Published in final edited form as:
Curr Clin Pharmacol. 2011 November ; 6(4): 260–273.

Metabolic Correction in the Management of Diabetic Peripheral Neuropathy: Improving Clinical Results Beyond Symptom Control

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Abstract

Current Clinical Management Guidelines of Diabetic Peripheral Neuropathy (DPN) are based on adequate glucose control and symptomatic pain relief. However, meticulous glycemic control could delay the onset or slow the progression of diabetic neuropathy in patients with DM type 2, but it does not completely prevent the progression of the disease. Complications of DPN as it continues its natural course, produce increasing pain and discomfort, loss of sensation, ulcers, infections, amputations and even death. In addition to the increased suffering, disability and loss of productivity, there is a very significant economic impact related to the treatment of DPN and its complications. In USA alone, it has been estimated that there are more than 5,000,000 patients suffering from DPN and the total annual cost of treating the disease and its complications is over $10,000 million dollars. In order to be able to reduce complications of DPN, it is crucial to improve or correct the metabolic conditions that lead to the pathology present in this condition. Pathophysiologic mechanisms implicated in diabetic neuropathy include: increased polyol pathway with accumulation of sorbitol and reduced Na^+/K^+-ATPase activity, microvascular damage and hypoxia due to nitric oxide deficit and increased oxygen free radical activity. Moreover, there is a decrease in glutathione and increase in homocysteine. Clinical trials in the last two decades have demonstrated that the use of specific nutrients can correct some of these metabolic derangements, improving symptom control and providing further benefits such as improved sensorium, blood flow and nerve regeneration. We will discuss the evidence on lipoic acid, acetyi-L-carnitine, benfotiamine and the combination of active B vitamins L-methylfolate, methylcobalamin and piridoxal-6-phosphate. In addition, we discuss the role of metforrnin, an

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DISCLOSURES

Authors have no potential conflicts of interest to disclose.

important drug in the management of diabetes, and the presence of specific polymorphic genes, in the risk of developing DPN and how metabolic correction can reduce these risks.

Keywords

Diabetic peripheral neuropathy; diabetes mellitus type 2; metformin; metabolic correction; methylenetetrahydrofolate reductase

INTRODUCTION

Diabetes Mellitus type 2 (DM2) is an increasingly prevalent disease with potential health disparity in Hispanic populations, showing a range of systemic complications that includes, among others, neuropathies. Diabetic neuropathies are neurological disorders resulting from microvascular injury involving small blood vessels that supply nerves (*vasa nervorum*). Diabetic neuropathy could affect all nerves: pain fibers, motor neurons, peripheral and autonomic nerves. Therefore, it may affect all organs and body systems since they all are innervated. Symptoms may vary depending on the nerve(s) affected. Symptoms usually develop gradually over years. Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and is responsible for substantial mortality and morbidity as well as deterioration in quality of life (QoL). DPN is characterized by damage to the peripheral nerves of the extremities and is usually associated with microvascular damage.

About 60% to 70% of people with diabetes have mild to severe forms of nervous system damage. The results of such damage include impaired sensation or pain in the feet or hands, difficulty swallowing, slowed digestion of food in the stomach, carpal tunnel syndrome, numbness and tingling of extremities, dysesthesia, diarrhea, urinary incontinence, facial, mouth and eyelid drooping, vision changes, dizziness, muscle weakness, speech impairment, fasciculation, impotence, erectile dysfunction and anorgasmia, burning or electric pain, among others [1]. The symptoms of DPN are often worse at night. Patients and clinicians mostly concentrate in symptomatology management and not in the constant progression of this devastating complication.

Almost 30% of people with diabetes beyond 40 years of age have impaired sensation in the feet [2]. DPN is occurring in up to 50% of population with diabetes and causes sensory, motor and autonomic dysfunction [3]. Several pathogenic mechanisms contribute to DPN etiology, including microangiopathy and oxidative stress, among others. The course and severity of DPN are further affected by a wide range of comorbid conditions and risk factors. In order to decrease the risk of vascular complications such as DPN, current standards for managing the patient with DPN include controlling blood pressure, lipids and blood glucose levels. However even with adequate control of these risk factors, many patients still develop complications. In order to improve outcomes, further slow the progression of disease, decrease complications and improve quality of life, it is necessary to target therapies to the underlying mechanisms of DPN. This review briefly discusses the underlying mechanisms of DPN development and progression, emphasizing on strategies that target correctable metabolic derangement.

PREVALENCE OF DPN

Neuropathies are among the most common chronic complications of diabetes, affecting up to 50% of patients [4-5]. There are many subgroups of neuropathies including sensory, motor and autonomic. Up to 50% of DPN may be asymptomatic, and patients are at risk of insensate injury to their feet. As many as 80% of amputations follow a foot ulcer or injury.

Early recognition of at-risk individuals, provision of education, and appropriate foot care may result in a reduced incidence of ulceration and consequently amputation. Treatment should be directed to correct the pathogenetic factors in addition to symptomatic management. Effective symptomatic treatments are available for the manifestations of DPN and autonomic neuropathy [1].

The prevalence of this complication depends on multiple factors which include: age; glucose control; duration of diabetes; method of diagnosis of DPN; simultaneous conditions or comorbidities; use of certain medications; elevated homocysteine and low cobalamin levels; among others. DPN appears that at least one manifestation of DPN is present in at least 20% of adult with diabetes. Prevalence data for DPN range from 1.6 to 90% depending on tests used, populations examined, and type and stage of disease [1]. Demographic studies in different countries give a range of prevalence of DPN. Several epidemiological studies assessed diabetic peripheral neuropathy among patients with diabetes and reported prevalence rates of 26-47% [5].

A previous study conducted in Bangladesh reported a DPN prevalence of 19.7% [6]. Factors associated with a higher risk of developing DPN included older age, lower socioeconomic status, treatment with insulin, longer duration of diabetes and poor glycemic control. Another study conducted in Belgium found that the prevalence of DPN was 43% (95% CI 40.1-45.9), and was higher in patients with type 2 diabetes (50.8%) than in those with type 1 (25.6%) [7]. The prevalence of painful DPN (P-DPN) was 14%. Physical and mental components of quality of life (QoL) were significantly altered by P-DPN, but not with asymptomatic DPN. Only half of the P-DPN patients were using analgesic treatment, while 28% were using anticonvulsants or antidepressants. These treatments are chemical compounds that bear significant risk of toxicity. The authors conclude that despite its profound impact on QoL, P-DPN remains undertreated [7].

In the United Kingdom, a study demonstrated that the prevalence of diabetes was 4.5% [8]. In this population the prevalence of P-DPN was 26.4%. Having P-DPN has a significant negative effect on quality of life, and increasing neuropathy is associated with an increasing risk of developing P-DPN. Eighty percent (80%) of them reported moderate or severe pain. The treatment options are limited, which may explain why up to 50% have not requested or received treatment for the condition [8]. The prevalence of DPN observed in the Chinese population with type 2 diabetes who were older than 30 years of age in Shangai reached up to 61.8% [9]. In Puerto Rico, the prevalence of diabetes is much higher than in the mainland USA and other countries, achieving levels of 12.9% during 2009. It is estimated that, from a total population of approximately 3.5 million, nearly 100,000 patients could have DPN [10-16].

COMPLICATIONS AND COSTS OF DPN

Common complications of DPN include increased risk of injury to the feet, secondary infections, ulcerations and amputations. The loss of sensation in DPN increases the chances of trauma and delayed treatment. In addition, these complications can produce loss in productive time and depression [5]. The increased risk of complications is a result of several mechanisms that ultimately decreases blood flow into the peripheral nerves tissues, depriving nutrient and oxygen supply. Endothelial health in these patients is compromised by numerous factors such as glycosylation, hyperlipidemias, hyperhomocysteinemia, hypertension, excessive platelet activity, smoking, reduced nitric oxide and excessive generation of reactive oxygen species. In addition, if the patient also has peripheral vascular disease, immune depression and decreased wound healing, regenerative processes will be compromised and improvement in metabolic and tissue functionality is less likely.

Comprehensive therapeutic plan should aim to improve all factors in an attempt to restore metabolic and tissue functionality to its maximum potential. However, in order to design a therapeutic regimen that achieves the best response, specific individual biochemical analysis must be made. It means going beyond a general protocol or guideline and considers the genetic variation of the population that produces biochemical derangements that are associated with specific health risks [17).

The total annual cost of DPN and its complications in the U.S. was estimated to be between 4.6 and 13.7 billion US dollars. Up to 27% of the direct medical cost of diabetes may be attributed to DPN [18]. Beside the cost of medical treatment, morbidity causes Loss in Productive Time (LPT). Costs of medical treatment of DPN and its complications are enormous and it can range from hundreds of millions to tens of thousands of millions per year depending on the Country. Thirty-eight percent (38%) of working adults with diabetes reported numbness or tingling in feet or hands due to DPN. Health-related LPT was 18% higher in the symptom ($p<0.05$) and 5% higher in the non-symptom ($p<0.05$) groups compared to those without diabetes. In the US, workers who have diabetes with neuropathic symptoms lose the equivalent of $3.65 billion/yr in health-related LPT [19].

When the cost of DPN and its complications is added to the cost in loss of productivity, the losses are astronomical. If we also consider quality of life issues, it becomes imperative that new and better treatment strategies targeted to reduce complications and its costs are needed. In summary, the cost of DPN and its complications are extremely high in both economical expenses of medical cost and loss of productivity and in addition in a dramatic reduction in quality of life. For this reason, therapeutic options that improve outcomes in DPN would have important economical and human impact.

PATHOPHYSIOLOGY OF DPN

The causes of DPN are thought to be a multi-factorial metabolic process that increasingly deteriorates tissues. Hyperglycemia, increased sorbitol and protein kinase C, elevated homocysteine, reduced nitric oxide and excessive Reactive Oxygen Species (ROS) damage endothelial tissue and produce a rheological change that increases vascular resistance and reduces blood flow to the nerves. Even though depletion of myoinositol has been considered an implicated factor in nerve fiber degeneration of the patient with DM, clinical studies revealed that myoinositol deficiency is not part of the pathogenesis of human diabetic neuropathy [20].

Microvascular Hypothesis

Vascular and neural diseases are closely related. Blood vessels depend on normal nerve function, and nerves depend on adequate blood flow. The first pathological change in the microvasculature is vasoconstriction. As the disease progresses, neuronal dysfunction correlates closely with the development of vascular abnormalities, such as capillary basement membrane thickening and endothelial hyperplasia, which contribute to diminished oxygen tension and hypoxia. Neuronal ischemia is a well-established characteristic of diabetic neuropathy [21]. High homocysteine along with decrease in Bl2 and folic acid has been related to decreased production of nitric oxide and, therefore, a state of vasoconstriction.

Advanced Glycated End (AGE) Products

Elevated intracellular levels of glucose cause a bonding with proteins that modifies their structure and inhibits their function. AGE products are a complex group of compounds formed *via* non-enzymatic covalent bonding between reducing sugars and amine residues on proteins, lipids, or nucleic acids. Glycosylated proteins have been implicated in the

pathology of DPN and other long term complications of diabetes [22-27]. AGEs can also originate from exogenous sources such as tobacco smoke and diet [28-31]. Higher levels of AGE have been documented in smokers and patients on high AGE diets [28-30]. Chronic hyperglycemia promotes AGEs generation and consequent accumulation, which could be impaired in patients with renal failure because AGE clearance is mainly trough kidneys. AGE accumulation in local tissues is promoted because AGE modified proteins may be more resistant to enzymatic degradation [32]. Advanced glycation occurs over a prolonged period, affecting long lived proteins. Glycated myelin is susceptible to phagocytosis by macrophages *in vitro* and can also stimulate macrophages to secrete proteases and this might contribute towards nerve demyelization in diabetic neuropathy [33-34]. Furthermore, the AGEs on myelin can trap plasma proteins such as IgG, IgM and C3 to elicit immunological reactions that contribute towards neuronal demyelization [35]. Elevated levels of AGEs have been documented in the peripheral nerves of subjects with diabetes [33]. The AGE pathway is a major pathophysiologic mechanism in the development of diabetic neuropathy and measures to reduce its formation can be a useful to conserve nerve function in the diabetic patient.

Polyol Pathway

The polyol pathway, also called the sorbitol - aldose reductase pathway, may be implicated in diabetic complications that result from microvascular changes that result in nervous tissue damage, as well to the retina and kidney [37]. Glucose is a highly reactive compound that must be metabolized to avoid reaction with other tissues in the body. When glucose levels increase, like those seen in poorly controlled diabetes, the polyol pathway activates as an alternate biochemical pathway, which in turn causes a decrease in glutathione and an increase in reactive oxygen radicals causing direct tissue damage. This pathway is dependent on the enzyme aldose reductase which is not activated in normoglycemic state. While most body cells require the action of insulin for glucose to gain entry into the cell, the cells of the retina, kidney and nervous tissues are insulin-independent. In the hyperglycemic state, the affinity of aldose reductase for glucose rises, creating much higher levels of sorbitol. The sorbitol do not cross cell membranes, and when accumulates, it produces osmotic stresses on cells by drawing water into the cell [36]. In summary, excessive activation of the polyol pathway by excess glucose levels leads to increased levels of sorbitol and reactive oxygen species and decreased levels of nitric oxide and glutathione, as well as increased osmotic stresses on the cell membrane. These elements lead to increased cell damage. In addition, increased levels of glucose cause an increase in intracellular diacylglycerol, which activates Protein Kinase C (PKC). PKC is implicated in the pathology of diabetic neuropathy. It could result in vascular damage and decreased neuronal blood flow [37]. These changes produce abnormal metabolism in neuronal, axonal and Schwann cell causing impairment in their function.

Homocysteine (Hcy), Folate and Vitamin B12

Elevated Hcy levels increases the risk of developing peripheral vascular disease [38]. Hcy levels have been shown to be elevated in patients with peripheral vascular disease and chronic, non-healing lower-extremity ulcers [39-40]. On the other hand, there are a growing number of articles documenting an increase in the blood Hcy levels and the risk of developing DPN in patients with DM2 taking metformin (Glucophage™) [41-48]. However, long-term, placebo-controlled data on the effects of metformin on Hcy concentrations in DM2 are sparse [43]. According to current medical guidelines, metformin is the first-line drug of choice for the treatment of DM2 particularly in overweight and obese people with normal kidney function [49]. Metformin reduces diabetes complications and overall mortality. As of 2009, metformin is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines [50].

Long-term use of metformin is associated with malabsorption of vitamin B12 (cobalamin) and elevated fasting Hcy and methylmalonic acid (MMA) levels, which may have deleterious effects on peripheral nerves in patients with DM2 [47]. Clinical (i.e., Toronto Clinical Scoring System and Neuropathy Impairment Score) and electrophysiological (i.e., nerve conduction) measures have confirmed this hypothesis, with cumulative metformin dose strongly correlating with such clinical neuropathic evidences. Moreover, it has been early shown that metformin increases total serum Hcy levels in non-diabetic male patients with coronary heart disease [48], and adding metformin to insulin therapy in DM2 reduces levels of folic acid and vitamin Bl2, which results in increase in Hcy levels within 12 weeks [44]. Metformin-treated patients are more commonly treated with glyburide and less commonly with insulin therapy. Insulin may be more beneficial in patients with diabetes with peripheral neuropathy because of mechanisms other than glycemic correction [51]. Insulin provides trophic support for distal regenerating axons and significant improvement in epidermal fiber density and length. In diabetic neuropathy, distal loss of axons is an important clinical and pathological feature [51-53].

MTHFR Polymorphism

Methylene-tetrahydrofolate reductase (MTHFR) is an enzyme that in humans is encoded by the *MTHFR* gene [54]. As depicted in Fig. (1), MTHFR catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the active form of folate and co-substrate for Hcy remethylation to methionine (Hcy is a potentially toxic amino acid and cardiovascular risk factor) [55]. *MTHFR* gene is located on chromosome 1, p36.3 in humans. Two of the most investigated single nucleotide polimorphisms (SNPs) in this gene are C677T (*rsl80ll33*) and Al298C (*rsl80ll31*). The 677T allele (C→T missense mutation at position 677 of the *MTHFR* cDNA, leading to a valine substitution at amino acid 222) encodes a thermolabile enzyme with reduced activity. Individuals with two copies of 677C (677CC) have the "*wild-type*" genotype. 677TT individuals (homozygous) are said to have MTHFR deficiency, which causes low level of active folate.

Prevalence of MTHFR Polymorphism—Previous studies of the C677T polymorphism have concentrated on European populations [56-59]. The C677T mutation has a relatively high frequency throughout the world. About ten (10%) percent of the White-Americans population are T-homozygous for this polymorphism. In a previous study conducted by García-Fragoso *et al.* [60], the prevalence of the TT genotype in a convenience sample of the Puerto Rican population (n=400, unrelated newborn individuals) was 14%. There is ethnic variability in the frequency of the T allele as frequency in Mediterranean/Hispanics > Caucasians > Africans/African-Americans. The T allele frequency in Europeans is 24%-40% [56], 26%-37% in Japanese populations [65, 61], and approximately 11% in an African American population [59). T allele frequency(%) in Amerindians has been determined as high as 44.9% [57].

Genotype - Phenotype Relationship—Individuals with *MTHFR* Al298 C variant, but not C677T, have been associated with metabolic syndrome. A group of 518 patients with schizophrenia and C/C genotypes had 2.4 times higher risk of developing metabolic syndrome than those with A/A genotypes [62]. Genetic variation in this gene influences susceptibility to many health conditions [63-71], and mutations in this gene are associated with methylenetetrahydrofolate reductase deficiency [65, 72-74].

Homozygotes for the C677T mutation (i.e., 677TT individuals) are predisposed to hyperhomocysteinemia (high blood homocysteine levels) and, therefore, increased risk of cardiovascular disease because they have less active MTHFR available to produce 5-methyltetrahydrofolate, which is used to decrease Hcy [64, 72]. Under such risk conditions,

low folate intake affects individuals with the 677TT genotype to a greater extent than those with the 677CC/CT genotypes. 677TT (but not 677CC/CT) individuals with lower blood folate levels are at risk for elevated plasma Hcy levels. The correlation between the frequency of myocardial infarction (MI) and neural tube defects with high T allele frequency is consistent with the hypothesis that the C677T mutation is a risk factor for these diseases [56].

Potential Effect of MTHFR Polymorphism on DPN—Metformin therapy for more than 6 months in patients with DM2 who are carriers of any dysfunctional *MTHFR* polymorphism could be considered a pharmacogenetic cause for exacerbation of DPN, suggesting an earlier switch to insulin. A clear understanding of its role necessarily awaits further research on this matter.

Several studies have reported increased risk of DPN with the use of metformin. Even though it is unknown the exact role of *MTHFR* polymorphisms on the manifestation of such risk, its use represents an important potential "*geneticiatrogenic*" contributor to the development and severity of DPN. Recognition of this readily identifiable (by genotyping) and potentially addressable (by adjusting therapy) component of disease severity might improve outcomes including reduction of complications and quality of life for this large population of patients with DM. Genotyping for treatment-modifying *MTHFR* variants as well as screening for elevated Hcy levels in blood should be considered upon initiation of, and during, metformin therapy to detect secondary causes of worsening peripheral neuropathy. Since serum folic acid and B12 levels are known to decrease during metformin therapy and, consequently, the Hcy levels might increase, this effect may be higher in those individuals who are carriers of the *MTHFR* 677T allele (677TT genotype).

Taken altogether, these reports suggest a potential clinical association between *MTHFR* genotypes and the metformin-treated patient's risk of worsening DPN. It leads us to postulate that carriers of *MTHFR* C677T and A 1298C variants in the population of metformin-treated patients with DM2 will demonstrate higher serum levels of Hcy and worsening of metformin-induced DPN as compared to *wild-type*. Noteworthy, there are no published data so far indicating clinical associations of *MTHFR* alleles with metformin-induced worsening of DPN in patients with DM2 [75]. However, the implications for the metabolic consequences of metformin on Hcy and the risk of neuropathy suggest a role for supplementation of active folate, especially in patients with some polymorphic variants.

Other Genetic Factors

According to early reports, there seems to be other genetic polymorphisms that may play a role in the development of DPN. Kolla *et al.* [76] findings indicate that the 'low-producer' IFN gamma +874 A/A genotype and 'high-producer' IL 10 -1082 G/G genotype, but not the TNF alpha - 308G/A variant, are significantly associated with cytokine-induced nerve damage in diabetic peripheral neuropathy in South Indian patients with DM2 [76]. Pitocco *et al.* [77] studied association between G1181C and T245G polymorphisms in osteoprotegerin with risk of Charcot neuroarthropathy and found patients to have a 2.4-fold greater likelihood of having the G allele and a 40% reduction in presence of the C allele, while there was no association with diabetic neuropathy alone [78].

Recently, Bazzaz and colleagues [79] concluded that polymorphisms in the *eNOS* gene may constitute a factor in the genetic propensity to diabetic retinopathy and neuropathy, suggesting a prognostic value for this gene variation in patients with diabetes. In diabetes, reduced NO production may be related to the development of diabetic retinopathy and neuropathy, where vascular endothelial growth factor (VEGF) levels are increased in a counter regulatory manner. Among the three NOS enzymes, most attention has focused on

eNOS because of its relevance to angiopathies [79]. Mehrab-Mohseni and co-workers [80] found that in patients with diabetic neuropathy the frequency of variable number of tandem repeat (VNTR) polymorphisms in the *eNOS* gene was significantly increased compared to the controls (*p=0.03*, OR=1.8; 95%CI: 1.0-3.7). Both genotype and allele frequencies were significantly different between patients who were complication-free compared to the controls (*p=0.007*, OR=2.6; 95%CI: 1.2-5.8 and *p=0.001*, OR=2.8; 95%CI: 1.4-5.9) respectively, with the A allele conferring the risk [80].

Moreover, Bazzaz *et al.* [81] also found that distribution of a *VEGF* gene polymorphism at promoter region (-7C/T) was significantly different between British-Caucasian diabetic subjects with vs. without neuropathy and the allele (C) conferred susceptibility to diabetic neuropathy (*p=0.02*; OR=1.78; 95%CI: 1.0-3.1). Therefore, they argued that this polymorphism might be implicated in the pathogenesis of diabetic neuropathy. However, further studies are required to determine the prognostic value of this polymorphism in diabetic neuropathy as a chronic complication of diabetes [81].

Another study in 130 Greek patients showed that patients with neuropathy exhibit a significantly higher frequency of the deletion polymorphism in the alpha2B-adrenoceptor gene located on chromosome 2, in comparison to those without neuropathy. Presence of the deletion is also associated with a higher neuropathic score. Results provide evidence for an association of this polymorphism with both presence and severity of neuropathy in patients with DM2 mellitus [82]. Many other reports of genetic polymorphisms associated with neuropathic complications have been published, but mostly in patients with DM1. The search for functional polymorphisms in genes involved in various metabolic and vascular pathways, with predisposition and/or a protective effect for diabetic neuropathy, is currently increasing but with contradictory results. An early article reviews main studies and data on this matter [83]. The identification of candidate-genes should allow, in the future, a better management of patients with DM at-risk for peripheral neuropathy.

DPN MANAGEMENT

Current treatment guidelines for the clinical management of DPN are limited to glucose control and symptomatic pain relief (Table **1**). However, there are other therapeutic options that have been studied as putative pathogenetic alternatives, which are shown in Table **2** [1]. Furthermore, some additional pathogenetic treatments have indeed shown promising results in ongoing clinical trials [1], including aldose reductase inhibitors, vasodilators, protein kinase C_β inhibitor (ruboxistaurin), etc, but they are beyond the scope of this review. Meticulous glycemic control delays the onset and slows the progression of DPN in patients with DM2, but it does not completely prevent the progression of the disease [84]. In a prospective multicentre study of 153 males with diabetes with an average duration of disease of 7.8 years, the baseline prevalence of DPN was 53% and 48% in the standard insulin treatment group (one morning injection per day) and the intensive therapy group, respectively. After two years, the prevalence of DPN rose significantly in both groups from baseline, but there was not a significant difference between both strategies [85].

The goal of the treatment guidelines for DPN management is to relief pain and improves QoL. With proper use of the medications in the guidelines many patients do not achieve more than 30% to 50% pain reduction. This response may result in the ability to return to work or participate in social activities and thus vastly improve their quality of life and mood. The limitations of the recommendation on this guideline are that it does not recommend any therapy that potentially change the underlying pathology and natural history of the disease process or contribute to improve the metabolic deficiencies related to neuropathy. It means that if the patient has lost sensation it will not come back and that pain will return soon after

the medication is discontinued. In addition, pain relief medications are often accompanied with adverse effects that include dizziness, drowsiness, gastrointestinal distress, constipation, headaches, dry mouth, and sexual dysfunction [1].

Advances in the medical fields over the last decades have improved our knowledge of the biochemical aspects that lead to disease mechanism or pathophysiology. It has allowed the development of effective and non-toxic strategies in the management of many conditions that often times can produce a positive impact, not only in the symptoms, but in improvement of function and structures leading to alter the course of the disease.

METABOLIC CORRECTION AND OPTIMIZATION

The therapeutic objective should not be just to improve symptoms, but to normalize or optimize all factors involved in nerve and vascular health to prevent DPN progression or even development. The ADA position statement recommends that treatment should be directed at underlying pathogenesis, even when effective symptomatic treatments are available for the manifestations of DPN and autonomic neuropathy [1].

Dietary minerals are the chemical elements required by living organisms present in common organic molecules, excluding carbon, hydrogen, nitrogen and oxygen. There are seventeen required minerals (essential minerals) to support cell structure and function in human biochemical processes. The optimum intake of micronutrients for each person varies according to age and genetic constitution, diseases and exposure to stress or toxins [86-87].

The Rate-Limiting Step (The Weakest Link)

Metabolic processes can be seen as links in a chain, where every element must be addressed in order for the body to perform at peak efficiency. The strength of the entire chain can be compromised by only one weak link. People who have been metabolically optimized will experience a faster recovery, greater strength, more endurance, higher lactate tolerance, an increased VO_2max, a reduction in injuries and illnesses, better performances and more energy [86-88]. The almost complete absence in USA of patients who present recognized nutrient deficiency diseases such as pellagra, rickets, scurvy, acute night-blindness or beriberi has probably led to a false sense of security and the belief that almost everyone gets enough vitamins from food. Vitamins and minerals have seemingly fallen off the screen for many health care professionals and supplementation is viewed as a fad. Interest now seems obsessively focused on toxicity. However, a significant fraction of the American population appears to not obtain even the Recommended Daily Allowance (RDA) of some critical nutrients from their food. Low levels of nutrients that fall between the RDA and the levels that produce recognized deficiency diseases (Subclinical Deficiencies or insufficiencies) can have serious health consequences. Supplementation with specific nutrients has been estimated to be cost effective in preventing disease [89]. Food alone may not provide sufficient micronutrients for preventing deficiency [90-91]. A large proportion of older adults do not consume sufficient amounts of many nutrients. Supplements compensate to some extent, but only an estimated half of this population uses them daily [92].

When one component in the metabolic micronutrient network is inadequate, repercussions are experienced in a specific biochemical process or even in a large number of processes and can lead to diseases. Many of the carriers of 50 human genetic diseases that are due to defective enzymes can be remedied or ameliorated by the administration of high doses of the vitamin component of the corresponding needed coenzyme, which raises the levels of the coenzyme and at least may partially restore the needed enzymatic activity.

Alpha-Lipoic Acid

Alpha-lipoic acid (ALA), also known as thioctic acid, is a naturally occurring compound, which function as cofactors for several important mitochondrial enzyme complexes. ALA contains two thiol (sulfur) groups, which may be oxidized or reduced. The reduced form is known as dihydrolipoic acid (DHLA), while the oxidized form is known as ALA. ALA is able to cross the blood-brain barrier. ALA can potently regenerate other antioxidants such as vitamin C, vitamin E and glutathione through redox cycling [93]. ALA is considered a potential therapeutic alternative for chronic diseases associated with oxidative stress [94]. Intravenous and oral ALA is approved for the treatment of diabetic neuropathy in Germany [95]. ALA seems to delay or reverse peripheral diabetic neuropathy through its multiple antioxidant properties. Treatment with ALA increases reduced glutathione, an important endogenous antioxidant.

A meta-analysis that combined the results of four randomized controlled trials, including 1258 patients with diabetes, found that treatment with 600 mg/day of intravenous racemic lipoic acid (LA) for 3 weeks significantly reduced the symptoms of diabetic neuropathy to a clinically meaningful degree [95]. A short-term study of 24 patients with DM2 found that the symptoms of peripheral neuropathy were improved in those who took 1800 mg/day of oral racemic LA for 3 weeks compared to those who took a placebo [96].

A much larger clinical trial randomly assigned more than 500 patients with DM2 and symptomatic peripheral neuropathy to one of the following treatments: 1) 600 mg/day of intravenous racemic LA for 3 weeks followed by 1800 mg/day of oral racemic LA for 6 months, 2) 600 mg/day of intravenous racemic LA for 3 weeks followed by oral placebo for 6 months, or 3) intravenous placebo for 3 weeks followed by oral placebo for 6 months [97]. Although symptom scores did not differ significantly from baseline in any of the groups, assessments of sensory and motor deficits by physicians improved significantly after 3 weeks of intravenous ALA therapy. Motor and sensory deficits were also somewhat improved at the end of 6 months of oral ALA therapy. In the longest controlled trial of oral ALA therapy, 299 patients with DPN were randomly assigned to treatment with 1200 mg/day of oral racemic LA, 600 mg/day of oral racemic LA or placebo [98]. However, after 2 years of treatment, only 65 of the original participants were included in the final analysis. In that subgroup, those who took either 1200 mg/day or 600 mg/day of oral ALA showed significant improvement in electrophysiological tests of nerve conduction compared to those who took the placebo. Overall, available data suggests that a treatment with 600 mg/day of intravenous ALA for 3 weeks significantly reduces the symptoms of DPN [98]. Based on previous reports, oral ALA (600-1800 mg/day) may be beneficial in the treatment of DPN and cardiovascular autonomic neuropathy [99-101].

Acetyl-L-Carnitine

Acetyl-L-Carnitine (ALCAR) is an amino acid derivative that the body manufactures by acetylating carnitine which in turn is synthesized from other amino acids. It serves as a cofactor facilitating the utilization of fats as energy source, especially within the electron-transport chain in the mitochondria. ALCAR promotes peripheral nerve regeneration and has been shown to have analgesic effects in patients with HIV-related or chemotherapeutic origin and DPN [102-104].

In a 52-week randomized placebo-controlled study of 1,257 patients with diabetic neuropathy two doses of ALCAR: 500 and 1,000 mg/day TID were tested and the results demonstrated significant improvements in sural nerve fiber numbers and regenerating nerve fiber clusters. Vibration perception improved in all patients and pain symptom showed significant improvement in those taking 1,000 mg ALCAR [105]. Treatment with ALCAR

in this study demonstrated significant pain reduction, nerve fiber regeneration and improvement in vibration perception in patients with established diabetic neuropathy.

In another randomized, multicentre, double-blind, placebo-controlled, parallel-group study a group of 333 patients with DPN 2000 mg of ALCAR were given to assess neurophysiological parameters and pain. After 12 months treatment with ALCAR significant improvement in nerve conduction velocity and amplitude compared with placebo ($p<0.01$) was observed. The mean pain Visual Analog Scale (VAS) scores were significantly reduced in ALCAR users (39% from baseline) compared to placebo (8%) [106].

Under certain conditions, the demand for L-carnitine may exceed an individual's capacity to synthesize it, making it a conditionally essential micronutrient [106]. Some individuals may have genetic defect that impairs the manufacture of carnitine and ALCAR. In addition, liver and kidney conditions as well as anticonvulsants like phenytoin and valproic acid, can reduce ALCAR blood concentration. These factors need to be considered when evaluating each individual case. Anticonvulsants are commonly recommended to treat painful DPN.

Benfotiamine

Benfotiamine is a synthetic lipid form of thiamin (B1) developed in Japan in the late 1950's to treat alcoholic neuropathy, sciatica and other painful nerve conditions. Benfothiamine increases intracellular thiamine diphosphate levels, which is a cofactor of transketolase. This enzyme reduces advanced glycation end products (AGE) and lipidoxidation end products (ALE) by directing its substrates to the pentose phosphate pathway. Reduction of AGE has been shown to contribute to the prevention of macro- and microvascular endothelial dysfunction in individuals with DM2 [107-109]. A study demonstrated that benfotiamine can lower AGE by 40% [110].

A study assessed 22 patients with DPN who were treated with the combination of benfotiamine and vitamin B6 during 45 days. Patients manifested significant improvement in pain and other symptoms, laboratory (glycosylated haemoglobin) and electrophysiological parameters (motor/sensory conduction). The authors concluded that the treatment resulted in significant subjective and objective improvement of the disease signs and symptoms, which suggest that benfotiamine is an effective starting choice for the treatment of diabetic polyneuropathy [111].

A randomized, double blind, placebo-controlled, phase-III-study including 165 patients with symmetrical, distal diabetic polyneuropathy received either benfotiamine 600 mg per day (n=47/43), benfothiamine 300 mg per day (n=45/42) or placebo (n=41/39). The improvement was more pronounced at the higher benfothiamine dose and increased treatment duration. After 6 weeks of treatment, Neuropathy Symptom Score differed significantly between the treatment groups (p=0.033) compared to placebo [112]. Other studies indicate that the most marked pain relief from benfotiamine occurred in patients with diabetic neuropathy after a three-week trial period [113-115].

Active B Vitamins

The water soluble B-complex vitamins L-methylfolate, methylcobalamin and pyridoxal-5-phosphate are key active metabolic cofactors in many metabolic reactions, especially in amino acid and carbohydrate metabolism (B6), nucleotide biosynthesis, remethylation of Hcy (folate), DNA synthesis and regulation, fatty acid synthesis, energy production and regeneration of folate (B12), which are often present in supplements in their inactive form. There are certain conditions for which there is an increased need of these nutrients. Certain genetic defects that are relatively prevalent render the inactive forms of these vitamins less effective and therefore increase the susceptibility to develop certain conditions. The

utilization of the most active forms of these nutrients is more efficient than using the vitamin precursor in facilitating these important metabolic reactions. The active forms of these vitamins are fundamental in patient management by correcting metabolic abnormalities leading to improvements that usually go beyond symptom relief. These nutrients improve structure and function thus facilitating the restoration of normal physiology. The best management of patients with conditions characterized by specific metabolic needs requires replacement of active vitamins that can help with the restoration of healthy physiological homeostasis.

L-methylfolate or (S)-5-methyltetrahydrofolate (5-MTHF), is the primary biologically active isomer of folate [116] and the form of folate in circulation [117]. It is also the form which is transported across membranes into peripheral tissues, particularly across the blood brain barrier [118]. The last step of the conversion of folate into L-methylfolate depends on the MTHFR enzyme [119]. Individuals with the *MTHFR* polymorphism have a compromised ability to perform this last step and may have insufficient levels of L-methylfolate. Reduced levels of L-methylfolate will reduce methylation and increase Hcy which in turn may increase the risk of myocardial infarction [120], stroke [121], depression [122, 123], migraine [124], birth defects [125], diabetic nephropathy [75] and memory loss [126, 127]. L-methylfolate is well tolerated even at large doses. Some people may present mild gastrointestinal symptoms which are less likely to occur iftaken with food.

L-methylfolate being the active form of folic acid, is 7 times more bioavailable than folate, and is 3 times more effective in the reduction of homocysteine levels than folic acid [39-40]. Although the role of folic acid in vascular disease is not well established, active folate (5-methyltetrahydrofolate) but not its inactive form (5, 10-methylenetetrahydrofolate) can regenerate the tetrahydrobiopterin (BH4). plays a role as enzymatic cofactor (Fig. **1**) for endothelial Nitric Oxide Synthase (eNOS) for conversion of guanidine nitrogen of L-arginine (L-Arg) into NO (a.k.a., vascular endothelium-derived relaxing factor). NO is an important messenger molecule involved in many physiological and pathological processes. Appropriate levels of NO production cause vasodilatation and are important in protecting an organ from ischemic damage and ischemic pain.

Methylcobalamin (Methyl-B12) is one of two forms of biologically active vitamin B_{12} (the other being adenosylcobalamin). Methyl-B12 is the principal form of circulating vitamin B12, hence the form which is transported into peripheral tissue. Methyl-B12 is absorbed by a specific intestinal mechanism which uses an intrinsic factor and by diffusion process in which approximately 1% of the ingested dose is absorbed [128]. Cyanocobalamin and hydroxycobalamin are forms of the vitamin that require conversion to Methyl-B12 *via* the intermediate glutathionyl-B12. As we age our bodies reduce the absorption of B12 and folate. Methyl-B12 is a cofactor of the enzyme methionine synthase, which functions to transfer methyl groups for the regeneration of methionine from Hcy. By increasing folate levels and maintaining normal levels of B12, we decrease Hcy and vascular risks including cognitive impairment [129]. Elevated levels of Hcy can be a metabolic indication of decreased levels of the methylcobalamin form of vitamin B12 [130]. Oral administration of methylcobalamin resulted in subjective improvement of burning sensations, numbness, loss of sensation, and muscle cramps. An improvement in reflexes, vibration sense, lower motor neuron weakness and sensitivity to pain was also observed [131]. Methylcobalamin has excellent tolerability and no toxic or adverse effects have been associated with large intakes of vitamin B12 from food or supplements [131].

Pyridoxal-5′-phosphate (P5P) is the active form of vitamin B6 and is used as the prosthetic group for many enzymes [132]. Pyridoxine, the parent compound of P5P and the most frequently used form of vitamin B6, requires reduction and phosphorylation before

becoming biologically active. P5P is necessary for the activation of glycine in the initial stages of heme production [133-135]. A direct correlation has been found between carpal tunnel syndrome (CTS) and a deficiency in P5P, and its use has been reported to be beneficial in CTS [136-138]. Vitamin B6 nutritional status has a significant and selective modulatory impact on the production of both serotonin and GABA, the neurotransmitters that control depression, pain perception and anxiety [139]. P5P is a cofactor in the synthesis of these neurotransmitters. Also, high plasma levels of Hcy are considered an independent risk factor for atherosclerotic disease and venous thrombosis. Hcy can be re-methylated to methionine or channeled down the trans-sulfuration pathway to cysteine, which requires two P5P-dependent enzymes: cystathionine synthase and cystathionase [140]. The use of supplemental P5P has not been associated with toxicity, although the inactive form, pyridoxine, has been associated with reports of peripheral neuropathy [141]. One hypothesis is that pyridoxine toxicity is caused by exceeding the liver's ability to phosphorylate pyridoxine to P5P, yielding high serum levels of pyridoxine which may be directly neurotoxic and may compete with P5P for binding sites, resulting in a relative deficiency [142].

There are several clinical trials [143-148] showing positive results with the use of L-methylfolate, methylcobalamin and pyridoxal combination (LMF-MC-PP), alleviating patients suffering from DPN with painful symptoms. A randomized, prospective study of 97 patients with P-DPN demonstrated that LMF-MC-PP can produce better pain control than acetaminophen in both 10 ($p<0.01$) and 20 weeks ($p<0.008$) [143]. In addition, these trials have achieved outcomes beyond those obtained with medications currently included in the accepted guidelines, such as recovery of sensorium [145], improved blood flow [146] and increased nerve fiber density [147], as depicted in Fig. (**2**). Indeed, an open label trial of 31 patients with DPN demonstrated that LMF-MC-PP combination, when used twice a day for 6 months and up to 12 months, a significant recovery ($p<0.001$) in the sensory loss from DPN as measured from baseline using the pressure specified sensory device [145]. All clinical trials have demonstrated a safety profile similar to placebo. Of further interest is the fact that metformin, one of the key medications in the treatment of DM2, has been found to decrease B12 and increase Hcy which results in endothelial dysfunction [42-44].

CONNECTION BETWEEN DEPRESSION AND DIABETES COMPLICATIONS

Increased rates of depression were reported in people with DM2; however, there is a need for well-controlled and better-reported studies to inform the development of effective treatments for depression in these patients [149]. Patients with DM2 with two or more complications, especially neuropathy or nephropathy, are at high risk of depression. Knowing this can help clinicians identify patients at risk for depression and facilitate timely and adequate treatment [150].

A study among Hispanic population from Puerto Rico found a correlation between depression and diabetes. Prevalence of diabetes appears to be significantly higher in Puerto Rican adults with major depression compared to those without this psychiatric disorder. Longitudinal prospective studies and randomized controlled trials are needed to shed light on the temporal or causal relationship and to test whether effective prevention and treatment can reduce the risk of developing diabetes [151].

Depression and DPN share some metabolic similarities, specially the increased levels of Hcy, a low level of folate and cobalamin and possibly a high prevalence of *MTHFR* polymorphisms [41-48, 122, 152-155]. Major depression is associated with a 2-fold higher risk of incidence of diabetic foot ulcers [156]. Future studies of this association should

consider possible confounders or mediators such as better measures of peripheral neuropathy and peripheral arterial disease.

L-MethyIfolate has demonstrated efficacy in achieving and maintaining control of symptoms of depression in patients partially controlled with an antidepressant. Such patients are usually treated with higher doses or another antidepressant, both of which increases cost and adverse events [157-162], Metabolic correction of the common biochemical/metabolic relationships in DPN and depression can have an important implication in the control of both conditions and in the prevention of complications. It means that treatment with L-Methylfolate combination product can improve outcomes of both conditions.

CONCLUDING REMARKS

In summary, metabolic correction of the biochemical derangements in DPN is an emerging treatment strategy for which the peer reviewed clinical evidence is growing and showing increasing efficacy and safety. This strategy can achieve pain relief, improvement of sensation and QoL. Metabolic correction is extremely safe and goes beyond symptom control to also provide improvement in circulation, sensation and restoration of nerve function and fiber density. Furthermore, based on available data, it would be expected that metabolic correction can either slow or stop the disease progression, and even reverse the damage of disease in some cases. Preliminary evidence suggests that metabolic correction can reduce costs, diminish adverse effects of treatment and change the course of the disease. As our knowledge of clinical pharmacogenetics expands we will have a better understanding of the role of metabolic correction in DPN and, therefore, improve its implementation.

Acknowledgments

The authors want to thank Dr. Hector M. Santos, Dr. Carmen L. Cadilla and Jessicca Y. Renta from the School of Medicine, University of Puerto Rico, for their help in this review.

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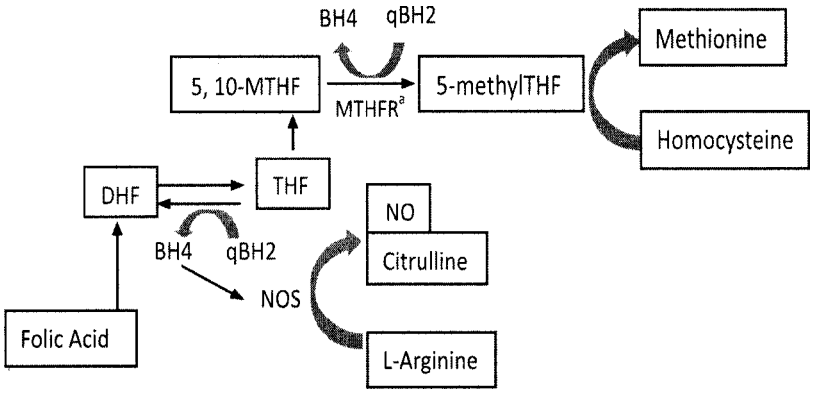

Fig. (1).

Metabolism of folic acid, homocysteine and nitric oxide (NO). Folic acid conversion to active 5-methyl THF requires several enzymes, adequate liver and gastro-intestinal function and B3, B6, B2, vitamin C and zinc as co factors. 50% individuals unable to fully convert folic acid into 5-methyl THF. [a]*MTHFR* C677T polymorphism seems to be involved, leading to hyperhomocysteinemia. MTHFR, methylenetetrahydrofolate reductase; 5, 10-MTHF, 5, 10 methy1ene-tetrahydrofolate; DHF, dehydrofolate; THF, tetrahydrofolate; NOS, nitric oxide synthase; BH4, tetrahydrobiopterin.

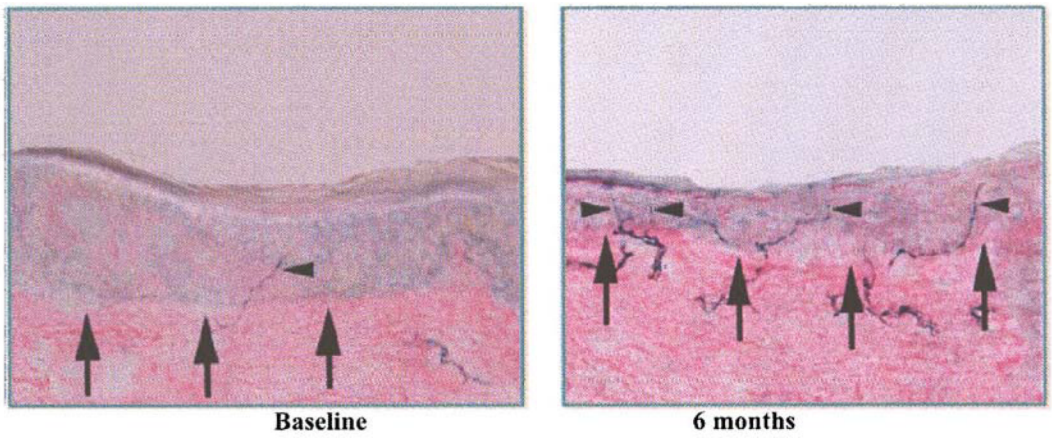

Fig. (2).

Microphotographs of baseline skin punch biopsy at right calf (left panel) and after receiving 6-month of twice daily LMF-MC-PP treatment (right panel) [147]. Arrows indicate epidermal nerve fiber density, whereas arrow heads represent nerve fibers. Patient average increase of 3.02 nerve fibers per mm after treatment.

Table 1

Painful Diabetic Peripheral Neuropathy Treatment Guidelines; Adapted with Permission from Boulton AJ *et al.* (2005) [1]

Agent Type	Class/Drug Names	Recommended Daily Dose (mg/d)	Side Effects	Reason for Recommendation
First Option	Duloxetine Oxycodone CR Pregabalin TCAs	60 to 120 10 to 60 150 to 600 25 to 150	+++ ++++ ++ ++++	>2 RCTs in DPN
Second Option	Carbamazepine Gabapentin Lamotrigine Tramadol Venlafaxine	200 to 4000 900 to 1800 200 to 400 50 to 400 150 to 225	+++ ++ ++ +++ +++	1 RCT in DPN; >1 in other painful neuropathies
Other Options	Bupropion Citalopram Paroxetine Topiramate	150 to 300 40 40 Up to 400	++ +++ +++ ++	>1 RCTs in other painful neuropathies or other evidence

RCT stands for randomized clinical trials; CR means controlled release; TCAs stand for tricyclic antidepressant drugs (e.g., Amitriptyline, Imipramine). “+” symbol represents the magnitude of the observed side effects: i.e., the greater the numbers of symbols “+”, the greater the magnitude.

Table 2

Alternative Strategies Based on Metabolic Correctors

Agent Type	Mechanism	Effect	Characteristics
Benfotiamine [ref. 111-115]	Blocks metabolic pathways of endothelial dysfunction and oxidative stress.	Improves in neuronal dysfunctions and symptoms.	No substantial adverse events. Most marked pain relief after 3 week period.
Alpha-Lipoic Acid [ref. 95-101]	Inhibition of oxidative stress and improvement of microvascular circulation	Significantly reduces neuropathic symptoms. Efficacy of intravenous dose of ALA on both the positive and negative symptoms.	Effective in RCTs, trials ongoing. Prominent safety profile.
Acetyl–L Carnitine [ref. 102-106]	Mechanism of action not clear. Pharmacological silencing of the nicotinic receptors has been suggested. May prevent inducement of nerve apoptosis.	Clinical trials have showed decreases in neuropathic pain scores. Seems to slow axonal degenerative changes.	Improvement in nerve conduction documented in one trial. Nerve fiber regeneration documented in one trial. Well tolerated.
*__Combination of Active B vitamins__ – [ref. 143-148]	Increase in Nitric Oxide Synthesis, increasing blood flow to the peripheral nerves	Increases blood flow to the peripheral nerves, improves cutaneous sensibility.	Has shown to improve pain control achieved with APAP or Gabapentin.

* L-methylfolate, Pyridoxal-5′-phosphate and Methylcobalamin. RCTs stand for randomized clinical trials; APAP stands for N-acetyl-p-aminophenol (acetaminophen).