The ΔF508 Mutation in the Cystic Fibrosis Transmembrane Conductance Regulator is Associated with Progressive Insulin Resistance and Decreased Functional Beta-Cell Mass in Mice

Running title: CFTR ΔF508 mutation and glucose homeostasis

Ghislaine Fontés\textsuperscript{1,2,*}, Julien Ghislain\textsuperscript{1,2}, Isma Benterki\textsuperscript{1,2,4}, Bader Zarrouki\textsuperscript{1,2}, Dominique Trudel\textsuperscript{2,5}, Yves Berthiaume\textsuperscript{2,3,*}, and Vincent Poitout\textsuperscript{1,2,3,4}

Montreal Diabetes Research Center\textsuperscript{1}, CRCHUM\textsuperscript{2}, and Departments of Medicine\textsuperscript{3}, Biochemistry and Molecular Medicine\textsuperscript{4}, and Pathology and Cell Biology\textsuperscript{5}, University of Montreal, QC, Canada

Corresponding author:

Vincent Poitout, DVM, PhD
CRCHUM
900 rue St Denis
Montréal, QC, H2X 0A9 - CANADA
Tel: (514) 890-8000 Ext: 23603
Fax: (514) 412-7648
Email: vincent.poitout@umontreal.ca

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\* Current affiliation: Department of Microbiology & Immunology, McGill University, Montréal, QC, Canada

\footnote{Current affiliation: IRCM, Montréal, QC, Canada}
ABSTRACT

Cystic Fibrosis (CF) is due to mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Cystic Fibrosis-Related Diabetes affects 50% of adult CF patients. How CFTR deficiency predisposes to diabetes is unknown. Herein we examined the impact of the most frequent cftr mutation in humans, ΔF508, on glucose homeostasis in mice. We compared ΔF508 mutant mice to wild-type (WT) littermates. Twelve-week old male ΔF508 mutants had lower body weight, improved oral glucose tolerance and a trend towards higher insulin tolerance. Glucose-induced insulin secretion was slightly diminished in ΔF508 mutant islets, due to reduced insulin content, but ΔF508 mutant islets were not more sensitive to proinflammatory cytokines than WT islets. Hyperglycemic clamps confirmed an increase in insulin sensitivity with normal beta-cell function in 12- and 18-week old ΔF508 mutants. In contrast, 24-week old ΔF508 mutants exhibited insulin resistance and reduced beta-cell function. Beta-cell mass was unaffected at 11 weeks of age but was significantly lower in ΔF508 mutants vs. controls at 24 weeks. This was not associated with gross pancreatic pathology. We conclude that the ΔF508 CFTR mutation does not lead to an intrinsic beta-cell secretory defect but is associated with insulin resistance and a beta-cell mass deficit in aging mutants.
Cystic Fibrosis (CF) is the most frequent autosomal recessive disorder in the Caucasian population. It results from loss-of-function mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Major improvements in the treatment of CF in the last decades has led to a remarkable increase in life expectancy of the patients, from 14 years in the 1980s to over 37 years today (1). This is associated with increased prevalence of complications and co-morbidities, such as Cystic Fibrosis-Related Diabetes (CFRD) which affects 50% of adult CF patients (1). Clinically, CFRD shares features of both types of diabetes, and gene variants associated with type 1 (2) and type 2 (3) diabetes increase the risk of CFRD. CFRD is considered its own clinical entity (4) and is believed to result primarily from defective insulin secretion from the pancreatic beta cell (5-12) with a secondary, aggravating effect of insulin resistance (5; 13-15) both in the liver (16; 17) and peripheral tissues (14; 18). Thus, the beta cell plays a key role in the pathogenesis of CFRD, yet surprisingly little is known regarding the mechanisms underlying its functional defect in CF. CFTR is expressed in islets including beta cells (19) but its functional importance in this tissue is unclear. Post-mortem examination of pancreata from CF patients has suggested that islet dysfunction might be secondary to fibrosis and fatty infiltration (20; 21) or amyloid deposits (22); however, islets from CF patients who develop diabetes are not more damaged than those who remain normoglycemic (23), and CF children exhibit impaired insulin secretion independent of pancreatic exocrine deficiency (24). These studies are in agreement with several observations in preclinical models suggesting that a primary, modest impairment of beta-cell function remains clinically silent initially and becomes more severe as systemic inflammation develops and the disease progresses (25). Accordingly, cftr-null mice are more susceptible to streptozotocin-induced diabetes (26), and cftr-null ferrets display an early beta-cell secretory defect which is already present at birth and precedes overt
pancreas pathology (27). Finally, recent studies suggest a role of CFTR in the regulation of insulin secretion (28; 29). Together, these findings indicate that defective CFTR function might affect pancreatic beta-cell function.

Although most preclinical studies on the mechanisms of CFRD used *cftr*-null models, the most frequent *cftr* mutation, which affects approximately 70% of CF patients, is a deletion of phenylalanine at position 508 (∆F508) resulting in misfolding and altered intracellular trafficking of the protein (30). This in turn results in endoplasmic reticulum (ER) stress (31) which, given the high susceptibility of pancreatic beta cells to ER stress, has been proposed as a possible cause of the insulin secretory defect (32). Thus, the impact of the ∆F508 mutation on the beta cell is likely different from that of complete deletion of the protein. Elucidating the impact of the ∆F508 mutation on beta-cell function has important clinical implications. However, to our knowledge ∆F508 mutant mice have not been characterized with respect to glucose homeostasis. In this study we tested the hypothesis that the ∆F508 mutation alters glucose homeostasis in an age-dependent manner. To this aim we systematically examined insulin secretion and sensitivity in ∆F508 mutant mice. Specifically, we asked the following questions: 1) Is the ∆F508 mutation associated with impaired glucose tolerance and defective insulin secretion and/or insulin sensitivity in mice? 2) Do islets from ∆F508 mutant mice have impaired insulin secretion under normal or pro-inflammatory conditions? 3) Do beta-cell function and mass and insulin sensitivity decline with age in ∆F508 mutant mice?
RESEARCH DESIGN AND METHODS

Animals and diet

All procedures using animals were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l’Université de Montréal (CHUM). Mice heterozygote (HET) for the ΔF508 mutation (cftr$^{WT/\Delta F508}$) backcrossed onto the FVB genetic background for more than 12 generations were kindly provided by Dr B.J. Scholte (Erasmus University, Rotterdam, Netherlands (33)) and bred to generate homozygous (ΔF508) mutants (cftr$^{\Delta F508/\Delta F508}$) and wild-type (WT) littermates (cftr$^{WT/WT}$). ΔF508 mutants were genotyped as described (33).

Male and female mice were housed under controlled temperature (21°C) on a 12-h light-dark cycle with unrestricted access to food and water. To prevent intestinal obstruction, CF mice were fed a high-protein diet. In a pilot study we compared male and female WT mice fed standard chow vs. a high-protein diet (59% carbohydrates, 26% protein, and 15% fat on caloric basis (Research Diets, Inc., New Brunswick, NJ); Suppl. Fig. 1). There was no effect of the diet on growth curves (Suppl. Fig. 1A & 1B), fat mass (Suppl. Fig. 1C & 1D), and fed or fasted blood glucose levels (Suppl. Fig. 1E & 1F) in either male or female mice. There was a tendency for glucose intolerance in high-protein-fed mice (Suppl. Fig. 1G & 1H), although the difference only reached statistical significance at the 60-min time point in female mice. For the remainder of the study both WT and ΔF508 mutant mice were fed the high-protein diet.

Body weight, body fat, and metabolic parameters

Body weight of male and female mice was determined weekly from weaning to 14 weeks-of-age. The percentage of body fat in 11-week old fed WT, HET and ΔF508 mice was assessed using an
EchoMRI-700 (Houston, TX). Respiratory Exchange Ratio (RER), heat production, food and water consumption, and locomotor activity were determined in 12-week old WT and ΔF508 mice using a Comprehensive Lab Animal Monitoring System CLAMS (Colombus Instruments, Columbus, OH). Body size, fasting blood glucose, plasma insulin and glucagon, triglycerides (TG), and total cholesterol were determined at 14 and 24 weeks-of-age.

**Mouse islet isolation and assessment of insulin secretion ex vivo**

Mouse islets were isolated as previously described (34), recovered overnight at 11.1 mmol/l glucose, and then cultured for 24 h in various experimental conditions as described in RESULTS. Insulin secretion was assessed in 1-h static incubations. Triplicate batches of 10 islets each were washed twice in Krebs-Ringer buffer containing 0.1% bovine serum albumin and 2.8 mmol/l glucose for 20 min at 37°C, and then incubated for 1 h at 37°C in either 2.8 or 16.7 mmol/l glucose. Secreted insulin was measured in the supernatant by radioimmunoassay (Millipore Corporation, Billerica, MA), and intracellular insulin content was determined after acidified ethanol extraction.

**Glucose and insulin tolerance tests and hyperglycemic clamps**

Oral glucose tolerance tests (OGTT) were performed in overnight-fasted mice at 10 weeks-of-age by administration of 2 g/kg glucose by gavage. Insulin tolerance was assessed in 5-hour fasted animals after intraperitoneal administration of 0.6 U/kg human insulin at 12 weeks-of-age. Insulin secretion in vivo was measured using hyperglycemic clamps. Mice underwent catheterization of the right jugular vein under general anesthesia. After a 5-day recovery, conscious mice were subjected to one-step hyperglycemic clamps. A 20% dextrose solution
(McKesson Canada Corp, Montreal, QC) was infused to clamp blood glucose at ~ 22 mmol/l for
60-80 min (Roche Accu-Check; Roche, Indianapolis, IN). Plasma samples were collected from
the tail for measurements of insulin and C-peptide using mouse ELISA kits (Alpco Diagnostics,
Salem, NH). The insulin sensitivity index (M/I) was calculated as the glucose infusion rate (M)
divided by the average insulinemia during the last 30 min of the clamp (I). The Disposition Index
(DI), an index of beta-cell function taking into account the prevailing level of insulin sensitivity
developed in humans (35) and validated in rodents (36), was calculated by multiplying M/I by C-
peptide levels during the last 30 min of the clamp.

Analysis of beta-cell mass and pancreas histology

Pancreata were trimmed of fat, weighed, fixed in 10% buffered formalin (v/v) and embedded in
paraffin. Five-µm paraffin sections including the head, neck and tail of the pancreas were made
and every 10th section, for a total of 6, was mounted on glass slides for immunohistochemical
and beta-cell mass analyses after insulin immunostaining with guinea pig anti-insulin IgG
(DAKO) and haematoxylin counterstaining as described (37). The beta-cell surface and whole
pancreas areas were determined using ImageJ software (NIH) and beta-cell mass was calculated
by multiplying the relative beta-cell surface area by the weight of pancreas. Pancreatic histology
was analyzed by light microscopy following hematoxylin phloxine saffron (HPS) staining of
paraffin sections.

Analytical measurements

Blood glucose was assessed using a hand-held glucometer, non-esterified fatty acid (NEFA)
levels using the Wako NEFA C kit (Wako Chemical, Osaka, Japan), glucagon by ELISA (Alpco
Diagnostics), TG by the GPO Trinder kit (Sigma Aldrich, Saint Louis, MO), and total cholesterol with the Amplex red assay kit (Molecular Probes, Eugene, OR). Plasma levels of an array of inflammatory cytokines were determined using the Mouse Inflammatory Cytokines Multi-Analyte ELISAarray™ Kit (Qiagen, Hilden, Germany).

**Statistical analysis**

Data are expressed as mean ± SEM and were analyzed by Student’s t-test or ANOVA followed by two-by-two comparisons with Bonferroni post-hoc adjustments, as appropriate, using GraphPad Instat (GraphPad Software, San Diego, CA). P<0.05 was considered significant.

**RESULTS**

**Energy metabolism in ∆F508 mutant mice**

Body weight was lower in both male (Fig. 1A) and female (Fig. 1B) ∆F508 mice compared to WT and HET controls from the beginning of the study (4 weeks-of-age) and this difference was maintained until 14 weeks-of-age (P<0.001). There was a non-significant trend in ∆F508 males (Fig. 1C) but not females (Fig. 1D) to gain less weight than controls. The percentage of fat mass was lower in male (Fig. 1E) but not female (Fig. 1F) ∆F508 mice. Body weight and fat mass in HETs were indistinguishable from the WT (Fig. 1). Body length was similar in all groups (data not shown). RER, heat production, food intake, and water consumption were similar between 12-week old WT and ∆F508 male mice (Suppl. Fig. 2A-D). Male ∆F508 mice exhibited a significant reduction in locomotor activity (Suppl. Fig. 2E).
Insulin secretion under normal and pro-inflammatory conditions in islets from ΔF508 mutant mice

Recent studies suggest that CFTR in beta cells is implicated in glucose-induced electrical activities governing insulin secretion (28) and the potentiating effect of cAMP (29). Furthermore, insulin secretion is compromised in islets from CFTR-null ferrets (27). Insulin secretion in response to glucose was assessed ex vivo in isolated islets from 14-week old WT and ΔF508 mice (Fig. 2). Basal insulin secretion was similar but glucose-induced insulin secretion was slightly but significantly reduced in islets from ΔF508 mice (Fig. 2A). This was associated with a reduction in intracellular insulin content (Fig. 2B), such that when expressed as a percentage of insulin content, glucose-induced insulin secretion was not significantly different between WT and ΔF508 mutant islets (Fig. 2C). These data suggest that under normal conditions, the mild insulin secretory defect observed in ΔF508 mutant islets is due to a decrease in insulin content.

The CFTR mutation in mice is associated with mild pancreatic insufficiency and inflammation (38; 39). Further, the ΔF508 CFTR mutation leads to ER stress (31). Therefore, it is possible that the ΔF508 mutation renders beta-cells more susceptible to inflammatory stress and induces a functional defect which is only revealed under pro-inflammatory conditions, normally not seen in mice bred in a highly controlled environment. To examine this possibility, insulin secretion in response to glucose was assessed ex vivo in isolated islets from 14-week old WT and ΔF508 mice after a 24-h exposure to pro-inflammatory cytokines (interleukin-1β + interferon γ; 1 and 5 ng/ml, respectively; Fig. 2). As expected, exposure to pro-inflammatory cytokines led to a significant reduction in glucose-induced insulin secretion (Fig. 2A) and intracellular insulin content (Fig. 2B) in WT islets. The inhibitory effect of pro-inflammatory cytokines was also
observed, but not more pronounced, in ΔF508 mutant islets (38 ± 6 % reduction vs. 47 ± 7 % reduction in WT islets, n=5, NS). Altogether, these data suggest that the ΔF508 mutation in mice is not associated with an intrinsic beta-cell secretory defect under normal or pro-inflammatory conditions.

**Glucose homeostasis in ΔF508 mutant mice**

Fed and fasting blood glucose levels were lower in 10-13-week old male (Fig. 3A) but not female (Fig. 3B) ΔF508 mutant mice. Both male and female ΔF508 mutant mice had improved oral glucose tolerance (Fig. 3C&D), as shown by a significantly lower area under the glucose curve during the OGTT (Fig. 3E&F). Intraperitoneal insulin tolerance test performed at 12 weeks-of-age showed a trend towards increased insulin sensitivity in male (Fig. 3G), but not female (Fig. 3H), ΔF508 homo- and heterozygous mutant mice. There was no significant difference in blood glucose levels or glucose tolerance in HET vs. WT animals (Fig. 3). Circulating glucagon and insulin levels were not significantly different between 14-week old ΔF508 and WT mice in males (Table 1) and females (data not shown). TG and total cholesterol levels were lower in male (Table 1) but not female (data not shown) ΔF508 vs. WT mice. Given their more severe phenotype, subsequent experiments were performed in male animals only. To assess insulin secretion in vivo, we performed one-step hyperglycemic clamps in 12- and 18-week old male ΔF508 and WT mice (Figs. 4 & 5). At 12 weeks-of-age, although blood glucose levels during the clamp tended to be lower in ΔF508 mice (Fig. 4A), the difference was not statistically significant (Fig. 4B). The average glucose infusion rate (GIR) was significantly higher (despite slightly lower blood glucose levels) in ΔF508 mice (Fig. 4C), while the insulin levels during the second half of the clamp were lower (Fig. 4D). C-peptide levels during the
steady-state were unchanged (Fig. 4E), and as a result the insulin/C-Peptide ratio was lower in ∆F508 mice (Fig. 4F), suggestive of increased insulin clearance. Accordingly, the M/I index of insulin sensitivity was higher in ∆F508 mice (Fig. 4G). The DI, which takes into account the degree of insulin sensitivity, was also higher in ∆F508 mice (Fig. 4H). Results from hyperglycemic clamps in 18-week old male ∆F508 and WT mice revealed that although blood glucose (Fig. 5A & B), insulin (Fig. 5D) and C-peptide (Fig. 5E) levels and the insulin/C-peptide ratio (Fig. 5F) were not significantly different, the average GIR (Fig. 5C), M/I index (Fig. 5G) and DI (Fig. 5H) were higher in ∆F508 mice. Taken together, these data indicate that 12- and 18-week old ∆F508 mutant mice are more insulin sensitive than their WT littermates and have normal beta-cell function in vivo.

The prevalence of CFRD increases with age, suggesting that a silent beta-cell defect might become apparent with age-related insulin resistance. To test this possibility in the ∆F508 mouse model, we examined glucose homeostasis in 24-week old males. Fasting blood glucose levels were lower in 24-week old male ∆F508 mice (∆F508: 3.7 ± 0.2 mmol/l vs. WT: 4.6 ± 0.1 mmol/l, n=10, P<0.01). Circulating insulin, glucagon, TG and total cholesterol levels were not significantly different between 24-week old ∆F508 and WT male mice (Table 1). In hyperglycemic clamps, blood glucose levels were similar in ∆F508 and WT mice (Fig. 6A&B), but in contrast to our observations in 12- and 18-week old animals (Figs. 4 & 5), the GIR was significantly lower in ∆F508 mutant than in WT mice (Fig. 6C). Insulin (Fig. 6D) and C-peptide (Fig. 6E) levels were not statistically different between ∆F508 and WT mice. Accordingly, the insulin/C-peptide ratio was unchanged (Fig. 6F). The M/I index was significantly lower in ∆F508 mutant mice (Fig. 6G), indicative of insulin resistance. The DI was also lower in ∆F508
mutant mice, suggestive of impaired beta-cell function in the context of insulin resistance (Fig. 6H). These data suggest that the ΔF508 mutation is associated with an increase in insulin resistance with age that is not compensated for by an increase in beta-cell function.

**Beta-cell mass and pancreas morphology in ΔF508 mutant mice**

Since we did not observe any intrinsic defect in insulin secretion in isolated islets (Fig. 2), the decrease in DI in 24-week old ΔF508 mutants (Fig. 6H) prompted us to measure beta-cell mass in 11-, 18- and 24-week old animals. Although beta-cell mass was similar in both groups at 11 weeks, it was significantly lower in ΔF508 mutants at 24 weeks (Fig. 7A&B). We then asked whether the exocrine pancreas is affected in ΔF508 mutant mice as has been described previously in CF humans (20; 21) and animal models (27; 40). HPS staining of pancreatic sections of 24-week old ΔF508 mutants revealed no fibrosis, inflammation or other pathological findings (Fig. 7C). There was no increase in circulating inflammatory cytokine levels in 18- and 24-week old ΔF508 mutants (data not shown). Overall these data suggest that the beta-cell defect observed in 24-week old ΔF508 mutant mice is due to a deficit in beta-cell mass that is not a secondary consequence of overt inflammation or exocrine pancreatic disease.

**DISCUSSION**

The objective of this study was to test the hypothesis that the ΔF508 cftr mutation alters glucose homeostasis in an age-dependent manner in mice. Systematic evaluation of beta-cell function and insulin sensitivity revealed that 1) islets from young ΔF508 mutants have a mild secretory defect ex vivo that can be accounted for by the decreased insulin content but are not more susceptible to pro-inflammatory stress; 2) the ΔF508 mutation in 12-18-week old mice is
unexpectedly associated with improved glucose tolerance and higher insulin sensitivity; 3) 12-18-week old ΔF508 mutants have adequate beta-cell function in vivo given their level of insulin sensitivity; and 4) as ΔF508 mutant mice age they become insulin resistant and have reduced beta-cell function in vivo associated with a marked decrease in beta-cell mass. Our interpretation of these findings is that the ΔF508 mutation does not lead to a *bona fide* insulin secretory defect, but is associated with insulin resistance and a beta-cell mass deficit in aging mutants.

Male ΔF508 mutant mice had lower body weight from the beginning of the study, which was associated with a lower fat mass and reduced locomotor activity, but no significant differences in weight gain or energy expenditure (Fig. 1 and Suppl. Fig. 2). Total food intake was not different, although since the ΔF508 mutant mice weigh less, their food consumption per unit of body weight was increased. This, together with lower locomotor activity, would be predicted to lead to a higher weight gain that was not observed. We suspect that this discrepancy is explained by reduced intestinal absorption in ΔF508 mutant mice (41). Unlike male mice, ΔF508 mutant females did not show a significant difference in body weight or fat mass compared to WT controls. Sex-specific modifier loci that have been shown to influence body weight in *cftr*-mutant mice (42) may be responsible for these differences.

Fed and fasted blood glucose was reduced and glucose tolerance was greatly improved in young ΔF508 mutant mice, particularly in males (Fig. 3). This can be due to reduced intestinal absorption of glucose (43), improved insulin secretion, and/or enhanced insulin sensitivity. Insulin tolerance tests (Fig. 3) and hyperglycemic clamps (Figs. 4 & 5) confirmed that 12- and 18-week old ΔF508 mutant mice were more insulin sensitive. We acknowledge that hyperglycemic clamps are primarily designed to assess beta-cell function, and that insulin
sensitivity is best measured using euglycemic-hyperinsulinemic clamps. However, given that it is essentially impossible to perform two separate clamps in the same mouse, the M/I index during a hyperglycemic clamp is an acceptable estimate of insulin sensitivity in rodents (36). In addition, the results of the insulin tolerance tests corroborate those of the clamps and show a trend for greater insulin sensitivity in male ΔF508 mutants. Accordingly, insulin clearance was increased in ΔF508 mutant mice (Fig. 4), as also observed in CF patients (44). The reasons for the increased insulin sensitivity in ΔF508 mutant mice is unknown, but has been suggested in humans to represent an adaptive process to the exocrine deficiency and energy deficit. In our model, it could also be due in part to the reduced fat mass of male ΔF508 mutants. Strikingly however, the difference between ΔF508 and WT mice was reversed at 24 weeks of age, with ΔF508 mutants showing insulin resistance as assessed by a lower M/I index (Fig. 6). The underlying cause of insulin resistance in aging ΔF508 mutants is unknown, but could be due to expression of the mutant protein in peripheral tissues. Clinically, conflicting results have been obtained regarding insulin sensitivity in CF patients, which has been reported as increased (14; 15; 45), unchanged (9; 10; 12; 44), or decreased (13; 17). Our results in mice suggest that these discrepancies might be due, in part, to the age at which insulin sensitivity is measured. In addition, a study in CF patients using hyperinsulinemic euglycemic clamps revealed a complex pattern with increased hepatic glucose production (i.e. liver insulin resistance) but enhanced peripheral insulin sensitivity (14).

Hyperglycemic clamps in 12- and 18-week old ΔF508 mutant mice failed to reveal any beta-cell secretory defect (Fig. 4 & 5). C-peptide levels were unchanged and the lower circulating insulin levels at 12 weeks-of-age were likely due to increased insulin clearance, consistent with
enhanced insulin sensitivity. DI, an index of beta-cell function that takes into account the prevailing level of insulin sensitivity, was actually higher in ΔF508 mutants. The similar C-peptide levels between ΔF508 mutant and WT mice suggest that beta-cell function is not reduced in mutants and is in fact high in light of the prevailing increase in insulin sensitivity. This possibly explains the increase in glucose tolerance in young ΔF508 mutants. Nonetheless, we can conclude from these experiments that there is no beta-cell defect in vivo in 12- and 18-week old ΔF508 mutant mice. This conclusion is also supported by the results of static incubations in isolated islet (Fig. 2).

The absence of a marked insulin secretory defect in young ΔF508 mutant mice in this study contrasts with reports of beta-cell dysfunction in newborn CF-pigs (40) and cftr-null ferrets (27). In the latter study, CF kits demonstrated impaired insulin secretion prior to the occurrence of overt pancreatic lesions. These differences can be explained by several factors. First, the impact of CFTR deficiency varies significantly between species (46). In that regard, our results are consistent with the normal glucose tolerance in 11- to 13-week old cftr-null mice (26), although islet function was not examined in that study. Second, since the ΔF508 mutant CFTR retains partial expression and function (47), the functional consequences of complete CFTR deletion in beta cells might be more severe. Nevertheless, recent studies suggest that CFTR in beta cells is implicated in glucose-dependent electrical activity (28) and the potentiating effect of cAMP (29). We acknowledge that the hyperglycemic clamps and 1-h static incubations used to measure insulin secretion may have masked a minor defect, however, our findings indicate that there is no major secretory defect in ΔF508 mutant mouse islets.
In 24-week old ΔF508 mutants we observed a significant decrease in DI (Fig. 6) indicating insufficient beta-cell function relative to the level of insulin sensitivity. Although beta-cell mass was normal in 11-week old male ΔF508 mutant mice, it was significantly reduced at 24 weeks (Fig. 7). In CF humans the defect in beta-cell mass is thought to be secondary to exocrine pancreatic disease (20; 21). Similarly, newborn cfr-null ferrets have smaller islets and beta-cell area decreases with time and correlates with increased severity of pancreatic pathology (27). In contrast to these studies, we did not observe any overt pathology in ΔF508 mutant pancreata at 24 weeks-of-age (Fig. 7).

In conclusion, our results show that the ΔF508 CFTR mutation is associated with increased insulin sensitivity and normal insulin secretion in vivo in young mice. As the mice age, however, they develop a deficit in beta-cell mass combined with insulin resistance relative to their WT counterparts. These results are consistent with the notion that an underlying beta-cell defect related to the ΔF508 mutation remains silent under normal conditions but becomes apparent in situations of increased secretory demand. This possibility is supported by the clinical observation that the prevalence of CFRD dramatically increases with age (1), and suggests that alleviation of insulin resistance might be beneficial in preserving glucose homeostasis in CF patients.

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TABLE 1

Table 1: Metabolic parameters of 14- and 24-week old male WT and ΔF508 mutant mice.

Data are mean ± SEM of 4-17 mice. *P < 0.05; ***P < 0.001 vs. WT mice.

|                     | 14-week old |            | 24-week old |            |
|---------------------|-------------|------------|-------------|------------|
|                     | WT          | ΔF508      | WT          | ΔF508      |
| Insulin (pmol/l)    | 141 ± 27    | 88 ± 24    | 205 ± 26    | 134 ± 21   |
| Glucagon (ng/l)     | 315 ± 32    | 398 ± 30   | 340 ± 32    | 268 ± 19   |
| TG (mmol/l)         | 11.9 ± 1.9  | 5.1 ± 0.7***| 9.6 ± 1.5   | 7.5 ± 1.3  |
| Total Cholesterol (mmol/l) | 1.84 ± 0.23 | 1.11 ± 0.28* | 2.10 ± 0.15 | 2.05 ± 0.80 |
FIGURE LEGENDS

Figure 1: Body weight and fat mass in ΔF508 mutant mice. A-D: Body weight (A, B) and weight gain (C, D) were measured from 4 to 14 weeks-of-age in male (A, C) and female (B, D) WT, HET and ΔF508 mice. Data are expressed as mean ± SEM of 7 to 15 mice per group. *P<0.05, **P<0.01, ***P<0.001 vs. WT. E, F: Fat mass content relative to body weight was assessed by EchoMRI in 11-week old WT, HET, and ΔF508 male (E) and female (F) mice. Data are mean ± SEM of 9 to 20 mice per group. **P<0.01 vs. WT.

Figure 2: Insulin secretion and content under normal and pro-inflammatory conditions in ΔF508 mutant islets. Insulin secretion (A), insulin content (B) and insulin secretion normalized by insulin content (C) as assessed in 1-h static incubations in isolated islets from 14-week old ΔF508 mutant and WT mice in response to 2.8 or 16.7 mmol/l glucose following a 24-h exposure to 16.7 mM glucose in the presence or absence 1 ng/ml interleukin-1β + 5 ng/ml interferon-γ. Data are mean ± SEM of 5 independent experiments. *P<0.05; **P<0.01; ***P<0.001.

Figure 3: Glucose and insulin tolerance in young ΔF508 mutant mice. A, B: Fed and fasting blood glucose levels were measured in 10- to 13-week old male (A) and female (B) WT, HET, and ΔF508 mice. Data are expressed as mean ± SEM of 9 to 32 mice per group. *P<0.05, **P<0.01 vs. WT. C-F: Oral Glucose Tolerance Tests (OGTT) were performed in 10-week old male (C) and female (D) WT, HET, and ΔF508 mice. The Area Under the Curve (AUC) of blood glucose levels was calculated during OGTT in male (E) and female (F) mice. **P<0.01 vs. WT. G, H: Intraperitoneal Insulin Tolerance tests (IPITT) were performed in 12-week old male (G)
and female (H) WT, HET, and ΔF508 mice. **C-H:** Data are mean ± SEM of 8 to 18 mice per group.

**Figure 4: Insulin secretion and sensitivity in vivo in 12-week old male ΔF508 mutant mice.**

Hyperglycemic clamps were performed in 12-week old male WT and ΔF508 mice. **A:** Blood glucose levels during the glucose clamp; **B:** Average blood glucose levels during the steady-state between 30 and 60 min; **C:** Glucose infusion rate; **D:** plasma insulin levels; **E:** plasma C-peptide levels; **F:** Insulin/C-peptide ratio; **G:** M/I index of insulin sensitivity; **H:** Disposition Index. Data are mean ± SEM of 10 mice per group. *P<0.05, **P<0.01 vs. WT.

**Figure 5: Insulin secretion and sensitivity in vivo in 18-week old male ΔF508 mutant mice.**

Hyperglycemic clamps were performed in 18-week old male WT and ΔF508 mice. **A:** Blood glucose levels during the glucose clamp; **B:** Average blood glucose levels during the steady-state between 30 and 60 min; **C:** Glucose infusion rate; **D:** plasma insulin levels; **E:** plasma C-peptide levels; **F:** Insulin/C-peptide ratio; **G:** M/I index of insulin sensitivity; **H:** Disposition Index. Data are mean ± SEM of 7-9 mice per group. *P<0.05, **P<0.01 vs. WT.

**Figure 6: Insulin secretion and sensitivity in 24-week old male ΔF508 mutant mice.**

Hyperglycemic clamps were performed in 24-week old male WT and ΔF508 mice. **A:** Blood glucose levels during the glucose clamp; **B:** Average blood glucose levels during the steady-state between 30 and 60 min; **C:** Glucose infusion rate; **D:** plasma insulin levels; **E:** plasma C-peptide levels; **F:** Insulin/C-peptide ratio; **G:** M/I index of insulin sensitivity; **H:** Disposition Index. Data are mean ± SEM of 3-4 mice per group. *P<0.05, **P<0.01 vs. WT.
Figure 7: Beta-cell mass and pancreatic histology in ΔF508 mutant mice. Pancreata were harvested from 11-, 18- and 24-week old male ΔF508 mutant and WT mice, and beta-cell mass was measured by morphomometric analysis after insulin immunostaining as described in RESEARCH DESIGN AND METHODS. A: Representative image from a WT (left) and ΔF508 mutant (right) mouse at 24 weeks-of-age. B: mean beta-cell mass ± SEM at the ages indicated of 4-7 animals in each group. *P<0.05. C: Representative HPS-stained pancreatic section from a WT (left) and ΔF508 mutant (right) mouse at 24-weeks-of age (n=7). Is, islets.
Figure 1

(A) Body weight (g) vs Age (week number)
(B) Body weight (g) vs Age (week number)
(C) Weight gain (g) vs Age (week number)
(D) Weight gain (g) vs Age (week number)
(E) Fat mass/Body weight for WT, HET, ΔF508
(F) Fat mass/Body weight for WT, HET, ΔF508
Figure 2

A

Insulin secretion (ng/ml)

|          | Control | Cytokines | Control | Cytokines |
|----------|---------|-----------|---------|-----------|
| WT       |         |           |         |           |
| ΔF508    |         |           |         |           |

B

Insulin content (ng/ml)

|          | Control | Cytokines | Control | Cytokines |
|----------|---------|-----------|---------|-----------|
| WT       |         |           |         |           |
| ΔF508    |         |           |         |           |

C

Insulin secretion (% content)

|          | Control | Cytokines | Control | Cytokines |
|----------|---------|-----------|---------|-----------|
| WT       |         |           |         |           |
| ΔF508    |         |           |         |           |
Figure 3

A Males

B Females

C

D

E

F

G

H

432x558mm (96 x 96 DPI)
Figure 6

Blood glucose (mmol/l) vs Time (min)

A

Blood glucose at 30-60 min

B

CIR (umol/kg/min)

C

Insulin (pM)

D

C-Peptide (nM)

E

Insulin/C-Peptide Ratio

F

M[1-index (umol/kg·min·1·GR/ml)

G

Disposition Index (C-Peptide x M[1-index)

H

432x558mm (96 x 96 DPI)
Figure 7

A

WT

ΔF508

B

|          | 11 weeks | 18 weeks | 24 weeks |
|----------|----------|----------|----------|
| WT       | 2        | 3        | 4        |
| ΔF508    | 1        | 2        | 6        |

C

WT

ΔF508
Supplemental Figure 1: Body weight, fat mass, blood glucose and glucose tolerance in WT mice fed a high-protein diet. A, B: Body weight was measured from 4 weeks to 14 weeks-of-age in male (A) and female (B) WT mice fed with a normal (ND) or a high-protein (HP) diet. Data are expressed as mean ± SEM of 7 to 11 mice per group. C-F: Fat mass content relative to body weight and blood fed or fasted glucose levels were assessed in male (C and E, respectively) and female (D and F, respectively) WT mice. Data are mean ± SEM of 7 to 20 mice per group. G, H: OGTTs were performed in male (G) and female (H) WT mice. Data are mean ± SEM of 7 to 11 mice per group. *P<0.05 vs. ND.
Supplemental Figure 2: Energy homeostasis in male ΔF508 mutant mice. A: Respiratory Exchange Ratio (RER); B: heat production; C: food consumption; D: water consumption; E: locomotor activity were measured in 12-week old male WT and ΔF508 mice during light (6 AM to 6 PM) and dark (6 PM to 6 AM) cycles. Data are expressed as mean ± SEM of 4-5 mice per group. *P<0.05 vs. WT.