p=0.037$ and 0.009, respectively). Subgroup analysis indicated that the IsoHypo PI(+) group had a more pronounced impairment in glucagon suppression ($p=0.045$), 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 ($p<0.001$), glucagon levels at 180.min did not significantly differ from those observed in the NGT and control groups ($p>0.05$), suggesting an inappropriate glucagon response. In regards to the presence of PI, glucagon response to meaningful low glucose at 180.min ($p=0.008$) 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 hypoglycaemia.

Kilberg et al. hypothesized that hypoglycaemia in PI(-) may not be differ significantly from that observed in the normal population as a functional experience in nature[8]. In our control group, although the frequency of hypoglycaemia was not significantly different from CF group, the severity were lower than IsoHypo PI(-). Additionally, two hypoglycaemic 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 pancreatic insufficient CF patients[21,22,27]. In our cohort, IsoHypo PI(-) and NGT PI(-) groups also had significantly higher CRP levels than healthy controls, indicating 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 is 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 analysed, as well as the relatively 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 hypoglycaemia in CF, and that these abnormalities can be observed even in the absence of pancreatic insufficiency. Isolated hypoglycaemia in paediatric CF patients appears to be common and may represent a predecessor for Hypo+AGT in pancreatic insufficient CF patients.

Author Contributions:

B.H. organized and designed the study, analysed data, and wrote the manuscript, T.S.M. participated in the conduct of the study and edited the manuscript, T.A, S.G.Y and T.I. analysed samples in the laboratory and edited manuscript, B.G.T. analysed data, S.D, T.G, Y.G, E.E. and B.K. contributed to discussion and reviewed the manuscript, A.B. designed the study, contributed to discussion, reviewed and edited the manuscript. All authors approved the final version of the manuscript.

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Conflict of Interest: There is no conflict of interest

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Table-1: Demographic features and OGTT results of the participants

	Hypoglycaemia (+)		Hypoglycaemia (-)				
	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
30. 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****,§,#,&
60. 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***,#,&
90. 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**#,&
120. 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
150. 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****
180. 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****,#,&
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
30. 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**#
60. 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
90. 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

120. 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
150. 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****
180. 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
60. 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**$^{\&}$
120. 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**$^{\S,\&}$
150. 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**$^{\&}$
180. 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, □NGT vs Controls

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
30. min	159±41 167 (93-201)	176±16 173 (161-210)	134±22 136 (83-167)	133±16 129 (115-163)	**0.002**[#,&]
60. min	144±61 164 (50-194)	157±31 161 (110-198)	120±24 118 (85-164)	126±26 119 (98-170)	ns
90. min	113±44 109 (68-167)	125±33 142 (77-155)	104±16 106 (77-126)	120±27 112 (93-174)	ns
120. min	90±32 84 (58-125)	106±13 110 (81-122)	101±10 103 (78-112)	101±20 103 (64-130)	ns
150. min	70±14 76 (53-83)	82±23 91 (42-105)	98±10 98 (80-114)	90±17 90 (62-110)	**0.029** [**]
180. 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
30. 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]
60. 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
90. 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
120. 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
150. min	17.3±7.9	16.0±7.1	37.6±18.5	31.5±35.3	**0.027**[#]

	14.7 (11.2-31.1)		16.6 (1.3-23.8)		28.2 (12.2-73.6)		24.1 (1.1-119.0)		
180. 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** [#]
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
60. 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** [&]
120. 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** [§]
150. 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** [§]
180. 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, □NGT PI(-) vs Controls									

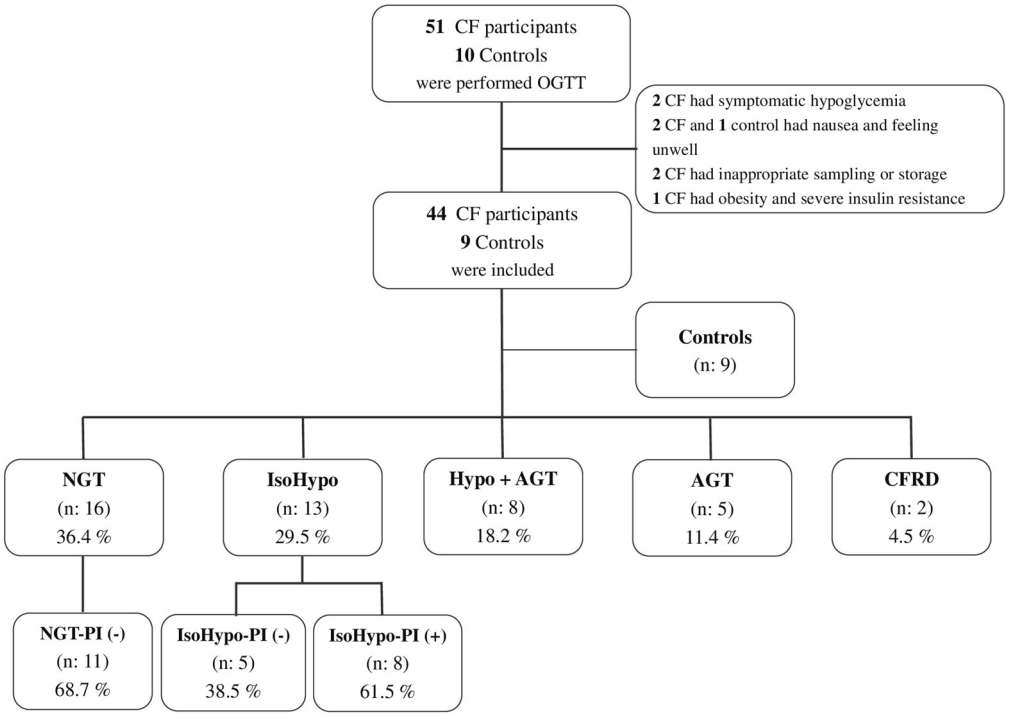

Figure 1: The distribution of the OGTT results

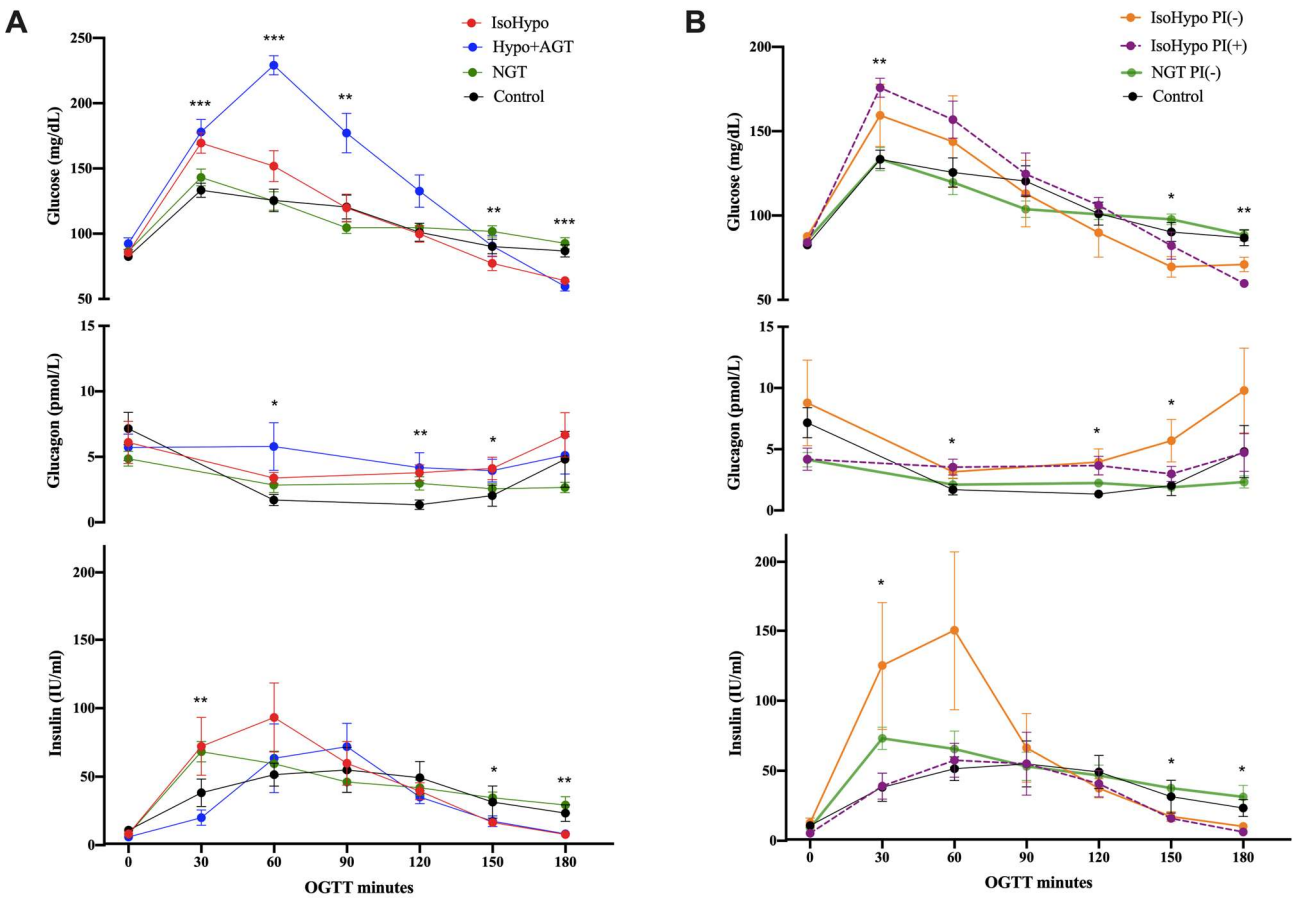

Figure 2: Glucose, insulin, and glucagon changes of the groups during OGTT. *In graph A*, red line shows the participants with isolated Hypoglycaemia (IsoHypo), blue line shows the participants with hypoglycaemia with abnormal glucose tolerance (Hypo+AGT), dark green line shows the participants with 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 $^{*}<0.05$, $^{**}<0.01$, $^{***}<0.001$