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Clinical Experience of a Diet Designed to Reduce Aging

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ESSENCE OF ARTICLE

“In the context of an outpatient medical clinic, a high-fat, adequate-protein, low-carbohydrate diet with nutritional supplementation led to improvements in serum factors related to the aging process. Further research regarding this dietary approach and its relationship to aging is in order.”

ARTICLE

Objective:

Aging is associated with elevated levels of glucose, insulin, and triglycerides. Our objective was to assess the effect of a nutritional program designed to reduce these correlates of aging.

Design:

This is a retrospective chart review of patients attending an outpatient metabolic management program including a high-fat, adequate-protein, low-carbohydrate diet, nutritional supplementation and periodic individual visits.

Outcomes measured at baseline and follow-up included body weight, fasting serum glucose, insulin, leptin, lipids, and thyroid hormone.

Results:

Thirty-one patients were identified with complete information. The mean age of patients was 57.6 ± 2.4 consisting of 53% female and 47% male patients. The average duration between follow up visits was 91.5 ± 8.5 days. Of the parameters measured at the follow-up visit, body weight, serum leptin, insulin, fasting glucose, triglyceride, and free T3 significantly decreased by $8.1 \pm 0.8\%$, $48.2 \pm 3.8\%$, $40.1 \pm 4.7\%$, $7.6 \pm 2.1\%$, $28.3 \pm 5.7\%$, and $10.8 \pm 1.8\%$, respectively. Furthermore, the triglyceride/high density lipoprotein ratio decreased from 5.1 ± 1.7 to 2.6 ± 0.5 .

Conclusions:

In the context of an outpatient medical clinic, a high-fat, adequate-protein, low-carbohydrate diet with nutritional supplementation led to improvements in serum factors related to the aging process. Further research regarding this dietary approach and its relationship to aging is in order.

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Since the discovery that caloric restriction extended the lifespan of rats 70 years ago (1), many model organisms such as yeast (2, 3), nematodes (4), fruit flies (5), and mice (6, 7) have been subjected to some form of food restriction that resulted in extended lifespan. These and many other studies regarding the impact of caloric restriction on lifespan has generated such compelling evidence that the National Institute on Aging has initiated studies in non-human primates (rhesus macaques) examining caloric restriction on a number of metabolic and biological parameters including lifespan (8).

Nevertheless, the mechanisms driving the increased lifespan by calorie restriction or weight loss are unclear. Data from *C. elegans* (9, 10), *Drosophila*, and yeast (11) and, more recently, rodents (12) implicate the insulin/Insulin-like growth factor signaling pathways as a potential regulator of this process. Moreover, the ongoing data collection on the physiologic effects of caloric restriction in rhesus macaques parallels rodent studies demonstrating reduced body and fat mass, improved gluco-regulatory function, decreased blood pressure and blood lipids, and decreased body temperature (13). Interestingly, centenarians have lower blood glucose, insulin, leptin, free T3 and serum triglycerides than those who do not live to be over one hundred years old (14). Therefore, the fundamental mechanism by which calorie restriction improves lifespan appears to alter these metabolic parameters.

In this paper, we examine the impact of a diet specifically designed to improve metabolic parameters independent of caloric intake. The diet is based on the premise that by reducing glucose and protein as substrates for oxidative metabolism and enhancing fatty acid oxidation, many of the same physiologic changes that are seen in calorie-restricted animals will also be seen in individuals following this type of diet.

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Design

This is a retrospective analysis of clinical information from patients attending a private practice. Patients were referred for the treatment of diabetes, cardiovascular disease, excessive weight, fatigue, and other chronic diseases of aging. All patients with sufficient baseline and follow-up parameters were included in this series.

Clinic procedures

On the first visit, a comprehensive history and basic physical examination was performed. Clinical information was obtained and dietary instruction was provided by a clinical team consisting of a naturopath, nutritionist, and physician. After the first visit, the patients were typically contacted by telephone after 2-4 weeks, and a return visit was scheduled for 2-3 months later for follow-up evaluation and labs.

Dietary recommendation

The diet included unlimited amounts of certain fats and oils, a restricted amount of protein, and a very limited amount of carbohydrate. Patients were told to eat when they were hungry. Calories were not explicitly restricted; calorie intake was determined only by levels of hunger. Recommended sources of fat included raw nuts and seeds, avocados, olives and olive oil, flax oil and cod liver oil. The intake of protein was told to be limited to

approximately 1.0 grams/kg lean body mass per day (increased for exercise to 1.25 grams/day). As a result, most patients were instructed to eat from 50-80 grams of protein per day. Recommended sources of protein included sardines, fish, eggs, tofu, chicken, turkey, wild meats, low-fat cheeses (cottage, ricotta, swiss), seafood, and veggie burgers. Only non-starchy, fibrous vegetables were acceptable: lettuce, greens, broccoli, cauliflower, cucumbers, mushrooms, onions, peppers, sprouts, asparagus, and seaweed. Though not explicitly stated, the general dietary intake as percent daily caloric intake from macronutrients for most people ended up by history to be approximately 20% carbohydrate, 20% protein, and 60% fat. For drinking, 6-8 eight ounce glasses of water and/or herbal tea were recommended. A written handout with a list of acceptable and unacceptable foods was provided.

Nutritional supplementation

Nutritional supplements to support fat metabolism and enhance insulin sensitivity were recommended to all patients to be taken on a daily basis: L-carnitine 2000mg, alpha-lipoic acid 400mg, coenzyme Q10 100 mg, 1 tbsp cod liver oil, magnesium 300mg, potassium 300mg, vitamin C 1000mg, vitamin E 800mg daily, and a multivitamin consisting of all essential B vitamins and minerals. (15, 16).

Medication adjustment

If an individual was taking lipid-lowering or sulfonylurea medications, these medications were discontinued at the first visit before starting the diet. In those patients taking blood pressure medication, medication was adjusted or, altogether discontinued, if low normal blood pressures were observed during the course of the intervention. Because these patients were seen in clinical practice, patients were responsible for purchasing their own supplements.

Outcome measures

At baseline and return clinic visits, body weight was measured on the same scale (Tanita Model TBF-300A, Tanita Corporation of America, Inc. Arlington Heights, Illinois). Baseline and follow-up laboratory measurements included body weight and sitting blood pressure. Laboratory parameters included serum glucose, insulin, leptin, total cholesterol, LDL, HDL, triglycerides (TG), free T3 and thyroid stimulating hormone (TSH) following a 12 hour fast. The clinical data were abstracted from the medical charts and entered into a computer database without identifying information.

Statistical Analysis

The primary analysis was a “pre-post” analysis comparing baseline to follow-up values using a paired t-test. Individual percent changes for each laboratory parameter were determined and used to calculate the mean percent change. This de-identified analysis of existing clinical data was approved by the Duke University Medical Center Institutional Review Board.

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- o AbstractBACKGROUNDMethodsResultsDiscussionREFERENCESResults

Patient demographics of those who had complete baseline and follow-up laboratory tests are displayed in Table 1a. The mean age was 57.6 ± 2.4 years consisting of 53% female and 47% male patients. The average duration between baseline and follow up measurements was 91.5 ± 8.5 days.

Table 1a

Baseline patient characteristicsSummary of patient demographics. This population of patients consisted of highly motivated, primarily Caucasian individuals. (Data is presented as \pm S.E.M.)

The recommendation of a high fat, adequate protein, low carbohydrate diet resulted in a significant loss of body weight by 7.1 ± 0.8 lbs in this patient population (Table 1b; Figure 1). Accompanied by the weight was a significant reduction in both systolic and diastolic blood pressure by $10.2 \pm 2.1\%$ and $11.4 \pm 1.8\%$ mmHg, respectively. Serum levels of leptin, insulin, fasting glucose, and free T3 significantly decreased from baseline levels by $48.2 \pm 3.8\%$, $40.1 \pm 4.7\%$, $7.6 \pm 2.1\%$, and $10.8 \pm 1.8\%$, respectively (Table 1b; Figure 1). In addition, despite the intake of predominantly fat, there was a significant decrease in triglyceride ($28.3 \pm 5.7\%$) in this patient group. The triglyceride/HDL ratio decreased from 5.1 ± 1.7 to 2.6 ± 0.5 . Serum creatinine and TSH did not change significantly.

Table 1b

Effect of Diet Program on Body Weight and Fasting Serum Levels
Effect of dietary and nutritional supplement therapy on gravimetric measurements and fasting serum levels. Patient physicals were performed at the initial and final, follow up clinic visits. (more ...)

Figure 1

Directional impact of dietary intervention on clinical parameters of aging. The percent change from baseline is indicated for the averaged data taken from each patient. (* $p < 0.001$; data presented as \pm S.E.M.) Abbreviations: Gluc, glucose; (more ...)

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This retrospective analysis of patients from a private clinic adhering to a high-fat, low carbohydrate, adequate protein diet demonstrated reductions in critical metabolic mediators including insulin, leptin, glucose, triglycerides, and free T3. Moreover, the patient group, on average, lost significant weight even though they were not instructed to reduce calorie intake. These findings are consistent with other studies showing weight loss and reductions in fasting serum insulin with a low carbohydrate, ketogenic diet (17, 18). The ability of the diet to lower these parameters, especially TGs and body weight, seems counter to standard thinking considering the increased ingestion of fats. A possible explanation is that the resultant lower leptin and insulin was indicative of increased leptin and insulin sensitivity, resulting in increased β -oxidation, hypothalamic mediated increased satiety, and possibly subconsciously lowered caloric intake (19, 20).

It has been known for some time that different dietary fats appear to have varying effects on insulin-sensitive tissues and this may differentially impact the metabolic parameters measured in this study (21, 22). Numerous studies have demonstrated that reduced glucose (carbohydrate) substrates in the diet suppress hepatic de novo fatty acid biosynthesis, triglyceride production, and triglyceride secretion, while enhancing hepatic, adipose, and skeletal muscle fatty acid oxidation (22-24).

Nonetheless, the impact of this dietary approach on aging mechanisms can only be implied from comparisons with longevity studies that have examined the same metabolic parameters. Many aging studies have used calorie restriction as the means to impact aging. These types of studies become difficult in humans for the obvious reasons of dietary compliance, human lifespan duration, and the multiple and complex confounding factors. For this reason, investigators have examined the effectiveness of weight loss as a surrogate for caloric restriction on human mortality rates (25) but have found an increased mortality rate and reduced lifespan (26). The reason is that many of these

studies did not distinguish between intentional and unintentional weight loss with the latter usually resulting from disease and, thus, increased mortality. It is now speculated that fat loss as opposed to weight loss decreases all-cause mortality in humans (25).

Patients in this study demonstrated a similar directional impact on the measured parameters when compared to studies using more established models of longevity such as caloric restriction. The patients in this study demonstrated significant weight loss along with a reduction in glucoregulatory mediators including insulin and leptin similar to those found in calorie-restricted primates (27, 28). Moreover, calorie restricted primates demonstrated a positive relationship between food intake and leptin levels (29). Thus, although patients were not told to restrict calories, there may have been a reduction in caloric intake secondary to reduced hunger, with the decrease in circulating leptin reflecting an increase in leptin sensitivity (19, 30). Interestingly, this study cohort exhibited a reduction in circulating free T3, the secreted form of thyroid hormone thought to mediate most of thyroid actions. Paralleling this reduction in circulating free T3, 9 patients of this study cohort that had basal body temperatures measured before and after intervention showed a significant decrease ($p=0.004$) in basal body temperature of 0.182 degrees C. Similar findings were reported in caloric restricted rodents, monkeys, humans, and centenarians (31-34). It has been suggested that the reduction in T3 and body temperature could alter the aging process by reflecting a reducing metabolic rate, oxidative stress, and systemic inflammation (35, 36).

It is important to note that the dietary recommendation in this study is unique from other ketogenic diets in that this dietary intervention limits protein intake as well as carbohydrates (though not total fat intake). It has been demonstrated that the longevity effects of calorie restriction can be partially attributed to the reduction in protein intake (37). It has been shown that limiting dietary amino acids, specifically methionine, inhibits signaling through mammalian target of rapamycin (mTOR) thereby decreasing mitochondrial damage and protein translation (38, 39). Future studies will be aimed at investigating the impact of a high fat, low carbohydrate with limited but adequate protein on mTOR signaling.

Because this was a retrospective analysis of a clinical practice, there may be bias introduced in the patient sampling procedure. This study reflects the effect of recommending this diet in a clinical practice, so food intake was not directly measured. In addition, this sample population may reflect the results in highly motivated individuals. Though the metabolic improvements occurred in patients who had both high and low weight loss, the improvements in metabolic parameters may be all or partially due to the weight loss. It should be noted, however, that the percent reduction in leptin particularly far exceeded the percentage of fat loss and may not be explained solely as a result of this fat loss. Also, it remains unclear as to the contributions of the suggested nutritional supplementation to the observed outcomes.

In conclusion, a nutritional program recommendation originally designed to treat chronic diseases of aging led to weight loss and metabolic changes currently thought to be beneficial in reducing the aging process. Further research regarding this dietary approach and its relationship to aging is in order.

ACKNOWLEDGEMENTS

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- Other Sections ▼

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Diet Soda, Sodium Tied to Kidney Trouble: Studies

It's more proof that what you eat and drink affects health, researchers say

HealthDay

By Robert Preidt

Monday, November 2, 2009

MONDAY, Nov. 2 (HealthDay News) -- A diet high in salt or artificially sweetened drinks increases the risk of kidney function decline, two studies show.

"There are currently limited data on the role of diet in kidney disease," researcher Dr. Julie Lin, of Brigham and Women's Hospital in Boston, said in a news release. "While more study is needed, our research suggests that higher sodium and artificially sweetened soda intake are associated with greater rate of decline in kidney function."

The first study looked at diet and kidney function decline in more than 3,000 women enrolled in the national Nurses' Health Study. The researchers found that "in women with well-preserved kidney function, higher dietary sodium intake was associated with greater kidney function decline, which is consistent with experimental animal data that high sodium intake promotes progressive kidney disease."

The second study looked at the association between sugar- and artificially-sweetened beverages and kidney function decline in the same group of women. The researchers found an association between two or more servings per day of artificially sweetened soda and a two-fold increased risk of faster kidney function decline. There was no connection between sugar-sweetened beverages and kidney function decline.

The association between artificially sweetened beverages and kidney function decline persisted after Lin and colleague Dr. Gary Curhan accounted for other factors, such as age, obesity, high blood pressure, diabetes, smoking, physical activity, caloric intake and cardiovascular disease.

Further study is needed to better understand how artificial sweeteners influence kidney function decline, the researchers said.

The studies were to be presented this week at the annual meeting of the American Society of Nephrology, in San Diego.

SOURCE: American Society of Nephrology, news release, Oct. 31, 2009

HealthDay

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Featured: Thyroid Peroxidase Test Main Article

The thyroid peroxidase test measures the level of an antibody that is directed against thyroid peroxidase (TPO). A presence of TPOAb in the blood reflects a prior attack by the body's immune system on thyroid tissue. A positive thyroid peroxidase test may signal chronic thyroiditis. Other autoimmune disorders, however, may have a positive TPOAb test, including lupus, rheumatoid arthritis, Sjogren's syndrome, or pernicious anemia.

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ARTICLES

Interferon-gamma reduces the thyroid peroxidase content of cultured human thyrocytes and inhibits its increase induced by thyrotropin

H Asakawa, T Hanafusa, T Kobayashi, S Takai, N Kono and S Tarui

Second Department of Internal Medicine, Osaka University Medical School, Japan.

ESSENCE OF ARTICLE

“ IFN gamma also inhibited the increase in TPO content induced by TSH. Thus, complex interactions appear to exist between IFN gamma and TSH or thyroid-stimulating antibodies in the modulation of hormone secretion and autoimmune phenomena in the thyroid.”

ARTICLE

To clarify the role of interferon-gamma (IFN gamma) in autoimmune thyroid diseases, we investigated the effects of IFN gamma on the content of thyroid peroxidase (TPO) and the expression of HLA-DR antigens in cultured normal human thyrocytes. The effect of TSH on the action of IFN gamma was investigated. Immunofluorescence staining

and photometric analysis showed that IFN gamma not only induced the expression of DR antigen, but also reduced the content of TPO in a concentration-dependent manner. The addition of TSH increased the content of TPO and enhanced the IFN gamma-induced expression of DR antigen. IFN gamma also inhibited the increase in TPO content induced by TSH. Thus, complex interactions appear to exist between IFN gamma and TSH or thyroid-stimulating antibodies in the modulation of hormone secretion and autoimmune phenomena in the thyroid.

<http://endo.endojournals.org/cgi/content/abstract/136/3/881>

Endocrinology, Vol 136, 881-888, Copyright © 1995 by Endocrine Society

ARTICLES

Tumor necrosis factor-alpha and interferon-gamma modulate gene expression of type I 5'-deiodinase, thyroid peroxidase, and thyroglobulin in FRTL-5 rat thyroid cells

KT Tang, LE Braverman and WJ DeVito

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ESSENCE OF ARTICLE

"These findings indicate that TNF-alpha and IFN-gamma in combination have a marked inhibitory effect on thyroid function, which is consistent with a decrease in thyroid hormone synthesis and metabolism. "

ARTICLE

Interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF- alpha) have many effects on a number of cell types, including thyrotrophs. In the present study, we used FRTL5 cells, a cultured rat thyroid follicular cell line, to examine the effects of IFN-gamma and TNF-alpha on type I 5'-deiodinase (5'D-I) activity and 5'D-I, thyroid peroxidase (TPO) and thyroglobulin (Tg) gene expression. Incubation of FRTL5 cells with the highest concentrations of TNF-alpha and IFN-gamma tested (1000 ng/ml or 1000 U/ml, respectively) for 72 h in the presence and absence of TSH had no effect on cell viability as assessed by trypan blue exclusion. In TSH-deprived FRTL-5 cells, TNF-alpha and IFN- gamma resulted in a small but dose-dependent decrease in 5'D-I activity. TNF-alpha or IFN-gamma blocked the TSH- or cAMP-induced rise in 5'D-I activity. 100 ng/ml TNF-alpha and 100 U/ml IFN-gamma completely blocked the TSH- or cAMP-induced rise in 5' D-I activity. However, when cells were incubated with TNF-alpha and IFN-gamma, in combination, there was a marked decrease in 5'D-I activity, with TNF- alpha (25 ng/ml) plus IFN-gamma (25 U/ml) completely blocking the TSH- induced rise in 5'D-I activity. Northern blot analyses were performed to examine the effect of TNF-alpha and IFN-gamma on 5'D-I gene expression. TNF-alpha had little effect on 5'D-I messenger RNA (mRNA) levels, while IFN-gamma resulted in a modest decrease in 5'D-I mRNA levels in TSH-deprived cells, and in TSH-stimulated FRTL-5 cells. However, when TNF-alpha and IFN-gamma were added in combination there was a marked decrease in 5'D-I gene expression with TNF-alpha (50 ng/ml) plus IFN-gamma (50 U/ml) decreasing 5'D-I mRNA levels by 89 percent in TSH-deprived cells. In TSH-stimulated cells incubated with 500 ng/ml TNF-alpha plus 500 U/ml IFN-gamma, 5'D-I mRNA levels were almost undetectable. We also examined the effect of IFN-gamma and TNF- alpha on TPO and Tg gene expression. As observed with 5'D-I mRNA levels, there was a synergistic effect of IFN-gamma and TNF-alpha on the inhibition of basal and TSH-stimulated TPO and Tg gene expression. These findings indicate that TNF-alpha and IFN-gamma in combination have a marked inhibitory effect on thyroid function, which is consistent with a decrease in thyroid hormone synthesis and metabolism.

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Recombinant Human Thyrotropin Is a Potent Stimulator of Thyroid Function in Normal Subjects

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[Abstract] [Full Text] [PDF]

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The role of interferon-gamma in lymphocytic thyroiditis: its functional and pathological effect on human thyrocytes in culture.

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ESSENCE OF ARTICLE

We have previously demonstrated that IFN-gamma suppressed thyroid-stimulating hormone (TSH)-stimulated thyroglobulin (TG) synthesis in human thyrocytes through inhibition of TG gene transcription. "

ARTICLE

Abstract

Interferon-gamma (IFN-gamma) has been recognized to possess diverse non-immunological effects on epithelial cells such as cellular growth and differentiation. We have previously demonstrated that IFN-gamma suppressed thyroid-stimulating hormone (TSH)-stimulated thyroglobulin (TG) synthesis in human thyrocytes through inhibition of TG gene transcription. To define the pathological mechanism involved in the action of IFN-gamma, we studied the ultrastructural changes of human thyrocytes cultured in monolayer. Stimulation of the thyrocytes with TSH 10 mU/ml for 2 days resulted in marked increase in TG release into the medium. This was accompanied by elongation of microvilli, increase in follicles and acinar formation, increase in secretory granules and prominence of Golgi apparatus and rough-surfaced endoplasmic reticulum. Addition of IFN-gamma (500 U/ml) resulted in marked degeneration with shrinkage of the cell membrane, vacuolation of cytoplasm, swollen mitochondria and presence of lysosomal granules. Co-culturing the thyrocytes with the IFN-gamma and TSH resulted in suppression of the morphological responsiveness to TSH. There was also suppression of TSH-induced TG secretion. However, at 500 U/ml IFN-gamma did not cause lysis of the thyrocytes as estimated by the cellular DNA content. Furthermore, binucleated cells were frequently encountered in those wells that were treated with IFN-gamma for either 2 or 5

days. The findings suggest that IFN-gamma resulted in de-differentiation and degeneration of the thyrocytes, which subsequently regained the growth potential and showed attempts at regeneration. This may explain why most patients with lymphocytic thyroiditis recover from the acute injury and do not suffer from permanent hypothyroidism.

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Am Heart J. 2009 Nov;158(5):761-7. Epub 2009 Sep 22.

Intake of total trans, trans-18:1, and trans-18:2 fatty acids and risk of sudden cardiac death in women.

Chiuve SE, Rimm EB, Manson JE, Whang W, Mozaffarian D, Stampfer MJ, Willett WC, Albert CM.

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ESSENCE OF ARTICLE

“However, trans fat intake may be associated with SCD risk among women with CHD, suggesting that trans fat intake may play a greater role in SCD risk among those with clinically manifest atherosclerosis.”

ARTICLE

BACKGROUND: Total intake of trans fat is associated with coronary heart disease (CHD), and recent reports in primarily male populations suggest that blood levels of specific trans isomers may have different effects on risk, particularly risk of sudden cardiac death (SCD). **METHODS:** We prospectively examined the association between dietary intake of trans fat and SCD among 86,762 women from the Nurses' Health Study. Coronary heart disease risk factors, including diet and lifestyle factors, were updated via questionnaires every 2 to 4 years, beginning in 1980. **RESULTS:** Over 26 years, we documented 317 SCD events. In the primary analysis, we found no significant association between intake of total trans fat, trans-18:1, or trans-18:2 isomers and risk of SCD. Compared to the lowest quintile of intake, the relative risk (95% CI) of SCD in the highest quintile was 1.28 (0.82-2.00) for total trans, 1.08 (0.64-1.83) for trans-18:1, and 1.19 (0.76-1.88) for trans-18:2. In a secondary prespecified analysis, total trans fat was significantly related to SCD among women who reported a diagnosis of CHD before SCD (relative risk 3.24, 95% CI 1.42-7.40 for the highest vs lowest quintile, P trend = .01); however, the test for interaction was not significant (P = .11). **CONCLUSIONS:** In this large prospective cohort of women, neither dietary intake of trans fat nor the individual trans isomers, trans-18:1 and trans-18:2, were significantly associated with risk of SCD. However, trans fat intake may be associated with SCD risk among women with CHD, suggesting that trans fat intake may play a greater role in SCD risk among those with clinically manifest atherosclerosis.

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<http://www.ncbi.nlm.nih.gov/pubmed/9267584>

Ann Nutr Metab. 1997;41(2):98-107.

Effect of micronutrient supplementation on infection in institutionalized elderly subjects: a controlled trial.

Girodon F, Lombard M, Galan P, Brunet-Lecomte P, Monget AL, Arnaud J, Preziosi P, Hercberg S.

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ESSENCE OF ARTICLE

"These results indicate that supplementation with low doses of vitamins and trace elements is able to rapidly correct corresponding deficiencies in the institutionalized elderly. Moreover, zinc and selenium reduced infectious events.:

ARTICLE

To determine the impact of a trace element and vitamin supplementation on infectious morbidity, a double-blind controlled trial was performed on 81 elderly subjects in a geriatric center during a 2-year period. Subjects were randomly assigned to one of four treatment groups, and received daily: placebo; trace elements/zinc 20 mg; selenium 100 micrograms); vitamins (vitamin C 120 mg; beta-carotene 6 mg; alpha-tocopherol 15 mg); or a combination of trace elements and vitamins at equal doses. (1) Before supplementation, low serum values in vitamin C, folate, zinc and selenium were observed in more than two thirds of the patients. (2) After 6 months of supplementation, a significant increase in vitamin and trace element serum levels was obtained in the corresponding treatment groups: a plateau was then observed for the whole study. (3) Subjects who received trace elements (zinc and selenium) alone or associated with vitamins had significantly less infectious events during the 2 years of supplementation. These results indicate that supplementation with low doses of vitamins and trace elements is able

to rapidly correct corresponding deficiencies in the institutionalized elderly. Moreover, zinc and selenium reduced infectious events.

PMID: 9267584 [PubMed - indexed for MEDLINE]

<http://www.medscape.com/viewarticle/467012>

www.medscape.com

From Journal of the American Geriatrics Society

Nutritional Formula Enhanced Immune Function and Reduced Days of Symptoms of Upper Respiratory Tract Infection in Seniors

Bobbi Langkamp-henken, PhD, Bradley S. Bender, MD, Elizabeth M. Gardner, PhD, Kelli A. Herrlinger-Garcia, BS, Michael J. Kelley, PhD, Donna M. Murasko, PhD, Joseph P. Schaller, PhD, Joyce K. Stechmiller, PhD, Debra J. Thomas, MS, Steven M. Wood, PhD

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ESSENCE OF ARTICLE

“RESULTS: Eighteen subjects in the control group and 16 subjects in the experimental group consumed an average of 7 ounces of formula daily and completed the 183-day study. Median days of symptoms of URTI were 3 (range 0-69, total days=156) and 0 (range 0-49, total days=78) for the control and experimental groups, respectively (P=.049). On Day 57, seven of 17 (41%) subjects in the control group and 13 of 15 (87%) subjects in the experimental group achieved a fourfold or greater increase in serum antibody titer to A/Beijing (P=.012). Lymphocyte proliferation to influenza vaccine components was greater in the experimental (median=1,365 cpm, range=0-14,955 cpm) than the control group (median=136 cpm, range=0-4,270 cpm) (P=.013).”

Abstract and Introduction

Abstract

Objectives: To assess whether an experimental nutritional formula, given as a supplement, would reduce days of symptoms of upper respiratory tract infection (URTI) and affect antibody and lymphocyte proliferative responses to influenza vaccine.

Design: A prospective, randomized, double-blind, controlled trial was conducted between October 1999 and April 2000.

Setting: Assisted- and independent-living facilities in North Central Florida.

Participants: Sixty-six individuals, aged 65 and older.

Intervention: Subjects received 8 oz/d of an experimental formula containing antioxidants, zinc, selenium, fermentable oligosaccharides, and structured triacylglycerol or an isoenergetic, isonitrogenous control formula for 183 days.

Measurements: Subjects recorded daily symptoms of URTI. Antibody titers and lymphocyte proliferation to three influenza vaccine components were measured on Days 57 and 183.

Results: Eighteen subjects in the control group and 16 subjects in the experimental group consumed an average of 7 ounces of formula daily and completed the 183-day study. Median days of symptoms of URTI were 3 (range 0-69, total days=156) and 0 (range 0-49, total days=78) for the control and experimental groups, respectively (P= .049). On Day 57, seven of 17 (41%) subjects in the control group and 13 of 15 (87%) subjects in the experimental group achieved a fourfold or greater increase in serum antibody titer to A/Beijing (P=.012). Lymphocyte proliferation to influenza vaccine components was greater in the experimental (median=1,365 cpm, range=0-14,955 cpm) than the control group (median=136 cpm, range=0-4,270 cpm) (P=.013).

Conclusion: Subjects consuming an experimental nutritional formula experienced enhanced immune function and fewer days of URTI symptoms.

Introduction

Advanced age is associated with increased risk of nutrient deficiency and altered regulation of the immune system.[1] Nutrient deficiencies in the young or elderly can impair immune function; nutrient supplementation can restore normal immune capacity.[2-4] Consequently, there has been interest in determining whether nutrient supplementation in seniors could attenuate age-associated declines in immune function.[3-8]

Well-documented clinical and experimental studies report an age-associated decrease in immune function, including antibody response to vaccination.[9-13] Influenza infections significantly affect morbidity and mortality in the aged, and when vaccinated, seniors experience a lower antibody response than that observed in young healthy cohorts.[9,14,15] One study noted that, in a population of 210 seniors hospitalized with influenza, 129 (61%) had been vaccinated against influenza, indicating a suboptimal response to the vaccine or less-than-adequate protection from the influenza vaccine.[16]

As early as the 1950s, single-nutrient deficiencies were known to suppress vaccine response.[17] More recently, it was shown that nutritional supplementation enhances antibody responses to influenza vaccination in seniors.[4,5,8] A few studies have investigated the influence of nutrient supplementation on immune function in seniors and found a clinically meaningful benefit, such as reduced infection rates or days of illness. A more recent study examined the effect of trace-element (zinc and selenium) supplementation on immune function and infections in institutionalized seniors (mean age 83).[8] Supplementation with trace elements corrected a selenium deficiency, enhanced antibody titers to influenza vaccination, and showed a trend toward fewer subjects with respiratory tract infections.[8] Another study reported better immune responsiveness and "fewer infection-related illnesses" with a multivitamin supplement than with a placebo in apparently healthy, independent-living elderly (mean age 75).[4] Other researchers have supplemented the diets of seniors and found no immunological benefit or reduction in acute respiratory tract infections.[18-20]

Research conducted in military personnel has shown immune dysregulation caused by stress that is similar to the immune dysregulation noted in the elderly (e.g., anergy and decreased proliferative response).[21-24] Several nutritional formulations had previously been tested until one was identified that minimized stress-induced immune dysregulation. The formula contained vitamins and minerals, with elevated levels of antioxidants (vitamins E, C, β -carotene), selenium, zinc, fructo-oligosaccharides (fermentable oligosaccharides), and structured lipids (a unique triacylglycerol containing long- and medium-chain fatty acids from canola and medium-chain triacylglycerols). Several of the nutrients included have been shown to enhance or support immune function in studies of seniors.[7,25-27] Fructo-oligosaccharides are readily soluble and fermentable to short-chain fatty acids in the large bowel, thus maintaining gastrointestinal health.[28,29] Structured lipids have been shown to enhance the absorption of lipid-soluble nutrients[30] and have the potential to be a unique energy substrate for metabolically active cells such as leukocytes.[31]

The objective of this study was to supplement the diet of relatively healthy independent- and assisted-living seniors with an experimental formula (containing protein, antioxidants (vitamins E, C, β -carotene), selenium, zinc, fructo-oligosaccharides, and structured triacylglycerol) and determine, in a prospectively designed study, whether seniors who were at risk of developing infections would have a reduced number of days of symptoms of upper respiratory tract infections (URTI) during the cold and influenza season (October 1999-April 2000). Antibody titers and lymphocyte proliferative response to influenza vaccine components were also measured as markers of immune function.

Methods

Subjects

Ambulatory men and women, aged 65 and older, were recruited from six assisted-living and seven independent-living facilities in Gainesville and Ocala, Florida, during September and October 1999. The study was conducted from October 1999 through April 2000. Subjects were excluded if they were taking vitamin K-dependent anticoagulant medication; allergic to any components of the study or control formula; ineligible to receive the influenza vaccine; currently being treated for Alzheimer's disease, insulin-dependent diabetes mellitus, or renal, hepatic, gastrointestinal, or immune system insufficiency or disease; receiving treatments known to affect the immune response; or receiving supplemental oxygen for at least 12 hours continuously per day. Subjects who would not agree to discontinue taking dietary supplements (other than calcium, vitamin D, iron, vitamin B12 injections, or fiber), had smoked within the past 5 years, or had a body mass index (BMI) of 32 kg/m² or greater were excluded. Informed consent was obtained from all subjects or their surrogate after the nature of the study and procedures had been explained. The study protocol and informed consent were in accordance with the Helsinki Declaration of 1975 and the 1983 revision and were approved by the institutional review board at the University of Florida.

Before randomization, a nurse practitioner recorded a detailed medical history, body weight and height were measured to calculate BMI, and subjects completed the Determine Your Nutritional Health questionnaire (NHQ).[32] Subjects were then stratified by age, sex, and score on the NHQ and randomized based on the stratification sequence to receive the experimental or an isoenergetic, isonitrogenous control formula (Table 1). Each independent- or assisted-living facility was stratified and randomized individually.

Study Design

A prospective, randomized, parallel, double-blind, controlled design was used. On study Day 0 (baseline), a nonfasting blood sample was collected and analyzed for vitamin E concentrations, clinical chemistries, and hematology, as well as lymphocyte proliferation and antibody titers to influenza vaccine components. Clinical chemistries and hematology analyses were performed at a central laboratory facility (SmithKline Beecham, Tampa, FL).

Randomization envelopes were opened to determine study group assignment, and all subjects were instructed to consume one 8-ounce can of control or experimental formula (Table 1); both groups received the same low-potency vitamin/mineral supplement tablet (Multi-Vites with Minerals, Vitamin Power, Freeport, NY (Table 1)) daily for the next 183 days. This was to ensure that all subjects received some supplemental vitamins and minerals along with their normal diets. Subjects completed daily case report forms for study formula and multivitamin intake, symptoms of URTI, and gastrointestinal tolerance for 183 days. Symptoms of URTI were analyzed from study Day 15 forward (169 days used for analysis). This design excluded any infections present but not yet symptomatic at enrollment and evaluated subjects' ability to complete daily case report forms. Subjects unable to record information on their own were identified within the first 15 days; thereafter, nursing personnel or study investigators verbally presented the questions on the case report forms to these subjects daily and recorded their responses.

On study day 15±2 days, subjects were vaccinated with a single lot of Influenza Virus Vaccine, Trivalent, Types A and B Fluogen (lot #03079P, Parkedale Pharmaceuticals, Rochester, MI). The U.S. Public Health Service formulated this vaccine for the 1999-2000 season; it contained 15 µg of each of the following hemagglutinin antigens: A/Beijing/262/95 (H1N1), A/Sydney/5/97 (H3N2), and B/Yamanashi/166/98 (B/Beijing/184/93-like). This antigen composition was the same as the 1998-1999 influenza vaccine. On study day 57±3 days (42 days postvaccination), a nonfasting, postvaccination blood sample was collected for determination of serum vitamin E, and influenza antibody titers and lymphocyte proliferation to vaccine components. This postvaccination point was selected based on other studies[33,34] and to ensure that antibody responses had peaked.[34] In addition, some seniors seroconvert to the influenza vaccine after 4 weeks.[35] Blood samples were obtained at the conclusion of the study (Day 183±3, final blood draw) for clinical chemistries, hematology, serum vitamin E, and lymphocyte proliferation and antibody titers to vaccine components.

Subjects were instructed to continue their previous life style (including diet and exercise) and to incorporate study formulas into their diet without changing body weight during the study. Subjects were weighed at baseline, once each month, and on Day 183. Adverse events were collected throughout the study and for an additional 30 days after cessation of formula consumption.

Outcome Variables

The primary variable was the number of days with symptoms of URTI recorded by the subjects daily. Possible symptoms included cough, running or congested nose, sore throat, stiffness or chills, fever, achiness, and headache. Combinations of two or more symptoms, including one or more of the first three listed symptoms, were defined as a day of URTI symptoms. An URTI was defined as symptoms lasting two or more consecutive days. A new URTI was defined as an initial infection separated from a new URTI episode by 5 or more symptom-free days.

Secondary variables included antibody response and lymphocyte proliferation to influenza vaccine components at baseline, postvaccination (Day 57), and final study time points (Day 183). Additional outcome variables included symptoms of gastrointestinal intolerance (diarrhea, constipation, bloating, flatulence, nausea, and vomiting). Serum vitamin E (α -tocopherol) was measured at baseline, postvaccination, and final study time points as a marker of the experimental group's compliance. The control formula contained no added α -tocopherol, whereas the experimental formula contained 135 mg α -tocopherol (all natural form, RRR- α -tocopherol) per 8-ounce serving.

Antibody Titers

Blood for determination of antibody response to the components of the influenza vaccine was collected in SST-Gel Clot Activator Vacutainer Tubes (Beckton Dickinson, Franklin Lakes, NJ). Serum was separated, frozen, and shipped on dry ice for analysis to Ross Products Division, Abbott Laboratories (Columbus, OH). Baseline, postvaccination, and final influenza antibody titers were quantitated simultaneously using a modified hemagglutination inhibition (HI) procedure used by the World Health Organization Collaborating Center for Influenza, Centers for Disease Control and Prevention (Atlanta, GA).[36] Modifications to the HI method included a doubling of the reagent dilution volumes (25-50 µL) and a 2-hour incubation (second hour at 4 °C) instead of 30 minutes for agglutination pattern development. HI antibody titers are reported as the reciprocal of the highest serum dilution causing complete inhibition of chicken red blood cell agglutination. Subjects with a fourfold or greater increase in antibody titer to a vaccine component were considered responders.

Lymphocyte Proliferation

Venous blood was collected at the same time of day in two 10-mL ethylenediamine tetraacetic acid-coated tubes and shipped overnight on ice to the Medical College of Pennsylvania Hahnemann School of Medicine (Philadelphia, PA) to assess lymphocyte proliferation. Blood samples were received at approximately the same time of day, and immediately upon arrival, lymphocytes were separated, and influenza-induced proliferation to previously dialyzed

1999-2000 trivalent influenza virus vaccine at a 1:200 dilution in complete media was assessed using the same lot of vaccine as administered to subjects, as previously described.[9] Proliferation was expressed as net counts per minute (cpm).

Preliminary studies showed that the proliferative response of samples held overnight was not less than 80% of the response of samples run immediately (within 4 hours of blood draw).

Vitamin E Concentrations

Blood for determination of vitamin E concentrations was collected in serum SST-Gel Clot Activator Vacutainer Tubes. Serum was separated, frozen, and shipped on dry ice for analysis by Craft Technologies, Inc. (Wilson, NC). High-performance liquid chromatography was used to identify and quantitate α -tocopherol using modifications of methods.[37] Serum vitamin E concentrations were determined and compared by normalizing the concentration to the total lipid (from the clinical chemistries) in the serum, because vitamin E is a lipid-soluble nutrient.

Statistical Analysis

Sample size determinations were based on the days of infection as described in a previous study.[4] By extracting midpoints of line segments of that study's figure, the number of days of URTI was determined to be 48 days with a standard deviation (SD) of 17 (the previous study reported a SD of approximately 6). The current study was different in that subjects were followed for 6 months (during cold and influenza season), versus 12 months in the previous study. Using the more conservative variance estimation, a sample size of 48 (24/group) would have 80% power to detect a 30% difference in mean days of symptoms of infections using a two-group t test with a .05 two-sided significance level.

The days with symptoms of URTI and several other variables were not normally distributed or ordinal in nature; therefore, the Wilcoxon rank sum test was used for analysis. Categorical variables with more than two categories, such as ethnicity and reason for exit, were analyzed using the chi-square test. Categorical variables with only two categories, such as sex and questions with yes/no responses, were analyzed using Fisher exact test. Variables that approximated a normal distribution, such as age, height, weight, BMI, NHQ total score, clinical chemistries, hematology (except for platelets and differential), log of influenza antibody, and α -tocopherol, were analyzed using the two-sample t test at each time point.

Subjects completing the 183 days of study were classified as study completers. All enrolled subjects were evaluated in an intent-to-treat analysis. Results from this group were also evaluated at each time point with all available data. (Individuals who dropped from the study did not provide follow-up blood samples. Nor were URTI symptom data collected from the time the subjects stopped consuming the assigned formula.) Results were considered statistically different at the two-tailed 0.05 alpha level. Statistical analysis was performed with SAS statistical software (SAS Institute, Inc., Cary, NC).

Results

Sixty-six subjects were enrolled in the trial. Twenty-three of the 32 subjects in the control group and 25 of the 34 subjects in the experimental group were still participating in the study at the Day 57 blood draw. Eighteen subjects in the control group and 16 subjects in the experimental group completed the trial and were designated study completers. The number of subjects who dropped from the study was not different between groups. Formula-related reasons for withdrawing were as follows: taste (one control, one experimental), gastrointestinal cramping (one experimental), diarrhea (one control), flatulence (one control, one experimental), vomiting (one experimental), and weight gain (four experimental). Three subjects were withdrawn from the study because of a suspected food-drug interaction between the protein concentration in the control and experimental formulas and the anti-Parkinson medication carbidopa-levodopa.[38] One 95-year-old subject in the control group died of natural causes. Three

subjects were withdrawn after they relocated after a stroke (one control, one experimental) or fall (one experimental). Fourteen subjects asked to be discontinued from the study for unspecified reasons (eight control, six experimental). In addition, because of technical problems or the unavailability of a subject due to a family emergency (one experimental), not all laboratory data were available for all subjects at all time points.

Average BMI was not different in the experimental and control groups at baseline (Table 2) or over the course of the study for all subjects (intent-to-treat) or study completers. Additionally, mean weight change and mean change in BMI were not different between the experimental and control groups over the course of the study for the intent-to-treat subjects or study completers (data not shown). The most frequent reason for withdrawing from the study was unwanted weight gain. One subject in the experimental group asked to be taken off the study when a weight gain of 3.6 kg (5.5% above baseline weight) occurred over the first 8 weeks of supplementation. At one assisted living facility, the staff physician removed all his subjects from the study because of weight gain. These subjects, all in the experimental group, gained an average of 6.1 ± 1.2 kg ($8.6\% \pm 2.1\%$ above baseline) over the first 11 weeks.

There were no differences in age, number of subjects who resided in assisted living facilities, or sex between the control and experimental groups for study completers and intent-to-treat subjects (Table 2). Health status, serum albumin (Table 2), NHQ responses, lymphocyte count, and total lipids (data not shown) were not different between the experimental and control groups for study completers and intent-to-treat subjects at any time point. Twelve of the 34 study completers (35%) and 29 of the 66 (44%) intent-to-treat subjects took a multivitamin/mineral supplement before baseline; this was not different between study groups.

The α -tocopherol/total lipid ratio ($\times 1,000$) was 5.95 ± 0.35 , 5.43 ± 0.41 , and 4.09 ± 0.23 of study completers fed control formula and 5.27 ± 0.56 , 6.91 ± 0.43 , and 6.08 ± 0.33 of study completers fed experimental formula at baseline, postvaccination, and final blood draws, respectively. There were no differences in baseline α -tocopherol/lipid ratio or α -tocopherol concentrations between groups (data not shown) for the study completers or intent-to-treat subjects (data not shown). The subjects who were fed the experimental formula achieved a higher mean serum α -tocopherol concentration and α -tocopherol/lipid ratio at postvaccination (study completers) and final blood draws (study completers and intent-to-treat) than the subjects who were fed control formula ($P=.019$).

The median days with cold and influenza symptoms per subject who completed the study were 3 (range 0-69) for subjects fed the control formula and 0 (range 0-49, $P=.049$, Figure 1) for subjects fed the experimental formula. For intent-to-treat subjects, median days with symptoms per subject were 1 (range 0-69) in the control group and 0 (range 0-49, $P=.076$) in the experimental group. Of the study completers, 13 of 18 subjects fed the control formula recorded a total of 156 days of symptoms, whereas six of 16 subjects fed the experimental formula recorded a total of 78 days of symptoms ($P=.08$). For the intent-to-treat subjects, 16 of 29 subjects fed the control formula recorded a total 172 days of symptoms, whereas nine of 30 subjects fed the experimental formula recorded 115 days of symptoms ($P=.07$).

The number of new infection episodes in study completers included 17 fed the control formula and 13 fed the experimental formula ($P=.24$). Eight subjects accounted for 17 new infections in the control group, and four subjects accounted for 13 new infection episodes in the experimental group. Nine of the new infections in the experimental group were from a single subject who was diagnosed during the study with swallowing problems resulting in frequent aspiration.

Antibody titers to each of the three components of the influenza vaccine were measured at baseline, postvaccination, and final blood draws. The percentage of subjects with protective influenza antibody titers (≥ 40 HI units) at any time for any of the three vaccine components was not different between the control and experimental groups for study completers or intent-to-treat subjects. However, of all 66 participants, fewer subjects ($P=.010$) had a protective titer at baseline to the A/Beijing component of the vaccine ($n=31$) than to A/Sydney ($n=45$) or B/Yamanashi ($n=65$) (Figure 2).

Figure 2. Geometric mean antibody titer with 95% confidence limits shown as error bars to the three components of the influenza vaccine at baseline (Day 0), 42 days postvaccination (Day 57), and final (Day 183) blood draws for study completers in the control (white bars) and experimental groups (black bars), * $P=.052$ for experimental versus control for corresponding day.

For subjects completing the study, there was a trend toward greater geometric mean antibody titer in the experimental group than in the control group for A/Beijing at the postvaccination time point (Day 57) in study completers ($P=.052$) (Figure 2). For intent-to-treat subjects, there were no significant differences in geometric mean titers between the control and experimental groups for any of the three vaccine components (data not shown).

The percentage of subjects who responded to A/Beijing as indicated as a fourfold increase in antibody titer at the postvaccination time point was higher for the experimental than the control groups for study completers (13 of 15 or 87% vs 7 of 17 or 41%; $P=.012$) and intent-to-treat subjects (17 of 23 or 74% vs 7 of 21 or 33%; $P=.014$). No differences were observed between the experimental and control groups in percentage of subjects who responded to A/Sydney and B/Yamanashi postvaccination or to any of the vaccine components at the final blood draw for study completers or intent-to-treat subjects (data not shown).

Thirteen of 15 (87%) subjects in the experimental group of the study completers had an influenza antibody titer of 40 or greater and a fourfold increase in antibody titer at the postvaccination blood draw for A/Beijing. By contrast, six of 17 (35%) subjects in the control group showed similar changes ($P=.004$). Similarly, 17 of 23 (74%) subjects in the experimental group and six of 21 (29%) subjects in the control group in the intent-to-treat analysis had an influenza antibody titer of 40 or greater and a fourfold increase in antibody titer at the postvaccination blood draw for A/Beijing ($P=.005$). No differences in these combined antibody measurements were observed between the experimental and control groups for the other two vaccine components in study completers or intent-to-treat subjects (data not shown).

Lymphocyte proliferative responses to vaccine components were measured at baseline, postvaccination, and final blood draws. Proliferation was significantly greater in the experimental group than the control group at the postvaccination blood draw ($P=.013$) (Figure 3) but not at baseline or final blood draws for study completers and intent-to-treat subjects (data not shown).

Figure 3. Lymphocyte proliferation to vaccine components at the postvaccination blood draw. The median proliferative response (denoted by the line on each dot plot) was significantly greater in the experimental group than control group (study completers (* $P=.013$) represent 16 controls and 15 experimental subjects; intent-to-treat ($\dagger P=.022$) represents 20 control and 23 experimental subjects). Proliferation is expressed as net counts per minute (cpms) after lymphocytes were stimulated with the same lot of vaccine and pulsed with tritiated thymidine. Cpms were measured using a scintillation counter detecting the incorporated tritiated thymidine in lymphocytes.

Clinical chemistry and hematology measurements were determined at baseline and final blood draws. Mean clinical chemistry and hematology measurements for all groups and at all time points were within normal ranges, with the exception of total cholesterol. Study completers had a total cholesterol of 5.6 ± 0.3 mmol/L (218 ± 12 mg/dL) and 4.8 ± 0.2 mmol/L (186 ± 8 mg/dL) at baseline in the control and experimental groups, respectively ($P=.040$), with 5.5 ± 0.3 mmol/L (213 ± 13 mg/dL) and 4.9 ± 0.1 mmol/L (188 ± 6 mg/dL) at the final blood draw in the control and experimental groups, respectively ($P=.09$). Total cholesterol was not different between groups at either time point for intent-to-treat subjects.

Gastrointestinal symptoms were evaluated as a measure of oral tolerance. There were no differences in gastrointestinal intolerance between subjects drinking the control and experimental formulas.

Discussion

The purpose of this study was to determine whether supplementing the diet of relatively healthy independent- and assisted-living seniors with a formula containing nutrients known to enhance immune function could reduce days of symptoms of URTI during cold and influenza season. It was found that subjects consuming the experimental nutritional formula had significantly fewer days of symptoms of URTI, better antibody response to influenza A/Beijing (H1N1), and greater lymphocyte proliferative response to influenza vaccine components postimmunization than control subjects.

Seniors are a heterogeneous population with respect to nutrient needs; therefore, subjects were stratified before randomization based on age, sex, and NHQ scores.[32] Nutritional status was not different between groups based on baseline BMI and serum albumin (Table 2). Mean BMI and serum albumin indicated that subjects were normal to slightly overweight with adequate protein status (Table 2). Although median nutrition scores for the NHQ showed good nutritional status for subjects in the control and experimental groups, there were subjects who scored at high nutritional risk within each group.

It is not uncommon for apparently healthy elderly populations with normal body weight and protein nutriture to have micronutrient deficiencies that impair immune function.[6,8,39] In a population of healthy independent-living subjects aged 90 and older, one study showed that the prevalence of micronutrient deficiency was highest for selenium, vitamin B6, and zinc, followed by vitamin A, and to a lesser extent, folate, vitamin E, and vitamin B12.[39] Another study found that, in apparently healthy, independently living subjects aged 59 to 85, zinc deficiency was common, whereas deficiency in β -carotene, ascorbate, and α -tocopherol was less common.[6]

Nutrient supplementation in excess of the dietary reference intakes modulates immune function in seniors. When the diet of healthy seniors who had normal serum vitamin E concentrations was supplemented for 4 months with four times greater (60 mg/d), 13 times greater (200 mg/d), and 53 times greater (800 mg/day) vitamin E levels than the current recommended dietary allowance (RDA),[40] serum vitamin E concentrations were greater than with placebo, and delayed-type hypersensitivity and antibody titers to hepatitis B were enhanced.[7] Optimal enhancement of immune parameters was observed with 200 mg/d vitamin E.[7]

In the current study, to meet the diverse nutrient needs of an elderly population, an experimental nutritional formula containing 360 kcal/237 mL and 13 g of protein plus vitamins, minerals, antioxidants, fructo-oligosaccharides, and structured triacylglycerol was formulated. Vitamins and minerals (zinc, selenium, α -tocopherol, vitamin C, and B vitamins) with known immune-enhancing properties in seniors were added at concentrations exceeding the dietary reference intakes.[2,7,8,19,27,41] The experimental formula may have been of benefit to subjects for different reasons; for example, one subject might have benefited from the zinc, whereas another might have benefited from the elevated concentrations of vitamin E, selenium, or other nutrients included in the formula. Another benefit of the experimental formula could have come from the structured triacylglycerol, which may have increased the absorption of fat-soluble nutrients important for immune function.[30] The design of the study does not allow for the identification of individual components but demonstrates an immune benefit of the combination of vitamins, minerals, structured triacylglycerol, and protein.

Serum α -tocopherol concentrations were measured at baseline, postvaccination, and final blood draws as an indication of compliance. Many of the subjects recruited for the study reported frequently taking vitamin E-containing multivitamin and mineral supplements before the study. On study Day 0, subjects were asked to discontinue any supplements and were given the study supplement along with a low-potency multivitamin/mineral supplement to consume daily (Table 1). The lack of a washout period before baseline may explain the elevated α -tocopherol concentrations. Average baseline α -tocopherol concentrations ranged from 33 to 46 $\mu\text{mol/L}$ (1.4-2.0 mg/dL) and were greater than the baseline concentrations of 25 to 27 $\mu\text{mol/L}$ reported by others.[7] Rise in serum α -

tocopherol concentrations and self-reported formula intake by subjects in the experimental group suggest good compliance for study completers.

A possible limitation of this study is that nutrient deficiencies may have occurred in the control group because subjects were asked to discontinue dietary supplements and consumed the control formula in place of items in their diet. However, this is not likely, because all subjects were given a low-potency multivitamin/mineral tablet daily that provided more than one-third of the RDA or adequate intake for vitamins A, D, C, B6, and B12, thiamine, riboflavin, pantothenic acid, and copper.

Another potential limitation of this study is the high dropout rate. Previous studies in seniors that administered a single-nutrient or multivitamin/mineral supplement and examined immune function reported a dropout rate in the range of 11% to 30% for 4 months to 2 years.[6-8] Unlike these previous studies that provided the nutrients in pill form, the experimental and control formulas in the present study were provided in the form of an 8-ounce liquid drink containing energy and protein in addition to the added micronutrients. Fourteen of the 32 subjects were withdrawn or dropped from the study for formula-related issues such as gastrointestinal problems (n=7), suspected protein-drug interactions (n=3), and weight gain (n=4). These additional formula-related concerns are not a problem with a micronutrient pill.

Four subjects in the experimental group dropped out because of unwanted weight gain. In a frail elderly population in which unintentional weight loss and undernutrition are a common problem, a nutritional supplement that produced weight gain would be a benefit, but there may be concern that increased body weight may suppress immune function.[42] To this end, antibody response (influenza antibody titer ≥ 40 and \geq fourfold increase in influenza antibody titer) in the intent-to-treat subjects with a BMI of less than 25 was compared between groups. In the subjects with BMI of less than 25, 12 of 13 (92%) subjects consuming the experimental formula responded to influenza vaccination, but only four of 14 (29%) of those consuming the control formula responded ($P=.034$), indicating a benefit beyond providing energy and extra protein, which is a common practice in nursing homes. In the intent-to-treat subjects with a BMI of 25 or greater, five of 10 subjects (50%) in the experimental group and two of seven (29%) in the control group responded to the vaccine, indicating that even elderly people with BMIs of 25 or greater (low risk of malnutrition) received immunological benefit from the experimental formulation.

Higher postvaccination antibody titers for the A/Beijing component of the vaccine were only observed in subjects consuming the experimental formula. Subjects in this study appeared to have protective antibody titers to A/Sydney and B/Yamanashi at baseline, making it difficult to determine formula-related differences with these components (Figure 2). Published evidence has shown that higher baseline antibody titers correlate with smaller increases in anti-influenza type-specific responses after vaccination.[43] Therefore, it is likely that a low baseline A/Beijing antibody concentration facilitated its use as an immunological marker of immune function and an opportunity to evaluate the immunological benefit of an experimental nutritional formula. Similarly, investigators in one study fed a complete nutritional supplement or a noncaloric placebo drink to seniors for 6 months and then vaccinated the subjects against influenza.[44] Seniors consuming the supplement experienced a larger mean increase in antibodies against A/Sydney than the control group, whereas there was no difference for the other two components. Clinical outcomes were not measured in another study.[44] The current study used the response to vaccination as a marker of immune function, using the conventional measurement (a fourfold increase in antibody or a postvaccine titer of 40 or greater) and found a difference in antibody response for one component. A clinically meaningful difference between the experimental and control groups (reduction in the number of days of symptoms of URTI) was also found.

Several published reports have described the effect of nutrient intake on immune parameters, but few studies have related nutrient intake to changes in immune function and clinical outcomes in seniors. Noteworthy findings of this study included the enhancement of humoral and cell-mediated immune markers (greater antibody response to the A/Beijing component of the influenza vaccine, increased proliferative response to the entire influenza vaccine), together with a clinically relevant reduction in total days of URTI symptoms in study completers consuming the experimental formula. These data suggest a strengthening of the immune system of seniors fed the experimental formula. Because antibody titers and lymphocyte proliferation to influenza vaccine components were higher in subjects consuming the experimental formula, it is possible that the fewer days of URTI symptoms were due to influenza infections, but no attempt was made to identify symptom-causing agents, and influenza is only one of several pathogens capable of producing acute URTI symptoms noted in this study.[45]

In conclusion, this is one of the first studies to demonstrate a clinically meaningful benefit of a complete nutritional formula on the immune system of independent- and assisted-living seniors. In an elderly population, in which only 30% to 70% of those vaccinated are likely to be protected from hospitalization due to influenza, it may be possible to modulate immune function and ultimately reduce URTI symptoms with nutritional supplementation.

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Vitamin K1 Supplementation Retards Bone Loss in Postmenopausal Women Between 50 and 60 Years of Age

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"If co-administered with minerals and vitamin D, vitamin K1 may substantially contribute to reducing postmenopausal bone loss at the site of the femoral neck."

ARTICLE

Abstract Although several observational studies have demonstrated an association between vitamin K status and bone mineral density (BMD) in postmenopausal women, no placebo-controlled intervention trials of the effect of vitamin K1 supplementation on bone loss have been reported thus far. In the trial presented here we have investigated the potential complementary effect of vitamin K1 (1 mg/day) and a mineral + vitamin D supplement (8 µg/day) on postmenopausal bone loss. The design of our study was a randomized, double-blind, placebo-controlled intervention study; 181 healthy postmenopausal women between 50 and 60 years old were recruited, 155 of whom completed the study. During the 3-year treatment period, participants received a daily supplement containing either placebo, or calcium, magnesium, zinc, and vitamin D (MD group), or the same formulation with additional vitamin K1 (MDK group). The main outcome was the change in BMD of the femoral neck and lumbar spine after 3 years, as measured by DXA. The group receiving the supplement containing additional vitamin K1 showed reduced bone loss of the femoral neck: after 3 years the difference between the MDK and the placebo group was 1.7% (95% CI: 0.35–3.44) and that between the MDK and MD group was 1.3% (95% CI: 0.10–3.41). No significant differences were observed among the three groups with respect to change of BMD at the site of the lumbar spine. .

Keywords Vitamin K - Bone loss - Osteoporosis - Minerals - Vitamin D

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Creatine Monohydrate and Conjugated Linoleic Acid Improve Strength and Body Composition Following Resistance Exercise in Older Adults

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ESSENCE OF ARTICLE

"Together, this data confirms that supervised resistance exercise training is safe and effective for increasing strength in older adults and that a combination of CrM and CLA can enhance some of the beneficial effects of training over a six-month period. Trial Registration. ClinicalTrials.gov NCT00473902"

ARTICLE

Aging is associated with lower muscle mass and an increase in body fat. We examined whether creatine monohydrate (CrM) and conjugated linoleic acid (CLA) could enhance strength gains and improve body composition (i.e., increase fat-free mass (FFM); decrease body fat) following resistance exercise training in older adults (>65 y). Men (N=19) and women (N=20) completed six months of resistance exercise training with CrM (5g/d)+CLA (6g/d) or placebo with randomized, double blind, allocation. Outcomes included: strength and muscular endurance, functional tasks, body composition (DEXA scan), blood tests (lipids, liver function, CK, glucose, systemic inflammation markers (IL-6, C-reactive protein)), urinary markers of compliance (creatinine/creatinine), oxidative stress (8-OH-2dG, 8-isoP) and bone resorption (N-telopeptides). Exercise training improved all measurements of functional capacity (P<0.05) and strength (P<0.001), with greater improvement for the CrM+CLA group in most measurements of muscular endurance, isokinetic knee extension strength, FFM, and lower fat mass (P<0.05). Plasma creatinine (P<0.05), but not creatinine clearance, increased for CrM+CLA, with no changes in serum CK activity or liver function tests. Together, this data confirms that supervised resistance exercise training is safe and effective for increasing strength in older adults and that a combination of CrM and CLA can enhance some of the beneficial effects of training over a six-month period. Trial Registration. ClinicalTrials.gov NCT00473902

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Abstract Top

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Competing interests: Dr. Tarnopolsky has received an investigator initiated grant to evaluate the absorption characteristics of a new form of creatine (creatine ascorbate) as compared with creatine monohydrate from Avicena (2006). The current submitted study was completed in 2005 and Avicena did not contribute any money to the current study, but did supply product. Dr. Tarnopolsky has not received any personal money or sponsored talks for either Avicena or Pharmanutrients.

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