Severe Iatrogenic Hypoglycemia Modulates the Fibroblast Growth Factor Protein Response

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Abstract

Introduction. There is evidence that fibroblast growth factor (FGF) levels may be implicated in hypoglycemia, with FGF-19 being a potential contributor to insulin-independent pathways driving postprandial hypoglycemia following bariatric surgery and basicFGF (FGF2) being elevated following the mild hypoglycemia occurring post-glucose tolerance test. However, their response following severe iatrogenic hypoglycemia is unknown and therefore this pilot exploratory study was undertaken.

Methods. A case-control study of aged-matched type 2 diabetes (T2D; n=23) and control (n=23) subjects who underwent a hyperinsulinemic clamp, initially to euglycemia in T2D (5mmol/l; 90mg/dl), and then to hypoglycemia (<2mmol/L; <36mg/dl) with subsequent follow-up timecourse to 24-hours. FGF proteins were determined by Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement.

Results. At baseline, FGF12 (p=0.006) was higher and FGF20 (p=0.004) was lower in T2D versus controls. Post-hypoglycemic levels of FGF18, FGF19, FGF20 and FGF23 were lower while FGF12 and FGF16 were higher in T2D versus control at different time points, though FGF2 did not differ throughout. At 24-hours post-hypoglycemia, FGF20 (p=0.01) differed between controls and T2D, while the levels for the other proteins measured returned to baseline. None of the FGF proteins altered from baseline to euglycemia when clamped in T2D subjects.

Conclusion. Severe transient hypoglycemia transiently modulated FGF proteins in T2D, several of which have been associated with diabetes related complications, suggesting that recurrent hypoglycemia may contribute to complication development through changes in FGF proteins.

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Introduction:

Type 2 diabetes (T2D) is characterized by insulin resistance and progressive loss of beta cell mass and function (*1, 2*), whilst endogenous glucose production and postprandial hyperglucagonemia are also major traits of the disease (*3-5*) The global prevalence of T2D is estimated to be ~9% of the total population, thereby affecting ~500 million people (*6*). The therapeutic paradigm in the management of T2D aims to achieve normoglycemia with pharmacological agents and insulin therapy may be required. Glycemic control is a delicate balancing act in diabetes treatment, with stricter control increasing the risk of hypoglycemic episodes, those patients treated with insulin having the greatest risk (*7*).

Hypoglycemia is also recognized as a complication post-bariatric surgery (*8*). The underlying mechanisms responsible for glucose dysregulation post-bariatric surgery are complex and not well defined, but are consequent upon an exaggerated appearance of dietary glucose in the circulation due to the altered upper gastrointestinal anatomy (*9*). Abnormal glucagon secretion is also involved in the pathogenesis of both hypoglycemia and hyperglycemia in diabetes (*4*). Regardless of cause, recurrent severe hypoglycemia is associated with cardiovascular and cerebrovascular disease and may result in death (*10, 11*).

Fibroblast growth factors function by binding to FGF receptors, and can act in an autocrine, paracrine or endocrine manner **(Figure 1)**. FGFs are broadly classified as mitogenic and metabolic based on their function (*12*). Mitogenic FGFs have affinity for extracellular matrix heparan sulfate (HS) and therefore become trapped at the site of secretion and exert their action locally, whereas

the metabolic FGFs, lacking an HS binding domain, enter the circulation and therefore function in a hormone-like manner **(Figure 2)** (*13*).

FGF2 (also called basic FGF) was found to be elevated by acute hypoglycemia in non-diabetics with symptoms of reactive hypoglycemia (*14*). FGF2 is correlated to BMI and synthesized by adipocytes (*15*) and has been associated with inducing angiogenesis (*16*), with diabetic retinopathy (*17*) and with wound healing (*18*). FGF16 is purported to have a cardioprotective role in animal models of diabetic myocardial infarction (*19*). FGF16 is also associated with high glucose-induced cell proliferation *in vitro* (*20*); however, its role clinically is unclear.

FGFs have a vital role in energy metabolism regulation and metabolic homeostasis of bile acids, lipids and glucose (*21, 22*). Levels of FGFs are found to be altered in different pathological conditions such as T2D (*23*), obesity (*24*) and non-alcoholic fatty liver disease (NAFLD) (*25*). FGF18 is a growth factor and stimulates proliferation in several different tissues, including the liver, and has been associated with the hair cycle (*26*), though no association with diabetes or diabetes-related complications has been described. FGF19 is a postprandial insulin independent protein that regulates hepatic protein and glycogen synthesis and is involved in lipid and energy metabolism (*27*). Lower levels of FGF19 have been reported in newly diagnosed T2D patients, FGF19 having a close relationship to β-cell function, as indicated by the insulin secretion-sensitivity index-2 (*28*). In recent studies, increased levels of FGF19 have been shown in patients with post-bariatric surgery hypoglycemia (PBH) versus asymptomatic post-bariatric surgery patients (*29*). A prospective study of obese T2D patients undergoing bariatric surgery also showed elevated levels of FGF19 which was associated with improved mitochondrial health in adipose tissues (*30*). A meta-analysis of patients undergoing bariatric surgery revealed an increase in circulating levels of FGF19, implying a beneficial role post-procedure. In patients with diabetes

and impaired renal function, FGF20 has been associated with protection against renal function and a decline in end stage renal disease (ESRD) (*31*). FGF23 has a role in phosphate, calcium and vitamin D homeostasis (*32*) and increased levels of FGF23 have been found in patients with chronic kidney disease (*33*). FGF23 is associated with an increased risk of cardiovascular events, as well as increased mortality in T2D in patients who have normal and mildly impaired renal function (*34*).

Based upon the important role of FGFs in T2D and its co-morbidities, and the altered levels of FGF19 in reactive hypoglycemia or PBH, we sought to determine the FGF protein response following severe induced iatrogenic hypoglycemia. Therefore, this pilot exploratory study was undertaken on FGF2 and FGF19 and other members of the FGF family. This study was designed with intent to mimic a hypoglycaemic episode as would be experienced in practice by a diabetic patient.

Methods

Study design

This prospective parallel study was performed at the Diabetes Centre at Hull Royal Infirmary in 46 adult Caucasian subjects, T2D (n = 23) and control (n = 23). Subjects were aged 40–70 years. In T2D, diabetes duration was <10 years and all had been a stable medication for the preceding 3 months (metformin, statin and/or angiotensin converting enzyme inhibitor/angiotensin receptor blocker). Only metformin as anti-diabetic therapy was allowed; in addition, inclusion criteria were: HbA1c < 10% (86 mmol/mol), no hypoglycemia or hypoglycemic unawareness during the prior 3-months, and no diabetes related complications. In the control group, an oral glucose

tolerance test (OGTT) was performed to exclude diabetes. Body mass index (BMI) was in the range of 18-49 kg/m^2; liver and kidney biochemical profiles were normal; no prior history of cancer; no contraindication for insulin infusion to hypoglycemia (epilepsy, seizure history, drop attacks, ischemic heart disease, history of adrenal insufficiency and treated hypothyroidism).

Study subjects

History, physical examination, routine blood tests and an electrocardiogram was performed in all subjects. Hypoglycemia was induced by a continuous insulin infusion as previously detailed (*35*) with blood samples taken at hypo (time 0), 0.5h,1h, 2h and 4h post-hypoglycemia. After 4-hours, participants were fed and the T2D subjects took their a.m. diabetes medications. Evening medication was taken by patients as per prescription. The following day (~24-hours post-hypoglycemia), patients delayed taking their medications and remained fasting until further blood tests were drawn. Following breakfast but prior to discharge, a blood glucose measurement was taken with a point-of-care glucose analyser (HemoCue glucose 201 +) to confirm levels within the normal range, plus normal vital signs.

Subjects gave their informed written consent. The North West-Greater Manchester East Research Ethics Committee (REC number:16/NW/0518) gave approval for this study; registration documented at www.clinicaltrials.gov (NCT03102801) on 06/04/2017; conduct of the study was according to the Declaration of Helsinki.

Insulin infusion

The infusion of insulin infusion was undertaken as previously detailed (*35*). “After an overnight fast, and 30–60 min before the start of the clamp (0830 h), indwelling cannulas were inserted

bilaterally into the ante-cubital fossae. Soluble intravenous (IV) insulin (Humulin S, Lilly, UK) was given using a pump to induce hypoglycemia; the starting dose was 2.5 mU/kg body weight/min, increasing by 2.5 mU/kg body weight/min after each 15 min period; two capillary blood glucose readings of ≤ 2.2 mmol/L (< 40 mg/dl) or a single reading of ≤ 2.0 mmol/L (36 mg/dl) were documented in each subject [using a glucose analyser (HemoCue glucose 201 +)]. Initially patients with T2D were clamped to euglycemia (5mmol/l; 90mg/dl), then subsequently to hypoglycemia. The timecourse for subsequent blood sampling was timed according to when hypoglycemia occurred. Immediately after hypoglycemia was achieved, IV glucose (150 ml of 10% dextrose) was administered, with a repeat blood glucose measurement 5 min later if the reading was < 4.0 mmol/L.

Biochemical markers

As previously described (*36*), blood samples were immediately centrifuged (2000 g, 15 min, 4 °C); within 30-min of collection, aliquots were stored (-80 °C), awaiting batch analysis. Fasting plasma glucose (FPG), high-density lipoprotein (HDL), triglycerides and cholesterol levels were analyzed with a Beckman AU 5800 analyser (Beckman-Coulter, High Wycombe, UK).

Slow Off-rate Modified Aptamer (SOMA)-scan assay

Slow Off-rate Modified Aptamer **(**SOMA)-scan technology (SomaLogic, Boulder, CO) was used for protein quantification, as described previously, and following manufacturer's instructions (*37-39*). The assay utilized 96-well plates (85 plasma samples, 5 calibrator samples, 3 quality control (QC) samples per plate). Diluted EDTA plasma samples (40%, 1% and 0.05% bins) underwent the following: 1) binding analytes plus primer beads (PB)-SOMAmers equilibrated; 2) Streptavidin-substituted support used to immobilise analyte/SOMAmers complexes, followed by

washing 3) Cleavage step to release analyte-SOMAmer complexes into solution using long-wave ultraviolet (UV) light; 4) Analyte-borne biotinylation selectively immobilised the analyte-SOMAmer complexes on streptavidin support; washing steps; 5) Disruption of analyte-SOMAmer complexes by denaturation; released SOMAmers act as surrogates for analyte concentration quantification; 6) Quantification accomplished by hybridization of SOMAmer-complementary oligonucleotides to custom arrays. Normalization steps were performed based on the included standard samples, as previously described (*37, 40*)

The SomaScan Assay (Version 3.1) was used to target FGF proteins and glucagon in the SomaScan panel. Baseline, hypoglycemia and post-hypoglycemia (0.5, 1, 2, 4 and 24 hours) timepoints were utilised.

Data processing/analysis

Agilent Feature Extraction Software (Agilent, Santa Clara, CA) provided the initial raw Relative Fluorescent Units (RFUs) from microarray intensity images. These RFUs were normalized and calibrated using SomaLogic software.

R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) was employed to perform statistical analysis on $\log_2$ RFU values; limma models were used to analyze differential protein expression, comparing between T2D and control subjects, as well as between timepoints (*41*). Random effects and batch effects were corrected for; the Benjamini–Hochberg method was used to correct P-values (*42*).

Statistical analysis

No published studies are available upon which to base a power calculation that detail changes in FGF proteins in response to hypoglycemia. A publication by Birkett and Day reviewed pilot study sample size (*43*); the authors stated that, at a minimum, 20 degrees-of-freedom is necessary for estimation of effect size and variability. To meet that criterion, we therefore needed to analyze plasma samples from a minimum 20 patients per cohort. Student's t-test was used for both within group (from one timepoint to the next) and between group (at each timepoint) comparisons; $p < 0.05$ was taken as significant. Statistical analysis and graph presentation utilised Graphpad Prism (San Diego, CA, USA).

Interaction networks

The Search Tool for the Retrieval of Interacting Genes (STRING 11.0) allowed visualization of known and/or predicted interactions for the FGF proteins reported here (https://string-db.org/).

Results:

Details of clinical characteristics for the study subjects (23 T2D patients; 23 normal individuals) are shown in Table 1. The glucose response during the study is shown in Supplementary figure 1A.

FGF response to euglycemia in T2D

There was no change in any of the FGF proteins (FGF 2, 12, 16, 18, 19, 20 or 23) in T2D from baseline levels to euglycemia (5mmol/l; 90mg/dl), prior to subsequent hypoglycemia (Supplementary figure 2).

Difference between T2D and control groups

Baseline differences between T2D and control groups (significance between control and T2D is denoted by the symbol '*' in Figure 3)

At baseline, levels of FGF12 were elevated ($p<0.01$) (Figure 3A) and FGF20 were reduced ($p<0.01$) (Figure 3B) in the T2D group. The levels of FGF20 were significantly lower in T2D at all the points (Figure 3B). Baseline levels of other proteins did not differ significantly between the groups (Figure 3C-G). Glucagon was elevated in T2D versus controls at baseline ($p<0.01$) (Supplementary figure 1B).

Differences at hypoglycemia between T2D and control groups (significance between control and T2D is denoted by the symbol '*' in Figure 3)

Significant differences between control and T2D subjects were observed in response to hypoglycemia for the following proteins: levels of FGF20 ($p<0.001$) (Figure 3B) and FGF23 ($p<0.01$) (Figure 3C) were lower in T2D subjects.

Post-hypoglycemia differences between T2D and control groups (significance between control and T2D is denoted by the symbol '*' in Figure 3)

Whilst there was no difference in FGF12 levels at hypoglycemia due to a transient increase in levels in controls, as control levels decreased post-hypoglycemia there was a trend for levels of FGF12 to be elevated at 30-minutes post-hypoglycemia in T2D, with significance at 1-hour ($p<0.05$), after which levels were comparable in the two groups (Figure 3A). Though the cohorts showed a remarkably similar pattern in FGF20 levels during the study, as noted above, the levels of FGF20 were reduced in T2D subjects throughout, including at all the post-hypoglycemia time points (0.5-hours, $p<0.001$; 1-hour, $p<0.01$; 2-hours, $p<0.05$; 4-hours, $p<0.05$; 24-hours, $p<0.05$) (Figure 3B). There was a clear trend for levels of FGF23 to be lower in T2D throughout the study with significance at 0.5-hours ($p<0.05$), 1-hour ($p<0.05$) and 2-hours ($p<0.05$) (Figure 3C) post-hypoglycemia. Levels of FGF18 followed a similar pattern in both groups, with only a difference at 1-hour post-hypoglycemia ($p<0.05$) (Figure 3D) where FGF18 was lower in T2D. Again, levels of FGF19 followed a similar pattern in both cohorts, with a divergence seen only at the 2-hour post-hypoglycemia timepoint where levels spiked higher in controls (2-hours, $p<0.05$) (Figure 3E). Increased levels of FGF16 occurred at 0.5-hours ($p<0.01$) in T2D (Figure 3F) due to a decrease in control levels, a trend that was maintained throughout the remainder of the study.

In view of the changes in FGF12 and its prediction of insulin resistance with adiponectin (*44*), baseline adiponectin levels were measured and shown to be significantly lower in T2D (Baseline:

1885±100 vs 3319±304 RFU, T2D vs control, $p<0.0001$), though there was no correlation between FGF12 and adiponectin.

At 24-hours post-hypoglycemia, glucagon was elevated in T2D versus controls ($p<0.05$) (Supplementary figure 1B).

Differences within groups at different time points

Baseline to Hypoglycemia and post-hypoglycemia in T2D (Significance is denoted by the symbol '$' in Figure 3)

FGF20 was reduced significantly at the 1-hour ($p<0.01$) and 2-hour ($p<0.001$) timepoints post-hypoglycemia (Figure 3B). A steep reduction in levels of FGF18 occurred at 2-hours post-hypoglycemia ($p<0.001$) which returned to baseline levels by 4-hours post-hypoglycemia (Figure 3D). The levels of all the FGFs returned to baseline by 24-hours post-hypoglycemia.

Glucagon decreased significantly from baseline to hypoglycemia ($p<0.01$) in T2D. Thereafter, levels of glucagon increased, revealing a significant increase over baseline at the 4-hour post-hypoglycemia timepoint ($p<0.01$) (Supplementary figure 1B).

Baseline to Hypoglycemia and post-hypoglycemia in controls (Significance is denoted by the symbol '#' in Figure 3)

Decreases in FGF20 levels occurred at 1-hour ($p<0.01$) and 2-hours ($p<0.001$) post-hypoglycemia in controls that returned to baseline by 24-hours (Figure 3B). FGF18 levels decreased significantly at 2-hours ($p<0.05$) (Figure 3D) but had returned to baseline by 4-hours post-hypoglycemia, a pattern closely matching that in the T2D group. FGF19 levels spiked upward at 2-hours ($p<0.05$) post-hypoglycemia (Figure 3E), returning to baseline thereafter. A decrease in levels of FGF16

occurred in controls at hypoglycemia ($p<0.05$) and remained low at all subsequent timepoints (0.5-hours, $p<0.05$; 1-hour, $p<0.01$, 2-hours, $p<0.05$; 4-hours, $p<0.01$; 24-hours, $p<0.05$) (Figure 3F). Glucagon levels did not change in response to hypoglycemia in controls (Supplementary figure 1B). In the post-hypoglycemia follow up period, glucagon levels rose in controls, reaching significance over baseline at the 1-hour, 2-hour and 4-hour timepoints (all $p<0.0001$), but reverting to baseline levels by 24-hours (Supplementary figure 1B).

Hypoglycemia versus post-hypoglycemia in T2D (Significance is denoted by the symbol '&' in Figure 3)

FGF20 levels were significantly decreased from hypoglycemia to the early post-hypoglycemia period (0.5-hours and 1-hour, $p<0.01$; 2-hours, $p<0.001$) (Figure 3B). Relative to hypoglycemia levels, FGF23 levels increased at 4-hours ($p<0.05$) though normalized by 24-hours (Figure 3C). FGF18 levels decreased from hypoglycemia to 2-hours post-hypoglycemia ($p<0.0001$) (Figure 3D), reverting thereafter to baseline.

Relative to hypoglycemic levels, glucagon was significantly elevated at the 1-hour, 2-hour, 4-hour (all $p<0.0001$) and 24-hour ($p<0.01$) timepoints (Supplementary figure 1B).

Hypoglycemia versus post-hypoglycemia in control (Significance is denoted by the symbol '^' in Figure 3)

FGF20 levels decreased significantly from hypoglycemia to the 0.5-4-hour timepoints (0.5h, $p<0.01$; 1h, $p<0.001$; 2h, $p<0.00$; 4h, $p<0.05$), returning to baseline by 24-hours (Figure 3B). Paralleling the changes in T2D, FGF18 levels decreased at 2-hours post-hypoglycemia in controls

296 ($p<0.01$) (Figure 3D), reverting thereafter to baseline whilst, by contrast, FGF19 levels spiked at
297 2-hours ($p<0.05$) (Figure 3E) before returning to baseline.
298 Relative to hypoglycemic levels, glucagon was significantly elevated at 30-minutes ($p<0.05$), 1-,
299 2- and 4-hours ($p<0.0001$ for all) and 24-hours ($p<0.05$) (Supplementary figure 1B).
300
301 **STRING analysis**
302 STRING analysis was performed to detail the relationship between the FGF proteins that
303 confirmed the close relationship of the FGF proteins to each other in this study (Supplementary
304 figure 3).
305

Discussion

Baseline elevations of FGF12 was seen in T2D, whilst FGF20 was lower; with hypoglycemia, further FGF changes were seen for FGF18, FGF19, FGF20 and FGF23, that were lower, while FGF12 and FGF16 were higher in T2D. At 24-hours, FGF20 remained lower in T2D.

Baseline FGF12 was initially higher in T2D but thereafter, with the exception of one timepoint (1-hour post-hypoglycemia), did not differ from control subjects, either at or following hypoglycemia. FGF12 lacks the N terminal signal sequence and has been thought of as being primarily intracellular, acting as a nuclear localization signal. Despite having the similarity in sequence and structure to other FGFs, FGF12 does not activate any of the 7 FGF receptors on cell surfaces (*45*). However, circulatory levels of FGF12 and its changes in response to glycemia have not been investigated extensively. Here, we report that plasma levels of FGF12 are higher in obese patients with T2D compared to control subjects. FGF12 levels did not alter in response to hypoglycemia or post-hypoglycemia in T2D, which may suggest an impaired glucose lowering effect in T2D (*44*). In a study in subjects with impaired glucose tolerance and using the SOMAscan proteomic platform (as was also used in this study), FGF12 was reported to be the top protein associated with a decline in pancreatic beta cell function; the other top ranked protein associated with decline in beta cell function was adiponectin, that was decreased in those with impaired glucose tolerance (*44*). The data reported by Belongie et al are in accord with the data presented here, with higher levels of FGF12 in T2D versus controls, also aligning with their findings. These data would therefore suggest the changes seen here for FGF12 may reflect beta cell function (*44*). That plasma levels of FGF12 were higher in this obese non-diabetic cohort relative to the T2D cohort is interesting and suggests that plasma levels of FGF12 should be considered for use as a biomarker

to study the progressive decline of beta cell function from normoglycemia, through impaired fasting glucose, to onset of T2D.

Baseline FGF20 was lower in T2D, at hypoglycemia and throughout the post-hypoglycemia time course. FGF20 has been associated with protection against renal function and a decline in end stage renal disease (ESRD) (*31*). The SOMAscan platform was used for proteomic analysis in two longitudinal exploratory studies of type 1 (T1D) and type 2 diabetes (T2D) patients with chronic kidney disease (CKD) stage-3 compared to those with normal renal function (*31*). Data from that study suggested that ANGPT1, TNFRSF12 and FGF20 showed a strong, additive protective effect against decline in renal function. The short duration of diabetes in the cohort here, with normal renal function, may be the reason that FGF20 was lower in T2D versus controls, but we did not have a reference population of T2D with CKD with which to compare. Nonetheless, it could be seen that FGF20 was reduced by hypoglycemia in both controls and T2D in a parallel fashion, though with recovery at 4 hours; hypothetically, this may mean that recurrent hypoglycemia could blunt its protective effect for CKD.

It was noteworthy that the change in blood glucose to euglycemia in T2D (5mmol/l; 90mg/dl) was not associated with any fluctuation in the levels of the FGF proteins. Given the changes in glucose variability reporting to affect oxidative stress and inflammatory markers, it was surprising that no changes were seen (*46*); however, this suggests that the changes in FGF are independent of glycemia and that their investigation does not require fasting samples.

There was no change in FGF2 for either the T2D or control groups, suggesting that FGF2 is not under the regulation of marked changes of glycemia nor of the inflammatory, oxidative stress or heat shock protein changes associated with hypoglycemia (*47*). FGF2 has been correlated to BMI

(*15*) and has been associated with inducing angiogenesis (*16*); in diabetes, it is related to the development of diabetic retinopathy (*17*) and improvement in wound healing (*18*). In vitro, fibroblasts cultivated in high glucose displayed increased FGF2 mRNA, protein synthesis and secretion compared with normal glucose (*48*), suggesting that local tissue FGF2 modulation rather than circulating levels may result in hyperglycemia..

FGF16 showed no change in T2D to hypoglycemia, but there was a decrease in control subjects with the levels at 24-hours being lower relative to baseline. FGF16 has been associated with a cardioprotective role in animal models of diabetic myocardial infarction (*19*). FGF16 is also associated with high glucose-induced cell proliferation *in vitro* (*20*) and its fall in controls may be protective by decreasing the drive for proliferation with a fall in glucose that is lost in T2D; however, its role clinically is unclear. The overall elevated level of FGF16 in T2D might be due to obesity of the T2D cases, because in rat, FGF16 has been reported as a unique growth factor involved in proliferation of embryonic brown adipose tissue (*49*). However, further studies with human adipocytes from patients with obesity and T2D are required to confirm this speculation.

FGF18 showed the same decrease, significantly so, at 2-hours in both T2D and controls with no difference between the cohorts. It has been suggested that FGF18 is a pleiotropic growth factor that can stimulate proliferation in several different tissues, including the liver, and has been associated with the hair cycle (*50*). These data are therefore novel, describing the decrease in FGF12 that reached a nadir at 2-hours after hypoglycemia, suggesting that it is potentially modulated by glycemia and, given its pleiotropic growth stimulation role, its decrease in response

to low glucose levels may be protective as hypothesized for FGF16; however, no role of FGF18 has been identified in T2D.

Fibroblast growth factor 19 (FGF19), a hormone secreted from the ileum, has been shown to act as a modulator of lipid and glucose homeostasis, and could well be important to metabolic recovery post-bariatric surgery (*51*). Clinical study data seems to implicate FGF19 as causative rather than consequent upon type-2 diabetes improvement (*52*). Moreover, direct administration of recombinant FGF-19 to obese mice resulted in an increased metabolic rate concurrent with increased fatty acid oxidation which, in turn, caused significant weight reduction and reversed dietary and leptin-deficient diabetes (*53*). In the current study, FGF19 was shown to peak in controls at 2-hours, while no change in the T2D cohort was seen, which may suggest the anti-obesity response of FGF19 in response to lowering glucose concentration in control subjects, but that response is blunted in T2D. Moreover, FGF19 is associated with insulin resistance, metabolic syndrome and non-alcoholic fatty liver disease, where lower levels are reported (*54*); that may again explain why there was no response to hypoglycemia in our T2D cohort, likely consequent upon the underlying insulin resistant state whereas, by contrast, the control group showed a significant response. In addition, as mentioned earlier, FGF19 expression has been associated with an increased metabolic rate in animal models (*53*) and, given the increase in metabolic rate associated with hypoglycemia (*55*), this may also help explain the increase in FGF19 with hypoglycemia seen in controls.

FGF23 was shown to be lower in T2D compared to controls at all timepoints and both T2D and controls were unaffected by hypoglycemia, suggesting that glycemic changes and the metabolic

response to hypoglycemia do not modulate FGF23 *in vivo*. FGF23 is associated with an increased risk of cardiovascular events and mortality in T2D in patients with both normal and mildly impaired renal function (*34*). In addition, in patients with T2D and chronic kidney disease, FGF23 has been associated with insulin resistance and inflammation (*56*). Here, FGF23 was lower than in controls and may reflect that these patients had no diabetes-related complications, and their diabetes was of short duration, raising the possibility that sequential measurement of FGF23 may indicate advancing cardiovascular disease risk if its levels increase.

The STRING analysis was undertaken to confirm the physical and/or functional interactions of the FGF proteins and showed the close relationship of the individual FGF proteins that were studied here to determine their response to iatrogenic hypoglycemia.

Overall, from the evidence detailed above, it can be seen that FGF2 showed no acute changes and therefore its reported correlation with diabetic retinopathy (*17*) is likely to be a consequence of long-term aberrations in circulating glucose and unrelated to acute hypoglycemic insults, as modelled here. Conversely, it can be seen that FGFs 16, 18, 19, 20 and 23 showed acute changes that were glycemia dependent. Whilst these results are intriguing, more studies are needed to establish the connection between FGF proteins and diabetes complication development in T2D, as the data on this relationship remains scant. Here, those FGFs associated with diabetes complications showed a complex picture; FGF16 is cardioprotective (*57*) and therefore the decrease seen here may be detrimental and, similarly, FGF20 is protective for renal function decline (*31*) and therefore its fall here may be detrimental. However, FGF23 elevation has been associated with increased cardiovascular disease events (*34*) and here was lower, suggesting a potential beneficial effect.

The T2D patients studied here had a short duration of disease and were relatively treatment naïve, adding strength to this study. Major study limitations include the small number of participants, as more subjects may have further highlighted FGF protein changes during the timecourse. However, such a severe hypoglycemic episode would be expected to make apparent any induced changes in FGF protein levels. The T2D subjects had an elevated BMI, though this is unlikely to have altered expression of FGFs to the hypoglycemic insult. This was an entirely Caucasian population; as such, the results presented here may not be generalizable to other ethnic groups. In addition, serum levels of FGF proteins may not reflect cellular levels or activity. Of note, the counter-regulatory response in both controls and T2D was robust and did not differ between the groups, indicative of the short duration of diabetes in the T2D group, as the response may be blunted in long standing diabetes.

In conclusion, severe transient hypoglycemia transiently modulated FGF proteins in T2D, some of which have been associated with diabetes-related complications, suggesting that recurrent hypoglycemia may contribute to complication development through changes in FGF proteins.

DECLARATIONS

Conflict of interest: No authors have any conflict of interest or competing interests to declare.

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Author contributions

MN, ASMM and AEB analyzed the data and wrote the manuscript. AA-Q performed the clinical studies. TS supervised clinical studies and edited the manuscript. SLA contributed to study design,

data interpretation and the writing of the manuscript. All authors reviewed and approved the final version of the manuscript. Alexandra E Butler is the guarantor of this work.

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Consent for publication: All authors gave their consent for publication.

Availability of data and materials: All the data for this study will be made available upon reasonable request to the corresponding author.

Abbreviations

FGF1 Fibroblast growth factor 1; FGF2 Fibroblast growth factor 2; FGF4 Fibroblast growth factor 4; FGF6 Fibroblast growth factor 6; FGF7 Fibroblast growth factor 7; FGF 8A Fibroblast growth factor 8A; FGF8B Fibroblast growth factor 8B; FGF9 Fibroblast growth factor 9; FGF10 Fibroblast growth factor 10; FGF12 Fibroblast growth factor 12; FGF18 Fibroblast growth factor 18; FGF19 Fibroblast growth factor 19; FGF20 Fibroblast growth factor 20; FGF23 Fibroblast growth factor 23;FGFR1 Fibroblast growth factor receptor 1; FGFR2 Fibroblast growth factor receptor 2; Fibroblast growth factor receptor 3; Fibroblast growth factor receptor 4 Fibroblast growth factor receptor 23; STAT3 Signaltransducer and activator of transcription 3**;** IL17RD Interleukin-17 receptor D**;** GREM1 Gremlin-1; PLCG1 1-phosphatidylinositol 4,5-bisphosphate

phosphodiesterase gamma-1; ITGAV Integrin alpha-V; YES1 Tyrosine-protein kinase; FGR Tyrosine-protein kinase; FLRT1 Leucine-rich repeat transmembrane protein;

References:

1. M. E. Cerf, Beta cell dysfunction and insulin resistance. *Front Endocrinol (Lausanne)* **4**, 37 (2013).
2. A. E. Butler *et al.*, Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102-110 (2003).
3. R. A. Rizza, Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. *Diabetes* **59**, 2697-2707 (2010).
4. P. E. Cryer, Minireview: Glucagon in the pathogenesis of hypoglycemia and hyperglycemia in diabetes. *Endocrinology* **153**, 1039-1048 (2012).
5. S. Haedersdal, A. Lund, F. K. Knop, T. Vilsboll, The Role of Glucagon in the Pathophysiology and Treatment of Type 2 Diabetes. *Mayo Clin Proc* **93**, 217-239 (2018).
6. N. H. Cho *et al.*, IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* **138**, 271-281 (2018).
7. L. C. Perlmuter, B. P. Flanagan, P. H. Shah, S. P. Singh, Glycemic control and hypoglycemia: is the loser the winner? *Diabetes Care* **31**, 2072-2076 (2008).
8. E. Suhl, S. E. Anderson-Haynes, C. Mulla, M. E. Patti, Medical nutrition therapy for post-bariatric hypoglycemia: practical insights. *Surg Obes Relat Dis* **13**, 888-896 (2017).
9. M. Salehi, A. Vella, T. McLaughlin, M. E. Patti, Hypoglycemia After Gastric Bypass Surgery: Current Concepts and Controversies. *J Clin Endocrinol Metab* **103**, 2815-2826 (2018).
10. K. Khunti *et al.*, Hypoglycemia and risk of cardiovascular disease and all-cause mortality in insulin-treated people with type 1 and type 2 diabetes: a cohort study. *Diabetes Care* **38**, 316-322 (2015).
11. P. E. Cryer, Severe hypoglycemia predicts mortality in diabetes. *Diabetes Care* **35**, 1814-1816 (2012).
12. X. Li, The FGF metabolic axis. *Front Med* **13**, 511-530 (2019).
13. A. Kharitonenkov, FGFs and metabolism. *Curr Opin Pharmacol* **9**, 805-810 (2009).
14. W. F. Eik *et al.*, Blood levels of pro-inflammatory and anti-inflammatory cytokines during an oral glucose tolerance test in patients with symptoms suggesting reactive hypoglycemia. *Braz J Med Biol Res* **49**, (2016).
15. N. Gaebler *et al.*, Age- and BMI-Associated Expression of Angiogenic Factors in White Adipose Tissue of Children. *International journal of molecular sciences* **20**, (2019).
16. M. Presta, G. Andrés, D. Leali, P. Dell'Era, R. Ronca, Inflammatory cells and chemokines sustain FGF2-induced angiogenesis. *Eur. Cytokine Netw.* **20**, 39-50 (2009).
17. L. Fang, A. J. Barber, J. S. Shenberger, Regulation of fibroblast growth factor 2 expression in oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci* **56**, 207-215 (2014).
18. J. Wu *et al.*, Heparin-Based Coacervate of FGF2 Improves Dermal Regeneration by Asserting a Synergistic Role with Cell Proliferation and Endogenous Facilitated VEGF for Cutaneous Wound Healing. *Biomacromolecules* **17**, 2168-2177 (2016).
19. Y. Hu *et al.*, FGF-16 protects against adverse cardiac remodeling in the infarct diabetic heart. *Am J Transl Res* **9**, 1630-1640 (2017).
20. C. Chen, C. Zhao, C. Gu, X. Cui, J. Wu, MiRNA-144-3p inhibits high glucose induced cell proliferation through suppressing FGF16. *Biosci. Rep.* **39**, (2019).

21. D. Struik, M. B. Dommerholt, J. W. Jonker, Fibroblast growth factors in control of lipid metabolism: from biological function to clinical application. *Curr Opin Lipidol* **30**, 235-243 (2019).
22. V. J. Nies *et al.*, Fibroblast Growth Factor Signaling in Metabolic Regulation. *Front Endocrinol (Lausanne)* **6**, 193 (2015).
23. L. M. Jaacks, K. R. Siegel, U. P. Gujral, K. M. Narayan, Type 2 diabetes: A 21st century epidemic. *Best Pract Res Clin Endocrinol Metab* **30**, 331-343 (2016).
24. B. Q. Farah, W. L. Prado, T. R. Tenorio, R. M. Ritti-Dias, Heart rate variability and its relationship with central and general obesity in obese normotensive adolescents. *Einstein (Sao Paulo)* **11**, 285-290 (2013).
25. M. Ocker, Fibroblast growth factor signaling in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Paving the way to hepatocellular carcinoma. *World J Gastroenterol* **26**, 279-290 (2020).
26. Y. Shwartz *et al.*, Cell Types Promoting Goosebumps Form a Niche to Regulate Hair Follicle Stem Cells. *Cell* **182**, 578-593.e519 (2020).
27. C. Degirolamo, C. Sabba, A. Moschetta, Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. *Nat Rev Drug Discov* **15**, 51-69 (2016).
28. M. J. Tang *et al.*, Serum fibroblast growth factor 19 and endogenous islet beta cell function in type 2 diabetic patients. *Diabetol Metab Syndr* **11**, 79 (2019).
29. C. M. Mulla *et al.*, Plasma FGF-19 Levels are Increased in Patients with Post-Bariatric Hypoglycemia. *Obes Surg* **29**, 2092-2099 (2019).
30. L. Martinez de la Escalera *et al.*, Impact of gut hormone FGF-19 on type-2 diabetes and mitochondrial recovery in a prospective study of obese diabetic women undergoing bariatric surgery. *BMC Med* **15**, 34 (2017).
31. Z. I. Md Dom *et al.*, Circulating proteins protect against renal decline and progression to end-stage renal disease in patients with diabetes. *Sci Transl Med* **13**, (2021).
32. T. Shimada *et al.*, FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* **19**, 429-435 (2004).
33. M. H. de Borst, M. G. Vervloet, P. M. ter Wee, G. Navis, Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol* **22**, 1603-1609 (2011).
34. S. M. H. Yeung *et al.*, Fibroblast Growth Factor 23 and Mortality in Patients With Type 2 Diabetes and Normal or Mildly Impaired Kidney Function. *Diabetes Care* **42**, 2151-2153 (2019).
35. A. Al-Qaissi *et al.*, Effects of acute insulin-induced hypoglycaemia on endothelial microparticles in adults with and without type 2 diabetes. *Diabetes, obesity & metabolism* **21**, 533-540 (2019).
36. A. S. M. Moin, A. Al-Qaissi, T. Sathyapalan, S. L. Atkin, A. E. Butler, Hypoglycaemia in type 2 diabetes exacerbates amyloid-related proteins associated with dementia. *Diabetes, obesity & metabolism* **23**, 338-349 (2021).
37. S. Kraemer *et al.*, From SOMAmer-based biomarker discovery to diagnostic and clinical applications: a SOMAmer-based, streamlined multiplex proteomic assay. *PLoS One* **6**, e26332 (2011).
38. K. Suhre *et al.*, Connecting genetic risk to disease end points through the human blood plasma proteome. *Nat Commun* **8**, 14357 (2017).

39. L. Gold *et al.*, Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One* **5**, e15004 (2010).
40. H. Kahal *et al.*, Effect of induced hypoglycemia on inflammation and oxidative stress in type 2 diabetes and control subjects. *Sci. Rep.* **10**, 4750 (2020).
41. M. E. Ritchie *et al.*, limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* **43**, e47 (2015).
42. Y. Benjamini, D. Drai, G. Elmer, N. Kafkafi, I. Golani, Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* **125**, 279-284 (2001).
43. M. A. Birkett, S. J. Day, Internal pilot studies for estimating sample size. *Stat. Med.* **13**, 2455-2463 (1994).
44. K. J. Belongie *et al.*, Identification of novel biomarkers to monitor β-cell function and enable early detection of type 2 diabetes risk. *PLoS One* **12**, e0182932 (2017).
45. S. K. Olsen *et al.*, Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs. *J Biol Chem* **278**, 34226-34236 (2003).
46. C. Watt, E. Sanchez-Rangel, J. J. Hwang, Glycemic Variability and CNS Inflammation: Reviewing the Connection. *Nutrients* **12**, (2020).
47. A. S. Atkin *et al.*, Impact of severe hypoglycemia on the heat shock and related protein response. *Sci. Rep.* **11**, 17057 (2021).
48. R. Vasko *et al.*, Role of basic fibroblast growth factor (FGF-2) in diabetic nephropathy and mechanisms of its induction by hyperglycemia in human renal fibroblasts. *Am J Physiol Renal Physiol* **296**, F1452-1463 (2009).
49. M. Konishi, T. Mikami, M. Yamasaki, A. Miyake, N. Itoh, Fibroblast growth factor-16 is a growth factor for embryonic brown adipocytes. *J Biol Chem* **275**, 12119-12122 (2000).
50. J. Zhao *et al.*, Suppression of FGF5 and FGF18 Expression by Cholesterol-Modified siRNAs Promotes Hair Growth in Mice. *Front. Pharmacol.* **12**, 666860 (2021).
51. I. Kyrou, M. O. Weickert, S. Gharanei, H. S. Randeva, B. K. Tan, Fibroblast growth factors: new insights, new targets in the management of diabetes. *Minerva Endocrinol* **42**, 248-270 (2017).
52. S. Sachdev *et al.*, FGF 19 and Bile Acids Increase Following Roux-en-Y Gastric Bypass but Not After Medical Management in Patients with Type 2 Diabetes. *Obes Surg* **26**, 957-965 (2016).
53. L. Fu *et al.*, Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology* **145**, 2594-2603 (2004).
54. T. C. Schreuder *et al.*, The hepatic response to FGF19 is impaired in patients with nonalcoholic fatty liver disease and insulin resistance. *American journal of physiology. Gastrointestinal and liver physiology* **298**, G440-445 (2010).
55. A. K. Rehni, K. R. Dave, Impact of Hypoglycemia on Brain Metabolism During Diabetes. *Mol. Neurobiol.* **55**, 9075-9088 (2018).
56. J. S. Garland *et al.*, Insulin resistance is associated with Fibroblast Growth Factor-23 in stage 3-5 chronic kidney disease patients. *Journal of diabetes and its complications* **28**, 61-65 (2014).
57. Y. Hu *et al.*, FGF-16 protects against adverse cardiac remodeling in the infarct diabetic heart. *Am J Transl Res* **9**, 1630-1640 (2017).

Table 1. Study participant demographic and biochemical data.
Data is Mean ± 1 SD (Median).

Baseline	Type 2 Diabetes (n=23)	Controls (n=23)	p-value	Statistically significant
Age (years)	64±8 (66)	60±10 (63)	0.15	No
Sex (M/F)	12/11	11/12	0.77	No
BMI (kg/m^2)	32±4 (32)	28±3(27)	0.001	Yes
Duration of diabetes (years)	4.5±2.2 (5.0)	N/A		N/A
HbA1c (mmol/mol)	51.2±11.4 (50.0)	37.2±2.2 (37.0)	<0.0001	Yes
HbA1c (%)	6.8±1.0 (6.7)	5.6±0.2 (5.5)	<0.0001	Yes
Total cholesterol (mmol/L)	4.2±1.0 (4.1)	4.8±0.67 (4.9)	0.02	Yes
Triglyceride (mmol/L)	1.7±0.7 (1.5)	1.34±0.6 (1.3)	0.06	No
HDL-cholesterol (mmol/L)	1.1±0.3 (1.1)	1.5±0.4 (1.4)	0.002	Yes
LDL-cholesterol (mmol/L)	2.27±0.8 (2.1)	2.7±0.7 (2.8)	0.06	No
CRP (mg/L)	3.0±2.7 (1.9)	5.1±10.3 (2.1)	0.33	No

BMI: Body mass index, BP: Blood pressure, HDL-cholesterol: High density lipoprotein cholesterol, LDL-cholesterol: Low density lipoprotein cholesterol, CRP: C-reactive protein. HbA1c: Haemoglobin A1c; N/A: not applicable

Figure Legends:

Figure 1: Classification of the FGF super- and subfamilies. The particular FGF proteins included in this study are depicted in bold red font.

Figure 2. Role of FGFs in regulation of energy metabolism and metabolic homeostasis. Different FGFs bind to their respective FGFR receptors on the cell surface with the help of heparan sulfate proteoglycans (HSPG) (in the case of paracrine FGFs) and co-receptor α/β-Klotho (in the case of endocrine FGFs) and activate the downstream pathways involved in a myriad of functions including morphogenesis, development, survival, proliferation, differentiation and glucose metabolism.

Abbreviations: FGF-Fibroblast Growth factor; FGFR- Fibroblast Growth factor receptor; HSPG-Heparan sulphate proteoglycan; PLCγ- phospholipase C gamma; IP3- Inositol trisphosphate or inositol 1,4,5-trisphosphate; DAG- diacylglycerol; PKC- Protein kinase C; RSK- Ribosomal S6 Kinase; P38- Mitogen-activated protein kinase; FRS2- Fibroblast growth factor receptor substrate 2; GRB2- growth factor receptor-bound protein 2; RAF- c-Raf; MEK- Mitogen-activated protein kinase kinase; ERK1/2- Extracellular signal-regulated kinase1/2; PI3K-phosphatidylinositol-3-kinase; AKT- Serine/Threonine Kinase; mTOR- mechanistic target of rapamycin; JAK- Janus kinase; STAT- signal transducer and activator of transcription. SMAD Sma genes-Mothers Against Decapentaplegic Family Member 1/5; CYP1A1-Cytochrome P450, family 1; SHP- small heterodimer partner; CREB- The cAMP-response element binding protein; MEF2A- Myocyte Enhancer Factor 2A; PGC1a- Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ALK1- Activin receptor-like kinase 1.

Figure 3: Comparison of blood glucose levels of FGF proteins at baseline, after induction of hypoglycemia and in the post-hypoglycemia follow-up period in control and type 2 diabetes (T2D) subjects. Blood sampling was performed at Baseline (BL), at hypoglycemia (0 min) and at post-hypoglycemia timepoints (0.5-hours, 1-hour, 2-hours, 4-hours and 24-hours) for controls (white circles) and for T2D (black squares). Somalogic proteomic analysis was undertaken for the following fibroblast growth factor proteins: FGF12 (A), FGF20 (B), FGF23 (C), FGF18 (D), FGF19 (E), FGF16 (F) and FGF2 (G) for which levels differed at baseline, at hypoglycemia and at different post-hypoglycemia time points when levels in T2D and control subjects were compared. Statistics: *, p< 0.05, **, p< 0.01, ****, p<0.0001, control vs T2D.

Changes in protein levels within group (control and T2D) relative to baseline and hypoglycemia are also shown. Statistics: T2D, baseline to subsequent timepoints: $$ p<0.01, $$$ p<0.001; T2D, hypoglycemia to subsequent timepoints: && p<0.01, &&& p<0.001; Control, baseline to subsequent timepoints: ## p<0.01, ### p<0.001; Control, hypoglycemia to subsequent timepoints: ^ p<0.05, ^^ p<0.01, ^^^ p<0.001.

RFU-relative fluorescent units; BG-blood glucose; Hypo-hypoglycemia

Supplementary figure 1. Glucose (A) and glucagon (B) response throughout the experimental time course for the control and type 2 diabetes [T2D] cohorts.

Statistics: (A) T2D versus control: **** p<0.0001.

(B) T2D, baseline to subsequent timepoints: $$ p<0.01; T2D, hypoglycemia to subsequent timepoints: && p<0.01, &&&& p<0.0001; Control, baseline to subsequent timepoints: #### p<0.0001; Control, hypoglycemia to subsequent timepoints: ^ p<0.05, ^^^^ p<0.0001.

RFU-relative fluorescent units; BG-blood glucose; Hypo-hypoglycemia

Supplementary figure 2. Levels of FGF proteins at baseline (BL) in both controls and type 2 diabetes (T2D) and in response to euglycemia (BM, in T2D only; blood glucose lowered to 5 mmol/L from a baseline level of 7.5±0.4 mmol/L). The data shows that FGF protein levels were not affected by glycemic fluctuation to the level of euglycemia. T2D vs control at baseline (BL): ** $p < 0.01$.

Supplementary figure 3. STRING interaction network showing the interactions of the FGF proteins.

The Search Tool for the Retrieval of Interacting Genes (STRING 11.0) was utilized to demonstrate both known and predicted FGF interactions (https://string-db.org/). Nodes=proteins; lines=physical/ functional interactions. (known interactions, light blue (from curated databases) and pink (experimentally determined); predicted interactions, dark blue (gene co-occurrence); relationships gleaned from text mining (lime green), co-expression (black) and protein homology (mauve).