T.C.
YEDITEPE UNIVERSITY
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THE ROLE AND EFFECT OF GLUCAGON IN GLUCOSE REGULATION IN CHILDREN WITH CYSTIC FIBROSIS

PHD THESIS

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for an award of any other degree except where due acknowledgment has been made in the text.

13.11.2021

BELMA HALILOGLU

DEDICATION

I dedicate my thesis to my mother Ifakat Haliloglu,

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Firstly, I want to express my best regards and gratitude to Prof.Dr.Turgay ISBIR for helping me on my way to becoming a PhD, by training me how to do study and leaving his personal imprint on my life. Professor Dr.Turgay ISBIR inspires me to become an educator and scientist.

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LIST OF SYMBOLS AND ABBREVIATIONS

ADA: American Diabetes Association
AGT: Abnormal Glucose Tolerance
BG: Blood Glucose
CF: Cystic Fibrosis
CFRD: Cystic Fibrosis Related Diabetes
CFTR: Cystic Fibrosis Transmembrane Regulator
CGM: Continuous Glucose Monitoring
CVS: Cardiovascular System
EASD: European Association for the Study of Diabetes
GLC: Glucagon
IGT: Impaired Glucose Tolerance
IHSG: International Hypoglycemia Study Group
INDET: Indeterminate
NGT: Normal Glucose Tolerance
OGTT: Oral Glucose Tolerance Test
T1D: Type 1 Diabetes
T2D: Type 2 Diabetes

ABSTRACT

HALILOGLU B. 2021. The Role And Effect Of Glucagon In Glucose Regulation In Children With Cystic Fibrosis. Yeditepe University Institute of Health Sciences, Doctorate Thesis, Istanbul

Post-oral glucose tolerance test-related hypoglycemia is common and important issue in Cystic Fibrosis (CF). The underlying mechanisms of hypoglycemia are still controversial. The current mice studies show that CFTR gene is identified in alpha cells and regulates glucagon secretion. The aim of the study is to determine of the role and effect of glucagon in CF patients with hypoglycemia. A 3-hour OGTT was performed in 45 non-diabetic children with CF and 9 age-matched healthy controls. The subjects were classified based on OGTT as isolated hypoglycemia, hypoglycemia with abnormal glucose intolerance (AGT), AGT, CF related diabetes (CFRD) and normal glucose tolerance (NGT). After CFRD subjects were excluded, the demographic characteristics and plasma glucose, insulin and glucagon levels obtained at 0,60,120,150 and 180.min during OGTT were assessed. Isolated hypoglycemia group did not differ from NGT and controls in terms of age, sex, BMI and genetics. The frequency of isolated hypoglycemia, hypoglycemia + AGT, AGT and NGT were 31%(n=14), 18%(n=8), 11%(n=5) and 35.5%(n=16), respectively. Level 2 hypoglycemia (<54 mg/dL) was observed in 15% of the subjects. Basal glucagon level in NGT was significantly lower than the controls ($p<0.05$) and glucose-stimulated glucagon response were not observed in NGT. The glucagon levels at 60, 120 and 150.min in isolated hypoglycemia were significantly higher than controls ($p<0.01$, $p<0.01$, $p<0.05$, respectively). However, the statistically significant difference did not observe at 180.min ($p=0.17$) despite of higher insulin levels. These findings can be interpreted as the glucagon secretion is still sensitive to glycemic changing in isolated hypoglycemia group but not properly, whereas it was blunted in NGT. It might be suggested that NGT is more pathologic state than isolated hypoglycemia in terms of glucagon secretion.

Keywords: Cystic Fibrosis, Hypoglycemia, Glucagon

ÖZET

HALİLOĞLU B, 2021, Kistik Fibrozisli Çocuklarda Glukagonun Glukoz Regülasyonundaki Rolü ve Etkisi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, İstanbul

Kistik Fibrozis (KF)'de oral glukoz tolerans testi (OGTT) sonrası hipoglisemi sık görülen önemli bir durumdur. Mekanizması halen bilinmemektedir. Son dönemlerde CFTR proteinin alfa hücrelerinde de bulunduğunu glukagon salınımda rol oynadığını gösteren fare çalışmaları yayınlanmıştır. Bu çalışma ile KF hipoglisemisindeki glukagonun rolü ve etkisinin araştırılması planlanmıştır. Bilinen diyabeti olmayan 45'i KF'li ve 9'u sağlıklı çocuğa 3-saatlik OGTT yapıldı. OGTT sonuçlarına göre izole hipoglisemi, bozulmuş glukoz toleransı ile beraber hipoglisemi, bozulmuş glukoz toleransı, KF ilişkili diyabet (KFİD) ve normal glukoz toleransı (NGT) olarak hastalar sınıflandırıldı. KFİD olanlar dışlandıktan sonra hastaların demografik özellikleri ve OGTT sırasında 0,60,120,150 ve 180.dk da alınan glukoz, insülin ve glukagon düzeyleri değerlendirildi. Yaş, cinsiyet, VKİ ve genetik sonuçlar açısından izole hipoglisemi grubu ile NGT ve kontroller arasında anlamlı bir fark saptanmadı. Hastaların %31(n=14)'nde izole hipoglisemi, %18(n=8)'de hipoglisemi ile beraber AGT, %11(n=5)'inde AGT ve %35.5(n=16)'de ise NGT saptandı. Düzey 2 hipoglisemi (<54 mg/dL) hastaların %15'inde görüldü. NGT'de bazal glukagon düzeyi kontrole göre anlamlı olarak düşüktü ($p<0.05$) ve glukoz değişimine bağlı glukagon salınımı ve baskılanması körelmişti. İzole hipoglisemi grubunda 60, 120 ve 150.dk glukagon düzeyleri kontrole göre anlamlı yüksekti (sırasıyla $p<0.01$, $p<0.01$, $p<0.05$) ve yine daha yüksek bir 180.dk insülin düzeyi olmasına rağmen istatistiksel anlam gözlenmedi ($p=0.17$). Bu çalışmada NGT olanlarda körelmiş glukagon yanıtı görülürken izole hipoglisemisi olanlarda glukagon yanıtı yeterli olmamakla beraber halen glukoz değişikliklerine duyarlılığın olduğu görülmüştür. Bu sonuçlarla glukagon salınımının NGT olanlarda izole hipoglisemisi olanlardan daha fazla etkilendiği öngörülebilir.

Anahtar Kelimeler: Kistik Fibrozis, Hipoglisemi, Glukagon

1. INTRODUCTION AND PURPOSE

Improved life-expectation in patients with Cystic Fibrosis (CF) has led to some additional consequences and complications of the disease. One of these complications is Cystic Fibrosis Related Diabetes (CFRD) and it has emerged as a major comorbidity affecting nearly 40% of adults aged >30 years (1).

In CF patients, a different type of glucose disturbance has also become apparent. Following oral glucose tolerance test (OGTT), hypoglycemia has been reported in both children and adults with prevalence varying from 7-30% depending on the definition of hypoglycemia and the duration of the OGTT (2-5).

The etiology of spontaneous hypoglycemia in CF is still unknown. Malnutrition combined with high energy expenditure because of underlying inflammation or acute infection has been linked to fasting hypoglycemia in CF patients. It was also linked to dysregulated basal insulin secretion in one research. This study found that in non-diabetic patients with CF, basal insulin and c-peptide levels were not appropriately decreased with lower fasting plasma glucose (2).

Post-prandial or reactive hypoglycemia might be caused by delayed and prolonged insulin release with blunted counterregulatory hormone response. In one study, the authors showed that CF patients with hypoglycemia during OGTT had a delayed insulin response and a greater insulin level at 120 min than patients without hypoglycemia (4). In a recent study, the authors found that patients who experienced hypoglycemia at 120 min had lower insulin levels than patients who did not. Furthermore, they speculated that abnormal glucagon and incretin hormones might cause reactive hypoglycemia (6).

In recent studies, the CFTR gene has been shown to regulate insulin and glucagon release from the pancreatic beta and alpha cell, respectively. Following CFTR inhibition in alpha cells, glucagon secretion was increased in response to glucose (7). However, contrary to the these findings, the authors also concluded that the observed reduction in insulin secretion was exactly proportionate to the decreasing in insulin content and did not develop as a result of a CFTR-induced beta cell insulin secretory dysfunction. However, results from human islets differ. On both sides, compelling and

strong data has been given, more research are being conducted to shed light on the subject (7-10).

Based on these findings, we performed a cross-sectional, exploratory study to understand the role and effect of glucagon in hypoglycemia in CF patients. Our initial hypothesis was that the post-glucose load hypoglycemia seen in patients with CF is caused by an excessive and delayed insulin response and/or a defective glucagon response. Since hypoglycemia in CF patients usually observe in late period of glucose loading, we performed 3-hour OGTT with frequent sampling to detect more hypoglycemia. Since, we also hypothesized that the CF patients with normal glucose tolerance (NGT) could not be “real” normal, a healthy control group was included in the study.

2. LITERATURE REVIEW

2.1 Cystic Fibrosis (CF)

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease in White population and was firstly described by Andersen in 1938 (11). The incidence varies in worldwide. In USA, the incidence is 1 in 3500 births, whereas is reported to be 1 in 2000-3000 births in European countries (12). CF is caused by mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (*CFTR*) protein. CFTR protein is a chloride/bicarbonate channel, which is found on the long arm of chromosome 7 and spans 230 kb with 27 coding exons (13). This channel is a member of the ATP-binding cassette membrane transporter gene superfamily that is a class of transporters that also includes the sulfonylurea receptor. The main function of the CFTR is the outward movement of Cl^- and the linear movement of water across cell membranes but also regulates some other membrane proteins such as chloride channel ORCC, Cl^- /HCO_3^- exchanger, the epithelial sodium channel ENaC. (14,15).

Ever since the discovery of the *CFTR* gene in 1989, approximately 2000 variants have been reported to the Cystic Fibrosis Mutation Database (16). CFTR mutations are categorized into five groups according to the amount of protein present at the cell surface membrane, structural abnormalities and its function. Class I, II and III mutations are associated with more severe phenotypes, whereas class IV and V mutations cause milder phenotype (17). Furthermore, the expression of dysfunctional CFTR gene changes in various tissues and this difference results with the heterogeneity of the clinical manifestations in individuals with same mutation (18).

CFTR dysfunction results with an abnormal ion and water transport in several organs including the lung, gastrointestinal tract, pancreas and liver. In addition to these major systems, sinusitis, nasal polyposis, osteoporosis and fertility issues might be seen in CF. Nearly 85% of CF patients have pancreatic involvement with early onset of clinical exocrine pancreatic insufficiency because of the high expression of CFTR

protein in pancreatic ducts. The impaired chloride and bicarbonate transport in the ducts cause high protein concentration, fluid volume reduction and alteration in pH. As a result of these impairments ductal obstruction, pancreatic destruction and atrophy are occurred and leads to both of exocrine and endocrine insufficiency (19,20).

2.2 Cystic Fibrosis Related Diabetes (CFRD)

As the lifespan of patients with CF continues to increase, comorbid conditions appear with age, especially CFRD. CFRD is the most common comorbidity in CF patients and there is an age-related increase with a frequency of approximately 20% in adolescents and 40-50% in adults (1). The deleterious effects of hyperglycemia on pulmonary function, nutritional status and survival usually occur before the diagnosis of overt CFRD (21). Considering that the insulin has anabolic effect, it was shown that early administration of insulin in CF patients with early glucose abnormalities improves the nutrition, lung function and survival (22).

2.2.1 Pathophysiology of CFRD

The main hypothesis for the development of CFRD is that islets are lost as a consequence of fibrotic progression. The reason of the development of fibrotic progression in pancreas is reduced chloride and bicarbonate secretion that leads to acidification of the pancreatic juices and increased production of mucins, causing to pancreatic ductal obstruction (20). Particularly class I-III mutations in CFTR gene cause exocrine pancreatic insufficiency and this processes lead to autodigestion of the pancreatic ducts and finally islet cell destruction. Other hypothesis for the development of CFRD are based on whether CFTR is expressed in the islet and mutations in CFTR gene lead to intrinsic or extrinsic defects that finally provoke reduction in insulin secretion (23). Boom et al reported that low-level CFTR mRNA could be identified in rat beta cells and alpha cells showing higher levels of the gene (24). In studies on CFTR-deficient cell lines and on islets from F508del mutant mice, insulin secretion was demonstrated decreased independently islet cell destruction (7,8).

The CFTR gene has been shown to regulate glucagon release from the pancreatic alpha cell in addition to insulin secretion. Following CFTR inhibition in

human cells, glucagon secretion was increased in response to glucose. On the other hand, depolarization-induced glucagon secretion was unaffected. The authors indicate that CFTR modulates alpha cell membrane potential primarily using a mathematical model of alpha cell electrophysiology. Increased glucagon release was also consistently seen in the islets of F508del mice. It was discovered through overexpression studies in the alpha TC1-9 cell line that wild type CFTR functions as a glucose-sensing negative regulator of glucagon release and that abnormalities in this mechanism may contribute to glucose intolerance (7,25).

However, contrary to the findings of the these findings, the authors also concluded that the observed reduction in insulin secretion was exactly proportionate to the decreasing in insulin content and did not develop as a result of a CFTR-induced beta cell insulin secretory dysfunction. Furthermore, the mice in this study developed insulin resistance as they aged (7). Hart et al found significant intra-islet inflammation and a 65% decrease in beta cell area in CF, indicating that these are the major reasons of the development of CFRD (26).

The main hypothesis proposes that pancreatic islets are destroyed as a result of pancreatic fibrosis, and it is a plausible explanation for how diabetes develops in older CF patients with established pancreatic disease. However, decreasing in beta cell mass (but not islet mass) has been documented in children, independent of the severity of pancreatic fibrosis, suggesting that additional mechanisms beyond autodigestion and fibrosis of the pancreas may be involved in controlling beta cell mass in CF. Furthermore, the recent findings from a study showed that over half of children tested have glucose responsiveness abnormalities, suggest that the main hypothesis may not fully explain diabetes onset in CF patients. According to the literature, CFTR is detectable in mouse islets and also rodent beta lines. However, results from human islets differ. On both sides, compelling and strong data has been given, more research are being conducted to shed light on the subject (7-10).

2.2.2 Clinical Features of CFRD

CFRD has been identified as a unique form of diabetes and the distinct features between type 1 diabetes (T1D), type 2 diabetes (T2D) and CFRD are summarized in Table-1. The classical characteristic feature of the CFRD is the partial loss or dysfunction of pancreatic islets causing insulin deficiency but not absolute absence of insulin secretion. In addition, insulin resistance due to chronic baseline inflammation and flares during infection contributes to the development of CFRD (27).

Table 1: Distinct features of CFRD, T1D and T2D

	Type 1 Diabetes	**Type 2 Diabetes**	**CFRD**
Onset	Acute	Insidious	Insidious
Peak age at onset	Children and adolescents	Adults	Young adults
Insulin secretion	Severely decreased to absent	Decreased	Severely decreased but not absent
Insulin sensitivity	Somewhat decreased	Severely decreased	Somewhat decreased*
Antibody positivity	Yes	No	Rare
Treatment	Insulin	Diet, OAD, insulin	Insulin
Diet	Monitoring of carbohydrates	Monitoring of carbohydrates	High calorie diet
Microvascular complication	Yes	Yes	Yes
Macrovascular complication	Yes	Yes	No
Cause of death	CVS disease and nephropathy	CVS disease	Pulmonary disease

* Severely decreased during acute illness

The earliest insulin defect in CF patients is loss of first phase insulin secretion and followed by progressive decreasing of insulin secretion to oral glucose (28). The continuous glucose monitoring (CGM) systems can detect early glucose abnormalities in CF patients with normal glucose tolerance (NGT) by oral glucose tolerance testing (OGTT) and more than half of CF patients with NGT have intermittent post-prandial glucose levels >200 mg/dL (29). Glucose tolerance in CF usually progresses through stages of indeterminate glycaemia (INDET), impaired glucose tolerance (IGT), CFRD without fasting hyperglycemia and CFRD with fasting hyperglycemia. Although

glucose tolerance in some patients may show fluctuations depending on the infectious status, the overall trend is towards diabetes (30).

2.3. Hypoglycemia in CF

2.3.1 Definition of Hypoglycemia

Hypoglycemia is a condition characterized by low plasma glucose that puts patients at risk for death or injury (31). In individuals that use insulin treatment or insulin secretagogue, the glycemic thresholds that trigger hypoglycemia symptoms and counterregulatory hormone response might be impaired. Patients with poorly managed diabetes and low exposure to hypoglycemia have a higher threshold, while patients with frequent hypoglycemia have a lower threshold (31). Therefore, establishing a single plasma glucose threshold value to define hypoglycemia would be inappropriate.

The International Hypoglycemia Study Group (IHSG) has published a position statement on the glucose level that should be used to define hypoglycemia in clinical studies (32) (Table 2). It has been endorsed by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Neuroglycopenic symptoms appear at plasma glucose level of 54 mg/dL and immediate treatment is necessary to avoid a severe hypoglycemia event.

Table 2: Definition of hypoglycemia

Level	Glycemic Definition
Level 1	Glucose <70 mg/dL
Level 2	Glucose < 54 mg/dL
Level 3	A severe event characterized by altered mental and/or physical status requiring assistance

2.3.2 Physiological Response to Hypoglycemia

In healthy people, decreasing in plasma glucose levels activates a hierarchy of physiological changes that are begun to restore normal glucose levels (33). In normal physiology, lowering glucose levels leads to firstly a decrease in insulin levels, which is followed by a rise in counterregulatory hormones such as glucagon, adrenaline, cortisol

and growth hormone. When glucose levels fall but remain within the physiological range (80 mg/dL), insulin levels begin to decline. A further decreasing in plasma glucose to 68 mg/dL induces increment in glucagon and epinephrine levels (33). Glucagon stimulates glycogenolysis and gluconeogenesis, inhibits hepatic glucose uptake, resulting in increased plasma glucose. Epinephrine promotes hepatic glycogenolysis and gluconeogenesis, inhibits insulin secretion and also insulin-induced glucose uptake, increases lipolysis. In addition to providing sources of energy, the sympathetic nervous system activation causes to release of catecholamine and results in development of typical neurogenic symptoms of hypoglycemia.

In the recovery from hypoglycemia, glucagon is considered to play a primary role and epinephrine a secondary, in addition to suppressing insulin levels (34). The effects of cortisol and growth hormone in hypoglycemia appear several hours later and they may not be essential for recovery from acute hypoglycemia (35).

2.3.3 Hypoglycemia in CF and Diabetes

The people with T1D and T2D have impaired physiological defenses against hypoglycemia. Exogenous insulin treated diabetic patients are unable to reduce insulin levels in the setting of falling glucose levels (36). The glucagon response to hypoglycemia is also impaired in T1D patients and this is linked to beta cell failure.

Hypoglycemia induced glucagon response has been demonstrated to be reduced in patients with CF and exocrine pancreatic insufficiency (37). However, the patients in this research recovered normally from hypoglycemia because of normal epinephrine response. The CFTR protein may be expressed in alpha cells and CFTR mutations may lead to dysregulated glucagon secretion in CF patients (26), even though this is controversial (25).

2.3.4 Hypoglycemia in CF in the Absence of Diabetes

In CF patients, spontaneous hypoglycemia in the absence of glucose lowering therapies can develop in both fasting and post-prandial state. It has been observed during OGTT and in continuous glucose monitoring (CGM) (4). The etiology of spontaneous hypoglycemia in CF is still unknown. Malnutrition combined with high energy expenditure because of underlying inflammation or acute infection has been

linked to fasting hypoglycemia in CF patients. It was also linked to dysregulated basal insulin secretion in one research. This study found that in non-diabetic patients with CF, basal insulin and c-peptide levels were not appropriately decreased with lower fasting plasma glucose (2).

Post-prandial or reactive hypoglycemia might be caused by delayed and prolonged insulin release with blunted counterregulatory hormone response. In one study, the authors showed that CF patients with hypoglycemia during OGTT had a delayed insulin response and a greater insulin level at 120 min than patients without hypoglycemia (4). In a recent study, the authors found that patients who experienced hypoglycemia at 120 min had lower insulin levels than patients who did not. Furthermore, they speculated that abnormal glucagon and incretin hormones might cause reactive hypoglycemia (6).

2.4 Syntheses and Secretion of Glucagon

Glucagon is a 29 amino acid peptide hormone secreted by pancreatic alpha cells in the islet and has an important effect to maintain glucose homeostasis by increasing hepatic glucose synthesis. High glucose levels suppress glucagon levels, whereas low plasma glucose concentrations are one of the most powerful glucagon secretory stimuli. As a result, strict normal glucose range is primarily dependent on the balanced secretion of insulin and glucagon by pancreatic beta and alpha cells, respectively (38,39).

Glucagon originates from the precursor proglucagon. Proglucagon is produced by pancreatic alpha cells, intestinal enteroendocrine L-cells and neurons in the brain. The processing enzymes prohormone convertase 1/3 (PC1/3) and prohormone convertase 2 (PC2) are responsible for the processing of proglucagon. PC2 converts it to glucagon, GRPP, IP1 and MPGF in the pancreas, whereas PC1 processes proglucagon to glucagon like peptide 1 (GLP-1), glukagon like peptide 2 (GLP-2), oxyntomodulin, IP2 and glicentin in the gut and brain (Figure 1) (38). Glucagon, oxyntomodulin and glicentin are products of proglucagone and shared same amino acides (residues 33-61). Therefore, measurements of these three glucagon-containing peptides come with some difficulties in terms of specificity.

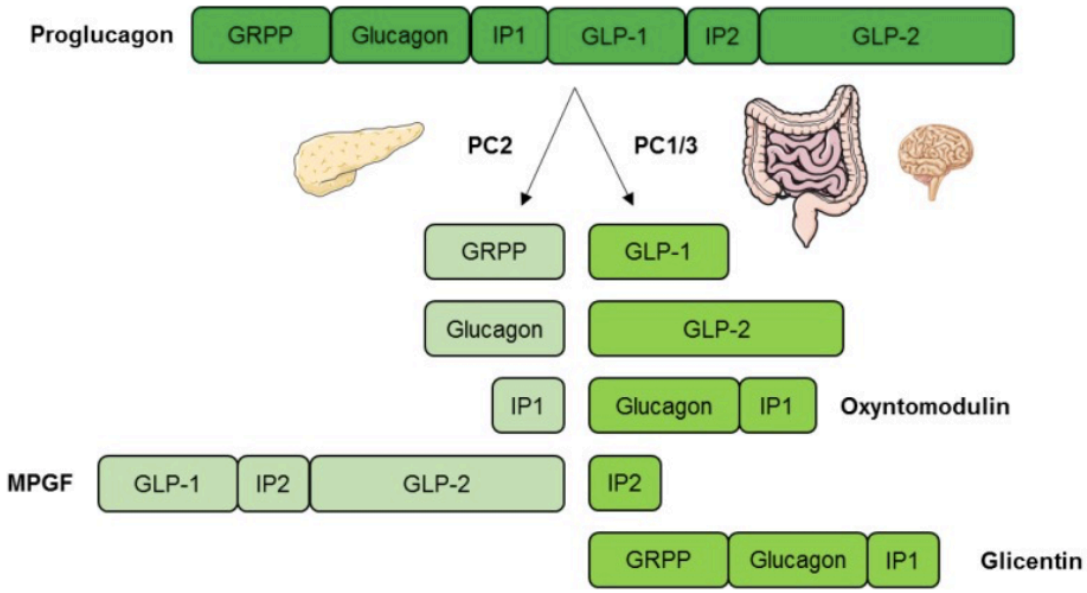

Figure 1: Processing of proglucagon.

Glucagon concentration in normal conditions is in the picomolar range and it is released in baseline levels resulting in plasma concentrations below 20 pmol/L in the fasting state with plasma glucose levels about 90 mg/dL. Circulating glucagon levels can rise to 3-4 times basal levels during exercise or hypoglycemia in healthy people (38,39).

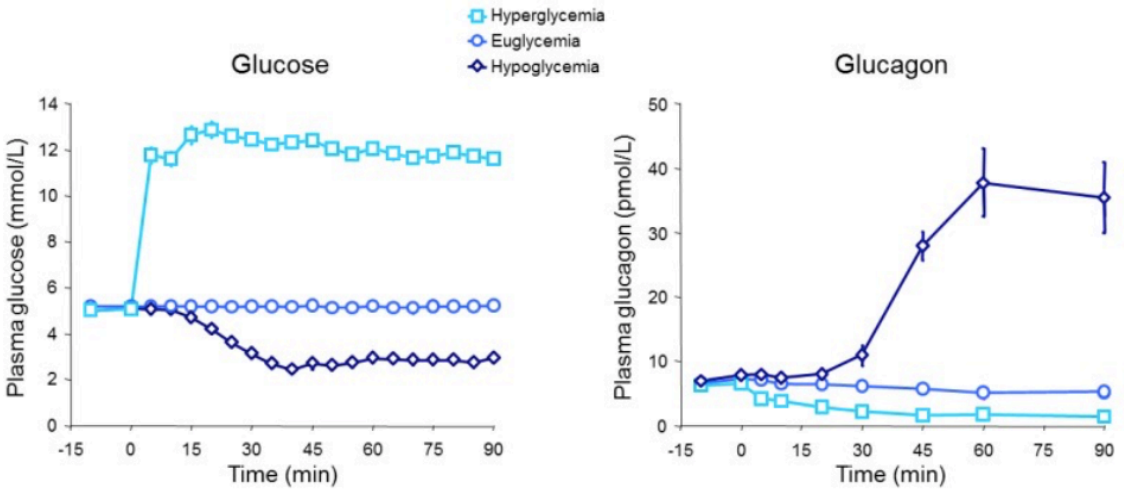

Figure 2: Physiological glucagon response to hypoglycemia, euglycemia and hyperglycemia.

3. MATERIALS – METHODS

3.1 The Patient Selection and The Study Protocol

3.1.1 Subjects and Controls

In the study, 50 children and adolescents aged 10-18 years with CF that were regularly followed-up at Marmara University, School of Medicine, Pediatric Pulmonology and Endocrinology Units, Istanbul, between January 2020 and December 2020 were included. The patients who used corticosteroid therapy or had acute exacerbation in the last 3 months and had previously diabetes diagnosis were excluded. Among 50 patients, 5 subjects were excluded due to sampling or storage problem and finally 45 subjects were included and underwent to the analysis. Height, weight and BMI were recorded.

The control group included 9 age-matched healthy non-diabetic siblings of patients with type 1 diabetes that were followed-up at our clinic. All the controls were negative for β-cell autoantibodies (anti-glutamic acid decarboxylase, islet cell antibody, and insulin antibody). The study protocol was approved by the Marmara University Ethics committee, and written informed consent was obtained from the parents and participants.

3.1.2 Oral Glucose Tolerance Test (OGTT)

OGTT was performed in the morning following overnight fasting of ≥8 h. All the participants (the CF subjects and controls) received oral glucose solution (1.75 g/kg; max: 75 g). Blood was collected at 0, 60, 120, 150 and 180 min for glucose, insulin and glucagon measurement. HbA1c levels were also measured via high-performance liquid chromatography (HPLC).

OGTT results were categorized as follows:

1. Normal glucose tolerance (NGT), fasting blood glucose ≥70 - 100 mg/dl; 120-min glucose <140 mg/dl;

2. Impaired fasting glucose (IFG), fasting blood glucose ≥100 - <126 mg/dl; 120-min glucose <140 mg/dl;

3. Indeterminate (INDET), fasting blood glucose <126 mg/dl, 120-min glucose <140 mg/dl; 60-min glucose ≥200 mg/dl;

4. Impaired glucose tolerance (IGT), fasting blood glucose <126 mg/dl; 120-min glucose ≥140 - <200 mg/dl;

5. CFRD, fasting blood glucose ≥126 mg/dl or 120-min glucose ≥200 mg/dl

Abnormal glucose tolerance (AGT) was used for the subjects who had IFG, INDET or IGT without hypoglycemia in OGTT.

The definition of hypoglycemia was performed according to the IHSG position statement as Level 1 Hypoglycemia (if any venous glucose level <70 mg/dL) and Level 2 Hypoglycemia (<54 mg/dL). The terminology of isolated hypoglycemia was used for the subjects who had hypoglycemia without AGT or CFRD.

3.2 Glucagon Analysis

3.2.1 Glucagon Sample Collection

Venous blood samples of the subjects and control groups were collected in 5 ml EDTA tubes and then each sample centrifuged immediately after collection. After centrifugation, plasma was separated and stored at 4°C until the OGTT was ended (180 min). Glucagon in EDTA plasma is stable at 2-8°C for at least 6 hours (35). Following the ending of OGTT, all samples immediately stored at -80°C until analysis. Plasma glucagon was measured using the Mercodia ELISA (cat. No. 10-1271-01) with standard manufacturer's protocol (detailed below).

3.2.2 Glucagon Measurement

Briefly, Mercodia Glucagon ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the glucagon molecule. During the first incubation glucagon in the sample reacts with anti-glucagon antibodies bound to microplate wells. After washing, peroxidase conjugated anti-glucagon antibodies are added. After a second incubation and a simple washing step, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB).

The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

This protocol was optimized to reduce cross-reactivity with other glucagon-related molecules. The lower limit of quantification (LLOQ) was 1.5 pmol/L, the upper limit of quantification (ULOQ) was 130 pmol/L, the detection limit was 0,75 pmol/L and with intra-assay and inter-assay CV of <10%.

The performance characteristics of the sequential protocol were established by the central biochemistry laboratory. The standard curve for glucagon had a range of 1.3–126 pmol/L, with an assay sensitivity of 1.1 pmol/L. Linearity of the assay was determined by serial dilution and was demonstrated to be between 89 and 129% up to a dilution of 1:64. Analyte recovery, determined with use of spiked human plasma samples, ranged from between 92 and 107%. Intra-assay coefficient of variation was 6.9%, 4.0%, and 1.9% on samples with average values of 1.9, 3.1, and 30.9 pmol/L, respectively. Inter-assay coefficient of variation was 8.3%, 7.5%, and 5.7% on samples with average values of 4.4, 13.9, and 41.6 pmol/L. Glucagon values #1.0 pmol/L (below lower limit of quantification) were assigned a value of 0.5 pmol/L for data analysis.

Before test procedure, the enzyme conjugate 1X solution was prepared. Following steps were performed step by step;

1. Calibrators were reconstituted with 1000 µL redistilled water
2. 25 µL each of calibrators, controls and samples were pipetted in duplicate into appropriate wells
3. 200 µL enzyme conjugate 1X solution was added to each well and attach the plate sealer
4. The plate was incubated on plate shaker (700-900 rpm) over night (18-22 h) at 2-8°C
5. They were washed 6x700 µL using an automatic plate washer with overflow wash function. The chosen program filled all the wells with wash buffer in each cycle and ensured that the wells were never left without wash buffer. Additional soak wasn't used. The plate was inverted and tapped firmly against absorbent paper

after final wash

6. 200 µL enzyme conjugate 1X solution was added to each well
7. The plate was incubated on plate shaker (700-900 rpm) 1 hour at room temperature
8. 200 µL substrate TMB was added per well
9. It was incubated on the bench for 15 min at room temperature
10. 50 µL stop solution was added to each well and the plate was placed on a shaker for approximately 5 seconds to ensure mixing
11. Optical density at 450 nm was read within 30 min

Commercial controls pools with low, intermediate and high glucagon concentrations routinely assayed as samples and results charted from day to day.

3.3 Statistical Analysis Method

All analyses were carried out using GraphPad Prism V.8.2.1 (GraphPad Software). Variables are summarized for each group using descriptive statistics. Data are expressed as mean ± SD. Comparison of means was performed using the non-parametric Mann-Whitney U test. The chi-squared test was used for cross tables. The level of statistical significance was accepted $p<0.05$.

4. RESULTS

The mean age of 45 CF subjects (17F/28M) was 13.57±1.96 years and the mean height, weight and BMI SDS were -0.85±1.1 SDS, -0.62±1.1 SDS and -0.20±0.9 SDS, respectively.

All subjects have CFTR gene analysis and the data was shown in Table 3.

Table 3: The results of CFTR gene analyses

Genetic Analyses		Number of Subjects	Genetic Analyses		Number of Subjects
F508del	F508del	6	N1303K	D1152H	1
F508del	R1159X	1	N1303K	c.2658-1G>C	1
F508del	E831X	1	W1282	R347P	1
F508del	G542X	1	Y515X	W1282	2
F508del	L732X	1	Y515X	CFTRdel	1
F508del	L571S	1	Y515X	c.579+3A>G	1
F508del	N1303K	2	Y515X	c.2988+1G>A	1
F508del	L467F	1	E92K	R117C	1
F508del	R347H	1	E92K	c.489+1G>T	1
F508del	P1175L	1	R170H	c.743+40A>G	1
F508del	E1044G	1	D110H	c.2988+1G>A	1
F508del	c.2909-15T>G	1	I1000L	c.1116+1G>A	1
CFTRdel	CFTRdel	3	G542X	c.3964-3C>G	1
Y515X	Y515X	1	R334W	E831X	1
M470V	M470V	1	L732X	IVS13+3A>G	1
I1000L	I1000L	1			
E92K	E92K	1			
c.2657+5G>A	c.2657+5G>A	4			

OGTT findings were completely normal in 16 (35.5%) of the 45 subjects. The number of the subjects with isolated hypoglycemia, INDET, IGT and CFRD in OGTT were 14 (31.1%), 8 (17.7%), 5 (11.1%) and 2 (4.4%), respectively, whereas IFG did not seem in any subjects.

Overall hypoglycemia was detected in 22 (48.8%) subjects but 8 of them (11.1%) were along with abnormal glucose tolerance (INDET and IGT) (the data is shown Table 4). According to the IHSG classification, the frequency of level 1 hypoglycemia (54-69 mg/dL) was 33% and level 2 hypoglycemia (<54 mg/dL) was 15%. Two subjects among 9 controls (22%) had level 1 hypoglycemia without hypoglycemic symptoms.

Table 4: The distribution of hypoglycemic patients

	Hypoglycemia (+)	Hypoglycemia (-)	Total
AGT (+)	8	5	13
AGT (-)	14	18*	32
Total	22	23	45

* 2 subjects CFRD, 16 subjects NGT

The demographic characteristics of the subgroups and controls are shown in Table 5. Although, the mean HbA1c level in isolated hypoglycemia was higher than NGT group, it was not statistically significant (p=0.06). However, the mean HbA1c level in isolated hypoglycemia was significantly higher than controls and lower than AGT group (p<0.001 and p<0.05, respectively).

Table 5: Demographics of the subjects and controls

	Hypoglycemia (+)		Hypoglycemia (-)			
	Only Hypo (n=14)	Hypo + AGT (n=8)	AGT (n=5)	NGT (n=16)	**Control** (n=9)	p
Age (mean)	13,5 ± 1,5	13,1 ± 1,8	13,4 ± 1,5	13,7 ± 2,2	13,2 ± 1,8	ns
Male (%)	11 (78%)	4 (50%)	3 (60%)	9 (56%)	4 (44%)	ns
BMI SDS	- 0,07 ± 1,2	- 0,35 ± 1,0	- 0,58 ± 0,5	- 0,19 ± 0,8	- 0,04 ± 0,7	ns
HbA1c (%)	5,68 ± 0,27	5,73 ± 0,22	5,94 ± 0,32	5,51 ± 0,31	5,22 ± 0,23	P<0,01
Genetic (%, ΔF508)	42%	50%	60%	25%		ns
Homozygote ΔF508	1	2	1	1		
Heterozygote ΔF508	5	2	2	3		
Others	8	4	2	12		
Glucose (mg/dL)						
0.min glucose	85 (75-93)	92 (80-116)	97 (91-103)	87 (80-98)	83 (79-89)	P<0,05
60.min glucose	150 (50-198)	229 (204-267)	202 (119-260)	125 (85-189)	126 (98-170)	P<0,01*
120.min glucose	100 (58-125)	132 (99-190)	146 (120-177)	105 (78-126)	101 (64-130)	P<0,05*
150.min glucose	78 (42-105)	91 (46-125)	128 (100-171)	102 (80-155)	90 (62-110)	P<0,05
180.min glucose	63 (52-82)	59 (42-68)	112 (72-154)	93 (70-128)	87 (59-102)	P<0,01

* The significance was based on AGT and Hypo + AGT group

The controls were compared with NGT group, glucagon response to glucose loading in NGT was blunted. Basal glucagon level in NGT was significantly lower than the controls and glucose-stimulated glucagon secretion/suppression were not observed in NGT (Figure 3) (Table 5). Insulin and glucose levels were similar in NGT and control groups.

When the isolated hypoglycemic group was compared with NGT and controls, the trend of glucagon response was similar with the controls but the glucagon levels at 60, 120 and 150.min were significantly higher ($p<0.01$, $p<0.01$, $p<0.05$, respectively).

Although, the glucagon response to hypoglycemia at 180.min was higher than control, it was not statistically significant (p=0.17). Also, there was statistically significant increment at 60.min insulin levels in isolated group (p=0.049).

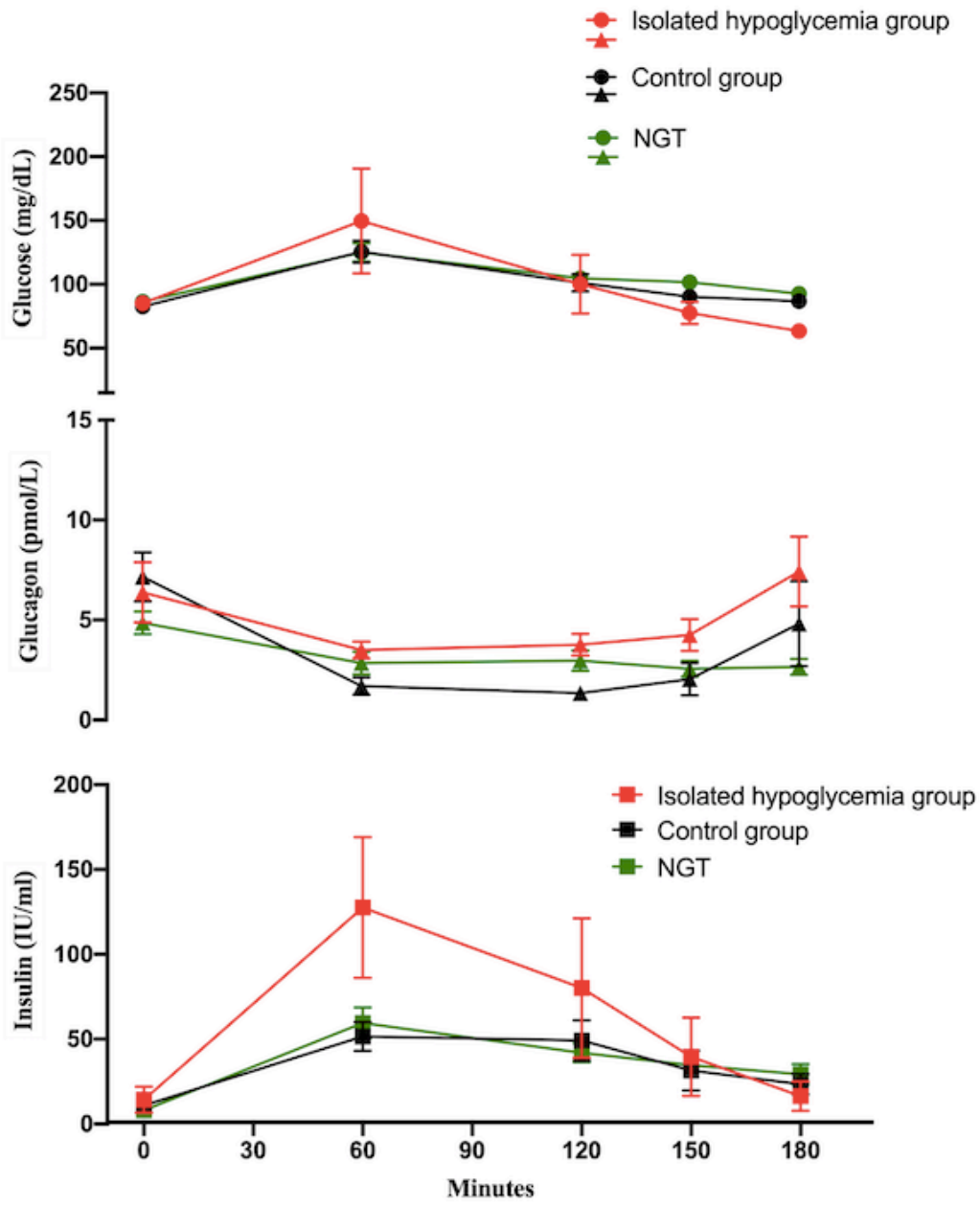

Figure 3: Comparing of glucose, insulin and glucagon levels in isolated hypoglycemia, NGT and controls

Table 6: Glucose, insulin and glucagon levels in isolated hypoglycemia, NGT and controls

OGTT (min)	**Isolated Hypo** (n=14)			**NGT** (n=16)			**Control** (n=9)			**P**		
	BG	Insulin	GLC	BG	Insulin	GLC	BG	Insulin	GLC	BG	Insulin	GLC
0.	85	14,3	6,3	86	7,9	4,85	82	10,9	7,1	<0,05	ns	<0,05
60.	149	127,6	3,5	125	59,4	2,85	125	51,5	1,69	<0,05	<0,05	<0,01
120.	100	80,1	3,7	104	41,8	2,96	101	49,2	1,3	ns	ns	<0,05
150.	77	39,5	4,2	101	34,5	2,56	90	31,2	2,03	<0,001	ns	<0,05
180.	63	16,4	7,4	92	29,3	2,6	86	23,4	4,8	<0,001	ns	<0,01

* BG: Blood Glucose, GLC: Glucagon

Glucagon levels were still significantly higher at 60, 120 and 150.min, when AGT + Hypoglycemia group (8 subjects) included to isolated hypoglycemia group (overall hypoglycemia group) (Table 7) (Figure 4).

Table 7: Glucose, insulin and glucagon levels in overall hypoglycemia, NGT and controls

OGTT (min)	**Overall Hypo** (n=22)			**NGT** (n=16)			**Control** (n=9)			**P**		
	BG	Insulin	GLC	BG	Insulin	GLC	BG	Insulin	GLC	BG	Insulin	GLC
0.	88	12,9	6,1	86	7,9	4,85	82	10,9	7,1	<0,05	ns	<0,05
60.	179	101,5	3,4	125	59,4	2,85	125	51,5	1,69	<0,01	<0,05	<0,05
120.	112	71,4	3,4	104	41,8	2,96	101	49,2	1,3	ns	ns	<0,05
150.	82	37,8	3,8	101	34,5	2,56	90	31,2	2,03	<0,01	<0,05	ns
180.	62	16	6,6	92	29,3	2,6	86	23,4	4,8	<0,001	<0,05	<0,05

* BG: Blood Glucose, GLC: Glucagon

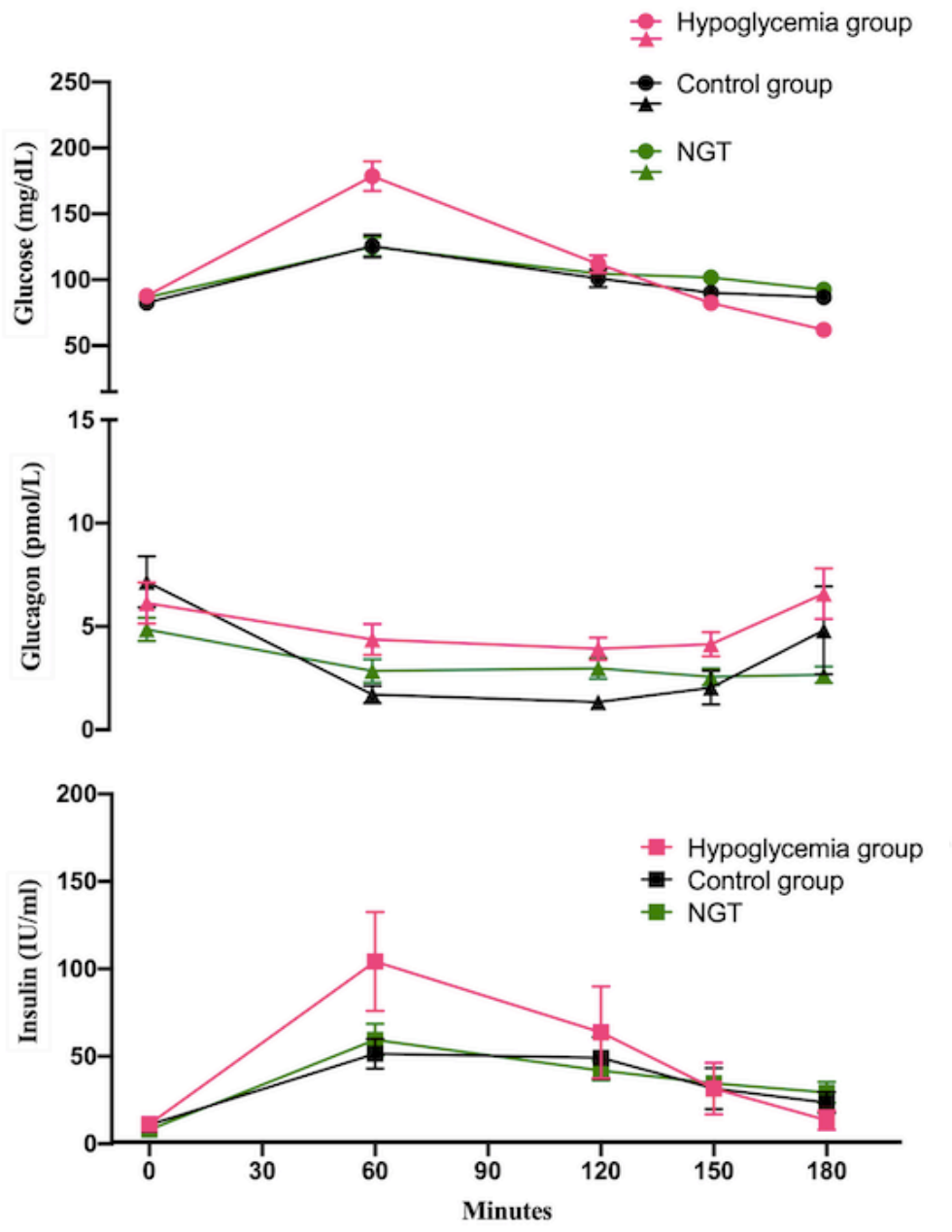

Figure 4: Comparing of glucose, insulin and glucagon levels in overall hypoglycemia, NGT and controls

In AGT group, glucagon levels at 0, 60,120 and 150.min were higher than NGT and controls ($p<0.01$, $p<0.05$, $p<0.01$, $p<0.05$, respectively) (Table 8) (Figure 5)

Table 8: Glucose, insulin and glucagon levels in AGT, NGT and controls

OGTT (min)	**AGT** (n=5)			**NGT** (n=16)			**Control** (n=9)			**P**		
	BG	Insulin	GLC	BG	Insulin	GLC	BG	Insulin	GLC	BG	Insulin	GLC
0.	97	8,3	9,67	86	7,9	4,85	82	10,9	7,1	<0,01	ns	<0,05
60.	202	46,4	5,5	125	59,4	2,85	125	51,5	1,69	<0,05	ns	<0,05
120.	146	49,5	4,2	104	41,8	2,96	101	49,2	1,3	<0,01	ns	<0,05
150.	128	37,7	4,3	101	34,5	2,56	90	31,2	2,03	<0,05	ns	<0,05
180.	112	22,1	3,75	92	29,3	2,6	86	23,4	4,8	ns	ns	ns

* BG: Blood Glucose, GLC: Glucagon

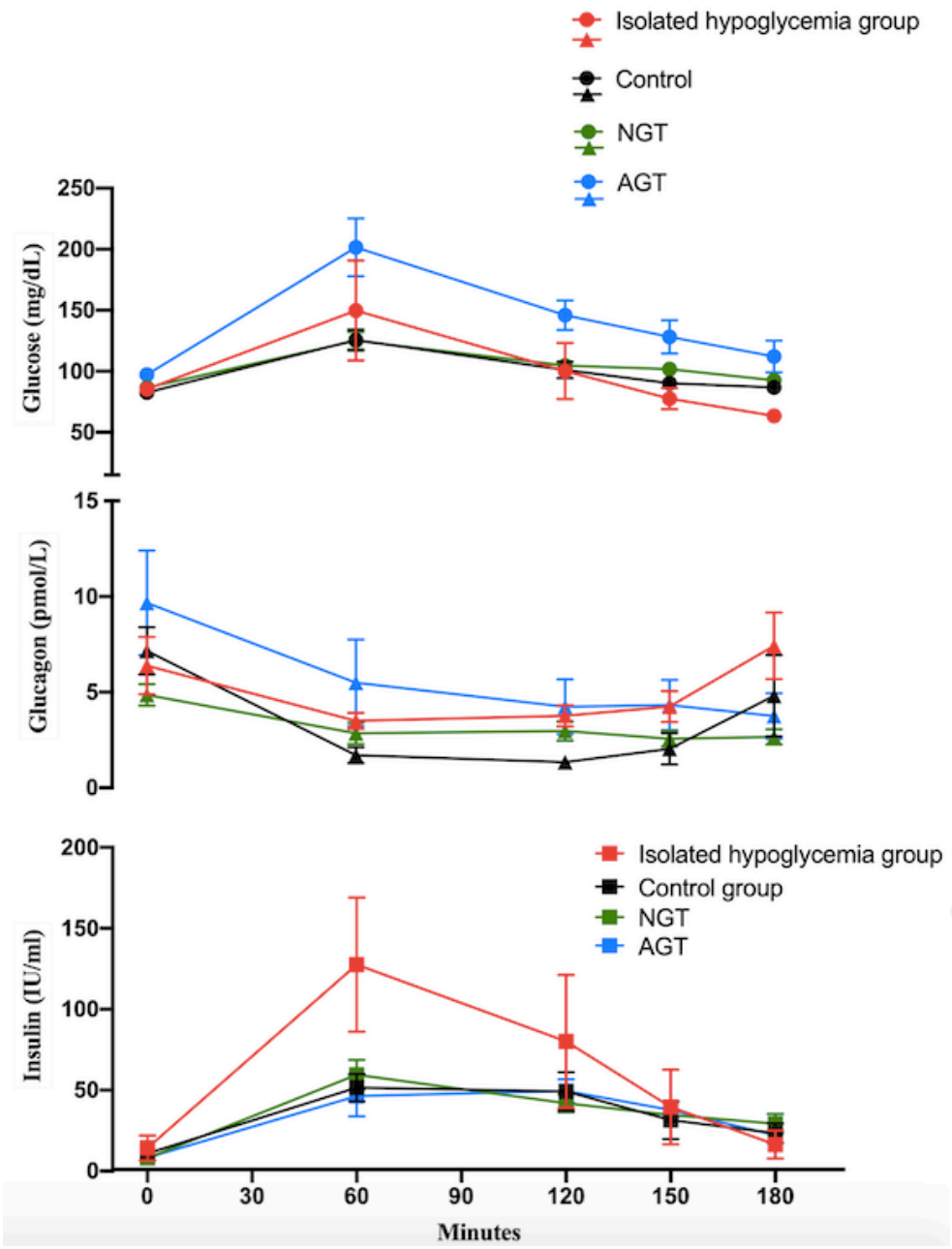

Figure 5: Comparing of glucose, insulin and glucagon levels in isolated hypoglycemia, AGT, NGT and controls

5. DISCUSSION

Insulin therapy is required for the treatment of CFRD and it has been linked to better nutritional and respiratory outcomes in CF patients. Insulin therapy's most prevalent and feared consequence is iatrogenic hypoglycemia (22,27,40). Iatrogenic hypoglycemia is a limiting factor in achieving the better glycemic control that has been demonstrated to avoid microvascular consequences in individuals with type 1 and type 2 diabetes. Patients with CFRD, like those with T1D and T2D, are at risk for hypoglycemia as a result of insulin treatment (41). Patients with CF, particularly those who are not on any glucose-lowering medications, are prone to spontaneous (reactive) and fasting hypoglycemia. In comparison to T1D and T2D, the literature about hypoglycemia in patients with CFRD/CF is lacking and this leads to limitations in our understanding of its impact on the lives of people with CF.

In patients with CF, reactive hypoglycemia is often detected during or after OGTT and is usually asymptomatic. In a review, Armaghanian et al (42) reported that incidence of hypoglycemia unrelated to diabetes in CF patients ranged from 7 to 69% in several studies. This variety in the prevalence of hypoglycemia between studies is because of different study designs and various definitions of hypoglycemia. When compared to 2-hour OGTT, 3-hour OGTT will more likely detect reactive hypoglycemia. In the present study, 3-hour OGTT was preferred to detect more hypoglycemia and by this way to understand the glucagon response to hypoglycemia in CF patients. The first such data were reported by Battezzati et al. (2), who observed that the frequency of fasting hypoglycemia (<60 mg/dl) and reactive hypoglycemia (2-h glucose <50 mg/dl) in OGTT were 14% and 15%, respectively. Another study that used the definition of hypoglycemia as <60 mg/dL reported that the frequency of reactive hypoglycemia was 7% in patients with CF (5); however, Hirsch et al. (3) noted that hypoglycemia in adults with CF was more common than expected by extending OGTT to 3 h, observing reactive hypoglycemia (<60 mg/dl) at 3 h in 45% of the patients. In the present study OGTT was 3-hours and the overall frequency hypoglycemia (<70 mg/dL) was 49%. It was similar with other 3-hour OGTT studies (3,4). In a systemic review, IHSG classification is suggested in future studies about hypoglycemia in CF.

According to the this classification, the frequency of level 1 hypoglycemia (54-69 mg/dL) was 33% and level 2 hypoglycemia (<54 mg/dL) was 15%. In healthy individuals, the average nadir glucose level was 63 mg/dL and nearly 25% of subjects had nadir glucose of 55 mg/dL during 5-hour OGTT (43). In a current CGM study, 1.1% (0.3-3.3%) of 24-hour glucose sensor values were <70 mg/dL (level 1 hypoglycemia) in healthy 6-12 years of age children, whereas 0% (0-0.2%) of values were <54 mg/dL (level 2 hypoglycemia) (44). In present study, the frequency of hypoglycemia in healthy controls was 22% and the nadir glucose was 59 mg/dL.

There is a strong linear relationship between average glucose and HbA1c in adults with type 1 and type 2 diabetes. In comparison to the OGTT, HbA1c has been regarded as insensitive for diabetes screening in CF patients. It has also been argued that HbA1c underestimates mean glucose in CF due to increased red blood cell turnover and high vitamin E consumption, which results in reduced glycosylation. However, the evidence for these theories is limited, the specific reasons why HbA1c may results differently in patients with CF remain unknown. In different studies, a relation between HbA1c and the presence of hypoglycemia did not detect (2,5,45,46). Similar with these studies, we did not find statistically significance in HbA1c between isolated hypoglycemia group and NGT ($p=0.07$), however the mean HbA1c level in isolated hypoglycemia group was significantly lower than AGT and higher than healthy controls ($p<0.05$). Despite of hypoglycemia, the significantly higher glucose levels at 60.min may explain the higher HbA1c in isolated hypoglycemia group than the healthy controls.

Gender differences and a female predominance in CFRD have been reported previously (1). Although the underlying mechanism remains unclear, Coriati et al. showed that adult females with CF have higher insulin secretion than adult males, but this finding seems to contradict the reported female predominance in CFRD (1,47). In addition, Battezzati et al. (48) reported that increased insulin clearance in adult females contributes to deterioration in glucose tolerance. In terms of the hypoglycemia in CF, the ratios are different. Mannik et al and Aitken et al founded male predominance in hypoglycemic CF subjects, whereas a female predominance was observed in our previous study (45,49). However, Kilberg et al did not found statistically significance

gender differences in hypoglycemic subjects despite of higher male ratio (46). In the present study, male predominance was detected but not statistically significant. It was compatible with the literature but opposite of our previous study. We attributed this to the different designs of our two studies.

By now, over 2000 different CFTR mutations have been identified and by far the most common is F508del mutation (50). In Europe, 82% of the patients had at least one F508del mutation, although the prevalence of F508del mutation is higher in Northern Europe than Southern Europe such as 83% in Denmark compared to 40-60% in Spain and Greece. All other mutations are seen in just a small percentage of European CF patients. However, the frequency of a specific mutation might range from 10-15% (51). In our cohort, 40% of the subjects had F508del mutation, 13% of them also was homozygote. The literature is quite controversial in terms of hypoglycemia. Aitken et al found that the frequency of hypoglycemia was higher in subjects with homozygote F508del mutation, whereas Mannik et al reported that hypoglycemia was more common in subjects with heterozygote F508del mutation (45,49). However, Kilberg et al did not find any significant difference the genetic analysis in terms of hypoglycemia (46). Similar with the last study, we did not identify statistical significance among groups.

In CF, the pathophysiology of hypoglycemia during the OGTT is not clearly understood. However, there has been some common explanations for the hypoglycemia. Hypoglycemia in OGTT (much like type 2 diabetes) (52) has been proposed as a possible precursor or predictor of CFRD development. This makes sense, as CFRD is most common comorbidity in CF and that even CF patients with normal glucose tolerance have insulin secretion abnormalities (53). Given that the pancreas in CF usually does not secrete insulin properly, it is expectable to think that hypoglycemia might be developed as a result of the dysregulated insulin secretion that precedes the onset of CFRD. Both condition are thought to have the same pathophysiology, namely reactive hypoglycemia, with delayed and then over-reactive insulin secretion occurring following oral ingestion of simple carbohydrate. In contrast to the above hypothesis, Mannik et al showed that the subjects who experienced hypoglycemia during OGTT had a reduced risk of progression to CFRD in following 10 years, despite being older and have higher median lung function (49). This result clearly supports a controversial

previous study by Radike et al, that hypoglycemia during the OGTT is not a predictor of CFRD or other severe morbidity in CF patients (5). In another study by Armaghanian et al, the subjects who experienced hypoglycemia had lower insulin levels at 2 hour in OGTT than those who did not (6). Consistent with this study, similar findings were detected in the present study. Although the insulin levels at 120.min were higher in isolated and overall hypoglycemic group than NGT and controls, it was not significant. All of these studies have pointed that different mechanisms may be involved the development of hypoglycemia in CF.

The CFTR gene has been shown to regulate glucagon release from the pancreatic alpha cell in addition to insulin secretion. The current findings indicate a suppressive function for CFTR in glucagon secretion control, revealing a new mechanism intrinsic to pancreatic cells (7). The results obtained from the CFTR/F508del-overexpressing alpha cells, in addition to those obtained from wild-type and F508del mice/isolated islets, have clearly demonstrated that glucagon secretion can be regulated intrinsically by cells, with CFTR as a key player in suppressing glucagon release. These findings are consistent with another study that detected an inhibitory effect of CFTR in regulating glucagon release in both mouse and human islet alpha cells (25). The suppressive effect of CFTR on glucagon secretion is attributed to a more hyperpolarized Vm and a lower degree of glucose-deprivation-induced depolarization and Ca+2 response observed in wild-type/CFTR-overexpressing cells compared to F508del/CFTR inhibited cells (7). Following CFTR inhibition in human cells, glucagon secretion was increased in response to glucose. On the other hand, depolarization-induced glucagon secretion was unaffected (25). The authors indicate that CFTR modulates alpha cell membrane potential primarily using a mathematical model of alpha cell electrophysiology. Increased glucagon release was also consistently seen in the islets of F508del mice. It was discovered through overexpression studies in the alpha TC1-9 cell line that wild type CFTR functions as a glucose-sensing negative regulator of glucagon release and that abnormalities in this mechanism may contribute to glucose intolerance and hypoglycemia (7,25).

Given the insulin levels in hypoglycemic subject in our study, the subjects with isolated hypoglycemia group were significantly higher insulin levels at 60.min in OGTT ($p<0.05$) and this difference was still detected in overall hypoglycemia group. Also, the insulin levels at 150 and 180.min were significantly lower in isolated hypoglycemia group, whereas there was no statistically difference in insulin levels at 120.min. This data showed that the subjects with hypoglycemia have higher insulin levels in early period of OGTT (over-secretion) and then lower but inappropriately higher insulin levels in late-hypoglycemic period. This meant that previous hypothesis (delayed and over-secretion in insulin) is observed again in our study.

In the present study, as expected, the glucagon secretion was suppressed by increasing in glucose levels and stimulated by decreasing in glucose levels during 3-hour OGTT in healthy controls. However the glucagon response to the glycemic waxing and waning was blunted in CF subjects with normal glucose tolerance. This finding shows that the subjects with normal glucose tolerance may not be exactly normal in terms of glucose-glucagon regulation. On the other hand, the trend of glucagon response in isolated hypoglycemia group was very similar with healthy controls but had higher glucagon levels than controls. Despite statistically higher levels, the response to hypoglycemia at 180.min in isolated hypoglycemia group was not sufficient and also the suppression of glucagon at 60.min in OGTT was inappropriate. It might show that alpha cells in isolated hypoglycemia group have relatively normal glucose sensing but insuffucient glucagon response. These statistical significances were still continuing in overall hypoglycemia group. The blunted glucagon response to the glycemic waxing and waning and the glucagon trend in NGT group was similar with AGT group but AGT had higher glucagon levels than NGT. It may be interpreted as blunted and dysregulation of glucagon might be the pioneer evidence for the progression of CFRD.

Glucagon, oxyntomodulin and glicentin are products of proglucagone and shared same amino-acides (residues 33-61). Therefore, measurements of these three glucagon-containing peptides come with some difficulties in terms of specificity. The dominating antigenic determinant of the glucagon corresponds to a mid-region of the peptide and antibodies against this location will also recognize oxyntomodulin and glicentin (54).

So, assays for any of these peptides must be properly characterized in terms of their specificity. Furthermore, because these peptides circulate at low concentrations, sensitivity is crucial. Unlike other glucagon studies in CF, we used ELISA 10-1271-01 (two-site enzyme immunoassay) that its sensitivity and specificity were approved by several studies (55,56). The assay has stated cross-reactivity with glicentin-related polypeptide (<0.0005%), GLP-1 (<0.3%), glicentin (0.8%) and 4.4% cross-reactivity with oxyntomodulin.

The strengths of our study are the only pediatric data, relatively large number of subjects with hypoglycemia, the prospective data collection, using of more specific glucagon kit and the presence of healthy controls. However, the lack of glucagon data at 15 ve 30.min is the main limitation of the study. Another limitation is that we have no long-term follow-up of the subject to understand of the role of glucagon response in OGTT on the future comorbidities or life-expectation in CF.

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55. Kramer CK, Zinman B, Choi H, Connelly PW, Retnakaran R. Impact of the Glucagon Assay When Assessing the Effect of Chronic Liraglutide Therapy on Glucagon Secretion. *J Clin Endocrinol Metab*. 2017 Aug 1;102(8):2729-2733.
56. Wewer Albrechtsen NJ, Veedfald S, Plamboeck A, Deacon CF, Hartmann B, Knop FK, et al. Inability of Some Commercial Assays to Measure Suppression of Glucagon Secretion. *J Diabetes Res*. 2016; 2016:8352957.

7. APPENDICES

7.1. Patient Application Form

Kistik Fibrozisli Hastalarda
Glukagonun Kan Şekeri Regülasyonundaki Rolü ve Etkisi
HASTA BAŞVURU FORMU

Adı-Soyadı: Başvuru Tarihi:
Cinsiyeti: Doğum Tarihi:
KF tanı yaşı: Yaşı:
Ek hastalık varlığı:
Kullandığı ilaçlar: İnhale steroid Beta-bloker
Creon Pulmozyme
A D E K
Diğer
Son oral/ıv steroid zamanı, süresi ve miktarı:
Son OGTT tarihi:
Genetik mutasyonu:

Boy: TA:
Kilo: VKİ:
Sistem muayeneleri:
Puberte evresi: TV/Breast ...../..... Pb .....

Laboratuvar:
HbA1c:

OGTT	0.dk	60.dk	120.dk	150.dk	180.dk
Glukoz					
İnsülin					
Glukagon					

7.2. Ethical Approval

Marmara Üniversitesi Tıp Fakültesi
Klinik Araştırmalar Etik Kurulu

BAŞVURU BİLGİLERİ	**PROTOKOL KODU**	**09.2019.933**
	PROJE ADI	Kistik Fibrozisli Hastalarda Glukagor Regülasyonundaki Rolü ve Etkisi
	SORUMLU ARAŞTIRICI ÜNVANI/ADI	Doç. Dr. Belma HALİLOĞLU

KARAR BİLGİLERİ	**Tarih : 01. 11. 2019** **Yukarıda başvuru bilgileri verilen araştırma başvuru dosyası ve ilgili belgeler araştırmanın gerekçe, an alınarak incelenmiş ve gerçekleştirilmesinde sakınca bulunmadığı için Kurulumuzca onaylanmasına oy sonrasında yapılacak her türlü proje değişiklikleri (katılımcılar, başlık vb.) veya protokol değişiklikler onayının yenilenmesi gerekmektedir.**

ÜYELER

Unvanı / Adı / Soyadı	Uzmanlık Dalı	Kurumu / EK Üyeliği	Onaylanan Proje ile İlişkisi	Toplantıya katılım
Prof.Dr. Haner DİRESKENELİ	Romatoloji	M.Ü Tıp Fakültesi/ Başkan	□ Var □ Yok	□ Evet □ Hayır
Prof.Dr. Tülin ERGUN	Dermatoloji	M.Ü Tıp Fakültesi/Başkan Yrd.	□ Var □ Yok	□ Evet □ Hayır
Prof.Dr. Atila KARAALP	Farmakoloji	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ EVET □ HAYIR
Prof. Dr. Şefik GÖRKEY	Tıp Tarihi ve Etik	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Prof.Dr. Handan KAYA	Patoloji	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Prof.Dr. M.Bahadır GÜLLÜOĞLU	Genel Cerrahi	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Prof.Dr. Semra SARDAŞ	Eczacı	M.Ü Eczacılık Fak./Üye	□ Var □ Yok	□ Evet □ Hayır
Prof.Dr. Başak DOĞAN	Diş Hekimi	M.Ü Diş Hekimliği Fak./Üye	□ Var □ Yok	□ Evet □ Hayır
Prof. Dr. Beste Melek ATASOY	Radyasyon Onkolojisi	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Doç. Dr. Elif KARAKOÇ AYDINER	Çocuk Sağlığı ve Hastalıkları	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Doç.Dr. Meltem KORAY	Diş Hekimi	İstanbul Üniv. Diş Hekimliği Fak./Üye	□ Var □ Yok	□ Evet □ Hayır
Doç. Dr. Gürkan SERT	Hukukçu	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Doç.Dr: Figen DEMİR	Halk Sağlığı	Acıbadem Üniv. Tıp Fak.	□ Var □ Yok	□ Evet □ Hayır
Doç.Dr. Pınar Mega TİBER	Biyofizik	M.Ü Tıp Fakültesi/Üye	□ Var □ Yok	□ Evet □ Hayır
Gözde Aynur MİRZA	Sağlık Mensubu olmayan kişi	Serbest	□ Var □ Yok	□ Evet □ Hayır

7.3. CV

BELMA HALILOGLU, MD, Assoc.Prof

H-Index: 10 (WEB of Science), 11 (Scopus)

EDUCATION AND QUALIFICATIONS

1997-2003 Medical Doctor, Istanbul University, Istanbul Medical Faculty (Istanbul, Turkey)

2004-2009 Resident in Pediatrics, Istanbul Goztepe Education and Research Hospital, Department of Pediatrics (Istanbul, Turkey)

2009-2010 Bingol Genc State Hospital, Department of Pediatrics (Bingol, Turkey)

2010-2014 Pediatric Endocrinology and Diabetes Clinical Fellowship – Marmara University (Istanbul, Turkey)

2013-2013 Research Fellowship, University of Exeter, Department of Molecular Genetics (Exeter, United Kingdom)

2014-2016 Diyarbakir Child Health Hospital, Department of Pediatric Endocrinology (Diyarbakir, Turkey)

2017-2019 PhD student (ongoing in thesis), Yeditepe University, Department of Molecular Medicine (Istanbul, Turkey)

2016-202 Yeditepe University Hospital, Department of Pediatric Endocrinology (Istanbul, Turkey)

2021- .. Marmara University School of Medicine, Department of Pediatric Endocrinology (Istanbul, Turkey)

Membership and Activities in Professional Associations

European Society of Pediatric Endocrinology (ESPE)

International Society for Pediatric and Adolescent Diabetes (ISPAD)

Diabetes Working Group (Ministry of the Health)

Turkish Pediatric Endocrinology and Diabetes Society

Turkish Medical Association

Awards-Prizes

2019- 1st best case report, published in JCRPE (Journal of Clinical Research in Pediatric Endocrinology)

2013- 2nd best oral presentation, 17th National Pediatric Endocrinology Congress

2012- ISPAD Research Visiting Fellowship Award

The project Title: Exploring new forms of MODY

Date and Place of Research Project: 01.02.13-01.08.13 (Department of Molecular Genetics, University of Exeter, Exeter, UK)

Speakers:

International:

1. 8th International Pediatric Endocrinology Symposium 2014 (Prague, Republic of Czech)

Workshop 3: Differential diagnosis of pediatric diabetes (with Prof.Tadej Battelino)

National:

1. Pediatric Endocrinology and Gastroenterology Joint Meeting 2021 (İstanbul, Turkey)
   "Cystic Fibrosis and Endocrine Problems "
2. PEGİK School 2020 (Ankara, Turkey)
   "Diabetic Ketoacidosis"
3. National Diabetes Team Meeting 2020 (Ankara, Turkey)
   "New Technologies in Self Monitoring Blood Glucose"
4. 23th National Pediatric Endocrinology Congress 2019 (Antalya, Turkey)
   Session 1: Yearbook 2019 (Diabetes)
5. 2nd National Symposium in Diabetes Technology 2019 (Ankara, Turkey)
   "Computer software systems in Diabetes Technologies"
6. 4th Marmara Pediatric Congress 2017 (Istanbul, Turkey)
   Session 2: New consensus in Diabetic Ketoacidosis
7. 8th Obesity Congress 2017 (İstanbul, Türkiye)
   "Complementary therapy in Childhood Obesity"
8. 19th National Pediatric Endocrinology Congress 2015 (Istanbul, Turkey)
   Session 4: Yearbook 2015 (Obesity and Diabetes)

9. 9th Nutrition and Metabolism Meeting (International attendance) 2007 (Istanbul, Turkey)

   Approach to a newborn with inborn errors of metabolism

Science Schools - Fellowships

2018- Diabetes School, ESPE/ISPAD/EASD, Prag, Rebublic of Czech

2015- Science School, ESPE, Anncy, France

2015- Diabetes and Obesity School, Lamberhg Chateau Begunje, Slovenia

2013- Summer School, ESPE, Milano, Italy

2012- Post fellowship programme, ESPE, Varna, Bulgaria

2012- Science School, ISPAD, Kyoto, Japan

2011- Winter School, ESPE, Ankara, Turkey

2008- Istanbul University, Istanbul Medical Faculty Pediatric Endocrinology, "Pediatric Endocrinology Courses – Thyroid and Obesity", Istanbul

2008- Istanbul University, Istanbul Medical Faculty Pediatric Endocrinology, "Symposium of Pediatric Endocrinologic Emergency", Istanbul

Thesis in Pediatrics: Haliloglu B. Complementary and alternative medicine in patients with Type 1 Diabetes Mellitus (Tip 1 Diabetes Mellitus tanılı çocuklarda tamamlayıcı ve alternatif tedavi kullanımı) 2009.

Thesis in Molecular Medicine (ongoing): Haliloglu B. Unmethylated insulin cfDNA levels in children with Type 1 Diabetes.

BOOKS

In International Books

1. **Haliloglu B**, Bereket A. Chapter 37: Central Control of Energy Metabolism and Hypothalamic Obesity. In book: Pediatric Obesity (2nd edition) edited by Michael Freemark. 2017 doi: 10.1007/978-3-319-68192-4_2

In National Books

1. **Haliloglu B**, Turan S. İskelet displazileri ve perioperatif yönetim. Editör Prof.Dr.Selim Kurtoğlu. *Çocuklarda Acil Endokrin Hastalıklar*. 2013 (Nobel Tıp Kitapevleri)

2. **Haliloglu B**, Aycan Z. Diyabet Tiplendirmesinde Zorluklar ve Ayırıcı Tanısısnda Yardımcı Yöntemler. Editör Prof.Dr.Zehra Aycan. *Çocukluk Çağı Diyabeti, Tanı ve Tedavi Rehberi*. 2018

3. **Haliloglu B**, Semiz S. Maturity Onset Diabetes of the Young (MODY). Editör Prof.Dr.Zehra Aycan. *Çocukluk Çağı Diyabeti, Tanı ve Tedavi Rehberi.* 2018

4. **Haliloglu B**, Bideci A, Döğer E, Evliyaoğlu O, Baş F, Gençer Ş. Çocuk ve Ergenlerde Diyabetes Mellitus Tanımı, Tanısı ve Sınıflandırması. Editör Prof.Dr.Rüveyde Bundak ve Prof.Dr.Damla Gökşen. *TC Sağlık Bakanlığı Birinci Basamak Sağlık Kurumlarında Tip 1 Diyabet Tanı Tedavi izlem Rehberi.* 2018

PUBLICATIONS

Publications In International Journals

1. De Franco E, Lytrivi M, Ibrahim H, Montaser H, Wakeling MN, Fantuzzi F, Patel K, Demarez C, Cai Y, Igoillo-Esteve M, Cosentino C, Lithovius V, Vihinen H, Jokitalo E, Laver TW, Johnson MB, Sawatani T, Shakeri H, Pachera N, **Haliloglu B** et al. YIPF5 mutations cause neonatal diabetes and microcephaly through endoplasmic reticulum stress. *J Clin Invest.* 2020 Nov 9:141455. doi: 10.1172/JCI141455.
2. Demirbilek H, Cayir A, Flanagan SE, Yıldırım R, Kor Y, Gurbuz F, **Haliloglu B** et al. Clinical characteristics and long-term follow-up of patients with diabetes due to PTF1A enhancer mutations. *J Clin Endocrinol Metab.* 2020 Dec 1;105(12):dgaa613

3. Bayrak NA, Volkan B, **Haliloglu B**, Kara SS, Cayir A. The effect of celiac disease and gluten-free diet on pubertal development: a two-center study. *J Pediatr Endocrinol Metab.* 2020 Mar 26;33(3):409-415.
4. Abalı S, Tamura M, Turan S, Atay Z, Isguven P, Güran T, **Haliloglu B**, Baş S, Isojima T, Kitanaka S, Bereket A. Hereditary vitamin D-resistant rickets: a report of four cases with two novel variants in the VDR gene and successful use of intermittent intravenous calcium via a peripheral route. *J Pediatr Endocrinol Metab*. 2020 Apr 28;33(4):557-562.
5. **Haliloglu B**, Tuzun H, Flanagan SE, Celik M, Kaya A, Ellard S, Ozbek MN. Sirolimus-induced hepatitis in two cases with hyperinsulinemic hypoglycemia. *J Clin Res Pediatr Endocrinol* 2018 Dec 8. doi: 10.4274/jcrpe.5335.
6. **Haliloglu B**, Abali S, Bugrul F, Celik E, Bas S, Atay Z, Guran T, Turan S, Bereket A. The distribution of different types of diabetes in childhood: A single center experience. *J Clin Res Pediatr Endocrinol* 2018 Nov 24. doi: 10.4274/jcrpe.5204.
7. Işık E, **Haliloglu B**, Doorn JV, Demirbilek H, Scheltinga SA, Losekoot M, Wit JM. Clinical and biochemical characteristics and bone mineral density of homozygous, compound heterozygous and heterozygous carriers of three novel IGFALS mutations. *Eur J Endocrinol*. 2017 Jun;176(6):657-667. doi: 10.1530/EJE-16-0999.
8. Aydin BK, Saka N, Bas F, Yilmaz Y, **Haliloglu B**, Guran T, Turan S, Bereket A, Yesiltepe Mutlu G, Cizmecioglu F, Hatun S, Bezen D, Tutunculer F, Cebeci N, Isguven P, Memioglu N, Ercan O, Poyrazoglu S, Bundak R, Darendeliler F. Evaluation, Treatment and Follow-up Results of Ovarian Cysts in Childhood and Adolescence: A Multicenter, Retrospective Study of 100 Patients. *J Pediatr Adolesc Gynecol*. 2017 Aug;30(4):449-455. doi:10.1016/j.jpag.2017.01.011.
9. **Haliloglu B**, Gokdemir Y, Atay Z, Abali S, Guran T, Karakoc F, Ersu R, Karadag B, Turan S, Bereket A. Hypoglycemia is common in children with cystic fibrosis and seen predominantly in females. *Pediatr Diab, 2016* Nov;18(7):607-613. doi: 10.1111/pedi.1247.

10. **Haliloglu B**, Hysenaj G, Atay Z, Guran T, Abali S, Turan S, Bereket A, Ellard S. GCK gene mutations are a common cause of childhood-onset MODY (maturity-onset diabetes of the young) in Turkey. *Clin Endocrinol (Oxf),* 2016 Sep;85(3):393-9. doi: 10.1111/cen.13121
11. **Haliloglu B**, Atay Z, Guran T, Abali S, Bas S, Turan S, Bereket A. Risk factors for mortality due to hypothalamic obesity in children with hypothalamic tumors. *Pediatr Obes*, 2016 Oct;11(5): 383-8. doi: 10.1111/ijpo.12076.
12. Nalcacıoglu H, **Haliloglu B**. A rare cause of polyuria and polydipsia in a patient with cystic renal disease: Mmaturity-onset diabetes of the young type 5. *World J Nephrol Urol.* 2016;5(3):67-70.
13. **Haliloglu B**, Bereket A. Hypothalamic obesity in children: pathophysiology to clinical management. *J Pediatr Endocrinol Metab*. 2015 May;28(5-6):503-13.
14. Bas S, Guran T, Atay Z, **Haliloglu B**, Abalı S, Turan S, Bereket A. Premature Pubarche, Hyperinsulinemia and Hypothyroxinemia: Novel Manifestations of Congenital Portosystemic Shunts (Abernethy Malformation) in Children. *Horm Res Paediatr.* 2015; 83(4):282-7
15. Turan S, Thiele S, Tafaj O, Brix B, Atay Z, Abali S, **Haliloglu B**, Bereket A, Bastepe M. Evidence of hormone resistance in a pseudo-pseudohypoparathyroidism patient with a novel paternal mutation in GNAS. *Bone* 2015 Feb; 71:53-7.
16. Guran T, Guran O, Paketci C, Kipoglu O, Firat I, Turan S, Atay Z, **Haliloglu B**, Bereket A. Effects of leukemia inhibitory receptor gene mutations on human hypothalamo-pituitary-adrenal function. *Pituitary*. 2014 Aug 22.
17. Abalı S, Turan S, Atay Z, Güran T, **Haliloglu B**, Bereket A. Higher insulin detemir doses are required for the similar glycemic control: comparison of insulin detemir and glargine in children with type 1 diabetes mellitus. *Pediatr Diabetes*. 2014 Jul 11.
18. Arman A, Bereket A, Coker A, Kiper PÖ, Güran T, Ozkan B, Atay Z, Akçay T, **Haliloglu B**, Boduroglu K, Alanay Y, Turan S. Cathepsin K analysis in a pycnodysostosis cohort: demographic, genotypic and phenotypic features. *Orphanet J Rare Dis.* 2014 Apr 26;9:60

19. Atay Z, Bereket A, **Haliloglu B**, Abali S, Ozdogan T, Altuncu E, Canaff L, Vilaça T, Wong BY, Cole DE, Hendy GN, Turan S. Novel homozygous inactivating mutation of the calcium-sensing receptor gene (CASR) in neonatal severe hyperparathyroidism-lack of effect of cinecalcet. *Bone*. 2014 Jul;64:102-7.
20. Atay Z, Bereket A, Turan S, **Haliloglu B**, Memisoglu A, Khayat M, Shalev SA, Spiegel R. A novel homozygous TMEM70 mutation results in congenital cataract and neonatal mitochondrial encephalo-cardiomyopathy. *Gene.* 2013; Feb 15;515(1):197-9.
21. **Haliloglu B**, Guran T, Atay Z, Abalı S, Mornet E, Bereket A, Turan S. Infantile loss of teeth: odonthohypophosphatasia or childhood hypophosphatasia. *Eur J Pediatr*. 2012; doi:10.1007/s00431-012-1868-4.
22. Turan S, Hughes C, Atay Z, Guran T, **Haliloglu B**, Clark AJ, Bereket A, Metherell LA. An Atypical Case of Familial Glucocorticoid Deficiency without Pigmentation Caused by Coexistent Homozygous Mutations in MC2R (T152K) and MC1R (R160W). *J Clin Endocrinol Metab*. 2012;97(5):E771-4.
23. **Haliloglu B**, Isguven P, Yıldız M, Arslanoglu I, Erguven M. Complementary and alternative medicine in children with type 1 diabetes mellitus. *J Clin Res Pediatr Endocrinol*. 2011;3(3):139-43.
24. Erguven M, Sahin K, Yilmaz O, **Haliloglu B**. Evaluation of Side Effects of Monotherapy and Combined Drug Therapies on Gastrointestinal System in Patients with Juvenile Idiopathic Arthritis. Calicut Medical Journal 2007; 5:e2.

ORAL PRESENTATIONS

In International Meetings

1. Lytrıvı M, De Franco E, Patel KA, Esteve MI, Cosentino C, Wakeling MN, **Haliloglu B**, Yıldız M, Godbole T, Hattersley AT, Cnop M. A novel Genetic Syndrome of Early-Onset Diabetes, Microcephaly and Epilepsy Due to Homozygous YIPF5 Mutations. ADA 2019, San Francisco.

2. De Franco E, Lytrıvı M, Patel KA, Esteve MI, **Haliloglu B**, Unal E, Godbole T, Yıldız M, Ellard S, Cnop M, Hatterslet AT. Mutations in YIPF5 are a novel cause of neonatal diabetes, highlighting the critical role of endoplasmic reticulum to Golgi trafficking in human beta cell survival. EASD 2018, Berlin
3. **Haliloglu B**, Akesen E, Atay Z, Guran T, Bereket A, Turan S. Diabetes Related Problems and Diabetic Controls Among the School Children With Type 1 Diabetes Mellitus Living in Istanbul. ISPAD 2012, Istanbul
4. Turan S, Hughes C, Atay Z, Guran T, **Haliloglu B**, Clark AJ, Bereket A, Metherell LA. An Atypical Case of Familial Glucocorticoid Deficiency without Pigmentation Caused by Coexistent Homozygous Mutations in MC2R (T152K) and MC1R (R160W). ENDO 2011, Boston
5. Turan S, Bereket A, Guran T, Akcay T, Atay Z, **Haliloglu B**, Bastepe M, Jüppner H. Autosomal recessive form of hypophosphatemic rickets related to Dentin matrix protein-1 (DMP-1) mutation. ESPE 2011 Working Group1-58, Glasgow

Publications in National Journals

1. **Haliloglu B**. Long Term Complications of Type 1 Diabetes. Türkiye Klinikleri; 2021;2(3):77-81.
2. Atmıs B, Engin M, **Haliloglu B,** Bayazıt AK, Anarat A. A case of congenital nephrogenic diabetes insipidus with aquaporin 2 gene mutation. Cukurova Med Journal 2018;43(4):1065-1067.
3. **Haliloglu B,** Tuysuz G, Tayfun F. Endocrinopathies in Turkish children with Thalassemia Major . J Pediatr Res 2017;4(4):216-9.
4. **Haliloglu B,** Tuysuz G, Kaya A. The effect of vitamin B12 deficiency on the GH-IGF1 axis. Med Science 2017;6(3):496-7.
5. Fedakar A, **Haliloglu B**. Leigh's Syndrome Presenting with Encephalopathy J Child 2010;2:94-97
6. Cakı S, Erguven M, **Haliloglu B**, Yoruk A, Saltık S. A-nine-year-old girl with diminished motor activity. Turkish Arch of Pediatr. 2007; 42:43-4.
7. Cebeci AN, Erguven M, Agachan N, Tekin M, **Haliloglu B**. Case report: Severe late neurotoxicity due to carbonmonoxide intoxication. J Child. 2007;7:197-200.

8. Haliloglu B, Kir Sahin F, Demirbasoglu S, Bozoklu O, **Haliloglu B**, Peker H. Neonatal outcomes of premature infants of severe preeclamptic mothers between 28-34 weeks' gestation. Kocatepe Tıp Der. 2005;6:35-39

Oral Presentations In National Meetings

1. **Haliloglu B**, Hysenaj G, Atay Z, Guran T, Abalı S, Turan S, Ellard S, Bereket A. GCK gene mutation frequency in patients with atypical diabetes. 17th National Pediatric Endocrinology Congress 2013.
2. **Haliloglu B**, Gokdemir Y, Atay Z, Abalı S, Guran T, Karakoc F, Ersu R, Karadag B, Turan S, Bereket A. Comparing glucose monitorisation by CGMS (Continue Glucose Monitorization System) vs standart OGTT in determining CFRD (Cystic Fibrosis-Related Diabetes). 16th National Pediatric Endocrinology Congress 2012.
3. Aydin BK, Saka N, Bas F, Poyrazoglu S, Bundak R, Yesiltepe Mutlu G, Cizmecioglu F, Hatun S, **Haliloglu B**, Turan S, Bereket A, Bezen D, Tutunculer F, Memioglu N, Guran T, Darendeliler F. Çocukluk çağındaki over kisti vakalarının incelenmesi: çok merkezli çalışma. 16. Ulusal Pediatrik Endokrinoloji Kongresi 2012. (Sözlü sunum)
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POSTERS

Posters in International Congress

1. 1**. Haliloglu B.** Somatostatine Analogue in Hypothalamic Obesity. 58th Annual Meeting of the ESPE 2019.
2. 2. **Haliloglu B.** A 45X0/46XY Girl Diagnosed with Prepubertal FSH Elevation. 57th Annual Meeting of the ESPE 2018.
3. **Haliloglu B**, Losekoot M, Kaya A, Wit JM. IGFALS Gene Deletion in a Family with Short Stature. 55th Annual Meeting of the ESPE 2016.
4. Abalı S, Tamura M, Atay Z, Isguven P, Guran T, **Haliloglu B**, Bas S, Isojima T, Turan S, Kitanaka S, Bereket A. Hereditery vitamin D-resistance rickets: Report of four cases with successful use of intermittent intravenous calcium via peripheral route. 54th Annual Meeting of the ESPE 2015
5. **Haliloglu B**, Tuzun H, Flanagan S, Celik M, Ellard S, Kaya A. The Effectiveness of Sirolimus in a Newborn with Hyperinsulinemic Hypoglycemia. 54th Annual Meeting of the ESPE 2015.

6. **Haliloglu B**, Turan S, Atay Z, Guran T, Abali S, Bas S, Bereket A. Younger age and BMI>3 SD are risk factors for mortality in children with hypothalamic obesity. 53rd Annual Meeting of the ESPE 2014.

7. Turan S, Zeynep Atay Z, Gokdemir Y, Bas N, **Haliloglu B**, Abali S, Bas S, Refika Ersu R, Bereket A. Sleep-related Breathing Disorders in Pycnodysostosis 53rd Annual Meeting of the ESPE 2014.

8. Atay Z, Abali S, Guran T, **Haliloglu B**, Bas S, Turan S, Bereket A. Final Height in girls with idiopathic central precocious puberty treated with GnRH anlog: comparison with untreated controls. 53rd Annual Meeting of the ESPE 2014.

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