THE ASSOCIATION BETWEEN SERUM VITAMIN D CONCENTRATION AND NEUROMUSCULAR FUNCTION IN PATIENTS WITH CROHN'S DISEASE

by

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Serum vitamin D concentrations are not typically assessed in patients with Crohn's disease, even though neuromuscular complaints are one of the most common complaints observed. Crohn's patients, especially with small bowel resections, are at risk for hypovitaminosis D and fatigue. **Purpose:** To determine if Crohn's disease patients have low serum 25(OH)D concentrations and to determine the association between 25(OH)D and measures of neuromuscular function, such as muscle strength, muscle fatigue, nerve function, and quality of health. **Methods:** Nineteen Crohn's patients (9 male and 10 female), with at least one small bowel resection were tested. Isometric muscle strength at 45° of knee extension and flexion, EMG fatigue rates of the rectus femoris (RF) and vastus lateralis (VL), and the total physical score (PCS) and total mental score (MCS) from the SF-36 were collected. **Statistics:** Pearson correlations were calculated to determine the association between vitamin D and the measures of neuromuscular function. **Results:** The mean 25(OH)D was 32ng/ml. The Crohn's patients tested were 43.16 $\pm$ 10.26 years with an average of 1.79 resections and 17.79 years with the disease. The mean peak torque (Nm) for knee extension was 75.24 $\pm$ 45.39, and 28.94 $\pm$ 12.76 for flexion. The mean average peak torque (Nm) for knee extension was 55.91 $\pm$ 35.55, and 20.96 $\pm$ 9.80 for flexion. The mean fatigue rates (Hz/sec) were -0.07 $\pm$ 0.05 for RF and -0.03 $\pm$ 0.04 for VL. Peroneal nerve latency mean was 4.28 $\pm$ 1.75 ms and the mean amplitude was 2.26 $\pm$ 2.03 mV. No significant differences at the $\alpha = 0.05$ level for muscle strength, fatigue rates, nerve function, and the MCS

were found. A significant ($p = 0.02$) positive correlation existed for the PCS ($r = 0.55$) of the SF-36. **Conclusions:** The average vitamin D concentrations were sufficient and vitamin D was not correlated to muscle strength, fatigue, or nerve function. The serum vitamin D concentrations were found to explain 30% of the variability of the PCS of their quality of health. Further studies are required to identify the exact mechanisms of the decreased strength and fatigue experience by Crohn's patients.

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1.0 INTRODUCTION

Within the past decade, several studies have revealed an increased incidence neuromuscular fatigue and low bone mineral density in patients with inflammatory bowel disease (IBD), with a higher prevalence in Crohn's disease (CD) patients (Stiffledeen et al., 2004; Harpavat, Klio, & Regueiro, 2004; Lamb, et al., 2002; Jahnsen, Samson, & Verhaar, 2002). The mechanisms underlying the neuromuscular fatigue are not clearly defined (Stiffledeen et al., 2004, Jahnsen et al., 2002). Although muscle pain and bone pain is a chief complaint of Crohn's disease patients (Jahnsen et al., 2002), the impairment in neuromuscular function is unknown. A number of factors are associated with the possible decreased function in Crohn's disease, such as disease activity, corticosteroid therapy, calcium and vitamin D deficiency, sex hormone deficiency, cytokines, and overall poor nutrition (Stiffledeen et al., 2004; Jahnsen et al., 2002).

Nutrient malabsorption and vitamin D deficiency related to small intestinal involvement and small bowel resections are commonly found in patients with Crohn's disease (Jahnsen et al., 2002). Vitamin D deficiency is associated with decreased serum calcium concentration, which also results in the resorption of bone. The reduction in vitamin D levels also causes a compensatory increase in the level of parathyroid hormone (PTH) (Jahnsen et al., 2002), which stimulates bone resorption and results in bone loss or osteoporosis.

Patients with Crohn's disease, especially those with small bowel resections, are at risk of developing hypovitaminosis D, secondary hyperparathyroidism, osteoporosis, osteomalacia, and

fatigue. Understanding the relationship between serum vitamin D levels and muscular strength, excessive fatigue, nerve conduction velocity, and quality of life in Crohn's patients will lead to proper treatment to increase vitamin D levels and neuromuscular function. There is currently not an objective basis for recommended dietary intake levels of vitamin D for adults, or for optimal levels of serum vitamin D (Vieth, 1999; Veith & Fraser, 2002), especially for those with Crohn's disease. Vitamin D supplementation may facilitate an increase in neuromuscular function and improve the quality of life in patients with Crohn's disease.

1.1 STATEMENT OF THE PROBLEM

Serum vitamin D concentrations are not typically assessed as a part of the treatment protocol in patients with Crohn's disease, even though neuromuscular complaints are one of the most common complaints observed in this patient population. The common levels of serum vitamin D concentration in Crohn's disease patients are unknown, although evidence of low bone mineral density in people with vitamin D deficiency has been well established. Therefore, inflammatory bowel disease patients may be at an increased risk of vitamin D deficiency, especially Crohn's disease patients with small bowel resections, affecting muscular strength, muscular fatigue, nrve function, and quality of life.

1.2 OBJECTIVE OF THE STUDY

The purpose of the study was to determine if patients with Crohn's disease have low levels of serum vitamin D concentrations and to examine the relationship between serum vitamin D concentrations and measures of neuromuscular function, specifically the muscular strength, muscular fatigue, nerve function, and quality of health in patients with Crohn's disease. Specifically, it is proposed to examine muscular strength, muscular endurance, nerve conduction velocity, and quality of life. Based on studies of elderly adults, it is hypothesized that there will be a logarithmic relationship between serum vitamin D and these outcome measures of neuromuscular function (Bischoff-Ferrari et al., 2004). Therefore, it is proposed that increased levels of serum vitamin D will be related to better muscular strength, muscular endurance, nerve conduction velocity, and quality of health.

1.3 SPECIFIC AIMS AND HYPOTHESIS

1.3.1 Specific Aims

The primary specific aim of the study is to determine if a relationship exists between serum vitamin D concentration and the following measures in a group of patients with Crohn's disease:

a. muscular strength
b. muscular fatigue
c. nerve function
d. quality of health

1.3.2 Research Hypothesis

1. It is hypothesized that there will be a positive relationship between serum vitamin D concentration and muscular strength in patients with Crohn's disease.
2. It is hypothesized that there will be a positive relationship between serum vitamin D concentration and muscular fatigue in patients with Crohn's disease.
3. It is hypothesized that there will be a positive relationship between serum vitamin D concentration and nerve function in patients with Crohn's disease.
4. It is hypothesized that there will be a positive relationship between serum vitamin D concentration and quality of health assessment scores with Crohn's disease.

1.4 DELIMITATIONS OF THE STUDY

The research study will be delimited by the following factors:

1. Only patients with at least one small bowel resection were selected for the study.
2. Only Crohn's patients with a diagnosed disease duration of 5 or more years were included in the study.
3. Only Crohn's patients taking less than 20 mg of prednisone were included in the study.
4. Only Crohn's patients with no known diagnosed neuropathy from flagyl (metrinadazol) were included in the study.
5. Only Crohn's patients with no known neuromuscular disorders, such as multiple sclerosis, were included in the study.

6. Only Crohn's patients participating in the UPMC Center for Inflammatory Bowel Disease (IBD) Research Registry were included in the study.
7. Only Crohn's patients who reported an idiopathic musculoskeletal pain or weakness score of at least three on a scale from one to ten were included in the study.
8. Only individuals not diagnosis with severe Psoriasis and Sprues were included in the study.
9. Only individuals who were not currently taking vitamin D supplements were included in the study.
10. Only individuals who were not exposed to indoor UVB tanning beds were included in this study.
11. Only subjects who were not taking anticonvulsant medications and asthma medications were included in the study.
12. Only individuals who do not have a pacemaker or an intravenous port were included in the study.
13. Only individuals who do not have diabetes were included in the study.
14. Only subjects who do not have knee pain or anterior cruciate ligament pathologies were included in the study.
15. Only individuals who do not have left side stroke were included in the study.

1.5 LIMITATIONS OF THE STUDY

The research study was limited by the following factors:

1. All subjects for this research were volunteers; no attempt was made to control the sample

for self-selection.

2. No attempt was made to control for genetics as a determinant of Crohn's disease or vitamin D deficiency.
3. No attempt was made to control for dietary intake as a factor of Crohn's disease or vitamin D deficiency.
4. No attempt was made to control for calcium intake.
5. The researcher assumed that all subjects answered the questionnaires honestly.
6. No attempt was made for lean mass as a determinant of Crohn's disease or vitamin D deficiency.
7. The study was limited by the reliability and validity coefficients of the measurement instruments.
8. All subjects entering the study were assumed to comply to the best of their ability with the methods of testing.
9. No attempt was made to control for cotricosteriods or glucocorticosteroid use.
10. No attempt was made to control for exposure to natural sunlight.

1.6 DEFINITIONS OF TERMS

Amplitude: The maximum voltage difference between two points, usually baseline to peak.

Crohn's disease: A genetically determined prolonged and severe mucosal inflammatory response of the gastrointestinal tract.

Distal: Far from the point of attachment or origin.

Electromyography (EMG): The sum of the median frequency, both positive and negative, in a

muscle contraction.

Inflammatory Bowel Disease: Immunoinflammatory response of the bowels

Muscular endurance: The capacity of a muscle to produce force over a series of consecutive contractions

Muscular strength: The ability of a muscle to exert force

Nerve conduction velocity: The assessment of the speed of propagation of an action potential along a nerve or a muscle fiber. The maximum nerve conduction velocity is calculated from the latency of the evoked potential at maximal from the intensity.

Osteoblast: A bone-forming cell.

Osteoclast: A large cell that absorbs or breaks down bone matrix.

Osteoporosis: A disease characterized by low bone mineral density and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and a consequent in fracture risk.

Proximal: Next to the point of attachment

2.0 LITERATURE REVIEW

2.1 CROHN'S DISEASE

Crohn's disease is a subcategory of disorders collectively known as Inflammatory Bowel Disease (IBD). Crohn's disease is a chronic, relapsing, transmural inflammation of any portion of the digestive tract (Head & Jurenka, 2004). The ulceration and inflammation of the intestinal wall is primarily found in the terminal ilieum and/or colon, and can be interspersed with healthy tissue (Berstein, Bector, & Leslie, 2004; Kocha, 2003)). A peak incident age has been revealed in the second through third decades of life (Bernstein & Blanchard, 2003).

Men and women are equally affected by Crohn's disease. Caucasians demonstrate the highest rate, with a prevalence approximating 50 per 100,000 population (Cohen, 2003; Kocha, 2003). However, the incidence of Crohn's occurring in women is increasing due to oral contraceptive use (Cohen, 2003; Kocha, 2003). The use of oral contraceptives for six years is equivocal to the effects of smoking, which is one of the highest risk factors, after genetics and environmental factors (Cohen, 2003).

Symptoms of Crohn's include: frequent diarrhea, abdominal pain in the right lower quadrant appearing soon after meals, anemia, fatigue, loss of appetite, weight loss, fever, stomatitis, perianal fistula or fissures, and rectal bleeding (Head et al., 2004; Kocha, 2003).

Another component of Crohn's disease is the perianal disease (Kocha, 2003), which is manifested as skin tags, skin lesions, anal canal lesions, and fistulae (Kocha, 2003).

Due to the fact that there is no cure for Crohn's disease, conventional treatment has been aimed at suppression of the inflammation and relief of symptoms of fever, diarrhea, and abdominal pain. Once the acute symptoms of the disease are stabilized through medication, drug therapy to manage the disease is administered with aminosalicylates, corticosteroids, immune-modulating agents, antibiotics, or a combination to decrease the frequency of disease flares, fistulas, and maintain remission (Head et al., 2004; Kocha, 2003). Medical management through medication is based on disease location and severity (Kocha, 2003).

Crohn's disease patients often demonstrate nutrient deficiency due to malabsorption in the small intestines, increased nutrient needs due to the disease activity, low nutrient intake, nutrient loss due to diarrhea, or nauseating effect of the medication (Head et al., 2004). Manifestations of malnutrition, including caloric and nutrient deficiencies, anemia, and electrolyte imbalance are frequently seen. The most common sequel to Crohn's disease is osteoporosis resulting from nutrient deficiencies and corticosteroid therapy. The disease itself, and some of the commonly used therapies, can result in low bone mineral density, increased fracture risk, and thus osteoporosis.

2.1.1 Crohn's Disease and Vitamin D

The pathogenesis of inflammatory bowel disease involves a complex interaction between genetic, environmental, and immunological factors. Although the etiology of Crohn's is still unknown, vitamin D may be an important environmental factor (Zhu, Mahon, Froicu, & Cantorna, 2005; Cantorna, Munsick, Bemiss, & Mahon, 2000). People that live in areas that

receive less sunlight have lower circulating vitamin D and have been found to have a higher prevalence of inflammatory bowel disease (Zhu et al., 2005). In addition, Crohn's patients have an increased incidence of vitamin D deficiency (Zhu et al., 2005; Harpavat, 2004). However, it is not known whether a low vitamin D status is a contributing factor or a consequence of the disease.

Patients with Crohn's disease are at additional risk for developing vitamin D deficiency for many reasons. Crohn's patients often experience gastrointestinal discomfort from lactose intolerance, which may lead to avoidance of dietary foods that are commonly supplemented with vitamin D. In addition, the disease may be associated with physical inactivity, which leads to reduced exposure to sunlight, thereby further comprising serum vitamin D concentrations (Head, 2004; Stiffledeen et al., 2004; Tajika et al., 2004; Janssen, Samson, & Verhaar, 2002). Crohn's disease patients often demonstrate nutrient deficiency due to malabsorption in the small intestines, increased nutrient need, low nutrient intake, nutrient loss due to diarrhea, or effect of the medication (Head et al., 2004).

Duration of the disease itself is thought to predict the occurrence of vitamin D deficiency. The disease duration is thought to be associated with multiple factors, including dietary health habits, physical activity levels, corticosteroid exposure, and surgical history (Tajika et al., 2004). Sufficient dietary intake of vitamin D is problematic because very few foods are naturally rich in vitamin D (Holick, 2003; Cantorna et al., 2000). Weight has been found to decrease in 65-75% of patients diagnosed with Crohn's disease (Cantorna et al., 2000). Without complaints of bone pain, muscle weakness, and neuromuscular weakness, it is difficult to detect vitamin D deficiency in these patients. Therefore, history of disease and surgeries, along with the length of

the active stage of the disease, may indicate the need to begin assessing serum vitamin D levels in patients with Crohn's disease.

Vitamin D is an important environmental factor in autoimmune diseases. Specifically, $1,25(OH)_2D_3$, the active form of vitamin D, is known to suppress the development of autoimmune diseases (Zhu et al., 2005, Cantorna & Mahon, 2004; Cantorna et al., 2000). Zhu et al. (2005) reported vitamin D deficient mice on low-calicium diets developed the most severe cases of inflammatory bowel disease. Cantorna et al. (2000) also reported that vitamin D deficiency exacerbates symptoms of inflammation of the colon in mice, and treatment with $1,25(OH)_2D_3$ for as little as two weeks ameliorated the inflammatory bowel symptoms in the mice. Therefore, improving vitamin D status in patients with autoimmune disorders should be a concern (Zhu et al., 2005; Cantorna & Mahon, 2004; Cantorna et al., 2000).

2.1.2 Crohn's Disease and Small Bowel Resection

Crohn's disease patients with small intestinal involvement or small bowel resections are at a greater risk for malabsortption and vitamin D deficiency (Janssen et al., 2002; Tajika et al., 2004). Deficiency in vitamin D, defined as a low serum vitamin D concentration, is common in patients with Crohn's disease, occurring in 30% of all patients and 62% of patients who undergo small bowel resections (Valentine & Sninsky, 1999). Janssen et al. (2002) have also reported Crohn's disease patients with small bowel resections have decreased serum vitamin D and 25-hydroxyvitamin D levels. The resection of the terminal ileum, which plays a role in the enterohepatic circulation, is a risk factor for vitamin D deficiency (Tajika et al., 2004). The reduction in vitamin D levels is due to impaired calcium absorption and a resulting increase in

parathyroid hormone levels. The increase in parathyroid hormone causes an increase in bone resorption, resulting in greater bone loss.

Janssen et al. (2002) and Tajika et al. (2004) reported the contributions responsible for vitamin D deficiency in Crohn's disease patients are malabsorption of vitamin D and 25-hydroxyvitamin D, lack of sun exposure, low dietary intake, and glucocorticoid medications. The resection of the small intestine leads to depletion of bile acids, which are essential for the absorption of vitamin D (Janssen et al., 2002). The magnitude of vitamin D malabsorption reflects the extent of the distal small bowel resection.

In a study by Janssen et al. (2002), secondary hyperparathyroidism was present in many Crohn's disease patients, all of whom were small bowel resection surgical patients. Vitamin D deficiency, renal dysfunction, and decreased calcium absorption are all related to secondary hyperparathyroidism. Therefore, because vitamin D deficiency is avoidable, heightened awareness and adequate intake is recommended to decrease the chance of secondary hyperparathyroidism and osteoporosis.

2.1.3 Crohn's Disease and Osteoporosis

Osteoporosis is a common extraintestinal manifestation of inflammatory bowel disease (Valentine & Sninsky, 1999; Lichtenstein, 2003: Janssen et al., 2002). The mechanisms contributing to the loss of bone mass in patients with Crohn's disease are undefined (Janssen et al., 2002). Specific factors may include the use of corticoisteroids or gluccorticosteroids, calcium deficiency, vitamin D deficiency, the disease state itself, smoking, and malnutrition (Lichtenstein et al., 2003; Janssen et al., 2002).

Patients with IBD may have up to a 40% greater risk of fractures than the general population (Litchenstein, 2003; Harpavart et al., 2004). Harpavat et al. (2004) reported that elderly patients with inflammatory bowel disease are at the highest risk for fracture. Lichtenstein (2003) reported that 40-50% of patients with IBD have osteopenia, and 30% of individuals have osteoporosis. Osteopenia, a precursor to osteoporosis, and osteoporosis are seen more frequently in patients with Crohn's disease than in healthy elderly (Lichtenstein, 2003; Bernstein & Leslie, 2004; Harpavat et al., 2004).

Treatment with glucocorticosteroids to reduce inflammation is common in practice for IBD. This treatment with glucocorticoids is known to contribute to the low bone density in patients with inflammatory bowel disease (Harpavat et al., 2004) by decreasing bone formation, gonadotrophin-releasing hormone, and estrogen, and increasing bone resorption. Corticosteriods affect both resorption and formation of bone, resulting in an increased loss of bone and fractures at sites with high content of trabecular bone (Valentine & Sninsky, 1999). Harpavat et al. (2004) reported that glucocorticoids suppress bone formation through a direct inhibitory effect on osteoblasts, inhibition of growth factors, and increased osteoblast apopotosis, and by increasing bone resorption and increasing the release of parathyroid hormone.

Over fifty percent of people treated with long-term glucocorticoids develop osteoporosis, irrespective of disease (Harpavat et al., 2004). In men, glucorticoids have been shown to decrease serum testosterone concentrations, leading to a similar effect on bone. Although age-related osteoporosis is predominantly a female problem, the incidence is equal between genders among patients with IBD (Valentine & Sninsky, 1999; Bernstein & Leslie, 2004).

For both men and women, glucocortoisteroids inhibit osteoblast maturation ability (Lictenstein, 2003; Berstein & Leslie, 2004). Glucocorticoids also inhibit intestinal calcium absorption and

promote calcium loss in urine. These effects can lead to secondary hyperparathyroidism and increased bone remodeling (Lictenstein, 2003; Berstein & Leslie, 2004). The observed loss of bone due to corticosteroid therapy is most rapid in the first few weeks of treatment, continues through the therapy, and remains for two to three months after the corticosteroid therapy has ceased (Pfeifer et al., 2002; Lictenstein, 2003; Berstein & Leslie, 2004). The resulting decrease in bone mineral density caused by the inflammatory bowel disease itself or the medications used to treat the disease has been found to increase nonvertebral fractures, morbidity, and even mortality. Therefore, the abnormal bone density and treatment of the condition in patients with Crohn's disease is an important concern.

2.2 VITAMIN D

Vitamin D is one of the oldest known hormones in the body, and is critically important for the development, growth, and maintenance of a healthy skeleton (Holick, 2003). In bone, vitamin D stimulates bone turnover while protecting osteoblasts from dying by apoptosis, or cell death, and maintains the function of type II muscle fibers, preserving muscle strength and preventing falls (Holick, 2003). Vitamin D acts as a regulator of bone mineral homeostasis by promoting the transport of calcium and phosphate to ensure that blood levels of these ions are sufficient for the collagen matrix in the skeleton (Holick, 2003).

Vitamin D maintains calcium homeostasis by increasing the efficiency of the intestine in calcium absorption. Vitamin D deficiency may cause a mineralization defect in the adult skeleton, resulting in osteomalacia (Holick, 2003). Osteomalacia is associated with muscle and bone pain (Grant & Holick, 2005). The binding of vitamin D to its receptor inside the nuclear

membrane will cause the expression of osteoblasts and the inhibition of osteoblast apoptosis. Vitamin D also opens the calcium and chloride channels, resulting in an increase in calcium in the blood. The increase in calcium in the blood will in turn cause an increase in the levels of stored calcium in the bones, and enhance the mobility and changes required for osteoblast function.

A severe deficit in vitamin D affects muscular function and strength, and is referred to as osteomalacic myopathy, or osteomalacia. The recommended daily dosage of vitamin D in children, 1,000 IU, was developed in response to the rickets epidemic in children who resided in European and American cities during the late 19th and early 20th centuries (Zittermen, 2003). Surprisingly, the current recommended guidelines for the minimum daily dosage of Vitamin D for adults is at the 400-600 IU level, which, although it prevents osteomalacia in adults, is not enough to maintain bone density in post-menopausal women (Zitterman, 2003).

Vitamin D deficiency in children causes rickets and prevents children from reaching their genetically programmed height and peak bone mass. For adults, the effects of vitamin D deficiency are more subtle. Many of the effects of vitamin D deficiency are on the skeleton. Secondary hyperparathyroidism mobilizes calcium from the skeleton and can reduce bone mineral density and ultimately cause osteoporosis (Holick, 2004). In addition to the skeletal effects, muscles have receptors for 1,25(OH)2D, and vitamin D deficiency may cause muscle weakness

Recent evidence indicates that vitamin D plays a role in neuromuscular function, although the exact mechanisms in which vitamin D affects the muscular and nervous systems are unknown (Mingrone, Grerco, Castagneto, & Gasbarrini, 1999; Schott & Wills, 1976; Janssen, 2002; Bischoff-Ferrari et al., 2004; Pfeifer et al., 2002; Pferifer, Begerow, & Minnie, 2002). In

addition, the levels of serum vitamin D required to prevent osteomalacia in adults are much less than the levels required for optimizing neuromuscular function or muscling weakness. There is currently not an objective basis for recommended dietary intake levels of vitamin D for adults, or for optimal levels of serum vitamin D (Zitterman, 2003; Heaney Dowell, Hale, & Bendich, 2003; Holick, 1994), especially for those with Crohn's disease.

2.2.1 Sources of Vitamin D

Humans obtain vitamin D primarily from two sources: sunlight, or more specifically UV B light (wavelength 290-315 nm), and oral ingestion (Zitterman, 2003). More than eighty percent of a person's vitamin D needs are obtained via sunlight exposure (Pfeifer et al., 2002; Heaney et al., 2003). According to Holick (1994), there is evidence of a plateau in vitamin D after thirty minutes of UV B irradiation during the summer months. Little vitamin D is produced in the skin during winter months at latitudes above 37°N through the months of November through February (Holick, 1994, 2004). Pittsburgh is at a latitude of approximately 40°, Boston at 42.5°, while Memphis, TN, is almost exactly 35° (Heaney, 2005). Therefore, a large percentage of Americans produce little or no vitamin D between the months of October or November to February or March.

Vitamin D is often taken for granted and is assumed to be plentiful in a healthy diet. Unfortunately, few foods are fortified with vitamin D and few contain vitamin D naturally. A 70 year old individual exposed to the same amount of sunlight as a 20 year old will only make roughly 25% of the vitamin D than that of the 20 year old (Holick, 2004). Vitamin D is fat soluble, meaning that it can be stored in the body fat. Therefore, any excess vitamin D that is

produced during exposure to sunlight can be stored during the winter months when little to no vitamin D_3 is produced in the skin (Holick, 2004).

Very few foods actually contain vitamin D_3. Oily fish such as salmon with the bones, mackerel, sardines, and herring are good sources of vitamin D_3. Cod liver oil is an excellent source of vitamin D_3 and is critically important for bone health (Holick, 2004). More than 90% of the vitamin D requirement comes from casual exposure to the sunlight. Holick (2005) reported that exposure to 20% of the body's surface to direct sunlight or that of a tanning bed radiation was effective in increasing the vitamin D_3 or 25-hydroxyvitamin D_3 ($25(OH)_2D_3$). Therefore, serum concentrations of vitamin D levels give a better indication of vitamin D levels than a dietary recall of vitamin D fortified foods.

2.2.2 Types of Vitamin D

The hormone vitamin D is produced in the skin after exposure to ultraviolet radiation and undergoes two hydroxilations in the liver and the kidney to become biologically active (Montero-Odasso et al., 2005). The two forms of vitamin D found are: ergocalciferol, also known as vitamin D_2, and cholecalciferol, or vitamin D_3 (Rapuri, Gallagher, & Haynatzi, 2004). The active form of vitamin D_3 is $1,25(OH)_2D_3$, and has a significantly higher affinity to the vitamin D receptor (VDR) than its inactive form, $25(OH)_2D_3$ (Montero-Odasso, 2005; Holick, 2003). Ergocalciferol, or $25(OH)_2D_2$, derived primarily from plant sources, is obtained via dietary intake (Vogeser, Hyriatsoulis, Huber, & Kobold, 2004).

Ergocalciferol is the primary form of vitamin D sold in multivitamins and foods fortified with vitamin D (Holick, 1994; Rapuri et al., 2004). Cholecalciferol is then made endogenously

from 7-dehydrocholesterol in the skin via UV radiation (Pfeifer et al., 2002). The cholecalciferol then is hydroxylated in the liver to 25-hydroxyvitamin D_3 ($25(OH)_2D_3$) (Pfeifer et al., 2002).

In the kidneys, $25(OH)_2D_3$ is then further broken by the enzyme 1, alpha- hydroxylase, down to the active metabolite 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) (Holick, 2003; De Manicor, 2004). 1,25-dihydroxyvitamin D3 was once believed to only be involved only in calcium and phosphate metabolism (Holick, 2003). However, we now know that 1,25-dihydroxyvitamin D_3 is also involved in muscle function, immunomodulation or the prevention of autoimmune diseases, and the regulation of cell growth or cancer prevention (Montero-Odasso et al., 2005; Holick, 2003), and not just calcium and phosphate metabolism.

2.2.3 Appropriate Levels of Vitamin D

Although the vitamin D separation techniques are novel and are only performed in a few well-equipped laboratories, most researchers only report data for vitamin D (25(OH)D), rather than its component parts of $25(OH)_2D_2$ (ergocaclciferol) and $25(OH)_2D_3$ (cholecalciferol). In adults, it has been suggested that a healthy level of vitamin D in the blood serum is a concentration greater than 50 nmol/L (Holick, 2004), which is a representation of a combined vitamin D_2 and D_3 concentration, but the literature is inconclusive and the optimal serum concentration of $1,25(OH)_2D_3$ required for muscle and bone health is unknown.

Individuals with a serum $25(OH)_2D$ concentration between 30 and 50 nmol/L (Holick, 2004) or 50 and 80 nmol/L or 20-30 ng/mL (Grant & Holick, 2005) are considered to have vitamin D insufficiency. Individuals with a vitamin D level of less than 30 nmol/L (Holick, 2004), now at levels of 50 nmol/L (Grant & Holick, 2005) have vitamin D deficiency, or

hypovitaminosis D. These values are based upon the amount required to maintain calcium homeostatis according to Glenndenning et al. (2003).

Evidence from Bischoff-Ferrari et al. (2004) indicates that a serum 25(OH)D concentration less than 40 nmol/L is related to marked decreases in neuromuscular function in elderly individuals, as seen in an 8-meter timed walk and a sit-to-stand test. Heaney (2005) reports concentration levels of vitamin D below 80 nmol/L to be unhealthy for maintaining bone health. Calcium and parathyroid hormone plateau around 30 ng/mL or 75-80 nmol/L (Grant & Holick, 2005). Although the optimal range is still the subject of debate around 30-50 ng/mL or 75-125 nmol/L and higher (Grant & Holick, 2005), and a consensus of scientific understanding is presented in Table 1.

Table 1 Health Implications of Various Levels of Serum 25(OH)D (Grant & Holick, 2005)

25(OH)D Level (ng/mL)	25(OH)D Level (nmol/mL)	Health Implications
<20	<50	Deficiency
20-33	50-80	Insufficiency
32-100	80-250	Sufficiency
54-90	135-225	Normal in sunny countries
>100	>250	Excess
>150	>325	Intoxication

The guidelines recommended for maintaining bone health for the United States is 5µg/day (200IU/day) for children and younger adults (Grant & Holick, 2005). For individuals over 51-70 years of age, 400 IU/day and 600 IU/day for those over 70 years are recommended (Grant & Holick, 2005). According to Grant & Holick (2005), the mean intake for vitamin D is 320 IU/day for age 50 and older with 200 IU/day from dietary sources. The average summertime

circulating blood 25(OH)D level for Caucasian adults is 30-50 ng/mL, which is reported to drop to 25 ng/mL in winter, therefore in the insufficient range (Grant & Holick, 2005).

The serum concentration of $1,25(OH)_2D_3$ is dependent on the availability of $25(OH)D_3$. It is therefore not surprising that individuals who are 25(OH)D deficient are also $1,25(OH)_2D_3$ deficient. Individuals with a $1,25(OH)_2D_3$ concentration of less than 57 pmol/L are reported to be at an increased risk of hip fracture (Grant & Holick, 2005).

Vitamin D levels are thought to plateau at levels above 80 nmol/L, which is similar with what is observed in the parathyroid levels (Heaney, 2005). The rising absorptive efficiency at vitamin D levels below 80nmol/L indicates a reduction in fracture risk. A randomized placebo-controlled vitamin D trial in the elderly raised serum vitamin D levels from 53 to 74 nmol/L and produced a 33% reduction in osteoporotic fractures (Heaney, 2005). Bischoff-Ferrari et al. (2003) also reported that improvements in vitamin D status increased bone mineral density rapidly and substantially.

The vitamin D level input needed to achieve optimal serum concentrations depends on both the starting value and the chosen target level. A committee of researchers, the Upper Limits Panel, defined the target of vitamin D levels to be set at 80 nmol/L for optimal bone health (Heaney, 2005). Healthy men and women with low dietary intakes of vitamin D at 41° latitude during the winter months and not supplementing vitamin D were studied. To obtain the induced rise in serum vitamin D, one would need to increase the additional daily oral input to 1720 IU (or 43μg) of cholecalciferol (Heaney, 2005). Grant and Holick (2005) estimated that 55 μg, or 2200 IU (Heaney, 2005) of daily doses of vitamin D needs to be supplemented in order to achieve a serum of 80 nmol/L, when baseline levels are between 20-40 nmol/L, which is within the insufficient and sufficient range.

Heaney (2005) claims that the key to vitamin D supplementation lies in the careful definition of the tolerable upper limit intake level, or the TUIL. There have been many studies supplementing 5,000 to 10,000 IU/day for up to 16 weeks, and 50,000 IU/day for 8 weeks, all with no evidence of toxicity. A continuing daily dose intake of 10,000 IU would produce a serum vitamin D level of what is naturally seen from the sun exposure in the summer months at mid latitudes (Heaney, 2005). Therefore, using 80 nmol/L as the desired target or 2,600 IU/day by the RDA would meet the needs of 97% of the population (Heaney, 2005).

Although the upper limits of vitamin D are still under debate, the upper limits of vitamin D_3 concentrations for most commercial assays is around 125 nmol/L (50 ng/mL) (Holick, 2004). Lifegaurds and sunbathers have reported blood concentrations greater than 250 nmol/L or 100 ng/mL, and they were not vitamin D intoxicated (Holick, 2004). Therefore, it is concluded that concentrations of at least 80 nmol/L are required to minimally satisfy that body's vitamin D requirement for bone, but levels lower than 30 nmol/L have been considered to be deficient for the requirement for muscle.

Understanding the appropriate levels of vitamin D is very confusing and even the experts contradict themselves and do not agree on a definitive point at which vitamin D is considered low. One reason for the inconclusive levels of vitamin D could be from the subjective measures of the functional assessment tests (Bischoff-Ferrari et al., 2004). Therefore, the purposed study will collect more objective measures than subjective measures.

2.2.4 Measurement of Vitamin D

Serum vitamin D can be measured in a variety of ways, although the accuracy of some of the techniques has been questioned. Use of a radioimmunoassay is a common and relatively easy

assessment method (Vogeser et al., 2004); however, while both 25(OH)D and $1,25(OH)_2D_3$ can be evaluated, the technique has insufficient accuracy in discerning levels of D2 and D3. Quantitative High-Pressure Liquid Chromatography (HPLC) assays have been developed based on ultraviolet detection and normal-phase separation, or reversed-phase separation alone (Vogeser et al., 2004).

HPLC is currently considered the gold standard for vitamin D assessment because it provides good recovery, precision, and reliable measurement of 25(OH)D and 1,25(OH)D as well as D_2 and D_3 (Vogeser et al., 2004). The amount of skill, laboratory time, and expense involved with the HPLC technique is greater with HPLC than with radioimmunoassay. A new established technique utilizing liquid chromatography/mass spectrometry (LC/MS) is becoming more popular because it combines excellent sensitivity with convenient sample preparation to the analysis (Masuda et al., 2004).

2.2.4.1 Vitamin D and Muscle Strength

Vitamin D is required for successful muscle development and growth. Vitamin D metabolites directly influence muscle cell maturation and function through a vitamin D receptor (Janseen et al, 2002). The effect of vitamin D in muscle includes the activation of protein kinase C (PKC) and the release of calcium into the cytosol (Montero-Odasso et al., 2005). Calcium is then transported into the sarcoplasmic reticulum by the calcium-adenosine triphosphatase (Ca-ATPase), which in turn increases the amount of calcium available for contraction. The activation of protein kinase C also has an effect on the protein synthesis in the muscle cell (Montero-Odasso et al., 2005).

Several studies have reported that higher serum vitamin D concentrations are associated with better lower-extremity function in older individuals. Severe vitamin D deficiency is also

associated with muscle weakness, which is termed hypovitaminosis D myopathy (Parbhala et al., 2000; Mingrone et al., 1999; Schott et al., 1976), or osteomalacic myopathy (Montero-Odasso et al., 2005). Both in the young and the elderly, prolonged vitamin D deficiency has been associated with severe muscle weakness, often leading to marked disability (Montero-Odasso et al., 2005). Montero-Odasso et al. (2005), also reported within several weeks of vitamin D supplementation, the osteomalacic myopathy that showed an abnormal electromyogram with signs of both myopathy and reduced nerve conduction velocity improved with the supplementation.

Since the type II fibers, or slow twitch fibers, are the first muscle fibers to be recruited to avoid falling, and that type II fibers are affected by vitamin D deficiency, it is hypothesized that vitamin D deficiency may increase the risk of falling (Montero-Odasso et al., 2005). Janseen, et al. (2002) reported that an intervention of vitamin D resulted in improved functional ability and a reduction in the number of falls in elderly with hypovitaminosis D (Janseen et al., 2002). Bischoff-Ferrari et al. (2004) studied active and sedentary people between the ages of 60 and 90 yrs in order to examine the relationship between serum 25(OH)D concentration in the times of a 8-foot walk test and a sit-to-stand test, and concluded the subjects with the highest 25(OH)D concentration demonstrated the fastest sit-to-stand times. Bischoff-Ferrari et al. (2004) also reported a strong logarithmic relationship between the serum vitamin D concentration and performance times, with the critical point of 40 nmol/L of 25(OH)D being the threshold for optimal function.

Vitamin D supplementation is suggested to reduce the risk of falls among both ambulatory and institutionalized elderly subjects (Sato, Iwamoto, & Kanoko, 2005). Sato et al. (2005) addressed the reduced risk of falls and hip fractures in patients with long-standing stroke

by vitamin D supplementation. Ninety-six elderly women with poststroke hemiplegia were followed for two years, where the patients were randomly assigned to one of the two groups, those 48 patients who received 1,000 IU ergocalciferol daily, and those who received placebo (Sato et al., 2005). The number of falls per person and incidence of hip fractures were compared between the two groups, along with strength and tissue ATPase of the skeletal muscles. The number and size of type II muscle fibers increased and improved muscular strength in the vitamin D-treated group, and accounted for a 59% reduction in falls (Sato et al., 2005). Sato et al. (2005) concluded that vitamin D may increase muscle strength by improving atrophy of type II muscle fibers, which may lead to decreased falls and hip fractures.

Supplementation with vitamin D, in addition to its protective effect against fractures, may reduce the number of falls by improving muscle strength (Loew & Manupetit, 2005; Gallagher, 2004). Most of the research on vitamin D and muscular strength has examined the risk of falls and not the risk of a decline in strength and increased incidence of fatigue. Little research has been published on the neuromuscular effect of vitamin D supplementation without regard to fall risk.

Glerup et. (2000) conducted a study on the effects of vitamin D on muscle function in veiled Arabic women in Denmark and Danish controls. At baseline, the 25-hydroxyvitamin D serum concentrations of the veiled women were extremely low at 6.7 ± 0.6 nmol/L in the veiled women and 47.1 ± 4.6 nmol in the Danes. The veiled women also demonstrated significantly less muscular power of the hip, knee, ankle, and shoulder at baseline. After the veiled women underwent six months of vitamin D treatments by intramuscular injections of 100,000 IU of $25(OH)_2D_2$, the measures of muscular power were similar between the veiled women and the controls (Glerup et al., 2000).

Vitamin D maintenance is a benefit for optimal muscle strength (Grant & Holick, 2005). Vitamin D deficiency leads to osteomalacia, which is associated with bone and muscle pain (Grant & Holick, 2005). Plotnikoff et al. (2003) reported of 150 patients at a Minneapolis hospital with musculoskeletal pain syndromes, 140 patients were vitamin D deficient with a mean of 12.1 ng/mL. Pferier et al. (2002) also reported decreased muscular strength with serum vitamin D or 25(OH)D levels less then 12 ng/mL.

2.3 OSTEOPOROSIS

Patients with Crohn's disease are at a high risk of developing osteoporosis. This may be in part, due to the purported low levels of serum vitamin D in their bodies. However, most of the research on osteoporosis has been on post-menopausal women because of the high incidence of low bone mineral density in this group.

Thirteen to eighteen percent of women aged 50 and over have osteoporosis (Healthy People, 2010), which is a disease characterized by low bone mineral density (BMD). BMD is a measure of the amount of calcium and phosphate within the bones (Merieb et al., 2000). The World Health Organization defines osteoporosis as having BMD more than 2.5 standard deviations below the normal (Looker et al., 1995). Low BMD has been found to lead to bone fragility and fractures.

Osteoporosis affects both males and females, but is more prevalent in females (Looker, 1995; Merieb et al., 2000). As age increases, bone mineral density decreases, which increases the risk of osteoporosis. Women tend to have less bone mass than men due to the smaller frames of females, and because women lose bone at an accelerated rate after menopause (Harpavat et al.,

2004). One in two women and one in eight men older than 50 will experience an osteoporotic bone fracture in their lifetime (Lichtenstein, 2003).

Osteoporosis affects an estimated 44 million Americans, and accounts for more than 1.5 million bone fractures annually (Healthy People 2010, 2000). There are many risk factors associated with osteoporosis: aged 50 and over, a low bone mass, and history of a first degree relative with osteoporosis. Being female, as does estrogen deficiency, amenorrhea (or absence of menstruation), and anorexia nervosa/bulemia (or presence of an eating disorder) also put one at a higher risk. Low calcium and vitamin D intake, bone resorptive medications such as corticosteroids and anticonvulsants, inactivity, excessive alcohol intake, being Caucasian or Asian, cigarette smoking, and low testosterone in men are also risk factors associated with osteoporosis (Warren & Shantha, 2000).

Due to the fact that postmeopausal women have the greatest incidence of osteoporosis, they also exhibit the most serious consequences of osteoporosis. (Gourlay & Brown, 2004). Hip fractures cause the most morbidity and mortality in white women, and the incidence increases as age increases. Each standard deviation decrease in femoral neck density increases the age-adjusted risk of hip fracture by 2.6% (Lui-Ambrose et al., 2004). Thus, exercise programs aimed at reducing falling risk through increased muscular strength and balance may be particularly important for older people with low bone mass (Lui-Ambrose et al., 2004).

At this time, osteoporosis can not be cured, but it can be prevented. The best way to prevent osteoporosis is through building strong bones during adolescence (Kell, Bell, & Quinney, 2004). During growth and development, the load placed on the bones from the skeleton supporting bodyweight alone is enough to cause bone modeling. Further increases in load are

related to additional deposition of bone mass. After peak bone mass is achieved, around age 30, body weight alone is not enough to maintain bone remodeling (Kell et al., 2004).

Weight-bearing exercise is known to increase BMD. Weight bearing exercise places high magnitudes of force and strain on the bone, which activates osteoblastic activity (Teegarden et al., 1996). Due to osteoporosis patients have low bone mineral density, the internal bone structure of trabeculae and matrix may be insufficient to withstand the weight bearing exercise needed to endure the loads which instigate remodeling. Therefore through resistance training, greater loads can be transferred onto the bone, thus stimulating the increased bone mineral absorption and bone remodeling (Kell et al., 2004), which makes for stronger bones.

A balanced diet rich in calcium and vitamin D will also aid in the prevention of osteoporosis. Supplementation with vitamin D and calcium led to a 43% decrease in hip fractures in older women (Pfeifer et al., 2002). Adding vitamin D and calcium to one's diet can decrease hyperpathyroidism and bone turnover rate, thereby increasing bone strength (Holick, 1996; Willett, 2005). Vitamin D and calcium supplementation has also been linked to reductions in body sway, increased balance ability and in turn decreased fall and fracture risk in a highly susceptible population of increasing age (Pfeifer et al., 2002).

Proper assessment of bone mineral density by dual energy x-ray absorptiometry (DXA) testing will help detect the disease before its occurrence, or from progressing once diagnosed. Many medications also aid in the prevention of the progression of osteoporosis. Medications such as bisphosphonates, calcitonin, and hormone replacement therapy (HRT) are prescribed once diagnosis of osteoporosis is established (Gourlay & Brown, 2004; Lewiecki, 2004). Other common medications given to osteoporosis patients are parathyroid hormone, which stimulates

bone formation in the spine and hip, and selective estrogen receptor modulators (SERMs), which prevent bone loss at the spine, hip, and total body (Gourlay & Brown, 2004; Lewiecki, 2004).

2.3.1 Dual Energy X-Ray Absorptiometry

A safe, accurate, and reproducible low dose examination of bone mineral density is necessary to test bone mineral density of individuals properly. Many methods for measuring densiometry have been used. Over the last several years, the continued refinement of the dual energy x-ray absorptiometry (DXA) has led investigators to adopt the measurement as the primary method (Kelly, 1998). Two types of DXA machines are currently available: a fan-beam and a pencil beam DXA (Kelly, 1998). Pencil beams generally require 20 minutes of scan time to pass a small beam through a large area. Fan-beam instruments result in testing time of 3 minutes. The increased speed and precision of the fan-beam method is due to several hundred detectors moving parallel to provide no overlap between adjacent pixels (Kelly, 1998).

Ultrasound (US) is also used to diagnose osteoporosis and to predict fractures (Ostlere & Gold, 1991). The calcaneous is often the site measured in the ultrasound due to the fact that only peripheral bone sites can be measured this way. Resch et al. (2000) stated that ultrasound is an accepted, short-lasting, radiation free method most widely used as screening tools.

Makan, Bayley, and Webber (1997) studied the precision and accuracy of total bone mass and body composition measurements in rats using the DXA. The two types of DXA machines used were Hologic QDR 1000W and QDR 4500A (Hologic Inc., Waltham, MA). The difference between the two machines is that the 1000W is a pencil-beam, and the 4500A is a fan-beam. The researchers concluded that the fan-beam based absorptiometry provided better and reproducible

measurements for bone content, bone mineral density, fat mass, and lean body mass, than the pencil beam.

The DXA has been established as the gold standard for diagnosis of osteoporosis. The DXA scan is not only used for diagnosis of the disease, but also as a predictor of fracture, determinant of medical therapy, and monitor progression of disease. The DXA is a quick, inexpensive, reproducibale way to measure bone density with minimal radiation.

The measured bone mineral density from the DXA is compared to reference ranges defined by the World Health Organization (WHO) (Looker et al., 1995). A normalized value based on peak bone mass, according to the WHO for young adults, is a T-score. A T-score between -1 and -2.5 is defined as someone having osteopenia, and osteoporosis is characterized by having a T-score of -2.5 and below. A Z-score is defined by the WHO as the amount of standard deviations away from the expected number for matched age, gender, and race, with the same standard deviations as the T-scores.

2.4 PARATHYROID HORMONE

The parathyroid hormone is an 84 amino acid chain polypeptide that is released secreted from cells of the parathyroid gland and finds its major target cells in bone and kidney (Sandler, Pattron, & Partain, 1986). Parathyroid hormone is the major endocrine regulator of calcium and phosphorus concentration in the blood (Sandler, Pattron, & Partain, 1986). The physiologic effects of increasing parathyroid hormone are with increasing calcium concentration and decreasing the concentration of phosphate ions within the blood. Parathyroid hormone stimulates four processes: inhibition of phosphate reabsorption by the proximal renal tubule, enhancement

of renal tubular calcium, osteoclastic stimulation with resultant resorption of calcium, and increased calcium absorption from the gastrointestinal tract (Sandler et al., 1986).

Parathyroid hormone secretion increases in response to a reduction in plasma calcium concentrations (Holick, 1996). The reduction in plasma calcium concentration can be either a result of low calcium intake or increased urinary calcium losses or other pathological states. Increased levels of calcium in the blood stimulate the thyroid to release more calcitonin. Calcitionin then inhibits the activity of the osteoclasts within the bone, and therefore decreases the blood calcium levels (Willett, 2005). The decreased levels of calcium in the blood will stimulate the release of excess parathyroid hormone. The effect of parathyroid hormone is to promote the resorption of bone matrix, which releases calcium into the blood and decreases the amount of calcium lost in the urine.

Parathyroid hormone is also stimulated in the kidneys to synthesize the active form of vitamin D, 1,25-dihydroxyvitamin D_3 (Holick, 1996; Willett, 2005). Together, the parathyroid hormone and 1,25-dihydroxyvitamin D mobilize monocytic stem cells to become osteoclasts and increase bone resorption, thus releasing calcium into the circulation (Willett, 2005). The 1,25-hydroxyvitamin D also increases the efficiency of intestinal calcium absorption by regulating active transport and aids the reabsorption of calcium in the kidneys (Willett, 2005).

The net effect is to raise the serum calcium concentration and reduce parathyroid synthesis and secretion from parathyroid glands (Holick, 1996). In a negative feedback reaction, 1,25-dihyroxyvitamin D also suppresses parathyroid production (Holick, 1996). The effects of 25OHD on parathyroid hormone secretion are believed to be indirect, whereby low 25(OH)D may limit intestinal calcium absorption, resulting in decreased plasma calcium concentration

(Willett, 2005). Persistently low 25(OH)D may result in prolonged elevation of parathyroid and a subsequent increase in bone resorption (Willett, 2005).

Increased and decreased secretion of parathyroid hormone is recognized as a cause of serious disease. Excessive secretion of parathyroid hormone is seen in primary and secondary hyperparathyroidism (Sandler et al., 1986). Primary hyperparathyroidism, in which excessive is the result of a parathyroid gland disease, most commonly due to a parathyroid tumor (adenoma) (Guyton & Hall, 2000). Some common manifestations of primary hyperparathyroidism disorder are chronic elevations of the blood calcium concentration (hypercalcemia), kidney stones, and decalcification of bone (Guyton & Hall, 2000; Willett, 2005).

Secondary hyperparathyroidism develops when a disease outside of the parathyroid gland leads to excessive secretion of parathyroid hormone, or high levels of parathyroid hormone occur as a compensation for hypocalcemia (Guyton & Hall, 2000). One common cause of secondary hyperparathyroidism disorder is kidney disease and vitamin D deficiency (Guyton & Hall, 2000; de Francisco, 2004). In secondary hyperparathyroidism, the kidneys are unable to reabsorb calcium and the blood calcium levels decrease as a result (de Francisco, 2004). Therefore, the kidneys are unable to produce sufficient amounts of the active form of vitamin D, 1,25-hydroxyvitamin D_3 (Guyton & Hall, 2000).

Through vitamin D deficiency, there is a resulting inadequate mineralization of the bones, called osteomalacia, and the continual secretion of parathyroid hormone is stimulated to maintain normal calcium levels in blood, and causes absorption of the bones (Guyton & Hall, 2000). Secondary hyperparathyroidism can also result from inadequate nutrition. Inadequate nutrition can result from diets that are deficient in calcium or vitamin D, or diets which contain excessive

phosphorus (e.g. all meat diets for carnivores), and malabsorptive diseases, such as Crohn's disease (de Francisco, 2004).

Under the conditions of hyperparathyroidism, one or more of the parathyroid glands produces excess hormones regardless of the level of calcium (Sandler et al., 1986). Individuals with persistently elevated calcium levels due to overproduction of the pararthyroid hormone can also experience bone pain. In the severe form, osteopenia and osteoporosis results from loses of an excessive amount of calcium and become brittle and break (Lane & Morris, 2005). The abnormal increase of parathyroid hormone production is even more common in older individuals, as production normally increases with age.

Other symptoms of hyperparathyroidism are the development of gastric ulcers and pancreatitis. High calcium in the blood can be dangerous to a number of cells in the body including the lining of the stomach and the pancreas. Both of these organs can become inflamed and painful, causing ulcers and or acute pancreatitis, and the development of kidney stones. Therefore, severe osteoporosis and osteopenia, bone fractures, kidney stones, peptic ulcers, pancreatitis, and nervous system complaints, are the potential dangers of hyperparathyroidism.

2.4.1 Vitamin D and Parathyroid Hormone in Crohn's Disease Patients

Serum parathyroid hormone (PTH) concentration is inversely related to calcium concentrations (Heaney, 2005, 2003; Montero-Odasso et al., 2005, von Mühlen, 2005). Therefore, vitamin D levels have been found to decrease with age, and parathyroid hormone levels to increase with age (von Mühlen, 2005). Calcium absorption is reduced at serum concentrations of 25OHD levels below 80 nmol/l (Heaney, 2003). Currently, there is debate as to the efficacy of calcium absorption at levels of 25(OH)D greater than 100 nmol/L (Heaney,

2005). Montero-Odasso et al. (2005) claimed that the point on the 25(OH)D continuum where parathyroid hormone becomes constant is an indication where calcium becomes constant. Due to the fact that calcium absorption testing is difficult and expensive, parathyroid hormone levels are frequently tested instead.

The relationship between parathyroid hormone and bone mineral density in patients with inflammatory bowel disease has been conflicting. Janssen et al. (2002) found patients with Crohn's disease have an increased parathyroid level, or hyperparathyroidism. The reason for the findings may have been due to inclusion of patients with renal failure (Janssen et al. 2002). Many studies have excluded these patients because of the chronic diarrhea caused by the intestinal disease and small bowel resections (Jahnsen et al., 2002). Jahnsen et al. (2002), in a study of sixty patients with Crohn's disease and sixty patients with Ulcerative Colitis, concluded that hypovitaminosis D and hyperparathyroidism were common findings in these populations.

2.5 EXPERIMENTAL TECHNIQUES

2.5.1 Muscular Strength

2.5.1.1 Historical Background

Strength refers to the physical ability to exert force. Perrin (1993) defines muscular strength as the capacity of the muscle to produce force through either a static or dynamic contraction. Strength is important not only in sport activities, but also as a means of improving health, improving activities of daily living, and maintaining or improving normal function.

Kulig, Andrews, and Hay (1984) defined strength in a more appropriate definition, as a muscle or group of muscles that has the magnitude of the variable force where the contractile entity exerts on the skeletal system at the attachment site. Therefore, Kulig et al. (1984) define maximum strength as magnitude of the maximum force that a muscle can exert on the skeletal system at the attachment point of interest under a specific set of exercise conditions. Maximum strength depends on the exercise environment and describe the muscle's ability to exert force.

The theory of strength development for variable resistance is based on the overload principle (Kulig et al., 1984). The overload principle states that gains in strength are achieved by taxing the muscle to its limits or by requiring it to exert forces that exceed the normal exertion. The muscle responds to the overload by attempting to retain the muscle's margin of safety or by increasing the capability of exerting a force (Kulig et al., 1984).

Through a set of exercise conditions, the strength of the contractile entity or maximum force may vary as a function of joint angles (Kulig et al., 1984). A strength curve is often presented as a plot of the variations in the muscle force versus joint angles. Strength curves are important indicators of muscular capability, determine variable resistance, and interpret findings.

2.5.1.2 Testing Procedures

Experimental testing of muscular strength is primarily performed under either isotonic or isometric conditions. In an isometric contraction, the muscle is prevented from shortening by fixing both ends or preventing joint movement (McComas, 1996). Isometric assessment of a muscle determines the amount of tension a muscle can generate against a resistance allowing no observable movement (Perrin, 1993). An isometric strength assessment measures the muscle's maximum potential to produce a static force as the muscle develops tension at its point of

attachment. An example of an isometric contraction in everyday is carrying a heavy object or maintaining one's posture against gravity.

An isometric contraction observed in a laboratory setting is stimulating the muscle and measuring the velocity of the shortening (McComas, 1996). The period when the weight is not moved is referred to as the latent period and the muscle is contracting isometrically (McComas, 1996). During an isometric contraction, the interaction between the actin and myosin filaments depends on the length at which the muscle is held prior to activation, where there is no action of the actin filaments (McComas, 1996).

The strength curve presented from the isometric testing is a plot of the muscles' maximum force capacity as a function of the joint angle (Kulig et al., 1984). In order to obtain a strength curve, the following restrictions need to be met: 1.) only one joint changes configuration during the exercise, 2.) the muscle or group entity of interest crosses the joint and appears to be the dominant cause of the joint motion, and 3.) the joint that changes its configuration only has one degree of freedom (Kulig et al., 1984).

Relative to the first restriction, if more than one joint has mobility during the exercise then multiple muscles are probably involved (Kulig et al., 1984). The second restriction is necessary due to the actions of voluntary or stabilizing muscles through out the body that may be active for a single-joint exercise. Therefore, the second restriction attempts to eliminate the possibility of the strength curve describing the nondominant or anatagonistic muscles at the involved joint, instead of the dominant muscle group action alone (Kulig et al., 1984). The third restriction is included because of the complexity that multiple joint degrees of freedom cause. In the case of more than one rotational degree of freedom for the joint in question, there are no

restrictions on the angular variables and the problems of identifying a characteristic strength curve for multiple-degree-of-freedom joint (Kulig et al., 1984).

Kulig et al. (1984) in a review of literature related to strength curves, determined the strength curve results for knee flexion and extension. A typical knee flexor strength curves is shown in Figure 1 (Kulig et al., 1984). To note in the figure, knee flexion angle of zero degrees denotes full extension.

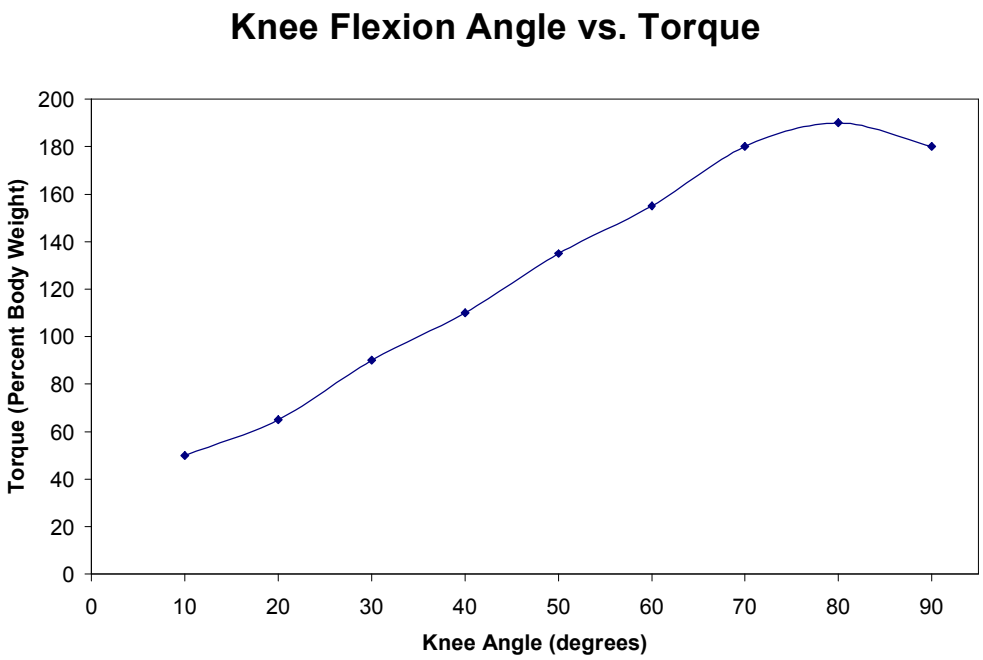

Figure 1 Knee flexion angles versus the peak torque to percent body weight.

The longer the knee flexor muscles, the greater the force and torques recorded (Kulig et al., 1984). For knee extension curves, the maximum strength value occurs at the extended middle range of motion (Kulig et al., 1984). Therefore, the maximum force or torque usually is recorded at a joint angle of 20-50 degrees of flexion.

2.5.1.3 Muscular Strength and Crohn's Disease

Malnutrition has been found to induce deleterious effects on muscle mass, function, and endurance. Wiroth et al. (2005) tested muscular strength, muscular endurance, and physical activity on forty-one outpatients with Crohn's disease and concluded that muscle performance in Crohn's patients was approximately 25% lower than controls. Geerling et al. (2000) also showed that Crohn's patients had lower isokinetic hamstring strength than controls.

It is well established that Crohn's disease patients have an increased energy expenditure, nutritional deficiencies, and express symptoms and complaints general fatigue (Wiroth et al., 2005). However the cause of the muscular fatigue in Crohn's disease patients is unknown. Wiroth et al. (2005) concluded that resistance training may reduce the deleterious effects of Crohn's disease on muscle function and enhance the quality of life. Therefore, research on muscular strength and fatigue in Crohn's disease patients is necessary.

2.5.2 Muscular Endurance

2.5.2.1 Historical Background

An electromyographm (EMG) is the electrical signal associated with a muscle contraction (Winter, 1990). Surface electromyography is widely used as a noninvasive measure. EMG reflects the electrophysiology of the muscle activity while the muscle fibers generate a potential field on the skin surface (Rau, Disselhorst, and Silny, 1997). EMG use has been well established in literature, and only technical restrictions make it difficult for correlations between EMG and the contraction of the muscle (Rau et al., 1997).

The EMG process uses monoploar or bipolar electrodes to assess fatigue. EMG is defined as the sum of action potentials, both positive and negative, occurring within its measurement

range (Hamill, 1995). The noninvasive EMG procedure has a high resolution and allows the detection of a single motor unit, unlike the use of other methods (Rau et al., 1997).

2.5.2.2 Testing Procedures

Electromyographic techniques can be preformed in two ways, invasive fine-wire electrodes and noninvasive surface electrodes. Both techniques of electromyography have limitations. Surface electrodes are limited by interference caused by skin movement and the inability of subcutaneous tissue to separate signals (Rau et al., 1997). Fine wire electrodes also have several limitations, such as pain caused from the invasive needle and interference or inability to perform movement activities with the needles (Rau et al., 1997). Surface electrodes have been found to be more accurate than fine wire electrodes for superficial muscle groups of the lower extremity.

Winter (1990) stated that certain specifications are necessary in order to acquire clean signals with no noise. Signals refers to the change in output due to the process itself, and noise is a change in the output of measurement not due to the process, but from errors or fluctuations of ionic currents and stochastic nature of action potentials (Winter, 1990). Winter (1990) describes four major considerations to be made when specifying the amplifier for EMG: gain and dynamic range, input impedance, frequency response, and common-mode rejection.

A biological amplifier is required in collection of EMG and necessary for clean signals. The gain of the amplifier is defined as the ratio of output to input voltage, and should be set within the range of the output signal. The input impedance, or the resistance, of the amplifier must be sufficiently high as to not attenuate the EMG signal as it is connected to the input terminals of the amplifier (Winter, 1990). Winter (1990) recommends a desirable input impedance as 1 MΩ or higher, and to prepare the skin to reduce the impedance to 1000 Ω or less for surface electrodes.

The frequency response of the frequency bandwidth of an EMG amplifier is preformed to amplify without attenuation all frequencies in the EMG (Winter, 1990). The recommended range for surface electrode is between 10-1000 Hz, where the majority of the signal will be concentrated between 20 and 200 Hz, with the lesser component extending up to 1000 Hz (Winter, 1990). Winter's (1990) final consideration was including the common-mode rejection due to the fact that human body is a good conductor and will therefore act as an antenna for electromagnetic radiation. The interference from power cords, fluorescent lighting, and electrical machinery can interfere with the EMG recording. Therefore, a common-mode rejection is required where a bipolar electrode is used for a common mode signal from each pole to be detected and subtracted from the EMG signal (Winter, 1990).

The process of muscular endurance testing is done by placing the bipolar electrode on the skin over the muscle to be investigated and an additional electrode for the ground. A signal is then gathered to enable researchers to quantify the muscular activation (Winter, 1990). Once the EMG signal has been amplified, it can be processed for biomechanical signals.

Muscle fatigue results when the muscle cannot supply the metabolism at the contractile element due to ischemia or insufficient oxygen or the local depletion of metabolic substrates (Winter, 1990). As the muscle contraction remains constant, fatigue begins as the muscle losses tension. The maintenance of the tension after onset of fatigue requires increased motor unit recruitment to compensate for the already reduced firing rate (Winter, 1990).

Muscle fatigue will reduce the force of contraction, but will also change the shape of the motor action potentials by decreasing the higher frequency components (Winter, 1990). The decrease in higher frequency components may result from lower conduction velocity and slower motor units with longer duration of the motor unit action potential (Winter, 1990). When surface

electrodes are used in the procedure, the EMG amplitude will then be increased because of the decreased conduction velocity which causes a widen pulse and increased area under the curve (Winter, 1990). Another change seen with fatigue is the tendency for the motor units to fire more synchronously, and isometric contractions will show evidence of tremors in the tracings (Winter, 1990).

A Fast-Fourier Transform technique will allow the data or analog signal in a time domain to be converted to a frequency domain to determine the power density spectrum (Winter, 1990). From the power density, median muscle activation frequency (MF) can be determined for every second of data. The data may then be plotted with time on the X axis and MF on the Y axis. A linear regression may derive the slope of this line, which is the rate of change of the median frequency, or the fatigue rate (Callaghan et al., 2001).

Muscular fatigue is a common compliant in patients with Crohn's disease. After an intensive search of literature, no studies were found evaluating the muscular endurance or muscular fatigue in Crohn's disease patients. Therefore one of the main purposes of the proposed research is to determine the muscular fatigue rate of patient's with Crohn's disease.

2.5.3 Nerve Function

2.5.3.1 Historical Background

Nerve conduction studies and needle electromyography are used to evaluate the peripheral nervous system, most specifically the motor unit (Lee, Claussen, & Oh, 2004). Muscle and nerve cells are excitable cells, and when the cell is electrically stimulated, the cell depolarizes, causing a change in the membrane potential (Lee et al., 2004). If the depolarization is within a specific threshold, an action potential is generated.

The ability of the external electrical stimulus to produce the depolarization and excitation depends on the intensity and the duration of the stimulus (Lee et al., 1997; Marieb et al., 2004). Therefore, the propagation of the action potential results in generation of a nerve action potential, or summated potential of all nerve fibers that have been stimulated by the electrical pulse. The intensity and duration of the electrical stimulus to produce the excitation of a nerve depends on the nerve's excitability. Injury or dysfunction of a nerve decreases the excitability of the nerve (Lee et al., 1997).

The motor nerve conduction (MNC) testing is performed by stimulating a peripheral nerve with a single supramaximal stimulus along the course of the nerve (Lee et al., 1997). The result of the stimulus is a compound muscle action potential (CMAP), which is recorded with a surface electrode from a muscle innervated by the stimulated nerve (Lee et al., 1997). The latency of the nerve, which is measured in milliseconds (ms), is the time required from nerve stimulation to the production of the CMAP.

Physiologically, the response of nerve to a stimulus requires axonal transmission of the impulse, neuromusclar transmission, and muscle fiber depolarization (Marieb et al., 2004). The terminal or distal latency is the time in milliseconds, required for the response from the distal point of stimulation (Lee et al., 1997). The conduction time is found by subtracting the terminal latency from the latency derived from the point of stimulation (Lee et al., 1997).

2.5.3.2 Testing Procedures

Nerve conduction velocity is the assessment of the objectivity and functional status of the peripheral neuromuscular system (DeLisa, Lee, Baran, Lai, & Spielholz, 1994). The reaction of the stimulated nerve is monitored with the recording electrode. The nerve conduction velocity is found by dividing the conduction time by the distance from the stimulating site (Lee et al.,

1997). The nerve conduction velocity is referred to as the maximum conduction velocity of the fastest fibers (Lee et al., 1997). According to Lee et al. (2004) the most common technical error made in nerve conduction velocity is obtaining an inaccurate distance measurement. The proper technique, including placement of the electrodes and stimulator as well as increasing the stimulus to maximal stimulation, is critical to the conduction process.

The most common factors that affect nerve conduction velocity are temperature, age, and distance measurements (Lee et al., 2004). Nerve conduction velocity increases linearly with increasing body temperature (Lee et al., 2004). As age increases, the nerve conduction velocity and compound muscle and nerve action potential exhibit a gradual decline (Lee et al., 2004).

The motor nerve conduction studies are interpreted on the basis of three parameters: latency, conduction velocity, and amplitude. Motor latency measures the transmission of time in the nerve fibers in milliseconds required for the nerve stimulus to travel from point of stimulus to the active electrode and cause a compound muscle action potential (CAMP) (Brevio, 2005). A nerve compression or neurological disorder can slow the transmission time below the established normal values.

The conduction velocity is measured when the latency value is determined between a proximal and a distal site divided by time. The amplitude reflects the number of intact muscle or nerve fibers within the muscle or group being tested. Amplitude is typically measured from baseline to the peak of the waveform (Brevio, 2005). The amplitude of a motor response of a normal healthy nerve from distal to proximal will remain nearly constant (Brevio, 2005). The sensory nerve conduction studies measure the sensory nerve action potential (SNAP) from the stimulation of a nerve (Brevio, 2005). A sensory nerve fiber carries cutaneous sensory information.

2.5.3.3 Nerve Conduction Velocity and Crohn's Disease

There have been sporadic accounts of neuralgic complications occurring in Crohn's patients throughout the literature (Lossos, River, Eliakim, and Steiner, 1995). Lossos et al. (1995) stated the neuralgic involvement associated with inflammatory bowel disease was rarely reported. The pathogenesis of nerve dysfunction is unknown and little has been reported about its incidence (Elsehety and Bertorini, 1997). Elsehety and Bertonin (1997) and Lossos et al. (1995) revealed there to be a 19% increase in the incidence of nerve dysfunction in Crohn's patients than the general population.

Patients with Crohn's disease have been found to have similar neuropathologies as those with multiple sclerosis (Purrman et al., 1992), which is defined as a chronic degenerative disease of the central nervous system in which gradual degenerative of myelin occurs in patients throughout the brain or spinal cord or both (Merieb et al., 2004). Multiple sclerosis interferes with the nerve pathways and causes the muscular weakness, loss of coordination and visual disturbances. Found chiefly in young adults, Multiple sclerosis is thought to be a defect in the immune system that may be genetic or viral in origin. Patients Crohn's disease and those with multiple sclerosis have been found to share similar epidemiological traits (Purrman et al. 1992).

Lindgren, Lilja, Rosen and Sundkvist (1991) evaluated nerve function in 33 patients with Crohn's disease, and reported that 48% showed signs of autonomic neuropathy. The nerve dysfunction was not attributed to the duration or severity of the disease, or the evidence of inflammation or malabsorption of vitamins or trace elements, but rather to the frequent disturbance in bowel function (Lindgren et al., 1991).

Lossos et al. (1995) reported motor nerve conduction studies of patients with Inflammatory Bowel Disease to show a prolonged distal latency up to 7 ms obtained over the

median and common peroneal nerves. Also reported were reduced velocities, normal compound action potentials, and absent F waves (Lossos et al., 1995). Lossos et al. (1995) concluded that neurologic disorders develops after the diagnosis of inflammatory bowel disease, and rarely coincides with exacerbations of the bowel disease and is more prevalent in the peripheral nervous system.

2.5.4 Short Form 36-item Health Survey (SF-36)

The Short-Form 36-item (SF-36) (see Appendix D) is a quality of life index instrument based on rating scales (Haywood, Garratt, & Fitzpatrick, 2005). The SF-36 has 36 items and assesses health across eight domains (Haywood et al., 2005). The eight subscales of the SF-36 consist of: physical functioning (10 items), bodily pain (2 items), vitality or energy level (4 items), social functioning (2 items), mental health (5 items), general health perceptions (5 items), role limitations because of physical problems (4 items), and role limitation due to personal or emotional problems (3 items) (Bernklev et al., 2005). An additional question on health transitions is asked, but is not included in the final score to assess change in health (Haywood et al., 2005). A health profile score is given from the eight domains, and two component summaries scores for physical and mental health can also be calculated (Haywood, 2005).

In a review of self-assessed health measurements, Haywood (2005) reported the SF-36 to have high reliability. The SF-36 survey is the most widely used health survey, and has been used in many patient populations (Haywood et al., 2005). Haywood (2005) also reported high levels of test-retest and internal consistency, and high levels of internal construct validity. The SF-36 was also found to have relatively good evidence of reliability, supporting application in group assessment, and in some instances individual assessment, and evidence of validity and

responsiveness (Haywood, 2005). Short Form 36-item Health Survey (SF-36) and Crohn's Disease

Bernklev et al. (2005) compared the health-related quality of life in patients with inflammatory bowel disease to a healthy control group, using the SF-36 questionnaire. Patients with Crohn's disease displayed a significant reduction in health related scores with increasing symptoms in all eight dimensions compared to the general population (Bernklev, 2005). The SF-36 has been recommended where a detailed and broad ranging assessment of health is required, particularly in community dwelling individuals with limited morbidity (Haywood, 2005) and in Crohn's disease patients (Bernklev, 2005).

3.0 RESEARCH DESIGN AND METHODS

3.1 RESEARCH DESIGN

This is a cross-sectional study that included 19 subjects (9 males, 10 females) aged 27-62 years. Nineteen subjects with Crohn's disease, having at least one small bowel resection, and who reported idiopathic musculoskeletal pain or weakness of a 3 (on a scale of 1-10) were recruited.

3.1.1 Subject recruitment

Nineteen individuals with Crohn's disease, having at least one small bowel resection and a disease duration of more than 5 years were recruited to participate. There were nine males and ten females between the ages of 27 and 62 years of age. The Crohn's disease patients were recruited from the Inflammatory Bowel Disease (IBD) Center at University of Pittsburgh Medical Center (UPMC) Presbyterian Hospital, under the clinical care of Dr. Miguel Regueiro.

Inclusion criteria for patients including having at least one small bowel resection and idiopathic musculoskeletal pain or weakness of a 3 on a scale of 1-10, no vitamin D supplementation or exposure to UVB tanning beds, no history of diabetes, no knee pain or anterior cruciate ligament pathologies, and no history of left side stroke. The Crohn's patients were only included in the study if they had a known diagnosis of Crohn's for at least five years.

The subjects were also be included if they reported not taking any anticonvulsant medications or asthma medications.

Exclusion criteria for the Crohn's patients were to exclude those individuals who were currently on a vitamin D supplement, individuals who use anticonvulsant medications because of the interference with vitamin D metabolism, currently taking more than 20mg of flagyl, and individuals who have a pacemaker or an intravenous port because of complications that may arise from the nerve function testing. Subjects were also excluded if they had been diagnosed with severe Psoriasis or Spures, have a known neuromuscular disorder such as multiple sclerosis, and any neuropathy.

3.1.2 Experimental Variables

The independent variable was serum Vitamin D concentration, specifically the concentrations of 25(OH)D ng/mL. Dependent variables are measures of isometric muscle strength of the quadriceps and hamstrings, quadriceps muscle endurance, peroneal nerve latency and amplitude, and measures of quality of health assessment from the SF-36. Glerup et al. (2000) conducted a study on the effects of vitamin D on muscle function in veiled Arabic women in Denmark and Danish controls. After the veiled women underwent six months of vitamin D treatments by intramuscular injections of 100,000 IU of vitamin D, the measures of muscular power were similar between the veiled women and the controls. Therefore in the current study, serum vitamin D concentration was the independent variable and the measures of neuromuscular function were the dependent variables.

3.1.3 Overview of Procedures

Subjects who met the inclusion/exclusion criteria reported to the University of Pittsburgh Inflammatory Bowel Disease (IBD) Center in Presbyterian Hospital, Pittsburgh, PA to participate in this study. Each subject completed an informed consent form approved by the University of Pittsburgh Institutional Review Board (Appendix A). After completing the informed consent, the subjects then were asked to fill out an Inclusion/Exclusion Verification form (Appendix B). Subjects had a blood draw by a trained technician at the IBD Center.
The subjects then were escorted to the Hand Research Laboratory in UPMC Biomedical Science Tower, Pittsburgh, PA where the neuromuscular data, such as the muscular strength and endurance tests, nerve function, and a current health assessment survey, were collected. Participation in the study lasted approximately one and a half hours.

3.1.4 Inflammatory Bowel Disease (IBD) Center, UPMC Presbyterian Hospital

Upon entering the IBD Center, the principal investigator met the subjects and explained the informed consent. Information confirming their medication status, duration of disease, and inclusion/exclusion criteria was given (Appendix B), along with the idiopathic musculoskeletal scale (Appendix C). Then following, at the IBD Center, a licensed phlebotomist obtained a sample of each subject's blood in order to assess the concentration of serum vitamin D. A resting venous blood sample (15 ml) was drawn from a forearm vein. The blood samples were stored at –70°C in a freezer (Revco Model ULT2186-5, 230 volt) until they were analyzed.

The serum samples were packed in dry ice and shipped to a laboratory at the Boston School of Medicine, under the direction of Dr. Michael Holick, for analysis. Through the use of

radioimmunoassay, a common and relatively easy assessment method for vitamin D analysis, blood serum was analzyed. The blood serum was utilized to measure indices of bone and mineral metabolism, specifically serum 25-hydroxyvitamin D. The laboratory at Boston University School of Medicine, directed by Dr. Michael Holick, PhD, MD, is one of the few laboratories in the United States that can accurately separate the forms of vitamin D using the gold standard HPLC technique.

3.1.5 Muscle Function Assessment, The Hand Research Laboratory, UPMC Biomedical Science Tower

3.1.5.1 Muscle Strength Assessment

After the blood draw, the subjects were escorted to the Hand Laboratory in the UPMC Biomedical Science Tower. At the Hand Laboratory, a custom built strength chair was utilized to assess the isometric strength of the knee flexors and extensors of the left leg. An example of the custom designed strength chair is shown in Figure 2.

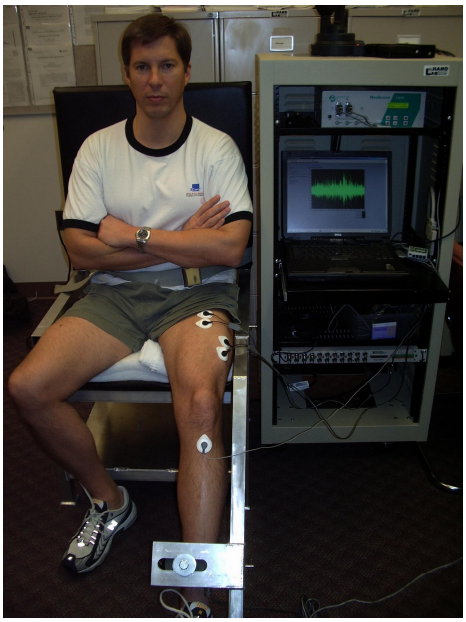

Figure 2 A subject in the custom designed strength chair.

Subjects were placed in a comfortable seated position on the strength chair, and were secured using a pelvic strap in order to minimize extraneous body movements. The maximal isometric strength of the quadriceps and hamstrings was measured using a customized strength testing device. The testing device included a tension/compression load cell (Kistler Instruments, Inc., Amherst, NY) attached to a custom-designed chair, which had been previously used to assess lower extremity strength of athletes of the 2005 National Senior Games, also called the "Senior Olympics," which were held at the University of Pittsburgh in June 2005. The load cell was connected to a National Instruments (National Instruments, Inc., Austin, TX) A/D board located inside a computer. Labview software (National Instruments, Inc., Austin, TX) was used to collect the data.

In the chair, the subject's hip was placed in 90º of flexion, and the knee in 45º of flexion. The load cell was attached to the adjustable bar so that the load cell can be positioned just proximal to the malleoli. A cloth strap was used to secure the subject's leg to the load cell device

and also around the subject's waist to control for extraneous movements.

Subjects were asked to perform three repetitions of maximal isometric knee flexion lasting five seconds each. Thirty seconds of rest was provided between contractions, and more time was allotted if needed. Subjects then were asked perform three repetitions, each lasting five seconds, of maximal isometric knee extension. Thirty seconds of rest again were provided between contractions.

The distance from the knee to the adjustable bar was recorded. The weight of the subject's leg was determined in order to calculate the gravity effect torque. For each test, torque was calculated as the product of the force in the load cell times the distance from the joint center to the adjustable bar. For each muscle group, peak torque and peak torque normalized to body weight ratio, and average peak torque and average peak torque normalized to body weight ratio was recorded.

3.1.5.2 Muscular Endurance Assessment

Fatigue rates of the vastus lateralis and rectus femoris were assessed by surface electromyography (EMG), in accordance with the methods of Montero-Oddsson et al. (2005) and Callaghan et al. (2001). The approach used simultaneously monitored median frequency from the electrode sites during a sustained sub-maximal contraction. All data was collected on the subject's left leg. EMG was assessed with the MyoSystem (Noraxon USA Inc., Scottsdale, AZ; Model "Telemyo"). The MyoSystem is a frequency modulated (FM) telemetry system. Electromyographic signals collected from the electrodes are passed through a single-ended amplifier (gain 500) to an 8 channel frequency modulator transmitter. A receiver unit collects the telemetry signals from the transmitter. The receiver amplifies (gain 500) and filters (500 ~ 1/2 Hz band pass Butterworth filter, common mode rejection ratio of 130dB) the signals. Signals

from the receiver are converted from analog to digital data via a BNC-2090 (16 channels, 12 bit) A/D board (National Instruments, Austin, TX) at a rate of 1024 Hz to allow for conversion to the frequency domain.

The subject was placed in a supine position, and the skin was prepared by shaving and scrubbing the area with isopropyl alcohol. Bipolar Ag/AgCl electrodes were applied to the skin parallel to the alignment of the muscle fibers with an inter-electrode distance of 20mm. For the vastus lateralis, the bipolar electrode was placed at 12-15° from the long axis of the femur, 15 cm from the superior lateral border of the patella. A bipolar electrode also was placed on the rectus femoris at the mid-point of the muscle belly, halfway between the anterior superior iliac spine and the superior pole of the patella. A ground electrode was placed on the skin superficial to the long axis of the tibia on the anterior shin.

All testing of muscular EMG occured on the custom built strength chair with the subject in the same position as described previously for the knee flexion/extension strength tests. For the contraction period, subjects were asked to perform a 60 second sub-maximal isometric contraction at 60% of their maximum knee extension torque as assessed previously in the muscle strength tests. The 60% level was shown to subjects via a computer-generated line on the monitor for the strength chair computer.

Data was imported into a custom written program in Matlab (Mathworks, Inc., Natick, MA). The EMG was collected for one minute and for each second, a Fast-Fourier Transform technique was used to convert the data from the time domain in millivolts (mV) to the frequency domain or number of waves. From the Fast-Fourier Transform, the median muscle activation frequency (MF) was determined for every second of data. Each muscle activation frequency was normalized against the initial muscle activation frequency, and was plotted with time on the X

axis and muscle activation frequency on the Y axis. From the graph, the slope of line was found which is equal to the fatigue rate.

For example, fatigue data for the rectus femoris are shown in Figure 3. In a previous pilot study of the investigators of this project, the rectus femoris fatigued at a greater rate in the recreationally active subjects (0.31% per second) than in competitively active subjects (0.002% per second). Figure 3 presents the fatigue rates of the rectus femoris in competitively active (n = 2) and recreationally active (n = 2) young subjects during a 60 second submaximal isometric contraction.

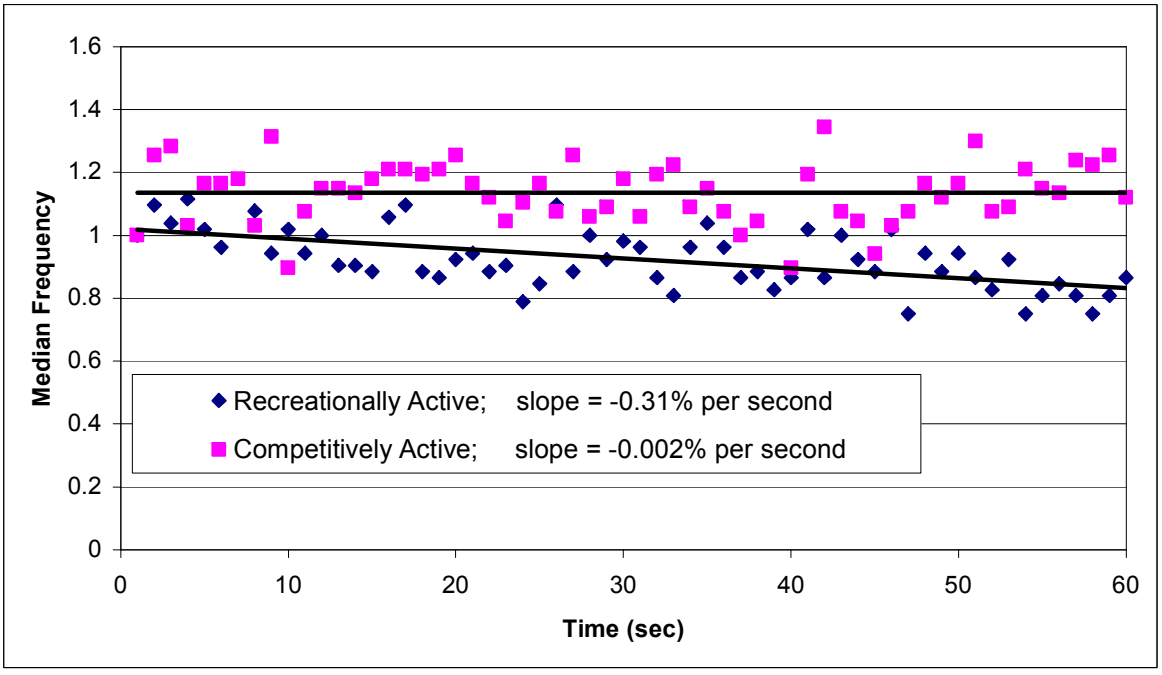

Figure 3 Fatigue rates of the rectus femoris.

3.1.5.3 Nerve Conduction Velocity

Assessments of lower extremity nerve conduction were performed with an automated electrophysiological neurodiagnostic device (Brevio® NCS-Monitor, NeuMed Inc., West Trenton, NJ). The Brevio® device has been shown to provide reliable measurements that are

comparable to those obtained on a traditional electroneurodiagnostic device and has the advantages of automated detection algorithms and prefabricated biosensors that allow for standardization of nerve conduction parameter measurement (Brevio, 2005).

Assessments were made of the peroneal nerves, and the side tested was the left side, to maintain consistency with the muscular strength and endurance testing. To test the peroneal nerve, the subject was asked to be seated in a comfortable position in a chair. Each test performed by the Brevio® required a specially designed Neuro-Sensor for single-patient use only. The Brevio® design integrates an active, a reference, and a ground electrode with a single connector (Brevio, 2005). The response captured from the Brevio® system is the actual time that elapses from the moment a nerve is stimulated to the detection of the evoked action potential.

The motor nerve conduction studies are interpreted on the basis of three parameters: latency, conduction velocity, and amplitude. The conduction velocity is measured when the latency value is determined between a proximal and a distal site divided by time. The amplitude reflects the number of intact muscle or nerve fibers within the muscle or group being tested, and is typically measured from baseline to the peak of the waveform (Brevio, 2005). The sensory nerve conduction studies measure the sensory nerve action potential (SNAP) from the stimulation of a nerve (Brevio, 2005).

A Brevio® neuro-sensor was applied to the lateral posterior aspect of the subject's leg, and then was connected to the Brevio® NCS-Monitor. An electronic pulse was then delivered by the Brevio® NCS-Monitor system. The following parameters were then obtained: distal motor latency and amplitude of the motor nerve action potential (Brevio, 2005), of the peroneal nerves.

To test the peroneal nerve, the area of the subject's foot and leg that was tested was cleansed with an alcohol solution, and wiped with a gauze pad. The active electrode was placed

over the belly of the extensor digitorum brevis muscle. The reference was then attached at the side of the small toe, distal to the extensor digitorum brevis muscle, and the ground was attached at the distal tibia. The stimulator probe was then placed over the peroneal nerve, on the anterior aspect of the leg and seven centimeters from the center of the active electrode. A small amount of conductive gel was applied to each probe of the stimulator. The stimulator was then placed over the dorsal aspect of the distal lower leg between the tendons of the tibialis anterior and the extensor hallucis, with the negative probe seven centimeters from the center of the active electrode.

3.1.5.4 SF-36 Current Health Assessment

The SF-36 Current Health Assessment is a survey asking the individuals views on their health. Questions are asked about how the individual feels and how well they are able to do their usual activities. The SF-36 survey is the most widely used health survey, and has been used in many patient populations. Haywood (2005) in a review of health-related quality of life surveys, reported low levels of test-retest variability and good internal consistency, and high levels of internal construct validity for the SF-36. The SF-36 was also found to have relatively good evidence of reliability, supporting application in group assessment, and in some instances individual assessment, and evidence of validity and responsiveness (Haywood, 2005).

The SF-36 consists of eight subscales: physical functioning (10 items), bodily pain (2 items), vitality or energy level (4 items), social functioning (2 items), mental health (5 items), general health perceptions (5 items), role limitations because of physical problems (4 items), and role limitation due to personal or emotional problems (3 items) (Bernklev et al., 2005). The toal physical score (PCS) and the total mental score (MCS) were calculated for the present study. The SF-36 has been recommended where a detailed and broad ranging assessment of health is

required, particularly in community dwelling individuals with limited morbidity (Haywood, 2005). The subjects were all asked to complete the SF-36 questionnaire. A copy of the SF-36 is shown in appendix D.

3.2 DATA MANAGEMENT AND BIOSTATISTICAL DESIGN

3.2.1 Data Management

All study subjects were assigned study identifiers that appeared on all data collection instruments, tapes, documents, and files used in the statistical analysis and manuscript preparation. Personal information needed for tracking and informed consent was stored separately from other data with only limited team members having access to that data. No personal information concerning study participants was released without their written consent.

3.2.2 Statistical Analysis

The initial analysis to determine the levels of serum vitamin D concentration in Crohn's disease patients began by describing the demographic (e.g., age, gender, height, weight) and health characteristics of the study population. Descriptive statistics, including measures of central tendency (means, medians, other percentiles) and dispersion (standard deviations, ranges) were computed for continuous data. Graphical displays including histograms and box plots were produced. Transformations were sought for variables to be included in further analyses to ensure

distributional assumptions are met. All statistical analysis were performed using SPSS 14.0 Statistical software (Lead Technologies Inc., Chicago, IL).

To test the hypotheses, the alpha level was set at 0.05. Separate analyses were performed for the each of the independent and dependent variables. Serum vitamin D concentration were the independent variable. Four Pearson correlations were calculated to determine if there is a relationship between serum vitamin D concentrations and muscular strength, fatigue rates, nerve function, and quality of health outcome measures in Crohn's patients.

4.0 RESULTS

The results of the present study are presented in two distinct sections. The descriptive statistics of the muscle strength, for both knee extension and flexion, are presented first, followed by the quadriceps fatigue rates, peroneal nerve function, and the quality of health data respectively. Secondly, the correlation statistics are presented in the aforementioned order of muscle strength, muscle fatigue, nerve function, and quality of health.

4.1 DESCRIPTIVE STATISTICS OF OUTPUT VARIABLES

The descriptive statistics for the demographic variables are summarized in Table 2. A total of 19 Crohn's patients were tested, 9 male and 10 female. The mean age of subjects tested was 43.16 $\pm$ 10.26 years. The Crohn's patients had a mean body mass of 77.295 kg $\pm$ 19.45 kg and a mean height of 66.37 cm $\pm$ 6.01 cm. Mean vitamin D concentration was 32 ng/mL $\pm$ 9.10 ng/mL. The number of small bowel resections and length of disease were obtained through the Inclusion/Exclusion verification form (Appendix B). The Crohn's patients reported having an average of 1.79 resections and an average of 17.71 years with the disease. All statistical analyses were preformed using SPSS 14.0 software (Chicago, IL), with the alpha level set at 0.05.

Table 2 Descriptive Statistics for Demographic Variables (n=19)

Variable	Mean	SD	Min.	Max.
Age (yrs)	43.16	10.26	27.00	62.00
Height (cm)	66.37	6.01	49.00	76.00
Weight (lbs)	170.05	42.79	114.00	260.00
Bowel Resections	1.79	1.80	1.00	7.00
Years with Disease	17.71	12.39	5.00	42.00

4.1.1 Muscle Strength

Examples of the output data collected for a single subject, for muscle strength, of both knee extension and knee flexion, are presented in Figures 4 and 5 respectively. The subjects started in a relaxed state, held their maximum extension or flexion for five seconds, and then ended in a relaxed state again. On these figures, the peak value and the average value were calculated. The descriptive statistics for muscle strength, knee flexion, and extension are presented in Table 3. The mean of the extension peak torque was 75.24 Nm $\pm$ 45.39 Nm and 0.06 Nm/Bw + 0.03 Nm/Bw for peak torque normalized to body weight. The mean for the extension average peak torque was 55.91 Nm $\pm$ 35.55 Nm and the mean for the extension average peak torque was 0.04 Nm/Bw $\pm$ 0.02 Nm/Bw. The mean of the flexion peak torque was 28.94 Nm $\pm$ 12.76 Nm and 0.02 Nm/Bw $\pm$ 0.01 Nm/Bw for flexion peak torque normalized to body weight. The mean for the flexion average peak torque was 20.96 Nm $\pm$ 9.80 Nm and the mean for the flexion average peak torque was 0.02 Nm/Bw $\pm$ 0.01 Nm/Bw.

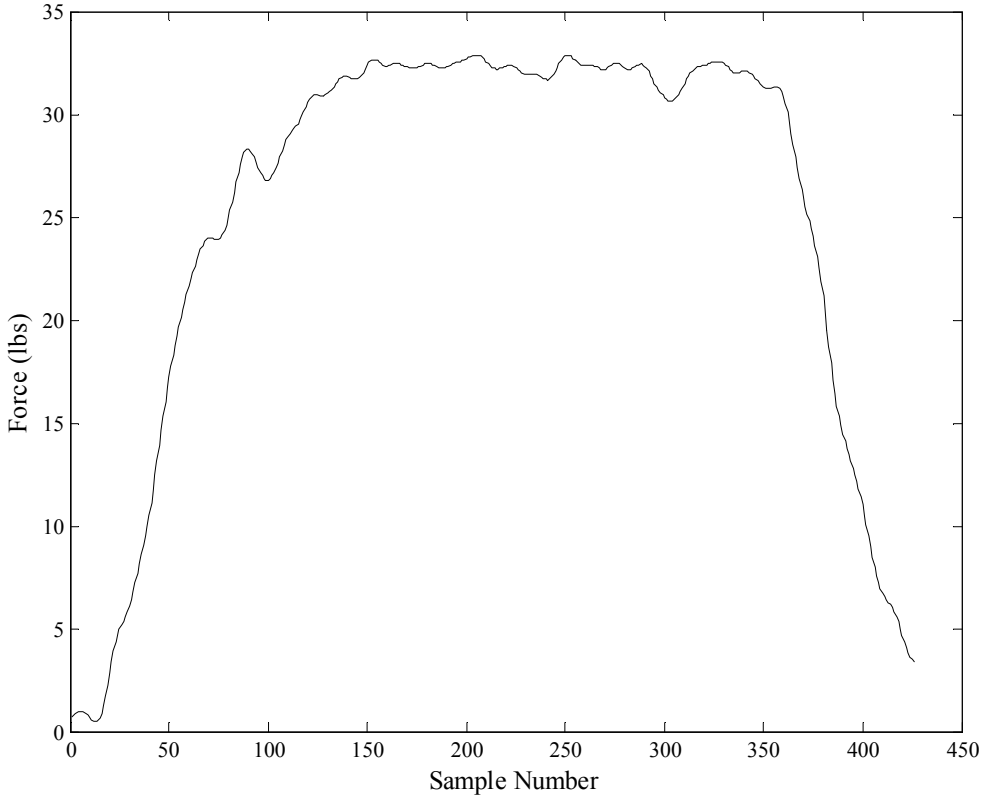

Figure 4 Example output of the knee extension torque raw data for a single subject.

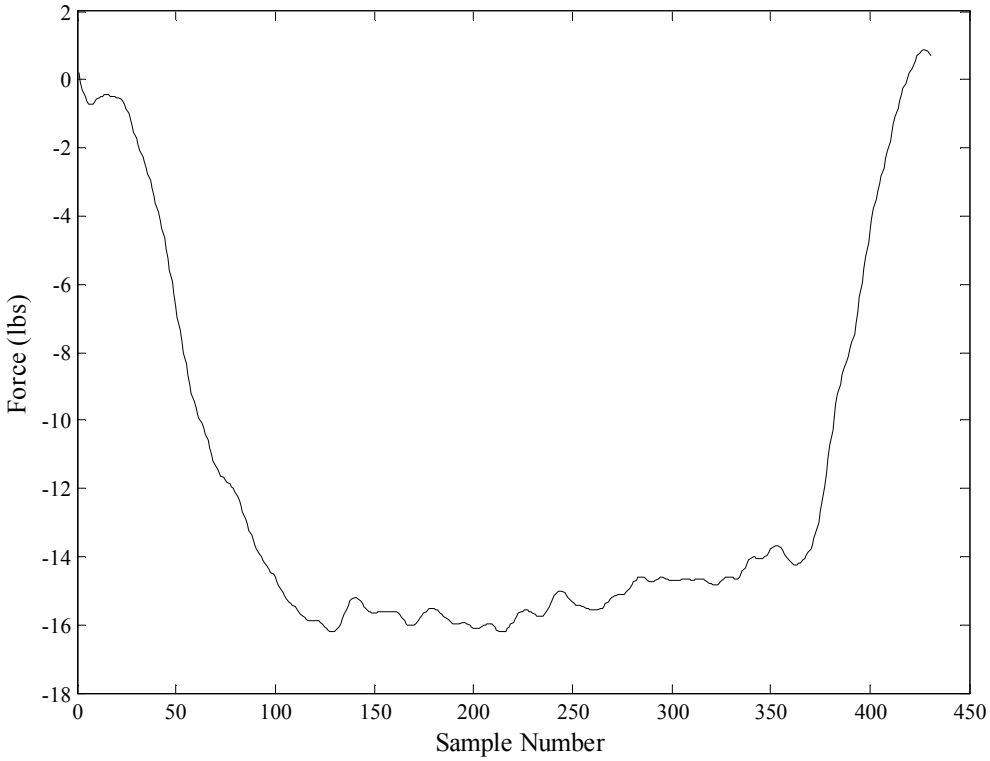

Figure 5 Example output of the knee flexion torque raw data for a single subject.

Table 3 Descriptive Statistics for Muscle Strength (n=19)

Variable	Mean	SD	Min.	Max.
Muscle Strength				
Extension				
Peak Torque (Nm)	75.24	45.39	13.30	206.00
Peak Torque (Nm/Bw)	0.06	0.03	0.02	0.12
Average Peak Torque (Nm)	55.91	35.55	9.30	163.00
Average Peak Torque (Nm/Bw)	0.04	0.02	0.01	0.09
Flexion				
Peak Torque (Nm)	28.94	12.76	5.72	59.70
Peak Torque (Nm/Bw)	0.02	0.01	0.01	0.04
Average Peak Torque (Nm)	20.96	9.80	3.83	45.3
Average Peak Torque (Nm/Bw)	0.02	0.01	0.01	0.03

4.1.2 Muscle Fatigue

Examples of the output data collected for a single subject for the muscle fatigue of the rectus femoris and vastus lateralis are presented in Figure 6. A best fit line was produced from the output data, and the slope of the line determined the fatigue rate. The descriptive statistics for the fatigue rates of the rectus femoris and the vastus lateralis are presented in Table 4. The mean of the fatigue rate for the rectus femoris was -0.07 Hz/sec $\pm$ 0.05 Hz/sec. The mean for the vastus lateralis was -0.03 Hz/sec $\pm$ 0.04 Hz/sec. Only sixteen subjects had complete raw data, due to equipment malfunction; therefore, not all Crohn's patients tested were included in the statistics.

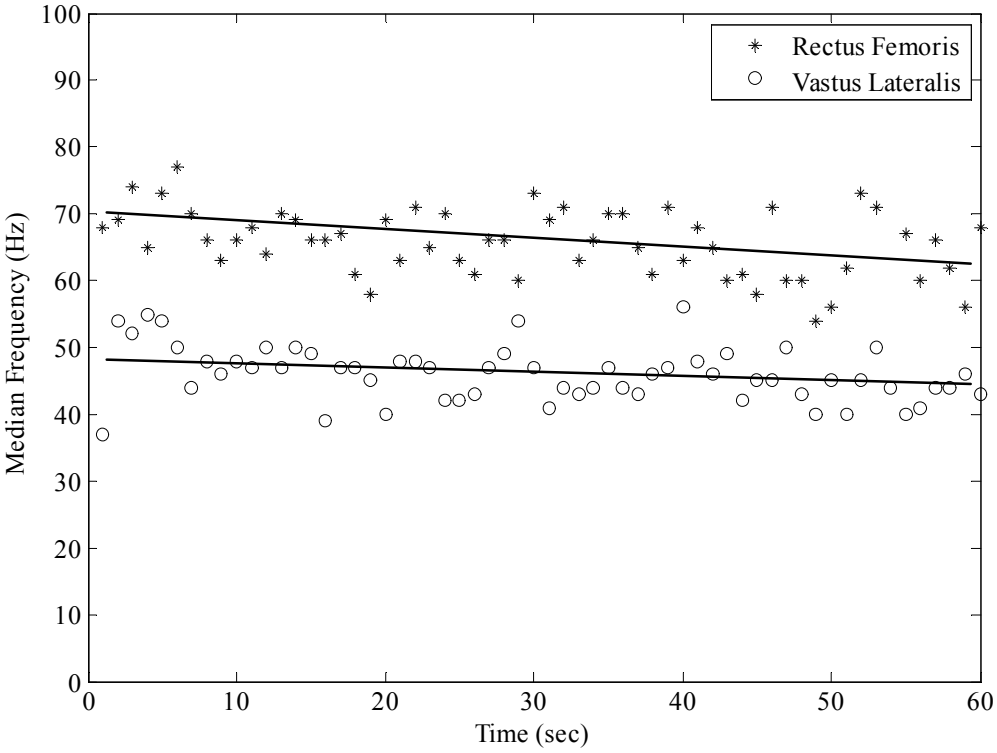

Figure 6 An example of muscular fatigue raw median frequency vs time data from a single subject.

Table 4 Descriptive Statistics for Muscle Fatigue (n=16)

Variable	Mean	SD	Min.	Max.
Fatigue rates (Hz/sec)				
Rectus Femoris	-0.07	0.05	-0.17	0.04
Vastus Larealis	-0.03	0.04	-0.12	0.54

4.1.3 Nerve Function

Data collection for only thirteen of the nineteen subjects tested for nerve function was successfully completed. All six missing subjects reported pain and asked to stop the test prematurely; therefore, there results are not included in the statistics. The mean of the latency for

the peroneal nerve was 4.28 ms ± 0.05 ms. The mean of the amplitude for the peroneal nerve was 2.26 mV ± 2.03 mV. Descriptive statistics for the nerve function are presented in Table 5.

Table 5 Descriptive Statistics for Nerve Function (n=13)

Variable	Mean	SD	Min.	Max.
Nerve Function				
Latency (ms)	4.28	1.75	1.20	7.70
Amplitude (mV)	2.26	2.03	1.40	7.70

4.1.4 Quality of Health

Descriptive statistics for the quality of health measures collected from the SF-36 are presented in Table 6. SF-36 data were collected on eighteen out of nineteen subjects. One subject accidentally missed the survey, therefore resulting in eighteen completed surveys. Specifically measured for the present study were the total physical score (PCS) and the total mental score (MCS). The mean of the PCS was 50.10 ± 10.75. The mean for the MCS was 50.22 ± 12.08.

Table 6 Descriptive Statistics for SF-36 (n=18)

Variable	Mean	SD	Min.	Max.
SF-36				
PCS	50.01	10.71	24.65	64.46
MCS	50.04	12.23	21.60	61.85

4.2 CORRELATIONS BETWEEN VITAMIN D AND OUTPUT VARIABLES

Correlations to determine the relationship between vitamin D levels, 25(OH)D specifically, and muscle strength, muscular fatigue, nerve conduction velocity, and the SF-36 measures were calculated. The alpha level was again set at .05 for all correlations. Vitamin D concentrations, specifically 25(OH)D, for all 19 Crohn's patients was determined. The mean 25(OH)D concentration was 32 ng/mL $\pm$ 9.10 ng/mL.

4.2.1 Muscle Strength

Correlation analyses were performed for both knee extension and knee flexion, and the results are presented in Table 7. No significant ($p = 0.56$) linear relationship was found between vitamin D levels and knee extension peak torque (Nm), in patients with Crohn's disease. No significant ($p = 0.45$) linear relationship was found between vitamin D levels and knee peak torque normalized to body weight. The scatter plot presenting the vitamin D concentrations and extension peak torque is presented in Figure 7. The plotted point for serum vitamin D concentrations at 25 ng/mL and 52 Nm has a frequency of two in Figure 7. No significant ($p = 0.51$) linear relationship was found between vitamin D levels and knee extension average peak torque in patients with Crohn's disease. No significant ($p = 0.39$) linear relationship was found between vitamin D levels and knee average peak torque normalized to body weight in patients with Crohn's disease.

The correlation statistics to determine the relationship between vitamin D levels and knee flexion are also presented in Table 7. No significant ($p = 0.56$) linear relationship was found between vitamin D levels and knee flexion peak torque in patients with Crohn's disease. No significant ($p = 0.34$) linear relationship was found between vitamin D levels and peak torque normalized to body weight in patients with Crohn's disease. The scatter plot for the vitamin D concentrations and flexion peak torque is presented in Figure 8. No significant ($p = 0.60$) linear

relationship was found between vitamin D levels and knee flexion average peak torque in patients with Crohn's disease. No significant ($p = 0.42$) linear relationship was found between vitamin D levels and knee average peak torque normalized to body weight in patients with Crohn's disease.

Table 7 Correlation Statistics between Vitamin D Levels and Muscle Strength (n=19)

	r value	p
Muscle Strength		
Extension		
Peak Torque (Nm)	0.14	0.56
Peak Torque (Nm/Bw)	0.19	0.45
Average Peak Torque (Nm)	0.16	0.51
Average Peak Torque (Nm/Bw)	0.21	0.39
Flexion		
Peak Torque (Nm)	0.14	0.56
Peak Torque (Nm/Bw)	0.23	0.34
Average Peak Torque (Nm)	0.13	0.60
Average Peak Torque (Nm/Bw)	0.20	0.42

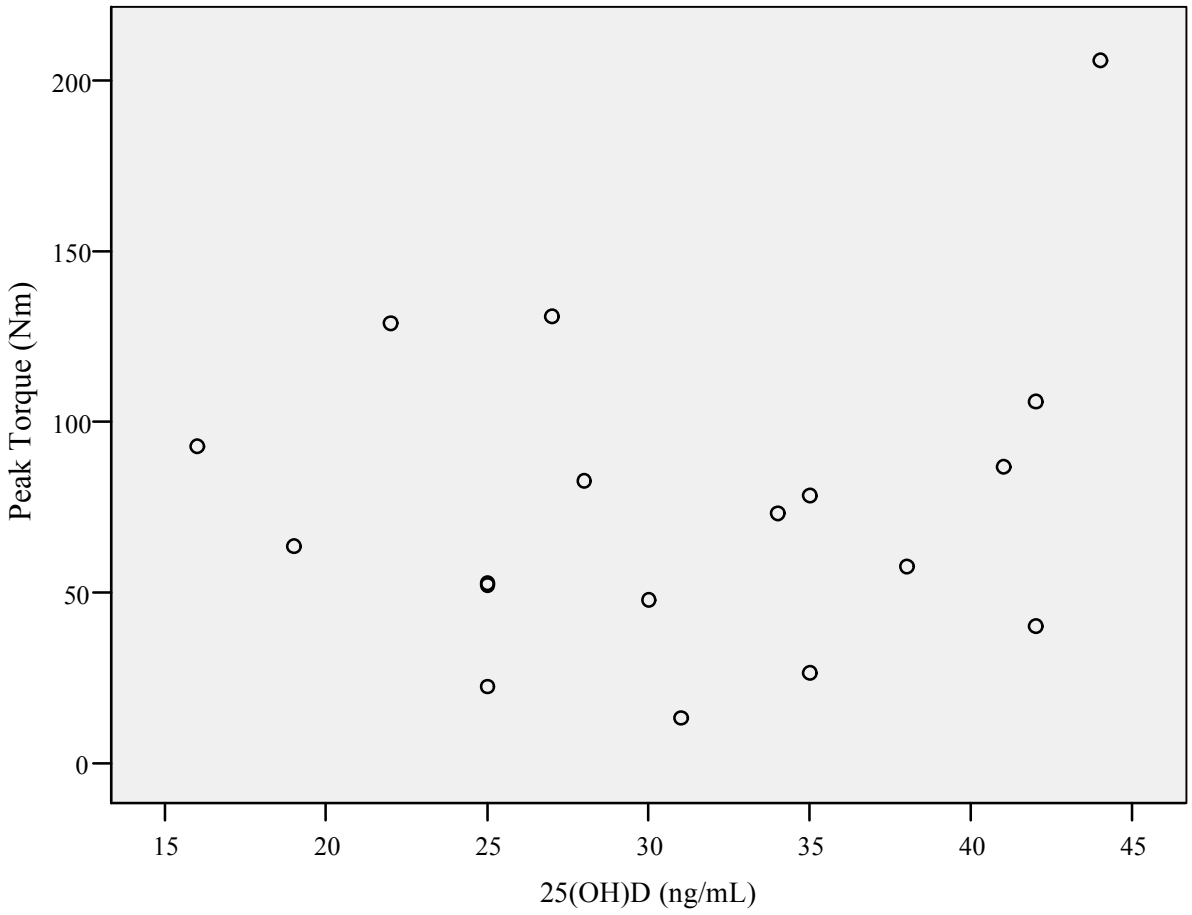

Figure 7 Scatter plot for vitamin D 25(OH)D levels and knee extension peak torque

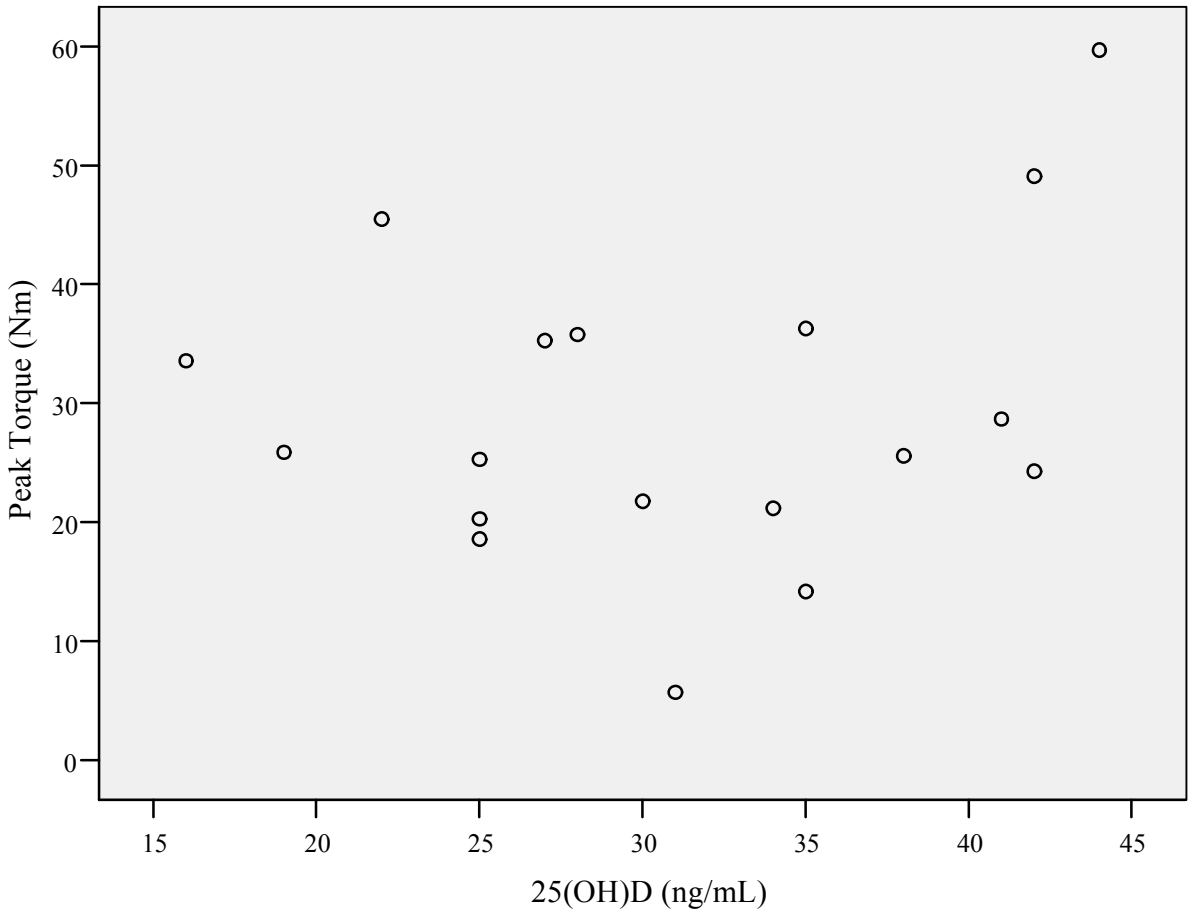

Figure 8 Scatter plot for vitamin D 25(OH)D levels and knee flexion peak torque

4.2.2 Muscle Fatigue

Correlation analyses were also performed for measures of vitamin D and muscle fatigue of the rectus femoris and vastus lateralis muscles. Results for the correlations of muscle fatigue are presented in Table 8. The assumption of normality was not met for the fatigue rates of the rectus femoris, therefore, the two outliers were removed. When the outliers were removed, the assumption of normality was met, however, the results of the correlation did not change. No significant linear relationship was found between the vitamin D levels and the fatigue rates of the rectus femors ($p = 0.62$) and the vastus lateralis ($p = 0.96$) respectively, in patients with Crohn's

disease. The scatter plots for the vitamin D concentrations and the rectus femoris and vastus lateralis are presented in Figures 9 and 10, respectively.

Table 8 Correlation Statistics between Vitamin D Levels and Muscle Fatigue

Variable	n	r value	p
Fatigue Rates (Hz/sec)			
Rectus Femoris	14	0.15	0.62
Vastus Lateralis	16	-0.02	0.96

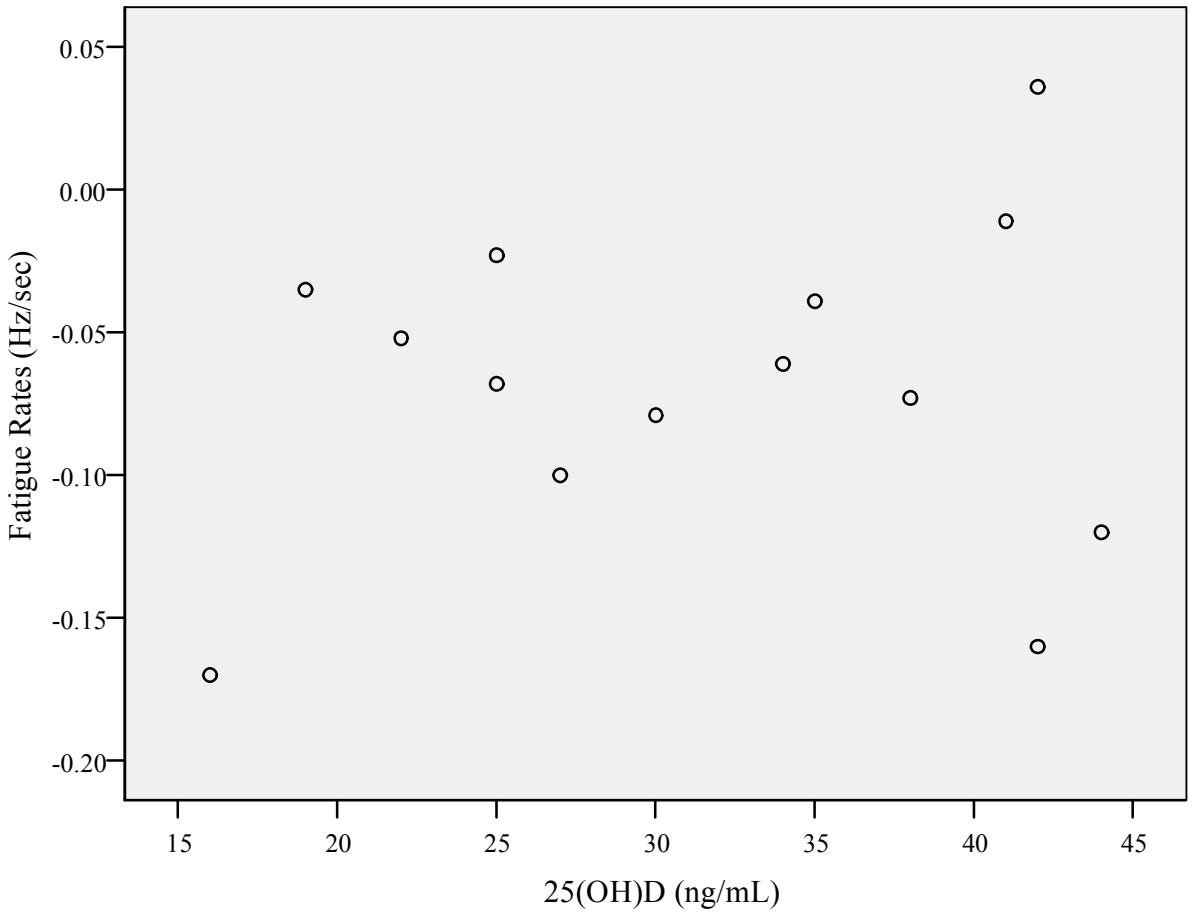

Figure 9 Scatter plot for vitamin D 25(OH)D levels and rectus femoris fatigue rate

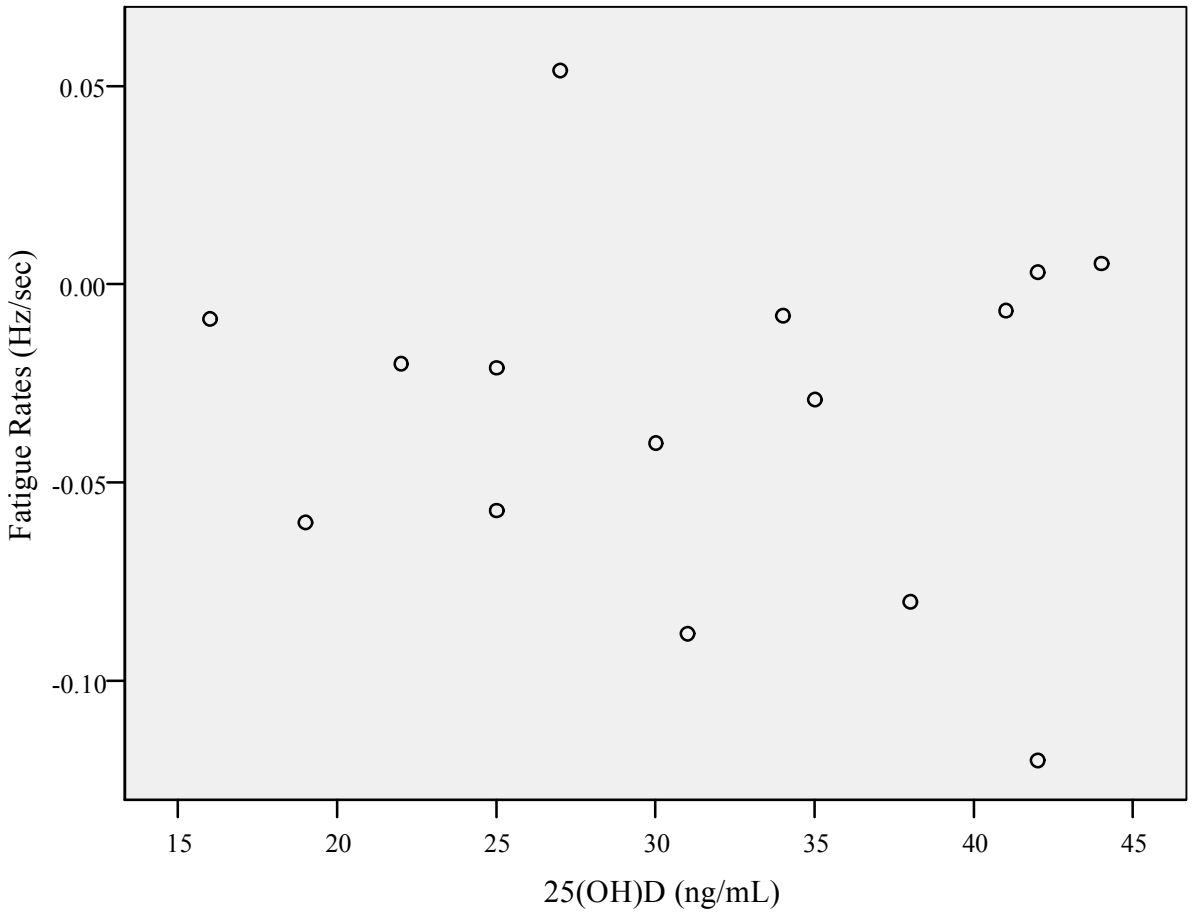

Figure 10 Scatter plot for the vitamin D and vastus lateralis fatigue rate data

4.2.3 Nerve Function

Correlation analyses for nerve conduction velocity were performed on the peroneal motor nerve for both latency and amplitude in 13 subjects and are presented in Table 9. Nerve conduction velocity was not available for all of the subjects tested; therefore, the analysis was run on the 13 subjects on which nerve function was successfully collected. No significant ($p = 0.40$) linear relationship was found between the vitamin D levels in patients with Crohn's disease and the latency of nerve conduction velocity. The data for the amplitude of the peroneal nerve did not meet the assumptions of normality; therefore, a log transformation was performed. No

significant (p = 0.99) linear relationship was found between the vitamin D and the log amplitude of the peroneal nerve in patients with Crohn's disease. The scatter plots for the vitamin D concentrations and the nerve function of the latency and log amplitude are presented in Figures 11 and 12, respectively.

Table 9 Correlation Statistics between Vitamin D Levels and Nerve Function (n=13)

Variable	r value	p
Nerve Conduction Velocity		
Latency (ms)	-0.26	0.40
Ampliturde (mV)	0.00	0.99

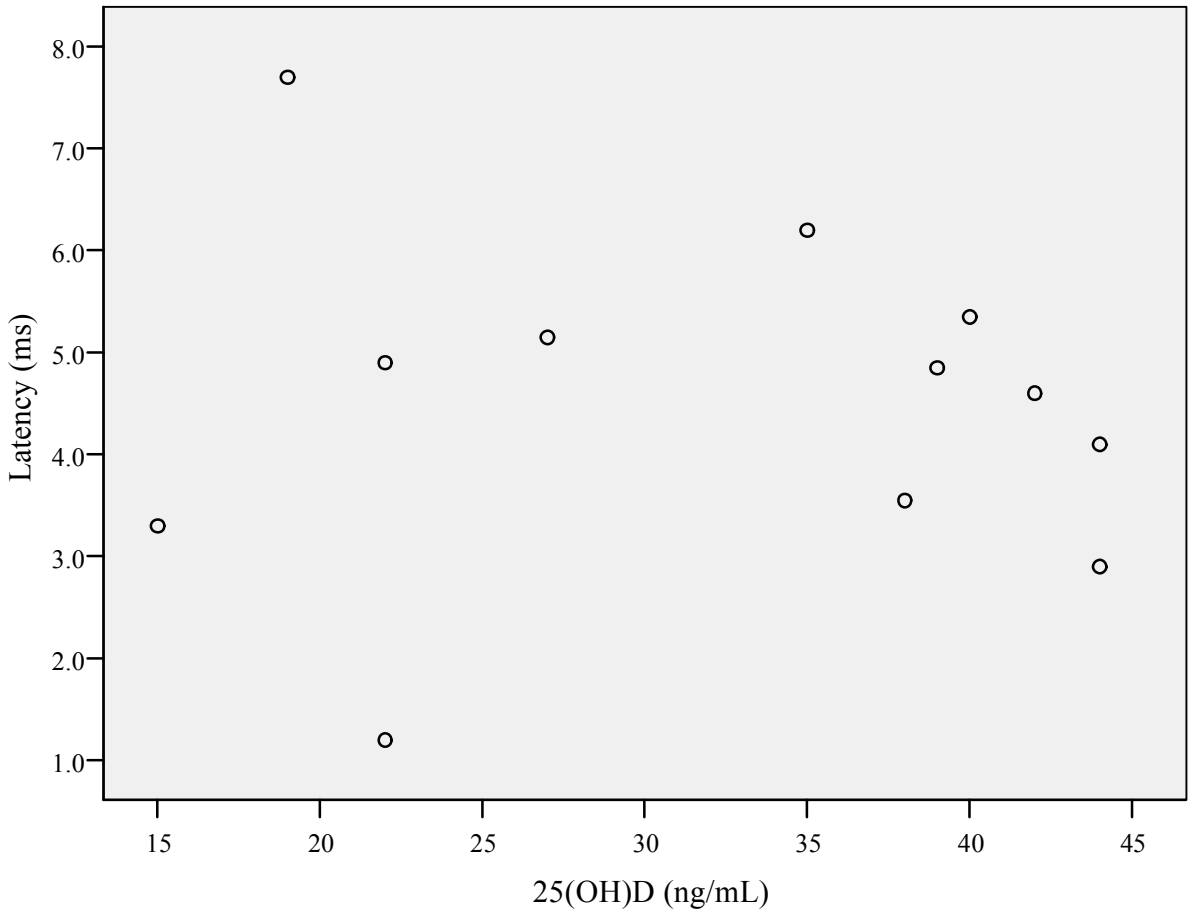

Figure 11 Scatter plot for vitamin D 25(OH)D levels and latency of the peroneal nerve

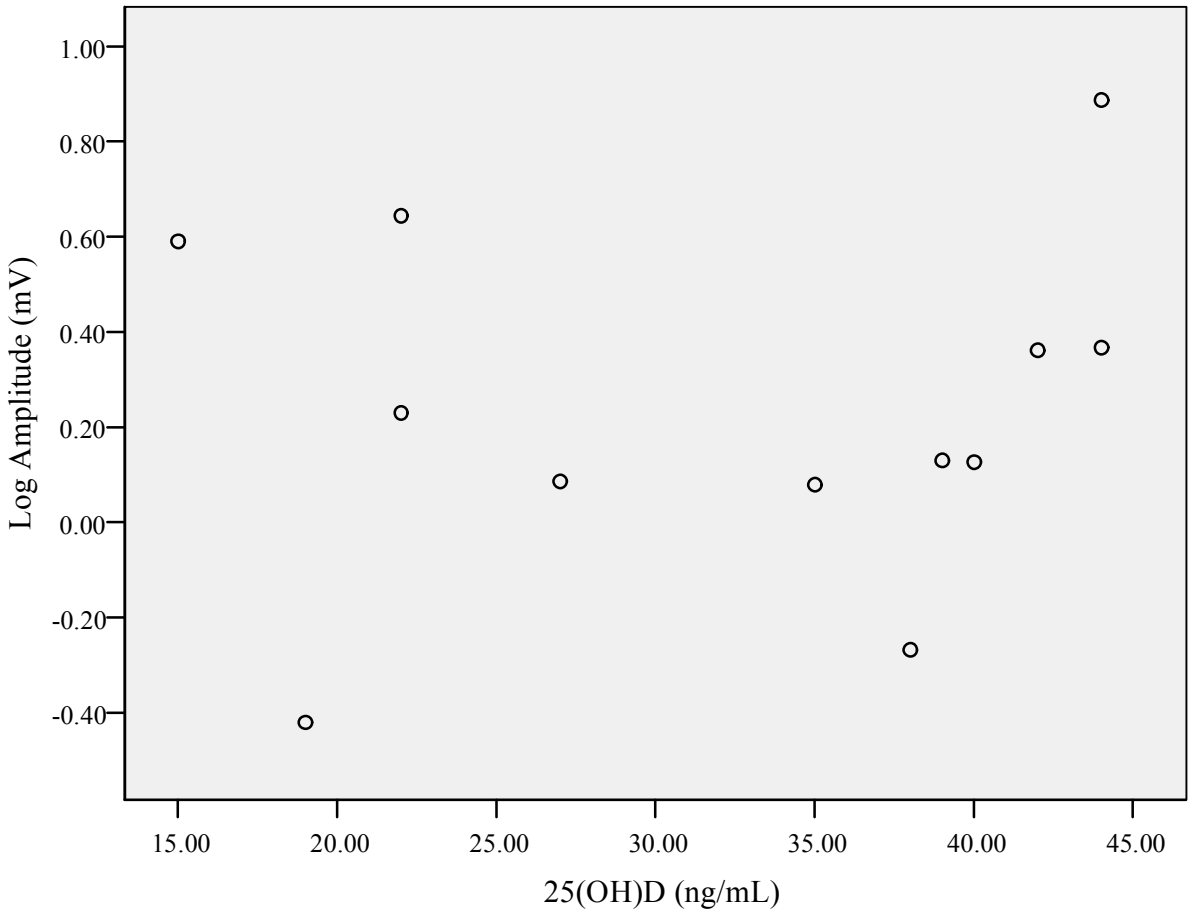

Figure 12 Scatter plot for vitamin D 25(OH)D levels and the log amplitude of the peroneal nerve

4.2.4 Quality of Health

Two correlation analyses were performed to look at the relationship between the levels of vitamin D and the SF-36 in Crohn's disease patients. Specifically measured was the total physical score (PCS) and the total mental score (MCS), respectively. A significant ($p= 0.02$) linear relationship was found between vitamin D and the total physical score in patients with Crohn's disease. No significant ($p = 0.70$) linear relationship was found between the total mental score in patients with Crohn's disease. The correlation statistics to determine the relationship

between vitamin D levels and outcome measures of the SF-36 are represented in Table 10. The scatter plots for the vitamin D concentrations and the PCS and MCS measures of the SF-36 are presented in Figures 13 and 14, respectively.

Table 10 Correlation Statistics between Vitamin D Levels and SF-36 Measures (n=18)

Variable	r value	p
SF-36		
Total Physical Score (PCS)	0.55	0.02
Total Mental Score (MCS)	-0.10	0.70

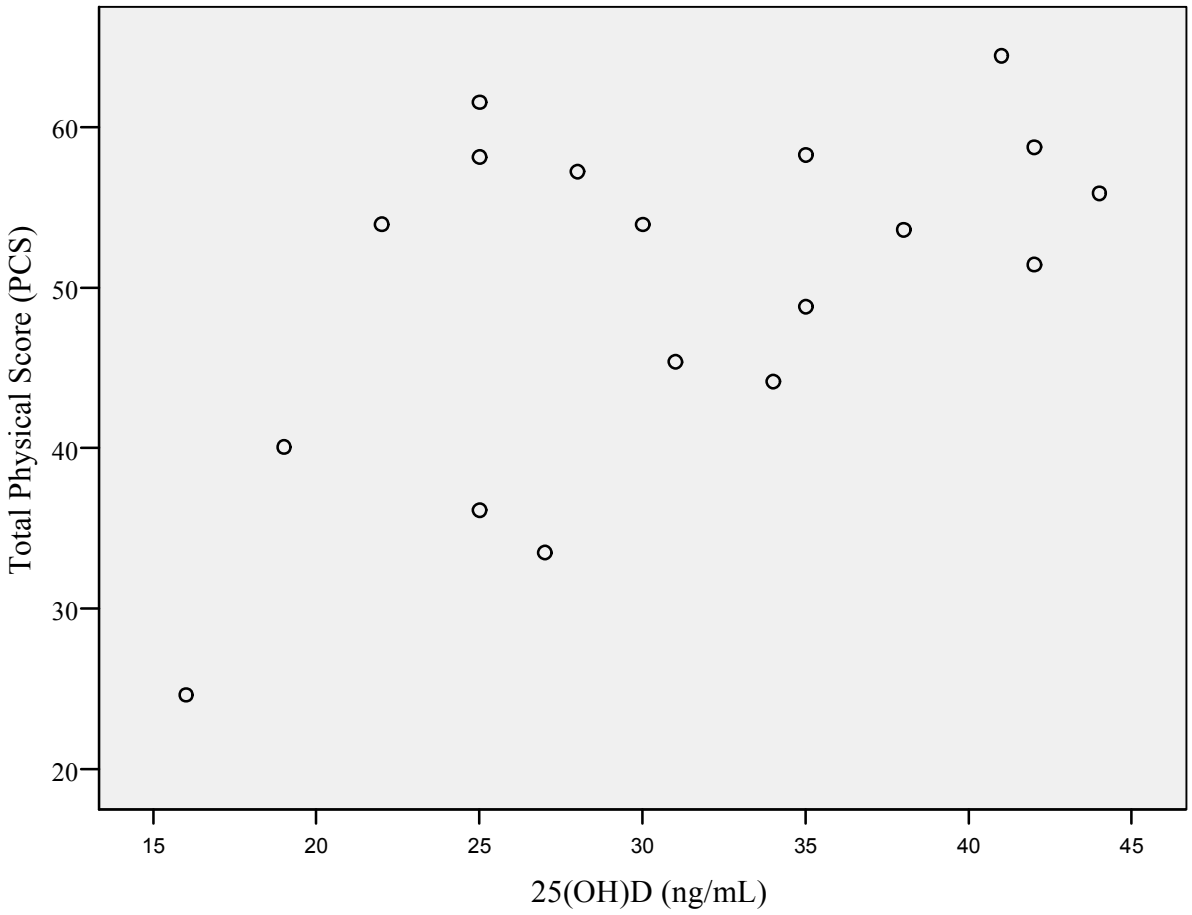

Figure 13 Scatter plot for vitamin D 25(OH)D levels and total physical score

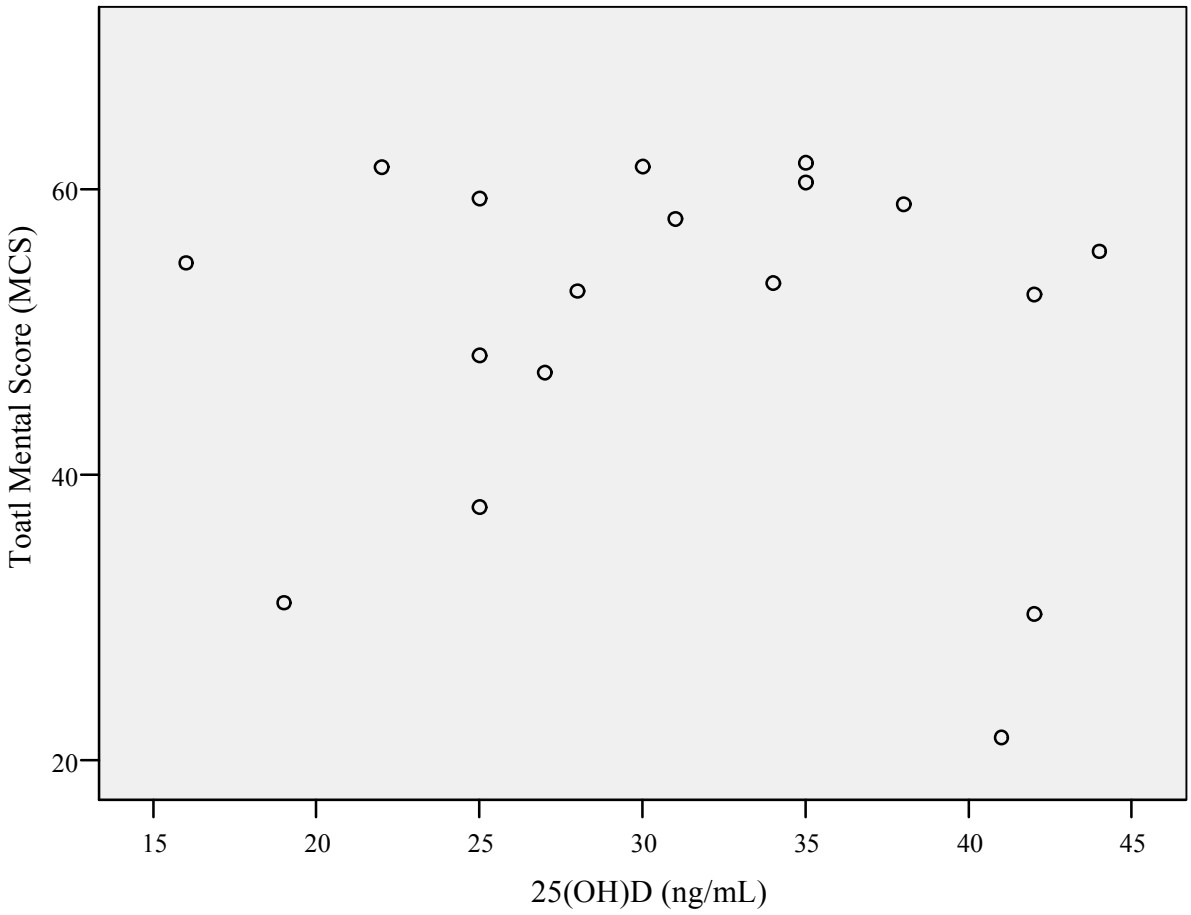

Figure 14 Scatter plot for vitamin D 25(OH)D levels and the total mental score

5.0 DISCUSSION

Low vitamin D status has been implicated in the etiology of autoimmune diseases (Cantora & Mahon, 2004). Deficiency of vitamin D, as assessed by low serum concentrations of the major circulating metabolite, 25-hydroxyvitamin D (25(OH)D), has been found to be common in Crohn's patients with small bowel resections (Haderslev et al., 2003). Hypovitaminosis D has also been related to poor neuromuscular function in the elderly and other populations (Bischoff-Ferrari, 2004; Janssen, 2002: Pfeifer, 2000 & 2004). Therefore, the purpose of this study was to determine if a relationship exists between vitamin D levels in patients with Crohn's disease and measures of muscle strength, muscular fatigue, nerve function, and qualities of health.

Serum 25(OH)D levels are the best indicators to define vitamin D deficiency, insufficiency, hypovitaminosis, sufficiency, and toxicity (Zitterman, 2003). The current study indicated that circulating 25(OH)D concentrations were generally not low in our sample of patients with Crohn's disease. These results are similar to the findings reported by Tajika et al. (2004) and Berstein and Leslie (2004). However, it is possible that significant relationships were not found due to the small sample size of the study.

The pathogenesis of vitamin D deficiency in Crohn's disease is considered to be multifactorial. These factors include (1) insufficient dietary intake and inadequate sun exposure, (2) malabsorption of the vitamin D metabolites, and (3) faulty conversion of these metabolites (Tajika et al., 2004). The current study showed that the average of vitamin D serum

concentrations was not deficient in the sample of Crohn's patients with small bowel resections. Tajika et al. (2004) concluded that no correlation was found between Vitamin D levels and the anatomical length of the small bowel resection, but the origin of where the small intestine had been injured may be a better indicator of deficiency. Origin of injury was not collected in the present study.

The incidence of vitamin D deficiency in the present study was found to be low. According to Grant and Holick (2005) (refer to Table 1), serum 25(OH)D concentration levels of less than 20 ng/mL are considered deficient, 20-32 ng/mL insufficient, and between 33-100 ng/mL sufficient. Therefore for the current study, only 10.5% of the Crohn's patients tested had deficient levels of vitamin D concentrations and 36.8% of the Crohn's patients tested were insufficient.

Although, the current study did not find a high rate of vitamin D deficiency in Crohn's disease patients, Jahnsen et al. (2002) concluded that hypovitaminosis D was a common finding in patients with Crohn's disease. Jahnsen et al. (2002) further suggested that Crohn's patients be examined for vitamin D deficiency regularly. The role of vitamin D in inflammatory bowel disease is controversial, and some data suggest that overt osteomalacia, the distinguishing characteristic of vitamin D deficiency, is infrequent (Bernstein, & Leslie, 2004). Nonetheless, the importance of vitamin D as a general approach to osteoporosis prevention, treatment, and cancer remission, still remains.

Many factors affect vitamin D deficiency, such as food intake, physical activity, and sun exposure. The design of the present study did not control many of these factors, resulting in investigative limitations. However, subjects were excluded from the present study if a tanning bed was used within six months prior to the beginning of the study. Exposure to natural sunlight

was not controlled. Vitamin D intake through fortified foods was also not included as a factor within the present study, which could be another limiting factor.

Other researchers have reported that Crohn's patients have decreased muscle function, especially in their lower limbs (Wiroth et al., 2005). Wiroth et al. (2005) concluded that Crohn's disease patients in clinical remission experience a decrease in skeletal muscle strength and endurance. Developing peak strength requires optimizing the neural drive to activate the maximum number of motor units. However, in the current study, there were no correlations between 25(OH)D serum vitamin D levels and muscular strength, fatigue, and nerve function in the Crohn's disease patients tested.

The present study did not compare Crohn's patients to healthy controls. Compared to a normal population, the muscular strength of the Crohn's patients tested would be considered low. Silva et al. (2003) tested knee isometric muscle strength at the same 45 degree angle as tested in the present study, and the average extension torque was 105.9 $\pm$ 35.2, and a flexion peak torque of 63.9 $\pm$ 32.8. The average knee extension torque for the Crohn's patients was 75.24 $\pm$ 45.39 and average flexion peak torque was 28.94 $\pm$ 12.76 (refer back to Table 3). Therefore, the fatigue rate of the patients tested may not have been as high because a subject in this study would have not exerted as much force to hold a percentage of 60% of their maximum strength. Recently collected data on community dwelling healthy individuals aged 65-70 years, tested with the same equipment used in the present study, had an average of extension peak torque of 94.0 Nm $\pm$ 28.1 Nm and an average flexion peak torque of 37.0 Nm $\pm$ 17.8 Nm. The Crohn's patients in the current study had a lower average for both flexion and extension peak torque (refer to Table 3). No correlation was found between vitamin D concentrations and muscle strength, however the Crohn's patients were low in strength, and the serum vitamin D was not responsible, therefore

something else was responsible for the decreased muscle strength. Future studies should compare normal healthy controls to patients with Crohn's disease to determine if a difference exists in regard to muscle strength, muscle fatigue, nerve function, and quality of health.

Although no correlations were found between serum vitamin D concentrations and most of the dependent variables, there was an association between the vitamin D concentrations and the total physical score from the SF-36. The vitamin D concentrations explained approximately 30 percent of the variability in total physical component of the quality of health in patients with Crohn's disease (refer to Table 10). Therefore, the lower the vitamin D, the lower the physical score of the Crohn's patients' quality of health.

Although vitamin D concentrations were not associated with muscle strength and muscular fatigue, they were correlated with the total physical score of the quality of health. The dependent variables tested in the current study should have explained the measures found in the SF-36, but vitamin D was not associated with muscle strength or muscular fatigue. For the purposes of this discussion post hoc correlation analyses were run to determine if the total physical score of the SF-36 is associated with our quantitative measures of physical function. Of these, only the fatigue rates of the rectus femoris ($p = 0.04$) was associated with the total physical score of the SF-36. The fatigue rate of the rectus femoris explained approximately 32 percent of the variability in the total physical component of the SF-36. Recall that there was no association between the fatigue rate of the rectus femoris and the serum vitamin D concentrations referred to in Table 8. The Venn diagram in Figure 15 illustrates the relationship between serum vitamin D concentrations, the total physical score on the SF-36, and the rectus femoris fatigue.

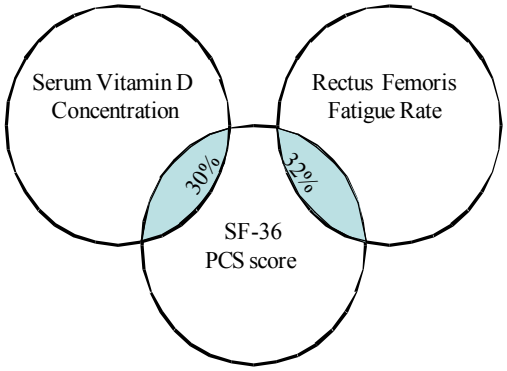

Figure 15 Venn diagram illustrating the relationship among serum vitamin D levels, the total physical score from the SF-36, and the fatigue rates of the rectus femoris.

Healthy controls were not included in the present study due to many confounding variables such as medications, surgery, and disease activities. These confounding variables differ between groups of healthy controls and patients with Crohn's disease. However, in comparing nine of the subjects that were deficient in vitamin D and the ten subjects tested that were sufficient, the difference in the confounding variables does not exist. Therefore, for the purposes of this discussion, the nine deficient serum vitamin D Crohn's patients were qualitatively compared with the ten sufficient Crohn's patients. Contrary to the expected result, the deficient group displayed 16.4% greater knee extension peak torque than the sufficient group. This relationship is noted in the other strength variables and shown in Figure 16 as well. The results were not compared statistically because of the low sample size and large dispersion.

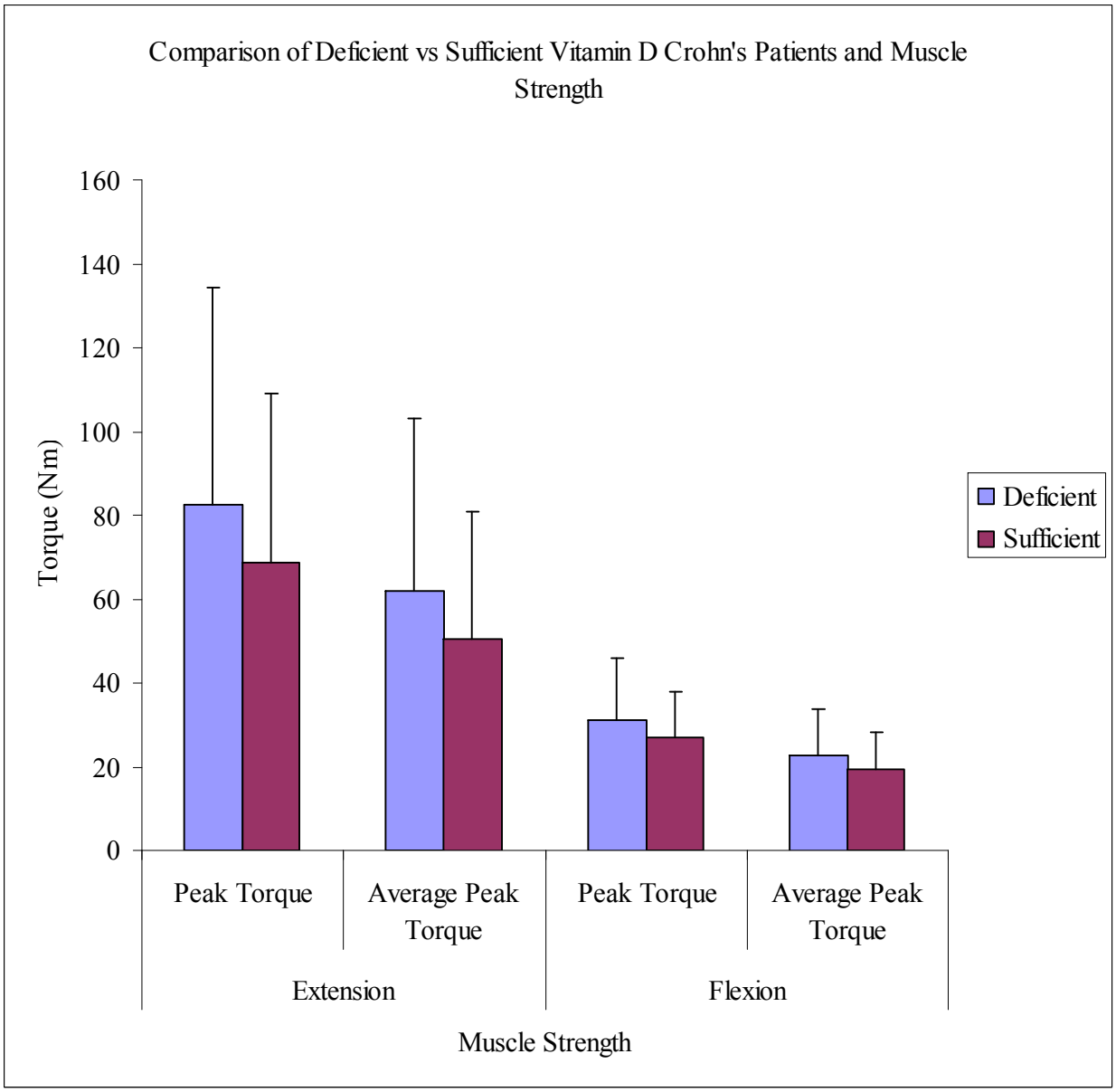

Figure 16 Comparison of deficient vs sufficient vitamin D levels in Crohn's patients and muscle strength.

Future studies should include an adequate number of subjects to determine correlations. The necessary sample size to obtain power of 80% was found for the current study using Power Analysis and Sample Size Software (PASS). Using the obtained r values, current sample size, and an alpha level of 0.05, the number of subjects needed for 80% power for each of the dependent variables was determined. Regarding the majority of the variables tested, the numbers

of subjects required to find significant correlations are feasible for a larger funded study, but were not feasible within the funding constraints of a dissertation. For example, extension, peak torque normalized to body weight and average peak torque normalized to body weight, and flexion, peak torque normalized to body weight and average peak torque normalized to body weight, would need an estimated sample size of 37, 30, 26, and 33 subjects respectively to decrease the chance of making a type II error. The estimated sample size for the latency of the peroneal nerve was estimated at twenty one subjects.

It was hypothesized that serum vitamin D levels would be correlated to muscular strength, fatigue and nerve function, all of which would increase the quality in life in patients with Crohn's disease. However, no correlations were found between serum vitamin D levels and the dependent variables, with the exception of total physical functioning. The average vitamin D levels of the Crohn's disease patients tested in the current study were not low. In may be that vitamin D only influence muscle function in severely low individuals. Future studies may need to prescreen Crohn's patients for low vitamin D status prior to testing the dependent variables in order to reveal a correlation.

According to the present study, there is currently no recommended basis for requiring the testing of vitamin D. However, as mentioned previously, this result may be due to the small sample size and many uncontrolled factors. As a result of the conflicting findings of other studies (Bernstein & Leslie, 2004) indicating low vitamin D status commonly found in Crohn's patients, more research on the serum vitamin D concentrations and muscular strength, muscular fatigue, nerve function, and qualities of health in patients with Crohn's disease need to be performed. Further research is required to identify the exact mechanism of Crohn's patients' decreased strength and fatigue.

APPENDIX A

CONSENT TO ACT AS A SUBJECT IN A RESEARCH STUDY

TITLE: The Association of Vitamin D on Neuromuscular Function in Patients with Crohn's Disease

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Why is this research being done?

The purpose of this study is to determine the association of vitamin D on nerve and muscular function in patients with Crohn's disease. Ultimately, we intend to determine if vitamin D levels are lower in patients with Crohn's disease and if there is a relationship between vitamin D and muscular strength, endurance, how hard you perceive the test to be, and nerve function.

Crohn's disease is a subcategory of disorders collectively known as Inflammatory Bowel Disease (IBD). Crohn's disease is a chronic, relapsing, transmural inflammation of any portion of the digestive tract. The ulcerations and inflammation of the intestinal wall caused by Crohn's disease is primarily found in the terminal ilieum and/or colon, and can be interspersed with healthy tissue. Neuromuscular fatigue is commonly found in patients with inflammatory bowel disease, especially in Crohn's disease patients.

The causes underlying the neuromuscular fatigue are not clearly understood. Although muscle pain and bone pain is a common complaint of Crohn's disease patients, the decrease in neuromuscular function is unknown. A number of factors are associated with the possible decreased function in Crohn's disease patients, such as disease activity, drug therapy, calcium and vitamin D deficiency, loss of nutrients through increased bowel movements, and overall poor nutrition.

Understanding the relationship between serum vitamin D levels and muscular strength, excessive fatigue, nerve conduction velocity, and quality of health in Crohn's patients will lead to proper treatment to increase vitamin D levels and neuromuscular function. There is currently not an objective basis for recommended dietary intake levels of vitamin D for adults, or for optimal levels of serum vitamin D, especially for those with Crohn's disease. Vitamin D testing and supplementation in the future may cause an increase in neuromuscular function and improve the quality of health and quality of life in patients with Crohn's disease.

Who is being asked to participate in the research study?

Forty six individuals between the ages of 30 and 65, twenty three with Crohn's disease and at least one small bowel resection and twenty three controls (healthy individuals who do not have Crohn's disease) will be asked to participate.

What procedures will be performed for research purposes?
Subjects will be screened for eligibility in the study by filling out an inclusion and exclusion verification form. In this form, questions will be asked whether you have any major illness preventing you from participating in the study. If you decide to take part in this research study, you will undergo the following procedures listed below:

Experimental Procedures:
Eligible subjects will report to the Inflammatory Bowel Disease (IBD) Center in the UPMC Presbyterian Hospital and will be escorted to the Hand Research Laboratory at the Biomedical Science Tower for a single testing session lasting approximately 90 minutes. After the experimental methods are explained to you, you will be asked to sign this informed consent which will take approximately fifteen minutes and agree to participate in the study. You are encouraged to ask questions as they arise.

Idiopathic Musculoskeletal Scale:

The idiopathic musculoskeletal scale is a visual scale used to determine if you experience pain in your muscles. You will be asked to mark an X on the line where you feel best describes your pain level. The scale will take approximately two minutes to complete.

SF-36:

You will be asked to complete a questionnaire called SF-36 survey. The questionnaire measures your qualities of health and general health perceptions. The SF-36 will take approximately 10 minutes to complete. The SF-36 will take approximately ten minutes to complete.

Blood Draw:

A person licensed to draw your blood from your arm at the Inflammatory Bowel Disease Center at Presbyterian Hospital, Pittsburgh, PA. The amount of blood taken is 15 ml, or 4 teaspoons. The blood draw procedure will take approximately five minutes.

Blood samples will be under the control of the principal investigator of this research project. Any personal identifiers such as name, social security number, and/or birth date will be removed and replaced with a specific code number. The information linking these code numbers to the corresponding subjects' identities will be kept in a separate, secure location or if the data will be anonymous. The investigators on this study will keep the samples. The principal investigator will keep the sample for the length of the study and will not be utilized in future studies. The samples will be sent to Boston University School of Medicine under the direction of Dr. Michael Holick, Ph.D., MD to be analyzed for vitamin D levels.

Subject Preparation:
Two small muscle activity sensors (surface electrodes) to measure the activity in your muscles will be attached to the skin over your quadriceps (the large muscle on the front of the thigh) on your left side. The surface electrodes are similar to small Band-Aids. A small square will be shaved (if necessary), scraped with an emery board, and wiped with rubbing alcohol to clean the area, allowing the transfer of signals between the skin and the electrodes. A third electrode will

be placed on your shin bone. Preparation will take approximately fifteen minutes and will be preformed by the principal invesigator.

The Strength Test:

You will be seated on the testing device with the force-sensing arm secured to your ankle and Velcro™ straps crisscrossing your body to eliminate any unnecessary motion. You will extend your left leg as hard as you can for five seconds, immediately after which you will rest for thirty seconds and repeat this action two times for a total of three. You will be asked to bend your left knee at your maximal effort level. Again, you will perform three five-second contractions with thirty seconds of rest between each contraction of the left leg. The strength test will take approximately ten minutes and will be performed by the principal investigator.

The Fatigue Test:

The next activity is to look at how your muscle tires during exercise. A small transmitter will measure the activity of your quadriceps muscle. It will transfer the signals from the electrodes to a receiver attached to a computer.

In order to test for fatigue, you will again be seated for a knee extension test in the strength chair. You will be asked to hold your left leg at 60% of your maximal strength (this value is determined by averaging the three extension values from the strength test). You will be seated facing a computer screen that will have two lines on it. The first line will be a completely horizontal line and represents the 60% level. The second line (the force line) moves as you extend your leg. When the trial begins, you will follow the force line until you reach the 60% line. You will then hold your leg position so that these two lines stay as close to one and other as possible for the sixty-second test. The length of time for the fatigue test is one minute, totaling ten minutes for explanation, set-up, and testing time and will be performed by the principal investigator.

Ratings of Perceived Exertion:

During the muscular fatigue test, we will ask you to rate your perceived exertion, or to rate how much effort you are exerting or giving. This will be done by choosing a number from one to ten on a scale with numbers and pictures to best describe the amount of effort you are giving. While seated in the chair, the scale will be described to you. We will ask you to rate your effort during the test at 0 seconds, 30 seconds, and 60 seconds of the muscular fatigue test. Length of time for the perceived exertion is included in the fatigue test.

Nerve function test:

Next we will be looking at the nerve function, or how fast your nerve sensations travel. Small electrodes will be placed on your leg and connected to a transmitter which will send small pulses to your nerves.

While seated in a comfortable chair, we will ask you to remove your shoe and sock of your left foot. We will clean and wipe the area where the electrodes will be placed on your left leg with an alcohol solution. Then when you are ready, we will send an impulse from the transmitter to the nerves in your leg and foot through the electrodes on your skin. The electrodes will measure the time it takes the nerve impulse to travel through your leg. We will perform this nerve test on two different nerves, a sensory nerve and a motor nerve, to find out how quickly the

impulse travels. The length of test will take around 12-15 minutes and will be performed by the principal investigator.

What are the possible risks, side effects, and discomforts of this research study?
We do not anticipate any injuries and the risks of participation are small. It is possible as with any experiment that harmful effects may occur. If in fact injury does occur, the investigators listed on the front page of this form will administer immediate and appropriate first aid care.

Risks for blood analysis:
There are a few possible risks of blood draw. Some common risks may include pain, bleeding, and the possibility of bruising at the site of the blood draw or the feeling of lightheadedness (occurs in 1-25%, or 1-25 people out of 100), and rarely infection at the site of the blood draw (occurs in less than 1% or less than 1 out of 100 people).

Risks for muscular strength testing:
The occurrence of slight muscle soreness is common because of the muscle contracting exercise. This mild soreness typically develops 2-3 days after the experiment and will last approximately 2-3 days.

Risks for muscle fatigue testing:
Skin irritation, redness, or chafing following removal of the electrodes is rare (occurs in less than 1% – less than 1 out of 100 people). In the event that skin irritation, redness, or chaffing does occur, the symptoms typically disappear within 24 hours.

Risks for nerve function testing:
The occurrence of slight nerve discomfort is common because of the small shock to your nerve. This mild shock typically lasts for a few seconds after the experiment.
You may become tired during the testing. If you do, you are permitted to rest as long as you need to, or to stop participating in this study, at any time.

What are the possible benefits from taking part in this study?
You may not derive personal benefit from this study, but knowledge may be gained about the association of vitamin D on neuromuscular function.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?
You will be promptly notified if any new information develops during the conduct of this research study that may cause you to change your mind about continuing to participate.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?
Neither you nor your insurance provider will be charged for participation in this study.

Will I be paid if I take part in this research study?
You will not be paid to participate in this study.

Who will pay if I am injured as a result of taking part in this study?
University of Pittsburgh investigators and their associates who provide services at UPMC recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or her advisor, Dr. McCrory, listed on the cover sheet of this form.
Emergency medical treatment for injuries solely and directly relating to your participation in this research will be provided to you by UPMC. It is possible that UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless otherwise stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

Who will know about my participation in this research study?
Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these numbers with your identity will be kept separate from the research records. Your research records will be maintained for at least 5 years following study completion, as per University policy. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission (release).

Who will have access to identifiable information related to my participation in this research study?
In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:
Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information for the purpose of monitoring the appropriate conduct of this research study.
In unusual cases, the investigators may be required to release identifiable information related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

Is my participation in this research study voluntary?
Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed, in general, to participate in the research study.) Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on

your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

If I agree to participate in this research study, can I be removed from the study without my consent?
You may be removed from this study if, for example, you are unable to perform any of the tests in this study.

VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668). By signing this form, I agree to participate in the research study. A copy of this consent form will be given to me.

_____
Participant's Name (Print)

_____ _____
Participant's Signature Date

CERTIFICATION OF INFORMED CONSENT
I certify that I have carefully explained the nature and purpose of this research study to the above named individual(s) and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise.

_____ _____
Printed Name of Person Obtaining Consent Role in Research Study

_____ _____
Signature of Person Obtaining Consent Date

APPENDIX B

INCLUSION/EXCLUSION VERIFICATION FORM

Name: _____ Date: _____

Please answer the following questions:

		Yes	No
1.	I am participating in the UPMC Center of Inflammatory Bowel Disease (IBD) Research Registry	❑	❑
2.	I have had at least one small bowel resection.	❑	❑
3.	I have had Crohn's disease diagnosed for at least 5 years.	❑	❑
4.	I am currently not taking 20 mg or more of prednisone.	❑	❑
5.	I have no known diagnosis of neuropathy from flagyl.	❑	❑
6.	I have no known neuromuscular disorder, such as neuromuscular disorders.	❑	❑
7.	I have no known documentation of having severe Psoriasis or Sprus.	❑	❑
8.	I am currently not taking any vitamin D supplementation.	❑	❑
9.	I am currently not indoor tanning.	❑	❑
10.	I am currently not taking any anticonvulsant medications or asthma medications.	❑	❑
11.	I do not have a pacemaker or an intravenous port.	❑	❑
12.	I do not have diabetes.	❑	❑
13.	I do not have knee pain or anterior cruciate ligament (ACL) pathologies.	❑	❑
14.	I have not had a left side stroke.	❑	❑
15.	I do not have parents with Crohn's disease (Controls only).	❑	❑

- Have you ever smoked cigarettes? ❑ Yes ❑ No
- If yes, and you are currently smoking, for how many years have you smoked? ___
- On average how many cigarettes do you smoke each day? _____
- If yes, and you are not currently smoking, when did you quit? _____ _____
  Month Year

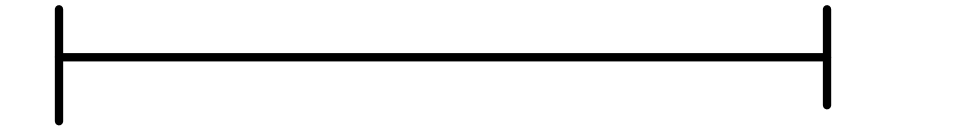
What types and dosage of medications are you currently taking?

Name of Medication	Dosage (mg)	Number of tablets per day	Duration on medication	Date last taken

APPENDIX C

IDIOPATHIC MUSCULOSKELETAL FORM

Please mark on the scale where your pain level is:



No Pain

Most Imaginable Pain

APPENDIX D

SF-36 CURRENT HEALTH ASSESSMENT

Instructions: This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Please answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is (check one):
   - ❑ Excellent
   - ❑ Very good
   - ❑ Good
   - ❑ Fair
   - ❑ Poor

2. Compared to one year ago, how would you rate your health in general now? (check one)
   - ❑ Much better now than one year ago
   - ❑ Somewhat better now than one year ago
   - ❑ About the same as one year ago
   - ❑ Somewhat worse now than one year ago
   - ❑ Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? (check one on each line)

Activities	Yes, Limited A Lot	Yes, Limited A Little	No, Not Limited At All
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	❑	❑	❑
Moderate activities, such as moving a table, pushing a vacuum cleaner,	❑	❑	❑

bowling, or playing golf			
Lifting or carrying groceries	❑	❑	❑
Climbing several flights of stairs	❑	❑	❑
Climbing one flight of stairs	❑	❑	❑
Bending, kneeling or, stooping	❑	❑	❑
Walking more than one mile	❑	❑	❑
Walking several blocks	❑	❑	❑
Walking one block	❑	❑	❑
Bathing or dressing yourself	❑	❑	❑

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? (check one on each line)

Problem	**Yes**	**No**
Cut down on the **amount of time** you spent on work or other activities	❑	❑
Accomplished less than you would like	❑	❑
Were limited in the **kind** of work or other activities	❑	❑
Had **difficulty** performing the work or other activities (for example, it took extra effort)	❑	❑

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)? (check one on each line)

Problem	**Yes**	**No**
Cut down on the **amount of time** you spent on work or other activities	❑	❑
Accomplished less than you would like	❑	❑
Didn't do work or other activities as **carefully** as usual	❑	❑

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups? (check one)
   - ❑ Not at all
   - ❑ Slightly
   - ❑ Moderately
   - ❑ Quite a bit
   - ❑ Extremely

7. How much bodily pain have you had during the past 4 weeks? (check one)
   - ❑ None
   - ❑ Very mild
   - ❑ Mild
   - ❑ Moderate
   - ❑ Severe
   - ❑ Very severe

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)? (check one)
   - ❑ Not at all
   - ❑ A little bit
   - ❑ Moderately
   - ❑ Quite a bit
   - ❑ Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. **How much of the time during the past 4 weeks…**
   (check one on each line)

	All of the Time	**Most of the Time**	**A Good Bit of the Time**	**Some of the Time**	**A Little of the Time**	**None of the Time**
Did you feel full of pep?	❑	❑	❑	❑	❑	❑
Have you been a very nervous person?	❑	❑	❑	❑	❑	❑
Have you felt so down in the dumps that nothing could cheer you up?	❑	❑	❑	❑	❑	❑
Have you felt calm and peaceful?	❑	❑	❑	❑	❑	❑
Did you have a lot of energy?	❑	❑	❑	❑	❑	❑
Have you felt downhearted and blue?	❑	❑	❑	❑	❑	❑
Did you feel worn out?	❑	❑	❑	❑	❑	❑
Have you been a happy person?	❑	❑	❑	❑	❑	❑
Did your feel tired?	❑	❑	❑	❑	❑	❑

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)? (check one)
    - ❑ All of the time
    - ❑ Most of the time
    - ❑ Some of the time
    - ❑ A little of the time
    - ❑ None of the time

11. How true or false is each of the following statements for you? (check one answer for each line)

	Definitely True	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
I seem to get sicker easier than other people	❑	❑	❑	❑	❑
I am as healthy as anybody I know	❑	❑	❑	❑	❑
I expect my health to get worse	❑	❑	❑	❑	❑
My health is excellent	❑	❑	❑	❑	❑

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