Nutritional intakes of patients with chronic pain and the effect of soy protein on neuropathic facial pain: a pilot study

Douna Maria Masmar

School of Dietetics and Human Nutrition
McGill University, Montreal
August 2012

A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Masters of Science

Abstract

Only scarce data exist about the nutrient intake and diet quality of subjects suffering from chronic pain as well as the effects of dietary nutrients on pain severity. We assessed nutrient intake adequacy of 79 patients with chronic pain and examined possible dietary correlates of pain levels (visual analog scale).Usual dietary intakes were estimated from three 24-h food recalls and compared to the US-Canadian Dietary Reference Intakes and with those of a healthy population of the same age and sex (Canadian Community Health Survey 2.2.). Intakes of many nutrients (notably vitamins D, E, K and potassium) were below Estimated Average Requirements and those of the general population in >39% of the patients, which suggests greater risks of nutrient deficiencies. Energy, carbohydrates and vitamin E intakes were weakly and negatively associated with pain levels ($r \leq -0.30$, $P \leq 0.043$). Among the various forms of chronic pain, neuropathic facial pain lacks effective management. Soy protein has been shown to decrease neuropathic pain in animal and few human studies. Thus, we tested the feasibility, compliance and effects of a diet enriched with soy protein against milk protein on chronic neuropathic facial pain, in a pilot study using an N-of-1 design. Participants were randomly and blindly exposed to a soy or milk protein powder during 3 week-intervals, in 3 paired treatment periods. Dietary intakes (24-h food recall and food frequency questionaire), pain intensity (Numerical Rating Scale), depression levels (Beck Depression Inventory-II), and quality of life (Pain Disability Index) indices were assessed at baseline and followed up during each period. The soy protein rich diet did not improve pain symptoms. The dietary intervention was a feasible but difficulties were encountered with recruitment, retention into the study and acceptability of the treatment products. Due to these barriers resulting in a small sample size, the absence of a soy effect is inconclusive. Future studies should consider other dietary approaches or better quality protein powder products to improve acceptability. The effect of soy protein on pain severity also warrants confirmation with future larger studies.

Résumé

Peu de données existent sur les apports en nutriments et la qualité de la diète des sujets souffrant de douleur chronique ainsi que sur les effets des nutriments sur l'intensité de la douleur. Dans cette thèse, l'adéquation des apports en nutriments de 79 patients souffrant de douleur chronique a été évaluée et les corrélations entre ces apports et la sévérité de la douleur (échelle visuelle analogue) ont été examinées. Les apports alimentaires habituels ont été estimés à partir de 3 rappels alimentaire de 24 h et comparés aux apports nutritionnels de référence (ANREF américains-canadiens) et à ceux d'une population de même âge et sexe (Enquête sur la santé dans les collectivités canadiennes 2.2.). Les apports de nombreux nutriments (notamment les vitamines D, E, K et le potassium) étaient au dessous des Besoins Moyens Estimatifs et de ceux de la population générale dans >39% des cas, ce qui suggère des risques de déficience nutritionnelle. Les apports en énergie, glucides totaux et vitamine E étaient faiblement et négativement associés aux niveaux de douleur ($r \leq -0.30$, $P \leq 0.043$). Parmi les différentes formes de douleur chronique, la douleur neuropathique du visage manque de traitements efficaces. Des études animales et quelques études humaines ont démontré que la protéine de soya peut diminuer la douleur neuropathique. Ainsi, la faisabilité, l'adhésion à la diète et les effets d'un régime enrichi en protéines de soya par rapport aux protéines de lait sur la douleur chronique neuropathique du visage ont été testés dans une étude-pilote (avec un devis « N-of-1 »). Les participants ont consommé une poudre de protéine de soya ou de lait, de façon aléatoire, pendant 3 périodes de 3 semaines. Les apports alimentaires (rappels de 24 h et questionnaire de fréquence alimentaire), l'intensité de la douleur (échelle d'évaluation numérique), la sévérité de la dépression (inventaire de Beck-II) et les indices de la qualité de vie ont été évalués avant et au cours de chaque période. Le régime enrichi en protéines de soya n'a pas amélioré les symptômes de douleur. L'intervention alimentaire s'est avérée réalisable mais des difficultés ont été rencontrées dans le recrutement et la rétention des participants à l'étude, et dans l'acceptabilité des produits. Ces obstacles ont résulté en un échantillon final de petite taille, invalidant ainsi

l'absence d'effet du soya. De futures études devraient adopter d'autres approches diététiques ou des produits de meilleure qualité pour en améliorer l'acceptabilité. L'effet de la protéine de soya sur la sévérité de la douleur mérite néanmoins d'être confirmé par de plus larges études.

Acknowledgements

My first and foremost thanks go out to my supervisor, Dr. Stéphanie Chevalier, for the high standards she set and her continuous guidance, dedication and encouragement throughout my studies. I believe I was lucky to work with a person like her. She always made sure that each student's work was perfect. Dr. Chevalier I have learnt so much from you and for that, I am grateful. Thank you for being so kind, helpful and for showing concern during the hard times I went through. I also thank you and Dr. Shir for providing me with a student stipend throughout my studies.

I would also like to thank my committee members, Dr. Louise Thibault and Dr. Yoram Shir, for contributing some of their valuable time to read my thesis in order to provide me with important insights and suggestions. I believe your comments contributed in improving my thesis writing.

I would like to extend my gratitude to Alexis Codrington, Sylvie Toupin and Dionne Codrington for helping in the data collection process and for being cooperative along the way.

I thank each lab member of the Nutrition and Food Science Center at the Royal Victoria. Although I did not spend a lot of time with you, I appreciate your kindness and help whenever I needed anything. I would like to thank you Dr. Marliss and Dr. Gougeon for providing me with helpful feedbacks on my proposal presentation, Marie Lamarche and Ginette Sabourin for providing me access to your computers for data analysis, and finally Cherise Labonte, Ayla Coussa and Rima Nasrah for giving me support whenever I needed anything.

My deepest appreciation and love goes out to my family who has provided me with an excellent education and ongoing words of wisdom and support. Without you none of this could have been possible. I would like to particularly thank my father for paying for my education and my mother for her emotional support every time I faced a challenge or obstacle along the way.

Dearest friends, thank you for your encouragement and support throughout my masters' degree.

Contributions of authors

The manuscripts included in this thesis were accomplished through collaborative efforts from the co-authors.

Manuscript 1

D.M. Masmar contributed to food intake data collection and analysis, performed all statistical analyses, data interpretation and drafted the manuscript. C. Naim contributed to patients' recruitment and data collection. The principal investigators were S. Chevalier and Y. Shir who designed the study and revised the manuscript.

Manuscript 2

A. Codrington, Y. Shir and S. Chevalier designed of the study. A. Codrington and Y. Shir participated in patient's recruitment. D.M. Masmar and A. Codrington equally contributed in baseline assessments, data collection and patients' follow-up. D.M. Masmar performed statistical analyses, interpretation of data and manuscript preparation under guidance from S. Chevalier.

The thesis supervisor (S. Chevalier) and committee members (Y. Shir and L. Thibault) reviewed and edited initial drafts of the whole thesis.

Table of contents

List of tables

Manuscript 1

Manuscript 2

List of figures

Manuscript 1

Manuscript 2

Appendices

Manuscript 1

Manuscript 2

List of abbreviations

AI: Adequate Intake

ALA: α-Linolenic Acid

AMDR: Acceptable Macronutrient Distribution Range

ANOVA: Analysis of Variance

BDI: Beck Depression Index-II

BIA: Bioelectrical Impedance Analysis

BMI: Body Mass Index

CCHS: Canadian Community Health Survey

CNF: Canadian Nutrient File

DHA: Docosahexaenoic Acid

DRI: Dietary Reference Intake

EAR: Estimated Average Requirement

ER: Estrogen Receptor

EPA: Eicosapentaenoic Acid

FFM: Fat Free Mass

FFQ: Food Frequency Questionnaire

LA: LinoleicAcid

MUFAs: Monounsaturated Fatty Acids

MUHC: McGill University Health Centre

NRS: Numerical Rating Scale

PDI: Pain Disability Index

PUFAs: Polyunsaturated Fatty Acids

REE: Resting Energy Expenditure

USDA: United States Department of Agriculture

VAS: Visual Analog Scale

1 INTRODUCTION

Chronic pain is a devasting condition with overwhelming consequences [1, 2]. In addition to a an overall reduction of quality of life, sufferers of chronic pain might experience a reduced appetite related to depression [3], medications' side effects [4] and pain itself [5]. They are also at an increased risk of morbidity; early mortality from chronic vascular diseases and certain cancers [6] that were associated with several factors including a poor diet [7]. Subjects with chronic pain reported eating less during painful episodes [8] and having an unhealthy diet, compared with healthy subjects [7], which places them at risk of nutrient deficiencies. Yet, no studies have examined the nutrient intake and quality of the diet of subjects suffering from chronic pain.

Most chronic pain patients have inadequate pain management despite the variety of pain treatment options [2]. This is why research on alternative intervention for pain is ongoing and interest in the role of complementary and alternative medicine is rising [2, 9]. Some dietary constituents were shown to have analgesic properties in animal [10, 11] and human studies [12-14]. But, studies examining a possible dietary role in improving pain symptoms are lacking [5, 9]. Thus, the aim of the first manuscript was to assess the nutrient intakes of patients with chronic pain and to examine possible dietary correlatesof pain levels.

One form of chronic pain lacking effective management is neuropathic facial pain. Patients can become refractory to conventional medications and research is ongoing for alternative treatment [9, 10]. Soy protein is one of the dietary constituents shown to modify neuropathic pain behaviour in animals [15-17] and humans [13, 14, 18, 19], in addition to its role in preventing various multifactorial illnesses. Pre-clinical findings have highlighted the beneficial effects of soy in some neuropathic pain patients [19] (but not all) and this supports

the notion that soy protein consumption could be a promising therapeutic approach for the clinical management of chronic neuropathic facial pain [19]. Therefore, the aim of the second manuscript was to determine the feasibility and compliance to a diet enriched with soy protein powder in neuropathic facial pain patients and to examine its efficacy compared to a placebo (milk) on pain, depression and quality of life scores based on individual assessments, and as a group.

2 BACKGROUND

2.1 Chronic pain

Chronic pain is a common devastating condition defined by a pain persisting longer than 3 to 6 months [1, 2]. Pain negatively affects many aspects of life and consequences are overwhelming [20]. The most common body locations affected by chronic pain include the low back, knee, head, leg and shoulder and the major causes are arthritis, osteoarthritis, traumatic injury, nerve damage and surgery [20]. Chronic pain mostly affected older adults, especially females [21]. In Canada, 18.9% of older adults are affected by chronic pain [21].

2.1.1 Chronic pain consequences

2.1.1.1 Impact on quality of life

Chronic pain is accompanied by an overall decreased quality of life including reduced physical functioning, disruption of social and family roles, and psychological distress [1]. Chronic pain in the majority of sufferers severely affects sleep, ability to exercise, ability to have healthy sexual relations and maintaining independent lifestyle [20].

2.1.1.2 Impact on emotional status and health

Most chronic pain patients suffer from depressive disorders. The prevalence of concurrent major depression in patients with chronic pain varied by study setting but ranged between 13% and 85% [22]. The main symptoms for depressive disorders involve decreased appetite [3]. Depression is also considered as the main common cause of malnutrition, under nutrition and weight loss [5].

In addition to depression, patients with chronic pain were found to be at an increased risk of morbidity; early mortality from cardiovascular disease and certain cancers [6]. The association between chronic pain, cancer and cardiovascular disease was partially explained by subjects' life style factors

including diet, body mass index (BMI) and smoking [7].

2.1.1.3 Impact on appetite

No systematic studies have been carried out to assess patients' appetite under different classes of pain including chronic pain. As already discussed in section 2.1.1.2, depression can result in a decreased appetite [3]. Pain medications' side effects (constipation, nausea, dry mouth and urinary retention) can alter appetite [4]. Also, pain experience has been related to a decrease or loss of appetite although the neural mechanism responsible is unknown [5]. A cross sectional survey of 65 subjects provided preliminary evidence that chronic pain is associated with self-reported appetite impairment in older adults [5]. Appetite was assessed with a questionnaire developped for the purpose of this study: it evaluated appetite in general and percieved interference of pain with appetite. The later was assessed using 3 statements: "Pain interferes with my eating", "I would eat more if I did't have pain" and "My appetite is reduced because of pain". Subjects were asked if they strongly disagreed, disagreed, were uncertain, agreed, or strongly agreed. Results showed that patients who reported that their pain interefered with their appetite (43%) had higher pain intensity levels than those who reported no appetite impairement ($P<0.001$). Also, general appetite and pain intensity were inversly correlated ($r=-0.45$, $P<0.001$). Yet, this association between pain and appetite impairement does not necessarily imply causation.

2.1.1.4 Impact on food intake

Decreased appetite might reduce the food intake of the subjects and consequently affecting their nutritional status [5]. In the literature, studies reporting the food intake or the nutritional status of subjects suffering from chronic pain are lacking. Yet, pain appeared to alter eating behaviours of these patients [8]. An exploratory study using a survey design evaluated the changes in eating patterns during pain episodes in 62 patients with sickle cell disease and showed that the majority reported eating less during episodes of pain and significantly decreasing their intakes of fats and proteins. Pain levels were measured by the Multidimensional Pain Inventory (MPI-2). Dietary pattern was

assessed by several questions developped for the pupose of this study. The questions included “When you are in pain, do you eat less, more or no change?” and “Do you eat more, less or experience no change in your consumption of fats, proteins, sugar and salt during episodes of pain”. A high proportion the participants (87%) reported they “eat less” during episodes of pain, 13% reported “no change” and none indicated an increase in food consumption. Also, the majority of the subjects indicated that they were likely to decrease their intakes of fats (χ^2=10.55, P<0.01) and proteins (χ^2=4.43, P<0.01) during pain episodes but not their intakes of food items containing sugar and salt. The major limitation of this study is the use of exploratory questions to assess dietary intakes under the influence of pain.These have not been used in previous studies and hence their psychometric properties are unknown. Food intakes should be assessed with validated questionnaires such as dietary recalls and food frequency questionaires [8].

Patients with chronic pain, especially women, reported an unhealthy diet, compared with healthy subjects in a nested case-control study. This study investigated the relationship between chronic pain and past diet and lifestyle using data from the 1958 British Birth Cohort study (n=17,000). At 45 years of age, pain was recorded using a self completion questionnaire in which subjects were asked if they experienced any pain that lasted for more than 3 months. At 33 and 42 years of age, data on diet were collected. Subjects recorded the frequency of consumption of fruit, vegetables, fatty foods, french fries or alcohol (more than once per day, once per day, 3 to 6 days per week, 1 or 2 days per week, less than once per week and never). Of the 8572 who completed the pain questionnaire, 12% reported having chronic pain. Results showed that women with chronic pain had increased odds of having rarely (less than once per week) consumed fruit and vegetables compared with women reporting no pain (OR 2.0 [95% CI 1.3 to 3.1]). They also had greater odds of having consumed high levels (at least once per day) of fatty foods (OR 1.7 [95% CI 1.1 to 2.7]. Although they were attenuated, these relationships remained after controlling for marital status and social class for rare

fruit and vegetable consumption (OR 1.5 [95% CI 0.9 to 2.5]) and for high consumption of fatty foods (OR 1.3 [95% CI 0.8 to 2.2]. However, smoking and income were not considered in these analyses [7].

2.1.2 Neuropathic facial pain

Neuropathic facial pain is one type of chronic pain characterized by pain manifesting in the facial area caused by tissue injury or nerve damage. The nerve involved is usually the fifth cranial nerve, the trigeminal nerve [23]. It is a rare disease with an incidence of 21.7 out of 100,000 persons per year and seen more commonly among women compared to men. Several types of neuropathic facial pain exist including trigeminal neuralgia, glossopharyngeal neuralgia, facial postherpetic neuralgia, occipital neuralgia and local neuralgia [24]. Trigeminal neuralgia is the most common form with an incidence of 4.3 per 100,000 persons per year [25].

2.1.2.1 Signs and symptoms

The disorder is characterized by episodes of pain described as sharp, burning, electric, and shock-like. The pain is most of the times unilateral, appearing suddenly in the form of attacks that last from seconds to minutes with pain free intervals in-between [23]. Symptoms of neuropathic pain are often chronic and affect the quality of life of the patients (physical and emotional well being). Many suffer from depressive symptoms after realizing that their untreated pain is lasting for long periods of time and is affecting their life quality [26].

2.1.2.2 Etiology and diagnosis

There are different proposed etiologies of neuropathic facial pain. Demyelinisation of the cranial nerves in the root entry zone is the main origin of the disease. Another proposed etiology is vascular nerve compression that might result from tumors [27]. Other less common but probable causes of neuralgia include inflammation, ischemia or multiple sclerosis [23]. In some cases, the origin of neuropathic pain is unknown and referred to as idiopathic [27]. The

disease is diagnosed using the 2004 International Headache Society guidelines [28].

2.1.2.3 Treatment

A variety of treatments options exist for neuropathic facial pain including pharmacology, neural blockade procedures, and surgery [27]. Pharmacological treatment is the first choice and preferred intervention type. Antidepressants with anti-nociceptive properties, anticonvulsants and anti-inflammatory agents are the most commonly used [23]. Nevertheless, their efficacy in neuropathic facial pain is questioned because many patients become refractory or intolerant to medications [23, 29]. For instance, a rapid failure of initial medical treatment regimen is common [24]. Plus, most patients spend months to years trying different drug regimen to pinpoint the most effective one for pain relief and with the least side effects [26]. Neural blockade is a procedure that consists of injecting local anesthetics (e.g. stellate ganglion blocks and sphenopalatine ganglion blocks) into the nerve to manage pain. The effects of this method in pain control are temporary. Pain is reduced for a short period of time with the longest response being 3 months [27]. Surgical procedures (glycerol rhizolysis/ injection, radiofrequency rhizotomy, gamma knife radiosurgery and microvascular decompression) aim at destroying the nerve or decompressing the trigeminal nerve at its root entry zone [23, 25, 29]. Most of the patients are still pain free after 1 year; but long-term relapse rate still exists [23, 25, 29]. Plus, these procedures are invasive: surgical risks exist and include sensory loss (5% risk of occurrence), stroke, meningitis and death (1% incidence rate) [25, 29]. In summary, although most procedures were found to be effective in providing short term pain relief, pain recurrence is common within several years for many patients [29].

2.1.3 Chronic pain management

Chronic pain management is challenging for health care professionals. Despite the existence of various treatments, pharmacological treatment is the first choice and preferred intervention type [1]. Medications to treat chronic pain

include antidepressants, anticonvulsants, opioids and non-steroidal anti-inflammatory drugs (NSAIDs). However, the rate of suboptimal treatments response and unwanted side effects from these medications is high [1, 2] leading patients to move toward using complementary and alternative medicine (acupuncture, therapeutic massage and herbal remedies) to treat their debilitating pain [9]. Nevertheless, the success of such treatment methods has not been confirmed or accepted due to lack of well-run controlled studies [30]. Diet is less explored, but not less plausible as a treatment. Today, diet is one of the possible analgesic modalities currently explored within the growing field of complementary and alternative medicine [9, 18]. Ongoing research is investigating the role of dietary constituents in pain treatment [9, 10].

2.2 Diet and its analgesic properties

Good dietary practices are important in preventing many chronic diseases such as cancer and heart disease. For instance, a diet low in saturated fatty acids (e.g. the Mediterranean diet) is related to a lower risk of coronary heart disease [18]. Accordingly, diet could play a role in chronic pain management [18]. Animal and human research revealed relationships between dietary constituents and analgesia.

2.2.1 Animal studies

Particular foods were shown to possess important pain relieving properties in animal models. Corn oil consumption reduced neuropathic pain levels compared to canola, hemp and sunflower oils in a study by Shir et al. (2005) assessing the anti-nociceptive properties of polyunsaturated fatty acids (PUFAs) in rats with a partial sciatic nerve ligation [31]. Vitamin B complex alleviated neuropathic pain in diabetic rats suggesting a potential use in treating diabetic neuropathy [11]. Tryptophan and taurine-rich diets decreased chronic neuropathic-like pain in rats undergoing hindlimb denervation following peripheral neurectomy [32]. Finally, tart cherry anthocyanins dietary supplements decreased nociception in multiple acute pain models [10].

2.2.2 Human studies

Some human studies evaluated the efficacy of different food constituents regarding their analgesic properties. Omega-3 fatty acids' beneficial effects in reducing joint pain associated with various inflammatory diseases (rheumatoid arthritis, inflammatory bowel disease, and dysmenorrhea) were highlighted in a meta-analysis of 17 studies [33]. Sucrose administration reduced procedural pain in newborn infants [34]. A Cochrane review concluded that sucrose can be an effective and safe analgesic agent in neonates undergoing painful procedures [34]. Vitamin C reduced the prevalence of complex regional pain syndrome (CRPS) type 1 in patients after wrist fractures in a randomized, controlled, double blind study [35]. Unfortunately, only scarce data concerning dietary analgesia exist. Little data has been provided with regard of this topic, as not much concern has been paid to consider chronic pain prevalence, its overwhelming physical, emotional and social consequences. This literature review will emphasize on soy protein, a promising dietary ingredient that was shown to possess analgesic properties.

2.3 Soy protein

Epidemiological studies highlighted the potential ability of soy consumption in preventing certain type of diseases since populations consuming large amounts of soybean foods (Asian populations) had lower risks of cardiovascular diseases [36-38] and certain cancers (prostate and breast cancers) [36, 37, 39-41] as well as enhanced menopausal symptoms (osteoporosis and hot flashes) [15-17, 31-34].

2.3.1 Soybeans

2.3.1.1 *Food products manufactured from soybeans*

Soybean (glycine max) is an ancient staple legume in Asians cultures and is used to produce non-fermented (green and whole dried soybeans and soymilk products) and fermented products (miso, tempeh and natto) [18, 37, 41]. There are also "second generation" soy products (soy flour and soy protein isolate)

produced following chemical extractions or other processing forms. Those are employed to provide other soy products such as dietary soy supplements, infant formulas [18] soy drinks, cereals and energy bars [41]. An average of 20 to 80 g per day of soy foods are consumed by Asians and only 1 to 3 g per day by Americans [37].

2.3.1.2 *Soybeans composition*

2.3.1.2.1 Macronutrient profile

Soybeans are composed of carbohydrates (30%), lipids (30-40%) and proteins (30-40%) [42]. Lipids are mainly made of unsaturated fat (24% monounsaturated fat, 61% polyunsaturated fat, and 15% saturated fat) [42]. Soybean protein is the most complete vegetable protein and almost equivalent to animal protein in essential amino acids [18, 36, 43]: it contains all essential amino acids except for methionine [44].

2.3.1.2.2 Micronutrient profile

Soybeans contain phytochemicals, which are bioactive ingredients that encompass saponins, phytates, protease inhibitors, phenolic acids, lecithin, phytosterols, and isoflavones [37]. Phytochemicals that received the greatest attention by the scientific research are soy isoflavones [36, 37].

Soybeans, particularly isolated soy proteins, contain high concentrations of isoflavones and are hence considered their main dietary sources [36, 37]. Isoflavone quantity fluctuates depending on bean diversity and growing circumstances [37, 41, 42], but in general, 100 g of soy protein contains an average of 200 mg of isoflavones [37]. Soy isoflavones are called phytoestrogens because they have both estrogenic and anti-estrogenic activity due their structural and functional similarity to the potent mammalian estrogen 17β estradiol [36, 37, 41, 42]. Soy isoflavones also possess antioxidant and anti-inflammatory activity, as described in section 2.c) [36,37, 42]. Accordingly, isoflavones were anticipated to be the active ingredients responsible for most of the beneficial effects of

soybean foods[36, 37]. However, studies examining the effects of isoflavones alone did not find any significant beneficial effects on cholesterol [41]. Also, few studies depicted the advantageous effects of soy protein without isoflavones [42]. Therefore, isoflavones seemed to act in conjunction with soy protein [36, 37]; yet, the interactions between these two components remain unclear [42].

2.3.1.3 Soy protein safety

2.3.1.3.1 Effect of soy consumption on health

Soy products and isoflavones consumption have no effects on reproductive health, glucose metabolism, as well as kidney and cognitive functions [38]. However, major concern relates to the consumption of soy protein and isoflavones by patients with breast cancer. The isoflavone genistein enhanced the proliferation of breast cancer cells in vitro and inhibited the efficacy of tamoxifen, a selective estrogen receptor modulator (SERM) used in the treatment of breast cancer, in rodent studies [43, 45]. In contrast, little clinical evidence suggests that isoflavones increase the risk of cancer or worsen prognosis in breast cancer patients [43]. The Shanghai Breast Cancer Survival cohort study concluded that soy consumption was related to a decreased death risk and cancer recurrence among breast cancer women regardless of tamoxifen use. Also, high soy intake and tamoxifen use may have a comparable effect on breast cancer outcomes [45].

2.3.1.3.2 Adverse events

Soy consumption is generally well tolerated and its related adverse events are minor and rare [18, 38]. Gastrointestinal side effects (bloating, nausea, constipation and flatulence) have been reported in adults [18, 38] and are mainly due to the inability to digest soybeans carbohydrates more specifically the oligosaccharides verbascose, stachyose and raffinose [46, 47]. Yet, nowadays >99% of soy carbohydrates are removed during the processing of isolated soy products [18,46, 47]. Other less common reported adverse events from consuming soy products are musculoskeletal complaints, headache, dizziness and rashes [38].

2.3.1.3.3 Soy protein allergies

Allergies to soy protein are relatively rare with an incidence of 0.5% [48-50]. They are mainly seen in infants and young children who will often outgrow their soy allergy as their immune systems mature [48, 49]. Soy protein products tend to produce less allergic reactions compared with other food proteins (e.g. milk, eggs, fish and peanut). For instance, soymilk has been shown to be an effective alternative to cow's milk in the case of lactose intolerance [49]. Finally, soy allergies are often reported in conjunction with other proven allergies because of cross-reactivity. For instance, patients with birch pollen allergy are at high risk of experiencing allergic reactions if they consume soy products [49].

2.3.1.3.4 Drug and nutrient interactions

Soy consumption was found to interact with warfarin (Coumadin), an anticoagulant used for the management of different cardiovascular conditions [51] and with levothyroxine (Synthroid®), a hormone replacement therapy for subjects with hypothyroidism [18]. In fact, soy reduced the anticoagulant effects of Warfarin [52] and decreased hormonal absorption if simultaneously consumed with levothyroxine. Therefore, it is recommended to avoid soy protein intake within one hour of taking the thyroid medication [18]. Soy protein is better not consumed with recent antibiotic use. Antibiotics usually alter the metabolism of the gut flora and might interfere with isoflavones absorption by decreasing their bioavailability [18].

A diet rich in soy may possibly reduce the bioavailability of some minerals such as zinc, iron and calcium. Yet, soy foods should not affect the mineral status if incorporated in a balanced diet [18].

2.3.2 Soy protein and analgesia

Soy protein was found to modify pain behaviours in animals [15-17] and humans [13, 14, 18, 19]. Nevertheless, the mechanism of soy-mediated analgesia remains unclear despite the identification of multiple analgesic constituents in soy protein [17, 18, 53].

2.3.2.1 Animal studies

Partial sciatic nerve ligation is an animal model of neuropathic pain that mimics features of clinical pain syndromes. Several studies used this model to examine the hyponociceptive effects of soy consumption on chronic neuropathic sensory disorders following partial denervation in rats and showed that soy enriched diets decreased chronic post-surgical pain [15-17]. Diets consisting of 20% soy protein suppressed chronic neuropathic like pain development (tactile and heat allodynia) in nerve injured rats [16]. Other series of experiments by Shir et al. (1998) depicted that soy omission from the diet, as opposed to casein addition, was responsible for neuropathic pain development in rats [15]. The pain suppressive properties of soy protein were established to be the result of preoperative rather than post surgical soy consumption [17].

2.3.2.2 Human studies

Until now, pre-clinical findings are still not applicable to humans and hence remain to be established. There is a lack of population studies comparing neuropathic pain prevalence in populations consuming soy-rich and soy-devoid diets. Yet, there are some studies investigating the relationship between soy protein and analgesia [18].

A randomized double blind crossover trial showed that daily consumption of diets enriched with soymilk for 3 months (34 g of soy protein/day) had mild analgesic effect on cyclical menstruation-related breast pain [13]. Eighteen premenopausal women with breast pain consumed either a soy protein drink or cow's milk (placebo) twice per day (total of 400 mL) for 3 months in a cross over design. The difference in pain relief between soy versus placebo was statistically significant (P=0.012). However, the study presented important limitations including a small sample size, a strong placebo effect with soy and milk preparations, and non-compliance of many patients (33%) to soy treatment as evidenced by the inability to detect elevated circulating levels of phytoestrogens.

Taking into account these factors, drawing definitive conclusions about the effectiveness of soy protein in breast pain alleviation is difficult.

A double blind, randomized placebo controlled trial highlighted the effectiveness of daily soy protein ingestion in relieving pain and discomfort associated with osteoarthritis. One hundred and thirty-five subjects diagnosed with osteoarthritis were randomly assigned to daily consume 40 g of either soy protein or milk-based protein supplementation for 3 months. Pain, knee range of motion and quality of life were assessed before the start of the intervention and every month thereafter. Biochemical markers of cartilage degradation including glycoprotein 39 (YKL-40), and insulin like growth factor (IGF-I), a growth factor related to cartilage synthesis, were measured at baseline and at the end of the trial. While both protein supplements significantly (P<0.05) improved the range of motion of the knee joint, work performance and productivity as well as reduced hindrance of activities of daily living and limitations to exercise, only soy protein supplementation significantly (P<0.05) reduced pain and use of medications. The improvements in the range of motion were significant after 3 month of supplementation while all the other beneficial effects were found as early as the first month of supplementation and persisted through the study. Subgroup analysis by gender revealed that in men, only soy protein supplementation resulted in beneficial effects while both protein supplementation engendered good effects in women. This was attributed to the lower estrogen levels found in men as compared to women, since isoflavones possess estrogen-agonist or antagonist activity depending on estrogen concentrations and the prevalence of estrogen receptors. Biochemical markers of cartilage metabolism further supported the efficacy of soy protein in men as evidenced by the significant (P<0.05) reductions in YKL-40 levels and increments in IGF-I levels. While this study presents preliminary evidence of a potential beneficial effect of soy protein supplementation in osteoarthritis management, predominantly in men, future clinical trials are required to verify the long-term effects of soy protein supplementation in improving symptoms of osteoarthritis [14].

In contrast, a randomized, double blind, placebo controlled, early phase trial showed that daily soy consumption did not improve fibromyalgia symptoms. In this study, 50 patients with fibromyalgia were randomized to daily receive either a soy shake (20 g of soy protein with 160 mg of soy isoflavones) or a casein (placebo) shake for 6 weeks. The scores of Fibromyalgia Impact Questionnaire (FIQ) and the Centre for Epidemiologic Studies Depression Scale (CES-D) were measured at baseline and at the end of the study. Between group comparisons using the intention to treat analysis indicated that FIQ scores were decreased by 14% in the soy group (P=0.02) and by 18% in the casein group (P<0.001). But, no significant difference (P=0.16) was found in the change in scores between the groups. CED scores decreased by 16% (P=0.004) in the soy group and by 15% (P=0.05) in the casein group, with no difference in the change in scores between groups. Within-group comparisons showed that both groups were subject to a significant but modest enhancement in total FIQ scores (soy group, P=0.02; placebo group, P<0.001) and CES-D scores (soy group, P=0.004; placebo group, P=0.005) between study entry and study completion. The findings of this early trial suggest that both protein shakes were related to improvements in fibromyalgia symptoms and depression. Future studies with larger sample size examining soy efficacy in fibromyalgia are not indicated since they are unlikely to detect the superiority of soy supplement over placebo [12].

A preliminary pilot, open-label study investigating the analgesic effects of soy enriched diets on neuropathic pain in humans showed that diets rich in soy protein reduced chronic post-traumatic neuropathic pain in some patients, and not on average. Twenty patients with chronic post-surgical or post-traumatic neuropathic pain presenting with tactile allodynia (a pain due to stimulus which does not normally provoke pain) regardless of its location and etiology, were recruited to participate. The study consisted of two periods of 6 weeks each. In the first period, patients incorporated isolated soy protein powder (30-55 g, proportional to body weight) in their usual diet (foods and beverages). They were

also asked to reduce by half all portion sizes of meat, fish, poultry and eggs and to omit dairy products in order to keep their diet isoenergetic and isonitrogenous. Also, patients had to replace their added fat with soy-derived products such as soy oil and margarine. In the second period, patients returned to their usual diet. Pain levels (Visual Analog Scale VAS), pain disability levels (Pain Disability Index) as well as mood changes (The Profile of Mood States Questionnaire) were determined at baseline and at the end of each period. Fifteen out of the 20 patients were able to complete the study despite the major dietary changes. Several improvements with no major adverse events were observed after the 6 weeks of soy protein intervention. Pain levels recorded for the whole group decreased from 71.8±4.8/100 (baseline VAS) to 66.8±5.0/100 (post-soy VAS). Although this decrease was not significant, a significant positive correlation (r=0.525; P=0.045) was depicted between the total amount of soy protein ingested and change in intensity levels. Disability levels were significantly (P<0.001) reduced after the 6 weeks of soy protein treatment. There were also positively correlated (r=0.631; P=0.012) to the total amount of soy protein consumed. Furthermore, the average size of the dynamic tactile allodynia decreased from 178±40 cm² to 99±25 cm² but this reduction failed to reach statistical significance (P<0.09). No significant changes in mood and static tactile allodynia were noted after 6 weeks of soy protein consumption. Finally, when patients were assessed individually, the area of tactile allodynia was measured in 8 patients only. It decreased by more than 30% in 5 patients and disappeared completely in one patient. The study concluded that certain patients did experience pain relief upon soy consumption but no common phenotype trait was identified [19].

Finally, soy rich diet consumption modified pain behaviour in a case report of complex regional pain syndrome type II, reported in [18].A 64 year-old woman was diagnosed with complex regional pain syndrome type II secondary to saphenous nerve injury following a surgery in her left foot. She suffered from sharp and burning pain at the medial aspect of her leg, radiating down to the 2nd, 3rd and 4th toes over the dorsum of the foot. Pain levels were “80/100 (VAS) with

tactile allodynia, atrophic skin changes and edema". Several pain management techniques were employed (e.g. physiotherapy, local lidocaine and depomedrol injections, and saphenous nerve blocks) but did not provide sufficient pain relief. Yet, after 6 weeks of soy consumption multiple improvements were observed. Her ongoing pain proximal to the ankle had completely disappeared and the pain located in the foot and the ankle was reduced as evidenced by the decrease in pain intensity levels from 8.1 to 4.5/10 (VAS). Also, "McGill Pain Questionnaire scores decreased from 48 to 8 and the area of dynamic tactile allodynia decreased from 346 cm to 56 cm." Because the patient noted a reoccurrence of her pain after two weeks of returning to her usual eating habits, she decided to follow a soy-rich diet. After three years of adopting it, she reported acceptable pain levels [18].

2.3.2.3 Hyponociceptive mechanisms of soy protein

The active component in soy protein responsible for pain elimination is still unidentified [17, 53]. Soy protein contains various bioactive constituents that may have analgesic properties such as phytoestrogens, phenolic acids, phytates, saponins and lipids [18].

Soy phytoestrogens, at certain plasma levels, were found to decrease neuropathic pain (tactile allodynia and mechanical hyperalgesia) in rats in a study by Shir et al [53]. Midrange plasma concentrations of phytoestrogens but not low or high levels were associated with the observed reductions [53]. The exact mode of action of phytoestrogens in pain behaviour reduction is not clear; yet several potential mechanisms appeared to be involved in their anti-nociceptive effects. First, their estrogen-like activity could be associated with their antinociceptive effects since steroid estrogens were demonstrated to produce antinociception [14, 15, 18, 53]. Plus, estrogen is one modulator of GABA receptor that enhances its antinociceptive effect and increases morphine analgesia [15]. Second, phytoestrogens were shown to inhibit numerous types of protein-kinase enzymes [18, 53] such as tyrosine kinase, which plays a role in processing pain sensation [53].

Genistein was identified to be the main isoflavone responsible for neuropathic pain relief after chronic constriction sciatic nerve injury in mice [54]. The suppression of neuropathic pain induced by genistein was related to the involvement of multiple mechanisms including its estrogen receptor-mediated activity, anti-inflammatory, anti-oxidant and immunomodulatory properties [18, 53]. First, the estrogen receptor- mediated activity of genistein was related to its weak estrogenic activity that enables it to bind to estrogen receptors (ER) with a greater affinity for those of beta subtype (ERβ), particularly expressed in neuronal and immune cells [54]. In neuropathic pain models, ERβ agonists were shown to possess anti-inflammatory effects on microglia and astrocytes that prevented allodynia and hyperalgesia development [55, 56]. Valsecchi et al. (2008) found that the administration of ERβ antagonists inhibited the effects of genistein in reversing hypersensitivity. Hence ERβ were proposed to be involved in the anti-nociceptive effects of genistein [54]. Second, the anti-inflammatory properties of genistein were related to its ability to prevent the over-activity of some neuropathic pain characteristics including nitric oxide synthase (NOS) and inflammatory cytokine over-expression in the peripheral and central nervous system [54]. Third, the antioxidative characteristics of genistein were demonstrated as it enhanced anti-oxidant enzymes (glutathione peroxidase and catalase) activity and reversed the increase in reactive oxygen species and lipoperoxide production in the paw tissues of mice. Since oxidative stress is involved in the pathogenesis of neurodegenerative diseases and neural tissue degeneration, the antioxidant activity of genistein can slow neural tissue damage or contribute in its repair. Finally, the immunomodulatory effects of genistein were related to its capacity to inhibit nuclear factor-κB activation, a mechanism involved in improving neuropathic pain [54].

Other potential bioactive components, phenolic acids (salicylic, chlorogenic, caffeic and ferulic acids) have anti-inflammatory and anti-oxidative actions that might account for their antinociceptive properties [18]. Finally,

saponins were shown to have antinociceptive effects in animal studies in addition to their anti-inflammatory properties [18, 57].

3 RATIONALE

Patients with chronic pain might have reduced appetite related to depression [3], medications' side effects [4], and pain itself [5] which may alter their eating behaviour [8]. In addition to nutritional deficiencies, they are also at increased risks of morbidity and early mortality from chronic vascular diseases and certain cancers [6, 7]. To our knowledge, no studies have reported the nutrient intake and quality of the diet of subjects suffering from chronic pain [8].

Chronic pain patients often try various treatment options with limited or no improvements [1, 2]. Research for alternative pain treatments is ongoing and diet could be a new therapeutic approach for pain attenuation [2, 9]. Some dietary constituents were shown to have analgesic properties in animal [10, 11] and human studies [12-14]; yet, little is known about the effects of dietary nutrients on chronic pain [9]. Correlation between nutrient intakes and pain levels could open a new research and therapeutic avenue for these patients, and could be the base for future large-scale nutrition studies in patients with chronic pain.

Soy protein is one promising dietary ingredients tested for its analgesic potential. Neuropathic facial pain is one form of chronic pain lacking effective management. Soy diets were demonstrated to be effective in preventing neuropathic pain development in animal models [15-17, 53]. Although its benefits for neuropathic disorders have not conclusively been demonstrated in humans, pre-clinical findings highlighted the beneficial effects of soy in some neuropathic pain patients (but not all) and supported that soy protein consumption as a promising therapeutic approach for the clinical management of chronic neuropathic facial pain [19]. More structured and controlled study designs are required to conclusively establish the efficacy of soy on neuropathic pain on an individual basis. It is thus important to determine the feasibility and compliance to a controlled intervention of diet enriched with soy protein powder (and isoflavones) in neuropathic facial pain patients.

4 MANUSCRIPT 1

Patients with chronic pain are at increased risks of specific nutrient deficiencies

Douna Maria Masmar, M.Sc. candidate[1,2], Cyrille Naim M.D.[3], Yoram Shir, M.D[3,4], Stéphanie Chevalier, Ph.D.[1,2]

[1]School of Dietetics and Human Nutrition, [2]Nutrition and Food Science Centre, [3]Department of Anesthesia, McGill University, [4]The Alan Edwards Pain Management Unit, McGill University Health Centre, Montreal, Quebec, Canada.

To be submitted to the Journal of the American Dietetic Association

4.1 Abstract

Background: Little is known about the nutrient intake and diet quality of chronic pain patients, as well as the effects of dietary nutrients on pain states.

Objectives: To assess nutrient intake adequacy of chronic painpatients and examine possible dietary correlates of pain levels.

Design: A cross-sectional survey of nutrient intakes. Dietary intake was assessed with three 24-h food recalls in 3-5 days. Pain levels were scored using a visual analog scale.

Participants: Seventy-nine adult subjects with chronic non-cancer pain of any type recruited from the Pain Centre of the MUHC- Montreal General Hospital, Canada.

Outcome measures: Dietary intakes and pain intensity levels.

Statistical Analyses: Nutrient intakes were compared to the Dietary Reference Intakes. The estimated average requirement cut point method was applied to determine the proportion of patients not meeting requirements. Nutrient intakes were also compared to appropriate gender and age groups of the Canadian Community Health Survey 2.2. (Quebec). Dietary correlates of pain scores were tested by bivariate or partial correlations (Pearson or Spearman rho coefficient).

Results: The distribution of macronutrients(carbohydrates, protein and fat) was within reference range in all patients. A high proportion of patients had inadequate micronutrient intakes (including supplements contribution): linoleic and linolenic acids, >44%; folate, 43%; vitamin D, 55%; vitamin E, 75%; vitamin K, 72%; calcium, 40% and potassium, 92%. Almost the majority (>49%) had nutrient intakes below the median intakes of the reference population except for carbohydrates, fibres and vitamin A. More than 39% of the subjects had nutrient intakes below the 5^{th} percentile of those from the healthy population except for fibres and vitamin A. Pain scores were negatively correlated with intakes of energy (r=-0.318, *P*=0.026), carbohydrates (r=-0.293, *P*=0.041), total vitamin E (r=-0.297, *P*=0.038) and vitamin E from food alone (r=-0.290, *P*=0.043).

Conclusions: Although macronutrient intake distribution was acceptable, subjects were at risk of many specific nutrient deficiencies. The weak negative correlations

found between pain levels and some nutrients and the lack of correlations with others could be attributed to the low narrow ranges of intakes, mixed etiologies of chronic pain and relatively small sample size. Nonetheless, improving diet quality could represent a potential therapeutic avenue for these patients.

Keywords: Nutrient intake, chronic pain, macronutrients, micronutrients, supplements.

4.2 Introduction

Chronic pain is a common devastating condition defined by a pain persisting longer than 3 to 6 months [1, 2]. The most common body locations affected include the low back, knee, head, leg and shoulder; and the major causes are arthritis, osteoarthritis, traumatic injury, nerve damage and surgery [20]. In Canada, 18.9% of older adults are affected by chronic pain and a higher prevalence was observed among women [21].

Chronic pain negatively affects many aspects of life and consequences are overwhelming [20]. It reduces the quality of life of the patients (decreased physical functioning, disruption of social and family roles, and psychological distress) [1] and is accompanied by depression which prevalence in chronic pain patients is reported to range between 13% and 85% [22]. Also, patients with chronic pain are at increased risks of morbidity and early mortality from cardiovascular disease and certain cancers [6] that were partially explained by subjects' dietary habits and other lifestyle factors (BMI and smoking) [7]. Furthermore, chronic pain might result in a decreased appetite related to depression [3], medications' side effects (constipation, nausea, dry mouth and urinary retention) [4] and pain experience itself, although the neural mechanism responsible is unknown [5]. No systematic studies have been carried out to assess patients' appetite under different classes of pain including chronic. A cross sectional survey provided preliminary evidence that chronic pain is associated with self-reported appetite impairment in older adults [5]. In this study, patients who reported that their pain interefered with their appetite had higher pain intensity levels than those who reported no appetite impairement [5].

Decreased appetite reduces food intake and pain seemed to alter eating behaviours of subjects suffering from chronic pain, which might affect their nutritional status [5, 8]. But studies reporting the food intake from these subjects are lacking. An exploratory study using a survey design showed that the majority

of patients with sickle cell disease reported eating less during painful episodes, significantly decreasing intakes of fats and proteins. However, dietary intakes under the influence of pain were assessed by exploratory questions developped for the purpose of this study. In addition, patients with chronic pain, especially women, reported an unhealthy diet, with lower consumption of fruits and vegetables and higher intakes of fatty food compared with healthy subjects in a nested case-control study [7]. Food intakes should be assessed with validated methods such as dietary recalls and food frequency questionaires for better interpretation of nutritional risks [8].

Chronic pain management is inadequate in most of the sufferers despite the existence of various treatments options. Pharmacological treatment is the first choice and preferred intervention type and include antidepressants, anticonvulsants, opioids and non-steroidal anti-inflammatory drugs (NSAIDs) [1]. However, the rate of suboptimal treatments response and unwanted side effects from these medications is high [1, 2]. Consequently, patients move toward using complementary and alternative medicine (acupuncture, therapeutic massage and herbal remedies) [9], but the success of these methods has not been confirmed or accepted due to lack of well-controlled studies [30]. Diet could be a new therapeutic approach [2, 9] since some dietary constituents were shown to have analgesic properties in animal [10, 11] and human studies [12-14]. For instance, omega-3 fatty acids reduced joint pain associated with various inflammatory diseases [33]. Sucrose administration reduced procedural pain in newborn infants [34]. Vitamin C reduced the prevalence of complex regional pain syndrome (CRPS) type 1 in patients after wrist fractures [35]. Yet, little is known about the effects of other dietary nutrients on pain severity and studies investigating a possible dietary role in improving pain symptoms are lacking. Therefore, this study was aimed to assess nutrient intake adequacy of patients with chronic pain and to examine possible dietary and anthropometric correlates of pain levels. We hypothesized that patients with chronic pain are at risk of nutrient insufficiencies, which would relate to pain levels.

4.3 Methodology

4.3.1 Study participants

This study is a cross-sectional survey of nutrient intakes of chronic pain patients. Patients were recruited over an eight-week period during their scheduled visit to the Alan Edwards Pain Management Unit at the MUHC-Montreal General Hospital and provided written consent to participate. Adult subjects (18 years or over) with chronic non-cancer pain of any type were included in the study. Excluded criteria were 1) past or present history of cancer, 2) diabetes mellitus, 3) any liver disease (resulting in AST levels more than 3 times the normal values), 4) any kidney disease (resulting in creatinine levels more than 133 μmol/L), 5) significant gastro-intestinal disease or malabsorption, 6) uncontrolled hypo- or hyper-thyroidism, 7) pace maker or metal prosthesis (due to interference with measuring body composition, see below). Recruitment was performed between July 1st and August 26th, 2005. Ethical approval was obtained from the Research Ethics Board of the McGill University Health Center, Montreal.

4.3.2 Data collection procedures and tools

During their clinical visit, socio-demographic and anthropometric data (height, weight, body fat, waist and hip) of the eligible patients were obtained. Height was measured to the nearest 0.1 cm, with a measuring tape mounted on the wall of the examination room. Waist and hip circumferences were measured with a non-elastic measuring tape at standard landmarks (WHO). Body weight and body fat percentage were measured on a scale/body fat monitor (UltimateScale^{TM}, Tanita®). Body fat percentage was estimated from standing bioelectrical impedance analysis (BIA).

The outcome measures were the patients' dietary intakes and pain intensity levels.

Pain levels were scored using a visual analog scale (VAS), which is a 10 cm line representing pain of increasing intensity from 0 on one end, representing "no

pain", and 10 on the other, representing the "worst possible pain" experienced **(Appendix 1)**. Subjects were asked to place a mark across the line in the position that best describes their average pain for the preceding week. VAS provides valid and reliable measures of self-reported chronic pain [58].

Dietary intake was assessed with three 24-hour food recalls **(Appendix 2)**, the first as a face-to-face interview, the other two by telephone 4 and 8 days after the interview. Posters illustrating two-dimensional portion sizes (NCE Food Portion Visual®) **(Appendix 3)** were used to improve the quantitative estimation of intakes. Supplements use (dosage, frequency and brand name) was recorded.

4.3.3 Data analysis

Dietary data was analyzed with Genesis R&D® Food Processor SQL Nutrition Analysis Software, Version 10.1.1 (ESHA Research, Salem, OR, USA). Genesis R&D® used the Canadian Nutrient File (CNF) version 2005 and the Food Processor software was updated with the most recent version of the CNF (2010). Most of the data in the CNF are derived from the United States Department of Agriculture (USDA) National Nutrient Database but the CNF excludes USDA foods that are not present on the Canadian market. Modification for Canadian levels of fortification and regulatory standards, along with addition of Canadian only foods or Canadian commodity data, as well as some brand name foods are included [59].

Underreporting of intakes was scrutinized and subjects with reported energy intakes less than their calculated resting energy expenditure (REE) [60] were removed from analyses.

Nutrient intakes from food and supplements were determined separately and combined. In case of the unknown dosage or brand name of a supplement, Centrum® micronutrients content was used. If omega-3 and omega 3-6-9 supplement brand names were not specified, the average content of

eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), LA (linoleic acid) and ALA (alpha-linolenic acid) from the most popular brand names in Canada (AG Natural Health®, Webbers Naturals®, Jamieson®, Life®, Swiss Natura®) were used.

Mean intakes of macronutrients (as a percentage of energy) were compared with the acceptable macronutrient distribution ranges (AMDR). Inadequacy was determined as the proportion of subjects with mean intakes below the AMDR. The acceptable AMDR for adults older than 18 years is 45% to 65% carbohydrates, 10% to 35% protein and 20% to 35% fat [61]. Micronutrients inadequacies were determined using the Estimated Average Requirement (EAR) cut-point method, in which the percentage of subjects with usual intakes below the sex and age specific EAR estimates the prevalence of inadequacy [62]. If the EAR was not established, nutrients were compared to the Adequate Intake (AI). This was the case for dietary fibre, LA and ALA fatty acids, vitamin D, vitamin K, calcium and potassium. Using the same method, mean intakes of LA and ALA fatty acids and micronutrients of supplements users and non-users were separately compared to DRIs.

Individual nutrient intakes were also compared with intakes of corresponding gender and age groups using Cycle 2.2 of the Canadian Community Health Survey (CCHS), province of Quebec. This cross-sectional survey provides reliable data (collected in 2004) about the food and nutrient intakes of Canadians (n=4,780), by province, and the relationship between diet and a wide range of health correlates. The survey comprises two parts: a general health questionnaire and a 24-h dietary recall done either by face-to-face interview or by telephone. Thirty percent of participants underwent two 24-hour recalls [63]. Data from our study were compared to the 5^{th} percentile and the 50^{th} percentile of intakes from the healthy, reference population.

4.3.4 Statistical Analyses

All statistical analyses were conducted with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL). Results were expressed as mean ± standard deviation or percentages. Normality of distribution was analyzed with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Differences in baseline characteristics, mean energy and nutrients intakes between men and women were tested by independent sample T-test (normal data distribution) or by Mann-Whitney U test (non-normal distribution). When significant differences in nutrient intakes were found, these were re-evaluated with covariate adjustment for energy intake. Between-group comparisons between supplements users and non-supplements users were conducted by Chi-squared test with Yate's corrections or Fisher's Exact test when an expected frequency was equal or less than 5. Bivariate analysis (Pearson's rho coefficient for normally distributed data and Spearman's for non-normally distributed data) was used to test for possible correlations of nutrients with pain levels. Significant correlations were controlled for energy intake by partial correlation. A *P*-value of <0.05 was considered significant.

4.4 Results

From the 189 persons who were approached to participate, 60 refused to participate and 14 could not be reached leading to a total number of 115 participants. During the study, 16 subjects could not be reached and hence were excluded. Twenty underreporting patients were excluded. Finally, 79 subjects (25 men and 54 women) with different chronic pain diagnostics **(Table 1)** were included in the analysis.

Subjects' baseline characteristics are presented in **Table 2**. Although both sexes were overweight (mean BMI greater than 24.9 kg/m²), men were significantly taller than women with a higher body weight and fat free mass. In contrast, women had a significantly higher body fat percentage and hip circumference. All other baseline characteristics were similar between sexes.

Fourty-seven subjects (60%) used at least one type of supplements. Vitamins and minerals were derived from multivitamins or from single vitamin/mineral supplements Supplements included multivitamins, single vitamin/mineral supplements, omega-3 and omega 3-6-9. Vitamin D and calcium were the most frequently used supplements in this study **(Table 3)**.

Mean energy and nutrient intakes from food only and from food and supplements of men and women suffering from chronic pain are described in **Table 4**. Although men had significantly higher energy and macronutrient intakes (carbohydrates, protein and fat) than women, there were no significant differences between their total energy and protein intakes when normalized to body weight. The distribution of macronutrients as percentage of energy intake was also similar between sexes except for monounsaturated fat (MUFAs): males consumed more than females. Greater intakes (total and from food only) of oleic acid (OA), linoleic acid (LA) and α-linolenic (ALA) fatty acids were observed in men while only total intakes of docosahexaenoic acid (DHA) were significantly greater in men **(Table 4)**. These differences did not remain significant after adjusting for energy intake except for total DHA intakes (P=0.021).

We also found differences in certain micronutrient intakes (whether from food only or from food and supplements) between sexes. Intakes from food only of vitamin B6, vitamin E, calcium, iron and zinc were greater in men (**Table 4**). These differences were no longer significant after adjusting for energy intake. Total intakes (from food and supplements) of thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, vitamin E, iron and zinc were significantly greater in men compared to women **(Table 4)**. These differences remained significant only for thiamin (P=0.043), niacin (P=0.029), folate (P=0.014), and vitamin E (P=0.022) after adjusting for energy intake.

Macronutrient intakes were compared to the acceptable macronutrient distribution range (AMDR). Thirty-nine percent of the subjects had adequate

intakes (within the AMDR) for carbohydrates, fat and protein. None had inadequate intakes (below the AMDR) for all 3 macronutrients. While carbohydrates and fat intakes exceeded the recommended levels in 13% and 42% of the subjects respectively, none of the patients exceeded the AMDR for protein.

Analysis of subjects' intakes showed that the same proportions of patients (67% and 66%) did not meet their age and sex-specific AI for LA fatty acid when dietary and total intakes were analyzed. In contrast, 72% and 42% of the patients did not meet their age and sex-specific AI for ALA when dietary and total intakes were analyzed respectively (data not shown). Dietary fibre intake was insufficient in only 11% of the subjects. Intakes below DRIs (EAR or AI) of vitamins D, E, K and potassium were prevalent in more than 72% of the patients when food only was analyzed and in more than 65% when total intakes were analyzed **(Figure 1)**. Intakes of folate, calcium and magnesium from food only and from food and supplements combined were also insufficient in a large proportion of subjects (folate: 48% and 43%, calcium: 54% and 40%, magnesium: 43% and 39%) **(Figure 1)**.

Dietary intakes of non-supplement users and total intakes of users were evaluated for adequacy. Suboptimal intakes of folate, vitamins D, E, K, calcium, magnesium, potassium, LA and ALA were prevalent in the majority of non-users. Vitamin K, potassium, LA and ALA intakes were also insufficient in more than 50% of supplements users **(Figure 2)**. Differences were found between users and non-users in prevalence of inadequate intakes for vitamin B6, folate, vitamin D, vitamin E, calcium and magnesium. Significant smaller proportions of supplement users than non-users had food intakes below the DRIs for those micronutrients **(Figure 2)**. Vitamin D supplement use significantly reduced the proportions of subjects with inadequate vitamin D intake (χ^2=50.164, P<0.001). Calcium supplementation also decreased the proportions of patients not meeting their DRIs for calcium (χ^2=10.390, P=0.001). Multivitamin use reduced the proportions of subjects with inadequate vitamin B6 (P=0.005 by Fisher's Exact

test), vitamin E (P<0.001 by Fisher's Exact test), folate (χ^2=8.721, P=0.003) and magnesium (χ^2=8.504, P<0.001). No association was found by Fisher's Exact test between supplement use and meeting DRIs for thiamin, riboflavin, niacin, vitamin B12, vitamin K, potassium, LA and ALA fatty acids.

In addition to DRIs, dietary intakes of patients with chronic pain were compared to those of a healthy Canadian reference population (n=4,864)[63]. Data showed that chronic pain patients of our study had macronutrient and micronutrient intakes below the 5th percentile of intakes from the healthy population except for fibre and vitamin A **(Figure 3)**. For instance, approximately one fourth of the patients had micronutrients intakes of riboflavin, vitamin B6, vitamin B12, vitamin C, iron, magnesium and potassium less than the 5th percentile of intakes from the healthy population **(Figure 3B)**. We also found that more than 65% of the subjects consumed monounsaturated fat, polyunsaturated fat, LA and ALA below the 50th percentile of intakes from the healthy population **(Figure 3A)**. Similarly, the majority of the subjects had micronutrients intakes below the 50th percentile of intakes from the healthy population, except for vitamins A and B12 **(Figure 3B)**.

Finally, possible correlates of pain levels were investigated among dietary intakes and body composition variables (n=49). The correlation analysis showed significant negative associations between pain VAS scores and energy intake (r=-0.318, P=0.026) and carbohydrates intake, both normalized to body weight (r=-0.293, P=0.041), total vitamin E intake (r=-0.297, P=0.038) and vitamin E intake from food only (r=-0.290, P=0.043). No statistical correlations were detected between all other nutrient intakes and VAS pain levels. When males and females were analyzed separately, energy and carbohydrate intakes normalized to body weight (r=-0.513 and -0.531, P=0.03) correlated with pain levels in males, but not in females. No significant correlations were found between pain levels scores and vitamin E intakes and body fat percentage in men and women, analyzed

separately. No correlations were found between pain scores and weight, BMI, waist to hip ratio and fat free mass.

4.5 Discussion

This cross-sectional survey was aimed to estimate the prevalence of nutrient inadequacies among subjects suffering from chronic pain and to examine possible correlates of nutrient intakes with pain levels. Results showed that specific nutrient intakes of 79 participants, assessed with three 24-h food recalls were inadequate in a large proportion of the cohort as per both official dietary recommendations (DRIs) and compared to intakes of a similar healthy population. Risk of nutrient deficiencies were found for LA and ALA essential fatty acids, vitamin D, vitamin E, vitamin K and potassium.

4.5.1 Differences in nutrient intakes between men and women

After adjusting for energy intake, nutrient intakes from food only were not different between sexes but total intakes (with supplements contribution) of thiamin, niacin, folate, and vitamin E were still greater in men than in women. These differences were most probably related to supplement use. Although the proportions of women using thiamin, niacin, folate and vitamin E supplements were higher than the proportions of men using the same supplements (thiamin: 7/11 vs4/11, niacin: 7/12 vs5/12, folate: 7/12 vs5/12, vitamin E: 19/19 vs8/19 respectively), the average quantity of each of these vitamins from supplements consumed by men was greater than that consumed by women (thiamin: 46±53 mg vs. 23±26 mg, niacin: 66±48 mg vs. 35±22 mg, folate: 1620±2100 µg vs. 400±0 µg, vitamin E: 80±109 mg vs. 36±77 mg) and could explain the differences in intakes between sexes.

4.5.2 Adequacy of energy and nutrient intakes and correlations with pain levels

4.5.2.1 *Energy*

The energy intake adequacy of a group is often assessed by the proportion of individuals with a BMI below, within or above the normal range (18.5-24.9 kg/m^2), reflecting inadequate, adequate and excessive energy intakes, respectively [61]. In our study, mean BMI was within the overweight category (men: 25±4 kg/m^2; women: 26±5 kg/m^2) with 54% of the subjects exceeding the normal range. These results indicate adequate and excessive energy intakes, considering the possibility of common under-reporting, even if true under-reporters were excluded [61]. This is not the first study to document a trend towards overweight and obesity in a sample of patients with chronic pain. Previous studies showed that individuals with chronic pain have high BMI and are more likely to be overweight [7, 8]. However, medication use for pain relief and physical activity could be possible confounding factors in estimating energy intake adequacy using the BMI method. Chronic pain patients use antidepressants and anticonvulsants as pain treatment modalities that have been found to increase body weight [64, 65]. We did not record the exact number of subjects using antidepressants and anticonvulsants in our study; yet, those are well documented as one of the widely used treatment options for chronic pain management [2, 23]. Also, patients with chronic pain have reduced physical activity [66] and consequently a lower energy expenditure and positive energy balance resulting in increased weight.

4.5.2.2 *Macronutrients*

Individuals who habitually consume intakes above or below the AMDR are at potential risk of chronic diseases that affect long-term health, and may also be at increased risk of inadequate intakes of essential nutrients [67]. In our study, none of the subjects had inadequate intakes (below AMDR) for carbohydrates, protein and total fat and a good proportion (39%) had adequate intakes for those 3 macronutrients. But 13% and 42 % had excessive intakes (above AMDR) of carbohydrates and total fat respectively, that could have contributed to an average BMI in the overweight category.

Carbohydrates intakes were adequate (within AMDR) in the all but 13% of subjects and intakes were approximately similar to those from the healthy population. Carbohydrate intakes were negatively correlated with pain scores, which mostly explains the negative correlation found between energy intakes and pain levels as carbohydrates intakes contributed strongly to energy intakes (r=0.768, $P<0.001$). Accordingly, greater carbohydrate intake was associated with less pain scores; but this finding was true for males but not for females. Whether this effect was due to total intake or different types of carbohydrates is unknown. For instance, no correlations were found between pain VAS scores and sugar or fibre intakes. Previous studies highlighted the analgesic effects of sucrose. Sucrose administration reduced pain in neonates undergoing painful procedures [68]. Sucrose analgesia was also reported in adults, more in males than in females [69]. However, in our study we assessed intakes of total sugars and not sucrose per se, which is not the only sugar. The efficacy of carbohydrates in pain reduction has been related to the hedonic experience of sugar consumption. Analgesia can be triggered by palatable food consumption that elicits pleasure [70]. For instance, sucrose administration directly into the stomach was ineffective in reducing pain sensations in premature infants [68]. This proposes that afferent signals from the mouth are possibly responsible for the analgesic characteristics of carbohydrates [71]. Nevertheless, because our subjects had adequate carbohydrates intakes and many were overweight, increasing intakes of carbohydrates to reduce pain would increase energy intake resulting in more weight gain. A high carbohydrate diet would have to be compensated by reduced fat intake to maintain diet isocaloric.

Protein intakes were adequate (within AMDR) in almost all subjects (96%) with an average intake of ~16% of energy in both men and women. Approximately one-fourth of the subjects had protein intakes below the 5th percentile of intakes from the healthy reference population. The finding that none of our subjects had excessive protein intakes (above AMDR) was not surprising because of the recently revised upper level of AMDR for protein at 35% of

calories [72]. No significant correlations were found protein intakes and pain VAS scores. In the literature, the analgesic effects of dietary protein as a whole were hardly studied; yet, certain amino acids such as tryptophan, arginine, D-phenylalanine, L-arginine and taurine, have been shown to possess anti-nociceptive characteristics. They were implicated in modifying the neurotransmitters serotonin, norepinephrine and dopamine which were implicated in altering pain threshold and behavioural responses to painful stimuli [73]. Specific amino acid intakes were not quantified in the present study.

Fat intakes were excessive (above AMDR) in 42% of the subjects. Also, mean fat intake as percentage of energy intake was high among our subjects (men: 35±9 %, women: 32±9 %). Women almost reached the highest AMDR for fat (35% of total calories), and men reached it. This was consistent with a previous study by Vandenkerkhof et al. (2011) showing that patients with chronic pain had high fatty food intakes [7]. However, when compared to the healthy population, some subjects had lower fat intakes: 1/5 consumed less than the 5^{th} percentile of intakes from the healthy population. However, we cannot precisely interpret the adequacy of intakes of saturated, mono- and polyunsaturated fatty acids separately due to several missing data in certain food items of the nutrient database. Notwithstanding, calculated intakes were lower than those of the reference healthy population: the majority of our study participants consumed below the 5^{th} percentile of intakes. Specifically, intakes of the essential fatty acids LA and ALA (total and from food only) were inadequate in a high proportion of subjects and below the 50^{th} percentile of intakes from the healthy population for the majority of our subjects. Of note, these prevalence of inadequacies could be overestimated due to the underestimated intakes of mono- and polyunsaturated fatty acids mentioned above. These findings suggest that although the majority had an adequate total fat intake, patients with chronic pain tend to make less healthy fat choices when compared to the healthy population. Fat intake has been shown to affect pain and nociception in previous studies. A high fat, low carbohydrates diet was superior to an isoenergetic high carbohydrates, low fat in

reducing experimental acute pain in 16 healthy subjects [74]. The pain relieving properties of fat were mainly attributed to the omega-3 polyunsaturated fatty acids that received a great attention in reducing inflammation-related nociception and pain. A meta-analysis of 24 articles concluded thatomega-3 fatty acids were effective in reducing joint pain associated with many inflammatory diseases (rheumatoid arthritis, inflammatory bowel disease, and dysmenorrhea). Their beneficial effects were related to the anti-inflammatory properties of the omega-3 polyunsaturated fatty acids: EPA and DHA [33]. It has been previously suggested that a dose of 2.7 g/day of EPA and DHA is required to achieve anti-inflammatory effects [75]. Our subjects with chronic pain had very low intakes (total omega-3: 0.9±1.3 g, omega-3 from food: 0.6±1.0 g, total EPA: 0.2±0.3 g, EPA from food: 0.1±0.2 g, total DHA: 0.3±0.5 g, DHA from food: 0.2±0.4 g) and no correlations with pain levels were identified.

4.5.2.3 *Micronutrients*

Intakes of folate from food only and from food and supplements combined were insufficient in 48% and 43% of the subjects respectively. Also, a large proportion (43%) of the subjects had intakes of folate below the 5th percentile of intakes from the healthy population. The prevalence of folate inadequacy in Canada has reduced since the folic acid fortification policy in 1998 [76] and supplement use have been found to contribute to excessive folate intakes [77]. Only 12 participants in our study used folate supplements. These findings suggest that our participants had low consumption of fruits, vegetables and fortified grains products, the major dietary sources of folates [77].

Vitamin D intake, total and from food only was inadequate in 67% and 87% of the subjects respectively. The high prevalence of insufficiency could be explained by a combination of the recent increase in vitamin D dietary reference intakes [78] and by a low consumption of major vitamin D sources including fortified dairy products (milk, yogurt and margarine), fortified fruit juices and fatty fish [78]. One study assessed nutrient inadequacies using the newly revised

DRIs for vitamin D and found a high prevalence of vitamin D (74 to 93%) inadequacy among Canadians [79]. Even before establishing the new DRIs, several studiesidentified a high prevalence of vitamin D insufficiency in healthy adults in North America [80]. Vitamin D intake was moderately low despite the vitamin D fortification policy [81]. This proposes that the high prevalence of inadequacy was most probably due to low consumption of vitamin D sources (natural and fortified). A major concern is the fact that almost half (43%) of the subjects consumed below the 5^{th} percentile of intakes from the healthy population. There is debate about the efficacy of vitamin D supplementation in relieving different types of chronic pain (e.g. low back pain and rheumatoid arthritis). Some studies showed a decrease in pain [82] while others did not [83]. Vitamin D treatments in these studies were of 25μg per day. Randomized controlled trials are too small and studies are insufficient to support the hypothesis that vitamin D supplementation is a useful treatment for chronic pain [84]. No relationship was found between vitamin D intakes and pain levels in the present study.

Vitamin E intakes (total and from food only) were insufficient in the majority of subjects. Vitamin E is a well-known antioxidant abundant in wheat germ, sunflower, and safflower oils [61]. In our study, higher intakes of vitamin E were associated with lower pain levels, though the association was weak. Analgesic effects of vitamin E and mechanisms of action have been previously examined in a rat model of neuropathic pain (spinal nerve ligation) [85]. Vitamin E injections decreased neuropathic pain behaviours in rats through reduction of central sensitization, induced by peripheral nerve injury. Chronic pain state is accompanied with abnormalities in both the central and peripheral nervous system. In the spinal cord, dorsal horn neurons become sensitized by peripheral tissue damage, a process known as central sensitization mediated by NMDA receptors. In the periphery, abnormal activities are produced and enter the spinal cord to initiate and maintain the central sensitization. Vitamin E was found to reduce the responsiveness of dorsal horn neurons and to decrease the levels of phosphorylated NMDA receptor subunit (pNR1) in the dorsal horn of the spinal

cord. Those analgesic effects of vitamin E were related to its antioxidant capacity. After nerve injury, the excessive oxidants build up in the spinal cord caused the central sensitization and hence increased oxidative stress in the spinal cord. Vitamin E was found to eliminate excessive oxidants and to restore the normal physiological condition and hence provides pain relief. The doses of vitamin E injections used were within the range of clinical use (2-5 g) but 200 times more the DRIs for vitamin E [85].

Inadequate intakes of vitamin K in 72% of the subjects suggests a low consumption of green vegetables, the main dietary sources of vitamin K. Plant oils such as soybean canola oil and margarine spreads are also important dietary sources of vitamin K [86]. We could not compare vitamin E and K intakes of our subjects with those of healthy subjects, as intakes of these macronutrients were not assessed in the CCHS survey.

Intakes of calcium and magnesium (total and from food only) were also insufficient in a large proportion of the subjects, most likely based on a limited consumption of dairy products. For calcium, it could be the recent increase in DRIs that resulted in a high prevalence of inadequacy [78]. However, even before revision of DRIs, low calcium consumption was reported in north America [80] and the prevalence of inadequate dietary calcium intake among adult Canadians was high (24-86%) [79]. Similarly, inadequacies in magnesium intake were also prevalent among adult Canadians (23-68%) [79]. Despite generalized inadequacies, the majority (63%-64%) of our patients had calcium and magnesium intakes below the median intake of the healthy population. Both calcium and magnesium play an important role in maintaining bone health, and magnesium was highlighted for its analgesic effects in both acute and chronic pain. A randomized, double blinded trial, showed that pre- and post-intravenous magnesium administration (15 mL pre and 2.5 mL post) was related to decreased pain and enhanced sleep in patients undergoing elective abdominal hysterectomy [87]. Magnesium supplementation (600 mg) for 2 months in patients with

fibromyalgia reduced their pain levels by 40% [88]. In our study, no correlations between magnesium intakes and pain levels were found.

Almost all subjects (92%) had inadequate potassium intakes from food only and from food and supplements combined. Inadequate potassium intakes have been reported in Canada. A study by Linda et al. (2010) assessed potassium intakes in 21 countries across North America, Europe, Asia and Oceania and found insufficient mean potassium intakes for Canadians (3 g/d) [89]**.** Despite the inadequate potassium intake of the healthy population, the majority of our subjects consumed even less, i.e. below the median intake of the healthy population. This is likely related to a diet lacking in fruits and vegetables, and rich in refined carbohydrates and processed foods, from which potassium is removed [90].

Most of the subjects with chronic pain had adequate intakes of thiamin, riboflavin, B6, B12, vitamin C and iron; yet, around 25% consumed below the 5^{th} percentile of intakes from the reference population. An even greater proportion (62%) had niacin intakes below the 5^{th} percentile of intakes from the healthy population. Among these micronutrients, vitamin C was highlighted for its potential analgesic properties. Vitamin C prevented neuropathic pain following fractures. A daily dose of 500 mg was found to reduce the risk of complex regional pain syndrome (CRPS) type 1 in patients after write fractures [35]. No correlations were found between vitamin C intakes and pain levels in our study.

The listed inadequacies depicted in our subjects suggest an unbalanced diet with low consumption of fruits, vegetables and dairy products. This would be consistent with findings from Vandenkerkhof et al. (2011) showing that patients with chronic pain (especially women), compared with healthy subjects, reported an unhealthy food intake with low fruit and vegetable consumption [7]. Moreover, the lack of correlations between pain levels and intakes of micronutrients that were previously shown to possess analgesic effects on dissimilar chronic pain

conditions could be related to the low and narrow ranges of intakes of our subjects. In fact, studies showing potential analgesic effects used supplementation (with high doses) as a treatment intervention. In our study, micronutrients intakes (even with supplements contribution) were much lower than the amounts of micronutrients provided by the supplements (e.g. mean total intake of vitamin C: 179±259 mg vs 500 mg, magnesium: 399±259 mg vs 600 mg, vitamin D: 8±7 µg vs 25µg). Plus, if supplementation of certain micronutrients were associated with lower pain severity, it could still be possible that a deficiency would exacerbate pain.

4.5.3 Supplement use and prevalence of nutrient inadequacies

The proportion of chronic pain subjects using supplements (60%) was higher than that of the healthy Canadian population (40%) [79]. As expected, non-supplement users had higher prevalence of inadequacies compared to supplements users. Supplement use significantly reduced the prevalence of inadequacies for certain micronutrients including folate, vitamin D, vitamin E, calcium and magnesium, a reduction similar to that reported in healthy Canadians [79]. Despite this effect, around 30% of patients still had inadequate intakes of vitamins E and calcium. Furthermore, the majority of supplement users still had inadequate intakes of vitamin K, potassium and essential fatty acids (LA and ALA). In fact, multivitamins contain very low quantities of potassium (80 mg) and vitamin K (10 to 30 µg) and omega-3 and omega 3-6-9 supplements have low content of LA (0.2 g) and ALA (0.004 to 0.4 g), such that these did not contribute importantly to achieve DRIs.

Methodological limitations are to be considered when interpreting results on supplement use from this study. The first is the approximation of fatty acid content of certain supplements (e.g. reported as “Omega-3”) since some subjects did not provide the exact brand names. Estimation was calculated based on an average content from supplements most commonly consumed by other subjects. Second, supplement use is subject to misreporting in terms of type, frequency and

dose. Because of these limitations, the proportion of supplements users not meeting DRIs may be higher or lower than reported in this study.

4.5.4 Nutrient inadequacy and assessment of nutritional status

Although our subjects had several nutrient inadequacies, we cannot conclude of any nutritional deficiencies, which would require confirmation by appropriate biochemical indices. However, insufficient dietary intakes indicate risks of nutrient deficiencies. Assessment of dietary adequacy is only one component of a nutritional status assessment; ideally, intake data are combined with clinical, biochemical, or anthropometric information to provide a valid assessment of nutritional status [61].

4.5.5 Diet quality and its relation to pain states

It is not possible to determine whether the diet quality of our participants was directly related to their pain states or not. There are other factors, such as socioeconomic background, that have been associated with lower diet quality and that were not taken into consideration while assessing diet quality of the participants. For instance, the nutritional quality of Canadians' food intakes has been found to be a function of their social position. Higher income and education levels were associated with increased consumption of milk and alternatives, vegetables and fruit, and significantly higher intakes of vitamins and minerals among adults [91]. Other studies reported that persons with low socioeconomic backgrounds tend to have poorer diets [7, 92].

4.5.6 Limitations and strengths

The cross-sectional design and modest sample size of the current study may present as a limitation to the generalizability of the current findings. Also, the disproportionate number of female vs. male participants limits the generalizability to the male chronic pain population. Despite these limitations, our study is currently the only one to provide an overview of the dietary intakes of patients with chronic pain.

Dietary 24-hour recalls are considered the strongest methods for dietary intake estimation and the method of choice for assessing the intakes of groups, especially when repeated multiple times. They have well-established reliability and validity [61]. Recalls are nonetheless subject to respondent biases, memory relapses and incorrect estimation of portion sizes [93]. We took several actions to obtain the greatest degree of accuracy of dietary intakes. Subjects with high intakes have been found to report lower than usual intakes leading to misleading results [94]. Also, underreporting has been found to be more frequent in persons with a higher BMI and was associated with hidden (forgotten) fat intakes [95, 96]. In our study sample, men and women had a mean BMI within the overweight category, hence increasing the chances of underreporting. Therefore, we corrected for underreporting by excluding from analysis subjects whose energy intakes were less than their calculated resting energy expenditure (Harris-Benedict equation) [60]. Most of these under-reporters (76%) had a high BMI ranging from 25 to 38 kg/m^2. The NCE Food Portion Visual® aid was taught at the first face-to-face interview and used by patients during subsequent phone calls, to estimate their portion sizes more precisely. Brand names of commercial foods and recipes of homemade foods were also recorded to refine the nutrient search upon analysis. Moreover, calculation of dietary intake from one 24-hour recall may give an inaccurate estimate of individual usual nutrient intake because of the existence of large intra-individual day-to-day variation due to factors such as appetite, food choices, day of the week and seasons [61]. To minimize daily variation, 3 dietary intakes were collected for each subject. In a small number of patients (n=14, neuropathic facial pain patients) only one dietary intake was collected as they were part of another study with a different experimental design.

The DRIs are set to meet nutritional needs of healthy populations, which is not the case for chronic pain patients. Chronic pain and its therapy might require higher nutritional requirements; therefore, our data may underestimate nutritional inadequacies for some nutrients.

The comprehensive nutrient database used, the Canadian Nutrient File, has certain limitations. First, some food items do not provide a full nutrient data set with missing values for certain micronutrients. To increase accuracy of micronutrient intakes, we corrected the nutrient content of all food sources for the micronutrients that were found inadequate in a high proportion of subjects (folate, vitamins D, E, K, magnesium and potassium) in presence of missing values. Choices of equivalency were made to ensure completeness of data for these micronutrients. Second, the database provides the average amounts of nutrients in foods available in Canada, taking into consideration different sources. Specific, local foods may have a different profile than the national average.

Not all subjects were included in the analysis when examining possible correlates of pain levels with nutrient intakes. Pain VAS scores were recorded for only 49 subjects (62%, 14 men and 35 women) leading to a smaller sample size. Finally, the assessment of pain is a subjective measure and could be biased even within a given patient by other concurrent issues (e.g. social and emotional distress) [97].

4.6 Conclusion

From the present study, we conclude that macronutrient intakes were sufficient or excessive with respect to individual energy expenditure, contributing to an average BMI in the overweight category. In contrast, intakes in essential fatty acids and many micronutrients, especially vitamin D, vitamin E, vitamin K and potassium were marginal and below those of the general population in high proportions of the patients with chronic pain. This is suggestive of a poor diet quality (low consumption of fruit, vegetables and dairy products) and greater risks of deficiency in these nutrients. Educating patients with chronic pain to achieve and maintain a healthy diet is important as these subjects have been shown to be at an increased risk of morbidity; early mortality from cardiovascular disease and certain cancers [6, 7] that were explained by past diet and lifestyle characteristics (BMI, smoking and physical activity) [7]. Higher intakes of energy, specifically

from carbohydrates, and vitamin E were associated with lower pain levels. Increasing energy intakes is not suggested due to a greater risk of weight gain; but carbohydrates and vitamin E werepreviously shown to exert analgesic effects in animal studies [85] and could open new research and therapeutic avenues for patients with chronic pain. Finally, the lack of correlations between pain levels and intakes of other nutrients that were previously shown to possess analgesic effects could be related to the low nutrient intakes of subjects, the mixed etiologies of chronic pain, and the relatively small sample size of our study.

Table 1. Diagnosis of chronic pain[a]	
Diagnostics	**n**
Facial pain and headache	21
Low back pain	10
Fibromyalgia and diffused body pain	12
Upper & lower limb pain	5
Peripheral neuropathy	3
Multiple sclerosis	2
Neck and shoulder pain	2
Pelvic pain	2
[a]Diagnosis of chronic pain are missing in 22 subjects	

Table 2. Subjects' characteristics at baseline[a]					
	n	**Males**	**n**	**Females**	***P* value**
Age (y)	25	47±12	54	50±16	0.379
Anthropometrics					
Height (cm)	25	174±5	54	163±6	<0.001
Weight (kg)	25	77±12	54	68±13	0.040
BMI[b] (kg/m^2)	25	25.4±3.8	54	25.8±5.2	0.726
Body fat (%)	22	22±7	50	33±9	<0.001
Fat free mass (kg)	22	60±7	50	44±4	0.037
Waist (cm)	22	91± 8	43	85±14	0.091
Hip (cm)	22	98±7	43	102±12	<0.001
Waist to hip ratio	22	0.93±0.05	43	0.86±0.06	0.726
Pain Characteristics					
Pain duration (y) †	24	6±6	52	9±9	0.075
VAS[c] (cm)	17	6.1±2.3	32	6.9±1.8	0.187

[a]Results are expressed as mean±SD. Differences between males and females were tested using independent sample T-test.

[b]Body Mass Index

† Differences between males and females tested by Mann-Whitney U test.

[c]Visual Analog Scale

P <0.05 is considered significant.

Table 3. Number of subjects using supplements[a]	
Supplements	**n**
Vitamin A	11
Thiamin	11
Riboflavin	13
Niacin	12
B6	13
Folate	12
B12	14
Vitamin C	14
Vitamin D	23
Vitamin E	18
Vitamin K	8
Calcium	27
Magnesium	14
Potassium	10
Omega 3	10
Omega 3-6-9	9

[a]Data reflect the 47 subjects who reported using supplements. Some subjects reported using combinations of multivitamin and individual supplements, such that, numbers sum to greater than total number of subjects in the subcategory.

Table 4. Energy and nutrient intakes of patients with chronic pain[a]			
Daily intake	**Males**	**Females**	***P* value**
Energy and macronutrient intakes			
Energy (kcal)	2387±675	1902±541	0.003
Energy (kcal/kg)	31±10	29±11	0.184
Protein (g)	101±44	76±23	0.030
Protein (g/kg)	1.3±0.6	1.2±0.5	0.393
Protein (%)	16.5±3.8	16.2±4.4	0.767
Carbohydrates (g)	294±86	249±84	0.030
Carbohydrates (%)	50±11	53±12	0.362
Total fat (g)	93±39	70±33	0.008
Total fat (%)	35±9	32±9	0.296
Polyunsaturated fat (g)	16±12	10±5	0.022
Polyunsaturated fat (%)	6±3	5±2	0.268
Monounsaturated fat (g)	33±16	22±13	0.003
Monounsaturated fat (%)	12±4	10±4	0.037
Saturated fat (g)	29±12	23±12	0.024
Saturated fat (%)	11.2±4.0	10.8±4.2	0.691
Fibre (g)	25±13	21±13	0.109
Sugar (g)	95±55	85±44	0.537
Cholesterol (mg)	303±177	264±193	0.264
Linoleic Acid (LA 18:2)			
Food[b] (g)	12.19±10.22	7.53±4.13	0.035
Total[c] (g)	12.23±10.22	7.55±4.12	0.038
α- Linolenic Acid (ALA 18:3)			
Food[b] (g)	1.35±1.12	1.11±1.20	0.047
Total[c] (g)	1.42±1.12	1.14±1.21	0.033
Eicosapentaenoic acid (EPA 20:5)			
Food[b] (g)	0.20±0.30	0.06±0.15	0.184

Total[c] (g)	0.33±0.49	0.12±0.22	0.056
Docosahexaenoic acid (DHA 22:6)			
Food[b] (g)	0.37±0.56	0.13±0.28	0.208
Total[c] (g)	0.44±0.65	0.17±0.31	0.049
Micronutrient intakes			
Vitamin A			
Food[b] (μg RE)	1363±1346	1300±1578	0.606
Total[c] (μg RE)	1573±1352	1367±1606	0.214
Thiamin			
Food[b] (mg)	2.0±1.2	1.4±0.7	0.055
Total[c] (mg)	11.4±28.6	4.4±11.9	0.006
Riboflavin			
Food[b] (mg)	2.3±1.1	1.8±0.7	0.094
Total[c] (mg)	11.7±28.5	11.3±54.9	0.019
Niacin			
Food[b] (mg)	24±15	18±8	0.214
Total[c] (mg)	37±34	23±18	0.030
Vitamin B6			
Food[b] (mg)	2.3±1.4	1.6±0.7	0.022
Total[c] (mg)	12.9±32.1	5.6±16.4	0.004
Folate			
Food[b] (μg DFE)	445±260	338±161	0.143
Total[c] (μg DFE)	770±1138	390±251	0.016
Vitamin B12			
Food[b] (μg)	6±5	5±6	0.053
Total[c] (μg)	22±38	10±14	0.021
Vitamin C			
Food[b] (mg)	140±131	125±78	0.788
Total[c] (mg)	160±133	184±303	0.817
Vitamin D			
Food[b] (μg)	6±5	3±3	0.245

Total[c] (μg)	8±6	8±9	0.531
Vitamin E			
Food[b] (mgα-tocopherol)	9±9	5±4	0.013
Total[c] (mgα-tocopherol)	10±9	5±5	0.003
Vitamin K			
Food[b] (μg)	79±63	94±129	0.768
Total[c] (μg)	83±69	96±131	0.476
Calcium			
Food[b] (mg)	1060±487	825±407	0.034
Total[c] (mg)	1141±483	1057±524	0.448
Iron			
Food[b] (g)	17.8±9.1	17.8±32.0	0.037
Total[c] (g)	19.4±9.6	18.3±32.1	0.013
Magnesium			
Food[b] (mg)	356±151	296±144	0.059
Total[c] (mg)	443±268	379±257	0.109
Potassium			
Food[b] (mg)	3345±1535	2759±1081	0.096
Total[c] (mg)	3371±1526	2770±1087	0.086
Zinc			
Food[b] (mg)	12±7	9±4	0.041
Total[c] (mg)	14±8	10±6	0.015

[a]Results are expressed as mean± standard deviation. Differences between males and females were tested using independent sample T-test for protein (%), carbohydrates (g), carbohydrates (%), total fat (%), monounsaturated fat (%) and saturated fat (%); Mann-Whitney U test for all other macronutrients and micronutrients. $P<0.05$ is considered significant.

[b]Dietary intakes from food only.

[c]Total intakes from food and supplements combined.

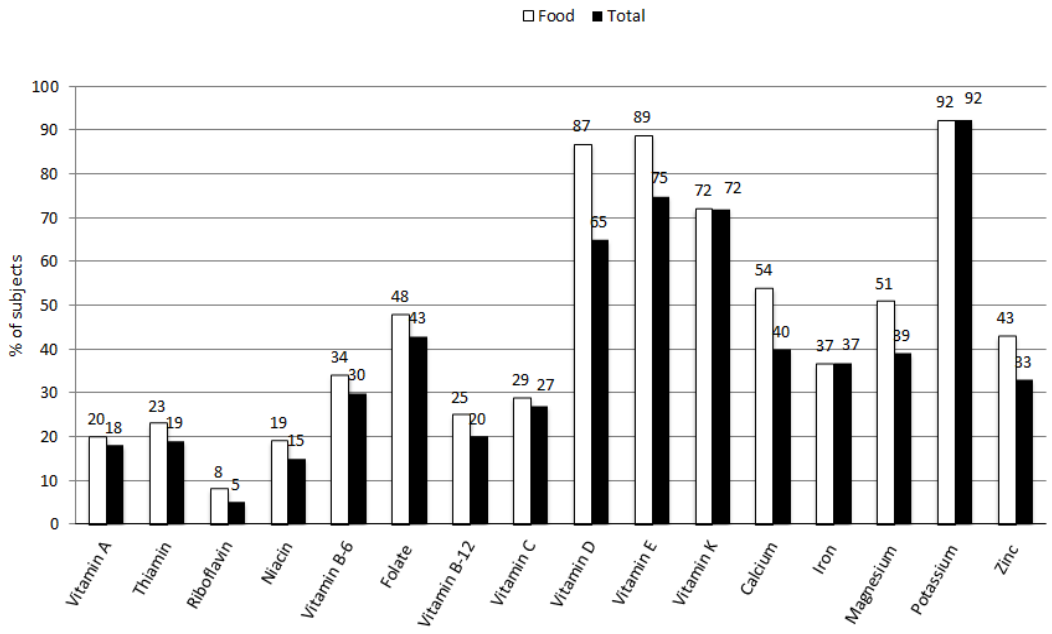


Figure 1. Percentage of subjects not meeting age and sex-specific Dietary Reference Intakes for micronutrients. Food: dietary intakes from food only. Total: intakes from food and supplements combined. Recommended intake levels used in the analysis were: vitamin A, 625 μg (for men), 500 μg (for women); thiamine, 1.0 mg (for men), 0.9 mg (for women); riboflavin: 1.1 mg (for men), 0.9 mg (for women); niacin: 12 mg (for men), 11 mg (for women); vitamin B-6: 1.4 mg (for men), 1.6 mg (for women); folate: 320 μg; vitamin B-12: 2.0 μg; vitamin C: 75 mg (for men), 60 mg (for women); vitamin D: 10 μg; vitamin E: 12 mg; vitamin K: 120 μg (for men), 90 μg (for women); calcium: 800 mg (19-50 y), 800 mg (for men, 51-70 y), 1000 mg (for women, 51-70 y), 1000 mg (≥70 y); iron: 6 mg (for men), 8.1 mg (for women, 19-50 y), 5 mg (for women, ≥51 y); magnesium: 330 mg (for men, 19-30 y), 350 mg (for men, ≥51 y), 255 mg (for women, 19-30 y), 265 mg (for women, ≥51 y); potassium: 4,700 mg; and zinc: 9.4 mg (for men) and 6.8 mg (for women, ≥31 y) [61, 78].

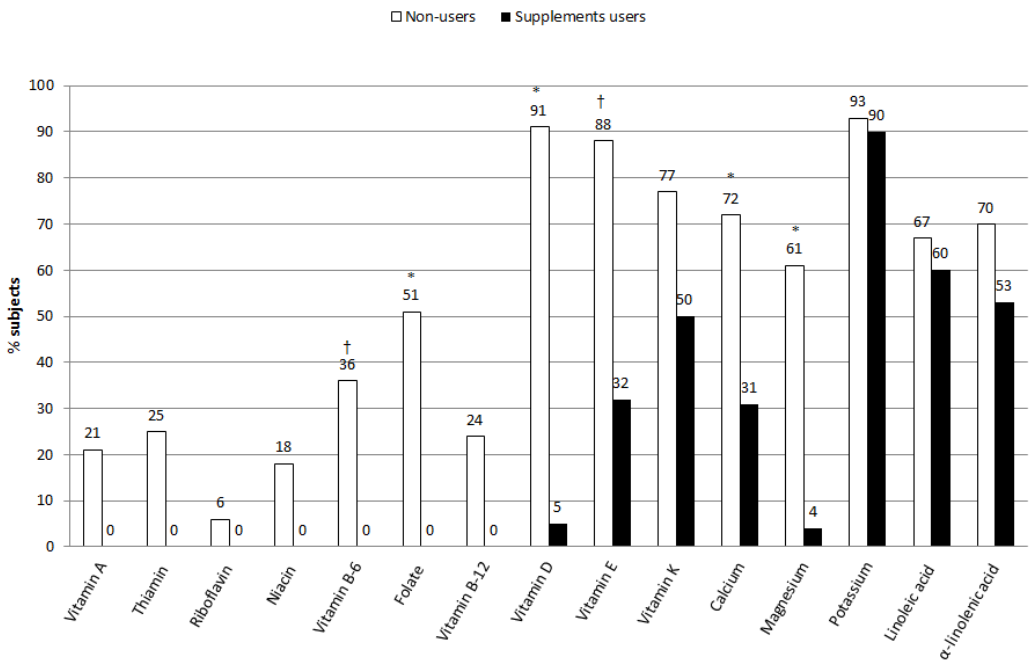


Figure 2. Percentage of non-users and supplements users not meeting age and sex-specific Dietary Reference Intakes for micronutrients. **P*<0.005 by Chi-squared test (χ^2). †*P*≤0.05 by Fisher's Exact test. Recommended intake levels used in the analysis were vitamin A: 625 µg (for men), 500 µg (for women); thiamine: 1.0 mg (for men), 0.9 mg (for women); riboflavin: 1.1 mg (for men), 0.9 mg (for women); niacin: 12 mg (for men), 11 mg (for women); vitamin B-6: 1.4 mg (for men), 1.6 mg (for women); folate: 320 µg; vitamin B-12: 2.0 µg; vitamin C: 75 mg (for men), 60 mg (for women); vitamin D: 10 µg; vitamin E: 12 mg; vitamin K: 120 µg (for men), 90 µg (for women); calcium: 800 mg (19-50 y), 800 mg (for men, 51-70 y), 1000 mg (for women, 51-70 y), 1000 mg (≥70 y); iron: 6 mg (for men), 8.1 mg (for women, 19-50 y), 5 mg (for women, ≥51 y); magnesium: 330 mg (for men, 19-30 y), 350 mg (for men, ≥51 y), 255 mg (for women, 19-30 y), 265 mg (for women, ≥51 y); potassium: 4,700 mg; and zinc: 9.4 mg (for men) and 6.8 mg (for women, ≥31 y) [61, 78].

Figure 3A.

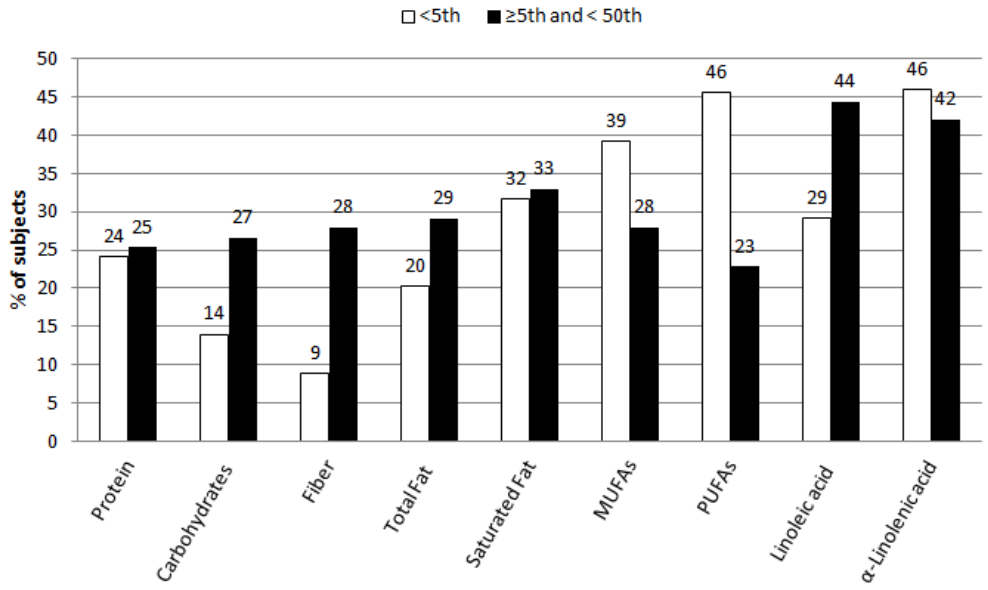


Figure 3B.

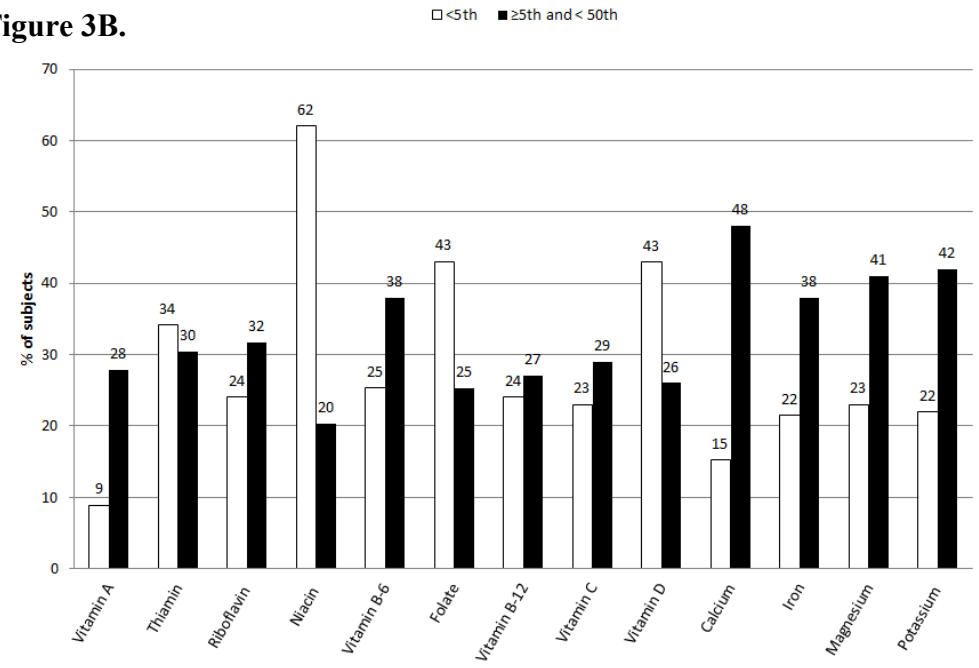


Figure 3. Percentage of subjects consuming (3A.) macronutrients and (3B.) micronutrients below the 5th percentile, and between the 5th and 50th percentile of intakes from the healthy population, of the Canadian Community Health Survey, Cycle 2.2., Nutrition (2004). Intakes were compared to sex and age relevant reference groups: men (31-51 y, 51-70 y and ≥ 70 y) and females (19-30 y, 31-51 y, 51-70 y and ≥ 70 y) [98].

5 BRIDGE

In the first manuscript we evaluated the dietary intakes of subjects with chronic pain and found that although macronutrient intake and distribution was generally acceptable, subjects were at risk of many, specific nutrient deficiencies. We also examined possible dietary correlates of pain levels and found negative association between energy, carbohydrates and vitamin E intake and pain scores. Correlations between other nutrients and pain levels were not found, possibly because of the low intakes of our participants, the mixed etiologies of chronic pain, and relatively small sample size. Thus, dietary strategies to improve diet quality and optimize nutritional status of patients with chronic pain are still warranted. Given the numerous insufficiencies, such strategies would imply changing many aspects of usual dietary habits, which represents a particular challenge for patients with chronic pain. Indeed, pain is often accompanied by depression, loss of appetite and general lack of motivation towards buying, preparing and eating food.

To maximize success, in the second study we tested the introduction of one dietary constituent, soy protein, to patients suffering from one form of chronic pain, the neuropathic facial pain. On the basis of studies indicating that soy protein reduced neuropathic pain in animals and humans, we hypothesized that soy protein could reduce pain in patients suffering from neuropathic facial pain. Thus, we conducted a pilot study testing the feasibility and compliance of a diet enriched with soy protein in patients with neuropathic facial pain. We also aimed to measure soy efficacy, compared to a placebo, on pain, depression and quality of life levels, in individual distinct patients (N-of-1 design) and as a group. The necessity for an assessment on an individual basis is important due to the large heterogeneity encountered within populations of chronic pain patient and consequent variability exhibited in response to analgesic medication. If soy was found to be superior, it would benefit afflicted patients as it is considered as a safe and available dietary ingredient as opposed to pharmacological agents.

6 MANUSCRIPT 2

Soy protein vs milk protein in the treatment of neuropathic facial pain: a randomized double blinded placebo-controlled N-of-1 pilot study.

Alexis Codrington, Ph.D.[1,2], Douna Maria Masmar, M.Sc candidate[3,4],Yoram Shir, M.D.[1,2], Stéphanie Chevalier, Ph.D.[3,4]

The first two authors contributed equally.

[1]Department of Anesthesia, McGill University, [2]The Alan Edwards Pain Management Unit,McGill University Health Centre, [3]School of Dietetics and Human Nutrition, [4]McGill Nutrition and Food Science Centre, McGill University, Montreal, Quebec, Canada.

To be submitted for publication in the Nutrition Journal

6.1 Abstract

Background: Neuropathic facial pain is one type of chronic pain lacking effective management. Research for alternative treatment is ongoing. Soy protein was shown to decrease neuropathic pain in animal and some human studies. This study aims to establish the feasibility and effects of a diet enriched with soy protein against milk protein on pain, depression and quality of life scores using an N-of-1, randomized, double-blinded, placebo-controlled design.

Methods: Participants were exposed to soy or milk protein powder during 3 week-intervals, in 3 paired treatment periods. Pain intensity (11-point Numerical Rating Scale), depression (Beck Depression Inventory-II), quality of life (Pain Disability Index) and dietary intakes (24-hour recall and food frequency questionnaire) were assessed at baseline and followed up throughout each period. Patients were provided with either soy or milk protein powder (30 to 50 g protein/d based on body weight), as a replacement of half of usual daily protein. Compliance was measured by weighting leftover powder. Differences between pain, depression and quality of life scores of the milk and soy interventions were tested using paired t-tests or the Wilcoxon signed ranks test. Differences between mean baseline, soy and milk outcome scores were tested by one-way ANOVA.

Results: Fourteen subjects participated but only 4 completed the study. Despite complaints in protein powder acceptance, average compliance (% powder consumed) was high (>85%). No significant differences in pain, depression and quality of life individual and group scores were found between the milk and soy interventions.

Conclusions: A diet enriched with soy protein is a feasible intervention but future studies should consider other dietary approaches or better quality protein powder products to improve acceptability. The absence of effect of soy protein on pain severity is inconclusive due to the small sample size of this pilot study. This warrants confirmation with future larger studies.

Keywords: neuropathic facial pain, soy protein, supplement, diet, feasibility, compliance, pain scores.

6.2 Introduction

Neuropathic facial pain is a type of chronic pain affecting the facial area and caused by tissue injury or nerve damage (usually the fifth cranial nerve, the trigeminal nerve) related to surgeries, dental procedures [23] and most often unknown origin [27]. Episodes of pain are described as sharp, burning, electric, and shock-like, most of the times unilaterally and lasting from seconds to minutes [23]. Symptoms affect the quality of life of the patients and many suffer from depressive symptoms [26]. Several types of neuropathic facial pain exist including trigeminal neuralgia (most common form) [25], glossopharyngeal neuralgia, facial postherpetic neuralgia, occipital neuralgia and local neuralgia [24]. It is a rare disease with an incidence of 21.7 out of 100,000 persons per year and seen more commonly among women than men.

Treatments options of neuropathic facial pain include pharmacology, neural blockade procedures, and surgery [27], These strategies are effective in providing only short term pain relief and recurrence is common within years [29]. Most of the patients become refractory or intolerant to medications (antidepressants, anticonvulsants and anti-inflammatory agents) [23, 29]. The effects of neural blockade, a procedure consisting of injecting local anesthetic products into the nerve, are temporary [27]. Side effects and long term-relapse rate still exist with surgical procedures that aim at destroying the nerve for pain control [23, 25, 29]. Therefore, patients move toward using complementary and alternative medicine (acupuncture, therapeutic massage and herbal remedies) to treat their debilitating pain [9], without confirmation of success [30].

Diet is a promising alternative treatment for pain [9, 18]. Specific dietary constituents were shown to have analgesic properties in animal [10, 11] and humans [12-14]. Soy protein is one of the promising ingredients tested for its analgesic properties although the mechanism of soy-mediated analgesia is unclear. It has been shown to modify neuropathic pain behaviors in animal studies [15-17]. In nerve injured rats, a 20% soy protein diet suppressed chronic

neuropathic like pain development (tactile and heat allodynia) [16]. A series of experiments by Shir et al. (1998) depicted that soy omission from the diet, as opposed to casein addition, was responsible for neuropathic pain development in rats [15]. Furthermore, the pain suppressive properties of soy protein were established to be the result of preoperative rather than post surgical soy consumption [17]. Soy protein also exerted analgesic effects in human studies of cyclical menstruation-related breast pain [13] and pain associated with osteoarthritis [14] but not fibromyalgia [12]. Finally, a preliminary pilot, open-label study showed that diets rich in soy protein (30-55 g, proportional to body weight) for 6 weeks reduced chronic post-traumatic neuropathic pain in some patients but not on average [19]. The main objective of the current study was to establish the feasibility and compliance to a diet enriched with soy protein powder in patients with neuropathic facial pain. We also aimed to evaluate the efficacy of this intervention compared to a control (milk) diet on pain severity, depression levels and quality of life indices, using an N-of-1 design. We hypothesize that a diet enriched with soy protein powder is a feasible intervention and superior to diet enriched with milk protein in decreasing the severity of neuropathic facial pain.

6.3 Methodology

6.3.1 Study Design

This study consisted of N-of-1 randomized, double-blinded, and placebo controlled trials. The N-of-1 trial is a multi-crossover study applied to a single patient. The participant undergoes pairs of treatment periods (one period of each pair with the treatment and one with placebo, randomly assigned). Both the investigator and the patient are blind to treatment allocation [99]. This study design allows the measurement of the treatment efficacy in individual, distinct patients and results from N-of-1 trials on several patients can be combined to estimate the treatment efficacy for the whole population [100, 101]. The N-of-1 trial is useful for chronic conditions for which the potential treatment has a rapid onset of action and ceases to act soon after it is discontinued [99].

Each patient was exposed to the soy protein powder and the control product, milk protein, for a total of 3 treatment pairs. Each pair lasted for 6 weeks (3-week periods of soy and milk protein each), and allocated randomly. All members of the research team were blinded except for the research assistant otherwise not involved in the study, who prepared the assignment codes and randomization schedule for each patient.

6.3.2 Subjects

Males or females over 18 years with neuropathic facial pain were recruited for this trial. Patients were eligible if (1) they were diagnosed with chronic neuropathic facial pain according to the 2004 International Headache Society guidelines, (2) had suboptimal pharmacotherapy, (3) their pain intensity score was more than 4 on a 10 cm VAS, (4) they used stable medication over 1 month before the start of the trial (current medication was maintained, but additional pharmacotherapy was not allowed). Subjects were excluded if they (1) had a history of heart, gastro-intestinal, liver or kidney disease, (2) used the anticoagulant warfarin (Coumadin), (3) had gastro-intestinal malabsorption of any type, (4) suffered from lactase deficiency, (5) had allergies to any dietary product, (6) consumed soy protein exceeding 10 g/day, (7) were strict vegans, (8) used antibiotics, (9) had a history of psychiatric disorder, and (10) had a body mass index (BMI) exceeding 35 kg/m².

Ethical approval was obtained from the MUHC-Research Ethics Board and the research team worked according to institutional policies governing human subjects' research, applicable research guidelines and in compliance with the relevant law.

6.3.3 Interventions (Appendix 2)

Soy protein isolate powder (PRO-FAM® 873; Archer Daniels Midland Co., Decatur, IL, USA) contained 85% protein, 4% fat, 2.2 mg/g of isoflavones, <5% ash and 4.4% moisture. Milk protein powder (Prodiet 85 dispersible;

Ingredia S.A., Arras, France; 81% protein, 5.5% fat, 7.5% ash, 5.5% lactase and 5% moisture) was used as the control since it has been shown to have minimal effects on nociception in previous animal studies [15, 16, 53], is widely consumed in Western diets and frequently served as a control in trials investigating the beneficial effects of soy protein [15-17]. Both powders were similar in color, had bland, clean-flavoured, readily dispersible in food or beverages. Patients received the amount of powder based on protein content and their body weight (30 g/d if <60 kg, 40 g/d if 60-70 kg and 50 g/d if >70 kg). These doses are well tolerated previously and are high enough to detect the desired effects of soy based on previous studies [19].

6.3.4 Outcome measures, experimental tools and study procedures (Appendix 3)

The primary outcome measures are pain intensity levels. Secondary outcome measures include patients' compliance, dietary intakes, depression levels, quality of life indices and adverse events.

After giving written informed consent **(Appendix4**), eligible subjects completed a medical history, baseline demographic, pain intensity, depression and quality of life questionnaires. Maximum and minimum pain intensity was measured using the 11-point numerical rating scale (NRS) **(Appendix 5)**, known to be sensitive to therapeutic-mediated changes [102]. Depression was measured with the validated Beck Depression Inventory (BDI-II) **(Appendix 6)** [103]. Life quality was assessed with the Pain Disability Index (PDI) **(Appendix 7)** [104]. Standing height was measured using a stadiometer (Seca®). Body fat percentage was estimated from bioelectrical impedance analysis (BIA) using a standing BIA instrument (Tanita®, Arlington Heights, IL), which was also used to measure body weight. A 24-hour recall **(Appendix 2)** and a food frequency questionnaire (FFQ) oriented towards energy and protein-dense foods were used to assess dietary intakes **(Appendix 8)** were performed by the study dietician. Patients were advised on how to dissolve the powder in their usual food and drinks according to

their preferences and were provided with different recipes. They were also instructed to maintain the same fat and carbohydrate consumption but to reduce by half their daily protein consumption of meat, fish, poultry and eggs to keep diet isoenergetic and isoproteic. Patients received 21 containers of the test powder every three weeks.

Follow-up 24-hour food recalls were completed by phone on days 5, 12 and 19 (±1 day) of each treatment period **(Appendix 9)** to complete and to improve adherence with adjustments to diet if needed. Compliance was measured by weighing any leftover powder not consumed every 3 weeks. Nutritional data was analyzed using the Food Processor SQL Nutrition Analysis Software, Version 10.1.1 (ESHA Research, Salem, OR, USA). Pain intensity (NRS) was recorded on days 15, 18 and 21 (±1 day) along with any change in medication or side effects **(Appendix 9)**. We chose to record maximum and minimum pain intensity levels for the preceding 24 hours twice during the third week of every treatment period because in the previous pilot study, pain scores returned to baseline after 2 weeks of stopping soy consumption [19].

6.4 Statistical Analysis

Formal sample size calculation was not performed due to the pilot feasibility nature of the study. For larger-scale N-of-1 study, 20 subjects would be required for detecting a within-patient decrease in pain intensity average of 2 cm on a 10 cm VAS with the soy protein treatment, with a within subject standard deviation σ=3 cm, α=0.05, 1-β= 0.8. Considering a 15 % drop out rate during each 6 week treatment period, a total of 33 patients would need to be enrolled.

Data are presented as mean ± standard deviation of the mean, standard error of the mean or percentages. The distribution of data was analyzed with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Differences between outcome scores (pain, depression and quality of life scores) during the milk and the soy intervention periods were tested for the subjects who completed the study (n=4)

using paired t-test (if the distribution of variables was normal) or the Wilcoxon signed ranks test (if the distribution was non-normal). The t-test approach takes into account the magnitude and consistency of the difference between milk and soy treatment [99, 105] while the Wilcoxon signed rank test enables us to incorporate the size of the outcome score differences [105]. For each subject, the average of mean outcome scores for the soy and milk periods were calculated. Differences between mean baseline, soy and milk outcome scores were also tested by one-way ANOVA. Differences between outcome scores of the two interventions were also evaluated for each subject by paired t-tests or the Wilcoxon signed ranks test, depending on the normality of the data distribution. The effect of protein replacement on energy, macronutrients and calcium intakes was tested for the subjects who completed at least one period (n=10) using one-way ANOVA and Games-Howell post hoc analyses. A P-value of less than 0.05 was considered significant. Statistical analyses were conducted with SPSS 19.0 for windows (SPSS Inc., Chicago, IL).

6.5 Results

Twenty-two patients suffering from neuropathic facial pain were screened between February and December 2011. Nine patients did not meet inclusion criteria and 14 (3 men and 11 women) (**Table 1)** participated in the study. Four patients (1 men and 3 women) completed the study (6 periods). Reasons for drop outs are shown in **Table 2**. Main reasons were deviance from study protocol such as participation in other studies for pain (n=1), surgery (n=2), change of medications (n=3) and voluntary discontinuing of the study (n=4).

Participants' baseline characteristics are presented in **Table 3**. Subjects were overweight on average (mean BMI 24.9-29.9 kg/m²) and had a high body fat percentage (31±12 % in men, 38±7 % in women). Minimum and maximum pain scores did not differ significantly. Depression levels were mild based on the BDI test scores[103]. The categories of life activity mostly disrupted by pain were

recreation, occupation and social life. PDI scores of those categories were mild (5/10) and mid-range for the rest of categories.

6.5.1 Soy and milk powder consumption and acceptance

Not withstanding manufacturers' claims, six subejcts enrolled reported difficulties in mixing the soy powder in their foods and beverages whereas one reported troubles with the milk powder upon unblinding. All 10 subjects who completed at least one period reported altered taste of their usual foods and most opted for mixing the powders in a blended shak or smoothie with yogurt or fruit juices. In all, 3/10 complained about boredom from the smoothies (n=1), bad taste (n=1) and powder quantity (n=1). One subject was satisfied with the new dietary approach and reported feeling more energetic. Four patients noted a taste difference and preferred the milk over the soy powder.

6.5.2 Compliance

Compliance (% of powder consumed) was assessed for the subjects who completed at least one period (n=10). One patient did not bring back the containers of the powder and was excluded from compliance analyses. From those who finished the study (n=4), three had a high (>80%) and one had a low compliance by the end of the study **(Table 4)**. Compliance of patients who completed one or few periods (n=5) was also high except for one. Compliance was greater during the milk than the soy intervention periods: 92±15 % vs 86±24 %, P=0.045 by Wilcoxon signed rank test.

6.5.3 Effect of protein replacement on energy, macronutrients and calcium intakes

In the 10 subjects who completed at least one period, calcium intakes were significantly higher during the milk (1598±253 g, P<0.001) than the soy intervention period (505±163 g) and baseline (717±253 g). Mean intakes of energy, protein, carbohydrates and fat were not significantly different between baseline, milk and soy intervention periods (data not shown).

6.5.4 Differences in pain scores between milk and soy intervention

Mean minimum and maximum pain scores did not change between the milk and soy intervention periods by visual inspection for the 4 subjects who completed the study **(Figure 1)**. This was supported by the absence of significant differences in pain scores, as well as in depression and quality of life scores between the milk and soy intervention periods **(Table 5)**. No significant effects of the milk nor the soy interventions on minimum and maximum baseline pain scores (4.8±1.2 and 7.9±0.4), baseline depression scores and baseline quality of life scores by one-way ANOVA (data not shown). On an individual basis, none of the 4 patients had differences in minimum and maximum pain scores **(Table 6)**, depression and quality of life scores (data not shown) between the interventions.

6.5.5 Adverse events

Seven out of 14 patients reported side effects during the soy intervention periods. These included decreased appetite (n=3), constipation (n=2) and bloating (n=1). Also, one subject had episodes of diarrhea, gases and felt noxious during the soy intervention period. Side effects from milk powder consumption were not observed. Three subjects complained of weight gain after starting the new dietary approach.

6.6 Discussion

The present pilot study tested the feasibility and effects of a diet enriched with soy protein against milk protein on chronic neuropathic facial pain, using a N-of-1 design. Participants were exposed to the soy or control diet during 3 week-intervals, in 3 paired treatment periods. Our data show that few participants completed the 18-week protocol. Introducing and maintaining dietary modifications was only possible for patients with chronic neuropathic facial pain as long as efforts were deployed to help participants consume the soy protein powder, on an individual basis.

6.6.1 Clinical effects of soy protein

This is the first study to test a possible analgesic effect of soy protein in humans with chronic neuropathic facial pain. Results showed that soy protein did not improve pain levels, depression or quality of life in the patients who completed the study (based on individual assessments and as a group). A previous preliminary pilot, open-label study showed that diet rich in soy protein reduced chronic post-traumatic neuropathic pain in some subjects but not on average [19]. In this study, subjects exchanged half of their protein and fat intake from soy sources (readily available shelf products) for 6 weeks. Patients were not blinded to the soy treatment and were not compared to a control group. Thus, the mild analgesic effects found could have been attributed to a placebo effect or to other ingredients present in the soy products. Moreover, the beneficial effects may have been related to soy protein and soy fat consumed together. Indeed, a previous animal study reported that dietary fat and protein interact in suppressing neuropathic pain-related disorders following a partial sciatic ligation injury in rats [106].

Other studies tested the effect of soy protein on other chronic pain conditions and found significant improvements with soy protein supplementation. A daily consumption of diets enriched with soymilk for 3 months (34 g soy protein/day) had mild analgesic effect on cyclical menstruation-related breast pain [13]. Soy protein ingestion was effective in relieving pain and discomfort associated with osteoarthritis [14]. Considering these positive studies, the very small sample size of participants who completed our pilot study (n=4) does not allow to draw firm conclusions as to the efficacy of soy protein.

6.6.2 Difficulties encountered

Challenges were faced while conducting this study from recruitment to retention of participants in the study. Though 9 medical or dentistry clinics of the Montreal metropolitan area were contacted and advertisements in local newspapers and online (Chronic Pain Association of Quebec) were posted. Only

22 subjects were recruited over 10 months. The low recruitment rate is most probably explained by the fact that chronic neuropathic facial pain is a rare condition with an incidence of 21.7 out of 100,000 persons per year [24].

Poor retention of the subjects in the study was faced. Fourteen eligible participants were enrolled but only 4 completed the study. Main reasons for exclusion were modification in pain treatment management during the course of study (n=6/10), considered important confounding factors of the study outcomes. The observed deviations from protocol could be attributed to the long duration of the protocol. Other subjects (n=4/10) chose to discontinue after few days of starting the new dietary approach because they reported side effects (vomiting, diarrhea, headaches, burping, constipation and palpitation) that they attributed to soy protein consumption, when indeed they were ingesting the milk powder (upon unblinding). However, these patients reported having no problems with milk consumption upon screening. Therefore, the causal relationship of the observed adverse events to the study intervention is questionable and might be side effects attributed to a placebo effect or to the milk protein powder quality.

6.6.3 High participant's burden

This study involved a high participant's burden, including important dietary modifications of usual intakes over a long period of time (4 months and a half) and numerous phone calls and visits to the clinic (every 3 weeks) for outcome measures assessment. We chose this rigorous N-of-1 study design because it had several advantages over a crossover trial design. First, it enables to assess the efficacy of soy protein on an individual basis. It was postulated that patients would be more inclined to participate in a N-of-1 trial rather than a crossover design as it can provide them with a personal feedback on their own responses at the end of the trial. This might increase the acceptability to potential participants and the clinicians responsible for referring them to the study [107]. Second, a series of individual trials administered in an identical way can estimate the treatment efficacy for the patient population comparable to a crossover trial

but requiring a smaller sample size [107]. In fact, results from N-of-1 trials on several patients can be combined using the hierarchical Bayesian random effects models that takes into consideration random effects including within-patient, between-patient and inter-observational variability [100]. Therefore, when recruitment is difficult because the studied clinical condition is rare, the use of the N-of-1 design is more advantageous over the crossover design that requires a large sample size making the study impractical.

Despite this demanding study design, none of the subjects wanted to discontinue because of fatigue or burden from the study protocol. The major objections were to the consumption of the milk and soy powders and inherent modifications of usual diet. Therefore, future studies should consider other intervention approaches or better quality protein powder to improve adherence. Previous studies investigating the analgesic effects of soy protein in other chronic pain conditions used soy shakes (and milk shakes as a placebo) [12], soy protein drink (vs cow's milk) [13] and powdered drink mix containing soy protein (vs milk protein) [14]. These provided 16 to 20 g of soy protein (or milk protein) and were consumed twice daily to obtain a total daily soy protein (or milk protein) intake of 34 to 40 g. Acceptance of these intervention products was not assessed in all but one study (soy and milk shakes) that reported 5/22 (23%) dropouts because taste dislike [12].

6.6.4 Compliance to intervention production

Despite the mentioned complaints, compliance of those who completed at least one period was high (>84% of the powder consumed) except for 2 subjects. This finding showed how motivated were participants to find a new treatment management for their debilitating pain. Some patients (n=4) mentioned their willingness to try anything to alleviate their pain. However, our compliance analysis is limited by no direct evidence of patients having actually consumed the powders (vs discarding it) and finishing all foods that contained it. More objective assessment for compliance would be measuring blood or urine isoflavones [13].

Because of the pilot nature of our study, no biochemical measurements were performed.

6.6.5 Adverse events

The soy protein powder was well tolerated by the majority of the subjects who were exposed to it during the study (n=10). Mild gastrointestinal side effects (constipation and bloating) were reported by 4 subjects consistent with another study [12]. These are considered as the most frequently reported adverse events related to soy consumption [18]. Side effects related to soy protein consumption in other previous human studies investigating the analgesic effects of soy on other pain conditions were not reported [12-14]. Other patients reported decreased appetite and weight gain. However, no changes in mean energy intakes from baseline were seen in these patients.

6.6.6 Limitations

One limitation of this study was that blinding to diet treatment was not reached. Half of the subjects who completed more than one period noticed a difference in taste between the soy and the milk powders. This is a recurrent limitation of nutritional studies, as a perfect placebo to a specific food or major component of a food does not exist due to the inherent taste, texture and palatability characteristics. This is especially true for well-known foods such as milk. Another limitation is pain assessment. Pain is a subjective measure and its assessment could be biased even within a given patient by other issues concurrent with pain assessment (e.g. social and emotional distress) [97].

6.7 Conclusion

Despite the difficulties encountered with recruitment and retention into the study, and acceptability of the treatment products and dietary changes, a diet enriched with soy protein was a feasible intervention for the participants who were not excluded due to protocol deviation and completed the study. Future studies should consider other study designs of shorter duration to minimize the risk of attrition. Other intervention approaches or better quality protein powder

products should be also considered to improve acceptability. Finally, the absence of effect of soy protein on pain severity in neuropathic facial pain patients is not conclusive due to the small sample size of this pilot study. This warrants confirmation with future larger studies.

Table 1. Neuropathic facial pain diagnosis and circumstances of onset	
Neuropathic facial pain diagnosis	**n**
Trigeminal neuralgia	3
Atypical trigeminal neuralgia	3
Burning mouth syndrome	2
Post surgical neuropathic pain	2
Other	4
Circumstances of onset	
Spontaneous	5
Surgery (dental, jaw alignment)	3
Dental therapy	2
Benign brain tumor	1
Meningioma and gamma knife therapy	1
Migraine	1
Stroke	1
n=14	

Table 2. Individual patients' protein powder intake, follow-up periods and reasons for withdrawal

	Total powder quantity[a] /day	Number of completed periods	Reasons for withdrawal	
1	50g	<1 period (2d, soy)	Deviance from protocol	Participation in another study (omega3 &pain)
2	40g	<1 period (3d, milk)	Voluntary discontinuation	Vomiting &diarrhea episodes[b]
3	40g	<1 period (11d, milk)	Voluntary discontinuation	Headaches &burping[b]
4	50g	<1 period (3d, milk)	Voluntary discontinuation	Headaches, constipation and palpitation[b]
5	50g	1 period (soy)	Deviance from protocol	Increase in medication dose
6	40g	1 period (milk)	Deviance from protocol	Started other medications
7	50g	1 period (milk), 2/3 period (soy)	Voluntary discontinuation	Difficulties of commitment to study protocol
8	30g	1 period (milk), 2/3 period (soy)	Deviance from protocol	Started antibiotics
9	50g	2 periods, 2/3 period (milk)	Deviance from protocol	Surgery (nerve block)
10	40g	4 periods	Deviance from protocol	Surgery (nerve block)

[a]based on protein content. n=10

[b]unknown relationship to study

Table 3. Baseline characteristics of subjects with neuropathic facial pain	
Age (yrs)	55±12
Gender ratio (F: M)	11:3
Anthropometrics	
Height (cm)	164±7
Weight (kg)	73±8
Body mass index (kg/m^2)	27.0±4.8
Body fat (%)	37±8
Fat free mass (kg)	47±6
Pain duration (yrs)	5±3
Pain scores	
Minimum NRS[a] (/10)	5±2
Maximum NRS[a] (/10)	7±1
Depression scores	
BDI[b] (/63)	15±8
Quality of life scores (PDI[c])	
Family responsibilities (/10)	4±3
Recreation (/10)	5±3
Occupation (/10)	5±4
Self-care (/10)	2±3
Social activity (/10)	5±3
Sexual behaviour (/10)	4±4
Life supporting activity (/10)	4±4
Data is presented as mean±SD	
[a]Numerical Rating Scale	
[b]Beck Depression Inventory-II	
[c]Pain Disability Index	
n=14	

Table 4. Compliance of subjects to the soy and milk powders

Soy powder[a]/ period	Milk powder[a]/ period	Percentage of powder consumed per period						
		Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Average
	35 g	98						98
34 g	35 g	93*	97	93*				94
34 g	35 g	92*	91	76*	81	83	83*	84
34 g	35 g	100	74*	87	42*	23*	45	62
	47 g	100						100
46 g	47 g	100	100*	100	100*	100	100*	100
	58 g	39						39
57 g	58 g	100*	100	100				100
57 g	58 g	100*	100	100*	100	100	100*	100

[a]The daily amount of powder ingested

*Soy intervention periods

n=9

Figure 1A.

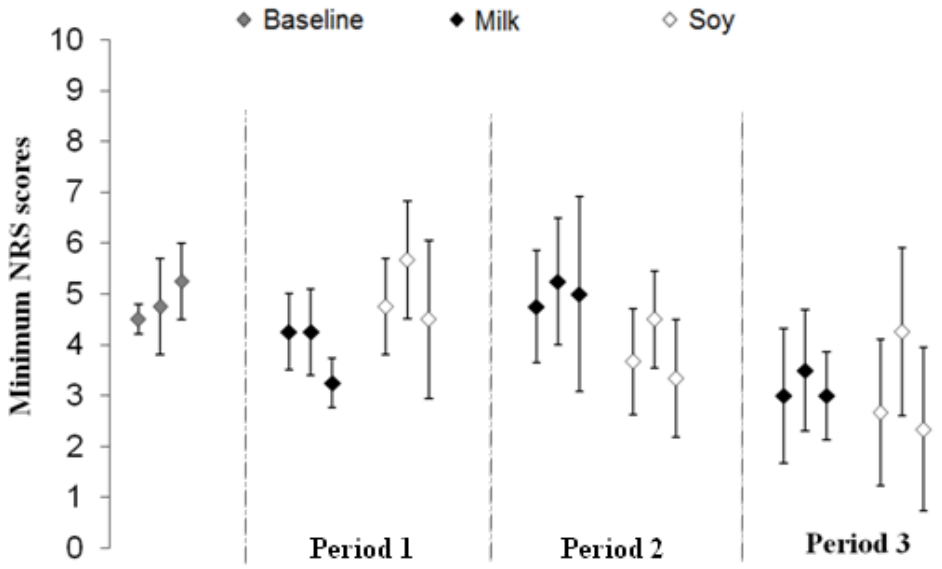


Figure 1B.

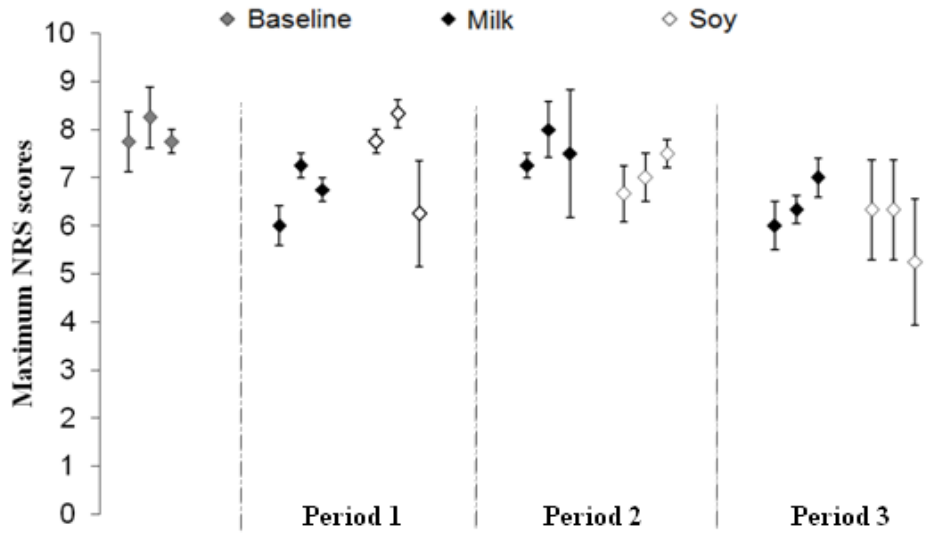


Figure 1. Graphical display of minimum (1A.) and maximum (1B.) numeric rating scale (NRS) scores over time in relation to the treatment being received (milk or soy). Data are presented as mean±SEM, computed for the subjects who completed the study (n=4). Each 3 week-paired intervention was randomly assigned, but reordered for graphical display.

Table 5. Mean differences between outcome scores of milk and soy intervention periods

	Milk	Soy	*P*-value
Pain scores			
Minimum NRS[a] scores	4±2	4±3	0.539
Maximum NRS[a] scores	7±1	7±1	0.696
Depression scores			
BDI[b]	13±13	14±11	0.501
Quality of life scores (PDI[c])			
Family responsibilities	3±3	2±3	0.348
Recreation	4±3	3±4	0.475
Social activity	4±3	3±3	0.284
Occupation	5±3	4±4	0.312
Sexual life	4±3	3±3	0.107
Self care	2±2	1±2	0.246
Life support activity	3±3	3±3	0.720

Data are presented as mean±SD. Differences were tested by paired sample t-test for pain and depression scores and by Wilcoxon signed rank test for quality of life scores (n=4).

[a]Numerical Rating Scale

[b]Beck Depression Inventory-II

[c]Pain Disability Index

Table 6. Mean individual differences between outcome scores of milk and soy intervention periods

	Minimum NRS[a] scores			Maximum NRS[a] scores		
	Milk	Soy	*P*-value	Milk	Soy	*P*-value
Patient 1	2.7±1.1	2.6±1.5	0.655	6.8±0.8	6.1±1.3	0.285
Patient 2	6.3±1.9	7.3±0.6	0.285	8.0±0.7	7.7±0.6	0.655
Patient 3	5.4±0.5	7.3±0.6	0.060	7.1±0.6	7.7±0.3	0.301
Patient 4	2.3±1.2	1.8±0.7	0.180	6.1±0.4	6.1±1.0	1.00

Data are presented as mean±SD. Differences were tested by paired sample t-test for patient 1 and by Wilcoxon signed rank test for the other patients.

[a]Numerical Rating Scale

7 FINAL CONCLUSION

Chronic pain is an overwhelming condition with devastating consequences. In addition to a reduced life quality, patients with chronic pain could be subject to a decreased appetite related to depression, medications' side effects and pain itself. However, very scarce information is available on the nutrient intake and diet quality of these subjects. Moreover, pain management is inadequate in most of the sufferers. Research is ongoing for alternative medicine to treat pain, including diet. Some dietary constituents were shown to have analgesic properties in animal and human studies. Yet, studies investigating a possible dietary role in maintaining pain states are lacking.

In the first study, nutrient intakes of patients suffering from chronic pain were assessed and possible dietary correlates with pain intensity levels were examined in a cross-sectional survey of nutrient intakes. A high proportion of subjects were found to have many, specific nutrient intake inadequacies and the majority had nutrient intakes below those of the healthy Quebec population. Thus, patients with chronic pain were at increased risks of nutrient deficiencies. Analysis of dietary correlates of pain levels showed that higher intakes of energy, carbohydrates and vitamin E were associated with lower pain levels. Previously shown to exert analgesic effects in animal studies, vitamin E and its role in analgesia is worth investigation in further research with human conditions pain. Other nutrients were not correlated with pain levels. This finding however does not preclude their involvement in pain states. Indeed, the lack of association could be explained by the low ranges of intakes of our patients, their mixed etiologies of chronic pain, and the relatively small sample size of the study. Therefore, dietary strategies are needed to improve the diet quality and to optimize the nutritional status of these patients. But improving many aspects of the usual dietary intake represents a challenge for these patients often suffering from depression, lack of appetite and motivation towards buying and preparing. Thus, involvement and follow-up by dietitians would be greatly needed in pain clinics.

The introduction of one dietary constituent, soy protein, to patients suffering from one type of chronic pain, the neuropathic facial pain, was tested in the second study. Patients with neuropathic facial pain can become refractory to conventional medications and soy protein was found to have pain relieving properties in rat models of neuropathic pain. The feasibility and compliance to a diet enriched with soy protein powder in patients with neuropathic facial pain were tested and its efficacy (compared to milk, the placebo) on pain, depression and quality of life scores based on individual assessments, and as a group was evaluated in a pilot study. Difficulties were encountered in retaining participants into the study. The main reason for exclusion was deviance from the study protocol, which was most likely due to the long duration of the study. Others chose to drop out because of side effects (unknown relationship to study) that they associated with the new dietary intervention. However, a diet enriched with soy protein was a feasible intervention for those who stayed in the study but could have been more successful if the time frame of the study protocol was shorter. Results showed no improvement of pain, depression and quality of life scores with the soy intervention; but these findings are not conclusive due to the small sample size of this pilot study and future studies are needed for confirmation of any effect.

References

1. Shelby RA, Keefe FJ: **Chronic pain and adherence** In *Improving Patient Treatment Adherence.* Edited by Bosworth H: Springer New York; 2010: 179-214
2. Hulisz D, Moore N: **Chronic pain management.***US Pharmacist* 2007, **32:**55-56.
3. Ohayon MM, Schatzberg AF: **Chronic pain and major depressive disorder in the general population.***Journal of Psychiatric Research* 2010, **44:**454-461.
4. Verdu B, Decosterd I, Buclin T, Stiefel F, Berney A: **Antidepressants for the treatment of chronic pain.***Drugs* 2008, **68:**2611-2632.
5. Bosley BN, Weiner DK, Rudy TE, Granieri E: **Is chronic nonmalignant pain associated with decreased appetite in older adults? Preliminary evidence.***Journal of the American Geriatrics Society* 2004, **52:**247-251.
6. Macfarlane GJ, Jones GT, Knekt P, Aromaa A, McBeth J, Mikkelsson M, Heliovaara M: **Is the report of widespread body pain associated with long-term increased mortality? Data from the Mini-Finland Health Survey.***Rheumatology* 2007, **46:**805-807.
7. VanDenKerkhof EG, Macdonald HM, Jones GT, Power C, Macfarlane GJ: **Diet, lifestyle and chronic widespread pain: Results from the 1958 British Birth Cohort Study.***Pain Research & Management* 2011, **16:**87-92.
8. Pells JJ, Presnell KE, Edwards CL, Wood M, Harrison MO, DeCastro L, Johnson S, Feliu M, Canada S, Jonassaint JC, et al: **Moderate chronic pain, weight and dietary intake in African-American adult patients with sickle cell disease.***Journal of the National Medical Association* 2005, **97:**1622-1629.
9. Tall JM, N. RS: **Dietary constituents as novel therapies for pain.***The Clinical Journal of Pain* 2004, **20:**19.

10. Tall JM, Seeram NP, Zhao C, Nair MG, Meyer RA, Raja SN: **Tart cherry anthocyanins suppress inflammation-induced pain behavior in rat.***Behavioural Brain Research* 2004, **153:**181-188.
11. Jolivalt CG, Mizisin LM, Nelson A, Cunha JM, Ramos KM, Bonke D, Calcutt NA: **B vitamins alleviate indices of neuropathic pain in diabetic rats.***European Journal of Pharmacology* 2009, **612:**41-47.
12. Wahner-Roedler DL, Thompson JM, Luedtke CA, King SM, Cha SS, Elkin PL, Bruce BK, Townsend CO, Bergeson JR, Eickhoff AL, et al: **Dietary Soy Supplement on Fibromyalgia Symptoms: A Randomized, Double-blind, Placebo-controlled, Early Phase Trial.** *Evidence-based complementary and alternative medicine* 2011, **2011**.
13. McFadyen IJ, Chetty U, Setchell KD, Zimmer-Nechemias L, Stanley E, Miller WR: **A randomized double blind-cross over trial of soya protein for the treatment of cyclical breast pain.***Breast* 2000, **9:**271-276.
14. Arjmandi BH, Khalil DA, Lucas EA, Smith BJ, Sinichi N, Hodges SB, Juma S, Munsona ME, Paytonb ME, Tivisc RD, Svanborgd A: **Soy protein may alleviate osteoarthritis symptoms.***Phytomedicine* 2004, **11:**567-575.
15. Shir Y, Ratner A, Raja SN, Campbell JN, Seltzer Z: **Neuropathic pain following partial nerve injury in rats is suppressed by dietary soy.***Neuroscience Letters* 1998, **240:**73-76.
16. Shir Y, Sheth R, Campbell J, Raja S, Seltzer Z: **Soy-containing diet suppresses chronic neuropathic sensory disorders in rats.***Anesthesia and Analgesia* 2001, **92:**1029-1034.
17. Shir Y, Raja SN, Weissman CS, Campbell JN, Seltzer Z: **Consumption of soy diet before nerve injury preempts the development of neuropathic pain in rats.***Anesthesiology* 2001, **95:**1238-1244.
18. Codrington A, Chevalier S, Shir Y: **Nutrition and pain management: dietary soy as an analgesic modality** In *Clinical Pain Management: A Practical Guide* 1st edition. Edited by Lynch ME, Craig KD, Peng PWH: Wiley-Blackwell; 2010: 105-113

19. Perez J, Shir Y: **The effect of soy-enriched diets on neuropathic pain in humans: preliminary results.***Pain Research & Management* 2005, **10:**95.
20. Breivik H, Collett B, Vittorio Ventafridda V, Rob Cohen R, Gallacher D: **Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment.***European Journal of Pain* 2012, **10:**287-333.
21. Schopflocher D, Taenzer P, Jovey R: **The prevalence of chronic pain in Canada.***Pain Research & Management* 2011, **16:**445-450.
22. Bair MJ, Robinson RL, Katon W, Kroenke K: **Depression and pain comorbidity: a literature review.***Archives of Internal Medicine* 2003, **163:**2433-2445.
23. Lincoff N: **Treatment of chronic facial pain.***Current Treatment Options in Neurology* 2006, **8:**69-79.
24. Koopman JSHA, Dieleman JP, Huygen FJ, de Mos M, Martin CGM, Sturkenboom MCJM: **Incidence of facial pain in the general population.***Pain* 2009, **147:**122-127.
25. Oberman M: **Treatment options in trigeminal neuralgia.***Therapeutic Advances in Neurological Disorders* 2010, **3:**107-113.
26. Arnstein P: **Chronic neuropathic pain: Issues in patient education.***Pain Management Nursing* 2004, **5:**34-41.
27. Graff Radford SB: **Facial pain.***The Neurologist* 2009, **15:**171-177.
28. International Headache Society Classification Subcommittee: **International Classification of Headache Disorders, 2nd edition.***Cephalalgia* 2004, **24** 1-160.
29. Krafft R: **Trigeminal Neuralgia.***American Family Physician* 2008, **77:**1291-1296.
30. Hoffer LJ: **Complementary or alternative medicine: the need for plausibility.***CMAJ: Canadian Medical Association Journal* 2003, **168:**180-192.

31. Perez J, Ware MA, Chevalier S, Gougeon R, Shir Y: **Dietary omega-3 fatty acids may be associated with increased neuropathic pain in nerve-injured rats.***Anesthesia and Analgesia* 2005, **101:**444-448.
32. Belfer I: **Dietary supplementation with the inhibitory amino acid taurine suppresses autotomy in HA rats.***NeuroReport* 1998, **9:**3103.
33. Goldberg RJ, Katz J: **A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain.***Pain* 2007, **129:**210-223.
34. Stevens B, Yamada J, Ohlsson A: **Sucrose for analgesia in newborn infants undergoing painful procedures (Review)** In *Cochrane Database of Systematic Reviews* 2010.
35. Zollinger PE, Tuinebreijer WE, Breederveld RS, Kreis RW: **Can vitamin C prevent complex regional pain syndrome in patients with wrist fractures? A randomized, controlled, multicenter dose-response study.***The Journal of bone and joint surgery American volume* 2007, **89:**1424-1431.
36. Xiao CW: **Health Effects of Soy Protein and Isoflavones in Humans.***The Journal of Nutrition* 2008, **138:**1244S-1249S.
37. Omoni AO, Rotimi AE: **Soybean foods and their benefits: potential mechanisms of action.***Nutrition Reviews* 2005, **63:**272-283.
38. Balk E, Chung M, Chew P, Ip S, Raman G, Kupelnick B, Tatsioni A, Sun Y, Wolk B, DeVine D, Lau J: **Effects of soy on health outcomes.***Evidence Report/Technology Assessment* 2005**:**1-8.
39. Dong JY, Qin LQ: **Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies.***Breast Cancer Research and Treatment* 2011, **125:**315-323.
40. Yan L, Spitznagel EL: **Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis.***The American Journal of Clinical Nutrition* 2009, **89:**1155-1163.

41. Sacks FM, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P, Winston M, Committee ftAHAN: **Soy Protein, Isoflavones, and Cardiovascular Health.***Circulation* 2006, **113:**1034-1044.
42. Cederroth CR, Nef S: **Soy, phytoestrogens and metabolism: A review.***Molecular and Cellular Endocrinology* 2009, **304:**30-42.
43. Messina MJ, Wood CE: **Soy isoflavones, estrogen therapy, and breast cancer risk: analysis and commentary.***Nutrition Journal* 2008, **7:**17.
44. Young VR: **Soy protein in relation to human protein and amino acid nutrition.***Journal of the American Dietetic Association* 1991, **91:**828.
45. Shu XO, Zheng Y, Cai H, Gu K, Chen Z, Zheng W, Lu W: **Soy food intake and breast cancer survival.***Journal of the American Medical Association* 2009, **302:**2437-2443.
46. Rackis JJ: **Flatulence caused by soya and its control through processing.***Journal of the American Oil Chemists' Society* 1981, **58:**503.
47. Calloway DH, Calloway: **Reduction of intestinal gas- forming properties of legumes by traditional and experimental food processing methods.***Journal of food science* 1971, **36:**251.
48. Cordle CT: **Soy Protein Allergy: Incidence and Relative Severity.***The Journal of Nutrition* 2004, **134:**1213S-1219S.
49. Mittag D, Vieths S, Vogel L, Becker WM, Rihs HP, Helbling A, Wuthrich B, Ballmer-Weber BK: **Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens.***The Journal of Allergy and Clinical Immunology* 2004, **113:**148-154.
50. Besler M, Helm R, Ogawa T: **Allergen Data Collection Update: Soybean.***Internet Symposium on Food Allergens* 2000, **2:**1S-35S.
51. Cambria-Kiely J: **Effect of soy milk on warfarin efficacy.***The Annals of Pharmacotherapy* 2002, **36:**1893-1896.
52. Izzo AA, Di Carlo G, Borrelli F, Ernst E: **Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction.***International Journal of Cardiology* 2005, **98:**1-14.

53. Shir Y, Ratner A, Raja SN, Campbell JN, Seltzer Z: **The correlation between dietary soy phytoestrogens and neuropathic pain behavior in rats after partial denervation.***Anesthesia and Analgesia* 2002, **94:**421-426.

54. Valsecchi AE, Franchi S, Panerai AE, Sacerdote P, Trovato AE, Colleoni M: **Genistein, a natural phytoestrogen from soy, relieves neuropathic pain following chronic constriction sciatic nerve injury in mice: anti-inflammatory and antioxidant activity.***Journal of Neurochemistry* 2008, **107:**230-240.

55. Lewis DK, Johnson AB, Stohlgren S, Simpson A, Sohrabji F: **Effects of estrogen receptor agonists on regulation of the inflammatory response in astrocytes from young adult and middle-aged female rats.***Journal of Neuroimmunology* 2008, **195:**47-59.

56. Myers RR, Campana WM, Shubayev VI: **The role of neuroinflammation in neuropathic pain: mechanisms and therapeutic targets.***Drug Discovery Today* 2006, **11:**8-20.

57. Suh HW, Song DK, Son KH, Wie MB, Lee KH, Jung KY, Do JC, Kim YH: **Antinociceptive mechanisms of Dipsacus saponin C administered intracerebroventricularly in the mouse* 1.***General Pharmacology* 1996, **27:**1167-1172.

58. Price DD, McGrath PA, Rafii A, Buckingham B: **The validation of visual analogue scales as ratio scale measures for chronic and experimental pain.***Pain* 1983, **17:**45-56.

59. **Health Canada. The Canadian Nutrient File: Compilation of the Canadian Food Composition Data** [http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/user_guide_d_utilisation01-eng.php]

60. Harris JA, Benedict FG: *A biometric study of basal metabolism in man.* Washington: Carnegie Institution of Washington; 1919.

61. Otten J, Pitzi Helliwig J, LD M: *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements.* Washington DC: The National Academies Press; 2006.

62. Murphy SP, Poos MI: **Dietary reference intakes: summary of applications in dietary assessment.***Public Health Nutrition* 2002, **5:**843-849.

63. **Statistics Canada. Canadian Community Health Survey- Nutrition (CCHS)** [http://www23.statcan.gc.ca:81/imdb/p2SV.pl?Function=getSurvey&SDDS=5049&lang=en&db=imdb&adm=8&dis=2 - b3]

64. Patten SB, Williams JVA, Lavorato DH, Khaled S, Bulloch AGM: **Weight gain in relation to major depression and antidepressant medication use.***Journal of Affective Disorders* 2011, **134:**288-293.

65. Jallon P, Picard F: **Bodyweight gain and anticonvulsants: a comparative review.***Drug Safety* 2001, **24:**969-978.

66. van den Berg-Emons RJ, Schasfoort FC, de Vos LA, Bussmann JB, Stam HJ: **Impact of chronic pain on everyday physical activity.***European Journal of Pain* 2007, **11:**587-593.

67. **Health Canada. Canadian Community Health Survey, Cycle 2.2, Nutrition (2004). A Guide to Accessing and Interpreting the Data** [http://www.hc-sc.gc.ca/fn-an/surveill/nutrition/commun/cchs_guide_escc-eng.php]

68. Ramenghi LA, Evans DJ, Levene MI: **"Sucrose analgesia": absorptive mechanism or taste perception?***Archives of Disease in Childhood Fetal and Neonatal Edition* 1999, **80:**F146-147.

69. Bhattacharjee M, Bhatia R, Mathur R: **Gender specificity of sucrose induced analgesia in human adults.***Indian Journal of Physiology and Pharmacology* 2007, **51:**410-414.

70. Foo H, Mason P: **Analgesia accompanying food consumption requires ingestion of hedonic foods.***The Journal of Neuroscience* 2009, **29:**13053-13062.

71. Pepino MY, Mennella JA: **Sucrose-induced analgesia is related to sweet preferences in children but not adults.***Pain* 2005, **119:**210-218.

72. Fulgoni VL, 3rd: **Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004.***The American Journal of Clinical Nutrition* 2008, **87:**1554S-1557S.

73. Seltzer S, Marcus R, Stoch R: **Perspectives in the control of chronic pain by nutritional manipulation.***Pain* 1981, **11:**141-148.

74. Zmarzty SA, Wells AS, Read NW: **The influence of food on pain perception in healthy human volunteers.***Physiology & Behavior* 1997, **62:**185-191.

75. Stamp LK, James MJ, Cleland LG: **Diet and rheumatoid arthritis: a review of the literature.***Seminars in Arthritis and Rheumatism* 2005, **35:**77-94.

76. Ray JG: **Folic acid food fortification in Canada.***Nutrition Reviews* 2004, **62:**S35-39.

77. Shakur YA, Garriguet D, Corey P, O'Connor DL: **Folic acid fortification above mandated levels results in a low prevalence of folate inadequacy among Canadians.***The American Journal of Clinical Nutrition* 2010, **92:**818-825.

78. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al: **The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know.***The Journal of clinical endocrinology and metabolism* 2011, **96:**53-58.

79. Shakur YA, Tarasuk V, Corey P, O'Connor DL: **A Comparison of Micronutrient Inadequacy and Risk of High Micronutrient Intakes among Vitamin and Mineral Supplement Users and Nonusers in Canada.***The Journal of Nutrition* 2012, **142:**534-520.

80. Calvo MS, Whiting SJ, Barton CN: **Vitamin D intake: a global perspective of current status.***The Journal of Nutrition* 2005, **135:**310-316.

81. Calvo M, Whiting S: **Prevalence of vitamin D insufficiency in Canada and the United States: importance to health status and efficacy of**

current food fortification and dietary supplement use.*Nutrition Reviews* 2003, **61:**107-113.

82. Al Faraj S, Al Mutairi K: **Vitamin D deficiency and chronic low back pain in Saudi Arabia.***Spine* 2003, **28:**177-179.
83. Warner AE, Arnspiger SA: **Diffuse musculoskeletal pain is not associated with low vitamin D levels or improved by treatment with vitamin D.***Journal of Clinical Rheumatology* 2008, **14:**12-16.
84. Straube S, Andrew Moore R, Derry S, McQuay HJ: **Vitamin D and chronic pain.***Pain* 2009, **141:**10-13.
85. Kim HK, Kim JH, Gao X, Zhou JL, Lee I, Chung K, Chung JM: **Analgesic effect of vitamin E is mediated by reducing central sensitization in neuropathic pain.***Pain* 2006, **122:**53-62.
86. Truong JT, Booth SL: **Emerging Issues in Vitamin K Research.***Journal of evidence based complementary and alternative medicine* 2011, **16:**73.
87. Tramer MR, Schneider J, Marti RA, Rifat K: **Role of magnesium sulfate in postoperative analgesia.***Anesthesiology* 1996, **84:**340-347.
88. Turan Y, Koçyigit H, Ekinci S, Bayram KB, Gürgan A, Altundal Y, Atay A: **Efficacy of oral magnesium treatment on primary fibromyalgia syndrom** *ADÜ Kakültesi Degrisi* 2009, **10:**11-15.
89. van Mierlo LAJ, Greyling A, Zock PL, Kok FJ, Geleijnse JM: **Suboptimal potassium intake and potential impact on population blood pressure.***Archives of Internal Medicine* 2010, **170:**1501-1502.
90. He FJ, MacGregor GA: **Beneficial effects of potassium on human health.***Physiologia Plantarum* 2008, **133:**725-735.
91. Tarasuk V, Fitzpatrick S, Ward H: **Nutrition inequities in Canada.***Applied Physiology, Nutrition, and Metabolism* 2010, **35:**172-179.
92. Dowler E: **Inequalities in diet and physical activity in Europe.***Public Health Nutrition* 2001, **4:**701-709.

93. US Food and Nutrition Board IoM: *Dietary Reference Intakes: Application in Dietary Assessment.* Washington, DC: National Academy Press; 2011

94. Margetts BM, Nelson M: *Design Concepts in Nutritional Epidemiology.* 2nd edn. Oxford; New York Oxford University Press 1997.

95. Singh R, Martin BR, Hickey Y, Teegarden D, Campbell WW, Craig BA, Schoeller DA, Kerr DA, Weaver CM: **Comparison of self-reported and measured metabolizable energy intake with total energy expenditure in overweight teens.***The American Journal of Clinical Nutrition* 2009, **89:**1744-1750.

96. Klesges RC, Eck LH, Ray JW: **Who underreports dietary intake in a dietary recall? Evidence from the Second National Health and Nutrition Examination Survey.***Journal of Consulting and Clinical Psychology* 1995, **63:**438-444.

97. Barkin RL, Barkin SJ, Barkin DS: **Perception, assessment, treatment, and management of pain in the elderly.***Clinics in Geriatric Medicine* 2005, **21:**465-490.

98. **Health Canada.Canadian Community Health Survey, Cycle 2.2, Nutrition (2004) - Nutrient Intakes from Food: Provincial, Regional and National Summary Data Tables, Volume 1,2 and 3** [http://www.hc-sc.gc.ca/fn-an/surveill/nutrition/commun/cchs_focus-volet_escc-eng.php - order]

99. Guyatt G, Sackett D, Adachi J, Roberts R, Chong J, Rosenbloom D, Keller J: **A clinician's guide for conducting randomized trials in individual patients.***CMAJ : Canadian Medical Association Journal* 1988, **139:**497-503.

100. Zucker DR, Schmid CH, McIntosh MW, D'Agostino RB, Selker HP, Lau J: **Combining single patient (N-of-1) trials to estimate population treatment effects and to evaluate individual patient responses to treatment* 1.***Journal of clinical epidemiology* 1997, **50:**401-410.

101. Zucker DR, Ruthazer R, Schmid CH, Feuer JM, Fischer PA, Kieval RI, Mogavero N, Rapoport RJ, Selker HP, Stotsky SA, et al: **Lessons learned combining N-of-1 trials to assess fibromyalgia therapies.***Journal of Rheumatology* 2006, **33:**2069-2077.

102. Farrar JT, Young JP, Jr., LaMoreaux L, Werth JL, Poole RM: **Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale.***Pain* 2001, **94:**149-177.

103. Richter P, Werner J, Heerlein A, Kraus A, Sauer H: **On the validity of the Beck Depression Inventory.***Psychopathology* 1998, **31:**160-168.

104. Pollard CA: **Preliminary validity study of the pain disability index.***Perceptual and Motor Skills* 1984, **59:**974.

105. Guyatt GH, Jaeschke R, Roberts R: **N-of-1 Randomized Clinical Trials in Pharmacoepidemiology.** In *Pharmacoepidemiology.* 3rd edition. Edited by Strom BL: Chichester, UK, John Wiley & Sons; 2000: 615-632

106. Pérez J, Ware MA, Chevalier S, Gougeon R, Bennett GJ, Shir Y: **Dietary fat and protein interact in suppressing neuropathic pain-related disorders following a partial sciatic ligation injury in rats.***Pain* 2004, **111:**297-305.

107. Nikles J, Mitchell GK, Schluter P, Good P, Hardy J, Rowett D, Shelby-James T, Vohra S, Currow D: **Aggregating single patient (n-of-1) trials in populations where recruitment and retention was difficult: The case of palliative care.***Journal of Clinical Epidemiology* 2011, **64:**471-480.

Appendices

Manuscript 1

Appendix 1. Visual Analog Scales (VAS)

A. The following line represents pain of increasing intensity from "no pain" to "worst possible pain". Place a slash (|) across the line in the position that best describes your average pain for the last week.

No pain ——————————————————— Worst pain

Visual Analog Score ☐☐.☐cm

B. The following line represents pain of increasing intensity from "no pain" to "worst possible pain". Place a slash (|) across the line in the position that best describes your pain now.

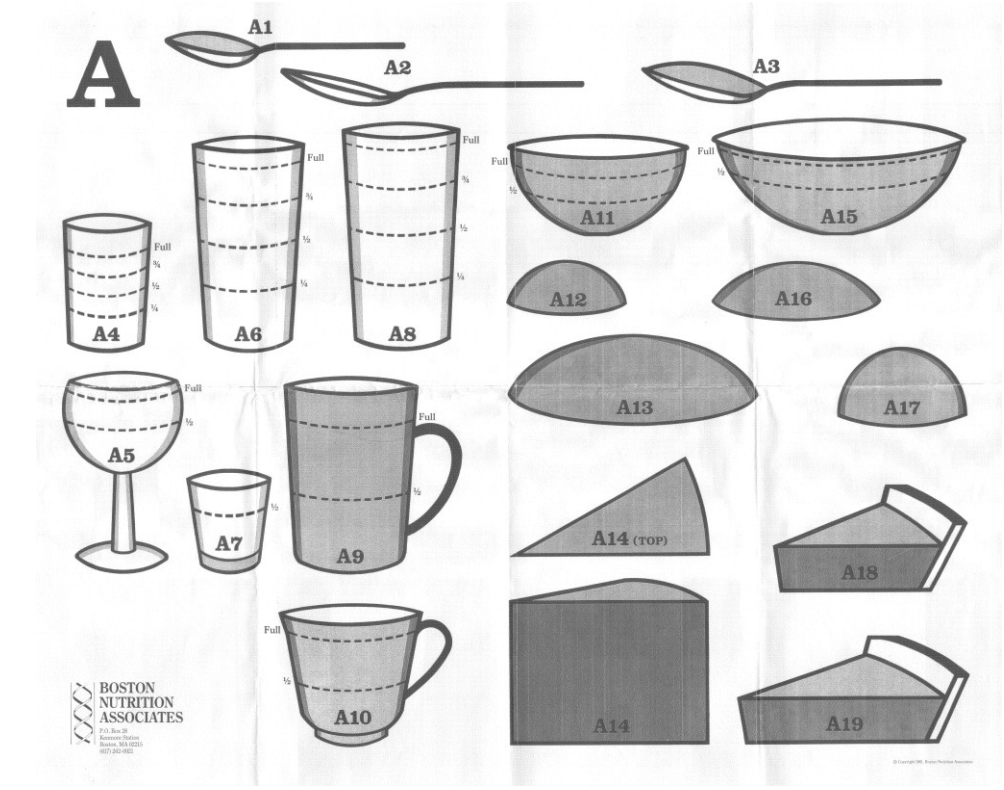
No pain ——————————————————— Worst pain

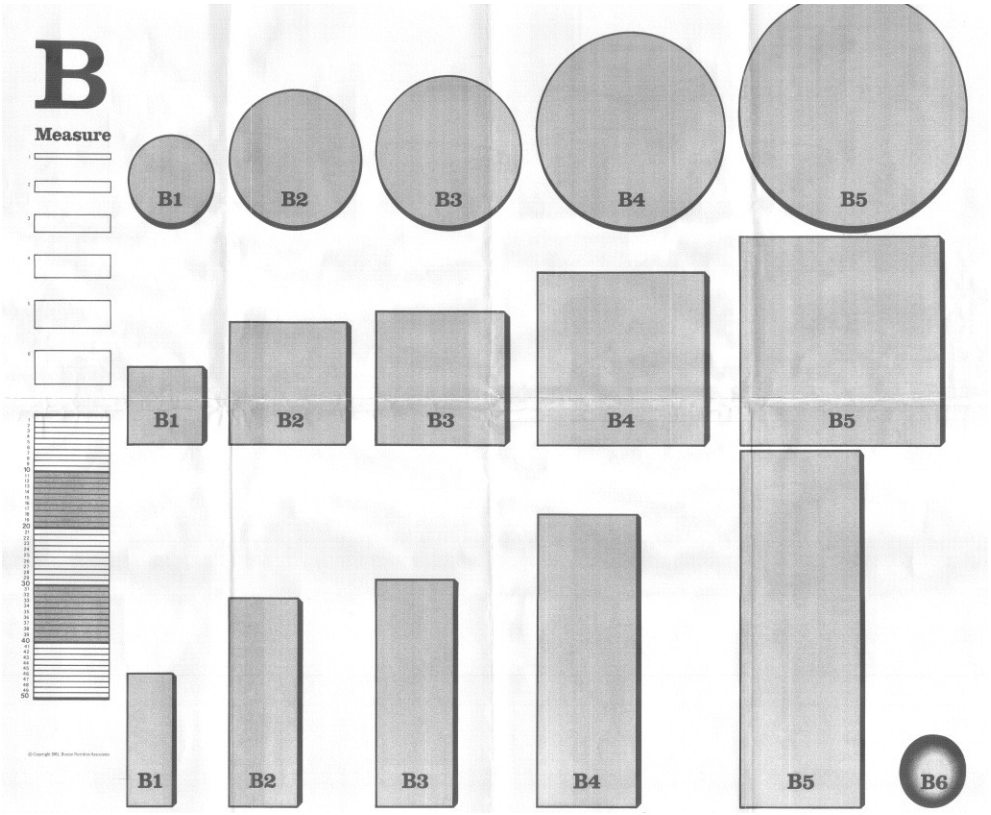
Visual Analog Score ☐☐.☐cm

Appendix 2. Dietary 24-hour recall

Subject number ☐☐☐ Date (dd/mmm/yyyy) ☐☐/☐☐☐/☐☐☐☐
Breakfast **Time:** ☐☐:☐☐☐ **AM** ☐ **PM**
Snack
Lunch **Time:** ☐☐:☐☐☐ **AM** ☐ **PM**
Snack
Dinner **Time:** ☐☐:☐☐☐ **AM** ☐ **PM**
Snack

Appendix 3. Two- dimensional visual aids





Manuscript 2

Appendix 1. Food frequency questionnaire – Soy protein

Foods (serving size)	Protein (g)	Number of portions /day	/week	/mo	Total g/day
Soy :					
Cooked soybeans, edamame (125 ml)	15				
Soy proteinpowder (20 g, 30 ml)	18				
Boiled soybeans (125 ml)	15				
Roasted soy nuts (30 ml)	8				
Soybean sprouts, raw (125 ml)	5				
Tofu :					
Hard tofu (1 slice of 4.5X4X4 cm)	13				
Soft tofu (1 slice of 6X4X4 cm)	9				
Beverage, cheese, dessert :					
Soymilk (250 ml)	7				
Soy cheese, curds (125 ml)	15				
Soycheese: mozzarella or cheddar (30 g)	6				
Soy cheese (1 slice)	4				
Tofu dessert, Sunrisetype(1 portion)	5				
Soy yogurt (100 g)	4				
Soy pudding (125 g/125 ml)	4				
Tofu « mousse », Soyummi type (125 g)	3,5				
Soy ice cream (125 ml)	2				
Meat substitutes (Yves Veggie type)					
Mixed dishes with soy or tofu (1 portion)	21				
Tofu dog or pattie (1 portion)	13				
Others :					
Soy cereals for breakfast (30g, 2/3 cup)	4				
Tempeh (15 ml)	2				
Tamari (15 ml)	2				
Natto (15 ml)	2				
Miso (15 ml)	2				
Soy sauce (15 ml)	1				
Hoisin (15 ml)	0,6				
TOTAL					

Appendix 2. Nutrient content of protein isolates

	Soy protein powder (Treatment)	Milk protein powder (Control)
Protein	85 %	81 %
Fat	4.0 %	5.5 %
Ash	5.0 %	7.5 %
Moisture	4.4 %	5.0 %
Calcium	50 mg/100 g	2100 mg/100 g
Isoflavones	2.2 mg/g	-

Appendix 3. Flow chart of study procedures

		Protein Consumption																	
		Period 1			Period 2			Period 3			Period 4			Period 5			Period 6		
		Week																	
	CV1*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Clinical Visits	X			X			X			X			X			X			X
Consent form	X																		
Inclusion/Exclusion	X																		
Demographic data	X																		
Anthropometric measurements	X																		
Pain intensity (NRS)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Depression (BDI)	X			X			X			X			X			X			X
Quality of life (PDI)	X			X			X			X			X			X			X
Nutritional Interview	X																		
Receive study protein	X			X						X						X			X
24-hr recall (phone)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Change in medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
*Clinical visit 1: first visit at the Montreal General Hospital																			

Appendix 4. Patient informed consent form

Principal Investigator:

Yoram Shir, MD, McGill University Health Centre (MUHC), Montreal General Hospital Pain Centre

Co-Investigators:

Alexis Codrington, PhD, Clinical Research Fellow, MUHC Montreal General Hospital Pain Centre

Stephanie Chevalier, PhD, MUHC Nutrition and Food Science Centre, Royal Victoria Hospital

José Correa, PhD, Department of Mathematics and Statistics, McGill University
Nathalie Rei, DMD, MSD, Faculty of Dentistry, University of Montreal
Russell Steele, PhD, Department of Mathematics and Statistics, McGill University
Mark Ware, MD, MSc (Epidemiology), MUHC Montreal General Hospital Pain Centre

INTRODUCTION

You are being invited to participate in this clinical study because you suffer from facial pain caused by a problem with one or more nerves in your face or mouth. Before deciding to participate in this clinical trial, you should clearly understand its requirements, risks and benefits. This document provides information about the study. Please read it carefully and ask the study nurse any questions you may have. She will discuss the study with you in detail. You may take this form with you and discuss the study with anyone else before making your decision. If you decide to participate, you will be asked to sign this form and a copy will be given to you.

BACKGROUND

Dietary ingredients, such as soy protein, could play a significant role in various diseases. For example, it has been shown that soy consumption decreases the severity of ailments including cardiovascular disease and osteoporosis. In addition, soy might possess promising pain-relieving properties; in experimental animals soy protein reduced neuropathic pain and in humans it reduced pain associated with osteoarthritis and cyclical breast pain in healthy women. Our research group has shown in a preliminary patient study that diets rich in soy could also decrease chronic post-traumatic neuropathic pain. Eating diets rich in soy is easy, safe and generally well tolerated.

PURPOSE OF THE STUDY

The purpose of this study is to determine whether soy protein can decrease pain in patients suffering from chronic neuropathic facial pain.

About thirty-three (33) subjects will take part in the study at the McGill University Health Centre (MUHC) Montreal General Hospital.

STUDY PROCEDURES

Study Duration:

The total duration of the study will be 19 weeks; 1 week of assessment and 6 treatment periods, each lasting three weeks. You will be asked to come to the clinic on seven different occasions. The first visit (screening visit) will take approximately 2 hours and all subsequent visits will last about 30 minutes.

If you agree to take part in this study, you will undergo the following procedures:

Screening Visit (Clinic Visit 1):

To confirm your eligibility to participate in the study:

- Your medical and medication history will be reviewed;
- You will undergo a pain assessment;
- You will undergo a nutritional interview;
- Your height and weight will be measured.

If you are eligible to take part in the study:

- You will be asked to fill in questionnaires about your personal data, pain levels, pain quality, quality of life and mood. It will take approximately 30 minutes to complete the questionnaires;
- You will receive a thorough explanation from the nutritionist of the dietary changes of the study;
- You will receive the first dietary protein supplement.

During the study you will receive two dietary supplements, the experimental product, soy protein and the control, milk protein. The study comprises of 6 periods of protein supplementation, each period lasting three weeks. During each of these three-week periods you will consume either the soy or the milk protein. The order of soy and milk protein consumption will be randomly decided (like a coin toss). Neither you nor the research team members will know the identity of the protein you receive in each period until the end of the study. This study design guarantees that you will be equally exposed to the experimental and the control protein.

The amount of protein supplement you will get depends on your body weight but will not exceed 50g a day. The supplement will be given to you in containers, each holding a one-day supply. You will receive 21 containers, for the next three weeks of therapy, at each clinic visit (see below) all containing the same protein powder. Protein powder containers are to be kept at room temperature. You will be advised on how to dissolve the powder in your usual food and drinks according to your preference (e.g. milk-shakes, pastry, soups, hot drinks).

Study Visits (Clinic Visit 2 – 7):

You will be required to come to the clinic every 3 weeks in order to return the previous 21 containers given to you and to receive your next set of 21 protein containers. As well you will fill in questionnaires similar to those completed at the first screening visit concerning your pain levels, pain quality, quality of life and mood. Each visit will last approximately 30 minutes.

Our research nurse will call you at home three times, approximately on the 7th, 15th and 18th day after starting each new treatment period in order to record your pain levels, the amount of pain relieving medications you use, any other medication use including natural health products, other therapies being used for pain and any side effects. This will last approximately 15 minutes.

On the 5th, 12th, and 19th day after starting each dietary supplement, the study dietician will call you at home to complete a twenty-four hour dietary recall questionnaire. This will last approximately 20 minutes.

POTENTIAL BENEFITS

You will be exposed to both the experimental and control protein supplements. The design of the study allows us to assess the efficacy of soy protein supplementation for your pain compared to the control milk protein. At the end of the study, your treatment code will be broken and we will determine the effect of the dietary protein supplementation on your pain during each three-week period. If proven to be effective, you can choose to continue consuming soy after the study. Soy protein is readily available on store shelves for you to use immediately. The study nutritionist could instruct you on how to incorporate soy protein into your diet with such commercially available shelf foods.

Please note that you may not benefit directly from participating in this study. However, the information collected from this study may benefit future subjects and provide a better understanding for physicians.

RISKS AND DISCOMFORTS

Soy protein is eaten in doses higher than what has been planned for this study, however the phytoestrogen content of the soy protein being given in this study is higher than what is consumed in our diets. Although there is inconclusive evidence suggesting that short-term consumption of soy protein increases the risk of breast cancer in healthy women, the United States National Cancer Institute considers it safe to consume moderate amounts of soy, such as those that are being used in this study. Limited mild side effects of soy consumption, including headaches, dizziness, bloating, nausea, constipation and gas, are possible yet rare. Soy allergy is rare in adults; actually it might reduce the incidence of allergies. The chances for allergic reaction to milk protein are 2-5%. Before joining the study we will verify that you have no known allergies to either protein. Soy protein may interact with the anticoagulant or blood thinner, warfarin (Coumadin®) and the synthetic thyroid hormone, levothyroxine (Synthroid®). Before joining the study we will verify if you are taking these medications. It is not necessary for hypothyroid patients to avoid soy protein; soy protein should not be consumed within one hour of taking thyroid medication. In general, soy should not be consumed a few hours before or after taking any medication or natural health products. Should you join the study, we advise you to notify your physician in order to monitor your drug levels. Should new information become available about soy protein, you will be notified.

PREGNANCY / CONTRACEPTION

If you are pregnant you will not be eligible for the study. If you are a woman of childbearing age, we ask that you take some form of contraception during the study such

as the oral contraceptive pill, or a barrier method (condom, diaphragm etc.) or abstinence. In the event of you becoming pregnant you must notify the study doctor immediately and your participation in the study will be discontinued.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM STUDY

Your participation in the study is strictly voluntary. You may refuse to participate or you may discontinue your participation at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you decide not to participate, or if you discontinue your participation, you will suffer no prejudice regarding your medical care or your participation in any other research studies. The study team is obliged to answer any question you have at any time before, during or after the study.

The study doctor may end your participation in the study if you experience excessive side effects, if you do not follow study procedures, if you need a medication that is not allowed during the study, or for administrative reasons unrelated to the purpose of the study. In addition, the McGill University Health Centre (MUHC) Research Ethics Board may terminate the study.

ALTERNATIVE TREATMENTS

Although there is currently no definitive treatment for the majority of neuropathic facial pain sufferers, you do not need to take part in this study in order to receive treatment for your pain. The study doctor is available to discuss all other alternatives that are available.

COMPENSATION

You will not be offered any compensation for your participation in this study. We do howeveroffer to pay your travel or parking expenses up to $20 with proof of payment for your study visits at the Montreal General Hospital.

INDEMNIFICATION

The MUHC, MUHC Research Institute and the investigators will not be able to offer compensation in the unlikely event of an injury resulting from your participation in this research study. However you are not giving up any of your legal rights by signing this consent and agreeing to participate in this study.

CONFIDENTIALITY

The team of researchers of the MUHC will consult your medical file to take note of the relevant data to this research project. All information obtained during this study will be kept strictly confidential. Your name will be coded and the code list will be kept at the study center at the Montreal General Hospital in a locked facility with limited access. The results from this study may be published, and other physicians participating in this research study may have access to your records related to this research study; however, your identity will not be revealed in the combined results. Data may be retained for 25 years after the completion of the study. In order to verify the research study data, monitors from the Quality Assurance Officer at the MUHC-Research Ethics Boards and Health Canada may review these records.

By signing this consent form, you give us permission to release information regarding your participation in this study to these entities. Your confidentiality will be protected to the extent permitted by applicable laws and regulations.

CONTROL OF THE ETHICAL ASPECTS OF THE RESEARCH PROJECT

The Ethics Research Board of the MUHC approved this research project and ensures the follow-up. In addition, it will first review and approve any amendment made to the informed consent form and to the study protocol.

FUNDING OF THE RESEARCH PROJECT

This research study is supported by the Alan & Louise Edwards Foundation and its affiliates and will be run by Dr. YoramShir. The study doctor is being awarded money to conduct the study, and to include you and look after you during your participation in this study.

STUDY RECORDS RETENTION POLICY

For security purposes, especially to be able to communicate with you rapidly, your family name, first name, coordinates and the start and end date of your participation in the project will be stored for one year after the completion of the project in a separate registry maintained by the researcher in charge of the project or by the institution.

This register is among some of the measures established, by the Ministry of Health and Social Services, for your protection. This will allow the hospital, should the need arise, to contact you. None of the information collected from this register will serve research and all information will be destroyed at the latest 12 months following the end of your participation in this research study.

QUESTIONS AND CONTACT INFORMATION

In case you develop side effects to the products administered you should contact Dr. YoramShir at 514-934-8558 (24-hour pager number 514-406-1151). If you have any questions regarding your rights as a study participant you should contact the Ombudsman at the Montreal General Hospital at 514-934-1934, local 48306.

DECLARATION OF CONSENT

I have read the contents of this consent form and I agree to participate in this research study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I understand that I will be given a copy of this signed consent form. By signing this consent form I have not given up any of my legal rights.

_____

Signature of the participant Printed name
Date signed

RESPONSIBILITY OF RESEARCHER

I certify that I have explained to the research subject the terms of the present form, that I have answered all questions that the research subject had to that effect and that I have clearly indicated that he/she remains free to terminate his/her participation, and this,

without prejudice and that I bind myself to respect what is in the consent form. A copy of the present consent form will be put in the medical file.

Signature of investigator signed	Printed name	Date

Appendix 5. 11-point Numerical Rating Scales (NRS)

In the **past 24 hours**, what were your maximum and minimum pain intensity levels?

A. Please circle the number on the scale that describes the **maximum** level of pain:

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst pain imaginable

B. Please circle the number on the scale that describes the **minimum** level of pain:

0	1	2	3	4	5	6	7	8	9	10
No pain										Worst pain imaginable

Appendix 6. Beck Depression Inventory (BDI) –II

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the one statement in each group that best describes the way you have been feeling during the past week, including today. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally, circle the highest umber for that group. Be sure that you do not chose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future.
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to enjoy.
- 3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I have lost confidence in myself.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself more than usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry any more than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying, but I can't.

____ Subtotal page 1

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.
2b I sleep a lot less than usual.

3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.
2b My appetite is much greater than usual.

3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

____ Subtotal page 1
____ Subtotal page 2
____ Total score

Appendix 7. Pain Disability Index (PDI)

The rating scales below are designed to measure the degree to which aspects of your life are disrupted by chronic pain. In other words, we would like to know how much your pain is preventing you from doing what you would normally do or from doing it as well as you normally would. Respond to each category by indicating the overall impact of pain in your life, not just when the pain is at its worst.

For each of the 7 categories of life activity listed, please circle the number on the scale that describes the level of disability you typically experienced. **A score of 0 means no disability at all, and a score of 10 signifies that all of the activities in which you would normally be involved have been totally disrupted or prevented by your pain.**

1) Family/home responsibilities: This category refers to activities of the home or family. It includes chores or duties performed around the house (e.g. yard work) and errands or favours for other family members (e.g. driving the children to school).

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

2) Recreation: This category includes hobbies, sports, and other similar leisure time activities.

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

3) Social activity: This category refers to activities that involve participation with friends and acquaintances other than family members. It includes parties, theater, concerts, dining out, and other social functions.

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

4) Occupation: This category refers to activities that are a part of or directly related to one's job. This includes nonpaying jobs as well, such as that of a housewife or volunteer worker.

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

5) Sexual behavior: This category refers to the frequency and quality of one's sex life.

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

6) Self Care: This category includes activities, which involve personal maintenance and independent daily living (e.g. taking a shower, driving, getting dressed, etc.)

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

7) Life-support activity: This category refers to basic life-supporting behaviours such as eating, sleeping, and breathing.

No disability 0 1 2 3 4 5 6 7 8 9 10 Total disability

Appendix 8.

FOOD FREQUENCY QUESTIONNAIRE – PROTEIN-RICH FOOD

Food	**Per Day**	**Per Week**	**Portion Size**
Red Meat			
Poultry			
Fish & Sea Foods			
Deli Meat, Sausages			
Eggs			
Dried Beans			
Tofu			
Peanut Butter			
Nuts, Seeds			
Milk (__% Fat)			
Cheese			
Yogurt			
Other			

FOOD FREQUENCY QUESTIONNAIRE- ENERGY-DENSE FOOD

Food	**Per Day**	**Per Week**	**Occasionally**
Fast-Food Restaurants			
Sweet Desserts			
Chips & Salty Snacks			
Candy Bars, Chocolate			
Soft Drinks			
Alcohol			
Other			

Appendix 9. Follow-up phone calls

	Periods 1-6																		
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Phone call					24-h recall		Adverse events, change in medications					24-h recall			NRS, adverse events, change in medications			NRS	24-h recall		NRS, adverse events, change in medications