

Chapter 22

Role of dietary factors in the etiology of fibrocalculous pancreatic disease and diabetes

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Summary

The pathogenesis of fibrocalculous pancreatic diabetes has been debated for years, and the role of dietary factors is at the forefront of proposed factors. In this article, the arguments for incriminating protein deficiency have been laid out in detail. Particularly important is the occurrence of protein deficiency in combination with a high starch diet. This can lead to chronic pancreatic damage, particularly mucoid vasculopathy in large amount. Overall, data seem to suggest that it is the fact that starch is ingested in large amounts, and not particularly its quality, which is linked to pancreatic damage. This data, based mainly on animal studies, needs proof with human studies before it is accepted as a truism.

Introduction

In the middle of the last century, a pattern of peculiar diseases suddenly started appearing in near-epidemic proportions in certain developing regions of the world mainly in the peri-equatorial tropics [1,2]. Grouped as “tropical diseases” these included tropical chronic calculous pancreatopathy (TCCP) also called fibrocalculous pancreatic disease (FCPD) with diabetes, endomyocardial fibrosis, non-atherosclerotic forms of vascular disease called mucoid arteriosclerosis and idiopathic aortoarteriopathy, goitre and idiopathic epidemic neuropathy [3,4,5,6,7,8,9]. Initially reported from Uganda, Jamaica, Brazil and South Africa in the 1940’s and 1950’s, similar conditions were sporadically reported from other places including Brazil, Argentina, Nigeria and in large numbers from Kerala in India in the 1960’s, 1970’s and 1980’s [1,10,11,12].

Clinico-pathological studies suggested toxico-nutritional factors, particularly dietary toxic factors in a background of low-protein high starch diets and deficiencies of vitamins and minerals, in the etiology of many of these diseases [6,7,13]. Various toxic factors suspected included cyanoglycosides from tapioca (cassava), a staple food in these regions, ergot-like toxins from plants and indigenous medicines, serotonin from bananas, trace heavy metals like thorium and cerium from the soil, and under-nutrition, associated with dietary deficiencies of protein and amino acids, especially tryptophan, cysteine and methionine,

minerals like iodine and magnesium [11,7,14,15,9]. However, there was no definite experimental proof available despite intense studies carried out on each of these diseases by several groups of research workers from different countries during the last five decades. It was however apparent that “cracking the etiogenic code” for any one of these conditions would help us understand the etiopathogenesis of all these diseases, since they seemed to have common clinico-epidemiological features and nutritional factors [16].

Autopsy studies at SCTIMST, Trivandrum, by this author, lead to the identification of a hitherto unrecognized acquired metabolic disorder presenting as an arteriosclerotic vascular disorder “mucoid vasculopathy”, with generalized deposition of abnormal acid mucopolysaccharides (proteoglycans) in the walls of blood vessels and in connective tissues [17,18,19]. Mucoid vasculopathy was similar to mucoid arteriosclerosis described from Uganda and intimomedial mucoid degeneration reported from South Africa and was responsible for a variety of occlusive and aneurysmal vascular diseases in Kerala [18,20]. The vasculopathy was associated with the pattern of “tropical diseases” found elsewhere and was therefore considered to be part of a metabolic syndrome, sharing common etiological factors, possibly, toxico-nutritional factors [17,2].

The autopsies also showed early FCPD-like pancreatic lesions associated with mucoid vasculopathy of the pancreatic blood vessels [under publication]. Similar pancreatic and vascular lesions were produced in bonnet monkeys by dietary means, with low-protein high-starch tapioca and cornstarch-based diets resembling that eaten by people from Kerala where mucoid vasculopathy was observed [2,21,22,23]. The experiments established a non-human primate model for nutritional pancreatopathies, mucoid vasculopathy and associated metabolic disorder [2,24,25]. The bonnet monkey model showed that protein carbohydrate malnutrition due to nutritional imbalances with such diets, not toxic dietary factors from tapioca, was responsible for these conditions [26]. The lesions were produced by both sources of carbohydrate, hence high levels of starch of any source, such as rice and tubers, in conjunction with mild to moderate protein-deficiency

may cause these specific cardiovascular and pancreatic lesions [2,24]. This article traces various hypotheses advanced during the last 50 years for understanding the role of toxico-nutritional factors in the etiopathogenesis of FCPD, a unique form of pancreatic disease that is fast disappearing from the Indian subcontinent.

Pathology of FCPD

Detailed descriptions of the pathology of FCPD were based on observations in a few autopsy studies [4,27] and from excised surgical specimens [28,29]. These cases represented a late and end-stage of the disease process when the pancreas had become "cirrhotic" and structurally damaged beyond repair. Incidental pancreatic lesions reflecting early FCPD-like changes in Kerala population were noticed in routine autopsies (both cardiovascular and neurological cases) carried out at SCTIMST, Trivandrum [under publication]. Such observations helped in understanding the nature of early lesions, including vascular lesions that lead to FCPD and its etiopathogenesis.

In human autopsies examined at SCTIMST, the pancreas was generally small- sized, firm, with small shrunken lobules separated by streaks of fibrous tissue and showing intraductal sand-like calculi also in an occasional case. The atrophy was more marked in the distal parts of the gland. The most important and constant feature observed was mucoid vasculopathy of pancreatic artery and vein in all these cases. The artery was firm and thick with narrow lumen and having large amounts of abnormal acid mucopolysaccharides in its wall [17]. Such changes were most obvious in the main artery and its larger branches. Pancreatic lobules showed moderate atrophy of acini, large hypertrophied islets and some nesidioblastosis. There was mucoid metaplasia of duct epithelium. Ducts and intralobular ductules were lined by tall columnar epithelium and contained mucin plugs admixed with eosinophilic secretions. Hypertrophied nerve bundles and autonomic ganglia were occasionally seen, similar to those described in other autopsies with mucoid vasculopathy [30]. There was a distinct periductal and periductular fibrosis with patchy atrophy of the lobules, replaced by adipose tissue, however there was no inflammation of the parenchyma. Consequently, in a Workshop on Diabetes in the Tropics, it was decided

that the term “pancreatopathy” should replace “pancreatitis” to emphasize the non-inflammatory nature of FCPD lesions [31].

In reports on surgically excised and autopsy specimens, the exocrine pancreas was described as a shrunken markedly atrophic gland, replaced by dense fibro-adipose tissue, with an extreme form called “lipomatous atrophy” in which remnant islands of islet tissue were all that remained of the gland [28,29,27]. Nesidioblastosis was a very important feature described by Balaraman Nair that suggested attempts at regeneration even in an atrophic gland [28,29]. Numerous calculi and inspissated eosinophilic secretions were seen within dilated ducts. Chronic inflammation was mainly around ducts containing calculi and was absent within the atrophic lobules [4,28,29]. Hypertrophied arteries with thick walls and mucoid degeneration were found within the gland [29]. Such changes were initially considered to be sequelae of chronic inflammation, scarring and ischemia. However since the main pancreatic artery was not available in the excised surgical specimen and autopsies in the population otherwise showed a generalized arteriosclerosis, not restricted to the pancreas, the occurrence of mucoid vasculopathy in the pancreatic vasculature and its role in the pathogenesis of FCPD were not recognized till recently [Balaraman Nair, and (late) JNP Davies, personal communications].

Salient clinical and epidemiological data regarding etiology of FCPD

- Found mainly in tropical and subtropical developing regions of the world [32].
- Seen in moderately nourished young individual with brittle diabetes, not prone to ketosis, associated with abdominal pain (therefore called pancreatitis), pancreatic calculi, varying degree of deficiency of exocrine pancreas [33]. FCPD was classified as a secondary form of diabetes mellitus and placed under Type 3 Diabetes in the recent WHO classification, clubbed with protein deficiency diabetes mellitus (PDDM), under malnutrition related diabetes mellitus (MRDM) [34].

Table 1. Dietary factors: summary of the evidence

- Association with the pattern of “tropical diseases,” in the region, and co-occurrence these diseases in the same individual [1,35].
- Patient did not have gross malnutrition or a history of severe malnutrition in childhood [36]. Obesity, macro-vascular disease and atherosclerosis were absent in most patients [10].
- Patient often had a characteristic facies, due to bilateral parotid swelling [37].
- Pancreatic adenocarcinoma was an important late sequel [38].
- FCPD was absent in places where kwashiorkor was common [39,4] and also in people with better nutrition subsisting on tapioca [40]; but found in certain rice eating populations from Orissa and Tamil Nadu in India [32,33].
- Patients of FCPD were not alcoholic [39]
- Characteristic diet consumed was a low-protein low-fat high-starch diet [39]. The patients mostly ate high starch monotonous foods comprised of tubers (particularly cassava or tapioca), rice and bananas that provided little protein [4,32].
- Histopathological features of FCPD did not resemble pancreatic changes in severe protein deficiency (kwashiorkor) in humans [41], nor in animal models for the same [42,43] and milder forms of isolated protein deficiency [44].
- Since tapioca was consumed as a staple, many considered a toxic etiology possibly due to cyanoglycosides from the tuber [45,46,47].
- Toxicity due to trace elements like thorium and cerium in association with magnesium deficiency, selenium deficiency, hyper-vitaminosis D with hypercalcemia, viral and genetic etiology, were some of the other aspects briefly studied in the patients from Kerala and elsewhere, but not proved to be causative for FCPD [10].

Reappraisal of experimental animal models for FCPD

A. Toxic etiology

To test if ingestion of toxic factors from cassava was the cause for FCPD, purified cyanoglycosides were injected into rats [45]. Raw tapioca tuber (bitter variety with high levels of cyanoglycosides) was fed to experimental animals like rats and dogs [48,49,50,51]. Combination with hyper-vitaminosis D was also studied in a few rabbit experiments [52]. Although transient hyperglycemia and a rise in serum levels of some of the pancreatic enzymes were observed in some of the animals, the pancreatic lesions consisted mainly of mild to moderate acinar atrophy but did not resemble the human disease exactly in any of the experiments. These results led to a failure of cyanoglycoside toxicity hypothesis for FCPD.

B. Dietary deficiency of protein

There were several experimental studies to test the effects of varying levels of protein deficiency on the pancreas in different species like rat, rabbit, dog, and rhesus and bonnet monkey [25,53]. With mild protein deficiency of 20% to 10% dietary protein, reduction of acinar enzyme content was directly proportional to degree of protein deficiency; below 10% it was inversely proportional [54], perhaps due to production of amylase by acinar cells in response to marginal increase in dietary starch.

Using diets with protein deficiency ranging from 2.5%, 5%, 7% and 10%, Wachstein et al [42] showed that the severity of acinar cell atrophy was related to the level of protein deficiency induced in experimental rats. In experiments with dietary protein below 2.5% [42,55], there was severe atrophy resembling changes described in kwashiorkor and starvation effects due to severe drought [41]. There was moderate acinar cell atrophy in rats and bonnet monkeys given diets with protein levels of 3.5 % to 4% [42,25]. Dietary protein content above 7% protected the pancreas in experiments by Wachstein et al [42]. Therefore moderate protein deficiency seemed to be necessary for significant histopathological changes to occur.

The islets, ducts and blood vessels were found to be relatively normal in all these protein deficiency experiments, as found in kwashiorkor by J.N.P. Davies [41]. In the rhesus monkey model for kwashiorkor and malnutrition-related diabetes mellitus, using 0% dietary protein, Bajaj et al reported "normal islet mass" with a hypo-functional gland producing inadequate insulin in their experimental animals [56]. The islet mass was calculated and expressed only by a mathematical extrapolation since they found the entire gland (acini and islets) to be severely atrophic [Dev MG, personal communication]. Hence protein deficiency per se as examined in all these experiments, did not produce lesions resembling human FCPD.

C. Low-protein high starch diets

Proximate analysis of tapioca tuber showed that it provided mainly starch and negligible amounts of protein [10]. Traditionally, in all cassava consuming regions, tapioca was served with fish or with pulses. Shaper had pointed out that FCPD patients usually ate a low-protein low-fat high-starch diet [39]. Hence it was considered that monotonous foods and dietary imbalances with low-protein high starch (not severe malnutrition with protein deficiency) might play a role in the development of FCPD as postulated for mucoid arteriosclerosis, endomyocardial fibrosis and goitre, also common in the same regions [2,25]. Experimental diets designed to resemble such diets, were used to develop an animal model for mucoid vasculopathy at SCTIMST, Trivandrum, with the support of DST, New Delhi. The bonnet monkey model also showed features of mucoid vasculopathy and early lesions of associated conditions, particularly cardiomyopathy resembling endomyocardial fibrosis, diffuse colloid goitre-like changes in the thyroid and pancreatopathy [2,24]. Sriramachari and Gopalan used similar low-protein high-starch diets to produce mucoid degenerative changes in the aorta of bonnet monkeys, however they did not study the pancreas in their animals [57].

A detailed study of the pancreas in the bonnet monkey experiments, carried out by the author with Prof. Balaraman Nair, demonstrated lesions ranging from pancreatic atrophy to early FCPD lesions, as described below [25].

Three groups of sub-adult bonnet monkeys were given the following diets, formulated and cooked each day: i) a protein deficient normal carbohydrate diet, ii) a protein deficient high carbohydrate diet, iii) a control diet with normal protein and carbohydrate, for 3 or 5 months experimental periods. Protein ranged from 20% in control diet to approximately 4% in the protein deficient diets that was given to all the test group animals. The experiments were conducted in two sets, using tapioca starch as the carbohydrate in the first set and cornstarch in the second set. Groundnut oil, vitamin and mineral mixture were dispensed equally in adequate quantities to all animals. After sacrifice, histopathological studies were carried out on the pancreas.

In all the test group animals given protein deficient diets, the pancreas showed atrophy, more in the tail end of the gland, particularly in animals fed low-protein high starch diets. There was lipomatous atrophy in some. Thick walled pancreatic arteries with diffuse narrowing of the lumen resembling human mucoid vasculopathy, were more prominent in protein deficient animals fed additional starch.

Microscopically, there were widespread changes and disarray affecting all parts of the pancreas in the test group animals. Lobular and acinar cell atrophy and loss of bipolar staining in acinar cells were more severe in animals fed low-protein diets. Whereas marked islet hypertrophy and hyperplasia (nesidioblastosis), mucoid metaplasia of ducts and arteries were found in protein deficient animals given additional starch diets, and for longer experimental periods. The ducts in these animals showed basal cell and goblet cell hyperplasia, epithelial stratification and papillomatosis with focal mild dysplasia in some. Proliferated and dilated ducts and ductules contained plugs of mucoid material admixed with eosinophilic secretions. There was fibrosis around ducts, ductules, blood vessels and within lobules. Enlarged autonomic ganglia and hypertrophied nerve bundles were seen in some. Inflammatory changes were not evident in any of the pancreas specimens. Both sources of carbohydrate, tapioca starch and cornstarch, produced identical lesions.

Table 2. Salient conclusions of the study

- Protein deficient animals developed pancreatic atrophy (resembling human PDDM lesions); in those fed protein deficient high starch diets, the pancreas showed changes akin to early changes of human FCPD. Muroid metaplasia of ducts and blood vessels were seen in both categories, more prominently in animals given the low protein high starch diets.
- Both tapioca starch and cornstarch based diets produced the same changes, proving that toxic factors from tapioca were not responsible for initiating the pancreatic lesions.
- Identical lesions were produced with both sources of carbohydrate, tapioca starch and cornstarch; hence tapioca consumption is not the sole causative factor for FCPD. A high level of dietary starch of any source is important.
- Muroid vasculopathy is an integral part of structural and functional pancreatic lesions of PDDM due to protein deficiency and FCPD due to nutritional imbalance with low-protein high carbohydrate diets. Hence it has a central role in the pathogenesis of these sub-types of malnutrition related pancreatopathies and diabetes mellitus.
- A non-human primate (bonnet monkey) model for FCPD and muroid vasculopathy was established by dietary means. Nutritional imbalance with mild to moderate protein deficiency and high carbohydrate, particularly starch, is responsible for changes characteristic of FCPD [2,25].

Discussion

Dietary ingredients and nutritional imbalances

The development of pancreatic lesions in the bonnet monkey model explains how FCPD can occur in persons with milder forms of malnutrition and not severe malnutrition or kwashiorkor in childhood. People consuming tapioca but having only mild protein deficiency did not develop FCPD [40]. Dietary protein was possibly still at a protective level in that population. Moderate protein deficiency is necessary for development of FCPD.

FCPD was also reported in people consuming rice, not tapioca as a staple food [32,33]. Since identical lesions were produced in the bonnet monkey model by either source of carbohydrate, tapioca starch and cornstarch, tapioca alone is not a causative factor. Excess rice starch and moderate protein deficiency may also give similar results. This explains how FCPD may occur in rice-consuming regions in India. Likewise, consumption of high carbohydrate from other tubers and starchy foods would have the same effects.

Malnutrition and vascular disease

Long-term effects of childhood malnutrition and under-nutrition (even transient phases) need to be studied, since these affect the structure and function of the exocrine and endocrine pancreas, the total size and micro-architecture of the gland and most importantly its vasculature. Muroid vasculopathic arteriosclerosis was the first lesion to occur in the bonnet monkey model, preceding the development of cardiomyopathy and pancreatopathy [24]. It was generalized, affecting all blood vessels (macro and micro-vasculature of all organs). FCPD patients did not have hypercholesterolemia and atherosclerosis [10], but absence of macro-vascular disease as shown in several reports [33] does not mean they had normal blood vessels. An accurate diagnosis of the vascular lesions and metabolic markers will help in understanding the pathogenesis of various pancreatic lesions and types of diabetes mellitus.

The severity of muroid arteriosclerosis limits optimum function of the pancreas. Repeated episodes of vasospasm may cause chronic ischemia and atrophy of the pancreas, especially at the tail end where most of the insulin-producing islets are located. Therefore with low-protein high starch diets the pancreas is subjected to a double insult – directly in the form of increased demands on the gland stimulating hypertrophy, hyperplasia and nesidioblastosis of the islets, and indirectly due to narrow blood vessels causing vascular compromise, relative ischemia and possible episodes of severe vasospasm in response to hyperglycemia. While mild vasoconstriction may stimulate release of insulin, severe vasoconstriction may also lead to focal necrosis of the pancreas that may be an important cause for episodes of acute abdominal pain. Antibodies described in some cases of FCPD [58] may have been

produced to pancreatic cellular antigens released from ischemic or necrotic tissue. Regenerative activity in FCPD seen as hypertrophy with marked variation in islet size and nesidioblastosis may be a reparative response to ischemia and necrosis following severe vasoconstriction. Hypertrophied nerve bundles and autonomic ganglia may influence vascular tone.

Pancreatic duct lesions, lithiasis and malignancy

Mucoid metaplasia of duct epithelium is an important feature of FCPD [29] and is also a direct effect of low-protein high-starch diets [25]. In the experimental bonnet monkeys, epithelial stratification, hyperplasia and papillomatosis in the larger ducts caused obstruction, the smaller inter- and intra-lobular ductules appeared dilated with pent-up eosinophilic and mucus secretions. Goblet cell metaplasia was seen throughout the duct system. Abnormal viscid mucus material produced by such cells may act as the nidus for stone formation, as described in human FCPD and certain forms of chronic pancreatitis [29]. Lithiasis is intraductal, there is no parenchymal calcification in FCPD. The term "tropical chronic calcific pancreatitis" was changed to "tropical chronic calculous pancreatopathy" or "fibrocalculous pancreatic disease" with diabetes, during a Workshop on Diabetes Mellitus in the Tropics [31].

FCPD patients had an increased risk of pancreatic malignancy, especially, adenocarcinoma, 10 to 15 years after onset of diabetes [38,59]. Prolonged metaplastic and dysplastic changes may predispose the duct epithelium to toxic factors and the development of malignancy.

Parotid and other exocrine glands

Bilateral parotid swelling described in FCPD patients [37] may be due to mucoid metaplasia affecting all exocrine glands, besides the pancreas. Similar changes were noticed in mucus glands of the trachea and bronchi in human autopsies with mucoid vasculopathy and in the bonnet monkey model (Sandhyamani, unpublished observations). In the animal model the parotid gland also showed mucoid metaplasia of duct epithelium [Sandhyamani, unpublished observations], however this aspect was not studied in detail.

Pathogenesis of nutritional pancreatopathies and diabetes mellitus

Structural integrity and the capacity of the pancreas to respond to metabolic demands will determine the onset of diabetes mellitus. The pancreas is small sized in lean persons with severe protein malnutrition in childhood and may be of normal size in those with obesity and continuous over-nutrition throughout [41,60]. In both categories, the gland has an apparent normal micro-architecture. In kwashiorkor and protein deficiency, islets showed initial hyperplasia and became normal sized after realimentation [41]. They did not have any abnormal cytological features. There were no permanent functional changes in most persons [10]. A few histopathological studies of the pancreas with maturity onset diabetes mellitus from the west showed degenerative changes with amyloid deposits and hyalinosis, possibly as ageing changes, causing impairment of the microvasculature of islets [61]. Such deposits were not described in PDDM and in FCPD from developing regions. Diabetes develops slowly in those with protein deficiency in childhood manifesting as PDDM in early adulthood and in those with over-nutrition and obesity, as maturity onset diabetes mellitus (MODM) by middle-age or later. The onset would depend on the degree of "relative-obesity" and dysmetabolic features in an individual and occurrence of precipitating factors like sudden changes in lifestyle and acute stress [18]. In both PDDM and MODM, there is a proportionate distribution of anatomical components. Structural disarray of the parenchyma, narrowness of the blood vessels and fibrosis are not as severe as in FCPD patients.

By contrast, in FCPD, pancreatic damage and metabolic changes occur at an accelerated pace because of the severity of abnormal and disproportionate cyto-architecture of the pancreas, fibrosis, disarray of the lobules and ducts, and marked arteriosclerosis. The FCPD patient may thus develop clinically manifest exocrine pancreatic disturbances, including lithiasis, and diabetes mellitus at a younger age, in childhood and adolescence itself, as reported in several studies [32,36]. Marked structural and functional disturbances of the pancreas are the hallmark of FCPD, as seen in cirrhosis of the liver. FCPD is rightly called cirrhosis of the pancreas.

In all three groups, namely, PDDM, FCPD and MODM, diabetes develops when a lean individual becomes "relatively obese" and there is a disparity between metabolic and functional demands of the body and the limited capacity of a structurally compromised pancreas. Fat-rich foods cause adiposity and atherosclerosis, as observed in developed regions in the west and in urban India. Obesity may be due to consumption of not only high-fat foods but carbohydrate-rich foods too, particularly high-starch foods that result in deposition of abnormal acid mucopolysaccharide material in the connective tissues and walls of blood vessels, typical of mucoid arteriosclerosis observed in Kerala [18]. Protein-carbohydrate malnutrition thus causes the pancreatic lesions, mucoid vasculopathy, the types of obesity, the patterns of dyslipidemia and diabetes mellitus more commonly seen in developing regions of the world. It is expected that long-term experiments and monitoring of insulin-glucose kinetics in the bonnet monkey model will establish the pathophysiology of nutritional pancreatopathies, particularly FCPD, and diabetes mellitus. The model may be used for understanding the development of late sequelae of FCPD, like lithiasis and cancer and for formulating preventive measures.

Fig. 1a,b,c. Pancreas from control bonnet monkey (fed normal-protein normal-carbohydrate diet) has sharp borders and grey-pink, fleshy lobules (a). Marked pancreatic atrophy is seen in animal fed a low-protein normal-carbohydrate diet (b). Lipomatous atrophy mainly in the distal half and mucoid vasculopathy of pancreatic (arrow) and superior mesenteric (arrow head) arteries are seen in pancreas (posterior view) of animal fed low-protein high-carbohydrate diet (c).

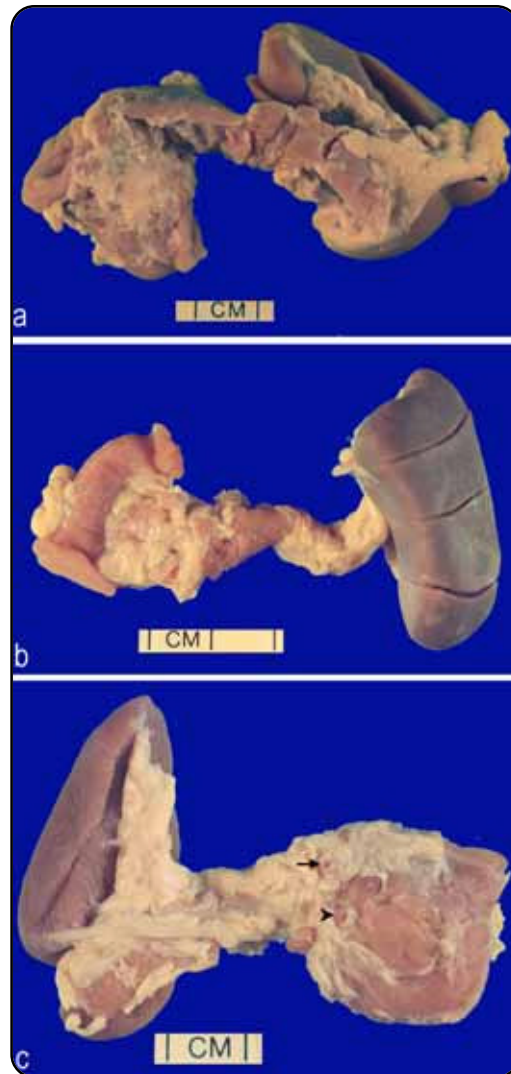
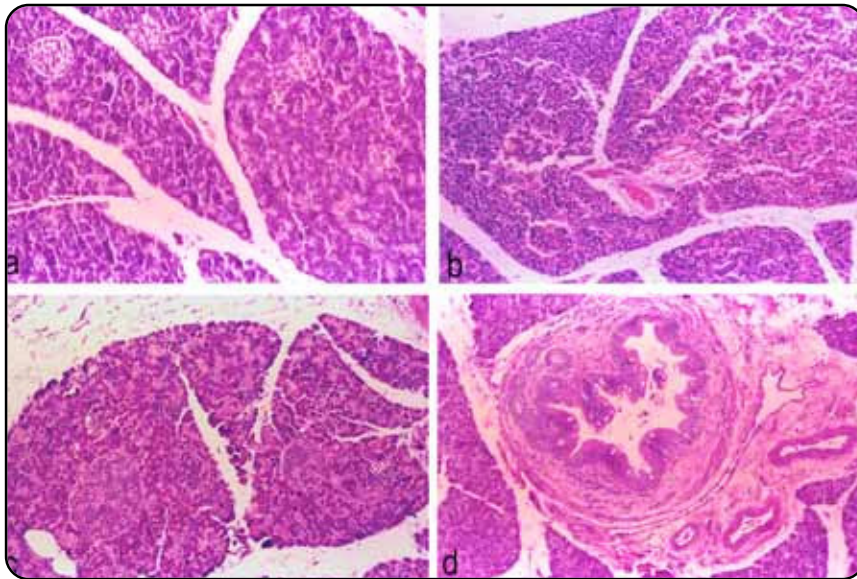


Fig. 2a,b,c,d. Pancreatic lobules from control animal (a), have sharp outlines, normal sized acinar cells with bipolar staining and small islets. Marked lobular and acinar cell atrophy and crowding of islets, is seen in protein-deficient animal (b). Pancreas from animal fed low-protein high-carbohydrate diet has moderately atrophic lobules with rounded outlines, hypertrophied islets and collections of pale eosinophilic secretions within dilated ductules. (c); the pancreatic duct (d) shows hyperplastic epithelium with mucoid metaplasia and inspissated mucoid secretions; thick-walled arteries are present in periductal fibrous tissue (d). [2a, b, c, d: H & E * 125].



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