Nutritional and Inflammatory Biomarkers in Chronic Pancreatitis Patients

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Abstract

Background: Chronic pancreatitis (CP) patients frequently experience malabsorption and maldigestion, leading to micro- and macronutrient deficiencies. Co-morbid diabetes and lifestyle habits, such as alcohol consumption, may impact nutritional status.

Methods: We compared micronutrient antioxidant, bone metabolism, serum protein, and inflammatory marker levels in 301 CP patients and 266 controls with no known pancreatic disease. We analyzed serum prealbumin and retinol binding protein, vitamins A, D, E, and B12, osteocalcin, tumor necrosis factor- α and C-reactive protein (CRP). We also evaluated biomarkers among subsets of patients, examining factors including time since diagnosis, body mass index, alcohol as primary etiology, diabetes mellitus, vitamin supplementation, and pancreatic enzyme replacement.

Results: After correcting for multiple comparisons, CP patients had significantly lower levels of vitamin A (40.9 vs. 45.4 µg/dL) and vitamin E (both α- [8.7 vs. 10.3 mg/L] and γ- [1.8 vs. 2.2 mg/L] tocopherol), as well as osteocalcin (7.9 vs. 10 ng/ml) and prealbumin 23 vs. 27 mg/dL), than controls. Both patients and controls that took vitamin supplements had higher serum levels of vitamins than those not taking supplements. Compared with controls, in controlled analyses, CP patients had significantly lower levels of vitamins A, D, and E (both α- and ϒ-tocopherol). CP patients also had significantly lower levels of osteocalcin, prealbumin and retinol binding protein, and higher CRP.

Conclusions: CP patients demonstrated lower levels of selected nutritional and bone metabolism biomarkers than controls. Diabetes and alcohol did not impact biomarkers. Vitamin supplements and pancreatic enzyme replacement therapy improved nutritional biomarkers in CP patients.

Introduction

The exocrine pancreas produces pancreatic enzymes to hydrolyze complex nutrients for further digestion and absorption by the intestinal mucosa while the endocrine pancreas secretes the hormones necessary for nutrient utilization. Chronic pancreatitis (CP) is a pathologic fibro-inflammatory syndrome of the pancreas in individuals with genetic, environmental, or other risk factors who develop persistent pathologic responses to parenchymal injury or stress.[1] CP causes progressive replacement of functional exocrine and endocrine tissue with fibrosis and manifests clinically by maldigestion, steatorrhea, weight loss, abdominal pain and diabetes mellitus.[2, 3] CP may cause severe malnutrition and metabolic derangements if proper treatment is not provided.[4-8]

Mechanistically, CP affects nutrition through at least four mechanisms. First, oral intake may be diminished because of sitophobia (fear of eating due to pain), or selective avoidance of nutrients such as fat due to steatorrhea, bloating, or diarrhea. Secondly, reduced pancreatic digestive enzyme secretion impairs the digestion of triglycerides, causing reduced absorption of fat and fat-soluble vitamins A, D, E, and K, while diminished proteases may cause protein malnutrition and vitamin B12 deficiency. Thirdly, chronic inflammation may produce an anabolic state that impairs protein utilization. Finally, the loss of islet of Langerhans cells results in metabolic disturbances due to diminished insulin, glucagon, and pancreatic polypeptide secretion. Individual CP patients may manifest any combination and degree of these mechanisms.

We previously reported significantly lower body mass index (BMI, kg/m^2) in patients with CP than those with recurrent episodes of acute pancreatitis or controls.[9] Low BMI correlates with severity indicators of CP and poor quality of life.[10] Change in BMI after the onset of CP may be an even more accurate measure of the effects of CP on nutrition. Active inflammation, regardless of pancreatic secretory function, can affect nutrition by decreasing appetite and driving catabolism. Indeed, inflammation and malnutrition have been shown to correlate, particularly when protein is deficient.[11-13] Levels of serum proteins, such as albumin, prealbumin and retinol-binding protein (RBP), are often lower in CP patients than in healthy individuals, and have frequently been used as a sign of protein-calorie malnutrition.[14, 15] However, levels of negative acute phase reactants such as albumin and prealbumin can be diminished when an individual has physiologic stress, such as during an infection, or in people with liver disease.[16] Combined consensus statements from Academy of Nutrition and Dietetics the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) as well as 2016 guidelines for nutritional support from the Society of Critical Care Medicine/A.S.P.E.N. have noted that prealbumin levels are inversely related to inflammatory cytokines.[17, 18]Although unreliable, physicians still often rely on these biomarkers as a clinical reflection of protein status. Malabsorption of nutrients and micronutrients and may contribute to elevated inflammatory cytokines and markers of oxidative stress.[19, 20] Thus, CP patients may enter a vicious cycle of antioxidant micronutrient deficiencies and inflammation-associated damage, where increased oxidative stress occurs simultaneously with decreased antioxidant protection, especially in diabetics.[19-22] Micronutrient deficiencies also predispose to oxidative stress in other systems, increasing the risk of illnesses such as cancer.[23-25]

Fat metabolism may be abrogated in CP. Lipase and apolipoprotein C-II deficiencies are two inborn errors of metabolism associated with CP.[26-28] Lipase deficiency also causes fat malabsorption, affecting fat-soluble vitamin status.[29] Low levels of serum lipids, fatty acids, and vitamins A and E often occur in CP patients.[21, 30-33] Vitamin D is essential to bone health and bone metabolism. Vitamin D depletion has been noted in men and women of all ages with CP.[29, 30, 34] A study of 73 patients (17 women and 56 men) in different stages of CP noted the presence of osteopathy in 39% of patients, observing osteopenia in 26%, osteoporosis in 5%, and osteomalacia in 8% of cases.[30] A 2014 meta-analysis that included 10 studies for a total of 513 CP patients, noted that patients had a pooled prevalence rate of osteoporosis of 23.4% (95% confidence interval, 16.6-32.0).[35] CP is associated with lower circulating levels of vitamin D3 and decreased bone mineral density, loss of skeletal mass, and an increased risk of diabetes mellitus.[36-38]

Pancreatic enzyme replacement therapy (PERT) is currently the most effective means of treating maldigestion and malabsorption in CP patients.[39, 40] Although guidance for treating pancreatic enzyme insufficiency in cystic fibrosis is established, variable etiology, diet, lifestyle factors, comorbidities and metabolic demands in adults with CP makes standardizing PERT guidance challenging. Controlled studies demonstrate that, with or without gastric acid suppression, some CP patients may require higher doses of PERT to achieve adequate absorption of dietary nutrients.[41]

To date, there have been a limited number of published investigations of malnutrition and vitamin deficiencies in CP.[33, 42] While many studies evaluating nutrition in pancreatitis have been of fairly high quality, most have been small in size, with fewer than 100 patients included in the analyses.[15, 43-48] Some have not included any female patients.[42, 48-50] We utilized the North American Pancreatitis Study II (NAPS2) studies with the primary aim of comparing micronutrient antioxidant, bone metabolism, serum protein, and inflammatory marker levels between a clinically relevant and well characterized CP cohort and controls. Given the extent of pancreatic compromise evident in CP patients, we hypothesized the CP patients would demonstrate more signs of malabsorption and malnutrition than individuals without pancreatic disease. Secondarily, we evaluated these same biomarkers among subsets of CP patients, examining factors that might influence inflammatory and nutritional status, such as time since diagnosis, BMI, alcohol as the primary etiology of CP, diabetes mellitus, and PERT usage. We analyzed the inflammatory biomarkers tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP), markers of inflammation and protein-calorie malnutrition serum prealbumin and RBP, nutritional variables vitamins A, D, E, and B12, and osteocalcin, a marker of bone turnover. Taken together, the insights gained from our study may provide better understanding of the effects of CP on nutrition and improve the process of assessing and optimizing the care of these patients.

Methods

Study cohort

CP patients and controls with no known pancreatic disease were ascertained from the North American Pancreatitis Studies, including the NAPS2-continuation and validation study (NAPS-CV) (2008-2012) and the NAPS2-AS (2011-2014). These studies were created following the success of the original NAPS2 study (2000-2006). Specific details of these studies have been published previously.[9, 51, 52] In brief, a subset of all CP patients and controls from these two NAPS2 studies that had serum samples available using strict standard operating procedures (SOPs) were included in the current study.

Participants were recruited based on having adequate serum samples and questionnaire completion. Primary entry criteria for CP were predetermined, definitive evidence of CP on imaging studies, primarily either endoscopic retrograde cholangiopancreatography (ERCP) using the Cambridge classification or cross-sectional studies (computed tomography, magnetic resonance imaging/magnetic resonance cholangiopancreatography), and endoscopic ultrasound (five or more criteria or presence of calcifications) or histology. Controls were spouses, family members, friends, or individuals unrelated to CP patients; controls needed to be free from pancreatic diseases and capable of completing a survey and undergoing blood draw. Genomic DNA was initially extracted from whole blood using the Flexgene DNA® kit (Qiagen, Valencia, Calif., USA) with a modified manufacturer's buffy coat protocol. Serum was isolated from 10mL whole blood in non-additive serum tubes. Serum and a whole-blood sample were stored in a freezer at –80°C.

Questionnaires

Physician Questionnaire

Physician case report forms for CP patients were completed by a recognized expert in pancreatic diseases at participating centers, usually with the assistance of a clinical research coordinator. The information in the questionnaire was supplemented by medical record documentation of key diagnostic, laboratory, and hospital reports. Information that was queried focused on disease phenotype (e.g. history of acute pancreatitis, age at pancreatitis diagnosis, presence of morphological and functional abnormalities, etc.), etiology and risk factors, treatment received, and their perceived outcome.

Patient and Control Questionnaire

All CP patients and controls completed the participant questionnaire. The questionnaire included information on demographic factors including current and maximum height and weight (to calculate BMI), personal and family history of a variety of medical conditions, pancreatitis-related symptoms, disability and quality of life.

Specific variables of interest for this study included age at enrollment, BMI, duration of disease (age at first symptoms or diagnosis of pancreatitis), physician-defined alcohol etiology; current alcohol consumption; drinking category derived from self-reported drinking history during the maximum drinker period in life; smoking status [past, current, or never]; vitamin/mineral supplement use PERT use, and physician reporting of diabetes diagnosis. Participant drinking categories included – lifetime abstainer (<20 drinks during lifetime), light (up to 3 drinks a week), moderate (up to 7 drinks a week in women and 14 drinks in men), heavy (8-35 drinks a week in women, and 15-35 drinks a week in men) and very heavy (35 or more drinks a week in women and men).[52] For the current manuscript, we combined heavy and very heavy drinking into one category. A number of controls (n=137) had missing information regarding drinking category, as they were enrolled in the NAPS2-CV and NAPS2-AS studies, when participant questionnaires for controls were abbreviated. Because participants took a large range of products, we used the broad term vitamin/supplement use (yes/no) as a covariate in analysis.

Laboratory testing

We analyzed the inflammatory markers TNF-α and C-reactive protein (CRP) as well as osteocalcin using a Luminex platform. An automated chemistry analyzer was used to measure levels of prealbumin; an enzyme-linked immunosorbent assay (ELISA) to measure RBP; a microbiological microplate assay for vitamin B12; and high-performance liquid chromatography (HPLC) to measure 25-OH Vitamin D3, vitamin E (α- and γ-tocopherol), and vitamin A.

Statistical Methods

Descriptive statistics are presented as proportions for categorical and as mean ± standard deviation or median (interquartile range) for continuous data. The distributions of the levels of vitamins, nutritional/inflammatory markers, and indicators of bone turnover were assessed for normality using graphical methods and the Shapiro-Wilks test. Correlation between continuous measures was assessed using Spearman's correlation. Comparisons in discrete baseline characteristics between the CP patients and controls were performed using the chi-square test. Comparisons for continuous variables between the CP patients and controls, and within subset of patients and controls were through the Mann–Whitney *U* test. Correlations between vitamin, nutrition, and biomarker levels were assessed using Spearman's rank correlation coefficient. To adjust for multiple comparisons, the Hommel's procedure was adopted for calculating adjusted p-values.[53]

Multivariable linear regression models were created to determine independent factors that were predictive of the vitamin, nutrition and biomarker level outcomes. Due to the skewed nature of serum markers, logarithm transformation was applied to all serum markers except for vitamin E γ-tocopherol and TNF-α, for which square root function was applied such that the transformed levels approximated a normal distribution. The log or square root transformed micronutrient and inflammatory marker values were included as the dependent variable in the multivariable models, and factors retained in the models as covariates, included diagnosis, age, gender, race, BMI, alcohol consumption, smoking status, and supplemental vitamin intake. Interaction term between race and diagnosis as well as sex and age were also included in the full model. Other first order interactions were also tested, but due to minimal changes in the model performance (i.e. in R^2) in some of the outcome variables, we have chosen to show simplified models with interaction term for race and diagnosis, and sex and age. No higher order interactions were examined due to the complexity of model interpretation. Separate multivariable analyses were also performed for CP patients only, where, in addition to the aforementioned variables, PERT use and diabetes status were also included as covariates. Backward stepwise selection was used for variable selection with a *P* value cutoff of $p < 0.20$ used for incorporation into the model while a *P* value of less than 0.05 was considered significant. For interpretation of the model, square root and log transformed data may be reverse transformed using a square or exponential function respectively to bring the data back to the original units with the understanding that not all components of the model (error terms, confidence intervals, etc.) will be correct in native units. Conversely, the coefficients in the log-transformed models correspond the percent change in the mean of the outcome variables. All statistical analysis was performed using the R Project software (www.r-project.org).

Results

Cohort

The final sample included 301 eligible CP patients and 266 controls (Table 1). When compared with controls, CP patients were significantly ($p<0.05$) older (50.8 vs. 45.6 years), male (50.1 vs. 40.2%), Caucasian (71.1 vs. 58.3%), past or current smokers (77.7 vs. 51.1%), and taking vitamin supplements (55.8 vs. 45.1%). CP patients were less likely to be current drinkers (20.6 vs. 66.2%), and amongst participants with available information, more likely to be heavy or very heavy drinkers (53.3 vs. 34.2%). CP patients were less likely to be overweight (25.9% vs. 36.5%) or obese (18.6% vs. 38.3%.) when compared with controls. Twenty-two of the 266 (9%) controls reported being diabetic (data not shown).

Among patients with CP, almost half (49.8%) had physician-defined alcohol etiology for their illness and more than one-third (38.5%) were identified as having diabetes per the enrolling physician. Just over one-half (55.1%) of CP patients were using PERT. About three-quarters (73.3%) of CP patients had a disease duration that was <5 years (Table 2).

Vitamin, nutrition and biomarker levels

Internal Validation

Several previously described relationships between biomarkers were evaluated as internal validation of the data.[5, 54-57] CP patients had significantly lower BMI than control patients, with 44.5% of CP patients being overweight or obese compared to 74.8% of controls (Table 1). Amongst all study participants (controls and CP patients), CRP levels correlated positively with BMI. Additionally, for all participants, there was a strong correlation between RBP and vitamin A levels (correlation 0.88, 0.89), between prealbumin and RBP (0.73, 0.70), and between prealbumin and vitamin A levels (0.72, 0.75). There were weaker positive correlations between fat-soluble vitamins A and E [α –tocopherol] (0.50, 0.58), and vitamins A and D3 (0.26, 0.34).

Effect of CP on vitamin and biomarker levels

Serum levels of vitamins, nutritional markers, and inflammatory biomarkers in CP patients and controls are provided in Table 3. After correcting for multiple comparisons, CP patients had significantly lower levels of vitamin A (40.9 vs. 45.4 μg/dL) and vitamin E (both α- [8.7 vs. 10.3 mg/L] and γ- [1.8 vs. 2.2 mg/L] tocopherol), as well as osteocalcin (7.9 vs. 10 ng/ml) and prealbumin 23 vs. 27 mg/dL), than controls. The median level of Vitamin B12 was significantly higher in CP patients when compared with controls (703 vs. 609.5 pg/mL). CP patients also had a significantly higher level of CRP than controls (0.3 vs. 0.2 mg/dL). Table 3 also shows the number and percentage of study participants that demonstrated vitamin and nutritional marker deficiencies according to standard laboratory thresholds. Compared to controls, a higher percentage of CP patients had deficiencies in vitamins A, D, and the α-tocopherol component of vitamin E, although the discrepancy between controls and CP patients for vitamin D was less pronounced. A greater proportion of patients than controls had lower levels of osteocalcin, and showed deficiencies in prealbumin and RBP with marked differences in rates of deficiency being noted for prealbumin (15.3% of CP patients showed deficiency vs. 2.3% of controls).

Effect of treatment with PERT and/or vitamin supplements

Among patients with CP, when compared with patients who were not on PERT, those receiving PERT had significantly higher levels of Vitamin A (median 43.3 vs. 36.6 μg/dl), prealbumin (median 24 vs. 22 mg/dl), and a trend towards higher levels of RBP (median 36.3 vs. 32.5 μg/dl) (Supplementary Table 1).

When compared with CP patients that were not taking vitamin supplements, those on supplements had significantly higher levels of vitamin A (median 45.0 vs. 38.3 μg/dl), α -tocopherol (9.5 vs. 8.0 mg/L) and γ-tocopherol (1.56 vs. 2.2 mg/L), and borderline higher levels of vitamin B12 (756.5 vs. 649 pg/ml) and RBP (36.3 vs. 32.2 μ/ml) (Supplementary table 2). No significant differences were noted in the serum levels of vitamins, nutritional markers, or biomarkers based on alcohol etiology or disease duration although a trend was observed for lower levels of prealbumin in CP patients with diabetes when compared to non-diabetic CP patients (data not shown).

Similarly, when compared with controls that were not taking vitamin supplements, controls that used supplements had significantly higher levels of vitamin B12 (median 653 vs. 587 pg/ml), vitamin D (27.2 vs. 16.1 ng/ml), α-tocopherol (11.60 vs. 9.70 mg/L) and a trend towards significantly higher levels of vitamin A (47.9 vs. 44 μg/dl) (Supplementary Table 3).

Multivariable regression models to assess the effect of CP and other co-factors on serum vitamins, nutrition and biomarkers

Independent predictors for serum levels of vitamins, nutritional markers and biomarkers in controls and CP patients are shown in Table 4. In this analysis, interpretation of parameter estimates for each of the covariates quantifies the independent effect of an attribute versus the comparison group (e.g. CP vs. controls) after adjusting for other variables. A positive parameter estimate suggests higher levels, while a negative parameter estimate indicates lower levels in reference to the comparison group.

After controlling for other factors, when compared with controls, CP patients had significantly lower levels of vitamins A and E (both α- and ϒ-tocopherol), but significantly higher levels of vitamin B12. CP patients also had significantly lower levels of osteocalcin, prealbumin and RBP. Additionally, CRP levels were significantly higher in CP patients compared to controls, suggesting that patients had increased systemic inflammation. As an example, the interpretation of parameter estimates for CRP will be that, on average, CRP levels in patients with CP are 0.681 mg/dL higher when compared with controls, after adjusting for other co-variates. A similar interpretation would be applicable for vitamins and other biomarkers, taking into consideration whether the parameter estimate has a positive or negative value.

Effects of demographics

The study cohort included a sufficient number of Caucasian (white) and African-American (black) patients for comparative analysis. When compared with Caucasians, African-Americans had significantly lower levels of vitamin D, α-tocopherol, and RBP. An interaction was noted between race and diagnosis for several vitamins and biomarkers. Lower levels of vitamin A and prealbumin levels in CP patients versus controls were observed among African-Americans study participants to a greater degree than among Caucasian participants. The increment of baseline levels of CRP in CP patients from controls is more evident in African-Americans than that in Caucasians. The interaction between diagnosis and race for vitamin B12 and TNF-a was interesting. Overall, African-American controls had significantly higher vitamin B12 levels when compared with Caucasian controls; however, there was no racial difference in vitamin B12 levels among CP patients. While TNF-α did not show a significant difference between Caucasian CP patients and Caucasian controls, it was significantly elevated in African-American CP patients when compared with African-American controls.

Significant associations with sex, age, BMI, alcohol and tobacco use were noted for a few comparisons (Table 4). Other than diagnosis and race, the only other clinically relevant interaction was noted between sex and age for osteocalcin levels – i.e. with increasing age, the level of osteocalcin decreased in males.

Effect of vitamin and PERT supplementation

Vitamin supplementation was associated with significantly higher levels of Vitamin A, D, E α-tocopherol, and B12, but not E ϒ-tocopherol. We evaluated the independent effect of PERT usage on serum vitamins, nutritional markers and biomarkers in regression models limited to CP patients only (Supplementary Table 4). PERT usage was associated with a significant increase in serum levels of vitamins A and ϒ-tocopherol and nutritional markers including RBP and prealbumin.

Discussion

Our study is the first of its kind to evaluate various nutritional and inflammatory markers in a sizeable, racially diverse group of CP patients and controls. The study is especially relevant as it reflects a cross-sectional assessment of the state of hundreds of well-characterized patients who are receiving care at pancreas disease centers across the United States. We assessed various parameters of nutritional status and inflammation amongst 301 CP patients and 266 controls. Previous studies of nutritional status are limited in number and have not factored in alcohol as an etiology, BMI, diabetes, vitamin/mineral supplementation and the use of PERT. Additionally, we are not aware of any U.S. studies that have examined whether race may play a role in the nutritional status of individuals who carry a diagnosis of CP.

Expected baseline differences between CP patients and controls included lower BMI, being current or previous smokers, taking vitamin supplements, and not being current drinkers. These effects were notable, since the duration of CP was fewer than 5 years in the majority of CP patients. Patients with a history of alcoholic CP did not have significantly different levels of nutritional markers than patients with other etiologies of CP. Counseling regarding the deleterious effects of alcohol may have accounted for fewer CP patients being current drinkers compared with controls. Nonetheless, even after adjusting for multiple confounders and multiple comparisons, significant differences between patients and controls and certain subsets of participants existed. Although the majority of patients in our study had been diagnosed with a chronic form of pancreatitis within 5 years of entering the study, most CP patients were using PERT. Steatorrhea and its associated symptoms are often the primary indicators for the need to prescribe PERT. However, steatorrhea may develop a decade after the diagnosis of CP but it can also be present at the time of diagnosis. According to the United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU), all newly diagnosed CP patients should be screened for pancreatic enzyme insufficiency.[58] When patient symptoms are not definitive, the guidelines advocate a 4-6 week trial of PERT.

Diminished symptoms of maldigestion (e.g. weight loss, flatulence, bloating, steatorrhea) and improvements in nutritional status reflect the efficacy of PERT.[58] A more recent diagnosis in a sizeable number of patients as well as PERT use likely explains similar serum micronutrient levels in CP patients using PERT when compared with those who were not taking PERT. Similarly, use of vitamin supplements in both controls and CP patients resulted in higher levels of certain vitamins or vitamin sub-units than in participants who did not supplement their diet.

Nutritional markers

The major form of vitamin E present in the diet of individuals living in the United States is ϒ-tocopherol, although α-tocopherol is the form found in human tissues as well as dietary supplements.[59] Our study found that CP patients had significantly lower levels of vitamin A and both forms of vitamins E (α- and ϒ-tocopherol) than controls although vitamin supplementation and PERT influenced these differences. A recent meta-analysis by Martinez-Moneo et al. evaluated fat-soluble vitamin deficiency in CP patients.[33] Among four studies that included161 patients, the pooled prevalence of vitamin A deficiency was 16.8% (95% CI, 6.9- 35.7%) and of vitamin E deficiency was 29.2% (95% CI 8.6 - 64.5%).[42, 45, 60, 61]

A 2014 study by Duggan et al. noted that 14.5% and 24.2% of CP patients had deficiencies in the fat-soluble vitamins A and E, respectively, although half of the patients in this study were either overweight or obese.[60] They also found that 19% of their patients had high serum levels of vitamin A. In our study, 74.8% of controls and 44.5% of CP patients were overweight or obese according to BMI. This large proportion of overweight or obese study participants raises the question of whether any participants had high levels of vitamins A or E. We found no correlations between overweight/obesity and elevated levels of vitamins A or E. Two CP patients had vitamin A levels in the toxic range (>120.0 mcg/dL) and both patients had normal BMI (21.1 and 23.0 m^2/kg). Just three CP patients had elevated vitamin E (α-tocopherol), two of whom were overweight (BMI of 26.5, and 28.7 m^2/kg) while one had normal BMI (23.8 m^2/kg). All CP subjects with an elevated vitamin A or E were taking vitamin supplements. No controls had elevated vitamin A or vitamin E levels regardless of supplement use. These data were not presented in our results due to the minimal number of patients with elevations and their lack of association with BMI. While the comparatively lower levels of fat-soluble vitamins A and E, and RBP in CP patients versus controls in our study may indicate that patients may require supplementation to reach adequate levels, a small number of patients may be taking an excessive dose of vitamins A or E. Universal or indiscriminate vitamin supplementation for individuals with CP is not advisable.[58] Each patient should be evaluated individually for vitamin deficiencies and appropriate supplementation should be recommended when dietary intake is inadequate to address nutritional deficits.

Osteopenia occurs when bone breakdown exceeds bone formation leading to lower bone mass. Diminished bone mass is a critical risk for morbidity in patients with CP.[30] We evaluated osteocalcin levels as a measure of osteoblast activity during bone formation with serum concentrations serving as a biochemical marker for bone formation.[62] Previous studies have noted low osteocalcin levels in CP patients.[47] Serum osteocalcin levels decline with age, reflecting decreased bone formation and turnover.[63] Osteocalcin may also function as a metabolic hormone, with multiple beneficial effects on glucose and fat metabolism.[62] Studies have shown that serum osteocalcin levels are associated with glucose and lipid metabolism in men and women, and lower levels have been shown to correlate to diabetes and metabolic syndrome.[64-66] In multivariable comparisons, osteocalcin levels were significantly lower in CP patients than controls. In regression analyses, there were no differences in osteocalcin levels in men and women. In adjusted analyses, levels of vitamin D and osteocalcin were lower in CP patients than in controls. Close to 40% of our CP patients had diabetes according to their physicians, compared to 9% self-reported by our controls. The high rate of diabetes in our patients, in part, may have accounted for their low osteocalcin levels. Vitamin D levels amongst all study participants were lower in African-Americans than in Caucasians.

Vitamin D is naturally present in a few foods, added to others, available as a dietary supplement, and produced endogenously when sunlight's ultraviolet rays strike the skin and trigger vitamin D synthesis.[67] Individuals with inadequate vitamin D absorption are at risk for all forms of osteopathy and have a significantly elevated likelihood of sustaining bone fractures.[68, 69] Given the high morbidity and mortality associated with fractures in older individuals, and previous findings of osteopathy amongst CP patients, vitamin D supplementation is advisable.[30, 49, 70]

An unexpected finding was modestly higher vitamin B12 levels in patients with CP than controls, with or without oral vitamin supplementation. Vitamin B12 is a dietary vitamin that binds to R-proteins in the stomach to form a B12-R complex.[71] Intrinsic factor (IF) synthesized by the stomach's parietal cells is secreted into the duodenum, where proteases secreted from the pancreas digest the R-proteins and release B12, which then binds to IF to form a B12-IF complex.[71, 72] Receptors on enterocytes in the terminal ileum are only able to recognize the B12-IF complex; therefore, the two must be bound to be absorbed.[72] Thus, vitamin B12 deficiency occurs in CP patients as a consequence of protease deficiency.[73] Although the majority of both CP patients and controls had normal range vitamin B12 levels, CP patients may have been receiving B12 injections, bypassing the gastrointestinal track altogether. This potential explanation cannot be confirmed because a history of B12 injections was not included in the NAPS2 case report forms. However, 20.3% of CP patients had a serum vitamin B12 level above 1000 pg/mL, which may indicate that they were receiving B12 injections (data not shown). By comparison, only 10.5% of controls had a serum vitamin B12 that surpassed 1000 pg/mL.

Inflammatory markers

CP exemplifies a chronic inflammatory disorder, but the amount and type of inflammation that a CP patient experiences at any specific time is typically unknown. Although inflammation plays a critical role in CP, the inflammation can be episodic or continuous while the disease itself is defined by morphology, pain and diminishing exocrine function in later stages. These features are not surrogates of each other.[74, 75]

Therefore, these factors should be assessed in relation to disease activity, progression and response to treatment.[76]

Previous research has focused on serum biomarkers of inflammation in pancreatitis linked to different outcomes and demonstrated that inflammation has a wide impact. For example, Rasch et al. conducted a systematic review of studies that measured inflammatory mediators in CP to determine the association between chronic inflammation, early mortality, and markers of accelerated biological aging, or "inflammageing."[77] Beyer et al. demonstrated that CRP, hemoglobin A1c, BMI, platelet count, and pain score were strong predictors of future hospitalization and length of stay.[75] An inverse correlation between log of CRP level and bone mineral density in patients with CP was noted by Duggan et al, suggesting that bone turnover is affected by systemic inflammation.[70] A study by Mroczko et al. demonstrated that both IL-6 and CRP levels are slightly but significantly higher in CP patients compared to controls, but markedly higher in patients with pancreatic cancer, with levels correlating with cancer stage.[78] Thus, systemic inflammation, as reflected by serum CRP levels, correlates with CP morbidity in systems outside the pancreas. Herein we extend these findings to a large, well-phenotyped cohort of CP patients with biomarkers of both nutrition and inflammation.

TNF-⍰ levels were below the level of detection in most CP patients, although some patients with CP were recruited during episodes of acute pancreatitis, which likely contributed to markedly elevated levels in a very small number of patients. CRP is an acute phase reactant produced by the liver in response to inflammation. Normal CRP levels are 3.0 mg/L (or 0.3 mg/dL) or less, requiring high sensitivity CRP testing for accurate measurement. CRP levels increased with BMI (Table 4), with obesity representing a known pro-inflammatory condition.[79] We confirmed that CRP levels are elevated in CP patients versus controls (Table 3), with CRP levels in our cases being similar to those noted by Duggan et al. (mean 3.0 vs. 3.15 mg/L), while levels in in our controls were higher (mean 2.0 vs. 0.9 mg/L).[70] Although our controls lacked pancreatic disease, they may have had other illnesses. CRP levels may be elevated due to chronic diseases and advancing age, with progressive increases being observed amongst individuals with multiple co-morbidities.[80, 81] The U.S. National Health and Nutrition Examination Survey (NHANES) cohort, for example, estimated multi-morbidity in 36.7% of adult Americans.[82] Compared to our CP patients, CRP levels in the NHANES cohort were 2.65 +/− 0.11 for controls, 3.92 +/− 0.11, for individuals with one chronic disease and 5.80 +/− 0.12 for the multi-morbidity cohort, including patients with asthma, arthritis, chronic liver disease, COPD, kidney failure, obesity and history of cancer – with obesity being a major contributor to elevation. CRP levels are often analyzed in conjunction with albumin levels to generate the Glasgow Prognostic Scores (GPS), a measure of chronic inflammation and a strong prognostic factor for survival in various cancers.[82] The severity score increases with CRP $\geq$ 10 mg/L, a value that was rarely seen in our cohort. Therefore, the GPS is unlikely to be a useful marker of chronic inflammation in CP, except in extreme cases. Similar to albumin, prealbumin may be diminished due to inflammation. The lower levels of prealbumin in our CP patients were likely to be a stronger reflection of systemic inflammation than protein-calorie malnutrition.[17,18]

Our study has numerous strengths. The study's large sample size could detect small differences in nutritional and inflammatory biomarker levels. Additionally, patients, patients' physicians, and controls each completed detailed questionnaires that resulted in well-defined CP etiology for patients and comprehensive information regarding lifestyle habits for all participants. Our serum samples were processed using meticulous SOPs, providing results that are likely to be accurate and reliable. Weaknesses of the study include the fact that our patients and controls were not precisely matched, with the median age of patients being 5 years older than controls and 50% of patients compared to 40% of controls being male. Advancing age may be associated with a decline in nutritional status due to medical, psychological, social, and other factors, such as limited food access.[83] Therefore, differences in nutritional markers may have reflected older age as well as disease status. Additionally, the NAPS2 study centers are tertiary care institutions. CP patients who have been evaluated at highly skilled centers may be diagnosed earlier and receive more comprehensive care, which could be reflected by the relatively high percentage of patients taking PERT, refraining from alcohol consumption, and using dietary supplements. Therefore, our findings may have been attenuated compared to the general population of CP patients. The type of vitamin supplementation used by study subjects varied widely from single vitamin (e.g. vitamin B12 or vitamin D) formulations to multivitamin preparations with various quantities of different vitamins, minerals and other nutriceuticals. The majority used combination products. Due to the vast array of ingredients and quantities ingested, we considered subjects using any form of supplement into the vitamin supplementation category. Our binary classification of supplement use may have impacted our conclusions. Finally, we were missing data for some lifestyle variables.

Overall, inflammatory markers were generally increased in CP patients and micronutrient levels were generally lower in CP patients when compared to controls, with CP patients showing higher rates of micronutrient deficiencies. The physician-defined etiology of alcohol does not appear to have a significant effect on the status of protein nutrition or micronutrients. Measures of protein nutrition and levels of micronutrients were clearly associated with vitamin supplementation and the use of

PERT. This indicates that PERT may help to minimize protein and vitamin deficiencies associated with CP, regardless of etiology, and therefore improve patients' micronutrient status. Our study also demonstrated that there are potential racial differences in nutrient and inflammatory markers, indicating the need for future studies of mechanisms and therapies.

References:

1. Whitcomb DC, Frulloni L, Garg P, Greer JB, Schneider A, Yadav D, et al. Chronic pancreatitis: An international draft consensus proposal for a new mechanistic definition. Pancreatology. 2016;16(2):218-24.

2. Steer ML, Waxman I, Freedman S. Chronic pancreatitis. N Eng J Med. 1995;332:1482-90.

3. Worning H. Chronic pancreatitis: pathogenesis, natural history and conservative treatment. Clin Gastroenterol. 1984;13:871-94.

4. Lankisch PG. Natural course of chronic pancreatitis. Pancreatology 2001;1:3-14.

5. Nakamura T, Takebe K, Imamura K, Tando Y, Yamada N, Arai Y, et al. Fat-soluble vitamins in patients with chronic pancreatitis (pancreatic insufficiency). Acta Gastroenterol Belg. 1996;59(1):10-4.

6. Pasanen AV, Tarpila S, Miettinen TA. Relationships between serum lipids and malabsorption of bile acids, neutral sterols, and fats in exocrine pancreatic insufficiency. Scand J Gastroenterol. 1980;15:503-7.

7. Petersen JM, Forsmark CE. Chronic pancreatitis and maldigestion. Semin Gastrointest Dis. 2002;13:191-9.

8. Whitcomb DC, Bodhani A, Beckmann K, Sander-Struckmeier S, Liu S, Fuldeore M, et al. Efficacy and Safety of Pancrelipase/Pancreatin in Patients With Exocrine Pancreatic Insufficiency and a Medical History of Diabetes Mellitus. Pancreas 2016;45:679-86.

9. Yadav D, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, et al. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis.[Erratum appears in Arch Intern Med. 2011 Apr 11;171(7):710]. Arch Intern Med 2009;169:1035-45.

10. Mokrowiecka A, Pinkowski D, Malecka-Panas E, Johnson CD. Clinical, emotional and social factors associated with quality of life in chronic pancreatitis. Pancreatology 2010;10:39-46.

11. Jensen GL. Malnutrition and inflammation-"burning down the house": inflammation as an adaptive physiologic response versus self-destruction? JPEN J Parenter Enteral Nutr. 2015;39:56-62.

12. Johnson AM. Low levels of plasma proteins: malnutrition or inflammation? Clin Chem Lab Med 1999;37:91-6.

13. Yamada S, Tokumoto M, Tatsumoto N, Tsuruya K, Kitazono T, Ooboshi H. Very low protein diet enhances inflammation, malnutrition, and vascular calcification in uremic rats. Life Sci. 2016;146:117-23.

14. Lasztity N, Biro L, Nemeth E, Pap A, Antal M, Lasztity N, et al. Protein status in pancreatitis--transthyretin is a sensitive biomarker of malnutrition in acute and chronic pancreatitis. Clin Chem Lab Med. 2002;40:1320-4.

15. Schrader H, Menge BA, Belyaev O, Uhl W, Schmidt WE, Meier JJ, et al. Amino acid malnutrition in patients with chronic pancreatitis and pancreatic carcinoma. Pancreas 2009;38:416-21.

16. Bharadwaj S, Ginoya S, Tandon P, Gohel TD, Guirguis J, Vallabh H, et al. Malnutrition: laboratory markers vs nutritional assessment. Gastroenterol Rep. 2016;4:272-280.

17. White JV, Guenter P, Jensen G, Malone A, Schofield M. Consensus statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: characteristics recommended for the identification and documentation of adult malnutrition (undernutrition). J Acad Nutr Diet. 2012;112:730-8.

18. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, et al. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). JPEN J Parenter Enteral Nutr. 2016;40:159-211.

19. Verlaan M, Roelofs HM, van-Schaik A, Wanten GJ, Jansen JB, Peters WH, et al. Assessment of oxidative stress in chronic pancreatitis patients. World J Gastroenterol. 2006;12:5705-10.

20. Shen J, Lai CQ, Mattei J, Ordovas JM, Tucker KL, Shen J, et al. Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. Am J Clin Nutr. 2010;91:337-42.

21. Braganza JM, Schofield D, Snehalatha C, Mohan V. Micronutrient antioxidant status in tropical compared with temperate-zone chronic pancreatitis. Scand J Gastroenterol. 1993;28:1098-104.

22. Quilliot D, Walters E, Bohme P, Lacroix B, Bonte JP, Fruchart JC, et al. Fatty acid abnormalities in chronic pancreatitis: effect of concomitant diabetes mellitus. Eur J Clin Nutr. 2003;57:496-503.

23. Qiao YL, Dawsey SM, Kamangar F, Fan JH, Abnet CC, Sun XD, et al. Total and cancer mortality after supplementation with vitamins and minerals: follow-up of the Linxian General Population Nutrition Intervention Trial. J Natl Cancer Inst.101(7):507-18.

24. Reyes-Ortiz CA, Ju H, Inniss A, Eschbach K, Kuo YF, Goodwin JS, et al. Acculturation and serum nutrients thought to be involved with cancer prevention among Mexican American men in the United States. Cancer Control. 2009;16:169-75.

25. Pelucchi C, Tramacere I, Bertuccio P, Tavani A, Negri E, La Vecchia C. Dietary intake of selected micronutrients and gastric cancer risk: an Italian case-control study. Ann Oncol. 2009;20:160-5.

26. Cox DW, Breckenridge WC, Little JA. Inheritance of apolipoprotein C-II deficiency with hypertriglyceridemia and pancreatitis. N Eng J Med. 1978;299:1421-4.

27. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. Arterioscler Thromb Vasc Biol. 1999;19:472-84.

28. Simon P, Weiss FU, Zimmer KP, Koch HG, Lerch MM. Acute and chronic pancreatitis in patients with inborn errors of metabolism. Pancreatology 2001;1:448-56.

29. Blecker U, Mehta DI, duPont AI, Davis R, Sothern MS, Suskind RM. Fat-soluble vitamin deficiencies. Pediatrics in Review 1999;20:394-5.

30. Dujsikova H, Dite P, Tomandl J, Sevcikova A, Precechtelova M. Occurrence of metabolic osteopathy in patients with chronic pancreatitis. Pancreatology 2008;8:583-6.

31. Nakamura T, Takebe K, Imamura K, Arai Y, Kudoh K, Terada A, et al. Changes in plasma fatty acid profile in Japanese patients with chronic pancreatitis. J Int Med Res. 1995;23:27-36.

32. Vaona B, Armellini F, Bovo P, Rigo L, Zamboni M, Brunori MP, et al. Food intake of patients with chronic pancreatitis after onset of the disease. Am J Clin Nutr. 1997;65:851-4.

33. Martinez-Moneo E, Stigliano S, Hedstrom A, Kaczka A, Malvik M, Waldthaler A, et al. Deficiency of fat-soluble vitamins in chronic pancreatitis: A systematic review and meta-analysis. Pancreatology 2016;16:988-994.

34. Teichmann J, Mann ST, Stracke H, Lange U, Hardt PD, Klor HU, et al. Alterations of vitamin D3 metabolism in young women with various grades of chronic pancreatitis. Eur J Med Res. 2007;12:347-50.

35. Duggan SN, Smyth ND, Murphy A, Macnaughton D, O'Keefe SJ, Conlon KC. High prevalence of osteoporosis in patients with chronic pancreatitis: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2014;12:219-28.

36. Mann ST, Stracke H, Lange U, Klor HU, Teichmann J, Mann STW. Alterations of bone mineral density and bone metabolism in patients with various grades of chronic pancreatitis. Metab Clin Exp. 2003;52:579-85.

37. Mann ST, Stracke H, Lange U, Klor HU, Teichmann J. Vitamin D3 in patients with various grades of chronic pancreatitis, according to morphological and functional criteria of the pancreas. Dig Dis Sci. 2003;48:533-8.

38. Peterlik M, Cross HS. Vitamin D and calcium insufficiency-related chronic diseases: molecular and cellular pathophysiology. Eur J Clin Nutr. 2009;63:1377-86.

39. Layer P, Keller J, Lankisch PG. Pancreatic enzyme replacement therapy. Curr Gastroenterol Rep. 2001;3:101-8.

40. Layer P, Keller J. Lipase supplementation therapy: standards, alternatives, and perspectives. Pancreas 2003;26:1-7.

41. Dominguez-Munoz JE, Iglesias-Garcia J, Vilarino-Insua M, Iglesias-Rey M. 13C-mixed triglyceride breath test to assess oral enzyme substitution therapy in patients with chronic pancreatitis. Clin Gastroenterol Hepatol . 2007;5:484-8.

42. Dutta SK, Bustin MP, Russell RM, Costa BS. Deficiency of fat-soluble vitamins in treated patients with pancreatic insufficiency. Ann Intern Med 1982;97:549-52.

43. Trolli PA, Conwell DL, Zuccaro G, Jr. Pancreatic enzyme therapy and nutritional status of outpatients with chronic pancreatitis. Gastroenterol Nurs. 2001;24:84-7.

44. Segal I, Gut A, Schofield D, Shiel N, Braganza JM. Micronutrient antioxidant status in black South Africans with chronic pancreatitis: opportunity for prophylaxis. Clinica Chimica Acta 1995;239:71-9.

45. Sikkens EC, Cahen DL, Koch AD, Braat H, Poley JW, Kuipers EJ, et al. The prevalence of fat-soluble vitamin deficiencies and a decreased bone mass in patients with chronic pancreatitis. Pancreatology 2013;13:238-42.

46. Klapdor S, Richter E, Klapdor R. Vitamin D status and per-oral vitamin D supplementation in patients suffering from chronic pancreatitis and pancreatic cancer disease. Anticancer Research 2012;32:1991-8.

47. Pezzilli R, Melzi d'Eril GV, Barassi A. Markers of Bone Metabolism in Patients With Chronic Pancreatitis and Pancreatic Ductal Adenocarcinoma. Medicine (Baltimore) 2015;94:e1754.

48. Moran CE, Sosa EG, Martinez SM, Geldern P, Messina D, Russo A, et al. Bone mineral density in patients with pancreatic insufficiency and steatorrhea. Am J Gastroenterol 1997;92:867-71.

49. Prabhakaran A, Bhasin DK, Rana SS, Bhadada SK, Bhansali A, Rao C, et al. Bone mineral metabolism and bone mineral density in alcohol related and idiopathic chronic pancreatitis. Trop Gastroenterol 2014;35:107-12.

50. Nicolas JM, Estruch R, Antunez E, Sacanella E, Urbano-Marquez A. Nutritional status in chronically alcoholic men from the middle socioeconomic class and its relation to ethanol intake. Alcohol & Alcoholism 1993;28:551-8.

51. Yadav D, Slivka A, Sherman S, Hawes RH, Anderson MA, Burton FR, et al. Smoking is underrecognized as a risk factor for chronic pancreatitis. Pancreatology 2010;10:713-9.

52. Whitcomb DC, Yadav D, Adam S, Hawes RH, Brand RE, Anderson MA, et al. Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis Study 2 (NAPS2). Pancreatology 2008;8:520-31.

53. Hommel G. A stagewise rejective multiple test procedure based on a modified Bonferroni test. Biometrika 1988; 75 (2): 383-386. doi: 10.1093/biomet/75.2.383.

54. Baeten JM, Richardson BA, Bankson DD, Wener MH, Kreiss JK, Lavreys L, et al. Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response. Am J Clin Nutr. 2004;79:218-25.

55. Bauernfeind JC, Newmark H, Brin M. Vitamins A and E nutrition via intramuscular or oral route. Am J Clin Nutr. 1974;27:234-53.

56. O'Doherty MG, Jorgensen T, Borglykke A, Brenner H, Schottker B, Wilsgaard T, et al. Repeated measures of body mass index and C-reactive protein in relation to all-cause mortality and cardiovascular disease: results from the consortium on health and ageing network of cohorts in Europe and the United States (CHANCES). Eur J Epidemiol. 2014;29:887-97.

57. Rode L, Nordestgaard BG, Weischer M, Bojesen SE. Increased body mass index, elevated C-reactive protein, and short telomere length. J Clin Endocrinol Metab. 2014;99:E1671-5.

58. Lohr JM, Dominguez-Munoz E, Rosendahl J, Besselink M, Mayerle J, Lerch MM, et al. United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). United European Gastroenterol J 2017;5:153-199.

59. Jiang Q, Christen S, Shigenaga MK, Ames BN. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr 2001;74:714-22.

60. Duggan SN, Smyth ND, O'Sullivan M, Feehan S, Ridgway PF, Conlon KC. The prevalence of malnutrition and fat-soluble vitamin deficiencies in chronic pancreatitis. Nutr Clin Pract. 2014;29:348-54.

61. Marotta F, Labadarios D, Frazer L, Girdwood A, Marks IN. Fat-soluble vitamin concentration in chronic alcohol-induced pancreatitis. Relationship with steatorrhea. Dig Dis Sci 1994;39:993-8.

62. Mizokami A, Kawakubo-Yasukochi T, Hirata M. Osteocalcin and its endocrine functions. Biochem Pharmacol 2017.

63. Vanderschueren D, Gevers G, Raymaekers G, Devos P, Dequeker J. Sex- and age-related changes in bone and serum osteocalcin. Calcif Tissue Int 1990;46:179-82.

64. Chen L, Li Q, Yang Z, Ye Z, Huang Y, He M, et al. Osteocalcin, glucose metabolism, lipid profile and chronic low-grade inflammation in middle-aged and elderly Chinese. Diabetic Medicine 2013;30:309-17.

65. Confavreux CB, Szulc P, Casey R, Varennes A, Goudable J, Chapurlat RD. Lower serum osteocalcin is associated with more severe metabolic syndrome in elderly men from the MINOS cohort. Eur J Endocrinol. 2014;171:275-83.

66. Lumachi F, Orlando R, Fallo F, Basso SM. Relationship between bone formation markers bone alkaline phosphatase, osteocalcin and amino-terminal propeptide of type I collagen and bone mineral density in elderly men. Preliminary results. In Vivo 2012;26:1041-4.

67. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80:1689S-96S.

68. Cauley JA, Lui LY, Genant HK, Salamone L, Browner W, Fink HA, et al. Risk factors for severity and type of the hip fracture. J Bone Miner Res. 2009;24:943-55.

69. Radcliff TA, Henderson WG, Stoner TJ, Khuri SF, Dohm M, Hutt E, et al. Patient risk factors, operative care, and outcomes among older community-dwelling male veterans with hip fracture. J Bone Joint Surg Am. 2008;90:34-42.

70. Duggan SN, Purcell C, Kilbane M, O'Keane M, McKenna M, Gaffney P, et al. An association between abnormal bone turnover, systemic inflammation, and

osteoporosis in patients with chronic pancreatitis: a case-matched study. Am J Gastroenterol 2015;110:336-45.

71. Gueant JL, Djalali M, Aouadj R, Gaucher P, Monin B, Nicolas JP. In vitro and in vivo evidences that the malabsorption of cobalamin is related to its binding on haptocorrin (R binder) in chronic pancreatitis. Am J Clin Nutr. 1986;44:265-77.

72. Srikumar K, Premalatha R. Effect of gastrointestinal proteases on purified human intrinsic factor-vitamin B_{12} (IF-B_{12}) complex. Indian J Biochem Biophys. April 2003;40:139-142.

73. Gueant JL, Champigneulle B, Djalali M, Bigard MA, Gaucher P, Hassouni A, et al. In-vitro test of haptocorrin degradation for biological diagnosis of exocrine pancreatic dysfunction using duodenal juice collected during endoscopy. Lancet 1986;2:709-12.

74. Wilcox CM, Yadav D, Ye T, Gardner TB, Gelrud A, Sandhu BS, et al. Chronic pancreatitis pain pattern and severity are independent of abdominal imaging findings. Clin Gastroenterol Hepatol 2015;13:552-60; quiz e28-9.

75. Beyer G, Mahajan UM, Budde C, Bulla TJ, Kohlmann T, Kuhlmann L, et al. Development and Validation of a Chronic Pancreatitis Prognosis Score in 2 Independent Cohorts. Gastroenterology 2017;153:1544-1554.e2.

76. Whitcomb DC. Better Biomarkers for Pancreatic Diseases. Pancreas 2015;44:1171-3.

77. Rasch S, Valantiene I, Mickevicius A, Beer S, Rosendahl J, Charnley RM, et al. Chronic pancreatitis: Do serum biomarkers provide an association with an inflammageing phenotype? Pancreatology 2016;16:708-14.

78. Mroczko B, Groblewska M, Gryko M, Kedra B, Szmitkowski M. Diagnostic usefulness of serum interleukin 6 (IL-6) and C-reactive protein (CRP) in the differentiation between pancreatic cancer and chronic pancreatitis. J Clin Lab Anal. 2010;24:256-61.

79. Han JM, Levings MK. Immune regulation in obesity-associated adipose inflammation. J Immunol 2013;191:527-32.

80. Stepanova M, Rodriguez E, Birerdinc A, Baranova A. Age-independent rise of inflammatory scores may contribute to accelerated aging in multi-morbidity. Oncotarget 2015;6:1414-21.

81. Salvioli S, Monti D, Lanzarini C, Conte M, Pirazzini C, Bacalini MG, et al. Immune system, cell senescence, aging and longevity--inflamm-aging reappraised. Curr Pharm Des. 2013;19:1675-9.

82. McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. Cancer Treat Rev 2013;39:534-40.

83. Hickson M. Malnutrition and ageing. Postgrad Med J. 2006;82:2-8.

Table 1: Baseline characteristics of controls and chronic pancreatitis patients

Characteristic	Controls (266)	Chronic pancreatitis Patients (301)	P value
Age at enrollment (Mean ±SD)	45.6 ± 13.4	50.76 ± 14.11	<0.001
Male – n (%)	107 (40.2)	151 (50.1)	<0.05
Race – n (%)			<0.01
Caucasian	155 (58.3)	214 (71.1)	
African-American	111 (41.7)	87 (28.9)	
Body mass index (BMI) – n (%)			<0.001
Underweight (<18 kg/m^2)	3 (1.1)	25 (8.3)	
Normal (18-25 kg/m^2)	64 (24.1)	141 (46.8)	
Overweight (≥ 25-30 kg/m^2)	97 (36.5)	78 (25.9)	
Obese (> 30 kg/m^2)	102 (38.3)	56 (18.6)	
Drinking category*			<0.001
Lifetime abstainer	34 (26.4)	42 (14.5)	
Light drinker	32 (24.8)	58 (20.0)	
Moderate drinker	24 (18.6)	36 (12.4)	
Heavy/very heavy drinker	39 (30.2)	154 (53.1)	
Missing data	137	11	
Alcohol consumption – n (%)			<0.001
Never	34 (12.8)	42 (14.0)	
Past	56 (21.1)	197 (65.5)	
Current	176 (66.2)	62 (20.6)	
Cigarette smoking – n (%)			<0.001
Never	130 (48.9)	67 (22.3)	
Past	74 (27.8)	78 (25.9)	
Current	62 (23.3)	156 (51.8)	
Vitamin Supplementation – n (%)	120 (45.1)	168 (55.8)	<0.05

SD= standard deviation

Missing data: BMI (1 CP), drinking category (CP 12, controls 152), vitamin supplementation (CP 1, controls 1);

Percentages shown are based on effective numbers.

*Based on self-reported drinking during the maximum lifetime drinking period

Table 2: Etiology, diabetic status, duration of disease and pancreatic enzyme replacement therapy (PERT) usage and duration of disease in chronic pancreatitis patients

Characteristic **N (%)**	**Chronic Pancreatitis patients (301)**
Physician-defined alcohol etiology	
No	151 (50.2)
Yes	150 (49.8)
Diabetes mellitus diagnosis	
No	185 (61.5)
Yes	116 (38.5)
PERT usage	
No	135 (44.9)
Yes	166 (55.1)
Time since diagnosis	
< 5 years	187 (62.1) (73.3)
5-10 years	40 (13.3) (15.7)
> 10 years	28 (9.3) (11.0)
Missing data	46 (15.3) (0.0)

Table 3: Serum vitamin, nutritional marker and biomarker levels (top) and deficiencies (bottom)

in controls and chronic pancreatitis patients

Serum vitamin, nutritional marker and biomarker levels			
Variable (reference range)	**Controls (266)**	**Chronic pancreatitis Patients (301)**	**p-value***
Vitamin A (30-105 μg/dL)	45.4 [37.1, 54.3]	40.9 [30.3, 51.9]	0.001
Vitamin B12 (cobalamin) (210-911 pg/mL)	609.5 [466.5, 794.5]	703.0 [565.0, 923.0]	< 0.001
Vitamin D (25-hydroxy vitamin D3) (10-55 ng/mL)	22.2 [12.3, 32.8]	20.7 [11.5, 32.8]	0.92
Vitamin E (α-tocopherol) (5.7-19.9 mg/L)	10.3 [8.4, 12.8]	8.7 [6.5, 12.2]	< 0.001
Vitamin E (γ -tocopherol) (≤4.3 mg/L)	2.2 [1.6, 2.9]	1.8 [1.1, 2.8]	< 0.001
Osteocalcin (9-42 ng/mL)	10.0 [7.3, 14.5]	7.9 [5.0, 12.4]	< 0.001
Prealbumin (transthyretin) (15-36 mg/dL)	27.0 [23.0, 30.0]	23.0 [19.0, 27.0]	< 0.001
Retinol Binding Protein (15-67 μg/mL)	37.0 [30.2, 44.1]	34.5 [25.6, 44.0]	0.055
C-reactive protein (CRP) (<0.7 mg/dL)	0.2 [0.1, 0.5]	0.3 [0.1, 1.0]	< 0.001
TNF-α (pg/mL)**	3.0 [2.0, 3.0]	3.0 [2.0, 4.0]	0.92
Serum vitamin and nutritional marker deficiencies†			

Variable	**Controls (266)**	**Chronic pancreatitis Patients (301)**

Vitamin A (<30 µg/dL) N(%)	20 (7.5)	75 (24.9)
Vitamin B12 (cobalamin) (<210 pg/mL)	2 (0.8)	1 (0.3)
Vitamin D (25-hydroxy vitamin D3) (<10 ng/mL)	49 (18.4)	63 (20.9)
Vitamin E (α-tocopherol) (<5.7 mg/L)	8 (3.0)	54 (17.9)
Vitamin E (γ -tocopherol) (>4.3 mg/L)	20 (7.5)	14 (4.7)
Osteocalcin (<9 ng/mL)	114 (42.9)	176 (58.5)
Prealbumin (transthyretin) (<15 mg/dL)	6 (2.3)	46 (15.3)
Retinol Binding Protein (<15 µg/mL)	4 (1.5)	14 (4.7)

*Adjusted for multiple comparisons using Hommel's procedure

** TNF-α does not have a standard reference range

† Unadjusted levels; Deficiency thresholds based on Quest Diagnostics Inc. laboratory, Madison, New Jersey

Table 4: **Multivariable linear regression analyses for predictors of serum levels of vitamins, nutritional markers and biomarkers**

	Vitamin A	**Vitamin B12**	**Vitamin D3**	**Vitamin E α Tocopherol**	**Vitamin E γ Tocopherol**	**Osteocalcin**	**Pre-albumin**	**Retinol Binding Protein**	**C reactive protein**	**TNF α**
(Intercept)	3.666 ‡	6.275 ‡	3.112 ‡	2.349 ‡	1.357 ‡	2.321 ‡	3.201 ‡	3.483 ‡	-2.356 ‡	1.648 ‡
Diagnosis (*CP*)	-0.105 *	0.237 ‡	-0.104 ^	-0.189 ‡	-0.083 *	-0.319 ‡	-0.100 *	-0.097 *	0.681 ‡	-0.034
Race (*African-American*)	-0.050	0.175 ‡	-0.508 ‡	-0.131 ‡		-0.124	0.074 ^	-0.102 †	0.133	-0.080
Sex (*male*)						0.031	0.043			0.084
Age (*centered*)	0.003 *			0.006 ‡		0.006 *		0.004 †	0.008 ^	
Vitamin Supplementation (*yes*)	0.127 ‡	0.148 ‡	0.233 ‡	0.133 ‡	-0.152 ‡			0.085 *		0.112
Current BMI										
Underweight				-0.171 *	0.089		-0.064		-0.522 ^	

Overweight				0.032	0.182 ‡		-0.001		0.466 †	
Obese				0.025	0.294 ‡		-0.104 †		1.035 ‡	
Alcohol consumption										
Former	0.093						0.031	0.117 *		
Current	0.138 *						0.101 *	0.151 †		
Smoking status										
Former			0.094	0.041	0.102 *		-0.061			
Current			-0.169 *	-0.090 *	0.139 †		-0.064 ^			
Diagnosis: Race Interaction	-0.180 *	-0.201 †				0.266 *	-0.129 *		0.528 *	0.319 *
Sex: Age Interaction						-0.019 ‡				

^ *p<0.10 * p<0.05 † p<0.01 ‡ p<0.001*

All serum levels underwent log transformation prior to modeling except for Vitamin E γ Tocopherol *and TNF* α, *which were transformed using a square root function.*