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Endocrine Parameters of Cystic Fibrosis: Back to Basics

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ABSTRACT

Dramatic changes in the life expectancy of cystic fibrosis (CF) patients are occurring, creating a cohort of aging individuals experiencing long-term complications of this chronic disease. The two most common of these complications include CF-related diabetes and CF bone disease. The clinical implications of each have become better understood, as have potential therapies. However, data obtained from the basic science studies of both diseases have not been widely recognized. In this review, we focus on the known and hypothesized pathogenesis of these two disorders. Additionally, the molecular underpinnings of CF will be explained along with the potential interactions with endocrine disease phenotypes. J. Cell. Biochem. 108: 353–361, 2009.

KEY WORDS: CYSTIC FIBROSIS; CFTR; DIABETES; BONE DISEASE

Recently the cystic fibrosis (CF) Foundation reported that the current median life expectancy for CF patients in the United States is now 37.4 years [Cystic Fibrosis Foundation Patient Registry, 2008]. This is remarkable, considering that 70 years ago the life expectancy was less than 1 year, and had only improved to 16 years by the mid-1970s [Orenstein et al., 2002]. Affecting one in 3,500 children born each year, currently there are about 30,000 individuals with CF in the United States [Cystic Fibrosis Foundation Patient Registry, 2008]. It continues to be the leading single gene life limiting disorder in Caucasians. Some manifestations of CF are due to defective chloride channel function of the CF transmembrane conductance regulator (CFTR), while others are believed to be the result of cellular responses to mutant CFTR [Orenstein et al., 2002; Sagel and Accurso, 2002; Virella-Lowell et al., 2004; Cystic Fibrosis Foundation Patient Registry, 2008; Stalvey et al., 2008]. As patients continue to increase in age, new complications are emerging. Presently, endocrine disorders associated with CF are more common than other disease related co-morbidities [Cystic Fibrosis Foundation Patient Registry, 2008]. This review will discuss the two most common endocrine complications observed in CF and the biological theories behind them.

Before beginning the discussion of the endocrine defects, one must first consider the basic metabolic abnormality in CF. The CFTR protein is a transmembrane glycoprotein from the ABC transporter superfamily [Guggino and Stanton, 2006; Cheung and Deber, 2008]. Its 1,480 amino acid sequence resides on chromosome 7 in humans. CFTR functions as an ATP-dependent regulator of chloride transport at the apical surface of the epithelium of the airways and many exocrine gland ducts. Normal CFTR function allows for the regulated flux of water into the lumen, bathing the epithelial surface. Abnormalities of this airway surface fluid are implicated in the thick viscous mucous accumulation observed in CF airways. Analogous dysregulation of chloride and water results in the blockage of the pancreatic ducts (thus earlier names: mucoviscidosis and mucoviscidosis of the pancreas). The activity of the CFTR channel has been shown to regulate the activity of other channels, such as ENaC, the calcium-activated chloride channel, and the outwardly rectifying chloride channel [Guggino and Stanton, 2006]. Although the significance of many of these activities is still unknown, we will discuss potential implications as they pertain to the endocrinologic system.

In the United States and Northern Europe, defects in CFTR most commonly result from one specific mutation (ΔF508), which demonstrates a strong founder effect [Rowntree and Harris, 2003; O'Sullivan and Freedman, 2009]. This particular mutation results in a misfolding of the CFTR protein, resulting in an endoplasmic reticulum (ER) overload response. Misfolded CFTR is a target for ubiquitination and proteosome mediated degradation. This interrupts the normal trafficking of CFTR from the ER through the Golgi. A small percentage of ΔF508 mutant CFTR molecules make it to the surface of the cell, and demonstrate partial transporter activity.

In addition to trafficking mutants like ΔF508, there are four other classes of mutations within the more than 2,000 that have been identified [Rowntree and Harris, 2003; O'Sullivan and Freedman, 2009]. An abbreviated description of the class mutations follows. Class I mutations are characterized by truncated and non-functional CFTR proteins stemming from large deletions or stop codons. Class II

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mutations (which include ΔF508) result in CFTR proteins incapable of proper folding, and are degraded by the cellular machinery, resulting in absence of the CFTR at the cell surface. Class III mutations are capable of reaching the cell surface, but are unresponsive to regulation by ATP–cAMP interactions. Mutants from Class IV also reach the cell surface, but do not demonstrate normal chloride conductance. Finally, Class V mutations demonstrate normal chloride conductance and regulation, but with a quantitative reduction in the synthesis of the protein, and subsequently a reduction in functional CFTR.

The discussion of class mutations illustrates one of the challenges in translational research in CF. The earliest mouse models of CF utilize a *Cftr* insertion, frame-shift and termination (an example of a Class I mutation) [Snouwaert et al., 1992], whereas the most common mutation seen worldwide in CF individuals are Class II (ΔF508 frequency varies from 87% in Northern Europe to 28% in Asia, worldwide 66%) [Dawson and Frossard, 2000]. The mutations G542X and G551D have the greatest frequency following the ΔF508 (worldwide 2.4% and 1.6%) but depend on the individual population background. Whereas the G542X mutation is a Class I defect (similar to the animal model genotype), the G551D mutation is a Class III defect [Rowntree and Harris, 2003; O'Sullivan and Freedman, 2009]. Given the potential for implications from the different mutation classes, throughout this review we will attempt to specify and relate back to them when relevant.

CYSTIC FIBROSIS RELATED DIABETES

A pediatric gastroenterologist named Harry Shwachman first reported CF related diabetes (CFRD) in 1955 [Shwachman et al., 1955]. At that particular time, the life expectancy of CF was less than 10 years. Since CFRD does not typically present prior to the second decade of life, it is not surprising that Dr. Shwachman described the case in a 5-year-old boy as follows:

> The appearance of diabetes mellitus in a patient with mucoviscidosis probably represents the superimposition of one serious disease on another. Because of the rarity of this combination of diseases, we present a case, perhaps the first recorded instance of diabetes mellitus occurring with mucoviscidosis [Shwachman et al., 1955].

Later, Rosan et al. [1962] reported 10 cases of CFRD out of 1,300 reviewed patients with CF. As the life expectancy of CF increased, CFRD increased in frequency as well. It is the leading co-morbidity associated with CF (20.6% of all individuals) [Cystic Fibrosis Foundation Patient Registry, 2008]. It is a disorder that increases in frequency as individuals age: 9% between the age of 5 and 9 years, 26% in 10 and 20 years of age [Moran et al., 1998], and 50% by the age of 30 years [Lanng et al., 1993a]. Even worse than the increasing frequency of CFRD, is the impact on the underlying health state. CFRD is associated with declines in weight, BMI, lung function and overall clinical health [O'Riordan et al., 2008]. Interestingly, the most devastating finding may be limited to women. In a study by Milla et al. (cohort of 1,081 subjects), they reported the mean survival of men with CFRD was reduced by 2 years from those unaffected, however a 16-year reduction in survival was found in women with CFRD [Milla et al., 2005]. In a separate, smaller study of 237 subjects, no difference in survival was found between sexes [Bismuth et al., 2008]. Although a rationale for these findings has not been established, there are other clinical and phenotypical examples of differences in men and women with CF.

The pathogenesis of CFRD (as well as the clinical characteristics) is unlike either type 1 diabetes (T1D) or type 2 diabetes (T2D). There is no correlation with the genes that convey susceptibility to T1D [Lanng et al., 1993a]. Additionally, unlike T2D, CF patients are insulin sensitive at baseline [O'Riordan et al., 2008]. This can change dramatically during exacerbations of endobronchial infection, pregnancy or other intercurrent conditions. Ketoacidosis is rare with CFRD, and diabetes-related complications are less frequent, as well. Microvascular complications are rare prior to 10 years into the disease, and macrovascular complications have not yet been reported.

Despite the description by Shwachman in 1955, it was not until 1969 that Handwerger et al. [1969] first described histologic findings of the pancreatic islets in CF. In that article, they describe an anatomic disruption of the islets, with total atrophy of the exocrine gland tissue due to enzymatic digestion. In contrast, they reported that the islets were intact, but condensed into small areas surrounded by scarring. Handwerger postulated that the scarring and disruption of the normal architecture resulted in decreased islet action. Later investigators [Iannucci et al., 1984; Abdul-Karim et al., 1986; Lohr et al., 1989] further described the CF chronic scarring and replacement of acinar tissue by adipose tissue [Iannucci et al., 1984; Abdul-Karim et al., 1986; Lohr et al., 1989]. Unlike the reports by Handwerger, these studies detailed variable changes in the islets, such as reductions in the insulin producing β cell numbers, while a relatively unchanged or increased percentage of non-β cells (α and δ cells, discussed further later). An example of the chronic scarring and adiposity, with close approximation of pancreatic islets is seen in Figure 1 [Lohr et al., 1989].

During this time period, studies into insulin production and sensitivity began. Wilmshurst et al. [1975] compared insulin secretion and responses in eight male subjects with CF. Despite a reduction in insulin levels during oral and intravenous glucose tolerance testing, they described normal glucose clearance in response to intravenous insulin [Wilmshurst et al., 1975]. Later Lippe et al. [1980] reported an increase in insulin receptors, but decreased receptor affinity, on peripheral monocytes. This finding was in the presence of diminished insulin secretion and mild hyperglycemia during oral glucose tolerance testing.

The primary characterization of CFRD is that of a partial *insulin deficiency and pancreatic islet β cell failure* that worsens with increasing age and glucose intolerance [Moran et al., 1991; Lanng et al., 1993b]. This defect in insulin secretion is further delineated by primarily a loss of first phase insulin, which precedes the diagnosis of CFRD by multiple years [Hamdi et al., 1993; Cucinotta et al., 1994; Garagorri et al., 2001]. Couce et al. describe an additional finding of β cell dysfunction (consistent with T2D), with the presence of amyloidosis in 69% of pancreatic samples obtained from individuals with CFRD. They reported the presence of amyloid in 17% of samples from patients with borderline CFRD and none in the non-diabetic CF patients [Couce et al., 1996].

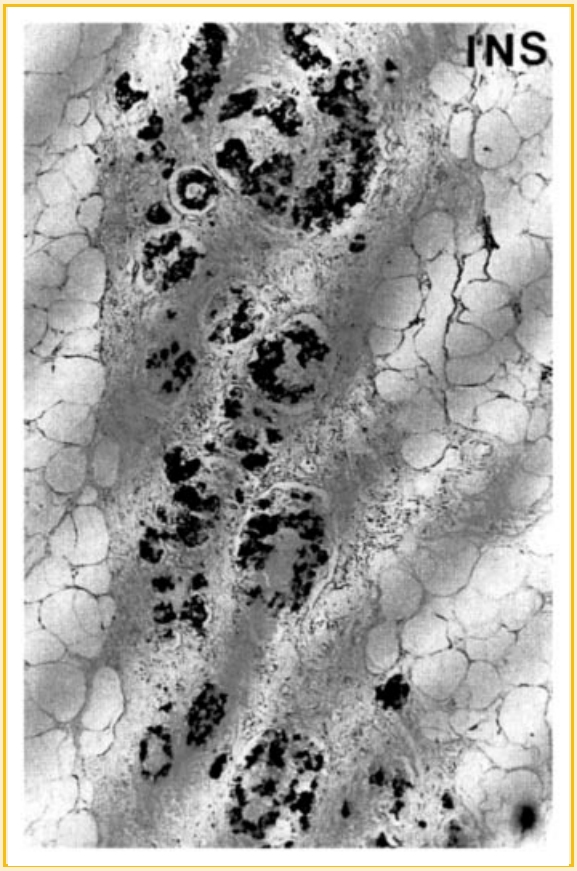

Fig. 1. Pancreas section from a 16-year-old CF patient that reveals grouping of islets, surrounded by fibrotic tissue and fat saponification. Immunohistochemical staining for insulin markedly demonstrates the dense collection of islets. Reproduced from Lohr et al. [1989] with permission.

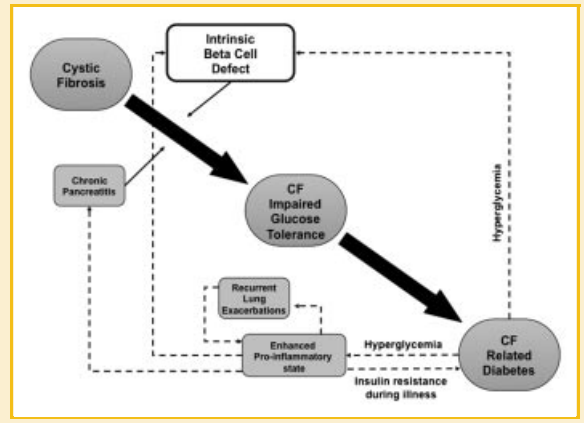

Fig. 2. Proposed mechanistic development of CFRD: The development of CFRD is a multifactoral process. It involves a complex dynamic of intermittent insulin sensitivity and resistance with decreased insulin production, complicated by the *Cftr*−/− mice to beta cell insult suggests an inherent defect in islet function. (Broken lines represent potential contributions to disease.) Reproduced from Stalvey et al. [2006] with permission.

More recently there is an emergence of research into the mechanisms associated with CFRD. We have turned for a more detailed understanding, from the cellular to the translational level. In 2006, our laboratory reported an increased sensitivity to injury of islets from *Cftr*-deficient (*Cftr*−/−) mice [Stalvey et al., 2006]. Following a low-dose chemical insult with streptozotocin (STZ), *Cftr*−/− mice had an increased fasting glucose and greater overall glucose concentrations after challenge with intraperitoneal glucose challenge compared to control strains. Additionally, the degree of hyperglycemia correlated with the number of residual islets in the *Cftr*−/− mice following STZ. This correlation was not seen in the control strains, maintaining normal blood glucose concentrations despite similar numbers of islets. Based on our data, we concluded an underlying defect in the *Cftr*−/− islets exists, that could predispose towards CFRD (Fig. 2) [Stalvey et al., 2006]. Previously, the assumption was the progressive pancreatic scarring resulted in an eventual loss of islets. Since *Cftr*−/− mice do not develop pancreatic scarring and loss, this could not be the sole mechanism.

That study further provoked a question long asked by investigators: *Is CFTR expressed on β cells?* An answer was finally supported by the identification CFTR in the islets of rat pancreas. Boom et al. [2007] described the expression of CFTR via mRNA from isolated islets as well as immunolocalization. This was further detailed by the flow cytometry-separated islet cells, demonstrating a greater expression of CFTR transcripts in non-β cell populations. In situ immunocytochemistry with CFTR, somatostatin and glucagon antibodies revealed the greatest accumulation of CFTR existing on glucagon secreting α cells, less expression on β cells, and no expression by somatostatin producing δ cells.

Given these recent findings of CFTR expression in the pancreatic islet, can the alterations in glucose metabolism found in CF be traced back to molecular alterations as an effect of an aberrant CFTR? Interestingly, the sulfonylurea receptor (SUR, which is integral to β cell function) is part of the same ABC transporter subclass as the CFTR, with high homology [Aguilar-Bryan and Bryan, 1999; Gadsby et al., 2006; Aittoniemi et al., 2009]. As implied by Boom et al. [2007], these findings have implications beyond the simple expression of CFTR on islets, or even β cells. Similarly noted in the lung of subjects with CF, the overall impact of dysfunctional transporters is not directly related to the quantity of its expression. This concept is further supported by the fact that CFTR coregulates the activity of other ion channels [Guggino and Stanton, 2006]. Although β cells do not express a large amount of CFTR, the ability to influence other channel activities could explain a significant role of CFTR in insulin secretion. The secretion of insulin is dependent on membrane depolarization, which is governed by ATP-sensitive potassium channels (formed by two subunit proteins, SUR1 and Kir6.2) [Nichols, 2006; Aittoniemi et al., 2009]. This depolarization results in an influx of calcium via the opening of voltage-dependent calcium channels, triggering the exocytosis of secretory granules containing insulin. Alterations in the membrane potentials due to defective CFTR, or other channels it regulates, could thus lead to aberrant regulation of insulin secretion.

Potentially correlating CFTR genotype with phenotype, there is a reduced frequency of CFRD in pancreatic sufficient patients [Marshall et al., 2005; O'Riordan et al., 2008]. Previous theories attributed this to the lack of pancreatitis and scarring. Not

surprisingly, there is an association of pancreatic disease and the "more severe" *Cftr* mutations (Class I, II, or III), resulting from either a homozygous or compound heterozygous state [Rowntree and Harris, 2003]. At least one "mild mutation" is found in pancreatic sufficient patients. As mentioned in the beginning of this review, Classes I, II, and III are consistent with absent or the least functional forms of CFTR. Could the class defect of the *Cftr* mutation directly relate to the degree of functionality and control of the β cells? Is the reduction in CFRD found in pancreatic sufficient patients a factor of reduced pancreatic damage, or a product of a less severe impedance of normal cellular function?

Furthermore, in the study of the pathogenesis of CFRD, we cannot overlook the need for an improved understanding of the consequences. As mentioned previously, evidence indicates a reduction in overall health, lung function and potentially survival, following the diagnosis of CFRD [O'Riordan et al., 2008]. This mechanism is not entirely clear, especially given the mild derangements of glucose control typically seen in CFRD patients. Additionally, these additional CF-related complications found with CFRD are not consistent with complications found in either type 1 or type 2 diabetes. Outside of the contribution to a catabolic state by hyperglycemia, or lack of an anabolic state by insulin deficiency, little is understood about the mechanism corresponding to the downward course seen after CF patients acquire CFRD. Earlier this year however, we demonstrated a connection between hyperglycemia and immunologic changes utilizing our CFRD mouse model [Stalvey et al., 2008]. In a comparison of *Cftr*−/− mice to C57BL/6J and FVB/NJ, both normoglycemic and with our hyperglycemic model, we found an increase in stimulated spleenocyte proliferation. This Con-A stimulated response further correlated with degree of fasting hyperglycemia found in the animal. Furthermore, we reported increased levels of stimulated IL-10 concentrations in the STZ-treated *Cftr*−/− compared to control strains and non-treated *Cftr*−/− mice. IL-2 concentrations were also increased in *Cftr*−/− mice, but were unrelated to STZ-treatment. Additionally, IL-10 has previously been noted to increase lymphocyte proliferation in the presence of IL-2. We concluded that newly increased IL-10 levels worked in conjunction with the pre-existing IL-2, resulting in the upregulation of the immune reactivity. Unfortunately, increased levels of IL-10 have also been reported to suppress macrophage activation and induce tolerance. In sum, we hypothesize that CFRD results in a proinflammatory state that is less effective at clearance of bacterial pathogens.

The further study of CFRD, outside of the clinical research arena, will focus on additional genotype characterization in mouse models and cellular pancreatic endocrine interactions (α, β, and δ). As we continue to learn more about the various CF genotypes, the interaction of the CFTR beyond the epithelial surface, and the embryological differentiation of the pancreas (as well as pancreatic stem cells), we must return our attention to the cellular aspects of the investigation. Outdated is the concept of simple long-term pancreatitis, architectural remodeling and eventual loss of the pancreatic islets by adipose replacement. New theories into pancreatic stem cells, β cell reserve, islet regeneration and cross-communication of pancreatic endocrine cells should instruct us to revisit our understanding of CFRD pathogenesis.

CYSTIC FIBROSIS BONE DISEASE

Mischler et al. [1979] detailed the first laboratory evidence, if not also clinical findings, of bone disease in CF. In their article, they describe the clinical observation of osteopenia in the routine chest roentgenograms of 15 (out of 25) patients with CF. This observation led to their study of bone mineral content (BMC) by direct photon absorptiometry of the nondominant arm. The authors compared 63 measurements, from 27 subjects with CF. They contrasted these findings to healthy control data obtained previously from 968 age and sex matched individuals. Twelve of the 27 individuals studied (44%) were found to have a BMC more than 2 SDS below control subjects. Since bone width was reduced in CF, a correction of BMC by bone width led to a reduction in this finding to nine of the CF subjects. Additionally, reduction in BMC was more prominent in females with (63%) than males (37%), although these differences were not statistically significant. The most convincing evidence of demineralization was found in the children greater than 13 years of age (thus adolescent girls being of greatest risk). Furthermore, short stature and delayed bone age complicated the picture with the findings in the adolescent girls. Lastly, reduction in BMC directly correlated with the extent of weight reduction.

The above study presented not only the first data consistent with CF bone disease, it also began to note the chronic problems and multiple systemic influences complicating the assessment of bone in CF still noted today. Since this early report, there are now hundreds of case reports, research articles and reviews. CF bone disease now closely trails CFRD for the leading comorbidity in CF at 20.6% in patients greater than 17 years of age [Cystic Fibrosis Foundation Patient Registry, 2008]. Bone disease appears to be related to lung health and malnutrition. Multiple studies describe the reduced bone mineral density in children and adults with CF, however controversy exists over the true risk of fracture for individuals with CF [Rossini et al., 2004; Rovner et al., 2005; Stephenson et al., 2006]. Occurring in males and females, conflicting data suggests that the risk of fracture is greater in females [Aris et al., 1998]. In an extreme case of pathologic fracture, Latzin et al. [2005] detail the fatal case of a 16-year-old girl with CF. Following a history of previous vertebral fractures, she presented with severe respiratory distress secondary to spontaneous sternal fracture.

CF bone disease is a classic example of a multifactoral disorder. The early focus of CF care, and subsequent comorbidity care, was on the *nutritional* aspect. Since more than 90% of CF individuals are pancreatic exocrine insufficient, improving nutrition impacts a large percentage of affected individuals [Cystic Fibrosis Foundation Patient Registry, 2008]. Despite correction by enzyme and vitamin replacement, increased demands and chronic catabolism still exist.

Deficiencies in *vitamin D and calcium* remain in CF patients, complicating the overall picture. Data provided by Schulze et al. [2003] demonstrated no difference in calcium absorption from the gut in clinically stable CF girls. Additionally, Haworth et al. [2000] examined postmortem vertebral samples from individuals with CF. The authors found no changes consistent with vitamin D deficiency osteomalacia, but severe osteopenia in trabecular and cortical bone. Furthermore, there was evidence of decreased osteoblast and increased osteoclast activity. In the next few factors of bone

disease, the major influence discussed will be the effects on those same cells.

Abnormalities in vitamin D and calcium will imply interactions with parathyroid hormone (PTH), and thus affect bone health in CF. In response to reduced total body calcium, whether from reduced vitamin D levels or increased calcium losses, increased levels of PTH have been detected [Aris et al., 1999, 2002; Greer et al., 2003; Rovner et al., 2007]. Increasing PTH will stimulate the conversion of the storage form of vitamin D (25-OH vitamin D) to the active form (1,25-OH vitamin D) and increase the resorption of bone to produce calcium for the internal total body stores.

For many years, chronic use of *corticosteroid* therapy was blamed for CF bone disease, a very rational point, especially in subjects following lung transplantation. However, this cannot be the only contribution to bone disease in CF, since it occurs across many individuals with CF who do not receive glucocorticoids. Corticosteroids reduce calcium absorption from the gut, and increase urinary calcium excretion. Additionally, there is suppression of gonadal hormone production, as well as osteoblast formation of new bone [Goodman et al., 2007]. Moreover, corticosteroids increase osteoclastogenesis via increased production of receptor activator of nuclear factor-kappa B ligand (RANKL) [Hofbauer et al., 1999].

Evidence suggests that the nuclear factor-kappa B (NFkB) pathway is overall upregulated in individuals with CF [DiMango et al., 1998; Rottner et al., 2007]. Thus, the influence of *inflammation*, and a chronic pro-inflammatory state, may be driving the increased osteoclast activity described by Haworth et al. [2000] above. On a gross level, bone mineral content correlates with mean IL-6 and CRP levels, as well as the number of days on antibiotics, corticosteroids and colonization with *Burkholderia cepacia* [Ionescu et al., 2000; Haworth et al., 2004; King et al., 2005]. At the cellular level, Shead et al. [2006] suggested an upregulation of osteoclast precursors in CF patients during infective exacerbations, specifically cells possessing the following molecular profile: $CD14^{+}CD33^{+}CD45^{+}CD34^{-}$. Another study by Haworth et al. [2004] reported increased levels of urinary markers for osteoclast activity related to serum levels of IL-6.

Influences on the osteoblast cell line are primarily pro-anabolic factors. *Insulin* stimulates endochondral bone growth, osteoblast proliferation and function. Insulin deficiency, whether autoimmune (in mouse or man) or chemically induced (in the mouse), it results in reduced bone density and an increased risk of fractures [Botolin and McCabe, 2007; Hamilton et al., 2009; Vestergaard et al., 2009]. The deficiency noted in T1D appears to include diminished linear bone growth during adolescence and puberty, decreased adult bone density, increased risk of fracture and reduced bone healing [Hadjidakis et al., 2006]. As described previously, CFRD is a chronically progressive state of insulin deficiency that increases in frequency and severity as patients age [Wilmshurst et al., 1975; Dobson et al., 2004; O'Riordan et al., 2008]. Further echoing the impact of insulin deficiency, data suggests there is an increase in BMD in individuals with type 2 diabetes, but still an increase in fracture risk [Hofbauer et al., 2007; Hadzibegovic et al., 2008; Rakel et al., 2008; Melton et al., 2008a,b].

Animal studies of bone mineral disease and induced diabetes have utilized the model of STZ-induced diabetes. Bone mineral density is significantly reduced in STZ models of diabetes [Hamada et al., 2007]. Intuitively, one might expect that this reduction in bone mineral density would be corrected by the replacement of insulin, and this was observed in the model. Amylin, another product of insulin producing β cells has been shown in improve bone mineral density in the animal model [Horcajada-Molteni et al., 2001]. Pancreatic transplants in STZ-induced diabetic rats, normalizes glucose tolerance and improves bone alkaline phosphatase (a marker of bone formation) [Kato and Nozawa, 1994]. Insulin therapy is associated with improved collagen production in these mice [Umpierrez et al., 1989]. Additionally, insulin receptors are present on osteoblasts and osteoblast-like cells with a high capacity for insulin binding [Thomas et al., 1996; Fulzele et al., 2007]. Osteoblast differentiation is decreased in STZ-induced diabetic animals [Goodman and Hori, 1984; Lu et al., 2003]. Inversely, osteoblasts increase proliferation when cultured with physiologic doses of insulin.

Another pro-anabolic factor that appears to be decreased in CF is insulin-like growth factor I (IGF-I). Laursen et al. [1999] reported decreased IGF-I concentrations, despite normal stimulated growth hormone (GH) responses, in a study of 20 individuals with CF. They speculated a condition of GH resistance was the cause. Later, in a multicenter trial by Hardin et al. [2005b], the authors reported reduced concentrations of IGF-I in CF children despite nutritional supplementation. GH supplementation, which effectively increases concentrations of IGF-I, improves bone mineral accrual in CF [Hardin et al., 2005a]. Furthermore, Gordon et al. [2006] demonstrated a correlation between IGF-I concentrations and bone density in CF. Similarly, Rosenberg et al. found decreased levels of IGF-I concentrations and IGF-I mRNA in *Cftr−/−* mice.

Finally, one cannot overlook the pro-anabolic component of the *sex hormones* on bone density in CF. Hypogonadism, whether primary (such as in post-menopausal women) or secondary (such as chronic disease or anorexia), is well associated with decreased bone density. Both men and women with CF suffer significantly from states of hypogonadism [Stead et al., 1987; Leifke et al., 2003]. Additionally, these findings are associated with an increase fractures in adults with CF [Rossini et al., 2004].

Modeling this clinical entity in the laboratory has enhanced both a direct and indirect understanding of disease. The first animal study to examine bone disease in CF was performed by Dif et al. [2004]. They examined 3-week-old female *Cftr−/−* mice. Mice utilized by this particular experiment develop intestinal obstruction and die when weaned to a normal chow diet. Evaluating the animals at 3 weeks of age allowed for the comparison of wildtype, heterozygote, and homozygote *Cftr−/−* animals maintained on the same diet. These investigators discovered a reduction in bone density in the *Cftr−/−* mice by Dual-energy X-ray absorptiometry (DXA). Although this observation was confounded by the reduced size and weight of *Cftr−/−* animals, further analysis through covariance analysis and bone histomorphometry revealed reduced bone density (as well as reduction in trabecular bone volume, thickness and increased trabecular separation) despite the small size. Additionally, the authors utilized a double fluorochrome labeling technique to demonstrate a reduction in bone formation.

Haston et al. [2008] later demonstrated the persistence of osteopenia into adulthood of *Cftr*−/− mice. In that study, the authors utilized a *Cftr*−/− mouse on a BALB background. Affected mice were not gut-corrected and required additional therapy to prevent intestinal obstruction. This therapy requires the addition of polyethylene glycol to the animal's drinking water. To control for effects of the polyethylene glycol on bone mass, it was added to the drinking water of the control animals as well. They evaluated three time points (8, 12, and 28 weeks). Haston et al. evaluated bone architecture through both micro-computed tomography (micro-CT) and histomorphology. The use of micro-CT nullifies differences in size and weight. *Cftr*−/− mice at all three time points were found to have a reduced bone density, based on micro-CT and histomorphometry. At 8 weeks of age, they utilized only female mice (six CF and six controls), whereas comparisons of BMD made at 12 and 28 weeks were made utilizing mixed male and female groups.

One major difficulty with studying mice at the age of 8 weeks is the variability of pubertal development in *Cftr*−/− mice. Much like the *pubertal delay* seen in humans with CF, *Cftr*−/− mice have delayed puberty as determined by vaginal opening. Jin et al. [2006] reported the mean timing of vaginal opening in wildtype animals was delayed by a mean of nearly 18 days (from an age of 31.3 to 48.9 days) in *Cftr*−/− mice. This delay would confound the analysis at 8 weeks of age. Wildtype mice will have 4 weeks of pubertal hormone exposure at 8 weeks of age, and CF mice may be only beginning puberty at that time.

In a recent investigation by our laboratory, we described reduced bone mineral density in *Cftr*−/− mice, despite the fact that this strain had undergone correction of the gut with the human CFTR [Pashuck et al., 2009]. In our study, we compared 14-week-old C57BL6 *Cftr(−/−) FABP-hCftr(+/+)* mice (male and female) against C57BL/6J mice, for bone mineral density (by peripheral quantitative computed tomography or pQCT and histomorphology) as well as dynamic states of bone. Both male and female *Cftr*−/− mice had reduced bone density compared to control mice (Fig. 3) [Pashuck et al., 2009]. Additionally, male *Cftr*−/− mice revealed an increased bone formation rate (via double-flourochrome labeling) when compared to both control male mice as well as female *Cftr*−/− mice. A major significance of this report, in addition to the findings of sex differences that is commonly seen in clinical CF care, was the finding of reduced bone density despite correction of the gastrointestinal defect of previous animals. Our animals are able to tolerate a normal diet, removing the confounding effects of nutritional inadequacies.

The summative findings of the murine data, given the ability to remove other clinical variables, imply an inherent defect in the *CF genotype*. These findings are further supported by detection of the CFTR transporter at the site of bone growth and metabolism. Shead et al. in *Thorax* [2007] reported expression of CFTR on both human osteoblasts and osteoclasts. This expression further echoes the increasing number of locations demonstrating CFTR expression beyond epithelial cells, and directly links the genotype to bone metabolism. The significance of this expression remains unclear. However, given the ability of CFTR to regulate other ion channels, the possibility of a direct effect on cellular differentiation, bone matrix maturation and calcification cannot be ignored.

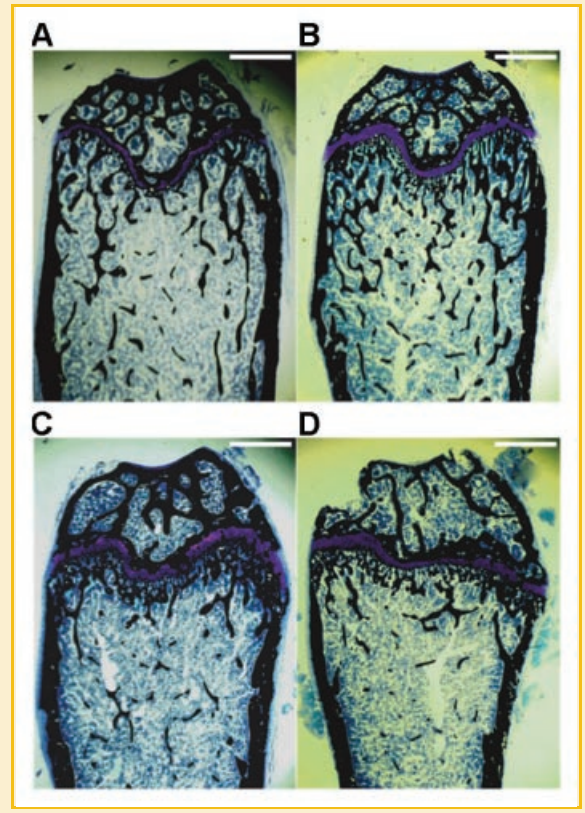

Fig. 3. Histomorphological sections obtained from distal femurs of 14-week-old male C57BL/6J (A), male C57BL/6J *Cftr*−/− (B), female C57BL/6J (C), and female C57BL/6J *Cftr*−/− (D) mice. C57BL/6J *Cftr*−/− mice have decreased trabecular bone volume as well as decreased trabecular width. The reduced number of black-stained cancellous bone spicules is especially apparent in the female C57BL/6J *Cftr* −/− mouse. Magnification = 20×, Scale bars = 1 mm. Reproduced from Pashuck et al. [2009] with permission.

The future of bone research in CF will focus on two major aims. The first major unknown is the influence and activity of the CFTR transporter on the osteoblasts and osteoclasts. This may be elucidated through in vitro analysis of osteoblast and osteoclast differentiation, cellular expression and activity. This question could be explored further by the use of CFTR inhibitors, as well as by correction of the CFTR defect. Secondly, the in vivo study of the CF phenotype and potential confounding variables should lead to an improved understanding of the impact and predictors of clinical disease. Additionally, new research techniques into limited expression of the CF genotype may provide further knowledge to the significance of in vivo expression.

OTHER ENDOCRINOPATHIES

Additional endocrine disorders are associated with CF, but much less descriptive data is available at this time. Disorders of sex-hormone production occur, as mentioned previously in regards to the impact on bone health, however limited data exists to the exact frequency or etiology. Leifke et al. [2003] reported a finding of low testosterone

levels in 28% of the CF men they studied, and years earlier Stead et al. reported 27% of adolescent and adult women had amenorrhea [Stead et al., 1987; Leifke et al., 2003]. These findings are likely a reflection of central hypogonadism, from chronic inflammation and/or total body wasting. Similarly, abnormalities in thyroid function are documented, but limited to mostly case reports and probably also driven by chronic inflammation and wasting as well [Segall-Blank et al., 1981; De Luca et al., 1982; Knopfle, 1985]. Furthermore, abnormalities in PTH production have a clear physiologic link to circulating calcium levels.

SUMMARY

As the life expectancy of individuals with CF continue to grow, the incidence of endocrinologic comorbid disorders will increase. This review focused primarily on the two most prominent, CFRD and CF bone disease. The frequency of both disorders appears to be directly related to the increasing age of the CF population. Moreover, both disorders appear to have a pathogenesis and overall impact on disease that is directly related to the genetic defect in CFTR. Additional studies are necessary to fully appreciate the molecular interactions and etiologies of these problems. In some cases, it is the next logical step in the understanding of the disorder, its systemic consequences, and the potential for intervention. In other instances, it will be necessary to reject our preconceptions about disease pathology, and embrace new understandings of molecular interactions on cellular differentiation. Only then will we be able to keep up with the growing problems associated with our expanding patient population.

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