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INSULIN-RESISTANCE, DIABETES AND RISK OF CANCER: WHICH IS THE LINK?

Cascio Maria[1], Ferrara Roberta[1], Martina Maria[1], Cereda Emanuele[1,2]

[1]Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy
[2]Nutrition and Dietetics Service, Fondazione IRCCS Policlinico San Matteo, Viale Golgi 19, 27100 Pavia, Italy

Email address: nutriketo@unisa.it

Abstract

The association between diabetes and cancer is very complex due to the heterogeneity of the two diseases and because there are multiple factors by which one pathology can interfere on the other. The increase of risk ranges from 20%, as for the breast cancer, up to 250% for liver and endometrium carcinoma, with a considerable association. A further confirmation of this variability is the absence of a clear association with other types of neoplasia as, for example, tumors of the respiratory system or even reduction of risk for prostate cancer. Factors and mechanisms responsible for onset/progression of the tumor in diabetic subjects are not known completely although some hypotheses have been discussed. Nevertheless, the maintenance of good glycemic compensation and the correction of other risk factors for cancer such as obesity, dyslipidemia, and chronic inflammation are an advisable strategy for all diabetic patients.

Keywords: *diabete mellitus, cancer, obesity, insulin resistance, hyperglycemia*

Introduction

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia resulting from defects in insulin secretion and production or an altered function of insulin. It includes a multiplicity of etiological and pathogenic conditions that lead to consider it as a syndrome, in which different genetic and environmental causes and different molecular and cellular mechanisms converge in a defective use of glucose that accumulates in the blood. Diabetes has been associated with a higher incidence of some types of cancers and an increased mortality from cancer. This association has considerable variability. Diabetes usually determines a greater risk of site-specific malignancies (with a risk ranging from +20% [breast cancer] to +250% [liver and endometrium carcinoma]), but there are also neoplasms in which the risk does not change or even reduces, albeit slightly, as in the case of prostate carcinoma. Regardless of the relationship between diabetes and the risk of neoplasia, there is also a negative effect of diabetic disease on tumor prognosis (1-3). A recent meta-analysis of 21 studies calculated a possible 40% increase in the risk of mortality from cancer in individuals with pre-existing diabetic disease (4). The deleterious effect of pre-existing DM on survival continues to be validated across multiple cohorts and across the entire spectrum of cancer types (5,6) and appears to be potentially correlated with the degree of hyperglycemia itself (7). Diabetes can facilitate the onset of neoplasms or, as is more likely, in diabetic patients it may be easier the progression to clinically evident cancers of precancerous lesions and in situ neoplasms, with the help of other environmental factors.

Methods

The association between diabetes and risk for cancer development

Several cohort or case-control studies have shown that subjects with DM have an increased risk of contracting certain types of tumors, such as liver (13), pancreas (14), kidney (15), endometrium (16), colorectal, bladder, breast and Hodgking's lymphoma. The strongest association between DM and specific site tumor is cancer of the liver and pancreas. The most frequent liver disease and the higher prevalence of hepatitis in diabetic patients means that they are more prone to developing cirrhosis, a condition certainly related to liver cancer. The relationship between DM and pancreatic cancer is very difficult to prove because it is difficult to determine which condition starts first between cancer and hyperglycemia. Regarding pancreatic cancer, it is obviously necessary to distinguish the patient with pre-existing diabetes in whom pancreatic cancer appears, compared to diabetes diagnosed in a patient because of pancreatic cancer (also unknown or very small). Pancreatic cancer, in fact, can cause diabetes not only for the mass destruction of pancreatic islets, but also for the local production of cytokines and toxic factors that prevent an adequate functioning of the pancreas and inhibit the secretion of insulin. For this reason, the appearance of diabetes at the age of 50 years old or more, in a non-obese and unfamiliar for diabetes, should always lead to a morphological evaluation of the pancreas. However, epidemiological studies limited to the only condition of pre-existing diabetes (diabetes already present for 3-5 years at the time of pancreatic cancer diagnosis) confirm that diabetes is a risk factor for pancreatic cancer. More in-depth research is needed to clarify the mechanism and implications of this correlation. Another tumor frequently associated with DM is breast cancer, the most common cause of cancer death in European women. Approximately one out of five women with breast cancer is suffering from DM and probably their coexistence may be explained by similar risk factors such as obesity, dyslipidemia and hyperinsulinemia, although the mechanism underlying this articulated relationship needs to be clarified. Several longitudinal and retrospective cohort studies show that the frequency of breast cancer is significantly increased in post-menopausal women with DM. Furthermore, they reveal that the prognosis of women with breast cancer and DM is worse, and overall mortality is greater, than in non-diabetic patients. There are other tumors related to diabetes, although in some cases studies are not so numerous or conducted with a strong design. Particularly, the most recent evidence points to an increased incidence of cancer and mortality from endometrial, colorectal and bladder carcinoma.

Nevertheless, there are neoplasms for which there is no correlation with DM, such as lung cancer, and even a neoplasm for which a negative association has been observed, such as prostate cancer, whose risk

appears to be modestly reduced. Perhaps, the lower frequency of cigarette smoking in diabetics has a beneficial effect on lung cancer, while the reduced frequency of prostate cancer could be explained by both reduced testosterone levels - a common finding in insulin-resistant subjects (3) - and the HNF1B gene polymorphism – conferring a negative and protective predisposition o type 2 diabetes and prostate cancer, respectively (4).

Pathogenic mechanisms

Most studies about the association between diabetes and cancer, concern type-2 diabetes and in any case, without an appropriate distinction between the two forms of diabetes, which is often based on surrogate indicators, such as young age or insulin treatment. However, since patients with type-2 diabetes account for approximately 90% of all diabetics and that cancer is primarily a disease of the older population, it is reasonable to assume that the studies mainly focused on type-2 diabetes. An important role in promoting initiation and/or progression of tumors in the diabetic patient is played by hyperglycemia, insulin resistance and consequent hyperinsulinemia, obesity, oxidative stress and the consequent chronic inflammation that are present individually or simultaneously in the diabetic pathology. Finally, a role could be also played by some antidiabetic drugs. With this background, there may be positive influences on the risk of onset/progression of neoplasms in the diabetic patient.

Hyperglycemia

The increase in circulating glucose concentrations could promote the growth of the neoplastic cell. Several mechanisms seem to be involved in this process. According to Warburg's hypothesis, the neoplastic cell is largely dependent on the energy generated by the glycolytic metabolism as also suggested by greater insulin-independent use of glucose. The incomplete use of glucose in aerobic glycolysis is not a very efficient process because it produces a smaller quantity of ATP compared to the ATP produced with the Krebs cycle. However, aerobic glycolysis saves the neoplastic cell lipids use for cell membranes and nucleotides for DNA synthesis. Neoplastic cells are so advantaged. However, this metabolic choice determines a reduced capacity of the neoplastic cell to adapt to micro-environmental stringent conditions and increases the susceptibility to the conditions of nutrient deficiency. So the superabundance of nutrients can promote cell proliferation, in reverse the conditions that lower blood sugar or activate AMP-activated kinase activity (AMPK), energy sensor, like the metformin, are effective in reducing the incidence of tumors. In addition to a metabolic effect, hyperglycemia is able to modulate the transcription of intracellular signals such as the thioredoxin-interacting protein (TXNIP) able to increase the synthesis of reactive oxygen species and therefore, generate potential damage at the DNA level.

Obesity

Over 80% of patients with type 2 diabetes are obese. The mechanisms proposed to explain how the expansion of the adipose tissue may promote the development of the neoplasia are numerous. The excess of adiposity is typically associated with a marked activation of the inflammatory response and a high release of adipokines. Some preclinical evidences indicated that leptin, a secreted cytokine from adipocytes, overexpressed in obese subjects, promotes cells proliferation. In obese subjects, moreover, the release of free fatty acids increases with worsening of insulin sensitivity, concomitant hyperinsulinemia and increase of oxidative stress. The increase in adipose tissue is also associated with a greater expression and activity of the aromatases resulting in an increased conversion of androgens into estrogens. Finally, an indirect role of dietary pattern – with Mediterranean diet being a protective factor – should be also taken into account due to its association with body weight excess and increased adiposity.

Obesity and cancer have VEGF in common

Obese and cancer patients have in common the presence in the blood of high levels of the endothelial growth factor VEGF; It is produced by tissues under hypoxic conditions (absence of oxygen). The adipose and the inflamed tissue such as the tumoral one are constantly found in hypoxia. It stimulates cells to produce and release in the blood VEGF which stimulates the cells of vessels to form new capillaries. An

obese person has the adipose tissue constantly in an oxygen deficiency which reacts by releasing large amounts of VEGF into the blood, stimulating the formation of new blood vessels in the adipose organ and tissues, including tumors. VEGF is in fact a growth promoter, that is a mitogen. Being obese carries a greater risk of developing cancer because in the blood of an obese circulate higher levels of pro-inflammatory cytokines and growth factors such as VEGF, which in case of a tumor can accelerate its development. Dr. Wan Iei-Gun subjected a group of obese mice and a group of mice with cancer to a pharmacological treatment with a VEGF inhibitor being able to reduce both the volume of adipose mass in obese mice and the volume of tumor mass in mice with tumor so in order to prevent both cancer and obesity it is good to reduce the growth factor VEGF in the blood.

Chronic inflammation and oxidative stress

The metabolic abnormalities that characterize diabetic patients, especially under conditions of poor metabolic control, increase the oxidative stress and sustain a chronic inflammatory background. This condition - which can last for years and decades - reduces the intracellular antioxidants capabilities, predisposing susceptible cells to malignant transformation. Indeed, higher concentrations of reactive oxygen species (ROS) can bring to DNA damage both through direct oxidation and interference with the mechanisms of DNA repair. ROS can also react with proteins and lipids, forming derivative products, which can alter intracellular homeostasis favoring the accumulation of mutations which, in turn, can contribute to the carcinogenic process. An additional factor related to obesity and insulin resistance is the proinflammatory cytokine TNF (tumor necrosis factor) released by the adipose tissue. TNF induces the development and progression of many tumors through the activation of NFkB (nuclear factor-kappa B) which mediates many of the photomurals effects of TNF.

Insulin and insulin resistance

Initially it was thought that the mitogenic effect of insulin was due to its interaction with the IGF-1 receptor (insulin-like growth factor 1 receptor, IGF-1R), a powerful growth factor with transforming and anti-apoptotic actions. The insulin receptor (IR) and the IGF-1 receptor (IGF-1R) are homologues and share many intracellular signalling pathways. Insulin, however, has a reduced affinity for IGF-1R and only at high concentrations, as it can be expected in the conditions of severe insulin resistance, may be able to activate this receptor too. Insulin, by binding to its membrane receptor, exerts metabolic and mitogenic actions by activating two main intracellular pathways: the predominantly metabolic pathways mediated by phospho-inositol-kinase 3 (PI3-K) and the predominantly mitogenic pathway, mediated by MAP-kinase (MAPK). Insulin resistance is present in many clinical conditions, including type 2 diabetes and obesity, but while these conditions are characterized by a selective defect of the metabolic pathway, the mitogenic one is substantially preserved. Therefore, compensatory hyperinsulinemia occurring with insulin resistance can be responsible for an activation of the mitogenic pathway, favoring cell growth and, potentially, cancer development.

Discussion

In conclusion, literature supports that the diabetic patient has an increased risk of cancer. Factors and mechanisms responsible for onset/progression of the tumor in diabetic subjects are not completely known although some hypotheses have been discussed. Nevertheless, the maintenance of good glycemic compensation and the correction of other risk factors for cancer such as obesity, dyslipidemia, and chronic inflammation are an advisable strategy for all diabetic patients. With this perspective, some dietary approaches (e.g. the ketogenic diet) and pharmacological treatments could have a therapeutic potential as they enable to lower blood sugar level and insulinemia, to improve insulin sensitivity, (8,9,10) chronic inflammation (11,12) and to reduce body weight which, in turn, is a valuable preventive strategy as well.

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ROLE OF HYDROLYZED COLLAGEN IN KETOGENIC DIET AND EFFECTS OF COLLAGEN ON BONE HEALTH AND SKIN AGEING

De Meo Paola, Cornacchia Amelia, Abbate Loredana, Lo Vuolo Marco, Paola Molettieri, Giuseppe Castaldo

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

This review deals with the role of collagen hydrolysate in the treatment of osteoarthritis and aesthetic flaws. Methods: Review of past and current literature on the metabolism of collagen hydrolysate and evaluation of clinical investigations on therapeutic studies in osteoarthritis and cellulite. Hydrolyzed collagen showed interest as a therapeutic agent of potential utility in the treatment of osteoarthritis. Its high level of safety makes it attractive as an agent for long-term use in these chronic disorders.

Keywords: *collagen hydrolysate, osteoarthritis, cellulite*

Introduction

Collagen is the most plentiful fibrillary protein of the body that conforms the conjunctive and connective tissues in the human body, essentially skin, joints, and bones. It holds all the living tissues together and ensures the integrity, elasticity, regeneration of the skin, cartilages and bones and maintenance of tonicity of the skin thanks to the serum proteins, the hydroxyproline, cystine and silicon amino acids. Collagen accounts for 80% of our connective tissues and 30% of proteins in our body. There are 28 different types of collagen in the literature depending on the tissue in which it is found: type I is more expressed in the skin, tendons and bones; cartilage is rich in type II. What changes is only the three-dimensional organization that collagen takes in space, influenced by the "cross-linking" induced by a specific amino acid: hydroxyproline. This is the reason why we find it elastic in the skin, hard and consistent in nails, filamentous and flexible in the fibrils of the bone. The human organism does not absorb collagen as such, but it is digested through the action of the gastric enzymes, in the constituent components, represented by more or less long chains of amino acids; it is superfluous for exogenous collagen, to speak of "different types", since the protein must necessarily be decomposed into fragments in order to "enter" the body and thus performs its precious physiological properties: the variation of the amino acid sequence of collagen, whatever it is, does not reflect changes in its physiological properties, but only a different conformational orientation in space. The daily integration of collagen should amount to about 1 gram per 10 kg of body mass (1).

The main types of collagen used to formulate food supplements are bovine and marine.Bovine collagen**:** industrially made from various animal tissues such as skin and bone.

Potential risks related to taking collagen supplements

Considering that collagen is obtained from carcasses and waste from animal slaughtering, there is concerns about the possibility that gelatine acts as a carrier for the transmission of TSE-BSE (transmissible spongiform encephalopathies, among which mad cow disease stands out). In theory, the processes of heating, filtration and alkalization of animal remains should be effective in eliminating, or at least reducing, the levels of infectious agents (prions) that transmit TSEs. Moreover, the law that regulates the production of supplements based on this protein provides for the execution of a series of strict controls, precisely in order to protect the health of the final consumer. Despite this, most producers (both food supplements and cosmetic products) prefer to include marine collagen (collagen of fish origin) or plant collagen (natto gum) in their formulations (2).

Marine collagen: obtained from the skin of fish and, unlike the animal, is characterized by permeability to sweat combined with resistance to aggression and spreadability on large and irregular surfaces, for this reason it is used for topical skin use.

Plant collagen: there are no sources of vegetable derived jelly, with absolutely no chemical correlation between collagen and other products mentioned as "vegetable jellies", extracted from algae and gums. Natto gum is a plant collagen obtained from soybeans through a particular fermentation method of the bacillus natto. The main component of phytocollagen is a polymer of glutamic acid (γ-pga).

Vitamin C: essential for the formation of collagen, it helps to maintain the integrity of substances of mesenchymal origin (connective tissue, osteoid tissue and dentin). It is a potent reducing agent which in the body is oxidized and reduced, functioning as a cellular redox system. In the absence of vitamin c, cells can not hydrogenate proline in y positions, resulting in an instability of the collagen leading to: scurvy.

Silicon is a structural element of connective tissue and enters the constitution of the main macromolecules such as elastin, collagen, proteoglycans and glycoproteins, promoting their regeneration. The silicon content is closely related to the optimal skin conditions, such as the rate of hydration, elasticity, the absence of wrinkles and expression lines, the ability to heal and regenerate. It also acts as a metabolic protector, acting at different levels: it opposes the lipid peroxidation responsible for the liberation of free radicals, it counteracts the cross-linking and non-enzymatic glycosylation of the connective tissue proteins that cause stiffness and sclerosis, it regulates and stimulates the mitosis of fibroblasts and for this property, it plays an essential role in the process of dermal and epidermal cells regeneration, and lastly it

is a co-factor of the elastin synthesis . Collagen synthesis is silicon-dependent, which influences the hydroxylation process of proline in hydroxy-proline.

It has been established that the collagen fibers are damaged over time: at the age of 25 cells begin to lose the ability to synthesize collagen, whose production decreases by 1.5% per year. This process is accentuated over 45 years old and when approaching 60, the production of collagen has decreased by over 35%, losing thickness and strength. In addition, there are other factors that intensify the loss of collagen: overuse (intense physical activity), trauma, menopause, overweight, hormonal therapies, incorrect diets, oncologicaltreatments, intense and continuous exposure to sunlight or to particular environmental conditions (pollutants -smoke -stress).

Collagen is an inert protein, but characterized by a continuous turnover. The aminoacidic-protein integration during the ketogenic diet and the integrated dissociated diet, besides the integration with the silicon contained in the horsetail, allow to respect this turnover and therefore to protect the skin. Collagen, which accounts for 70% of the dry weight of the dermis and which is produced by fibroblasts, under physiological conditions, is constantly renewed. In the course of ketogenic diet and integrated dissociated diet, the constant supply of amino acids allows the continuous turnover of the collagen, ensuring the maintenance and improvement of dermis and hypodermis tone.

Dietary supplementation with specific collagen peptides has a body mass index-dependent beneficial effect on cellulite morphology

A clinical trial has been investigating the efficacy of specific bioactive collagen peptides (bcp) on the cellulite treatment of normal and overweight women. In total, 105 women aged 24-50 years old with moderate cellulite were randomized to orally receive a daily dosage of 2.5 g bcp or a placebo over 6 months. The degree of cellulite was evaluated before starting the treatment and after 3 and 6 months of intake. In addition, skin waviness, dermal density, and the length of subcutaneous borderline were assessed. Bcp treatment led to a statistically significant decrease in the degree of cellulite and a reduced skin waviness on thighs *($p < 0.05$)* in normal weight women. Moreover, dermal density was significantly improved ($p < 0.05$) compared to placebo. The subcutaneous borderline showed a significant shortening after bcp intake compared to the beginning of the study, indicating cellulite improvement. The efficacy of bcp treatment was also confirmed in overweight women, although the impact was less pronounced in comparison with women of normal body weight. The results of the study demonstrated that a regular ingestion of bcp over a period of 6 months led to a clear improvement of the skin appearance in women suffering from moderate cellulite. Based on the current data, it can be concluded that a long-term therapy with orally administered bcp leads to an improvement of cellulite and has a positive impact on skin health (3-4). (Fig.1-2)

Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women-a randomized controlled study.

Investigations in rodents as well as in vitro experiments have suggested an anabolic influence of specific collagen peptides (scp) on bone formation and bone mineral density (bmd) (1). The goal of the study was to investigate the effect of 12-month daily oral administration of 5 g scp vs. Placebo (cg: control group) on bmd in postmenopausal women with primary, age-related reduction in bmd. Methods: 131 women were enrolled in this randomized, placebo-controlled double-blinded investigation. The primary endpoint was the change in bmd of the femoral neck and the spine after 12 months. In addition, plasma levels of bone markers-amino-terminal propeptide of type I collagen (p1np) and c-telopeptide of type I collagen (ctx 1)-were analysed. Results: a total of 102 women completed the study, but all subjects were included in the intention-to-treat (itt) analysis (age 64.3 ± 7.2 years; body mass index, bmi 23.6 ± 3.6 kg/m^2; t-score spine -2.4 ± 0.6; t-score femoral neck -1.4 ± 0.5). In the scp group (n = 66), bmd of the spine and of the femoral neck increased significantly compared to the control group (n = 65) (t-score spine: scp +0.1 ± 0.26; cg -0.03 ± 0.18; ancova p = 0.030; t-score femoral neck: scp +0.09 ± 0.24; cg -0.01 ± 0.19; ancova p = 0.003). P1np increased significantly in the scp group (p = 0.007), whereas ctx 1 increased significantly in the control group (p = 0.011). These data demonstrate that the intake of scp increased bmd in postmenopausal women with primary, age-related reduc-

tion of bmd. In addition, scp supplementation was associated with a favorable shift in bone markers, indicating increased bone formation and reduced bone degradation (5).

Skin aging is a progressive and degenerative process caused by a decrease in the physiological functions of the skin tissue. In addition, environmental factors as well as concomitant diseases and lifestyle (nutrition, sedentary lifestyle, smoking, etc) negatively impact the aging process. An association between oral administration of collagen peptides combined with vitamin c and extracts of *Hibiscus sabdariffa* and *Aristotelia chilensis* (delphynol®) (eximiafirmalize age complex®) on dermal thickness was studied and the improvement in aging signs was evaluated (6).

Female adult patients received an oral nutritional supplement containing collagen peptides, vitamin C, H. *sabdariffa*, and *A. chilensis* (delphynol) in a sachet and were instructed to consume 1 sachet diluted in 200 ml of water once daily for 12 weeks. They were evaluated clinically, by high frequency ultrasound and cutometry.

There was a significant improvement of firmness and elasticity and an increase in dermal thickness by ultrasound after 3 months of use. (Fig.3 *Notes:* Observe the measures in triplicate in each image.)

The effects of collagen peptides on body composition and muscular power output have not been investigated previously. Thus far, studies have mainly focused on the effects of collagen peptides on skin health and degenerative joint diseases such as osteoarthritis. The impact on body composition has not been in the focus, as it is generally believed that the relatively low biological value of collagen would not favour a significant improvement on muscular net protein synthesis.

In a recent study, Zdzieblik et al (7) have investigated the effect of post-exercise protein supplementation with collagen peptides on muscle mass and muscle function during a 3-month resistance training programme.

In this study, 60 healthy subjects (age>65 years), who experienced a considerable loss in muscular strength or physical performance within the last 3–4 years, were enrolled. Subjects needed to be able to participate in the 3-month resistance training and to be free of acute diseases or illness-related cachexia.

The participants of the study were randomly assigned to the treatment group (TG) (collagen peptide supplementation) or to the placebo group (PG).The primary outcome measure was the change in FFM before and after the intervention, which lasted for 12 weeks.

The subjects assigned to the TG (n 30) were given 15 g of collagen peptides/d. The test product with a mean molecular weight of approximately 3kDa is derived from a complex multi-step procedure by the degradation of type I collagen. Subjects in the PG (n 30) received silicon dioxide. Collagen peptides as well as placebo were given in powder form and were dissolved by the participants in 250 ml water.

The resistance training consisted of a 12-week guided training programme on fitness devices (pull down, leg press, bench press, back press, etc.). Subjects took part in the resistance training programme in the afternoon three times a week over a time period of 60 min.

Body composition was measured before and after the 3-month training period using DXA (Stratos DR 2D Fan Beam; Degen Medizintechnik). Muscular strength was tested by measuring isokinetic quadriceps strength of the right leg before and after the training programme (Con-Trex) and sensory motor control (SMC) was determined using a standardised one-leg stabilisation test (Posturomed; Haider-Bioswing).

In both groups, a statistically significant ($P<0 \cdot 001$) increase in FFM and a significant loss in fat mass (FM) ($P<0 \cdot 001$) could be observed after 3 months (Fig. 4). Moreover, muscle strength and SMC improved significantly ($P<0 \cdot 001$) in both groups. Moreover, data for bone mass (BM) revealed a statistically significant ($P<0 \cdot 001$) increase in both groups at the end of the study. (Fig.4)

In both groups, the loss of FM correlated with an increase in FFM; in the collagen-supplemented group, the correlation coefficient (r 0·72; $P<0·001$) was more pronounced than in the control group (r 0·55; $P<0·003$) (Fig 5-6).

The main finding of this study is that collagen peptides further increased the benefits of the 3-month

resistance training in older subjects with sarcopenia. Compared with placebo, subjects in the collagen-supplemented group showed a higher increase in FFM and muscle strength as well as a higher reduction in FM.

An explanation for the observed effects could be that collagen is rich in arginine and glycine, both known to be important substrates for the synthesis of creatine in the human body. Creatine supplementation has been shown to improve both muscle mass and muscular function in some but not all studies (8).

In addition, collagen peptides have shown to positively influence microcirculation that might cause an additional beneficial effect in promoting muscle growth compared with other protein sources.

In conclusion, the study has demonstrated that the combination of resistance exercise and collagen peptide supplementation resulted in a more pronounced improvement of body composition, as indicated by a significant increase in muscle mass and decrease in FM, compared with placebo. In addition, muscular strength was significantly improved after collagen peptide intake compared with the training programme plus placebo.

Rapid muscle loss, cachexia (literally, 'poverty of flesh'), is a serious complication to most cancer diseases and is a significant contributor to mortality and disability associated with cancers. Furthermore, loss of muscle mass may also manifest as a consequence of treatment, for example, radiotherapy or chemotherapy.

Biomarkers are another way to assess muscle mass. An important study has shown that the serum levels of the Collagen type III propeptide correlate well with whole body lean mass. As do, the circulating levels of Collagen type VI peptides containing the IC6 epitope.

Nedergaard et al. (9) showed that the anabolic response to reloading the following immobilization was inversely related to the levels of the matrix-metalloproteinase-generated Collagen type VI fragment C6M.Both Collagen types III and VI are known to be important constituents of the extracellular matrix of skeletal muscle. Therefore, fragments produced during muscle tissue turnover may be correlated with lean body mass. They studied blood samples and DEXA-data from 41 patients with head and neck cancer as part of the Danish Head and Neck Cancer Group (DAHANCA). They studied the collagen fragment serum markers Collagen type III propeptide (ProC3), Collagen type VI peptides containing the IC6 epitope, and Collagen type VI fragment C6M but were unable to show any correlation between biomarkers and lean body mass. It appears that these biomarkers, which worked as biomarkers of lean body mass in healthy individuals of both genders, do not work in the cancer patients investigated in this study. However, they did find that the biomarkers IC6, IC6/C6M, and ProC3 are biomarkers of LBM in the control group subjects (R^2/P of 0.249/0.035, 0.416/0.007 and 0.178 and P = 0.057, respectively).

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Figure 1: Skin surface profile of tight skin before and after 3 and 6 months of oral supplementation with bioactive collagen peptide (BCP) or placebo, measured by PRIMOS® Pico. Skin waviness of thigh was statistically significantly decreased ($P < 0.05$) after 6 months of BCP daily intake in the overall study subjects in comparison with placebo treatment (mean ± standard error of mean, n.s. not statistically significant)

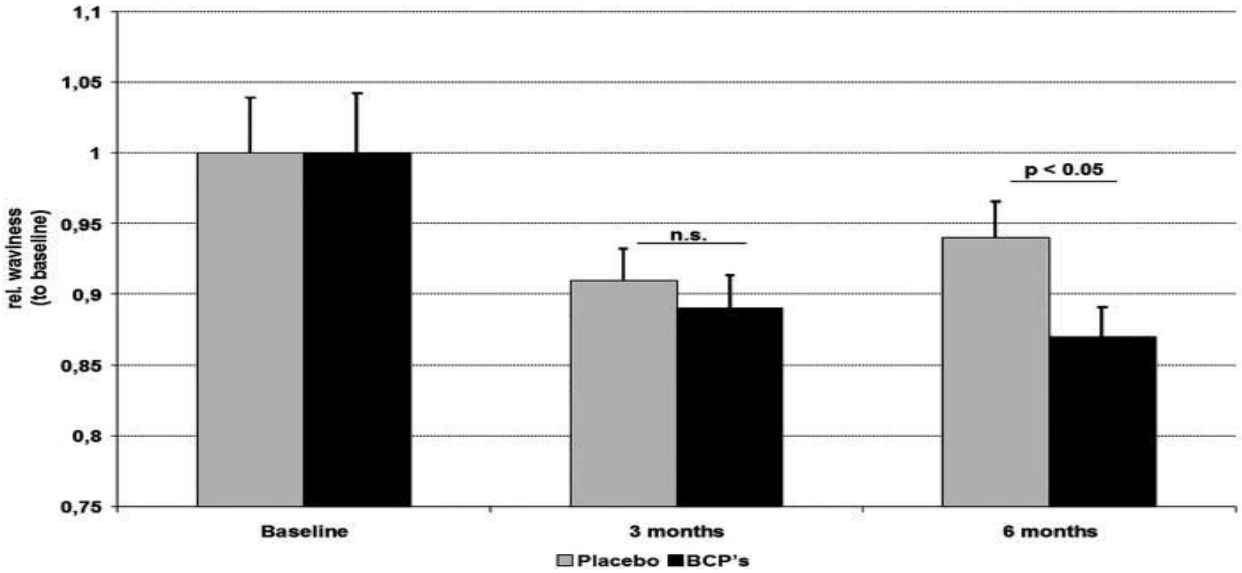


Figure 2: Representative 3D color-coded height images of tight skin surface, before (t0) and after 6 months (t6) of oral supplementation with BCP or placebo; measured by PRIMOS Pico. Each color is related to a height with *red* and *yellow* areas, indicating the skin depression and relief as for skin surface, respectively. At the external margins of the measuring fields, the resolution is limited seen by lower intensity in *green* and *blue*. **(A)** Placebo group. **(B)** BCP group. A notable improvement in skin waviness can be seen in the BCP group, showing more homogeneous skin surfaces.

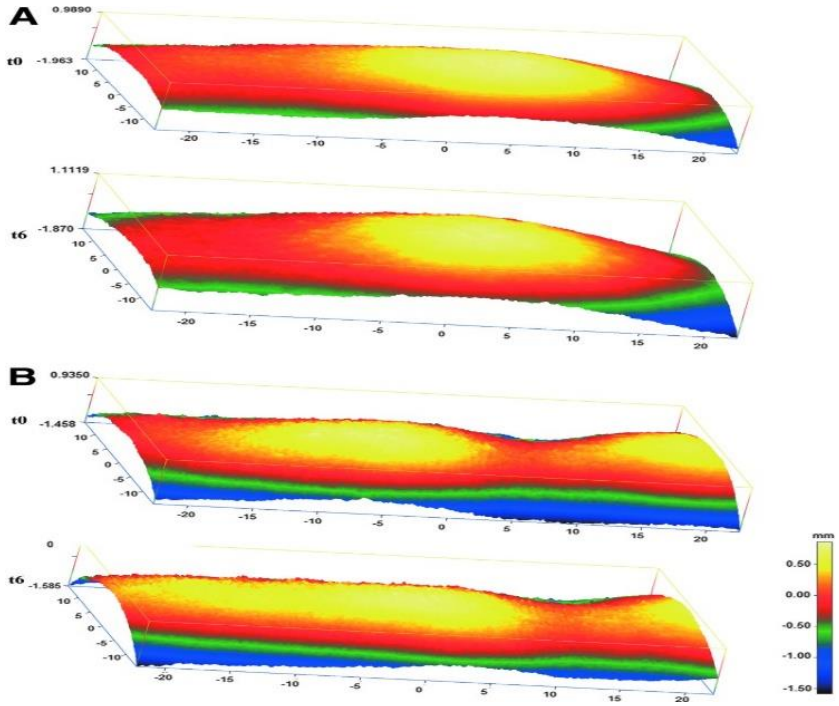


Figure 3: The association of collagen peptides, vitamin C, *H. sabdariffa* and *A. chilensis* (delphynol) could improve the signs of dermal skin aging.

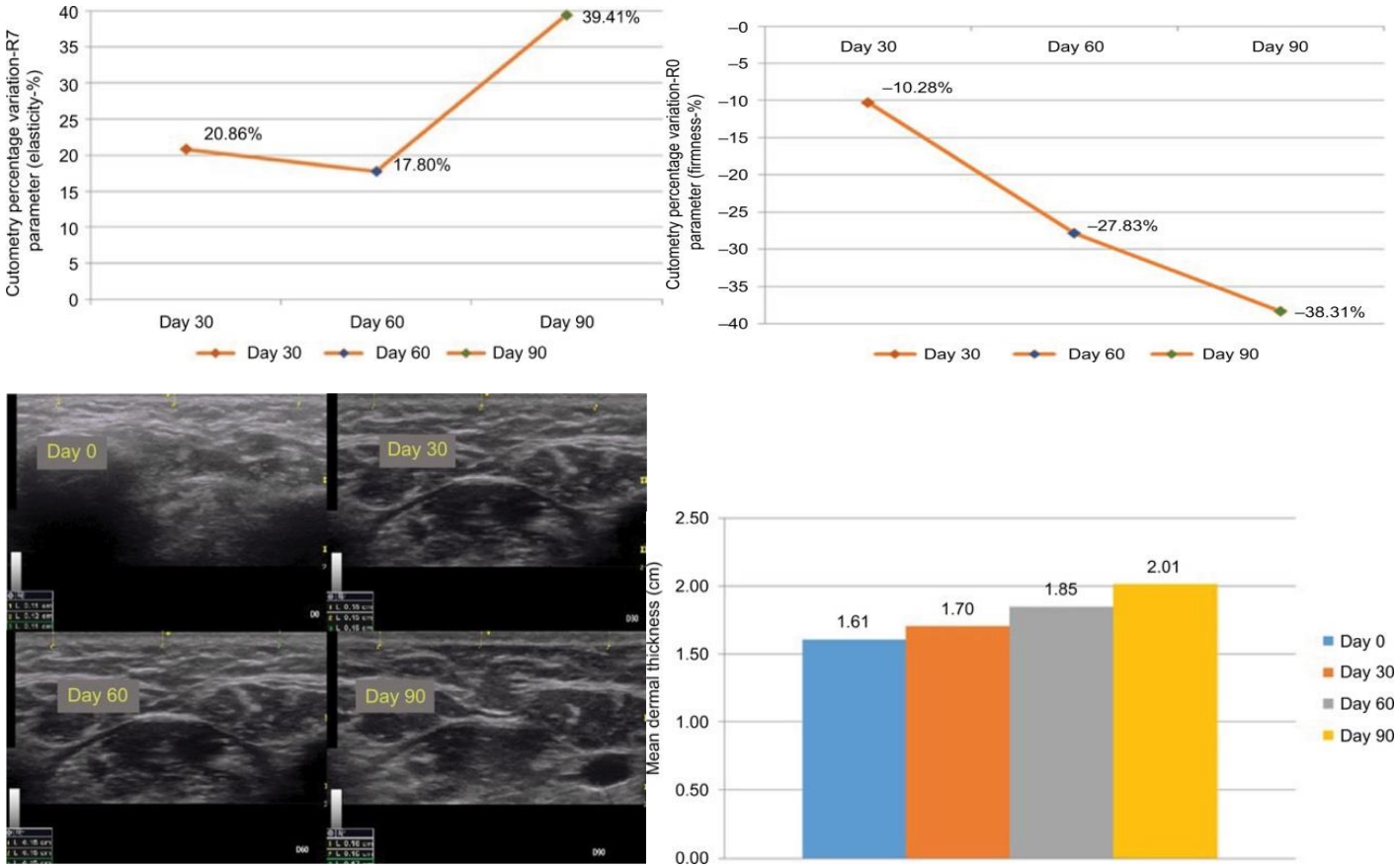
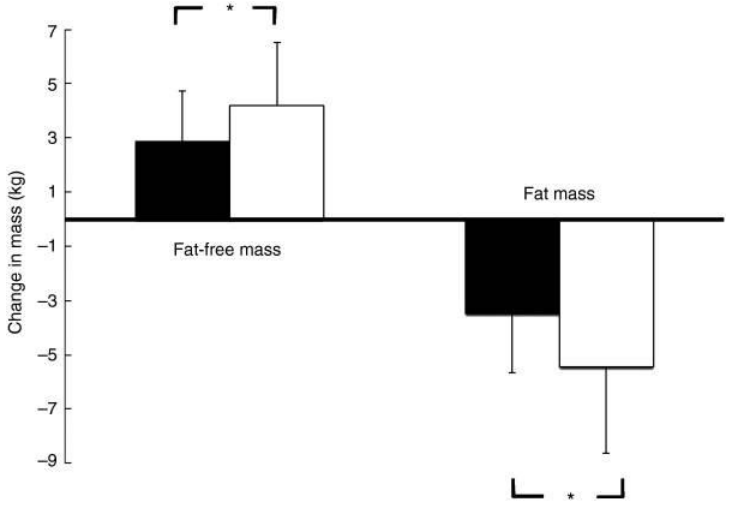


Figure 4: Change in fat-free mass and fat mass after 12 weeks of resistance training in elderly men (age>65 years) with collagen peptide supplementation or placebo. Values are means, with their standard errors represented by vertical bars. Significance was tested by ANOVA considering time × treatment interactions. * Mean value was significantly different from the placebo group one (P<0.05).



Black: Placebo group **White:** Treatment group

Figures 5: Correlation (Pearson's r) between fat-free mass and fat mass changes after 12 weeks of resistance training in elderly men in combination with a daily dosage of 15 g collagen peptides.

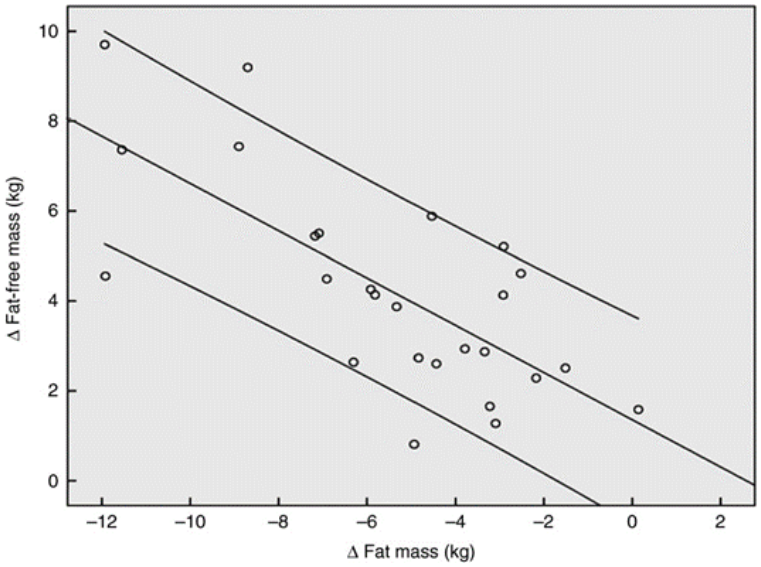
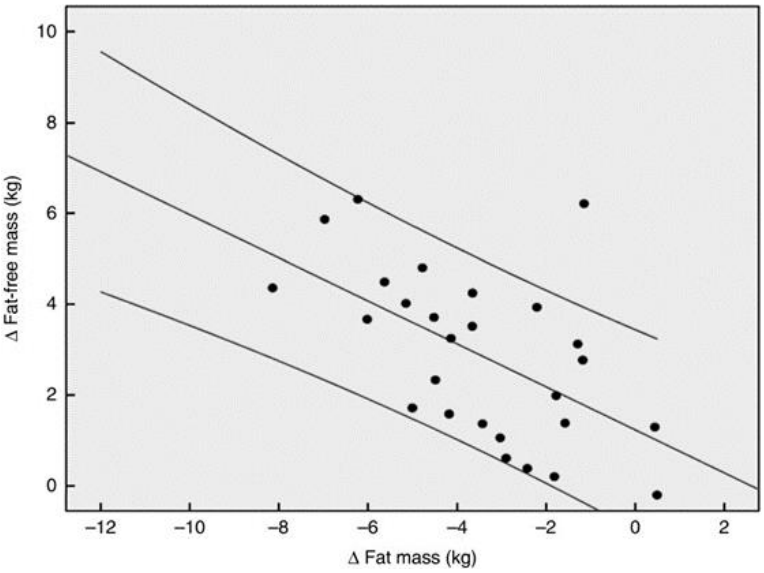


Figure 6: Correlation (Pearson's r) between fat-free mass and fat mass changes after a 12 weeks of resistance training in the placebo group.



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THE KETOGENIC DIET AS A NEW THERAPEUTIC OPTION IN THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE

Alizzi Silvia, Perricone Marco, Proto Maria Giuseppina, Mento Consolato, Sorrentino Paolo

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

Non-alcoholic fatty liver disease (NAFLD) has been identified as one of the most prevalent chronic liver disease in adults and children populations. NAFLD is usually associated with the metabolic syndrome (MS) which is chiefly related to insulin resistance and its consequences. Very few studies of the effects of different diets on NAFLD (as the reduction of liver fat) have been performed. In this paper we review current literature about the utilities of ketogenic diet (SKMD, Spanish ketogenic Mediterranean diet) for the treatment of NAFLD, showing how it could represent a valid alternative to the Mediterranean diet in the treatment of patients affected by NAFLD.

Keywords: *ketogenic diet, fatty liver disease, Mediterranean diet, steatosis*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic stress related liver disease defined as hepatic lipid accumulation, mainly triglyceride, in the absence of substantial alcohol consumption (<20 g/day) or other secondary causes. It encompasses a spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which increases the risk of cirrhosis and hepatocellular carcinoma.(1)

Currently, NAFLD is the main cause of chronic liver disease worldwide. NAFLD has been identified as one of the most prevalent chronic liver disease in adults and children populations. The high prevalence of NAFLD is probably due to the contemporary epidemics of obesity, unhealthy dietary pattern, and sedentary lifestyle. Its prevalence is much higher in diabetic or obese individuals. NAFLD is usually associated with the metabolic syndrome (MS) which is chiefly related to insulin resistance and its consequences. Insulin resistance has a crucial role in the pathogenesis of hepatic steatosis and potentially non-alcoholic steatohepatitis (NASH). Because of the contemporary epidemics of MS and obesity, the burden of NAFLD is also expected to rise unhealthy diets as the so called western diet, which are enriched in fructose trans fatty acids and saturated fat and seem to be associated with the development of NAFLD. Certain dietary sugar, particularly fructose, are used as a substrate for lipogenesis leading to hepatic fatty infiltration, inflammation, and possibly fibrosis. Other investigations have shown that fat consumption especially cholesterol and trans/saturated fatty acids is also steatogenic and seem to increase visceral adiposity. The identification of specific dietary components favoring the development of NASH could be important for the management of this disorder. (2)

As pharmacotherapy is not effective and safe enough and obesity is intimately associated with hepatic steatosis, lifestyle modification is the first line of treatment.

According to the AASLD (American guidelines for the diagnosis guidelines for the diagnosis and management of NAFLD) loss of at least 3%-5% of the initial weight through hypocaloric diet (alone or associated with increased physical activity) reduces liver fat, however more expressive weight loss (up to 10%) might be necessary to determine improvement in necroinflammation. The usual steps for the management of NAFLD are gradual weight loss and increased physical activity.(3)

Besides weight loss, dietary and lifestyle, no specific guidelines exist pertaining to diet. Very few studies regarding the effects of different diets on NAFLD have been performed.

The goal of this paper is to review current literature and evaluate the ketogenic diet for the treatment of NAFLD.

Methods

Review of papers

Results and Discussion

Recent studies have reported clear evidence for weight independent effect diets, rich in MUFA and PUFA and low in CHO, particularly fructose, on steatosis, liver test and insulin resistance (3).

Several studies suggest that CHO restriction can improve insulin resistance through reducing glycemic load and beta cell insulin secretion(4). Low CHO diets could also reduce serum triglycerides, insulin and glucose and increase high density lipoprotein (HDL)(5). A randomized study of 22 obese patients comparing a low-CHO (<50 g/day) versus a high-CHO (>180g/day) hypocaloric diet, showed a greater reduction of liver glucose production and hepatic steatosis at 48 hours in patients on the low-CHO diet. Nonetheless, the difference in liver steatosis was significant after achieving >7% weight loss, irrespective of the CHO composition of the diet (6). This data proposes that in spite of an early weight independent effect of low CHO diets on liver steatosis and insulin resistance, this effect surpassed by significant weight loss.

Glycemic index (GI) is defined as the proportion of food converted and absorbed as glucose, and it is expressed as a percentage. A recent meta-analysis of studies on dietary regimens based on GI, concluded that higher glucose and insulin exposure is associated with long-term complications, such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Likewise, a Cochrane metanalysis of six trials with a follow-up time between 1 month and 6 months concluded that a diet based on low-GI products induces a significantly higher weight loss (−1.1 kg) and fat mass reduction. (7) In a cross-sectional analysis of 257 healthy individuals, Valtueña et al demonstrated

that there is an association between high-GI food intake and the presence of liver steatosis (assessed by ultrasound). However, longitudinal prospective studies are needed to explore the effects of such diets on NAFLD and hepatic inflammation(8).

During the past decades fructose intake has considerably increased. Fructose metabolism increases lipogenesis, production of free radical oxygen species gut permeability, bacterial overgrowth and serum lipopolysaccharides level, furthermore it reduces lipid oxidation. Studies in animals showed that some of these mechanisms have been suggesting to play a role in NASH and insulin pathogenesis. Although a direct role of fructose in human NAFLD pathogenesis remains to be elucidated, patients should be advised to restrict food and beverage containing fructose.(1)

Impacts of polyunsaturated fatty acids (PUFAs) on NAFLD have been extensively studied. Omega 3 fatty acids (-3FAs), particularly docosahexaenoic acid and eicosapentaenoic acid, reduce liver steatosis by up-regulation of peroxisome proliferator-activated receptor PPAR, resulting in higher FA oxidation and lower lipogenesis.(9)

A multicenter randomized study (n-773 overweigh adults) demonstrated that a fair increase in protein intake (15,4%of total calorie intake) combined with a low GI diet, is associated with improved weight loss maintenance.(10) This finding may be associated with the satiating effect and increased energy expenditure relating in protein metabolism. However long term effects of this type of diet should be considered(1). In two long-term follow up cohorts (n°129, 716 and 10-16 years of follow up; n 38,094 with 10 years of follow up) higher consumption of animal derived protein significantly increases the incidence of diabetes and cardiovascular mortality, although this association was only significant for processed meat.(11)

Moreover, a further analysis of one of these cohorts showed that nuts, low-fat dairy, fish and poultry actually reduce cardiovascular risk.(12)

Concisely, high protein diets can be an alternative for weight maintenance but caution should be given to the protein source, giving priority to fish, poultry, nuts and protein derived from legumes.

The AASLD guideline (13) not specifically address protein intake, and the ADA recommends a protein intake similar to the general population, based on the recommended dietary allowances (RDA) and dietary reference intakes. However, similar to carbohydrates, the definition of protein varies widely in the literature. High protein diets have been classified anywhere from 27% to 68% of daily energy intake or about 90 to almost 300 g/day in absolute amounts. The ADA has suggested, in the absence of diabetic Kidney disease, higher protein eating patterns (30% of calories) may or may not improve HBA1c; however, they appear to improve one or more CVD risk measures (14,15).

Although the Mediterranean diet is widely recommended for weight loss and maintenance, ketogenic diets may be a viable alternative except in some cases that should be considered.

The greatest concerns about the ketogenic diet arise from management difficulties in patients with diabetes mellitus and renal failure as well as the use of processed red meat that is not recommended according to the guidelines.

According to the guidelines, patients suffering from NAFLD must reduce weight gradually by reducing refined carbohydrates as well as processed meats. A ketogenic diet which is proposed as a new therapeutic approach for fatty liver is the Spanish ketogenic Mediterranean diet (SKMD). Mediterranean diet is well known to be one of the healthiest diets, being the olive oil, red wine and vegetables the basic ingredients. This diet has been called "Spanish ketogenic Mediterranean diet" or SKMD because it involves the use of virgin olive oil as the main source of fat (≥ 30ml/ day), moderate red wine, green vegetables and salad as the main source of carbohydrates, and fish as the main source of protein.

In Spain olive oil, red wine, fish and vegetables are essential components of the diet. Olive oil is considered the pillar of the Mediterranean diet, since it improves the major risk factors of cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism and antithrombotic profile. Some of these effects are attributed, besides the mono-unsaturated fatty acids (MUFA), to the minor components of virgin olive oil. MUFA rich diet prevents central fat redistribution and the postprandial decrease in peripheral adiponectin gene expression and insulin resistance induced by a carbohydrate- rich diet in insulin resistant subjects.

The combination of ethanol and phenolic compounds in red wine is thought to be responsible for

the apparent protective cardiovascular effect, showing olive oil and red wine antioxidant polyphenols anti-atherogenic properties. In Spain the fish is an important component of such diet. Two long chain omega 3 polyunsaturated fatty acids (n3 PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the active constituents of it. Low rates of death from coronary heart disease has been reported among individuals with very high consumption of fish although these people should limit intake of species highest in mercury levels. Larger and longer-lived predators (swordfish, shark) have high mercury concentrations in their tissues, whereas in smaller or shorter living species concentrations are very low (anchovies, mollusks, salmon, sardines) (6).

Perez-Guisado and Munos Serrano administered the SKMD (≤ 30gr of carbohydrates per day from green vegetables and salad, at least 30 mL of olive oil, 200-400 ml of red wine, no protein or calorie restriction) to 14 obese men over a 12 week period and observed significant improvements in body weight, low density lipoprotein cholesterol, metabolic syndrome parameters and degree of hepatic steatosis.(16)

It was an unlimited calorie diet, nevertheless subjects were encouraged to consume per day: a maximum of 30 g of carbohydrates in the form of green vegetables and salad (such as alfalfa sprouts, lettuce, escarole, endive, mushrooms, radicchio, radishes, parsley, peppers, chicory, spinach, cucumber, chard and celery); the minimum of 30 ml of virgin olive oil was distributed unless in 10 ml per principal meal (breakfast, lunch and dinner); 200-400 ml of red wine; the protein block was divided in "fish block" and "no fish block". The fish block included meat, fowl, eggs, shellfish and cheese. Both protein blocks were not mixed in the same day and were consumed individually during the day on the condition that at least 4 days a week were for the "fish block". No more than two cups of coffee or tea and at least 3 liters of water were intake each day.

The SKMD has been shown to be an effective and safe way to cure patients suffering from metabolic syndrome. Moreover, Perez-Guisado and Munos Serrano found an improvement in NAFLD proved through normalization of transaminase levels and regression of steatosis degree. They also showed that the results were faster than those obtained through a ketogenic diet or a diet supplemented with Omega-3 fish oil for the improvement of fatty liver disease. Perhaps because of the ketogenic nature of SKMD, its richness in omega 3 fish oil and virgin olive oil exerts a synergistic effect.

The authors agree in stating that the different components of olive oil through different mechanisms of action have a beneficial effect in individuals with NAFLD. All patients after the study were free of the metabolic syndrome and all had shown a reduction in fatty liver in 92.86% of cases, and 21,4% had a complete fatty liver regression(18,19).

Conclusion

Currently, NAFLD has been considered the most common chronic liver disease in the western World. Its prevalence is much higher in diabetic or obese individuals. Patients with NAFLD should be treated for steatohepatitis and the association metabolic comorbidities, whereas patients with simple teatosis only need to treat the associated conditions to prevent hepatic and metabolic complications (1). The usual steps for the management of NAFLD are gradual weight reduction and increased physical activity which ameliorate the disease in different aspects.

According to the dietary advice and the recommendations of the AASLD, SKMD can represent a valid alternative to the Mediterranean diet in the treatment and management of patients affected by NAFLD, showing valid results that however need to be further confirmed.text

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FACTORS IMPACTING ON DURATION OF KETOGENIC DIET THERAPY IN CHILDREN WITH INTRACTABLE EPILEPSY

Barilà Valeria Maria, Longobardi Vincenzo, Serpico Arianna, Coppola Giangennaro

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

Epilepsy is a serious neurological condition that effects almost 1% of the world population. Despite pharmacological progress of past years in developing new drugs and reducing associated side effects, 25% of children continue to have uncontrolled seizures. One possible solution, when even surgery and vagal nerve stimulation aren't applicable or proven ineffective, is the Ketogenic Diet therapy (KDT) developed in 1920 and consisting of high in fat, moderate in protein and low in carbohydrates nutritional protocol. According to recent scientific literature, success rate for patients treated with KDT is impressive, often increasing if therapy is continued for prolonged time. As clinical treatment, even KDT is not free from side effects that could be short-to-mid-term KDT side effects or long-term KDT side effects. Most frequent short-term side effects, usually transitory, are gastrointestinal disorders. Long-term instead, include permanent effects on child growth, fractures (caused by bone depletion) and kidney stones (especially when KD is combined with carbonic anhydrase inhibitors). It seems that KDT can be kept longer than two years especially in children who show a seizure decrease > 50%, (unless there are serious adverse side effects leading to its withdrawal). KDT prolongation for much more than two years is desirable in patients who show a seizure recurrence when attempting the gradual re-introduction of dietary carbohydrates. With therapeutic success and side effects, other variables that reduce the effectiveness of KDT in terms of duration is the scarce adherence to the diet (which often cause an early treatment discontinuation) and different diets with a different KD-ratio.

Keywords: *ketogenic diet, children, duration, refractory epilepsy, side effects.*

Introduction

Epilepsy is a serious neurological condition that effects almost 1% of the world population. Generally, seizures are treated with one anticonvulsant drug or with a combination of more drugs. Despite the pharmacological progress leading to the development of new anticonvulsant drugs in the last fifteen years, 25% of children continue to be subject to uncontrolled seizures. One possible solution, when even surgery and vagal nerve stimulation aren't applicable or proven ineffective, is the Ketogenic Diet treatment (KDT) developed since 1920. KDT includes different diet regimens that have in common the production of ketone bodies for energetic purposes. The classic KD is a normoproteic hypoglucidic and hyperlipidic diet with long chain Triglycerides (TG), caloric and liquids restriction and induction of ketosis with fasting.

KD-ratio $\frac{0.9\,x\,FAT+0.46\,PRO}{CHO+(0.1\,x\,FAT)+(0.58\,x\,PRO)}$ (1) can change from 4:1 up to 2.5:1. KD can also be supplemented with medium chain TGs (MCT diet), ketosis is induced without fasting, without caloric and liquids restriction. Modified Atkins Diet (MAD) (2) is a less restrictive variation of KDT, it allows proteins and fats ad libitum and does not restrict calories, KR is 1:1 allowing about 20g of CHO daily. Another KDT is the Low Glycemic Index Therapy (LGIT)(3) which allows a higher amount of Low GI carbohydrates. The KD was abandoned all over the world in the mid 70-80' due to development of anticonvulsant drugs. Recent studies demonstrate that, with respect to specific pathology (4) or in case of pharmacological resistance, KDT remains the only alternative and it is perhaps more effective than most of the newer medications. The rediscovery of this effective therapy for childhood epilepsy has, within the past decade, had a major impact on the most difficult-to-control seizures of childhood and promises to have an impact on adults with epilepsy as well. Furthermore, KD is nowadays being assessed in neurological or psychiatric disorders other than epilepsy. New research into its mechanism of action shows promise to change our knowledge about cerebral metabolism and our understanding in the control of epilepsy.

Methods

A review of the most relevant retrospective and prospective studies was carried out, to systematically evaluate major factors impacting on KDT duration. For the purpose of the study, a 3 to 24 month duration was considered as short to mid-term treatment while a KD duration longer than 24 months was considered as long term. Therapeutic success was achieved with a 50% seizure decrease.

Results

While efficacy of KDT has been accepted by scientific community, guidance on the duration is limited and still debated. Most epilepsy centers suggest use of the diet up to 3 years if it is effective in controlling seizures, followed by a withdrawal of anticonvulsants. In most cases, the ketogenic diet has been the only successful therapy for many children with epilepsy, this better parent's compliance and these families continue KDT for longer periods.

Short and long term therapeutic efficacy and adverse effects of the KD as major factors for a good compliance, are analyzed in the next paragraph.

Discussion

Despite a single retrospective study (5) performed in 2015 reporting a limited efficacy of the ketogenic diet in the treatment of highly refractory epileptic spasm, most of the studies in the past 90 years conclude KDT to be an effective and safe treatment option for children with intractable epilepsy. Since the 1920s, positive reports have been consistent across all age groups as for seizure control, worldwide (6). In general, 10%–15% of children who started the diet were spasms free 1 year later, 30% had a 90% reduction in spasms, and 40% to 50% found that the diet was either too difficult to continue or insufficiently effective and therefore discontinued it during the first 6 months. Despite a dramatic increase in the number of anticonvulsant drugs in the past decade, KD continues to be an effective alternative therapy, even in children whose seizures are refractory to these newer medications (7).

Short-Mid term side effects (3 to 24 months) (8)

All studies analyzed, reported adverse effects of the dietary interventions in the short and mid-term including those diet regimens (i.e. MCT, LGIT and MAD) with a lower KD-ratio than classical KD. For those studies investigating the classical KD, the main adverse effects were gastrointestinal symptoms, including vomiting, constipation and diarrhea. Some

studies (9) found gastrointestinal symptoms to be significantly worse in the 4:1 ratio compared with the 3:1 ratio KD while others (10) reported vomiting to significantly affect more participants in the classical KD (45%) compared with the MCT group. Weight loss was reported upon by two KD studies (11): more significant among participants in the 4:1 ratio KD group than in the 2.5:1 ratio KD group. Gradual onset KD participants lost significantly less weight than the fasting-onset KD group. Statistical significance was reported with regards to a lack of energy at three months, affecting 36% of participants in the classical KD group compared to 14% of participants in the MCT group (12). Other adverse effects reported by the studies in lower numbers were respiratory tract infection, infectious disease (pneumonia and sepsis), acute pancreatitis, decreased bone matrix density, gallstones, fatty liver, nephrocalcinosis, hypercholesterolemia, status epilepticus, acidosis, dehydration, tachycardia, extended hospital stay, hunger and abdominal pain.

With specific regard to MAD protocol, three studies reported constipation to affect the dietary intervention groups, with 20% to 46% of participants affected (13). One study in particular reported constipation to affect 15.4% of participants in the MAD group and 25% of participants in the classic group, but no significance was reported. Two studies reported vomiting to affect 10% of participants in the MAD group and 30% of participants in the classic group. Furthermore, was reported diarrhea to affect more of the MAD participants than the classic KD participants (15.4% in the MAD group, 12.5% in the classic group). No significant difference between median weight change in the 10 g and 20 g carbohydrate MAD groups in the first three months. Other adverse effects were anorexia, lethargy, lower respiratory tract infections and hyperammonaemic encephalopathy.

Long Term Side Effects (≥24 months)

Most of the studies with regard to adverse effects, focus on the short period limiting the analysis to maximum 24 months, that is the average recommended maximum time for KDT. Only one retrospective analysis describes the long-term effects of the ketogenic diet in children who have been on the diet for over 6 years.

In synthesis, the most relevant and serious side effects regard long-term growth in children. Although 14 children were at less than the 10th centile for weight before starting the diet, this number had increased to 23 at their most recent follow-up. Similarly, 10 were at less than the 10t^{h}centile for height at diet initiation, whereas this number was 23 at the most recent follow-up. In the 20 children below the 5th centile for height, 11 had large urinary ketosis at the most recent follow-up, compared with 3 of the 8 children above the 5th centile. Children were overall proportional in measured height and weight centiles. BMI overall did not change at the most recent follow-up. The median BMI centile decreased slightly, from 25 to 50% to 10 to 25%; 16 had a BMI in a normal range of 10 to 90% at the most recent follow-up. Three of the four children with a baseline BMI of more than 90% had been treated with adrenocorticotropin in the previous 2 years. Seven patients developed kidney stones. The latency period from diet initiation to a symptomatic kidney stone ranged from 1 month to 6 years (median 2y). None of the children who developed kidney stones was receiving carbonic anhydrase inhibitors (topiramate, zonisamide, or acetazolamide) at the time, nor did the six children taking carbonic anhydrase inhibitors develop kidney stones.

Six children had skeletal fractures while on the diet; four had fractures at two or more separate locations and times. All these children were receiving calcium supplementation, as routinely recommended for ketogenic diet patients. Five of the six children with fractures were at the 10th centile or less for height at the most recent follow-up, in comparison with 18 of 22 without fractures. Dyslipidemia was rare; two children had significantly elevated cholesterol levels but overall lipid profiles were generally within the normal range. Cholesterol did not increase significantly and neither did triglycerides. Constipation occurred in 15 children. A single child died while on the diet after 7 years; autopsy revealed no clear etiology or relationship to the diet.

Other factors impacting on KD duration.

Despite medical suggestion and therapeutic success achieved, discontinuation and poor compliance(14) have been found as another key driving factor for overall therapy duration. The education level and care of the patient's family, the knowledge of epilepsy treatment, and the complicated recipe for daily

diet treatment as well as the side effects (15) of KD play an important role resulting often in poor compliance. Kessler et al. reported that adverse effects are usually transient and the most common reason for discontinuation of treatment is lack of efficacy (16). But at the same time, in order to be clinically significant, persistence or compliance would be quite important in the KDT. To improve therapeutic success of the ketogenic diet, some measures such as effectiveness evaluation, health education, supervision of compliance, awareness of epilepsy treatment, side effects minimizing, user-friendly recipes developing should be taken into consideration. Another factor affecting duration is the type of dietary option. Classic KD is no longer the only option for the treatment of drug-resistant epilepsy (17). Less strict and more palatable diets such LGIT and the MAD are currently under trial in order to avoid classic KD strict rules in which each food is rigidly calculated and weighed as well as the total amount of the daily calorie intake. As a consequence, KD is often poorly accepted by patients and hard to abide by family. Initial experience with the LGIT (18) confirms that can be beneficial to some patients with refractory epilepsy syndromes proving also that compliance is improved with respect to classical protocol.

Lower KD-ratio diet are *de facto,* a valid option to patients over the age of 2–3 years, as first dietary treatment in combination with the anti-epileptic therapy.

Effects of the diet have been reported in patients who were on it for as long as 3 to 6 years' duration (19), but still lots of questions on long-term use of the ketogenic diet are to be answered thoroughly. Is it feasible for patients to remain on the protocol for prolonged periods of time? Does time alter the efficacy or diet became more tolerable with it? Do families eventually tire of the strict regimen of calculating ratios and weighing out foods or become more familiar with it? Only one surveyed study (20) looked into the effect of the KD after its discontinuation, observing reduced benefit after cessation of treatment. Unfortunately, no such a data was found on children.

Many studies have shown the efficacy of ketogenic therapy in children with epilepsy, where the percentage of children who have had a reduction in attacks exceeds 60%. Ketogenic diet is a therapy and as a therapy is possible to have side effects. There are short-term complications, medium (3-24 months) and long-term complications (3-24). Short term side effects depend by ketosis induction, long-term side effects include growth retardation, alteration of bone mineralization and kidney stones. Delay in growth rate has been known for a long time and may depend on insufficient caloric and protein intake. Children on antiepileptic therapy are at risk of osteoporosis; this effect can be exacerbated by the ketogenic diet if not adequately supplemented with calcium and vitamin D. Kidney stones were found in 5-7% of cases with increased risk especially in very young children, familiarity with nephrolithiasis and urinary calcium/creatinine ratio > 0,2. Oral supplementation of potassium citrate was found to be useful in the prevention of kidney stones. In conclusion, it seems that in children who get a>50% seizure decrease, KDT can be kept for at least two years (unless there are side effects that counter the continuation).

KDT prolongation, more than two years is desirable in patients who show seizure recurrence when trying KD discontinuation. In these cases it is important to strictly control KDT (even with oral supplementation) in order to avoid growth retardation, kidney stones, osteoporosis and hyperlipidemia.

Today research takes giant steps on KDT, many studies have highlighted side effects, in order to minimize them. Currently, classical ketogenic diet and its variants (supplemented with MCT, MAD and LGIT) are used in 45 countries all over the world, which means that is the best seizure therapy for Drug-resistant epilepsy in children, even if mechanisms of action are still being studied and only with time and work of many researchers can be clarified. In synthesis, KDT duration is a combination of multiple non-linear factors that usually play in circle and influence each other, where compliance and different KD-ratio have an important role as well and should be investigated more.

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Outcomes of a 150-Patient Cohort Using the KD at Johns Hopkins Hospital

	3 mo	6 mo	12 mo	3–6 y and Longer
Seizure reduction, *n* (%)				
Seizure free	4 (3)	5 (3)	11 (7)	20 (13)
90%–99%	46 (31)	43 (29)	20 (20)	21 (14)
50%–89%	39 (26)	29 (19)	34 (23)	24 (16)
<50%	36 (24)	29 (19)	8 (5)	18 (12)
No. (%) remaining on the KD	125 (83)	106 (71)	83 (55)	18 (12)

Sources: Freeman JM, Vining EP, Pillas DJ, Pyzik PL, Casey JC, Kelly LM. *Pediatrics*. 1998;102:1358–1363; and Hemingway C, Freeman JM, Pillas DJ, Pyzik PL. *Pediatrics*. 2001;108:898–905.

Figure 1: Therapeutic success as reported by John M. Freeman et al.

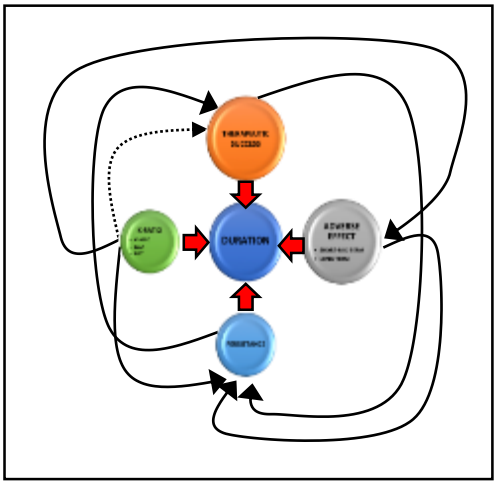

Figure 2: Major factors influencing KDT duration

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PROTEIN AND LIPIDIC FRACTION OF "COLATURA DI ALICI"

Pepe Vincenza[1]; Sauro Francesca[2]; Consoli Lina[1]; Di Luccia Aldo[3]; Rastrelli Luca[1]

[1]Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

[1]Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II - Fisciano (SA), Italy

[2]Servizio Prevenzione, Veterinaria e Sicurezza Alimentare della Regione Molise

[3]Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, University of Foggia

Email address: nutriketo@unisa.it

Abstract

Colatura di alici produced in Cetara is an amber liquid obtained using a traditional ancient procedure that the local fishermen have passed form father to son. It starts by using anchovies under salt, and the drippings are derived from it: the freshly fished anchovies, during the spring time, are rid of the head and the interiors and then laid in a container, covered in sea salt and left for 24 hours. After the first salting, they are put in a small barrel and covered with a wooden disc with weights on it. The liquid secreted for effect of the pressure and the aging of the fish starts coming to the surface. This liquid is the base for the "colatura": it is progressively collected, poured in big glass bottles and exposed to direct sunlight for about 4 to 5 months, to allow the water to evaporate and increase the concentration. It is then recovered through a hole and transferred to another container and filtered using linen cloths. The final result if a clear liquid with a strong amber, almost mahogany brown colour, with a decisive and bodily flavor. In Cetara it is used as a spaghetti sauce.

Keywords: *Colatura di alici, Garum, Protreins, Unsaturated fatty acids*

Introduction

The Garum preparation dates from Neolithic people who inhabited the Western countries of Francia and it was improved from Celtic Druids [1]. They used the Great Bleu Fish, a fish that lived in deep water and that migrated yearly to the "Armonica" region (the contemporary England), and they fermented this fish with salt and some aromatic herbs obtaining the so-called "Garum Armoricum". The Roman people used this product like a wide range "elisir" and so they drank it to re-form, relieve depression, headache and other problems and also soldiers drank it to prepare for marching and before a battle. After the "Garum" was introduced in Rome as a seasoning for every dish. By virtue of their active ingredients it was reputed as a healthy food since time immemorial and recent clinical works show the "Garum" as a real modern and legendary treatment to treat the anxiety, the depression and the tiredness. The best healing properties of "Garum Armoricum" by comparison with others fishes are related to the aptitude of this fish to live in the ocean deeps, where others fishes are not able to live because of considerable stress arising from the very low temperatures and oxygen amount and from the extreme pressures. Moreover the "Garum Armoricum" is endowed with a system of anti-oxidant, fatty acids and neuron-peptides that, forbidding the oxidative reactions, allow his to live to the surface too, where there is more oxygen [2].

A preparation similar to the Garum come down from the Roman's time and today is named "colatura di alici". This product, initially reputed as a by-product of anchovy in oil production, today is represent a typical product of Cetara, that is a little town in Amalfitana coast where the "colatura di alici" is a seasoning of everyday use like olive oil [3]. Differently from the "Garum" produced in ancient time, the "colatura" is a more poor sauce than "Garum" because it is only constituted of anchovies, that are easily findable, but as much good because it is obtained at the perfect ripening time of these. Moreover in the "Garum" spices and flavours was mixed into the salt and fish in the holder so these was subjected to the entire ripening process and produced a mixture of taste and scent that sometimes was not appreciated. Contrarily in the "colatura" spices and flavours are cleverly mixed, so this sauce has an incomparable bouquet that makes it an excellent first and second course and cake dressing.

The production process of "colatura di alici" provides first the beheading and by hand evisceration of fresh anchovies. These are stored in sea salt for 24 hours and after pressed for 48 hours at least to pull away water and fats [4, 5]. The liquor that first leaks is removed because it is abounding in water; further liquor is stored in glass bottles and exposed to the light to evaporate the remaining water. The ripening progress during some months and it is a decisive factor for flavour, appearance, and qualitative and hygienic-medical safety of the product. Moreover complicated modifications in muscular proteins (proteolysis) occur during ripening, and these modifications drag on all storage time of product. When the ripening process is terminated (after 4-5 months) the liquor, that was collected and stored, is finally discharged in the anchovy holder. This way the liquor, running down the holder, take up the best anchovy flavours, after it is collected, moved to another holder and finally filtered. Today this typical seasoning of local gastronomy obtains the right reward: the "colatura di alici" is included in the special national list of traditional food products to protect and preserve (Ministry of Agricultural Politics D.M. 18/7/2000). This condiment showed high protein content and average 20-25% of of the dry substance, whereas fats present in the raw material values around 3% increased to 6% in the finished product

This work wants to characterize the protein and lipid fraction of the "colatura di alici" by electrophoresis and chromatographic analysis to identify and enhance the nourishing and healthy properties of this seasoning arising from the ancient Garum recipe [6].

Methods

Lipid extraction

Liposoluble fraction is extracted from 50 ml of "colatura di alici" (3 samples) by n-hexane (1:1), so the hexane fraction is concentrated. Instead the water-soluble fraction has been subjected to another extraction by n-butanol (1:1); the butanol fraction is concentrated and stored at 4°C to after analyse organic compounds.

The 3 samples of fresh anchovies have been subjected to the toilet, that is these are decapitated and

eviscerated, so 50 g of these are homogenised with 10 ml of bi-distilled water. Therefore we extracted the liposoluble fraction by 100 ml of n-hexane. After the hexane fraction are got together and concentrated.

Muscular proteins extraction

10 g of anchovies are homogenized with bi-distilled water (1:2) to extract the water-soluble fraction containing the sarcoplasmatic proteins and low molecular weight components, peptides and amino acids, arising from both sarcoplasmatic and myofibrillar fraction. After the homogenate is centrifuged at 4500 g/min and 5°C for 20 min to separate the water-soluble phase, containing fractions required from the pellet, containing not water-soluble fractions. Therefore the water-soluble fraction is filtered (0,45 µm HV Millipore) and stored at –80°C. Instead the pellet is diluted with bi-distilled water, shaken and centrifuged again at 4500 g/min and 5°C for 20 min, to remove some remaining water-soluble fraction. Finally 2 g of pellet, containing myofobrillar proteins, are diluted with 5 ml of extraction buffer [Urea 8 M, 2% Nonidet P-40, 10% 2-mercaptoetanolo, 10% Glicerolo] [7] and shaken all night long at 5°C. After this is centrifuged at 12000 g/min at 5°C for 5 min and the supernatant, containing myofibrillar proteins, is stored at –80 °C and finally analysed.

Electrophoresis analysis of muscular proteins

The water-soluble fraction of "colatura di alici" and myofibrillar and sarcoplasmatic proteins of fresh anchovies are analysed by SDS-PAGE (16x18x0,15 cm) with concentration gradient (9 - 18%) and basic pH. We load 5 µl of molecular weight standard (Broad range) and 15 µl of samples. Samples undergo the following process:

- 50 µl of "colatura di alici" water-soluble fraction +50 µl of O'Farrel buffer [8];
- 50 µl of anchovy myofibrillar proteins + 50 µl of O'Farrel buffer;
- 50 µl of anchovy sarcoplasmatic proteins diluted with H2O (1:1) + 50 µl of O'Farrel buffer.

The voltages are 200 and 400V into the stacking and running gel respectively. The gel of anchovy protein fraction is stained with Coomassie Brilliant Blue R-250, instead for the gel of "colatura di alici" we use the silver stain.

Chromatographic analysis of "colatura di alici" protein fraction

The "colatura di alici" without fats (1 ml) is fractionated by low-pressure liquid chromatography with a column 90x2,6 cm (Amersham Pharmacia Biotech, Uppsala, Svezia) packaged with a molecular exclusion resin (SEC) Sephacryl S-100 High Resolution (Pharmacia). Peaks are eluted with a TRIS-HCl pH 7,5, 50mM buffer, containing NaCl 0,25M, the flow rate is 0,4 ml/min and the wavelength is 280 nm. Fraction collected are separated again by RP-HPLC, with a column C18, 250x4.60 mm (Phenomenex) and wavelengths are 220 and 280 nm. The elution gradient goes from the 0% of A (water-TFA 0.1%) to the 28% of B (CH3CN-TFA 0.1%) in 40 min.; the flow rate is 1 ml/min. The hexane extracts of "colatura" and fresh anchovy samples are analysed by TLC with Silica Gel slabs 20x20 and 0,25 mm thickness (Merck). Elution solvent is constituted of hexane (60 ml), diethyl ether (40 ml) and formic acid (1 ml). After we spray 2',7'-dichlorofluorescein 1% solution on slabs and detect the fatty acid band by means of the Wood lamp and then the band is removed and extracted with chloroform. The extract is now dried at 40°C and fatty acids are recovered with 2 ml of petroleum ether and then treated with BF3 (boron-trifluoride-methanol complex) [9]: 2 ml of petroleum ether containing fatty acids and 2 ml of BF3 are put in a test tube with cap and then heated in a double boiler for 30 min; after the tube is cooled and we put in 1 ml of water to stopped the reaction and after centrifugation we recover the supernatant containing methyl esters of fatty acids. 1,5 µl of these methyl esters are analysed by GLC, using a gas-chromatograph Fisons Ins. HRGC MEGA 2, a column SP-2340 60m x 0,25mm and 0,25 µm thickness. The oven temperature is 140°C, after 10 min it goes from 140 to 220°C (rate: 1°C/min) and finally it continues at 220°C for 10 min; the helium flow rate is 20 cm/sec; the FID temperature is 240°C; the Split injector temperature is 220°C and the split ratio is 1:50.

Results

Proteins

Electrophoretic courses of "colatura" (Fig. 1) show the absence of 97 and 50 kDa proteins that probably

represent sarcoplasmatic proteins and a total absence of myofibrillar proteins. (Fig.2)

The molecular exclusion chromatography of "colatura" (Fig. 2) shows a first peak (relative abundance 9,8%) which estimated molecular weight is 10 kDa; a second peak (31,2%) which estimated molecular weight is 8 kDa; a third peak (10,7%); a fourth peak (2,5%) 6300 Da; the fifth (35,9%) 4600 Da; and finally the sixth (9,8%) smaller than 1000 Da. The absence of some protein components and the presence of some lower molecular weight components denotes an high proteolysis. This process is related to the muscular enzymes action that, liberated during the rigor-mortis process, act during the anchovy fermentation period. These enzymes break down the muscular fibre and hydrolyse myofibrillar proteins. The absence of some sarcoplasmatic proteins into the electrophoretic courses of the "colatura" should be related to the high salt concentration that denature these proteins. Moreover the high salt concentration break down the cells liberating cytoplasmic enzymes that go out with the meat juice and contribute to the fermentation.

Results obtained by RP-HPLC analysis of each SEC peak (Table 1) show the prevalence of peptides, instead the not peptic organic components predominate only in few peaks (e.g. peak 4). However last peaks show a bigger quantity of not peptic organic components. The considerable presence of peptides confirm the remarkable protein hydrolysis that happen during the anchovy fermentation period. (Fig.1)(table.2)

Fatty acids

The fatty acid composition of the "colatura" and fresh anchovies is showed in Table 2. The more represented acids among total fatty acids of fresh anchovies are C16:0 (18.5%), C18:1 (10,8%), C20:5 (1,9%) and C22:6 (28,1%). Instead the more represented acids among free fatty acids are C16:0 (18,7%), C18:0 (12,6%), C18:1 (20,1%) and C22:6 (10,5%). Regarding total fatty acids of the "colatura", the more represented acids are C18:0 (16,3%), C16:0 (17,5%), C18:1 (35,7%) and C22:6 (4,16 %). (Tab. 1)

Instead regarding free fatty acids the more represented are C16:0 (10.3%), C18:1 (29.4%) and C20:5 (32.3%). The different composition in the "colatura" and fresh anchovy samples is probably related to the different solubility of lipidic fraction in salt solution and to the different fermentation processes. Moreover the fatty acid composition of fresh anchovies shows that the unsaturated/saturated ratio is 2,05; Nevertheless among the free fraction the quantity of saturated fatty acids is 66% and the unsatured/satured ratio is 0,78; this shows the lipolytic action that happens during of muscle into meat. Regarding the "colatura" the U/S ratio is 1,5 for total fatty acids, instead it is almost 4 for free fatty acids.

This is a very important result because this characteristic free fatty acid composition, related to the different solubility of fatty acids in the salt solution, is a functional factor of man health because it can reduce cardiovascular disease hazard [10, 11]. Moreover, the fatty acid composition of the "colatura" show an equilibrate proportion between healthy fatty acids and a larger quantity of monounsaturated fatty acids [12]. Finally the immediate access to these active ingredients, arising from the proteolytic process action, make the "colatura" a particularly healthy food.

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Table 1: HPLC peak area corresponding to peaks collected by SEC

SEC peaks	HPLC peaks	Area at 220 nm (mm^2)	Area at 280 nm (mm^2)
1	Fronte	41464	1300
2	1	28434	458
	2	3596	1577
	4	10266	13809
	5	8673	7494
	6	2751	3932
	7	2646	2082
	8	4329	1889
	9	10432	2458
	10	4360	1408
	11	6665	6549
	12	18910	/
3	8	19494	8106
	9	15949	3476
4	Fronte	12399	/
	3	13130	3618
	11	/	732
	13	729	9473
5	Fronte	13058	/
	4	4448	2712
	9	5672	9052
	10	2829	5009
	13	38193	14438
6	Fronte	12532	268
	3	4058	5292
	14	4285	4312

Table 2. Total and free fatty acid composition (TFA-FFA) of fresh anchovies and "colatura" (%)

Fatty acids	Anchovies		Colatura	
	TFA	FFA	TFA	FFA
Saturated	32.4	66.1	39.3	20.1
Monounsat.	20.5	26.6	44.3	41.2
Polyunsat.	46.1	25.2	16.3	38.7
U/S	2.05	0.78	1.54	3.97

Fig. 1

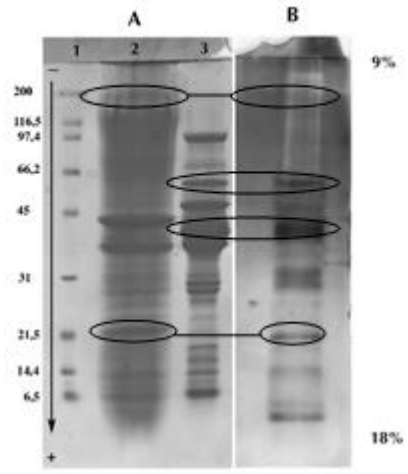

Fig. 1 *SDS-PAGE of fresh anchovies and "colatura". Gel A: course 1, molecular weight standard; course 2, myofibrillar fraction of anchovy muscular proteins; course 3, sarcoplasmatic fraction of anchovy muscular proteins. Gel B: corse of "colatura".*

Fig. 2

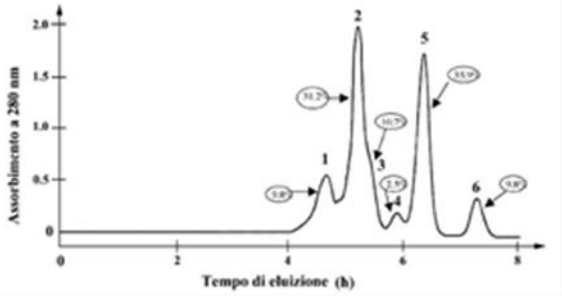

Fig. 2 *Molecular exclusion chromat. (10^3÷10^5 Da) of "colatura"*

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VEGETARIAN PROTEIN SOURCES: CHENOPODIUM PALLIDICAULE (CAÑIHUA) AND CHENOPODIUM QUINOA (QUINOA) SEEDS

Zoura Despina[1], Sauro Francesca[2], Borzi Elio[1], Fiore Valentina[1], Rastrelli Luca[1]

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

[1]Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

[2]Servizio Prevenzione, Veterinaria e Sicurezza Alimentare della Regione Molise

Email address: nutriketo@unisa.it

Abstract

Quinoa (*Chenopodium quinoa* Willd.) and cañihua (*Chenopodium pallidicaule* Allen) are native Andean food plants of high nutritional value used as food by the Incas and previous cultures. The chemical composition in proteins, lipids and carbohydrates can be compared to that of cereals more common as wheat and corn. Protein content (15%) is very interesting as well as the nutritional value of proteins, with good content in essential amino acids and with high values found for lysine, sulfurated amino acids and aromatic amino acids. The high content of polyunsaturated fatty acids (72.5%), compared to those defined as saturated (22.8%), recommended and searched for all foods, can ensure good activity in prevention hypercholesterolemia and cardiovascular disease.

Keywords: *protein, quinoa, cañihua, Andean crop.*

Introduction

The Andean people selected before of the Spanish conquest, a considerable number of plants for food purposes. In their vast empire In fact, the Incas had developed agriculture extremely diversified, suitable for the remarkable variety of ecosystems and able to meet the needs feeding a population of about 12 million of the people living there. Some of the cultivated plants, such as maize, potato and tomato were imported from the Spanish conquerors and found fast and wide diffusion, so as to change the eating habits and agriculture in Europe and later in the rest of the world world. It was not the same for many others who, disdained from the "conquistadores", went irreparably lost and their agriculture fell into disuse. Also, like quinoa and other seeds and tubers andin, continued to be cultivated, consumed and marketed in the markets of small villages on Andean highlands.

Quinoa and cañihua are original cereals of the Andean Cordillera. Traditionally it grows in arid and semiarid lands and has a large genetic variability with more than three thousand ecotypes. It also presents an ability to adapt to adverse climatic and climatic adversities different heights; from 4000 meters altitude to the level of sea. Quinoa and cañihua are a strategic alternative food and potential to solve the protein decifit in many populations of the worl (1-5).

Food Uses for *Chenopodium pallidicaule*

Chenopodium pallidicaule is a native plant of the Andes, for a long time it was considered a variety of the Chenopodium quinoa and only from 1929 it was classified as a different species. It is distributed in Peru and Bolivia, in the highest semi-arid regions of the central Andes, above 3500-4000 m altitude. Its cultivation is not widespread outside the borders of the highlands of Peru and Bolivia; in these areas the cañihua has established itself for its agronomic characteristics of greater resistance to low temperatures. It is a plant used not only for its edible seeds but also for its ashes. The indigenous people of the Peruvian and Bolivian plateau burn the residual biomass after mincing the seeds and with the ashes they process a paste called "llipta" rich in calcium and used by the coca leaves chewers. The cañihua is a traditional food of indigenous families whose preparation requires a laborious process; is considered of high nutritional value, with the seeds are also prepared cakes, hot drinks, food for children as well as characteristic breads that are the main food of the Campesine populations inhabitants of the highest lands of the highlands of Peru and Bolivia.

Food Uses for *Chenopodium quinoa*

It is a pre-Columbian plant whose cultivation is carried out in drastic climatic conditions at altitudes above 3000 m, it meets in the high regions of Bolivia, Chile, Ecuador and Peru and in the south of Colombia. It is native to the western slopes of the Andes. *Chenopodium quinoa* has been used by Andean populations for food purposes since prehistoric times. In the diet of the ancient peoples of America quinoa represented the almost exclusive alternative to animal proteins, in fact the consumption of meat, milk and eggs was not common among the Campesine populations and in many areas quinoa was the main protein component of the diet . During colonization, the use of quinoa was almost exclusive of the native societies, considering that wheat was a food for the Indians that related it to a low social position. Currently the quinoa is consumed in the highlands of Peru and Bolivia, with a minor use in Ecuador, the cereal is completely unknown in the Andean regions of Colombia, Chile and Argentina where in ancient times however it had a food importance. The edible part is represented by the seeds used for the preparation of small loaves called "quispiña", soups and fermented drinks (6). The by-products of the quinoa, stems, leaves and ears are used in the feeding of livestock producing a feed with high nutritional value, excellent digestibility and easy storage (7).

Nutritional value

We reported the chemical and nutritional characterization of cañihua and quinoa in relationship with wheat, corn, rice, rye, as sources of dietary fiber and other bioactive compounds in human and animal. *C. quinoa* and *C. pallidicaule* present an excellent nutritional value with high (14-18%) protein content, balanced amino acid composition, trace elements and vitamins and contain no gluten (8) (Table 1). This food species presented rich flavonol and triterpene glycosides fractions that include different compounds (3,4). *C. quinoa* and *C. pallidicaule* are an excellent example of functional foods that aims to prevent the risk of various diseases. In pseudocereals, such as quinoa, albumins and globulins are the major protein

fraction (44–77% of total protein), which is greater than that of prolamins (0.5–7.0%). Quinoa is considered to be a gluten-free grain because it contains very little or no prolamin. Quinoa provides a nutritional, economical, easy-to-prepare, flavourful food source which is of particular relevance for people with gluten intolerance, such as those with celiac disease (7). Quinoa is a food that lends itself to most food regimens. In medium portions and with daily consumption frequency, it could be included in the customary diet - as it happens in the areas where it comes from. Being a dried starchy seed, quinoa has a supply of carbohydrates and considerable calories. It should however be specified that, due to its fiber richness, this pseudocereal has a high satiating power and a medium-low level glycemic and insulin index. This feature makes it more suitable - compared to most cereals, especially refined - in the diet against the diseases of the exchange and overweight. In case of obesity, type 2 diabetes mellitus and hypertriglyceridemia, it is however advisable to reduce the average portion of both the raw food and its derivatives. Having a valuable lipid distribution and being cholesterol-free, quinoa has no contraindication for hypercholesterolemia. On the contrary, thanks to the abundance of fibers, this pseudocereal is able to sequester a small part of food cholesterol and bile salts, reducing intestinal absorption / reabsorption. This promotes the decrease in cholesterolemia. Quinoa has no implications for food therapy against hypertension, although indirectly - by helping to reduce caloric excess, load and glycemic-insulin index and increasing fiber intake - could prevent or even favor the decrease in overweight improving a possible primary arterial hypertension. The protein quality of quinoa and cañihua have proved excellent, due to the high content of essential amino acids with chemical indexes comparable to those recommended by FAO-WHO (Table 2).

Quinoa and cañihua haves a good supply of some vitamins and minerals. In the body, the water-soluble B group - especially B1, B2 and PP - mainly play the role of coenzymatic factors. Vitamin E instead has the role of antioxidant and protects cell membranes from the action of free radicals. Calcium and phosphorus are essential for bone, magnesium and potassium are two alkalizing and participate in the conduction of neuro-muscular membrane potential, while zinc forms some strongly antioxidant enzymes and keeps thyroid gland healthy.

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Table 1. Analytical Composition of cañihua and quinoa and other cereals.

%	Cañihua	Quinoa	Wheat	Corn	Rice	Rye
Water	10.8	14,7	12	12	12	12
Proteins	12.8	11,7	12,2	9,2	7,4	11
Lipids	7.0	12,4	2,3	3,9	0,5	1,9
Hydrolyzable carbohydrates	59,9	55,3	71,8	73,7	80	73,1
Whole fibre	6,3	2,2	2,1	1,6	0,4	-
Ash	3,1	3	-	-	-	-

*Documenta Geigy

Table 2. Aminoacid composition of *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (Quinoa)

Rt	Aminoacid	mg/g protein in Cañihua		mg/g protein in Quinoa		Essential aminoacid pattern (FAO-WHO)	Chemical Index Cañihua	Chemical Index Quinoa
2,8	Aspartic acid	67.5		66.8		-	-	-
3,9	Glutamic acid	169.1		16		-	-	-
				166.7				
9,8	Serine	36.3		38.3		-	-	-
14,2	Histidine	16.7		19.9		-	-	-
15,1	Glycine	63.5		60.9		-	-	-
15,6	Threonine	37.2		34.9		40	93	87
22,4	Arginine	87.4		84.3		-	-	-
24,5	Alanine	568		57.1		-	-	-
25	Tyrosine	29.0	} 68.8	31.3	} 74.7	60	115	125
37,8	Phenylalanine	39.8		43.4				
	Cystine	20.3	} 41.7	22.1	} 44.6	35	119	127
30,8	Methionine	21.4		22.5				
31,1	Valine	48.2		60.0		50	96	120
35,7	Isoleucine	37,5		743		41	94	81
37,8	Leucine	67.2		75.0		70	96	107
46,9	Lysine	58.3		45.8		55	106	83
	Tryptophan	n.d.		n.d.		10	n.d.	n.d.
42,9	Proline	18.4		22.6		-	-	-

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AKKERMANSIA *MUCINIPHILA* AND KETOGENIC DIET: TWO PLAYERS TO FIGHT METABOLIC DISEASES

Buono Giuseppina[1], Gallucci Sonia[1], Gentile Anna[1], Guastaferro Maria Giovanna[1], Ruggiero Alessandra[1], Di Nardo Veronica[2]

[1]Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy
[2] Adjunct Professor, University of Salerno Department of Farmacology

Email address: nutriketo@unisa.it

Abstract

In the last decades the importance of gut microbiota for the well-being of the individual and in the onset of pathologies has been re-evaluated, infact alteration of the composition and the activity of the gut is thought to be involved in many metabolic diseases. These kinds of affections are now worldwide epidemic and there are different kind of approaches to invert this bad trend, that is the first cause of death in the occidental world. Among the next-generation beneficial microbes that have been identified, Akkermansia muciniphila is a promising candidate. Akkermansia muciniphila is a commensal anaerobe that is found to reside in the intestinal tract of more than 80% of human population, constituting 1–4% of the total bacterial cells in the healthy adult feces. It is a mucin degrading bacteria and this feature makes it a modulator for gut homeostasis via strengthening the integrity of the epithelial cell layer and regulating the gut barrier function. Studies demonstrated that Akkermansia muciniphila is inversely correlated with body weight, diabetes, colorectal cancer, inflammatory bowel disease and autism. Akkermansia muciniphila treatment appears to reverse high-fat-diet-induced obesity and metabolic disease, to attenuate atherosclerotic lesions by ameliorating metabolic endotoxemia-induced inflammation and to improve glucose homeostasis by metformin treatment. These results provide a rationale for the development of a treatment that uses this human mucus colonizer for the prevention or treatment of obesity and its associated metabolic disorders. Akkermansia muciniphila appears linearly affected by the dietary treatment. The dietary regulation exerts influences on microbial metabolism and host immune functions through several pathways and a proper nutrition is the first approach both in inherited and not inherited metabolic diseases. Comprising of a high proportion of fat, adequate protein, and low carbohydrates, the ketogenic diet (KD) causes a drastic shift in host metabolism by mimicking the fasting state and promoting ketone body production and utilization. The KD is an effective treatment for refractory epilepsy, autism spectrum disorder, Alzheimer's disease, metabolic syndrome and cancer. KD consumption triggers gut microbiota remodeling then this provide insight into the therapeutic potential of KD manipulation by influencing gut microbial composition. There is an interaction between gut microbiome richness, certain metagenomic species and Akkermansia muciniphila, and higher abundance of Akkermansia muciniphila at baseline is associated with greater improvement in glucose homeostasis, blood lipids and body composition after calorie restriction. So, in our review we summarize how Akkermansia muciniphila may be identified as a medium through which KD can reverse metabolic diseases and it could represent a diagnostic or prognostic tool to predict the potential success of KD or the best candidate to support KD in inverting metabolic diseases that are related to excess feeding.

Keywords: *Akkermansia muciniphila, gut microbiota, ketogenic diet, metabolic disease*

Introduction

In recent years it has increasingly highlighted the importance of microbiota role in the individual's health. Gut microbiota is now considered as a pathogenic factor affecting host metabolic balance and disorders, modulating nutrition and energy harvest, influencing intestinal epithelial homeostasis, host immune system and drug metabolism. So, the alteration of the composition and the activity of the gut is thought to be involved in many metabolic diseases as obesity, diabetes, metabolic syndrome and cardiovascular diseases that have epidemic proportions in the world's population.

Methods

Several studies have been cross-examined to demonstrate the importance of A. *muciniphila* and KD in treatment of metabolic diseases and their possible interaction and correlation.

Results

Akkermansia *muciniphila* is a commensal anaerobe that resides in the intestinal tract of more than 80% of human population, constituting 1–4% of the total bacterial cells in the healthy adult feces. In the intestinal tract A. *muciniphila* uses intestinal mucins, highly glycosylated proteins of the epithelial mucus layer, as its major carbon and nitrogen sources and produces acetate and propionate, which are the important energy sources of human intestine epithelial cells. This unique mucin-degrading feature makes A. *muciniphila* a modulator for gut homeostasis via strengthening the integrity of the epithelial cell layer and regulating the gut barrier function.

Several analyzed studies demonstrated that A. *muciniphila* is beneficial to host health: higher A. *muciniphila* abundance is associated with a healthier metabolic status in overweight and obese humans; this bacterium appears inversely correlated with body weight, diabetes, colorectal cancer, inflammatory bowel disease and autism, it resulted an effective treatment to reverse high-fat-diet-induced obesity and metabolic disease, to attenuate atherosclerotic lesions by ameliorating metabolic endotoxemia-induced inflammation and to improve glucose homeostasis by metformin treatment.

A. *muciniphila* appears linearly affected by the dietary treatment. In fact, the dietary regulation exerts influences on microbial metabolism and host immune functions through several pathways and a proper nutrition is the first approach both in inherited and not inherited metabolic diseases.

The ketogenic diet (KD) is a very low-carb, high proportion of fat, adequate protein diet characterized by a metabolic state called ketosis; in ketosis the body becomes incredibly efficient at burning fat for energy and turning fat into ketones in the liver. KD can cause massive reductions in blood sugar and insulin levels with numerous health benefits. It is an effective treatment for refractory epilepsy, autism spectrum disorder, Alzheimer's disease, metabolic syndrome (comprising obesity, type 2 diabetes, polycystic ovary syndrome and non-alcoholic liver steatosis) and cancer. Mimicking the fasting state and promoting ketone body production and utilization, KD causes a drastic shift in host metabolism trigging gut microbiota remodeling.

The composition and function of the gut microbiota appears a key intermediary between diet and host physiology, modulating several metabolic and neurological pathways in the host that could be relevant to KD mediated effects.

Discussion

The aim of this review is to synthesize the literature data related to gut microbiota and A. *muciniphila* as medium through which KD can reverse metabolic diseases and to candidate A. *muciniphila* to support KD in inverting metabolic diseases that are related to excess feeding. Numerous studies have demonstrated that our dietary habits strongly influence the composition and function of the gut microbiota and eventually may contribute to the onset or the protection against metabolic disorders. Among the next-generation beneficial microbes that have been identified, A. *muciniphila* is a promising candidate and may be identified as a diagnostic or prognostic tool to predict the potential success of dietary interventions. Moreover, dietary changes and KD can profoundly impact the gut microbiota triggering gut microbiota remodeling and providing insight into the therapeutic potential of KD in influencing gut microbial composition. Furthermore, these literature results pro-

vide a rationale also for the development of a treatment that uses this human mucus colonizer for the prevention or treatment of metabolic disorders in association to KD.

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HEMP SEED: A DIETARY SOURCES OF PROTEIN FOR SPORT NUTRITION

Antonietta Carillo, Luca Rastrelli

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II - Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

Seeds of the plant cannabis sativa, hemp seed, contain all the essential amino acids and essential fatty acids necessary to maintain healthy human life. The hemp seeds showed a protein content about 25% and a lower content of carbohydrates; becoming a potential sport supplement candidates.

Ketogenic diets appear to be beneficial for endurance athletes and in sport nutrition. The higher protein and amino acid diet and the relative increased ratio of p-mTOR/mTOR uptake has a well-known effect on protein synthesis but also exert signaling effects on muscle protein deposition.

In this literature review we compared hemp seeds with others vegetal and animal source of protein, we underlined hemp seeds properties and their applicability in low carb and ketogenic diet as well in sport nutrition.

Keywords: *Hemp seed, Hemp protein, Ketogenic Diet, Sport Nutrition*

Introduction

Ketogenic diet (KD) is a nutritional approach consisting of high - fat and adequate protein content but insufficient levels of carbohydrates for metabolic needs (20 g/d or 5% of total daily energy intake), thus forcing the body primarily to use fat as a fuel source. An energy-sufficient KD diet with an adequate amount of protein (minimum 1.3-1.5 g/kg of body weight) is not an "extreme" diet apart from the very low carbohydrate levels (20 g/dof carbohydrates) (1)as such, it does not lead to metabolic imbalances that can have irreversible effects, if restrictive weight loss diets are repeated on a regular basis (1)

It should be underlined that, because of the intense physical activity of competitive athletes, there is an increased demand for protein (approximately 2.8protein kg1d-1). This is a fundamental point: an insufficient protein intake would be likely to negatively affect performance. (1)

We are considering appropriate an external support of vegetal protein supplement, for two different reasons. First of all, high quantity of animal proteins is considered responsible for increased risk of many diseases, as type 2 diabetes, cardiovascular disease, colorectal cancer (2, 3), whereas plant proteins have shown significant protective effects.(4). However, it is difficult to isolate the role of plant or animal protein on CVD and other risks (5). Additionally vegetable proteins are used in diets and as an ingredient in various food supplements for vegans, generating great interest from the companies in these sectors.

Role of Protein

Proteins represent the major structural component of muscle and they allowsynthesis of hormones, enzymes and hemoglobin. Proteins are also a source of energy even if they are not the primary choice.The 12nonessential amino acids (11 in children) can be synthesized by our body; the other ones, the essential amino acids, must be introduced by diet. The absence of any of these amino acids compromise the tissue growth, repair and maintenance. In addition, dietary proteins have a fundamental role in the various anabolic processes of the body and, as a result, high intensity training creates a greater protein requirement. This stems from the notion that if more protein or amino acids were available to the exercising muscle it would enhance protein synthesis.

The composition of various proteins may be so unique that their influence on physiological function in the human body could be quite different. The quality of a protein is vital when considering the nutritional benefits that it can provide. Protein is available in a variety of dietary sources. These include foods of animal and plant origins as well as the highly marketed sport supplement industry.A nimal protein are also associated with high intakes of saturated fats and cholesterol. Plant proteins, when combined to provide all the essential amino acids, provide an excellent source of protein, whereas on the contrary they reduce the intake of saturated fats and cholesterol, being instead rich in polyunsaturated fatty acids.

Casein

Caseins and whey proteins are the two main protein groups in milk. Caseins, representing about 80% of the protein content in bovine milk, are isolated from milk by acid precipitation.A1 casein is responsible of intestinal discomfort because, unlike the A2 casein, undergoes a proteolytic cut producing opioid peptides.

The milk allergy is mainly linked to casein and sometimes lead to anaphylactic shock. This kind of allergy is difficult to manage because of ubiquitous presence of caseinsusually applied in the food industry as an ingredient (6)

The caseins are made up mostly of hydrophobic amino acid residues therefore, having to subsist in the aqueous environment offered by the milk, they are dispersed in solution in the form of micelles. These are in turn formed by microglobules consisting of the hydrophobic association of the 4 types of milk casein.

The speed of absorption of dietary amino acids by the gut varies according to the type of ingested dietary protein, Because of their micellar nature, caseins are more difficult to digest and so they are considered slow-absorbing proteins (7).

Poorly digested residues alter the selective intestinal permeability going towards the lymph node stations. This event is the trigger for atopic diseases (like infant dermatitis, eczema, urticaria), inflammatory

(like hay fever, asthma, otitis, tonsillitis) and autoimmune deseases in genetically predisposed subjects. Finally, vegans do not use caseins, since they are an animal derivative product.

Soy Proteins

Thanks to the absence of gluten, soy proteins are suitable for celiac intolerant, even though it is also an allergen. In addition, as a protein supplement, soy proteins are distinguished in soy protein concentrate and isolated soy protein (with higher protein content), depending on the level of refining.

Soy protein concentrate contains at least 65% protein; his fitting is based on 3 steps:

1) acid extraction 2) extraction with water and alcohol 3) denaturation with steam and extraction with water. Soy protein concentrate has a low solubility. Isolated edible soy proteins, derive from the defatted flour with a high solubility in water (high NSI).

The aqueous extract is brought to a pH lower than 9, generating problems such as the isomerization of amino acids. Finally, the protein isolate is separated with centrifugation and multiple washes from soluble oligosaccharides.

Soybeans are particularly abundant source of phytoestrogens. Both concentrated and isolated proteins could contain genistein and daidzein isoflavones, diphenol compounds that bind estrogen receptors and are able to exert some similar effects to estrogen hormone, so they are classified as phytoestrogens. Approximately 27% of isoflavones are extracted from soy, while a residual remains. It becomes legitimate to claim that soy integrators could contain a low or high concentration of isoflavonoids that play a functional role on the organism (8).

Hemp seeds proteins

The seed of Cannabis sativa (hemp seed)is an expanding source of proteins (around 20-25%) easily digested, absorbed, and utilized. The amino acid composition may be influenced by genotypic variability or agronomic conditions such as soil fertility and processing that alters the ratio of seed components. Galasso et al., analyzed the proximate composition of 20 different genotypes of Cannabis s., from various countries, were analyzed. (9)

The authors showed that there is a mean protein content of 337g kg-1, with the higest concentration in Korean CAN20 (356) and Italian CAN40 (354) varieties. (9)

Italian variety had also lowest quantity of phytic acid (inositol hexaphosphate, IP6), that reduces protein digestibility; this data suggested that Italian CAN40 might be used to improve oil and protein content and, at the same time, to reduce the content of phytate in hemp through hybridization and selection. (9)

Mattila et al. analyzed nutritional value of various processed vegetal protein sources, the highest content of crude protein was found in faba bean, blue lupin and rapeseed press cake. The carbohydrates content in the hemp seeds was lower when compared with the others vegetal seeds and this data appears to be significant to validate the use of hemp seed and hemp seed protein in the ketogenic diet protocols and in sport nutrition (10).

The two main proteins in hemp seeds are the globulin edestin and albumin; edestin accounts for about 60–80% of the total protein content. This storage protein, which contains exceptionally high levels of arginine and glutamic acids, is easily digested and rich in all essential amino acids. (11-13)

In hemp isolated protein edestin usually composes about 70 % of total protein. Its solubility is lower with respect to isolated soy protein since in this case, the protein aggregation is foregone, possibly due to free sulfhydryl group and disulfide bond exchange between individual protein components.

Xian-Sheng Wang et al. investigated enzymatic hydrolysis of HPI with protease not only to modify the properties of food proteins, but also to increase their added value as potential health promoting products. Enzymatic hydrolysis resulted in the formation of more hydrophobic peptides: hydrolysate obtained exhibited antioxidant activities (DPPH radical scavenging ability, reducing power and Fe2+ chelating ability) related to higher contents of hydrophobic amino acids (13). In another paper twenty three peptide sequences were identified from a hemp seed protein hydrolysate. Identified sequences were found to contain about 80% hydrophobic amino acids that were responsible to enhances antioxidant activ-

ities and ACE- and renin- inhibitory properties. The hydrophobic properties of peptides could enhance their interactions with lipid targets or entry of them into target organs through hydrophobic associations with the cell membrane lipid bilayer.

WYT (Trp-Tyr-Thr), SVYT (Ser-Val-Tyr-Thr) and IPAGV (Ile- Pro- Ala Gly-Val) showed dual inhibition of ACE and renin while WVYY (Trp-Val-Tyr-Tyr) and PSLPA (Prp- Ser-Leu- Pro -Ala) only ACE inhibition (12).

Hemp seeds, soy seeds and casein final comparison.

Hemp isolated proteins (HPI) showed similar or higher levels of aspartic acid, glutamic acid, serine, arginine, leucine, phenylalanine, and lysine,in comparison to isolated soy protein (SPI), HPI also presented higher levels of arginine, methionine, and cystine and lower levels of aspartic acid, glutamic acid, and lysine, while the content of other ones was similar. Except lysine, HPI had higher levels of other essential amino acids than SPI. In comparison to casein, HPI had similar or higher levels of all amino acids except tyrosine, valine, leucine, methionine, and lysine. Compared with casein, vegetal seeds as hemp seeds and soybean showed greater aminoacidic content and HPI also had good profiles of essential amino acids, sufficient for the FAO/WHO suggested requirements for 2-5 year old children (Table 1).

Maki et al. reported that, by comparison between 25 g/d of SP insoluble fraction and 25g/d of total milk protein (TMP) for 4 weeks, SP and TMP respectively reduced total cholesterol (-7.4 and -3.6 %), LDL (-10.9 and -5.9 %), and non-high-density lipoprotein cholesterol (-10.8 and -3.9 %). Soy proteins were more effective in reducing cholesterol in relation to milk proteins; HDL cholesterol increased with soybean plant proteins and the level of triglycerides decreased compared to baseline, while for milk the trend was opposite (14).

Jason E. Tang et al. compared the acute response of mixed muscle protein synthesis (MPS) to rapidly (i.e., whey hydrolysate and soy) and slowly (i.e., micellar casein) digested proteins both at rest and after resistance exercise. The authors conclude that the feeding-induced simulation of MPS in young men was greater after whey hydrolysate or soy protein consumption than casein both at rest and after resistance exercise; moreover, despite both being fast proteins, whey hydrolysate stimulated MPS to a greater degree than soy after resistance exercise. These differences may be related to how quickly the proteins are digested (i.e., fast vs. slow) or possibly to small differences in leucine content of each protein (15).

Proteins from animal foods, such as eggs, dairy and meat, are typically harder to digest than most plant-based proteins. Easy digestibility involves better absorption of most of proteins while losing only a little to waste. This directly affects health and recovery in the human body as it pertains to the "bioavailability" of food ingested (16).

Hemp is a complete protein, providing all 20 amino acids, including the nine essentials. Soy contains a bit more protein than hemp, but hemp contains more digestible protein than soy. Hemp offers an eco-friendly, more easily digestible source of protein that supports muscle gain, provides fiber and adds to overall optimal nutrition. (table.1)

THC in hemp proteins

Cannabis sativa cultivation in most of countries has been prohibited due to the presence of the phytochemical drug component ?-9-tetrahydrocannabinol (THC). A low THC form of industrial hemp is now legal to grow in Canada and China, and the global market for low THC hemp is increasing rapidly (9).

All hemp plants considered in the study of Incoronata Galasso et al. were analyzed for THC content; they showed THC concentrations between 0.07 and 0.19% in their inflorescences, which are below the authorized limit of 0.20% on a DM basis set in Italy. The possibility of using industrial hemp with low levels of THC (<0.20% THC on a DM basis as established by the European Union and also by Italian law) is leading to a re-introduction of this plant in to the Italian production systems (9).

Leson et al. (19) evaluated the impact of extended daily ingestion of THC via hemp oil/seeds on urine levels of its metabolite 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) for four distinct single daily THC doses ranging from 0.09 to 0.6 mg. ingested in 15 THC-naive adults. None of the subjects

who ingested daily doses of 0.45 mg of THC screened positive at the 50-ng/mL cutoff. At a daily THC dose of 0.6 mg, one specimen screened positive. The highest THC-COOH level found in any of the specimens was 5.2 ng/mL, well below the 15-ng/mL confirmation cutoff used in federal drug testing programs. A THC intake of 0.6 mg/day is equivalent to the consumption of approximately 125 mL of hemp oil containing 5 microg/g of THC or 300 g of hulled seeds at 2 □g/g. These THC concentrations are now typical in Canadian hemp seed products. These concentrations appear to be sufficiently low to prevent confirmed positives from the extended and extensive consumption of hemp foods(19-20).

Discussion

Hemp protein is an industrial byproduct obtained after oil extraction of hemp seeds, the residue of extraction is high in protein and then processed into hemp protein supplements. A ketogenic diet D protocol establishes less than 5% of total daily energy from carbohydrates or less than 20 g of carbohydrate daily; in athletes, to preserve lean body mass, the daily requirement for protein should be in the range of 1.2 to 1.7 g kg-1 body weight.

This amount is needed to ensure the minimum quantity for body protein replacement and for gluconeogenesis. A lower quantity of protein may impair performance. On the other hand, an excessive protein intake (92.5 g kg-1 body weight or more than 25% - 30% of daily energy expenditure) might suppress ketogenesis. (1)

These data confirm that a rational protein supplementation has a central role in sport nutrition.

The quality of protein consumed is a modulating factor in determining postprandial resting and post-exercise muscle anabolism in young healthy men, both at rest and after resistance exercise. Thus, it appears that when providing an optimal dose of protein (10 g EAA), a rapid increase in EAA is important for supporting maximal rates of skeletal MPS(15).

Peptides derived from natural food sources have attracted growing interest because of their potential healt benefits associated with their low molecular weight, high biodisponibility, high activity, easy absorption and little or no negative side effects because of fast clearance from the blood.

Several studies have shown that peptides of plant and animal origin obtained through enzymatic hydrolysis can scavenge free radicals in addition to inhibiting physiologically relevant enzymes and may be effective antioxidant or antihypertensive agent.

Furthermore, hempseed has been consumed as a source of food throughout recorded history; the seed typically contains over 30% oil, with over 80% in polyunsaturated fatty acids (PUFAs), and about 25% protein, especially edestin and albumin, which are easily digested and rich in all essential amino acids (11).

The ketogenic diet is able to provide adequate amounts of energy and protein to athletes (16), with a dual purpose, to avoid protein deficiency and to induce a state of fasting, resulting in changes in the metabolic pathways and processes such as autophagy and stress resistance (17). In comparison to the others seeds, hemp seeds have a lower carbohydrates content (2), which makes them particularly usefulin the ketogenic protocols in sports and performance.

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Table.1

Content of amino acid (mg/g of protein) in hemp protein isolate (HPI), soy protein isolate (SPI) and casein.

Aminoacids	HPI	SPI	CASEIN
Asp	98.0	118.1	63.1
Glu	168.1	212.9	190.0
Ser	54.0	54.8	46.0
Gly	41.7	38.6	16.1
His	29.3	29.0	27.2
Arg	103.2	75.7	33.3
Thr	47.6	41.1	37.2
Ala	47.0	38.3	27.1
Pro	47.2	52.9	-
Tyr	38.2	37.1	55.0
Val	51.8	44.1	60.2
Ile	41.5	44.8	49.1
Leu	69.1	70.0	84.2
Met	14.5	9.3	26.4
Cys	1.7	0.6	0,ì.4
Phe	49.6	53.1	45.5
Lys	43.3	53.9	71.2

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MITOCHONDRIAL DYSFUNCTION AND FIBROMYALGIA: KETOGENIC DIET AS A POSSIBLE THERAPEUTIC APPROACH

De Vizio Deborah; Onnembo Maria Carmela; Parisella Chiara; Castaldo Giuseppe
Post Graduate Universitary course master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II, Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

Fibromyalgia (FM) is a worldwide diffuse musculoskeletal chronic pain condition that affect approximately 5% of the population worldwide. The etiology is unknown; however, recent studies suggest that mitochondrial dysfunction has been involved in the pathophysiology of this condition.
It is supposed a possible relationship between mitochondrial dysfunction, oxidative stress, and inflammation in FM.
It is hypothesized that reactive oxygen species (ROS), caused by oxidative and nitrosative stress, by inhibiting mitochondrial function can be involved in muscle pain and central sensitization as typically seen in these patients.
Mitochondrial dysfunction has been shown in muscle cells of FM patients, which could explain the muscle pain.
Additionally, if mitochondrial dysfunction is also present in central neural cells, this could result in lowered ATP pools in neural cells, leading to generalized hypersensitivity and chronic widespread pain.
Therefore, targeting increased ROS by antioxidants and targeting the mitochondrial biogenesis could offer a solution for the chronic pain in these patients.
It has been reported that mutation in cytochrome b gene of mitochondrial DNA (mtDNA) in a family with FM with inflammasome complex activation cause mitochondrial dysfunction.
Some evidence suggests that mutation in UNG1 gene causes oxidative stress, and mitochondrial dysfunction and that exposure the organism to ketogenic diet, or of cells to ketone body *in vitro*, elicits compensatory mechanisms acting to augment mitochondrial mass and bioenergetics via the PGC1α-SIRT3-UCP2 axis.

Keywords: *Fibromyalgia, Mitochondrial dysfunction, Ketogenic diet.*

Introduction

Mitochondrial dysfunction is a major cause of neurodegenerative and neuromuscular diseases of adult age and of multisystem disorders of childhood.
However, no effective treatment exists for these progressive disorders.

Mitochondria are essential organelles present in eukaryotic cells.
One of the primary functions of mitochondria is ATP production via the oxidative phosphorylation (OXPHOS) system through a series of reactions mediated by complexes encoded by both nuclear DNA and mitochondrial DNA (mtDNA) [1,2].
Moreover, mitochondria play crucial roles in many other metabolic, regulatory and developmental processes and in a variety of pathological mechanisms [2,3].

Fibromyalgia (FM) is a common chronic pain syndrome accompanied by other symptoms such as fatigue, headache, sleep disorders and depression.
Despite the fact that it affects up to 5% of the general population worldwide, its pathogenic mechanism remains still largely elusive.
Oxidative stress and mitochondrial dysfunction have been found to be important in FM pathogenesis [4-6].
Supporting these hypothesis, cells from patients with FM have reduced mitochondrial mass, reduced mitochondrial respiratory chain activity and impaired bioenergetics [6-12].
A limited number of studies about muscular mitochondrial function in FM patients are available and data are conflicting.
Some did not found any difference in mitochondrial enzyme activity compared to a matched control group, but only a decreased mitochondrial content[13].
Other have shown abnormal mitochondrial degeneration [14-16] and severe deletions of oxidative genes in mitochondrial DNA (mtDNA) [14].
These changes result in reduced ATP synthesis and increased lactate concentrations [15].

Patients with fibromyalgia may experience high levels of lactic acid at rest [16,17] or during aerobic physical activity [18]. However, other studies show that changes in glycolysis and lactic acid at rest or during physical activity were slight increased among fibromyalgia patients [19,20].

The ratio of lactic acid production in patients with fibromyalgia, as well as the influence of this metabolite in the pathophysiology of these diseases, is controversial or nonexistent.

In fibromyalgia there is an inappropriate switch from aerobic mitochondrial production of energy (via oxidative phosphorylation) to glycolysis (very inefficient anaerobic production of energy, not requiring oxygen, but with a large build up of lactic acid). Lactic acid in the short term causes immediate muscle pain. Normally this is remedied by the person slowing down or stopping because of the pain, cells switch back into aerobic metabolism and the lactic acid is quickly cleared away and got rid of.

This does not happen in fibromyalgia because the sufferer can not make ATP quickly enough to shunt lactic acid back to acetate and the sufferer is completely pole axed by ongoing lactic acid burn with inability to move and possibly secondary damage from lactic acid which, for example, is good at breaking down the collagen matrix which holds cells together. That is to say, the lactic acid may cause microscopic muscle tears, which would present as local areas of soreness and would trigger a process of healing and repair by the immune system. There would also be excessive release of free radicals as the immune system repairs. This may well cause further muscle damage in people with poor antioxidant system.

The most obvious reason for the switch into glycolysis is mitochondrial failure.

If mitochondria cannot supply sufficient energy to cells, cells will switch into glycolysis with a resultant build up of lactic acid.
Furthermore, cytokines, especially IL-1β, may play a role in FM [15,21-23] and reactive oxygen species (ROS) have also been shown to be an important activator of inflammasome-mediated inflammation[24].
A ketogenic diet (KD) is a high fat, low carbohydrate and restricted protein diet that is beneficial for treatment of drug-resistant epilepsy [25-30].

This diet has also been proposed as a therapeutic diet in several neurological diseases such as autism [31], amyotrophic lateral sclerosis [32], Huntington disease [33], Alzheimer`s disease [34], and Parkinson`s disease [35].

A KD mimics the metabolic state of long-term fasting by fatty acids being oxidized to ketone bodies. Ketone bodies produced in the liver act as an alternative energy source, compensating for limited availability of glucose during fasting [36].

KD increases mitochondrial mass and levels of proteins mediating electron transport (nuclear DNA encoded complex II), antioxidative defense (SOD2), mitochondrial fission (FIS1), and a longevity-associated deacetylase (SIRT1) in mutUNG1-expressing mice [37].

Both KD and βHB change the expression of mRNAs and proteins (UCP2, PGC1α, Drp1 and Mfn1) involved in mitochondrial dynamics and biogenesis.
So probably the PGC1α-SIRT3-UCP2 axis is activated by KD or βHB treatments to rescue mitochondrial function.

The diet decreased the amount of cytochrome c oxidase negative muscle fibers, a key feature in mitochondrial deficiencies, and prevented completely the formation of the mitochondrial ultrastructural abnormalities in the muscle.

Results

Mitochondrial dysfunction in FM

Several hypothesis have been proposed around the etiological origin of FM and its symptoms. Clinical studies have produced evidence that FM may be associated with immune dysregulation of circulatory levels of pro-inflammatory cytokines, affecting the neural dysfunction of pain-related neurotransmitter. Cytokines, depending on their concentration, induce symptoms, such as fatigue, fever, sleep, pain, and myalgia, all of which usually developed in FM patients. Alterations in pro-inflammatory cytokine levels have been observed in serum and biopsies of FM patients. In addition, increased levels of IL-1Ra and IL-6 have been found in the supernatants of cells from FM patients *in vitro* [38].

Both mitochondrial dysfunction and inflammation have been implicated in the pathophysiology of FM. Clinical trials have investigated the possible relationship between mitochondrial dysfunction, oxidative stress, and inflammation in FM.

They studied 30 women diagnosed with FM and 20 healthy women. Blood mononuclear cells (BMCs) from FM patients showed reduced level of coenzyme Q10 (CoQ10) and mtDNA contents and high level of mitochondrial reactive oxygen species (ROS) and serum tumor necrosis factor (TNF)-alpha and transcript levels [39].

Mutation in cytochrome b gene of mitochondrial DNA.

To assess if mitochondrial dysfunction is associated with mutations in mitochondrial genes in FM, mtDNA from blood cells of five patients with FM and intolerance exercise were sequenced.
After mtDNA sequence in patient 3 with FM, were found a mitochondrial homoplasmic mutation m. 15804 T>C in the mtCYB gene in a patient and family, which was maternally transmitted [40].
The new mutation was detected in a complex III protein, skin fibroblasts from patient 3 showed reduced levels of complex III activity. associated with coenzyme Q10 (CoQ10) deficiency reduced ATP levels, high levels of mitochondrial ROS, reduced mtDNA contents and low rate of proliferation of the cells.
Inhibition of complex I or III of the mitochondrial respiratory chain has been shown to induce ROS production and NLRP3-inflammasome activation [41-43].Therefore, they analyzed the activation of inflammasome-related proteins increased NLRP3, active caspase 1 (p20) and IL-1β (p17) protein expression levels in patient 3 fibroblasts (figure 3A) and increased levels of IL-1β and IL-18 in serum from the patient suggesting inflammasome activation.

A ketogenic diet increases mitochondrial biogenesis and dynamics

Ketogenic diet increases mitochondrial mass in a mouse model in which an induced mutated version of UNG1 (mutUNG1) causes mitochondrial dysfunction [44].

Furthermore a ketogenic diet increases the level of UCP2 in the CA1 pyramidal cell somas and axon terminals of mutUNG1-expressing mice.
In the WT fed SD, the UCP2 density was higher in the terminals than in the somas. The density of UCP2 in somas and in axon terminals was raised significantly in mutUNG1-expressing mice fed a KD compared to mutUNG1-expressing mice (or WT littermates) fed a SD.

Clinical trials have shown that hippocampal pyramidal neurons in mutUNG1 mice, as well as cultured rat hippocampal neurons and human fibroblasts with H_2O_2 induced oxidative stress, improve markers of mitochondrial biogenesis, dynamics and function

when fed on a KD, and when exposed to the ketone body β-hydroxybutyrate, respectively, by upregulating PGC1α, SIRT3 and UCP2, and (in cultured cells) increasing the oxygen consumption rate (OCR) and the NAD+/NADH ratio [40].

Scientists explored the proteins in rat hippocampal neurons and human fibroblasts treated with βHB (7 mM, 24 h and 48 h respectively), after being challenged with H_2O_2 treatment (50 μM, 48 h) and in hippocampi of mutUNG1 mice on a KD [45]. Western blotting analysis showed that there was a significant increase of PGC1α protein, the "master regulator" of mitochondrial biogenesis, in mutUNG1-expressing mice fed a KD ($p < 0.008$) compared to the model mice fed a SD. Furthermore, PGC1α protein content was decreased in human fibroblasts and in rat hippocampal neurons treated with H2O2, a pharmacological way to induce OS and damage mitochondrial function *in vitro*, and the decrease was rescued by co-treatment with βHB and H2O2. An increased level of mitochondrial UCP2 has previously been reported to be involved in mitochondrial biogenesis [46]. The level of UCP2 was increased in mutUNG1- mice fed a SD, compared to WT fed a SD, and further raised in the transgenic model mice fed a KD. The tendency to increased level of UCP2 in response to KD did not reach statistical significance, but was supported by *in vitro* experiments, which suggested that βHB could reverse the reduction of UCP2 by H2O2 treatment. As expected, βHB treatment showed the ability to increase the level of UCP2 in hippocampal neurons and the level of UCP2 followed the same expression pattern with H2O2 and βHB treatments.

Studies investigated key proteins controlling mitochondrial fusion, such as mitofusion 1 (Mfn1), and dynamin-related protein 1 (Drp1), which mediates fission dynamics. A significant increase of Drp1 expression was found in mutUNG1-expressing mice fed a KD compared to WT littermates on SD ($p < 0.013$), which might shift more mitochondria towards fission in response to a KD diet. The same tendency was also noticed when fibroblast and hippocampal neuronal cells were treated with H2O2 and βHB elevated the level of Drp1 [47].

Discussion

Mutation in Cytochrome b gene of mitochondrial DNA

Many symptoms associated with mitochondrial diseases are commonly presented in patients with FM: exercise intolerance, fatigue, myopathy and various neurological complaint [48]. Moreover, mitochondrial diseases have been usually related to maternal inheritance [49]. However, in patients with FM, although the inheritance factor has been documented, the absence of known pathogenic mechanisms complicates family studies. Familial aggregation studies revealed high possibilities of inherited factors in FM. Thus, Arnold and Coworkers [50] compared FM with other pain diseases such as rheumatoid arthritis and they found a higher inherited factor in the FM group. In this respect, Buskila and Coworkers [51] observed a high prevalence with FM among offspring of FM mothers. Supporting these findings, in this study it has shown for the first time a mutation in mtDNA in a family diagnosed with FM. As expected, the mutation was maternally transmitted and was present in all family members with typical clinical manifestation of FM. Pathophysiological studies showed evidences of a mitochondrial dysfunction in patient cells that was restored by CoQ10. The mutation found in this study converted a highly conserved valine at position 353 to alanine in the transmembrane functional domain of mtCYB, which could affect transfer of electrons from ubiquinol (reduced CoQ10) to cytochrome c, impair the utilization of energy to translocate protons from inside the mitochondrial inner membrane to the outside and increase ROS production [52].
In this study it was proposed the implication of the inflammasome complex in the pathophysiology of mitochondrial diseases. Inflammasome has emerged recently as an unexpected sensor for metabolic stress and it has been implicated in several diseases in which mitochondria play a central pathogenic role [53]. Inhibition of complex I or III of the mitochondrial respiratory chain has been shown to induce ROS production and NLRP3-inflammasome activation [54]. Furthermore, inflammation has been proposed to participate in the pathophysiological mechanisms of mitochondrial disorders [55]. In addition, inflammatory events have been observed in patients with a 3251 A>G mutation supporting the hypothesis that mitochondrial diseases induce a pathological inflammatory response [56]. Another interesting result showed in this study is the effect of the NLRP3 inhibition on the fibroblasts from the patient. A correct cell growth is an important marker of metabolic status in

the cell. Fibroblasts from mitochondrial diseases have been shown to have a relatively reduced growth, [57] so the increment of cell growth of the fibroblasts from patient 3 shows a possible improvement in cellular metabolism. Furthermore, this improvement demonstrates that inflammasome activation has a pathophysiological role in the phenotype of fibroblasts from patient 3. As inflammation has been shown to induce mitochondrial dysfunction, [58] an inhibition of several cytokines could prevent mitochondrial damage and cell death.

According to this, the inhibition of inflammasome could be a new pharmacological target in mitochondrial diseases. The most significant implication of these data could be the potential role of inflammasome complex activation and the subsequent IL-1β release in the different affected tissues in mitochondrial diseases (brain, muscle, kidney, heart, eye) [59, 60]. Probably, a high content of defective mitochondria could induce NLRP3-inflammasome activation by increased ROS production. In this sense, inflammasome inhibition could represent an interesting new therapeutic option in mitochondrial diseases. However, taking into account that ROS serve as important inflammasome-activating signals, antioxidant treatment can also be effective in these diseases [61]. Pharmacological approach in mitochondrial diseases is based on removing noxious metabolites, using ROS scavengers and administering different compounds as CoQ10 [62].

Indeed, mitochondria-targeted lipophilic antioxidants selectively block mitochondrial oxidative damage and prevent some types of cell death. Consequently, inflammasome complex which is activated by mitochondrial ROS production could be selectively inhibited by lipophilic antioxidants. In conclusion, it was proposed the indication of mtDNA sequence analysis in patients with FM with evidences for maternal inheritance. The presence of symptoms similar to mitochondrial myopathies could unmask mitochondrial diseases among patients with FM who could benefit from genetic diagnostic testing by mtDNA sequence analysis and be eligible for treatment with CoQ10 and/or another mitochondrial targeting compound. Furthermore, the inflammasome complex as a stress sensor could be implicated in the damage and the symptoms of the mitochondrial diseases.

In conclusion, it is possible to assert that an inhibition of several cytokines could prevent mitochondrial damage, that the inhibition of inflammasome could be a pharmacological target in mitochondrial disease and that KD and βHB are demonstrated to be involved in mitochondrial biogenesis.
In fact both KD and βHB change the expression of mRNAs and proteins (UCP2, PGC1α, Drp1 and Mfn1) involved in mitochondrial dynamics and biogenesis probably by activating the PGC1α-SIRT3-UCP2 axis to rescue mitochondrial function.

A KD increases the level of the "master regulator" for mitochondrial biogenesis, PGC1α and ketones upregulate the mitochondrial deacetylase, sirtuin 3 (SIRT3) in mice. The level of UCP2 increases protein which helps to reduce oxidative stress (OS). Due to the complex network of interactions, the open arrows do not represent a simple sequence of events. For example, UCP2 may increase SIRT3 to reduce mitochondrial OS and activate PGC1α. A KD also tends to induce a higher level of mitochondrial fission by increasing the level of Drp1, and to reduce fusion through reducing Mfn1. In cultured human fibroblasts and rat hippocampal neurons (green and purple respectively, right part of gure), β-hydroxybutyrate (βHB) shows similar molecular effects as a KD, increasing the levels of PGC1α, SIRT3 and UCP2. The level of NAD^+/NADH ratio, a measure of mitochondrial activity was significantly elevated by βHB and the opposite effect was exhibited by H_2O_2. The mitochondrial respiration was rescued by βHB in H_2O_2-exposed fibroblasts. The effects of KD and βHB were beneficial in rescuing mitochondrial damage. (However, KD in sum aggravates mitochondrial dysfunction (grey arrow) in mutUNG1-expressing mice, presumably due to the self-propagating action of mutUNG1, which means that enhanced formation of mitochondria results only in larger masses of malfunctioning mitochondria, which generate increased amounts of ROS when exposed to KD.) Thin solid arrows indicate increase or decrease.

Concluding Remarks and Future Directions

Mitochondrial dysfunction in FM

Most studies that have up to now examined the role of inflammation in FM have been incomplete

and contradictory showing several discrepancies. However, according to these results, the correlation between TNF-alpha, CoQ10, and mitochondrial ROS levels may explain that inflammation, in several FM patients, could depend on mitochondrial dysfunction, thus identifying a new subgroup of patients in FM. Increased mitochondrial ROS in FM would result from enhanced oxidative phosphorylation. Recently, it has been demonstrated that oxidative stress could be implicated in the severity of clinical symptoms in FM, therefore, antioxidant therapy should be examined as a possible treatment in FM. Blockade of ROS production by mitochondria would provide a new therapeutic strategy to decrease symptoms in FM and other inflammatory states. Moreover, CoQ10 treatment could be used as an alternative therapy in FM and this should be the aim of further studies.

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SAFETY AND TOLERABILITY OF THE KETOGENIC DIET IN PEDIATRIC PATIENTS WITH REFRACTORY EPILEPSY

D'Elia Maria, Conte Giovanna, Busillo Alfonso, Castaldo Giuseppe

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

It has been known for centuries that fasting can suppress seizure activity in patient with epilepsy. Ancient Greek texts contain references to dietary therapies for epilepsy, stemming from the observation that starvation stops seizures. In 1921, it was suggested that a ketogenic diet, designed to induce and sustain the metabolic effects of fasting, might have the same beneficial effects. Actually the Keto diet is gaining popularity among famous actors who want to lose weight and feel much better and online health sites tout the low-carbohydrate, high fat ketogenic diet.

However, this diet high in fat and low in carbohydrates remains the most important non-pharmacologic treatment strategy for children with epilepsy whose condition is resistant to medication. This article provides a general overview of the ketogenic diets and then focuses on the issues relating to the use of medicines in patients on these diets.

Keywords: *Ketogenic Diet, Epilepsy, Pediatric*

Introduction

The ketogenic diet is a strict diet consisting of minimal carbohydrate and protein intake and increased fat intake. It is used as a nonpharmacologic mechanism to control refractory epilepsy in children. Ketogenic diets mimic the body's response to starvation by using fat as the primary energy source in absence of an adequate dietary carbohydrate source. As we know, under normal metabolism, the body metabolizes carbohydrates into glucose, the fastest source of energy for the body and the sole energy source for the brain. In a fasting state, amino acids cannot provide an adequate energy source for the brain and fatty acids cannot cross the blood brain barrier. The liver uses the fatty acids to make ketone bodies, which can cross the blood brain barrier and substitute for glucose as an energy source. (1) The mechanism of how ketosis control seizures is unknown, however, one theory is that ketones have an anticonvulsant effect when crossing the blood brain barrier. Scientists observed that when the body is deprived of glucose, ketone bodies, acetoacetic acid (Ac Ac) and ?-hydroxybutyrate (BHB) are formed from the breakdown of fat and cross the blood-brain barrier where they can be used by the brain for energy. So that, the Ketogenic diet's aim is to simulate the body's response to starvation by inducing production of these ketone bodies. (2) As a matter of fact, the ketogenic diet consists in a high fat, adequate-protein (1 gram/kg) and low-carbohydrate diet with precisely calculated proportions so that 75 to 90% of calories are from lipid source.

Multiple theories have been postulated, considering the optimization of brain energy metabolism, changes in neurotransmitter concentrations, changes in cell membrane properties and the direct effect of ketone bodies, as well as in cognitive and behavioral improvements. (3)

Many researchers believe that ketosis is not the primary way ketogenic diets work but rather the metabolic shift that occurs with the treatment. In a recent review, evidence for four possible mechanisms was summarised: carbohydrate reduction (2-deoxy-D-glucose, a glucose analogue, partially inhibits glycolysis and has demonstrated antiseizure activity in animals), activation of ATP-sensitive potassium channels by mitochondrial metabolism, inhibition of glutamergic excitatory synaptic transmission and inhibition of the mammalian target of rapamycin. (4)

As Simon Heales, a clinical chemist at University College London has showed, the decanoic acid increases mitochondrial numbers in brain cells.

The mitochondria produce ATP, which helps transmit signals along the neurons, and its increased production could provide better control of potassium channels related to the seizure gates that Yellen mentions (Gary Yellen at Harvard Medical School, has identified a protein that switches a cell's fuel glucose to ketone bodies and in so doing opens a type of potassium ion channel in neurons that can dampen electrical activity) (5).

The ketogenic diets survey

Multiple variations of ketogenic diets exist, but the most commonly prescribed are the classic ketogenic diet, the modified Atkins diet, the low-glycemic index treatment diet, the medium-chain triglyceride (MCT) diet, and the modified MCT diet. The classic ketogenic diet is the oldest of the diets and it is one of the strictest of the diets. Russel wilder, a metabolic-disease expert at the Mayo Clinic in Rochester, Minnesota, devised the classic ketogenic diet in 1921. Wilder described a diet high in fat content (i.e. long -chain satured fats) with a low percentage of both proteins and carbohydrates (a distribution of 85-90% long chain fatty acid, 6-8% protein, and 2-4% carbohydrates. (6)

Because the classical 4:1 diet was considered unpalatable and, hence, associated with poor compliance, in the 1960s, researchers discovered that medium-chain triglycerides (MCTs)- found in coconut oil-provide greater ketogenic effects than normal dietary fats, which are mainly long-chain triglycerides. The MCT diet, created by the late University of Chicago neurologist Peter Huttenlocher, is restrictive but incorporates more carbohydrates and protein because MCTs are absorbed more easily by the body than long-chain triglycerides. The MCT diet 3:1 (fat-nonfat) ratio, is easier to prepare with a greater flexibility with protein and carbohydrate allowance. (7)

Kossoff, for his part, designed the modified Atkins diet (MAD) in 2003. Kossoff had the idea for the diet observing similar results in people who relaxed the

restrictions of the ketogenic diet. In common with the Atkins weight-loss diet, MAD does not involve calorie counting, but limits carbohydrates and encourages fat consumption. The modified Atkins dietary requirements are comprised of 60-70% long-chain fatty acid, 25-30% protein, and 5% carbohydrate. (8)

In 2005, the low-glycemic index treatment (LGIT) came from observation that patients on a ketogenic diet had extremely stable glucose level. In addition to high fat, the LGIT includes only carbohydrates with a glycemic index lower than 50, which means that these foods do not tend to increase blood glucose levels. The diet offers more variety- permitted low-glycemic-index foods include whole grains green vegetables and berries. The glycemic index scores individual carbohydrates based on each food item's effect on raising blood glucose within two hours of consumption. The diet's dietary distribution is 60-70%long-chain fatty acid, 20-30% protein, and 10% carbohydrate. (9)

The success of the MCT diet suggests another pathway to seizure-protection. Normal dietary fat contains mostly long-chain triglycerides- Medium chain triglycerides (MCTs), such as decanoic and octanoic acid, are absorbed more effectively and are more ketogenic than LCTs because they generate more ketones per unit of energy when metabolized. The modified MCT diet combines the use of both long-chain and medium-chain fatty acids and it distributes the calories as 30% MCT oil; 40-50% long-chain fatty acids, 10-20% protein, and 5-10% carbohydrates. The MCT diet is disfavored since MCT oil is more expensive than other fats and it is not covered by insurance companies. (10)

Efficacy and adverse effects of the ketogenic diets

Ketogenic diets have been used to treat seizures that are both idiophatic and symptomatic. Patients with the following seizure types have been evaluated for efficacy of the diet: myoclonic, focal motor, atypical absence generalized tonic and tonic clonic. All kinds of the diet formulations are equally efficacious overall efficacy ranges from 33-67%. There is no agreement on how a ketogenic diet can help control epileptic seizures. It's likely that there isn't a single mechanism involved and it may work in a different way in different children.

Most of the studies that have been published use seizure frequency, intensity and compliance as end points of the study. It appears that children younger than 10years old, respond the best to the diet from a physiologic perspective. These children tend to be more prone to ketosis than older children or adults. The brain's ability to extract ketone bodies and utilize them as an energy source decreases with age. Younger children are also more dependent on someone else preparing their diet than are older children or adults. Surprisingly, a majority of the children do not mind the taste of the high-fat diet, especially if they are involved in the selection of foods and their parents explain the importance of the special diet to them.

The seizure protection afforded by the ketogenic diet may be reversed in as little as 1 hour of a patient receiving a glucose infusion. This highlights the importance of strict adherence to the diet. (11)

But ketogenic diets, like any other treatment, are not without risk and require monitoring of complications- Short-term adverse effects include dehydration; mild metabolic acidosis, and hypoglycemia during fasting Long-term adverse effects include nephrolithiasis, constipation, vitamin and mineral deficiences and decreased bone mineral density. (12)

So that, all ketogenic diet's patients should receive a daily multi-vitamin as well as calcium supplementation- Carnitine, found in milk and red meats, has also been found deficient in patients placed on these diets. Levocarnitine is available as a supplement in tablet form the liquid base is sucrose syrup. (13)

The ketogenic diet and the implications for use of medicines

Children who start on the ketogenic diet are often already taking at least one anticonvulsant as well as various other medications. So that it is very important not only to determine the carbohydrate content of these medications, but also to consider the potential of the diet to affect drug serum levels and effects. In a retrospective cohort study including 115 children, those on phenobarbital in combination with the ketogenic diet were significantly less likely to have >50% seizure reduction (p=0.003) than those combining the diet with other anticonvulsants.

Conversely, those on Zonisamide at onset of the ketogenic diet were more likely to have >50% reduction in seizures (p=0.04). (14)

There is a historical perception that Valproate should not be used with the ketogenic diet. This stems from concerns for idiosyncratic reactions such as hepatotoxicity and given the fact it is a short-chain fatty acid. (15)

Knowing these ketonic diet anticonvulsant interactions may be valuable to clinicians when deciding which anticonvulsant to discontinue, maintain or possible start in a child with a suboptimal response to the ketogenic diet.

There is limited evidence that a type of ketogenic diet is more effective in reducing the frequency of seizures than another. The decision to apply one type of diet or another and when to use the ketogenic diet together or alone and in polytherapy, should also be based on costs, preferences and safety of the treatment.

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USE OF SUCROSE SUBSTITUTES IN DIABETIC PATIENT: ARE THEY GOOD?

Zanna Adolfo G. R.[1], Vanacore Antonio[1], Cereda Emanuele [2]

[1] Post Graduate Universitary Course in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

[2] Clinical Nutrition and Dietetics Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Email address: nutriketo@unisa.it

Abstract

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Sweeteners, both naturals and artificials, are thought to be beneficial for diabetics where sucrose can be a problem. These low-calorie sweeteners seemingly safe, provide sweetness without calories, and provide a choice of sweet food to those who otherwise cannot consume them. However, while sweeteners may indeed restrict calories, the question is "Are they good for diabetic patient?". It seems that most of them (especially artificial sweeteners) have no beneficial effects on glucose control of diabetes, rather possibly they increase its risk. Recent safety concerns about artificial sweeteners on glucose control came from a large epidemiological study and physiological studies in human. However weight gain and glycemic control is still linked to total energy consumption. Furthermore, they may have other safety concerns like the increased risk of developing cancer. In respect to natural sweeteners, excessive fructose intake can be a risk factor of the development and progression of diabetic nephropathy (DN) in diabetic patients and it is also known as an inducer of obesity, dyslipidemia, due to stimulation of triglyceride synthesis and fat deposition in the liver. Nevertheless, a recent study has pointed out to the anti-diabetic properties of Stevia.

Keywords: *Diabetes, Sucrose substitutes, Natural and Artificial Sweeteners, Safety Concerns.*

Introduction

Sweeteners play an important role in human diet as they are of great importance to the food industry and dieticians. Among sweeteners there are compounds which have a sweet taste and contain no calories or which sweetness is so intense to enable a use at very low concentrations. They can be classified based on their origin (natural or synthetic), the technological function (sweeteners and fillers), texture (powders and syrup), and nutritional value (caloric and non-caloric). Natural sweeteners include carbohydrates (glucose, fructose, maltose and lactose), sugar alcohols (erythritol, isomalt, lactitol, maltitol, mannitol, sorbitol and xylitol), thaumatin and Stevia, while synthetic sweeteners include acesulfame-k, aspartame, cyclamate, saccharin and sucralose. (1)

Carbohydrates are the most important and widely recognizable natural sweeteners, which are also a source of simple and quickly digestible energy. Can be divided into i) digestible, which are a source of energy, and ii) undigested in the gastrointestinal tract, i.e. some component of dietary fiber. Among the digestible carbohydrates, there can be found complex compounds such as starch and glycogen, and sugars, namely mono- and disaccharides. The nutritional requirements for carbohydrates are based on the average minimum amount of glucose that is utilized by the brain. Recommended Dietary Allowance (RDA), which is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all healthy individuals in a group, for dietary digestible carbohydrates amounts up to 130 g/day.

Sugar alcohols (polyols) are low digestible carbohydrates, which occur naturally in fruits, vegetables and human organism. These compounds have lower nutritional value than sugar table (sucrose), and supply only 2 kcal/g versus 4 kcal/g of the common sugars included in carbohydrates, due to incomplete digestion in the human organism. The glycemic index of sugar alcohols is substantially smaller than common sugars, thus, they are frequently used to sweeten food products for diabetics. Unlike common sugars, polyhydric alcohols are recognized as food additives, which may be used in accordance with good manufacturing practice (GMP) as acceptable daily intake (ADI) was not specified for them. (2)

Thaumatin is a mixture of two proteins (thaumatin I and thaumatin II) that is extracted from the arils of the fruit of *Thaumatococcus daniellii*. It characterizes with sweetness about 2000 times higher than sucrose (on a weight basis) and a licorice after-taste. According to JECFA (Food and Agriculture Organization/World Health Organization's Joint Expert Committee on Food Additives) and EFSA, thaumatin is safe for use as a sweetener with no ADI specified, which means it can be used according to GMP. There is no data of thaumatin mutagenic, allergenic or teratogenic effects. (3)

Stevia rebaudiana Bertoni, which is a shrub of the Asteraceae family originating from the northeast part of Paraguay, is the source of non-caloric sweetening compounds, i.e. steviol glycosides. Stevia is 200 times sweeter than sucrose. Besides sweetening function, it has been also found that stevioside expresses antimicrobial activity. Furthermore, in clinical studies, stevia glycosides expressed antihyperglycemic, insulinotropic, glucagonostatic and anti hypertensive effects (4,5,6). According to JECFA steviol glycosides are safe for human consumption as a non-medical ingredient up to 4 mg/kg b.w./day. Up to date, there is scarce information on Stevia extracts allergenicity.

Acesulfame-K is the generic name for the potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2dioxide. It was approved in the USA in 1988 for specific uses; in 1998 the FDA approved acesulfame-K for use in beverage and in 2003 it was approved for general use in food, excluding meat or poultry (FDA, 2006). Acesulfame-K is 200 times sweeter than sucrose and has no calories. A study conducted by Mukherjee and Chakrabarti (7) on the cytogenicity of this sweetener indicated that, when the dosage administered to mice was within the ADI of 15mg/kg of body weight, the number of chromosomal aberrations was not significant compared to control mice. However, at higher doses (up to 2.250mg/kg), acesulfame-K was found to be clastogenic and genotoxic.

The dipeptide aspartame (L-aspartyl-L-phenylalanine methyl ester) was first approved by the FDA in 1981 as a tabletop sweetener. In 1966 it was approved as a general-purpose sweetener in all foods and drinks (FDA, 2006). It is 200 times sweeter than sucrose. In humans, aspartame doses of 2 to 100 mg/kg resulted in dose-related increases in phenylalanine without an

observed effect on behavior or cognitive performance (8,9). In sub-chronic dosing studies, aspartame doses of 30 to 77 mg/kg/d over 13 weeks in 126 children and adolescents (and a similar study in young adults with a dose of 36 mg/kg/d) showed no significant impact on renal or hepatic function, hematologic status, ophthalmic examinations, or plasma lipid profile (10,11).

Cyclamate (cyclamic acid) has been approved by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982 and by the Scientific Committee on food (SCF) of the European Commission in 2000 and by the European Food Safety Authority (EFSA). The Cancer Assessment Committee of the US Food and Drug Administration confirmed the safety of cyclamate in 1984 and the US National Academy of Sciences did the same in 1985. The Acceptable Daily Intake (ADI) for cyclamate has been set at 11 mg/kg/b.w. by JECFA and at 7 mg/kg/b.w. by the SCF.

Saccharin is formed by initial reaction between toluene and chlorosulfonic acid, it is then converted to a sulfonamide with ammonia, then oxidized to a benzoic acid and heated to form the cyclic imide. Saccharin has no calories and is 300 times sweeter than sugar (FDA, 2006). Exposure studies of saccharin provided both positive and negative results, including the potential to induce cancer in rats, dogs and humans. In 2004, Weihrauch and Diehl (12) published a review on artificial sweeteners. They reported that more than 50 studies had been published about saccharin in laboratory rats. Their review of first-generation rats indicated that none of the groups demonstrated significantly higher incidence of neoplasm in the saccharin-fed animals over controls.

Sucralose is a sucrose molecule in which three of the hydroxyl groups have been replaced by chlorine atoms. It is 600 times sweeter than sucrose and contains no calories. Sucralose was approved by the FDA in 1998 for use in 15 food categories, and in 1999 the FDA expanded its use as a general purpose sweetener in all foods. Toxicology studies of sucralose show little effect, the most significant finding being shrunken thymus glands with diets providing 5% of sucralose in the daily intake. Review and critical evaluation of available data, including a special immunotoxicity study, clearly demonstrated that thymic changes were not a manifestation of intrinsic toxicity, but rather an exacerbation of the normal process of involution resulting from a nutritional deficit to reduced food intake (13).

In recent past there has been a growing pandemic of obesity in all populations and ethnic groups and because of aggressive marketing campaigns within food industry, the role of sweeteners has evolved from sucrose substitutes to health substitutes. Therefore, these substances are being marketed to the masses as healthy alternative to sucrose especially for diabetic population. Although these health claims appear promising, they have never been confirmed in clinical trial or epidemiological study. (14) On the other hand, recent safety concerns about artificial sweeteners came from a large epidemiological study and physiological studies in human. Nevertheless, even some natural sweeteners, such as fructose, have aroused some suspicion.

Methods

Several studies have been examinated:

In one of these, researchers examined the relationship between non-caloric artificial sweeteners (NAS) consumption and glucose intolerance by alteration of the gut microbiota. In physiological studies of artificial sweeteners both aspartame and sucralose were associated with significant post-prandial hyperglycemia in comparison to stevia. Post-prandial insulin level was also high with artificial sweeteners suggesting that artificial sweeteners may be associated with metabolic abnormalities. Physiological evidence included consumption in the form of in diet soda/soft drinks. In a large meta-analysis of prospective studies (17 cohorts with 38 253 cases) it has been shown that artificial sweeteners were associated with risk of type 2 diabetes mellitus (T2DM), and may not be as healthy alternative to beverage with sucrose as projected. Even though publication bias cannot be ruled out for artificial sweeteners, a recently published E3N EPIC Cohort study was unique in that it collected to year data among more than 10 000 women among consumers of artificial sweeteners. This study actually demonstrated an association between artificial sweeteners usage and risk of diabetes. More importantly they were also able to show a gradation of risk depending upon year of consumption and amount consumed per day. Another explanation given for a probable association between artificial

sweeteners and T2DM in observation studies is reverse causation bias because of increase intake of artificial sweeteners among obese. However in this study even after excluding cases of incident T2DM during first 5 years of follow-up there was a positive association between artificial sweeteners and T2DM suggestive of no significant reverse causation effect in the study. Strenght of the study was that reverse causation was not found to be a confounder and risk of T2DM was indipendent of traditional diabetic risk factors. However in a large study, Dekoning et al., (15) who prospectively analized more than 40 000 participants, reported that in white men, beverage with sucrose were significantly associated with T2DM although artificial sweeteners per se may not. Nevertheless the possible risk of diabetes cannot be ignored as these substances are being marketed extensively as an alternative to sucrose especially among diabetic population. In a double blind, randomized controlled trial 477 healthy school going children received sugar containing beverage with sucrose and Aspartame combination. At 18 month of follow-up artificial sweeteners were significantly associated with reduced weight gain as compared to sucrose. They concluded that decreased sugar consumption may lead to decrease in insulin level and satiety, leading to less weight gain. Regarding natural sweeteners, in a recent study, fructose has been reported to have the potential to progress diabetes and diabetic nephropathy (DN) in humans even though fructose itself does not increase post-prandial plasma glucose levels. In this study they investigated the effects of high fructose intake on the kidney of the Spontaneously Diabetic Torii (SDT) rats, are known a useful model for non-obese type 2 diabetes and spontaneously develops hyperglycemia and glucose intolerance resulting from impaired insulin secretion due to β cell degeneration in the pancreas, which have renal lesions similar to those in DN patients and compared these with the effects in normal SD rats.

Results

Some studies showed benefits for artificial sweeteners consumption and little induction of a glycaemic response, whereas others demonstrated associations between artificial sweeteners consumption and weight gain, and increased type-2 diabetes risk. However, interpretation is complicated by the fact that artificial sweeteners are more typically consumed by individuals already suffering from metabolic syndrome manifestations. Most artificial sweeteners pass through the human gastrointestinal tract without being digested by the host and, thus, directly encounter the intestinal microbiota. Interestingly, it emerged that artificial sweeteners consumption increases the risk of glucose intolerance and that these adverse metabolic effects are mediated by modulation of the composition and function of the microbiota. Notably, several of the bacterial taxa that changed following artificial sweeteners consumption were previously associated with type 2-diabetes in human. (16) Studies results showed that artificial sweeteners could be used as a method of dietary modification to reduce the consumption of sucrose. Particularly, they have been introduced to improve the management of obesity and diabetes mellitus, but weight gain and blood glucose control are still linked to total energy consumption. Although they may reduce the caloric intake, per se they may not have any beneficial effects on control of diabetes because as they may themselves alter the insulin sensitivity. Regarding natural sweeteners, digestible carbohydrates, in particular added sugars, are often indirectly related to noncommunicable disorders such as overweight and obesity, metabolic syndrome, cardiovascular disease or diabetes. (17,18) Therefore, it has become important to develop standards regarding the consumption of added sugars. The World Health Organization (WHO) issued a recommendation that the consumption of added sugars should not exceed 10% of the diet total energy in order to prevent non-communicable diseases, obesity and tooth decay. In 2014, the WHO has announced a public consultation on dietary recommendations for added sugars, which supports earlier findings and considers reduction of dietary energy intake by 5% (19). Also a recent study revealed that a high-fructose diet increases the urinary excretion of markers of kidney injury namely tubular injury and accelerated mainly tubular and interstitial lesions in SDT (Spontaneously Diabetic Torii) rats but not in normal rats. The progression of nephropathy in the SDT rats was considered to be related to increased internal uric acid and blood glucose levels due to the high intake of fructose. In addition to the above mechanisms, it has been reported that fructose can induce renal injury through other mechanisms including high blood pressure, depletion of

ATP and production of inflammatory molecules and pro-inflammatory mediators. Furthermore, fructose is also known as an inducer of obesity and dyslipidemia due to stimulation of triglyceride synthesis and increases fat deposition in the liver. (20) Regarding future perspective, a recent animal-model study by Ahmad et al., (21) has evaluated the anti-diabetic property of aqueous extract of Stevia in rats. Stevia natural (*Stevia rebaudiana*) is a valuable source of the pharmacologically important glycoside stevioside, which is linked to the pathology and complications of diabetes. The study concluded that aqueous extract of Stevia (at the concentration of 500 ppm/Kg of body weight) showed positive effect on several nutritional parameters (body weight, blood glucose, glycogen level, glycosylated hemoglobin and serum insulin). It is likely that stevia extract may have anti-diabetic effects – at least in rats – and, therefore, its use as natural anti-diabetic drug could be hypothesized.

Discussion

In conclusion, artificial sweeteners have been in vogue for a long time and are now constituents of many processed foods. They have been introduced for the management of obesity and diabetes mellitus. Although they enable to reduce the calorie intake, per se they may not have any beneficial effects on glucose control of diabetic patients as they may themselves alter the insulin sensitivity. In addition, they have arisen safety concerns like cancer. Regarding natural sweeteners, due to the fact that obesity and diet-related chronic diseases are international health problem, it is recommended to consume small amounts of sugars especially those added during technological processing. Qualitative composition of carbohydrates in the diet is of pivotal importance considering differences in fructose and glucose metabolism in the human body. However, nutrition surveys show that most adults and children eat more sugars both of natural origin and added than is recommended as part of a healthy balanced diet. This can be a therapeutic target, as sugars can be replaced by other natural sweeteners such as polyols, stevia or thaumatin that provide the taste wanted by many people without adding unnecessary calories. The use of artificial sweeteners should be probably limited as well due to potential safety concerns. Nonetheless, recent studies have also suggested a therapeutic activity, which applies at least to the anti-diabetic properties of Stevia.

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REVIEW OF FUNCTIONAL VEGETABLE OILS IN KETOGENIC THERAPIES – CHARACTERISTICS AND APPLICATION IN DIETING PROTOCOLS

Napoli Serena; Infante Raffaele; Oliva Antonio; Dell'Omo Maria Pia; Conte Aurelio; Castaldo Giuseppe; Rastrelli Luca

Post Graduate University Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

The intent of this work is to review the characteristics of selected functional vegetable oils and their strong antioxidant power in order to assess the benefits of their use in a ketogenic diet. Ketogenic diets need a considerable amount of healthy fats in their protocols, so we looked at oils like extra virgin olive oil, walnut oil or avocado oil because of the supporting literature about their potential in countering all the inflammatory processes of the metabolic syndrome. Reaching the fat quota mainly by use of these oils could provide an excellent multifaceted strategy for achieving weight loss in the patient, along with improvements in insulin sensitivity and lipid profile.

Keywords: *ketogenic, diet, oil, olive, walnut, avocado, pumpkin, argan*

Introduction

The ketogenic diet (KD) has been found to be effective in reducing the inflammatory biomarkers of patients with insulin resistance (IR) and metabolic syndrome. KD is a low-carb diet that substantially changes the energetic substrates for the body, where the most evident change is a major shift in macronutrients from carbohydrates to fats. Among fats, one should greatly enhance those beneficial for the body, such as polyunsaturated fatty acids (PUFA). Healthy fats are increased at the expense of the amount of carbohydrates in the patient's diet, forcing the body to activate ketosis processes in order to obtain ketones as the main energetic substrate. Among these healthy fats, great importance has been given in literature to functional vegetable oils, like extra virgin olive oil, walnut oil, avocado oil, pumpkin seed oil, both for the peculiar nature of their fatty acids content and the anti-inflammatory, strongly antioxidant properties of their specific polyphenols. We have also added argan oil to our review because of surfacing new studies highlighting its similar potential against IR and the metabolic syndrome. A patient under a classic protocol of KD would have to severely diminish his carbohydrates quota, at the same time introducing a good amount of vegetables, proteins and – most importantly – fats, among which those defined as "healthy" and high in PUFA contents. The oils examined are all well known for their great PUFA content and their beneficial effects on health, and so we looked for their potential in being both the major component of the fat quota in a KD protocol and a powerful beneficial supplement aimed at the inflammatory processes and biomarkers of IR, non-alcoholic fatty liver disease (NAFLD), hypertension and the metabolic syndrome in general.

Methods

We reviewed available literature with a systematic approach, with the intent of finding a common denominator between the studies favouring a high intake of the aforementioned oils in dieting protocols. General reduction of IR inflammatory biomarkers, and improvement of general health conditions in the patients were our discriminating factor. Final objective was to assess the possibility of employing these oils as the main fat source in KD protocols.

Discussion

Specific literature highlighted common properties among these oils and their active compounds capacity in reducing IR and all of the related inflammatory pathways.

A variety of studies exist in literature regarding positive effects of a ketogenic Mediterranean diet with phytoextracts on some cardiovascular/metabolic risk factors and inflammatory state, so we decided to evaluate the results of extra virgin olive oil studies first in antagonizing the effects of metabolic syndrome [1].

Extra virgin olive oil being the staple fat of the Mediterranean diet, we also looked for confirmation that olive oils high in their polyphenolic content could have a strong antioxidant impact on the body. Evidence on the health benefits of extra virgin is ample and always increasing with new studies, to the point where the European Medicine Agency drafted guidelines for the health claims of extra virgin olive oil, by which the antioxidant content and the nutraceutical properties could be indicated on bottles only in case of total polyphenol content being equal or above 5 milligrams for 20 grams of olive oil. Total polyphenol content in olive oil is expressed as derivatives of tyrosol and oleuropein-aglicone. Mono-unsaturated fatty acids along with these various phenolic compounds, such as oleocanthal, oleuropein, hydroxytyrosol, and tyrosol, are the main nutraceutical components of extra virgin olive oil. These substances have been suggested to have the ability to modulate aging-associated processes. It was observed that hydroxytyrosol and oleocanthal inhibit the cyclooxygenases (COX-1 and -2) responsible for prostaglandin production; oleuropein is a radical scavenger that blocks the oxidation of low-density lipoproteins [2]. Polyphenols in olive oil can also positively influence glycemia and type-2 diabetes through specific and distinct mechanisms, such as promoting the uptake of glucose in tissues, and therefore improving insulin sensitivity [3]. There is also convincing evidence to show that olive polyphenols, independently of olive lipids, reduce risk factors for metabolic syndrome, in particular by improving blood sugar and blood pressure control, and in reducing low-density lipoprotein oxidation. Lipid profile is generally enhanced with consumption of certain oils and polyunsaturated fats [4]. Another study concluded that acute intake of extra virgin olive oils rich in polyphenols was able to ame-

liorate glycaemia and insulin sensitivity, and to modulate the transcription of genes involved in metabolism, inflammation and cancer, switching peripheral blood mononuclear cells to a less deleterious inflammatory phenotype [5].

In the context of a varied approach regarding the choice of oils in a KD protocol, we then moved to search for literature regarding specific activity of walnut and avocado oil on IR and metabolic syndrome biomarkers.

Nuts in general and walnuts in particular have been the subject of extensive evaluation for the association of their consumption with improved health outcomes. Walnuts have an optimal composition in bioactive nutrients and recent clinical and experimental studies have uncovered a number of beneficial effects of walnut micronutrients, working in isolation or in concert, on metabolic pathways and clinical outcomes. Alpha-linolenic acid, a critical walnut component, which is metabolized into bioactive oxylipins, has been shown to protect microglial cells from inflammation, and is associated with lower fatal myocardial infarction rates through a putative antiarrhythmic effect. Phytosterols relate to the cholesterol-lowering effect of nut consumption. Non-sodium minerals are associated with better cardiometabolic health. Walnut phytomelatonin has anticancer effects that are shared by the main walnut polyphenols and their metabolites, ellagitannins and urolithins, respectively [6]. Even if literature about walnut oil polyphenols is not as specific as the one focusing on extra virgin olive oil, we collected interesting results from a study in which preliminary indications showed that dietary lipids from walnut oil are modulators of hepatic steatosis as the initial step of progressive NAFLD pathogenesis. NAFLD is strictly correlated to IR and it is always one of the prime targets in any therapy aimed at ameliorating health conditions in metabolic patients [7]. More evidence of a hepatoprotective effect from walnut compounds came from another study where the polyphenol-rich fraction (WP, 45% polyphenol) prepared from the kernel pellicles of walnuts was assessed for its hepatoprotective effect in mice. A single oral administration of WP significantly suppressed serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) elevation in liver injury. Among the constituents, ellagitannins with a galloylated glucopyranose core, such as tellimagrandins I and II, and rugosin C, suppressed hepatocyte damage significantly. Telliamgrandin is likely the principal constituent for the hepatoprotective effect of WP [8].

Although studies on the metabolic impact of avocado oil are not very abundant, the ones available are quite indicative of the avocado fats potential in combating metabolic syndrome. Avocado has quickly become a staple fat in a great number of diet protocols for its health benefits, in no small measure thanks to its ability to act as a butter substitute among conscious consumers or people who adhere to restrictive diets for ethical reasons, such as vegetarians and vegans.

One study conducted on rats highlighted the avocado oil potential on blood glucose levels. Glucose tolerance and insulin resistance induced by high sucrose diet in Wistar rats could be reduced by the dietary addition of 5-20% avocado oil [9]. In another study, virgin avocado oil treatment could partially recover the metabolism dysfunction induced by hypercholesterolemia mainly via lipid, energy, amino acid, and gut Microbiota metabolism [10]. Avocado oil supplementation has, in general, shown positive outcomes with metabolic patients because it reduces inflammatory events and produces positive changes in the biochemical indicators studied, related to the development of metabolic syndrome [11]. Another study taken into our review focused on the effects of avocado fats on blood pressure. Avocado oil is a source of antioxidants and oleic acid, and is known to improve mitochondrial function, but it also showed striking anti-hypertensive effects, which appeared to be, comparable to that exerted by common drugs for the treatment of hypertension, especially kidney hypertension. It has been proposed that the effects of avocado oil on hypertension might be mediated by decreasing the actions of angiotensin-II on mitochondria. These results suggest that avocado oil intake might be a nutritional approach to attenuate the deleterious effects of hypertension on kidney [12].

Our attention was subsequently shifted towards pumpkin seed oil, in the context of providing more choices for patients on a KD protocol. Oral administration of pumpkin seed oil in rats revealed significant diminution in body weight gain, glucose and insulin levels, which altered the activity of lipid profile and restored the pathological alterations. It demon-

strated that the oil had considerably altered these parameters when evaluated with obese control rats. In conclusion, this study established that pumpkin seed oil prevents induced obesity in rats by altering the markers that are important to lipid metabolism [13]. These findings complement the results of a similar study, which revealed that administration of pumpkin seed oil could cause significant diminution in expressions of lipid marker enzymes and inflammatory markers. This study suggested that improvement of induced obesity compared to control rats had happened because the oil altered the enzymes and mRNA expressions that are at the root of the inflammatory process [14]. Pumpkinseed oil was given in combination with simvastatin, as anti-hypercholesterolemic drug, to high cholesterol-fed rabbits, for three weeks. Concomitant administration of simvastatin and the oil succeeded to cause marked reduction of the aortic contractile response to norepinephrine and to normalize the most adverse effects observed during HC. These effects were explained by the potentiating effects of simvastatin with antioxidants and essential fatty acids in pumpkin seed oil [15].

So far we considered oils that are mostly common in our diets in order to have a clear picture of what could be best introduced in a dieting protocol without having particular sourcing problems for a patient, i.e. oils that are difficult to find for sale or not very popular in certain markets. In recent years, though, work has been conducted on a lot of other seed oils with interesting results for our focus. Another oil we considered for our review is Argan oil because of a reported specific metabolic activity, from a study examining the effects of argan oil on the three main cardiovascular risk factors associated with metabolic syndrome (hypertension, insulin resistance and obesity) and on one of its main complications, neuropathic pain. Glucose-fed rats showed increases in systolic blood pressure, epididymal fat, plasma levels of triglycerides, leptin, glucose and insulin, insulin resistance. Argan oil prevented or significantly reduced all those anomalies with an induction in plasma adiponectin levels. In contrast, the same treatment with corn oil had a positive impact only on triglycerides, leptin, adiponectin and insulin resistance. These data are the first to suggest that argan oil is an effective nutri-therapeutic agent to prevent the cardiovascular risk factors and complications associated with metabolic syndrome [16]. This study followed up a previous one that investigated whether a 5-week treatment with argan oil could reduce arterial hypertension, hyperglycemia, insulin resistance, and enhanced basal superoxide anion production and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in the aorta of glucose-fed rats.

Oxidative stress was evaluated by measuring the superoxide anion production and the NADPH oxidase activity using the lucigenin method. The findings from the present study demonstrated that argan oil treatment reduced elevation of blood pressure, hyperglycemia, and insulin resistance through its antioxidative properties in glucose-fed rats. Hence, argan oil, which is now increasingly available in the market as a consumable food, may be of potential therapeutic value in the treatment of arterial hypertension and insulin resistance [17].

All the oils examined in our literature review show promising effects against the inflammatory bio-markers of metabolic syndrome. In fact, goal of a KD protocol is to ameliorate all of these parameters in the context of a safe and consistent weight loss. KD protocols may be fine-tuned according to pathological conditions of the patient, like diabetes onset for example, but this regards the carbohydrate intake most often than not, so it is generally safe to assume that the oils taken into consideration might very well be a consolidated base for most KD regimes, given their high PUFA content across the board. We hereby provide a comprehensive table of the fat composition for the oils taken into our review (TABLE 1). Previously, studies have also shown that PUFA can also elicit a greater response in satiety after a single-meal challenge compared with other types of fats. Controlling appetite is crucial during the initial stages of any dieting protocol, in order to achieve compliance from the patient. Cravings for sugar are especially frequent in metabolic patients with uncontrolled eating habits.

Due to the consumption of foods or snacks with a high glycemic index, these cravings can delay the sense of satiety in the patient and compromise adherence to protocol. Even in this regard supplementation with PUFA could be providing precious support in the future, as the study below suggested, examining PUFA effects on target markers involved in hunger and satiety.

Twenty-six, healthy weight sedentary adults were randomly assigned to either a 7 days PUFA-rich diet or a 7 days control diet. After a 3 day lead-in diet, participants reported for the baseline visit where anthropometrics, fasting visual analog scale measurements, and a fasting blood sample were collected. Then, two high-fat meals (breakfast and lunch) were consumed. From pre- to post-PUFA-rich diet, there was a decrease in fasting ghrelin. However, there were no changes in fasting insulin or leptin concentrations. The postprandial response for fasting peptide YY was higher after the PUFA-rich diet visit compared to baseline. A PUFA-rich diet consumed for 7 days favorably altered fasting and postprandial physiological markers of hunger and satiety; yet, did not alter subjective ratings of hunger or fullness [18].

These evidences all point to the precious role that PUFA may play in a KD protocol, thanks to their intrinsic ability to be excellent substrates for achieving a desirable ketogenic fat quota, and also for their multitude of active compounds with a strong antioxidant capacity responsible for lowering or antagonizing most of the inflammatory bio-markers and processes that take place in the body after the onset of metabolic syndrome, IR and NAFLD. All of the oils taken into our review showed similar characteristics in terms of antioxidant power. Aside from the vast literature regarding the general health-protecting activity of antioxidants, in the context of IR and metabolic syndrome, this radical-scavenging capacity plays a major role in preventing blood lipids oxidation, a cornerstone in the onset of hypercolesterolemia and NAFLD. At the same time, all of these PUFA-rich oils also possess intrinsic ability to ameliorate many other parameters and this is probably due to unique active compounds in each one of them, mostly found in the spectrum of secondary metabolites such as polyphenols. Target applications are undoubtedly there or will be in the near future for most of these active molecules; notable examples are walnut oil polyphenols for their significant activity on blood LDL and HDL levels, or studies that the polyphenol oleocanthal – found in extra virgin olive oil – is undergoing for its effects on the respiratory system. At any rate, though, given the well acknowledged safety profile for each of these oils, in the context of a KD protocol aimed at combating or ameliorating all of the inflammatory bio-markers associated with IR, this group is of the idea that simultaneous and synergic employment of all of them in a balanced ketogenic protocol would provide an excellent choice as staple fats. Cold-pressed and uncooked oils preserve the most of their active polyphenol content, and patients should be instructed about this by the health practitioner when recommending particular dishes and meal preparation. Blending these oils together – using the most available ones like extra virgin olive oil or walnut oil as the main fat component – when seasoning cold dishes like salads, or using them generously on cooked meals, would be the best way to achieve tangible results from their beneficial activity, at the same time improving the levels of satiety markers for the patient, which lead to a reduced appetite for carbohydrate-rich foods, both during the KD protocol and in the long run.

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Table 1. Vegetable oils reviewed – fat composition

		Types of oils			
		Extra virgin olive Oil	**Walnut Oil**	**Avocado Oil**	**Pumpkin seed oil**
Total Fat (100 g)	**Saturated Fat**	13,8 g	9,1 g	11,6 g	20,2 g
	Monounsaturated Fat	72,9 g	22,8 g	70,6 g	24,7 g
	Polyunsaturated Fat	10,6 g	63,3 g	13,5 g	55,1 g

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THE ROLE OF DIET IN ACNE AND ATOPIC DERMATITIS

Sellitti Anna[1], Mauro Antonella[2]; Galdo Giovanna[1],[2]

[1]Post Graduate University Course Master in Diete e Terapie Nutrizionali Chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy
[2]IRCCS, centro di riferimento oncologico della Basilicata, via Padre Pio 1, 85028 Rionero in Vulture, Potenza, Italy

Email address: nutriketo@unisa.it

Abstract

Diet has an important role to play in many skin disorders, and dermatologists are frequently faced with the difficulty of separating myth from fact when it comes to dietary advice for their patients.
Numerous studies were published over the last 50 years to investigate whether diet is associated with the etiology of acne and atopic dermatitis. Although older studies well known by dermatologists that refute the association between acne - atopic dermatitis and diet exist, their scientific foundation is weak. New articles have recently brought to light evidence contrary to previous findings.
Acne vulgaris is a chronic dermatosis. It is a disease of the pilosebaceous follicle with four fundamental etiopathogenic factors: sebaceous hyperproduction, follicular hyperkeratinization, increase of Propionibacterium acnes colonization, and periglandular dermal inflammation.
There are a number of recent articles that have reexamined the role of diet in acne. The general view held by modern-day dermatologists with regard to diet and acne is that diet is unrelated to the etiopathogenesis of acne. In the early 1950s, the major US textbooks of dermatology, popular, contained elaborate prose regarding specific foods to avoid. The admonition to avoid chocolate, fats, sweets and carbonated beverages was commonly given to patients as part of acne therapy.
Atopic dermatitis (AD) is a bothersome and common skin disease affecting ~10.7% of children in the United States. This skin condition significantly decreases quality of life not only patients, but in their families as well.
AD is complex, multifactorial, and has historically had limited therapeutic options, but now the landscape of this disease is rapidly changing.
The role of diet in the cause and treatment of AD is very controversial. Pediatricians and allergologists are convinced of the causative role of food in the onset of AD, while dermatologists are convinced of the contrary.
Arguments in favor of the role of diet in AD include the fact that some foods provoke AD, an elimination diet can heal AD, diet manipulation can prevent allergy in newborns at risk for atopy.
Based on data from the literature that relate acne vulgaris and atopic dermatitis with diet, we have written this review to clarify the hypotheses about this topic.

Keywords: *Obesity, diet milk-free, diet chocolate-free, diet rich in vitamin D*

Introduction

Patients often seek dietary advice from their dermatologist, as they frequently link many of their health problems, including diseases of the skin, to their diet. While some of these concerns are unfounded, research has uncovered significant evidence that dietary considerations may be important in some dermatological conditions. While a number of well-known conditions are linked to diet, such as food allergies and dermatitis, in this article, the authors focus on conditions for which the role of diet has traditionally been an underappreciated aspect of therapy. In some cases, dietary interventions may influence the course of the skin disease, as in acne. In others, dietary change may serve as one aspect of prevention, such as in skin cancer and aging of the skin. In others, dermatological disease may be linked to systemic disease, and dietary changes may affect health outcomes, as in psoriasis. Lastly, systemic medications prescribed for dermatological disease, such as steroids, are known to raise the risk of other diseases, and dietary change may reduce this risk. In this article, the authors review studies that have examined the link between diet and dermatology. Many patients come in with questions due to misconceptions that they themselves have developed or due to information they have received from friends, family members, or the media (1)

Acne vulgaris

Acne is a chronic inflammatory disease of the pilosebaceous unit resulting from androgen-induced increased sebum production, altered keratinisation, inflammation, and bacterial colonisation of hair follicles on the face, neck, chest, and back by Propionibacterium acnes. Although early colonisation with P acnes and family history might have important roles in the disease, exactly what triggers acne and how treatment affects the course of the disease remain unclear. Other factors such as diet have been implicated, but not proven. Facial scarring due to acne affects up to 20% of teenagers. Acne can persist into adulthood, with detrimental effects on self-esteem. There is no ideal treatment for acne, although a suitable regimen for reducing lesions can be found for most patients. Good quality evidence on comparative effectiveness of common topical and systemic acne therapies is scarce. Topical therapies including benzoyl peroxide, retinoids, and antibiotics when used in combination usually improve control of mild to moderate acne. Treatment with combined oral contraceptives can help women with acne. Patients with more severe inflammatory acne usually need oral antibiotics combined with topical benzoyl peroxide to decrease antibiotic-resistant organisms. Oral isotretinoin is the most effective therapy and is used early in severe disease, although its use is limited by teratogenicity and other side-effects. Availability, adverse effects, and cost, limit the use of photodynamic therapy. New research is needed into the therapeutic comparative effectiveness and safety of the many products available, and to understand better the natural history, subtypes, and triggers of acne. (2)

Acne vulgaris is an epidemic inflammatory disease of the human sebaceous follicle and represents the most common skin disease affecting about 85% of adolescents in Westernized populations. Acne vulgaris is primarily a disease of wealthy countries and exhibits higher prevalence rates in developed compared with developing countries. No acne has been found in non-Westernized populations still living under Paleolithic dietary conditions constraining hyperglycemic carbohydrates, milk, and dairy products. The high prevalence rates of adolescent acne cannot be explained by the predominance of genetic factors but by the influence of a Western diet that overstimulates the key conductor of metabolism, the nutrient- and growth factor–sensitive kinase mTORC1.(3)

For years, dermatologists have denied a connection between acne and diet, based partially on earlier research. In fact, the latest research has provided strong support for diet as a potential cause of acne. Research has substantiated the role of specific foods, such as dairy products, as well as dietary patterns, including the high glycemic load diet typical of the Western diet. The Western diet has also been researched as a potential cause of acne. It has been noted that acne is absent in native non-Westernized populations.

Therefore, investigators have examined the role of the Western diet, which typically corresponds to a high glycemic load diet.(4) Glycemic load takes into account the quantity of carbohydrates consumed as well as the rate of carbohydrate absorption. Foods with a high glycemic index, such as sugar, white bread, and white rice, are rapidly absorbed, leadingto

higher serum glucose levels and corresponding elevated levels of insulin.(1)

Atopic dermatitis (A.D.)

Atopic dermatitis is a common inflammatory skin condition characterized by relapsing eczematous lesions in a typical distribution. Atopic dermatitis (AD), commonly referred to as eczema, is a chronic, relapsing, and often intensely pruritic inflammatory disorder of the skin.(5) Although it most often starts in infancy and affects two of ten children, it is also highly prevalent in adults. It is the leading non-fatal health burden attributable to skin diseases, inflicts a substantial psychosocial burden on patients and their relatives, and increases the risk of food allergy, asthma, allergic rhinitis, other immune-mediated inflammatory diseases, and mental health disorders. Originally regarded as a childhood disorder mediated by an imbalance towards a T-helper-2 response and exaggerated IgE responses to allergens, it is now recognised as a lifelong disposition with variable clinical manifestations and expressivity, in which defects of the epidermal barrier are central. Present prevention and treatment focus on restoration of epidermal barrier function, which is best achieved through the use of emollients. Topical corticosteroids are still the first-line therapy for acute flares, but they are also used proactively along with topical calcineurin inhibitors to maintain remission. Non-specific immunosuppressive drugs are used in severe refractory cases, but targeted disease-modifying drugs are being developed. We need to understand the heterogeneity of the disease and its subtypes, the role of atopy and autoimmunity, the mechanisms behind disease-associated itch, and the comparative effectiveness and safety of therapies.(6)

A recent epidemiologic study using national data suggested that the pediatric prevalence is at least 10% in most of the United States.(1) AD primarily affects children, and disease onset occurs before the ages of 1 and 5 years in 65% and 85% of affected children, respectively.

The pathogenesis of AD is complex and multifactorial. Skin barrierdysfunction, environmental factors, genetic predisposition, and immunedysfunctionall play a role in its development and are closely intertwined.

The relationship between AD and food allergy is complex but likely overemphasized. More than 90% of parents incorrectly believe that food allergy is the sole or main cause of their child's skin disease.(22) The resulting focus on food allergy can result in elimination diets; potential nutritional concerns, such as protein or micronutrient malnutrition or deficiencies; and misdirection of treatment away from the skin, thereby leading to undertreatment. Effective treatment of the skin tends to allay parental concern regarding food allergy. (5)

Atopic dermatitis (AD) characterized by a chronic or relapsing course, with periods of disease exacerbations alternating with various degrees of remission. The skin is dry and pruritus represents the hallmark symptom; it is disturbing and causes skin excoriations, substantially affecting the quality of life of patients and their relatives . The precise etiology, pathophysiology, and pathogenesis of AD are not yet fully understood. It can be considered the result of a complex interaction between genetic and environmental factors.

The disease is associated with abnormalities in skin barrier molecules and in cells of the inflammatory response.(7)

Results

Acne and Obesity

Acne and Weight.—There is less unanimity concerning the value of diet in the treatment of acne, but most writers recommend the elimination of chocolate and many advise reduction of carbohydrates, fats, and fried foods. Andrews (1954) says that acne is aggravated by ' excess of fats, sweets, starchy food in the diet or overeating." Sutton (1941), proposing acne as a ' pustular lipoidosis," even suggests that acne is worse in summer, owing partly to " gross increase in oil intake on account of the popularity of ice-cream." Mitchell Heggs (1950) advises correction of obesity; but MacKenna (1952), while recommending reduction of carbohydrate and fried food, counsels regular weighing to avoid undernourishment.(8)

Atopic dermatitis and obesity

Obesity has been associated with atopic dermatitis (AD); however, the results have been conflicting. Our aim was to provide an update on current knowledge from observational studies addressing the possible

association between obesity and AD. Systematic literature review was performed by identifying studies addressing a possible link between AD and overweight/obesity from PubMed, EMBASE and the Cochrane Library in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. The quality of the included studies was assessed using the Newcastle–Ottawa Scale. A total of 45 studies (comprising more than 90 000 individuals with AD) fulfilled the criteria and were included in the present review. The available studies revealed inconsistencies, but the majority indicated that obesity is associated with AD. Studies addressing obesity in infancy or early childhood (age < 2 years) and AD reported a positive association. From childhood into adulthood, there is a discrepancy in the observations, as the more recent prospective studies found a positive association, whereas this was not observed in older cross-sectional studies. The inconsistency might be explained by the difference in study design, the diagnostic criteria of AD, regional differences, and by the varied definitions of overweight and obesity used in the studies. In Conclusion, overweight/obesity is associated with an increased risk of AD. Large prospective cohort studies are required to confirm the association between AD and obesity and the possibility that weight control in childhood may help to mitigate or reverse AD symptoms. (9)

Acne and Milk

The papers from the Harvard School of Public Health establish an association between milk consumption and acne. But how could milk cause acne?

Because drinking milk and consuming dairy products from pregnant cows exposes us to the hormones produced by the cows' pregnancy, hormones that we were not designed to consume during our teenage and adult years.(10) An association exists between acne and skim milk consumption. This association made a division between dermatologists. In older studies, milk was shown to cause a disproportionate increase in insulin levels despite having a relatively low glycemic index, producing an insulinemic response 3 to 6 times higher than its corresponding glycemic index.

The role of dairy and its effects on overall health are complex. Many recent clinical studies examining the association between total dairy consumption and metabolic syndrome, insulin resistance, and even cardiovascular risk and stroke, found dairy consumption to be beneficial and protective, regardless of fat content. Other studies found that participants consuming a diet higher in dairy fat actually had a lower risk of obesity and cardiovascular disease.

The data regarding acne support a positive association specifically for skim milk. There are differences between full-fat and skim milk, including the overall composition of proteins, fatty acids, and cholesterol molecules.(29) Milk fat, although containing saturated fats, also contains numerous medium-chain fatty acids beneficial in promoting healthy metabolism and decreasing insulin resistance.

Low-fat/skim milk consumption was positively associated with acne, whereas other types of milk and dairy products had no association.

Particularly, studies showing the impact of skim milk elimination are needed before making specific dietary recommendations for patients with acne. (11-12)

Acne vulgaris and Vitamin D

Concerning dietary factors, studies have been conducted to show the role of zinc in treatment of drug-resistant cases of depression (13). Also these kinds of performances have done for vitamin D and its effects on acne vulgaris (14). Vitamin D, as an antioxidant agent, would have positive impression on the disease (15-16).

Otherwise, antimicrobial role of vitamin D is the other issue to be raised since ancient era by many authorities and social cultures (17) and focused now as a treatment hint in acne vulgaris. This study headed to compare serum levels of 25 (OH) vitamin D between cases of acne vulgaris and age-and sex-matched control group to find any relationship between the vitamin and the occurrence of the disease besides comparing the two groups in terms of vitamin D deficiency conditions. The aim of the present study is to compare the serum level of 25-hydroxy vitamin D in patients with acne vulgaris and healthy controls and to assess the association between disease severity and vitamin D deficiency.(18)

Acne vulgaris and Chocolate

Chocolate has always been blamed as an aggravating factor of acne. Patients often report the development of pustule a few days after the ingestion of this food.(19) For this reason, many patients ask us for a scientific position on this subject. Detailed below is what we know so far.

In 1969, 65 individuals ingested a chocolate bar daily, rich in chocolate liqueur and cocoa butter, for 4 weeks; after this period, the regimen was changed and patients ingested a control-bar, with the same weight, without chocolate liqueur and cocoa butter, for 4 more weeks. Lesions were classified at the start and end of the study into three different categories: aggravated, improved and unaltered. Since there was no significant difference between the ingestion of chocolate and control bars in the three categories of classification, the authors concluded that the ingestion of a great amount of chocolate does not interfere in the course of acne vulgaris or in sebum composition.

According to Cordain et al., the study mentioned above comes to a wrong conclusion. In the control bar, cocoa, in the form of butter and liqueur, was substituted by hydrogenated vegetable fat (28% of the weight). Moreover, both the modified and the chocolate bar had a high sucrose concentration (53% and 44.3% of the weight, respectively), which predisposed the individual to hyperglycemia and insulinemia, factors that are involved in the development of acne.

Therefore, to Cordain et al.(20) the results from the study cannot be generalized to assume that chocolate is not associated with acne, since other ingredients that may be involved in the etiology of acne are used in its manufacture. In another study, conducted by Anderson,(21) patients who said they did not tolerate chocolate because it aggravated their acne were selected. These patients ingested a great amount of chocolate for seven consecutive days, and no alteration in the number or severity of lesions was observed. Unfortunately, pre or post-experimental lesions were not considered, and there was no control group or statistical analysis of the study. Compared with the reference study, the analysis period of chocolate ingestion was very short (one and four weeks, respectively).

Although with mixed conclusions, the theory of the association between acne and chocolate is almost entirely confirmed by clinical findings from well designed studies that have been conducted with competence by study groups in nutrition and nutrology.

A study carried out by a group of Australian scientists compared the plasmatic profile of patients after the ingestion of food with and without chocolate. Interestingly, an increase of post-prandial insulinemia in slim young adults who ingested chocolate products (average 28% higher) was observed; the highest levels occurred with the ingestion of chocolate milk (average 48% higher as compared with plain milk) and milk enriched with dark chocolate as compared to white (13% higher).(22)

An explanation for the findings of the Australian group may be that chocolate is rich in biologically active compounds, such as caffeine, teobromine, serotonine, phenylethylamine, triglycerides, and cannabinoid like fatty acids, which increase secretion of THβCs (Tetrahydro-â-carbolines) and peripheral resistance to insulin.(23) Moreover, the aminoacids present in chocolate (such as arginine, leucine, and phenylalanine) are extremely insulinotropic when ingested with carbohydrates;(24) other aminoacids (valine, lysine, and isoleucine), found in other types of food, especially those rich in lactose, can also cause this plasmatic behavior.(25)

Based on what has been described so far, it would-not be impertinent to suggest that the ingestion of chocolate-based food products may be associated with the development or aggravation of acne vulgaris. It is important to stress that commercial chocolate bars, especially those with a high milk content, have a great amount of carbohydrates (refined sugars, so they have a high glycemic index), which increase the post-prandial plasmatic levels of IGF and IGF binding protein (IGFBP), having an insulinotropic profile.(26-27) This is worth to consider if you have been convinced of the comedogenic effect of a high glycemic index diet.

Conclusion

Over the last 37 years, many studies were conducted about the influence of diet in the pathogenesis of acne and atopic dematitis, with indication that food may indeed influence this dermatosis.(28)

Dietary interventions have traditionally been an underappreciated aspect of dermatological therapy. Recent research, however, has found a significant association between diet and some dermatological diseases. Dietary interventions may be recommended as therapy, as in acne and atopic dermatitis. Dietary change may help to prevent skin disease, as in aging of the skin or skin cancer. Dietary change may also be an important aspect of prevention of associated systemic disease, as in CVD and other systemic diseases associated with psoriasis or the use of systemic steroids. Dermatologists must be well-educated to the evidence linking diet and dermatology and must be able to counsel patients appropriately.

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CASE STUDY: KETOGENIC DIET AND ATHLETIC PERFORMANCE IN POWERLIFTING

Marco Magliano, Luca Rastrelli

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

This case study has been designed to report the direct effects of a ketogenic diet on sport and in particular on a sport of power like anaerobic no lactacid type. We presented the case of an athlete Master in competitive preparation for the European Championship of Powerlifting subjected to a ketogenic diet rich in polyunsaturated fatty acids. The athlete has well supported the diet, showing a significant loss of fat mass with decreased weight, without reducing the load capacity. Moreover Ketogenic diets may be useful in sports that include weight class divisions.

Keywords: *Poverlifting, anaerobic alactacid metabolism, ketogenic diet*

Introduction

The ketogenic diet is a nutritional approach consisting of high-fat and adequate protein content but insufficient levels of carbohydrates for metabolic needs (<20 g/dayor 5% of total daily energy intake). This type of nutritional approach can correct various metabolic states including the insulin resistance. The ability to generate explosive muscle power and strength is critical to success in Olympic weightlifting and powerlifting, as well as throwing events, including javelin, discus, shot put and hammer, plus sprints (100-200 m) in track and field.

The Sport of powerlifting that is the maximum strength sport expression of strength in non-Olympic weightlifting; the powerlifting consists in lifting in three types of risers such as the deadlift from the floor, bench and squat, every with the barbell in a very fast time with a maximum load (1 Rep Maximum). In this sports it is well known that the ratio of carbohydrates is a fundamental step for the energy supply, but there are numerous scientific evidences that contrast with this, recentely some studies pay attention to the fact that it is possible to practice sports physical activity in the absence of carbohydrate intake also in anaerobic sports.

We are presenting here the case of an athlete Master in competitive preparation for the European Championship of Powerlifting discipline bench press, in NBBUI Federation Junes 2018 subjected to a ketogenic diet rich in polyunsaturated fatty acids.

Case presentation

The athlete is a 47 years old male, master in competitive preparation for the European Championship of Powerlifting discipline bench press, in NBBUI Federation Junes 2018.

Diet Profiles

1800 KCAL, 6% carbohydrates, lipids 67%, proteins 1.2G / kg, fiber 22gr.

Supplements

Creatine 5gr breakfast + 5gr post workout
Multivitamin 1cps breakfast + 1 cps at dinner
10gr Glutamine per day (2 x 5gr docs)
Vitamin D 5000ui
Omega 3 (6 cps / day)
Bcaa 6gr post workout
Selenium 1 cps breakfast
Zinc 1 cps for breakfast

Results

From bibliographyc analysis emerged numerous results regarding ketogenic nutritional approaches of sport but above all short-term sportslike team sports like basketball , like as cycling or individual sports such as boxing (1-3), therefore, sports that are predominantly aerobic or mixed aerobic - anaerobic lactacids. The aim of this study was to demonstrate the usefulness of a ketogenic protocol in sports of very short duration and characterized by an anaerobic alactacid metabolism, with maximum and submaximal efforts.

In the literature there are no studies of which correlate the state of ketosis with the physiological weight loss in terms of fat for sports in which a specific weight category is required. In our case the athlete subjected to ketosis has found a good physical performance with more attention and lucidity and a good physical recovery after training.

The aim of this study was to verify the applicability of the ketogenic diet in the sport of powerlifting. To this end, a Master athlete was analyzed in preparation for the European benchmark championship in the NBB federation. With the goal of weight loss to enter its weight category.

As a supplement to the ketogenic diet, the athlete used creatrine monohydrate (Watt, Creapure ©).

The ketogenic diet was performed for 5 weeks, at 8 weeks of the race. In the first two weeks there was an adaptation of the athlete with carbohydrate reduction to a daily quota of 100 grams, from the third to the seventh week, the carbohydrates were brought to 30 g, with a fat protein ratio of 4: 1. The athlete has well supported the diet, starting from an initial body weight of 104 kg with a fat mass percentage of 23%, at the seventh week he reached 19% fat with a body weight of 98 kg, reaching categories weighing less than 99 kg.

The week before the competition there was a caloric reintroduction of of low-glycemic fruit-based carbohydrates in the early part of the day (100 grams). The workouts in the last precompetitive week went into one exhaust phase the athlete

started with a personal record of 118 kg in the bench specialty : the day of the the competition that was on Sunday .

At the end of the eighth week of study you have performed the three raised in the competition with the respective loads of 115-120 and 125 kg So surpassing their personal record of about 7%, reaching fourth overall in their category .

Conclusion

It can be concluded that a ketogenic approach can be followed by a sportsman in activity for various reasons including weight loss for falls within the weight category of his own discipline. It can be concluded from the evidence of the reported case that the ketogenic diet can be predicted for maximal and submaximal sports leading not only to a decrease in fat mass but to an increase in performance.

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CLINICAL IMPACT OF KETOGENIC DIET ON OBESE PATIENTS SCHEDULED FOR BARIATRIC SURGERY: A MINI-REVIEW

Domenica Mancusi[1]; Marilena Infernosa[1], Luigi Schiavo[2]

[1]Post Graduate University Course Master in Diete e Terapie Nutrizionali Chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy
[2]Department of Thoracic and Cardio-Respiratory Sciences, University of Campania Luigi Vanvitelli, Naples, Italy

Email address: nutriketo@unisa.it

Abstract

Surgery is the only effective treatment for people with a body mass index (BMI) greater than 40 Kg/m2 or even greater than 35 Kg/m2 when some diseases like diabetes or hypertension appear. In order to minimize surgical risk and improve postoperative results, preoperative preparation is very important. "Acute" preoperative weight loss just before surgery plays a crucial role in that preparation and can be achieved through different ways like a low -calorie diet or with a very low -calorie diet.

Keywords: *Low-carbohydrate diet, Ketogenic diet, Obesity, Bariatric surgery.*

Introduction

Obesity, both independently, and in association with other obesity-related diseases, leads to adverse physiological effects of many aspects of health including sleep, cognitive function, and cardiovascular health. A reduction in body fat is a significant treatment target used to improve obesity-induced adverse effects on each of these physiological health variables. However, studies investigating the effect of diet on the physiological aspects of health independent of the loss of weight (whether total mass or fat mass) are lacking. Diets low in carbohydrate and high in fat, or ketogenic diets (KD), are conventionally used to treat epilepsy and neurodegenerative disorders, but have been relatively popular for weight loss since the 1800s. Studies on KDs vary in their daily limit of carbohydrate intake; carbohydrate consumption may be restricted to as low as 4% up to 40% of daily caloric intake. The fundamental principle of the KD, however, is severe restriction of dietary carbohydrate consumption, with a concurrent increase in dietary fat to compensate for the energy deficit, resulting in the promotion of lipid oxidation to produce ketones as an energy source (as opposed to glucose), and thus, a metabolic state of nutritional ketosis.

Methods

Relevant studies were identified by computerized searches on PubMed, and review of bibliographies of selected articles. We have used preoperative weight loss, low calorie diet, very low-calorie diet as keywords. We included in the search reviews, meta-analysis, clinical trial, randomized controlled trials and practice guidelines.

Results

However, a meta-analysis on 23 controlled trials involving 1141 obese patients, reported that the KD diet in fact has favorable effects on cardiovascular health. In particular, the KD is associated with significant reductions in plasma triglycerides, fasting plasma glucose, glycated hemoglobin, plasma insulin, C-reactive protein, systolic and diastolic blood pressures, total body mass, abdominal circumference, as well as significant increases in high-density lipoprotein (HDL) cholesterol, with no significant changes in low-density lipoprotein (LDL) cholesterol (1).

In a prospective pilot study, a cohort of morbidly obese patients (n = 27, 17 females, 10 males) with a mean body mass index (BMI) of 45.2 kg/m2 scheduled for bariatric surgery underwent a 4-week preoperative KMED (ketogenic micronutrient-enriched diet) indicating it is safe and effective in reducing body weight, left hepatic lobe volume, and correcting micronutrient deficiency in obese patients scheduled for bariatric surgery (2).

In a randomized multicenter study, single-blind study, patients were randomly allocated to a2-week preoperative VLCD regimen or no preoperative dietary restriction (control group). The VLCD used in the study was a very low-energy diet (Optifast 800; Nestle´ HealthCare Nutrition GmbH, Frankfurt, Germany) designed to replace 3meals per day during a 14-day period.

To replace 3meals, 5 shakes per day were consumed, which provided 1906 kJ (800 kcal, including 70 g of protein, 15 g of fat, and 100 g of carbohydrates) plus the recommended daily allowance of essential vitamins, minerals, and trace elements.

Patients who were allocated to a normal diet were instructed to have their regular diet until the day of the procedure. All patients were instructed to avoid the intake of solids from midnight the day before the procedure. The intake of clear fluids was allowed until 2 hours before the induction of anesthesia.

In the present study, we demonstrated that, with modest preoperative weight reduction induced by the use of a 14-day VLCD regimen, perceived difficulty of the surgical procedure is reduced. Although this does not seemto have a major influence on operating time, risk of intraoperative complications, or short-term weight reduction, the risk of postoperative complications, in particular infections, was found to be reduced (3).

A systematic review assessed feasibility and effectiveness of preoperative meal replacements to improve surgical outcomes for obese patients. Fifteen studies (942 participants including 351controls) were included, 13 studies (n = 750) in bariatric patients. Adverse effects and dropout rates were minimal. Ten out of 14 studies achieved 5–10% total weight loss. Six of six studies reporting that liver volume achieved 10% reduction (4).Endpoints for perioperative risks and outcomes were too varied to support definitive risk benefit.

Another study from 2015 have assessed the safety, efficacy, and acceptability of avery low-calorie ketogenic diet in patients before bariatric surgery (5). This study standardized 30 days sequential preoperative diet regimen, optimizing metabolic response with gradual carbohydrate reintroduction. Patients were given a dedicated Keto Station kit, for used during the first 10days.

The scheme was followed by a hypocaloric diet for 20 days. The study group underwent routine laboratory tests and anthropometric measurements (percent weight loss, body mass index, waist circumference) at enrollment (T0), after 10 days (T1), and after 30 days (T2). Ketone body levels were measured in the plasma and urine. Between January 2015 and September 2015, 119 patients were included in the study. Mean body mass index was 41.5 ± 7.6 kg/m2. Weight, body mass index, and waist circumference at T0 and T1, T0 and T2, and T1 and T2 decreased significantly (P <.05). A bioelectrical impedance assay determined a significant reduction in visceral fat at T1 and T2. We observed a significant (P <.05) improvement in several clinical parameters, including glycemic and lipid profile parameters. We also observed a mean 30% reduction in liver volume. The majority of patients declared satisfied. The adverse effects were mild, of short duration, and not clinically relevant (5)

Discussion

After choosing the surgical technique to use (restrictive or malabsorptive) and after making appropriate pre-anaesthetic evaluation for the proper optimization of the patient in the context of a multidisciplinary team (surgeons, endocrinologists, psychologists, nutritionists) preparation of both physical and psychological aspects is essential. Weight reduction VLCD regimen before laparoscopic bariatric surgery seems to reduce the perceived difficulty of the procedure, only minor effects on operating time, intraoperative complications, and short term weight loss could be expected. However, the finding of reduced postoperative complication rates suggests that such a regimen should be recommended before bariatric surgery. VLCKD interventions are increasingly used for preoperative weight loss in obese patients undergoing elective surgery.

Overwhelmingly, the literature on the effectiveness of VLCKDs reflects its use in bariatric surgery to achieve preoperative weight loss in order to reduce visceral fat and the size of the fatty liver, thus facilitating the technical aspects of surgery and improving the safety of the surgical procedure for this high-risk population (4, 6).

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PREVENTIVE ACTION OF KETOGENIC DIET ON PCOS, BENIGN PROSTATIC HYPERPLASIA AND FERTILITY

Di Lauro Teresa, Guidotti Serena, Marino Antonella, Medio Anna, Castaldo Giuseppe
Post Graduate University Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

It is been long established in literature that the consumption of high quantity of complex carbs, a high intake of sugar, and being overweight are disadvantageous for fertility in male and female. In particular obese and overweight people with metabolic syndrome (MetS) are more predisposed to developing pathologies like polycystic ovarian syndrome (PCOS) and prostatic hyperplasia (BPH) that can affect the individual's fertility. Ketogenic diet is one of the protocols that possibly improve fertility status. A diet that is low in carbohydrates and high in fat not only results in weight loss for overweight people, but it also balances insulin levels, which play a positive role on male and female fertility and conception.

Keywords: *ketogenic diet, MetS, insulin resistance, PCOS, prostatic hyperplasia, female fertility, male fertility, overweight, obesity, insulin levels*

Introduction

Metabolic syndrome (MetS) is a complex and worldwide epidemic disorder with a high socioeconomic impact. It includes central obesity, impaired glucose tolerance, insulin resistance, hypertension, and dyslipidemia with high plasma concentrations of triglyceride and low concentrations of high density lipoprotein cholesterol [1].
Scientific studies continue to underline the exacerbating role of MetS-induced metabolic and endocrine derangements in the development of PCOS and BPH [2-3] both pathologies that affects human fertility and conception.

PCOS, MetS and female fertility

Polycystic ovary syndrome (PCOS), characterised by oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and/or polycystic ovaries [4], is one of the most common causes of ovulation dysfunction in women of reproductive age. The prevalence of PCOS has been found to range from 2 to 20% in the general population worldwide [5].
PCOS can be diagnosed by clinical and laboratory findings including irregular menstruation or amenorrhea, hirsutism, acne and/or enlarged polycystic ovaries in women with infertility.

In addition to abnormal clinical and biochemical signs of hyperandrogenism, insulin resistance, compensatory hyperinsulinaemia and impaired glucose tolerance could increase the risk of type 2 diabetes mellitus and hyperlipidaemia in women with PCOS [6-7].

Since the report by Burghen et al. in 1980 that PCOS was associated with hyperinsulinemia [8], it has become clear that the syndrome has major metabolic as well as reproductive morbidities. The recognition of this association has also instigated extensive investigation of the relationship between insulin and gonadal function [9-10]. Moreover, also obesity plays a crucial role in the pathogenesis of PCOS in presence of insulin resistance [9]. In fact it is common in 30–70% women with PCOS [7].

Additionally, in PCOS there is selective insulin resistance on the ovary, as reported for other tissues: insulin action on steroidogenesis is preserved, while insulin action on glucose metabolism is significantly decreased in granulosa-lutein cells from PCOS patients [11]. Furthermore, insulin inhibits the hepatic synthesis of sex hormone-binding globulin (SHBG), thus increasing free testosterone levels [12].

It is well documented in scientific literature that pre-conception weight is one of the major risk factors for female fertility outcomes and that weight loss improves fertility in overweight and obese women. Women with a body mass index (BMI) >30 kg/m2 are considered obese and this condition is often associated with an alteration of the natural menstrual cycle at a rate of almost three times higher than women with a BMI <25 kg/m2 [13]. Moreover, it has been demonstrated a positive relationship between pre-conception BMI and the time needed to fall pregnant in women who are overweight and obese [13]. In this scenario it has been estimate that approximately 7% of women of reproductive age have some form of sub-fertility [14]. Many overweight women with sub-fertility conditions, show co-morbidities. One of the major condition that affects fertility is exactly PCOS [15]. Furthermore, weight loss can improve fertility and pregnancy outcomes in women undergone in vitro fertilisation [16-17] thanks to a more regular menstrual cycles, a better quality of available embryos, a less hormonal dosage and fewer treatment cycles [18]. It has been demonstrated that weight loss of 5%–10% can significantly improve hormonal imbalances, reducing the rate of miscarriages and spontaneous abortions [18].

BPH, Mets and male fertility

Clinical benign prostatic hyperplasia (BPH) is one of the most common diseases in ageing men which can lead to lower urinary tract symptoms (LUTS). The prevalence of BPH increases after the age of 40 years. Recent reports suggest the strong relationship between BPH and metabolic syndrome but the exact biological pathways are still unclear yet. Key factors that play a fundamental role in this pathophysiological process are insulin resistance and increased visceral adiposity. Insulin is a mitogen and acts as a growth factor for prostatic epithelial cells [19-20]. Hyperinsulinaemia is also associated with lower SHBG, thus increasing the amount of androgen and estrogen entering prostatic cells, thereby increasing the risk of BPH [21]. Insulin-like growth factor 1 (IGF-1) was found to be associated with BPH risk as insulin receptor has homology with IGF receptor and can bind to IGF receptor activating the IGF signaling pathway to promote prostatic growth. In addition, insulin

lowers with insulin-like growth factor binding protein 1 (IGFBP-1) and further increases IGF-1 bioavailability [22]. Hyperglycaemia may increase cytosolic-free calcium in smooth muscle cells and neural tissues, leading to sympathetic nervous system activation. This activation may contribute to the increased smooth muscle tone of the prostate and may eventually worsen LUTS [23].

In Baltimore Longitudinal Study of Aging, each kg/m^2 increase in BMI corresponded with a 0.41 mL increase in prostate volume. Obese patients had a 3.5-fold increased risk of an enlarged prostate compared to non-obese participants [24]. Obesity results in an increased aromatase activity, leading to increased estradiol production, which further inhibits gonadotropin secretion and the production of testosterone. This hypogonadal obesity cycle results in a progressive enhanced estrogen to androgen ratio. Visceral adipose tissues secrete various bioactive substances known as adipocytokines, which can induce insulin resistance and related proinflammatory and proatherogenic effects. The reduction of adiponectin upon visceral fat accumulation stimulates glucose metabolism and fatty acid oxidation in the muscle, also enhances insulin sensitivity [25-26]. There are many evidences from epidemiological studies that obesity impacts negatively on male fertility. Sperm from obese men used for in vitro fertilization is associated with a greater number of pregnancy losses and experimental and epidemiological data show that male fertility, and offspring health, can be improved by weight loss in obese and overweight males [27]. Moreover has been demonstrated that in a case-controlled male cohort, MetS is associated with seminal inflammatory cytokines and reproductive dysfunction [28].

Methods

A literature revision was performed by using PubMed. The search was carried out for these eywords: "ketogenic diet", "MetS", "insulin resistance", "PCOS", "prostatic hyperplasia", "female fertility", "male fertility", "overweight", "obesity", "insulin levels".

Results and Discussion

The purpose of this review is to analyze the literature about the effects of ketogenic diet on the correction not only of the overweight, but also of the insuline resistance, both conditions related to MetS, PCOS, BPH and that can affects fertility outcomes.

Ketogenic diet (KD) is characterized by the reduction of carbohydrate intake (below 20 g per day) that induces a metabolic condition named "physiological ketosis". In this condition the body's glucose reserves become insufficient for the supply of glucose to the central nervous system, which cannot use fatty acids as an energy source. After 3–4 days of fasting or a very low carbohydrate diet the central nervous system starts to use an alternative energy source called ketone bodies (KB): these molecules are acetoacetate (AcAc), β-hydroxybutyric acid (BHB) and acetone. This process is called ketogenesis and occurs principally in the mitochondrial matrix in the liver [29].

Today Metabolic syndrome (MetS) is a major global public health problem. It is a combination of clinical, metabolic, and biochemical abnormalities including central adiposity, hypertension, insulin resistance, and dyslipidemias, closely associated with an increased risk of forthcoming cardiovascular disease and type 2 diabetes mellitus [30].

MetS is defined by distinct diagnostic criteria. The most commonly utilized criteria are the guidelines set by the National Cholesterol Education Program Adult Treatment Program III (ATP 3) in 2005. This definition includes: central obesity (defined as a waist circumference ≥88 cm for women and ≥102 cm for men), hypertriglyceridaemia (TG ≥150 mg/dL), reduced high density lipoprotein cholesterol (HDL-C < 50mg/dL for women and < 40 mg/dL for men), raised blood pressure (≥130/85 mmHg) and raised fasting plasma glucose (FPG ≥ 100mg/dL) [31]. People having three or more of the five components will be regarded as having MetS.Among these definitions, central obesity remains the important components of MetS. The increased visceral adipose tissue is associated with a range of metabolic abnormalities, reduced insulin sensitivity and adverse lipid profiles, all of which are risk factors for fertility [32-33].

Emerging evidence indicates that women with PCOS and men with BPH have an increased risk of developing metabolic syndrome over the course of their lives [2-3]. The prevalence ranging from 26.7% to 55.4% for the BPH and 33.4% to 53% for the PCOS [34-35].

Recent research provides substantial evidence of the therapeutic potential of ketogenic diets as clinical utility for prevention, reduction and reversal of MetS.

The majority of recent studies seem to amply demonstrate that a ketogenic diet can actually lead to significant benefits in weight loss, total cholesterol reduction, increases in HDL, reduction of blood triglycerides and improvements in insulin resistance. Yancy WS et al. conducted a pilot study on 120 overweight community volunteers with MetS who were randomly assigned to follow a low-carbohydrate ketogenic diet (<20g. CHO/die) or a low-fat - low-cholesterol reduced-calorie diet (<30% Fat/die and <300 mg/die of cholesterol). After 24 weeks of follow-up, the mean change in body weight was - 12.0 kg for those on the low-carbohydrate diet and - 6.5 kg for those on the low-fat diet. Both groups had more fat mass loss than fat-free loss, with the mean change in fat mass being - 9.4 kg for the low-carbohydrate group and - 4.8 kg for the low-fat group. The percentages of total weight loss from fat mass were similar, with 78% for the low-carbohydrate group and 74% for the low-fat group. Triglyceride levels, HDL-cholesterol levels, and ratio of triglycerides to HDL cholesterol changed statistically more with the low-carbohydrate diet than with the low-fat diet. LDL cholesterol levels increased by more than 10% in 30% of the 44 subjects on the low-carbohydrate diet and in 16% of 31 subjects on the low-fat diet [36].

In 2006 Dashti et al. conducted a trial study where obese individuals with MetS followed a VLCKD (Very Low Calory Ketogenic Diet) for 56 weeks; significant improvements in both weight/fat loss and metabolic parameters were evidenced at 10 weeks. At the end of 56 weeks they found improvements in fasting levels of glucose (-51%) and lipid markers triglycerides (-41%) [37].

Recently, Feinman et al., conducted a critical review of several studies comparing carbohydrate restriction to traditional low-fat diets as the first line of treatment for diabetes. They have shown that dietary carbohydrate restriction is the most effective method (other than starvation) of reducing serum triglycerides, increasing HDL and decreasing blood glucose levels [38]

Most of the women affected by PCOS seems to follow a high carbohydrate diet, i.e. they are more dependent on soft drinks, fast-food, sweets, cured and smoked meats, salted nuts, canned and processed vegetables, meats, marinades and sauces and less on traditional dietary habits. The sedentary lifestyle and unhealthy dietary patterns have mostly contributed to the prevalence of PCOS [39]. In a study performed by Yancy and coll. It has been demonstrated that a Low Carbohydrate Ketogenic Diet (LCKD) led to improvement in body weight, per cent free testosterone, LH/FSH ratio, fasting serum insulin, and symptoms in women diagnosed with PCOS. Hyperinsulinemia in women affected by PCOS appears to increase ovary androgen secretion as well as decrease circulating sex hormone binding globulin (SHBG). A Low Carbohydrate Ketogenic Diet led to a reversal of these processes [36]. The reduction of hyperinsulinaemia due to the LCKD would decrease stimulation of ovarian androgen production as well as increase SHBG levels, synergistically limiting the amounts of circulating free-androgens in the serum.

Moreover, a low carbohydrate diets may optimise fertility in overweight and obese women with PCOS [13]. A period of low carbohydrate ketogenic diet may improve fat oxidative metabolism and therefore reduce body weight. There are many evidences that reducing carbohydrate load can regulate circulating insulin levels, improve hormonal imbalance and result in a resumption of ovulation to ameliorate pregnancy rates.

Chavarro and colleagues performed a prospective study of 18,555 women founding that the quality of carbohydrate in the diet impacted the risk of ovulatory infertility with a 78% greater risk for women with higher carbohydrate consumption [16]. A study conducted on overweight and obese women with metabolic syndrome found that low carbohydrate diets achieved an improvement in waist circumference, fasting glucose, total-cholesterol and serum insulin. Furthermore, low carbohydrate diets resulted in considerable improvements in weight, high density lipoprotein cholesterol and triglycerides [40]. Moreover weight loss before pregnancy has historically been centred on the traditional low fat, energy restricted diet plan. However in the last few years the use low carbohydrate diets are increasingly being used to allow more favourable weight loss and fertility outcomes [44].Mavropoulos et al. performed a research on 11 women affected by PCOS with body mass index >27 kg/m^2 . They found that after 24 weeks of VLCKD (< 20g. CHO/die) there were significant reductions from baseline in body weight (-12%), percent free testosterone (-22%), LH/FSH ratio (-36%), and fasting insulin (-54%). Two women became pregnant despite previous infertility problems [41].

In both young and aged men, prostatic diseases, an unhealthy prostate, MetS and overweight can affect spermatozoa functioning and, therefore, male fertility. Randomized controlled trials are yet to be carried out to ascertain the association between exercise, diet and weight loss on the prevention, development or progression of LUTS. Epidemiological data show a favourable relationship between increased physical activity and weight loss with decreased risks and progression of BPH and LUTS [42]. A small number of studies evaluated the association between diet and BPH; the results of these researches were heterogeneous and inconclusive, but it seems that consumption of fruit and vegetables rich in b-carotene, lutein and vitamin C was inversely related to BPH [43]. Lifestyle emerges as a novel opportunity for the prevention and treatment of BPH and LUTS, infact promotion of healthy lifestyle is definitely beneficial in the context of MetS [44].

In a recent study has been reported that serum and seminal levels of TNF-α, IL-1β, IL6 and IL8 were all significantly increased in the MetS group. Ejaculation volume, sperm concentration, total sperm count, progressive and total motility and vitality were significantly decreased and sperm with abnormal MMP and DF were increased in the MetS group [32], so MetS seems to be correlated to male fertility. Unfortunately, there are no studies that correlate the benefits of the ketogenic diet with BPH, but it is known in the literature that the ketogenic diet positively impacts the metabolic syndrome and therefore it could be used for preventive purposes.

Conclusions

Data reported in literature confirmed that all the treatments aimed to reducing insulin levels, such as weight loss and insulin sensitizers, improve female and male reproductive health. In this scenario ketogenic diet can be considered as an effective strategy to prevent metabolic and endocrine disorders associated with PCOS, BPH and infertility by acting on weight loss, insulin regulation and improving fertility outcomes. Future studies could be focused on the link between Ketogenic diet and patients affected by BPH.

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THE KETOGENIC DIET AS AN ADJUVANT THERAPY IN CANCER TREATMENT

Mobilia Alessandro[1], Intintoli Luigia[1], Gioella Giuliana[1], D'Ursi Anna Maria[2]

[1]Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

[2] Associate Professor, Department of Pharmacy, University of Salerno, Italy

Email address: nutriketo@unisa.it

Abstract

Standard cancer treatments include chemotherapy, radiotherapy, or their combination. Most cancer therapies are designed to take advantage of the metabolic and physiological differences existing between cancer cells and normal cells. Cancer cells demonstrate significant alterations in metabolism that are proposed to result in increased steady-state levels of mitochondrial-derived reactive oxygen species (ROS) such as O_2 and H_2O_2.

The modulation of cellular metabolism by carbohydrate depletion via ketogenic diets has been suggested as an important therapeutic strategy to selectively kill cancer cells. Ketogenic diet is a high-fat, adequate-protein, and low-carbohydrate diet that leads to nutritional ketosis. Changes in the metabolic pathways and cellular signalling as well as increased mitochondrial biogenesis and improvement of mitochondrial function are some of the cellular effects observed after the adoption of a ketogenic diet.

Keywords: *Ketogenic Diet, Cancer, Cancer Nutrition, Low Carbohydrate, High fat, Oncology*

Introduction

This review focuses on how oncological disease have been targeted by ketogenic diets, their metabolic effects, the possible mechanisms of action on mitochondrial energy homeostasis and its role in enhancing cancer cell therapeutic responses.

Methods

Literature review. Articles published in scientific journals have been assessed texting words related to ketogenic diet and cancer.

Results

This review focused essentially on the impact of ketogenic diets on human health and how these types of diets can be applied as co-adjuvant therapies for cancer.

Since glucose is the main source of energy for cancer cells (the Warburg effect), a reduction in the availability of this fuel can be beneficial, controlling the proliferation and metastatic capacity.

Changes in the metabolic pathways and cellular signalling as well as increased mitochondrial biogenesis and improvement of mitochondrial function are some of the cellular effects observed after the adoption of a ketogenic diet.

Metabolic reprogramming from oxidative phosphorylation toward increased glycolysis is a hallmark of cancer cells, there is increasing evidence that the ketogenic diet may also be beneficial as an adjuvant cancer therapy by potentiating the antitumor effect of chemotherapy and radiation treatment.

In conclusion, ketogenic diets can be a good option as a co-adjuvant therapy, depending on the situation and the extent of the disease.

Discussion

The modulation of cellular metabolism by carbohydrate depletion via ketogenic diets has been suggested as an important therapeutic strategy to selectively kill cancer cells.

In the majority of normal cells with functional mitochondria, pyruvate generated via glycolysis is shuttled to the tricarboxylic acid cycle (TCA) for mitochondrial oxidative metabolism.

Cancer cells, on the other hand, use pyruvate mostly in the lactic acid fermentation pathway and exhibit alterations in mitochondrial oxidative metabolism that are believed to be the result of chronic metabolic oxidative stress (1,2,3).

Mitochondria are involved in the regulation of cellular energy production through the process of oxidative phosphorylation where electron transport chain (ETC) activity is used in the generation of cellular ATP (4).

In the mitochondrial ETC, electrons are shuttled down (Complexes I–IV), resulting in the generation of transmembrane proton gradient that is coupled to ATP production through ATP synthase (Complex V). Studies have shown increased prevalence of mitochondrial DNA mutations as well as alterations in the expression of nuclear encoded mitochondrial proteins in many human cancers (5,6,7) including head and neck (8), prostate (9),ovary (10) and liver cancer (11).

Previous data suggests that the susceptibility of mitochondrial DNA to mutations is largely due to the increase in ROS levels in this organelle (12,13,14,15,16,17).

Furthermore, recent studies have shown that breast and colon cancer cells demonstrate significantly increased steady-state levels of ROS relative to normal colon and breast cells (1).

These differences were even more pronounced in the presence of mitochondrial ETC blockers, suggesting dysfunctional mitochondrial ETCs as the major source of elevated ROS production in cancer cells (1).

Lipid metabolism limits the availability of glucose for glycolysis restricting the formation of pyruvate and glucose-6 phosphate which can enter the pentose phosphate pathway forming NADPH necessary for reducing hydroperoxides.

Additionally, lipid metabolism forces cells to derive their energy from mitochondrial metabolism. Because cancer cells are believed to have dysfunctional mitochondrial ETCs resulting in increased one electron reductions of O_2 leading to ROS production, cancer cells will be predicted to selectively experience oxidative stress, relative to normal cells, when glucose metabolism is restricted in the case of feed-

ing ketogenic diets. Overall, there is substantial literature indicating that there is a significant increase in intracellular $O_2^{\bullet -}$ and H_2O_2 in cancer cell mitochondria relative to normal cells and that this could represent a target for enhancing cancer therapy (18, 19,20 ,21 ,22 ,23).

The metabolic phenotype explicated above provides several advantages to cancer cells.

First, it allows for a more efficient generation of carbon equivalents for macromolecular synthesis than oxidative phosphorylation (OXPHOS), which is suitable for a proliferative phenotype (24).

Second, it bypasses mitochondrial oxidative metabolism and its concurrent production of reactive oxygen species (ROS). This confers a survival advantage since cancer cells display higher steady-state levels of oxidative stress relative to normal cells, which renders them more sensitive to ROS-mediated apoptotic stimuli (1).

Finally, an elevated glycolytic flux promotes acidification of the tumour site, which facilitates tumour invasion and progression (25).

Dysfunctional mitochondria may upregulate some oncogenes of the phosphatidylinositol 3-kinase/Akt mammalian target of rapamycin signalling pathway (26).

Akt, a downstream of insulin signaling (27), is involved in changes in tumour cell metabolism and increases resistance to apoptosis; it also decreases β-oxidation and increases lipid synthesis in the cytosol (28).

Since ketogenic diets stimulate mitochondrial biogenesis, improve mitochondrial function, decrease oxidative stress (29,30) and contribute to reducing the glycolytic rate due to increases in lipid oxidation and mitochondrial respiration (31).

It seems a reasonable possibility that a very-low-carbohydrate diet could help to reduce the progression of some types of cancer.

These diets have also been proposed as a possible treatment for mitochondrial disorders (32,33,34), in fact, Jarret et al. (35) demonstrated that a ketogenic diet affords protection to the mitochondrial genome against oxidative insults, increasing the levels of mitochondrial GSH, stimulating de novo biosynthesis of GSH, and improving mitochondrial redox status.

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THE INTESTINAL BENEFITS OF TRIPHALA ACCORDING TO THE AYURVEDIC MEDICINE, DURING THE KETOGENIC DIET

Lauricella Noemi Martina; Marcantonio Roberta; Muro Rita; Scala Francesco
Post Graduate Universitary course master in diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II, Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

The herbal remedies are among the most used in traditional medicine. According to the ayurvedic medicine the Triphala, a polyherbal formed by three species of ayurvedic plant, has therapeutic effects for the treatment of IBDs.

Keywords: *Ayurvedic, Boswellia Serrata, Triphala, Ibds*

Introduction

The ayurveda medicine

The Ayurveda medicine, ancient medical of India, teaches that health is maintained by the balancing of three subtle energies known as "doshas". Individually they are called Vatha (sometimes spelled Vata), Pitta and Kapha. The Ayurvedic medicine is based on an individual's characteristics and body frame rather than oriented toward treating disease or sickness. Each of us is made up of a combination of the three types of doshas. The doshas as a group are comprised of these five universal elements:

1. space (ether)
2. air
3. earth
4. fire
5. water

In particular, Vatha is a combination of air, space, Pitta is mostly fire with some water, and Kapha is mostly water with some earth.

Overall well-being depends on maintaining your health in order to keep your doshas balanced. Any imbalance among the tridoshas causes a state of unhealthiness. The factors that can bring about the balance of the tridoshas include diet, exercise, good digestion, and elimination of toxins.(1)

The concepts of ayurveda medicine are also applicable in daily diet, in fact according to this ancient science the "Six Tastes", or "Shad Rasas" so called in Ayurveda, are made up of the five elements; sweet is earth and water, salty is water and fire, sour is earth and fire, pungent is fire and air, astringent is earth and air, and bitter is air and space.

When all six tastes are included in the diet, all five elements are fed and your body is fully nourished.

Most importantly, the balanced application of the Six Tastes builds healthy tissue, increases energy, strengthens your immune system, promotes lightness, comfort, and supports mental clarity, concentration and contentment.

Each taste has an intimate relationship with the doshas and personal balance, for example: the taste sweet, or madhura, is a builder of tissues that are formed from earth and water. Hence, sweet substances strengthen Kapha, but an overload of sweets, on the other hand, can create a Kapha imbalance, which needs to be corrected with pungent, bitter and astringent tastes and warming foods.(1)

Salty, sour and pungent tastes strengthen Pitta, or rather all those functions associated with a rise in temperature (metabolic processes). An excess of these tastes, however, aggravates Pitta having to be balance out accordingly with sweet, cooling foods.

Pungent, bitter and astringent tastes increase Vatha and all phenomena to do with movement, penetration and cleansing of channels. If you need to pacify Vatha, therefore, you need to focus on the sweet, sour and salty tastes and eat more warm foods.

From the chemical point of view, the tastes are associated with biological components that the body needs:

- Sweet: carbohydrates, sugars, fats, amino acids
- Sour: organic acids
- Salty: salts
- Pungent: volatile oils
- Bitter: alkaloids, glycosides
- Astringent: tannin (1)

Approximately 90% of ayurvedic preparations are plant based.

Ayurvedic plants have a stronger action on the body than either food or spices. Such actions enable the plant to reverse pathophysiological processes and stabilize the doshas.

Polyherbal combinations have also proven lastingly effective than single herbs. In ayurveda, most of the classical preparations are polyherbal, with a combination of 3 to 30 plants involved.(9) These constituents are combined accurately, in such a way that the formula is balanced and reproducible. One or two of the plants in these combinations will be active and the others will play a supporting role. The supporting herbs will each have different actions, acting as catalysts to help proper absorption, transportation, and

to reduce toxicity. If an ideal combination is delivered, then the result can be excellent, but such outcomes are based on thorough plant knowledge.(9)

Methods

Side effects of a ketogenic diet

Like any significant change to your diet, when starting a ketogenic diet, it is normal to experience one or more side effects as the body adapts to a new way of eating.(1)

When going on a ketogenic diet, the body has to switch its fuel source from the glucose in carbohydrate to using its own fat stores, and this can lead to experiencing some of the following side effects:

- Loss of salts
- Keto-flu
- Changes in bowel habitus
- Leg cramps
- Bad breath
- Loss of energy

Usually these side effects are temporary and can usually be remedied.

Changes in bowel habits

Changing to a ketogenic diet may bring about changes in bowel habits such as constipation; this is one of the case that the body's own gut bacteria will need to adapt to handle different foods in different amounts.

For people who switch to keto, IBS issues are among the most common side effects.

Constipation, diarrhea, bloating, abdominal cramps, pain, and all the kinds of other unpleasant digestive symptoms are unfortunately problems that keto dieters often have to consider.

The ayurveda phytotheraphy offers multiple remedies for headaches caused by the ketogenic diet.

In particular, the "Triphala" has many clinical applications in Ayurveda; it is considered of special importance for gastrointestinal disorders ranging from constipation to colitis, exerts enteroprotective effects and promotes health of the gut epithelium and villi through the nutrient absorption.(1)

The Triphala and its utilization

Triphala translated as "three fruits", as it consists of the fruits of the amalaki, bibhataki, and haritaki trees. To prepare Triphala, the fruits are first dried, ground into powder form and then combined in three equal parts.

The compounds in each of the three fruits of Triphala are thought to have beneficial effects on the human body.

Let's take a look at each one in turn:

Amalaki (Emblica officinalis): The fruit of the amalaki has a very high vitamin C content. In Ayurvedic medicine, it is lauded for its antioxidant and anti-aging effects.

Haritaki (Terminalia chebula): The fruit of the Haritaki tree contains high tannin levels. Tannins have been shown to have natural antibacterial, antifungal, and antiviral qualities. Haritaki is viewed in Ayurvedic medicine as providing immune system support and is often recommended as an overall body panacea. In the area of digestion, haritaki is thought to have antispasmodic effects, and therefore would be recommended for use in easing abdominal pain and normalizing bowel movements.

Bibhitaki (Terminalia belerica): The fruit of the bibhataki tree contains levels of gallic acid, tannic acid, and glycosides. These compounds are thought to give bibhataki antioxidant and antispasmodic qualities.(6) (Fig.1)

Results

Ayurvedic Uses for Triphala

According to the Ayurvedic system, Triphala is most generally used as an overall body tonic, thought to be effective in cleansing and detoxifying the system. It may be recommended for arthritis, headaches, and liver problems. In terms of digestive health, Triphala can be helpful in addressing abdominal pain, bloating, dyspepsia and constipation.

Triphala may also promote proper digestion and absorption of food, reduce serum cholesterol levels, improve circulation, relax bile ducts, prevent immunosenescence, maintain homeostasis of the endocrine system, and increase production of red blood cells and hemoglobin.

Stress-reducing potential of Triphala

Recent studies have confirmed the beneficial use of triphala for stress disorders; stress is a state of disharmony caused by perceived threat that is counteracted by an adaptive response to reestablish homeostasis and is associated with many chronic diseases. Studies on animals have shown that Triphala protected against cold-induced stress and reversed stress-induced behavioral alterations and biochemical changes such as increased lipid peroxidation and corticosterone levels.

Antiobesogenic and antidiabetic potential of Triphala

Studies have demonstrated the potential of TLP as therapeutic agent for weight loss and reduction of body fat. Triphala treatment decreased the percentage of body fat, body weight, and energy intake, also decreased total cholesterol, triglycerides, and low-density lipoprotein cholesterol.(3)

Triphala exerts hypoglycemic effects. Patients with type 2 diabetes are likely to have high postprandial blood glucose levels, especially after consuming carbohydrates. High blood glucose levels results from the breakdown of carbohydrates by the digestive enzymes, alpha-amylase and alpha-glucosidase, and the reduced ability of cells to take in glucose from the blood.

Past studies report that Triphala may exert similar actions to diabetic pharmaceutical drugs by inhibiting digestive enzymes and may decrease absorption of glucose through inhibition of glycolytic enzymes, thereby reducing blood glucose levels. One study demonstrated the inhibitory potential of Triphala on pancreatic glycolytic enzymes, namely alpha-amylase and alpha-glucosidase, which break down larger polysaccharides into glucose molecules that enter the blood stream. It possible to state that the role Triphala plays in inhibiting starch digestion and absorption, thereby decreasing postprandial hyperglycemia, is similar to the one of diabetes pharmaceutical drugs, such as miglitol and acarbose, which also target these glycolytic enzymes.(3)(Fig. 2)

Triphala and cardiovascular health

Cardiovascular disease is a leading cause of mortality worldwide, and hypercholesteremia is an important risk factor.

Studies have reported the hypercholesteremic effects of Triphala. In one study, Triphala reduced the total cholesterol, low-density lipoprotein, very low-density lipoprotein, and free fatty acid levels.

Another study has revealed that Haritaki (one of the herbs in Triphala) induced hypolipidemic effects.

Anti-inflammatory effects of Triphala

Triphala has showed anti-inflammatory effects better or equivalent to nonsteroidal anti-inflammatory drugs.

Triphala reduced expression of inflammatory mediators such as IL-17, COX-2, and RANKL through inhibition of NF-kB activation. Another study found that Triphala increased antioxidant levels and decreased lipid peroxidation in the tissues of arthritic rats.(3)

Studies carried out on the gastrointestinal effects of Triphala

Triphala is perhaps most well-known for its use in general gastrointestinal health. A first consideration should be done on the content of Triphala FODMAP. FODMAPs are carbohydrates found in ordinary foods that can contribute to GI symptoms. (6)

Studies on animals have shown that both aqueous and alcohol-based extracts of Triphala prevent diarrhea. Triphala also induces enteroprotective effects, which are likely due, at least in part, to the high antioxidant content. In a rodent model, Triphala replenished depleted protein in the intestinal villi of the brush border as well as glutathione and phospholipid levels; the formula simultaneously decreased myeloperoxidase and xanthine oxidase levels in intestinal epithelium. In rats, Triphala exerted a gastroprotective effect on stress-induced ulcer.

One human clinical trial that investigated the use of Triphala in patients with gastrointestinal disorders reported that treatment reduced constipation, mucous, abdominal pain, hyperacidity, and flatulence while improving the frequency, yield, and consistency of stool. Triphala also reduced colitis in a mouse model and the treatment effects are attributed to antioxidant effects and high levels of flavonoids contained in Triphala.(3)

It is known that phytochemicals in Triphala such as quercetin and gallic acid promote the growth of

Bifidobacterium and Lactobacillus species while inhibiting the growth of undesirable gut residents such as E. coli.(3)

The presence of *P. emblica*, TLP inhibits the growth of pathogenic microorganisms in the intestines, which may aggravate intestinal symptoms. Polyphenols in TLP can modulate the human gut microbiota by promoting the growth of beneficial *Bifidobacterium* and *Lactobacillus* species and inhibiting the growth of undesirable intestinal residents, such as *Escherichia coli*, which may induce the inflammatory reaction.(3) Another study showed ythe activity of. *chebula*, on digestion, constipation and intestinal cramp. The effect of TLP depending on the proportions of *T. chebula*, *T. bellerica* and *P. emblica,* the level of the injuries varied. It was suggested that unequal formulation of the ingredients in TLP i.e. the highest proportion of *P. emblica*, rather than its equal formulation provided better protection of intestinal microvilli.

In addition, the lactic acid bacteria possess enzymatic activity (e.g., tannase) to degrade plant tannins such as gallic acid contained in Triphala. For example, Triphala-derived polyphenols, such as chebulinic acid, are transformed by the human gut microbiota into metabolites such as urolithins, which have the potential to prevent oxidative damage.(3)(Fig.3)

The results of studies conducted on groups of rats suggest that Triphala has a considerable and reliable effect in reducing colitis, probably for its antioxidant activity and the good presence of flavonoids.

Colitis is an inflammation of colon, which is often used, in medical context to describe an inflammation of the large intestine (colon, caecum and rectum). Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Antioxidants thus play an important role to protect the human body against tissue damage by reactive oxygen species.(4)

The group of rats, treated with DSS (a polyanionic derivative of dextran, produced by esterification with chlorosulfonic acid) able to induce colitis in rodents, has developed colitis as human ulcerative colitis both histologically and topologically.

The results suggest that *Triphala* (300 mg/kg) has a considerable and reliable effect in reducing colitis in rats. This effect can be attributed to its antioxidant activity and well presence of flavonoids. The above study also suggests the positive role of *Triphala* in suppression of inflammatory mediators leading to the suppression of colitis.(4) Earlier studies on *Triphala* proved it as anti-inflammatory, cytoprotective and immunomodulatory in nature and support the usage of *Triphala* in the treatment of colitis.(4)

Discussion

The information contained in this review was collected to evaluate the effects of the ketogenic diet on human organism and how to avoid or treat the gastric symptoms, through ayurveda phytotherapy, derived from it.

The ketogenic diet is a real therapy and as such can have related side effects to the effects on the gastrointestinal tract of a diet very rich in fats and poor in slag (dyspepsia, slowing down of transit) and the metabolic picture resulting from its use. Usually the disturbances are transitory, and are distinguished in short term complications (during the induction of the ketosis) and medium (3-6 month) and long-term complications.(5)

The former depend in part on the type of induction of the ketosis used. In fact, in case of fasting is more frequent than the appearance of dehydration, hypoglycemia, lethargy or rarely acidosis, complications that are significantly reduced or absent in case of gradual induction. The gastrointestinal disorders diseases are common and result from the intake of very high fat meals. In each case is generally mild and transient.(5)

The ayurvedic phytotherapy plays an important role in contrasting the gastrointestinal effects that occur during the ketogenic diet; this study focuses on the Triphala.

The health benefits of triphala are well documented; however, the impact of these herbal medicines on the human gut microbiota has been only scarcely studied. Triphala is a cornerstone treatment for gastrointestinal health and disease in Ayurveda and other traditional systems of medicine.

Triphala is considered a *rasayana* medicine, which classically categorizes it as a tonic that provides immunity and rejuvenation. In the context of gut health for which it is most renowned, the formula is often used to treat ulcers, constipation, abdominal pain, and colitis.(3)
The beneficial effects of the triphala were evaluated through various studies *in vitro,* anaerobic fecal cultures offers several advantages, including the ease and low-cost cultivation highly representative of human microbiota.(7)

Herbal medicine supplementation of human fecal cultures resulted in profound changes in the relative abundance of many phylogenetically diverse gut species. Each herbal medicine evaluated in this study uniquely altered gut bacterial communities; however, the microbial composition of triphala supplemented cultures more closely resembled one another.

Through these studies it was possible to state that human consumption of triphala, will induce similar alterations in gut microbiota since prebiotic effects are largely microbiologic processes driven by glycan microbial metabolism. The magnitude of alterations observed *in vitro* may be significantly dampened *in vivo* owing to inherent resilience of gut microbiota.(7) It is predicted that in humans these herbal medicines have a strong potential to elevate butyrate and propionate levels.

Butyrate is known to promote anti-inflammatory responses through activation of IL-10 producing Tregs, an improved gut barrier, and the accompanying reduction of circulating lipopolysaccharide, microbial and food antigens. In addition, butyrate is a HDAC inhibitor that increases expression of Muc2 and other mucins, which further promotes proper gut barrier function and global alterations of gene expression. Propionate is transported to the liver where it serves as substrate for gluconeogenesis, lipogenesis, and protein synthesis. Moreover, both butyrate and propionate are GPR41 ligands expressed by L cells inducing GLP-1 production that drives pancreatic β cells to secrete insulin and control blood glucose levels. Thus, the induction of gut microbiota SCFA production confers diverse health benefits to the host.(7)

These results, for the first time, provide evidence that the health benefits of Ayurvedic herbal medicines may be mediated by the glycan catabolic activities of the human gut microbiome. These studies are novel in highlighting the significant prebiotic potential of herbal medicines and suggest that the health benefits of these herbs are due, at least in part, to their ability to modulate the gut microbiota in a manner predicted to improve colonic epithelium function, reduce inflammation, and promote protection from bacterial opportunistic pathogenic infection. Forthcoming studies in human clinical trials will test the concordance of the results generated *in vitro* and the predictions made by genome analyses.(7)

Triphala is considered as one of the most important formulation in Ayurvedic therapeutics for its multiple organ protective effects including gastroprotection

Oxidative damage is considered causes of gastric ulceration in humans. Through studies on various mucosal cells of rats, it is possible to conclude that *Triphala* formulations possess significant anti-ulcer activity. This activity depends mainly on inhibition of free radical generation due to restoration of free radicals scavenging enzymes, enhancing the stability of gastric mucosal barrier and gastric cytoprotection against stress-induced gastric ulceration.(8)

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4. Vinay Rayudu, Akondi B. Raju1 Department of Pharmacology, St. Peters Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, Andhra Pradesh, India, 1 Department of Pharmacology, Ibn Sina National College for Medical Studies, Al Mahjar, Jeddah, Kingdom of Saudi Arabia. Effect of Triphala on dextran sulphate sodium induced colitis in rats AYU | Jul-Sep 2014 | Vol 35 | Issue 3

5. Prof.ssa Anna Tagliabue, Docente di alimentazione e nutrizione umana, Direttore Centro Interdipartimentale di Studi e Ricerche sulla Nutrizione Umana e i Disturbi del Comportamento Alimentare, Università degli Studi di Pavia, Dott.ssa Claudia Trentani, Dietista Dietista Responsabile del Centro Interdipartimentale di Studi e Ricerche sulla Nutrizione Umana e i Disturbi del Comportamento Alimentare, Università degli Studi di Pavia. La Dieta Chetogenica Manuale informativo per il medico e il personale sanitario. Nutricia Metabolics

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7. Christine Tara Peterson, PhD,1 Vandana Sharma, PhD,2 Sasha Uchitel,3 Kate Denniston, BS,4,Deepak Chopra, MD,1,5 Paul J. Mills, PhD,1 and Scott N. Peterson, PhD2 AbstractPrebiotic Potential of Herbal Medicines Used in Digestive Health and Disease THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE Volume 24, Number 7, 2018, pp. 656–665 JACM Mary Ann Liebert, Inc. DOI: 10.1089/acm.2017.0422

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Table 1. The main constituents of Triphala and their potential therapeutic effects

Botanical (Hindi name, English)	Phytochemicals	Percentage of phytochemical in each Triphala constituent	Indication for therapeutic usage
Terminalia chebula (Harad, Chebulic Myrobalan)	Gallic acid Tannic acid Syringic acid Epicatechin Ascorbic acid Chebulinic acid Anthraquinone Phosphoric acid	0.024% (w/w) 0.011% (w/w) 0.009% (w/w) 0.006% (w/w) 0.020% (w/w Unknown Unknown Unknown	Constipation, hemorrhoid, skin disease,asthma,dysentery, uterine debility, anemia, diabetes, leukoderma, tumors and heart disease
Terminalia bellerica (Baheda, Belliric Myrobalan)	Gallic acid Tannic acid Ascorbic acid β-sitosterol Ellagic acid Chebulic acid Mannitol Oxalic acid Galloyl Galactose Fructose	0.005% (w/w) 0.004% (w/w) 0.023% (w/w) Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown	Cough, asthma, anorexia, vomiting, arthritis, fever, epilepsy, splenomegaly, piles, diarrhea, leprosy, brain tonic and laxative
Phyllanthus emblica (Amala, Emblic Myrobalan)	Ascorbic acid Gallic acid Nicotinic acid Ellagic acid Linoleic acid Linolenic acid Oleic acid	0.036% (w/w) 0.081% (w/w) Unknown Unknown Unknown Unknown Unknown	Diabetes, hysteria, jaundice, eczema, piles, diarrhea, menorrhagia, scurvy, rebuilds and maintains new tissues and increases red blood counts

Table 2. Indication and contraindication for the use of ketogenic diet

Main indications	Contraindications
Metabolic diseases • Glucose transport protein deficiency • (GLUT-1) • Pyruvate dehydrogenase deficiency (PDHD) • Complex mitochondrial disorders • absolute • Carnitine deficiency	**Absolute** • Carnitine deficiency • Deficiency of carnitine palmitoyl transferase I or II • Deficiency carnitine translocase • Deficit beta oxidation of fatty acids • Pyruvate carboxylase deficiency • Porphyria
Epilepsy • Severe myoclonic epilepsy of childhood (Dravet syndrome) • Refractory epilepsy • Severe intolerance to antiepileptic drugs	**related** • poor motivation and collaboration of the patient

Table 3. Complication of the ketogenic diet

SHORT TERM	LONG TERM
Nausea and vomiting, diarrhea rejection of food, lack of appetite transient lethargy hypoglycemia dehydration acidosis	Alvo alterations (constipation, diarrhea) hyperuricemia, hypoproteinemia, hyperlipidemia, hypocalcemia, osteopenia kidney stones recurring infections acidosis delay growth

Figure 1:

Figure 2:

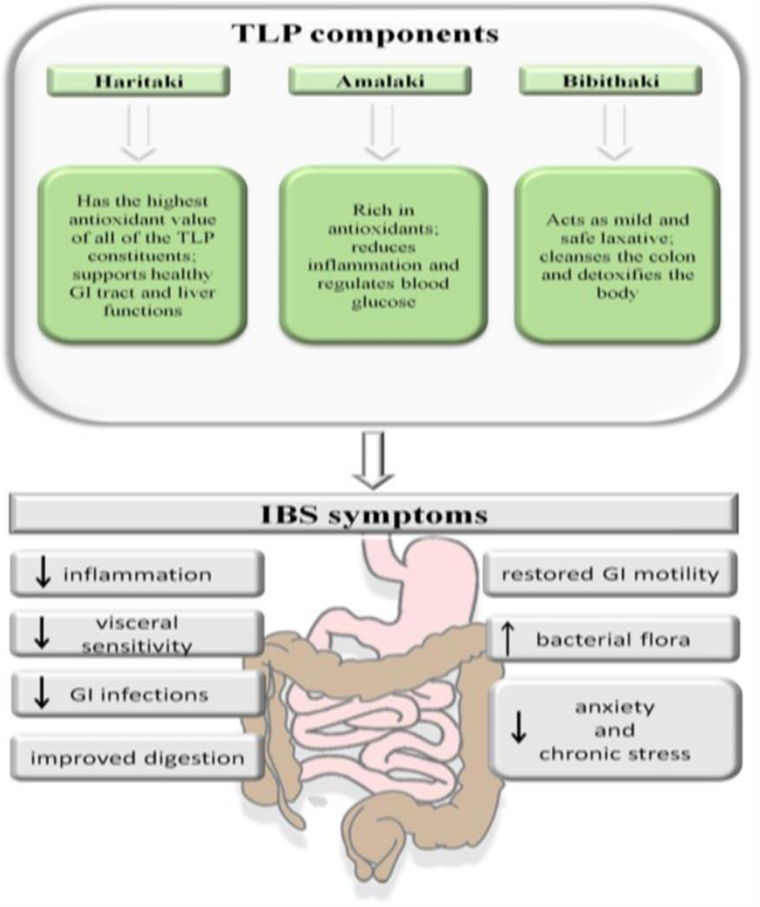
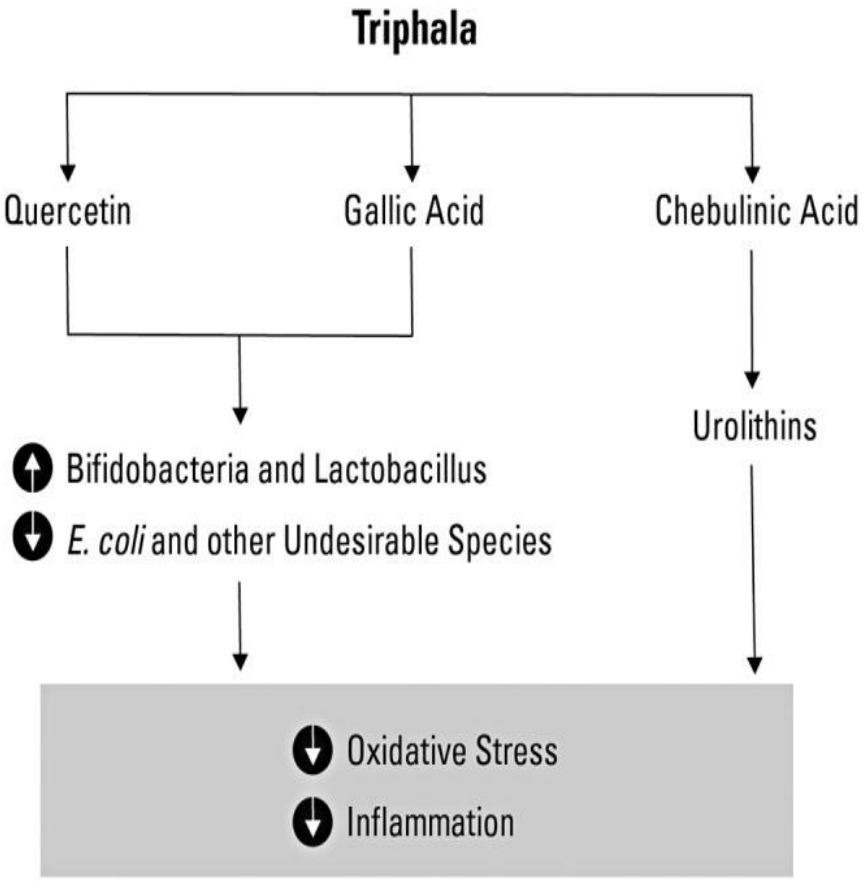


Figure 3:


Triphala
Quercetin
Gallic Acid
Chebulinic Acid
Urolithins
Bifidobacteria and Lactobacillus
E. coli and other Undesirable Species
Oxidative Stress
Inflammation

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THE ANTIOXIDANT ROLE OF ALPHA-LIPOIC ACID IN DIABETIC NEUROPATHY AND IN INSULIN RESISTANCE

Simeone Luciana; Sorrentino Denise; D'Amico Lucia; Rastrelli Luca

Post Graduate Universitary course master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II, Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

Alpha-lipoic (ALA) acid is a non-vitamin coenzyme that carries out important metabolic and antioxidant functions in the body, it is involved in energy metabolism of proteins, carbohydrates and fats, has physiological functions in blood glucose disposal, and is able to scavenge a number of free radicals. It functions in the body much like a B-vitamin, since it is involved in energy production. As part of several multi-enzyme complexes located in the mitochondria, alpha-lipoic acid is essential for metabolizing carbohydrates, proteins, and fats, and for the conversion of their energy into ATP.

A related metabolic function of alpha-lipoic acid is its role in blood glucose disposal. This important coenzyme appears to be necessary for the normal transport of blood glucose into the cell. For this reason, alpha-lipoic acid is used as a dietary supplement to improve the antioxidant state of the organism and regulate glucose metabolism, also because this molecule is efficiently absorbed, transported to the tissues, and readily taken up by cells. ALA is approved for the treatment of diabetic neuropathies, a common and potentially serious complication of diabetes that involves the peripheral nerves of the body, as a result of high blood glucose, or hyperglycemia.

For the treatment of diabetes, the recommended dose of ALA is 300-600 mg per day. For the general antioxidant support, the dosage is 20-50 mg per day.(23) ALA intravenously and orally are approved for the treatment of diabetic neuropathy in Germany. Based on the evidence presented above, ALA accelerates the removal of glucose from blood, at least partly improving insulin function, and reduces insulin resistance, which is a basis of many cases of coronary heart disease and obesity.

Keywords: *Alpha-lipoic (ALA), diabetic neuropathy, insulin resistance*

Introduction

Alpha-lipoic acid, what is it and what are its functions?

Alpha-lipoic acid is a fatty acid containing sulfur, which is produced by the liver and other tissues of our body. It is also absorbed by some foods introduced with the diet and accumulates in some tissues (liver, heart, muscle, brain, nerves and others). The main sources of the lipoic acid are liver, kidney, heart, spinach, broccoli.

It was chemically isolated for the first time in 1951 by Reed and Gunsalus from liver extracts. It is a small molecule, endogenously produced (in relatively low quantities) at the mitochondrial level starting from cysteine and octanoic acid, but mainly introduced with diet. The ALA is a nutritional coenzyme that is involved in energy metabolism of proteins, carbohydrates and fats, it is a fat- and water-soluble, sulphur containing coenzyme, formed of eight carbon atoms, two of oxygen in the carboxylic group and two of sulfur. In nature it exists in two forms, such as cyclic disulphide (oxidized form) or as an open chain with the name of dihydrolipoic acid, which shows two sulfhydryl groups in position 6 and 8 (Fig.1) and has physiological functions in blood glucose disposal, and is able to scavenge a number of free radicals.

It functions in the body much like a B-vitamin, since it is involved in energy production. It works as a cofactor that binds acyl groups and transfers them from one side of the enzyme complex to the other. During this process the lipoic acid is reduced to dihydrolipoic acid (DHLA) which is then subsequently reoxidized by the enzyme lipoamide dehydrogenase with the formation of NADH.

In heavy metal intoxication, lipoic acid and its metabolites are useful for capturing metals and eliminating them from the circulatory stream. It is also important to remember how DHLA has the ability to regenerate some endogenous antioxidants such as Vitamin C, E and glutathione. In diabetic neuropathy, lipoic acid reduces both the oxidative damage induced by glucose in nerve endings and inflammation, through a series of inhibitory mechanisms on the neuronal transcription factor NF-kB (Lee at all 2008), which is at the basis of biosynthesis of numerous inflammatory cytokines including IL-1 and IL-6.

The action on energy metabolism characterizes an important effect on the resistance of tissues to insulin. In fact, supplementation with alpha lipoic acid:

- Increases the synthesis of ATP and cellular energetic disponibilities;

-has a hypoglycaemic effect because it stimulates glucose uptake in muscle cells mimicking the action of insulin (jacob et all 1996);

- increases glucose uptake at the nerve level, thus increasing the energy available to the nerve cell: an important effect in diabetic neuropathy where there is a deficit in nerve energy availability.

As part of several multi-enzyme complexes located in the mitochondria, alpha-lipoic acid is essential for metabolizing carbohydrates, proteins, and fats, and for the conversion of their energy into ATP.

Alpha-lipoic acid (ALA), a naturally existing compound that acts as a coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, plays a key role in bridging glycolysis, the citric acid cycle, regulates glucose and lipid metabolism, and as such assume a central role for general energy production. Another lipoic acid containing enzyme complex, BCKADH (branched-chain keto-acid dehydrogenase), is involved in deriving energy from the branched chain amino acids, leucine, isoleucine, and valine.

A related metabolic function of alpha-lipoic acid is its role in blood glucose disposal. This important coenzyme appears to be necessary for the normal transport of blood glucose into the cell. This may be explained by its functions in the glucose metabolizing enzymes, PDH and alpha-KGDH, but some researchers suspect a more direct role in cellular glucose uptake at the cell membrane. Alphalipoic acid also protects from the oxidation of LDL-cholesterol (the "bad" cholesterol), thus acting as a factor in preventing the risk of cardiovascular disease. Furthermore, some studies have documented the ability of ALA to chelate metals or to capture and requisite mainly copper and iron, which in some cells would lead to the formation of strongly oxidizing substances. The studies have shown a hypotensive and anti-inflammatory action that is still being studied. Alpha-lipoic acid is considered a memory-enhancer, a substance that can improve memory in old age and

counteract the aging of the brain thanks to its powerful antioxidant action.

The antioxidant role of alpha-lipoic acid

As early as 1959, alpha-lipoic acid was suggested to be an antioxidant, since it could extend the actions of vitamin C in guinea pigs, and those of vitamin E in rats. It is only recently, however, that the specific actions of alpha-lipoic acid in free radical quenching, metal chelation, and antioxidant regeneration have been investigated. Body cells and tissues are threatened continuously by damage caused by toxic free radicals and reactive oxygen species (e.g., peroxides) which are produced during normal oxygen metabolism, and by toxic agents in the environment.

Free radicals, once formed, are capable of disrupting metabolic activity and cell structure. When this occurs, additional free radicals are produced which, in turn, can result in more extensive damage to cells and tissues. The uncontrolled production of free radicals is thought to be a major contributing factor to many degenerative diseases. Alpha-lipoic acid is unique among biological antioxidants, because it is soluble in both water and lipids. This allows it to neutralize free radicals just about everywhere in the body, inside and outside the cells. Due to its unique sulfur-containing structure, alpha-lipoic acid can scavenge several types of free radicals, such as the highly reactive hydroxyl, and singlet oxygen free radicals. It is also capable of suppressing the generation of free radicals in the first place, since alphalipoic acid chelates transition metals, such as iron and copper.

In addition to being a universal scavenger of free radicals, alpha-lipoic acid can also regenerate other endogenous antioxidant systems, extending the activity of vitamins C and E and regenerating glutathione.

ALA fights free radicals in every part of the neuron (but also in other cells) and is the only antioxidant to do so. Among the unique characteristics of alpha-lipoic acid, there is, in fact, that of being able to act both inside the cell, in an aqueous (hydrophilic) environment such as the cytoplasm of the cells (where other antioxidants such as vitamin C also act, both in an oily environment that is rich in lipids (lipophilic) as the membrane of neurons and cells, whose integrity is essential for the transmission of nerve impulses. Ultimately, the ALA can reach all the compartments of a cell protecting it in and out of the incessant attack of free radicals.

The role in nerve Self-repair processes

Alpha-lipoic acid can recycle and regenerate natural, water-soluble antioxidants such as vitamin C and glutathione and also fat-soluble ones such as vitamin E and coenzyme Q, amplifying their effects. In this way, it protects nerves and blood vessels from dangerous ROS attacks, promoting the well-being of nerve fibers and carrying out a general anti-aging action. Ultimately, ALA protects its integrity and keeps it younger for a long time and therefore results more able to efficiently transmit nerve impulses, essential for neuro-muscular conduction.

At the level of the nerve cell, some studies have documented that the alpha-lipoic acid acts in synergy with other antioxidants and so-called neutrophic substances thanks to the strong neuroprotective action they perform, able to accelerate the repair and regeneration of the nerve, improving the speed of nerve conduction.

Among the other antioxidants, with which ALA acts, above all superoxidodismutase or SOD, a powerful anti-radical agent, which acts at the origin of oxidative stress and which has shown an anti-inflammatory and neuroprotective action synergistic with ALA, must be mentioned; vitamin E and selenium that contribute to the natural protection of nerve cells from oxidative stress caused by free radicals.

Among the best known and beneficial neurotrophs there is gamma-linolenic acid (GLA), an essential polyunsaturated fatty acid (Essential Fatty Acids or EFA), which our body is unable to synthesize on its own and which must be taken through an appropriate daily diet. GLA is a component of the membrane of nerve cells where it performs a reparative activity and is essential to maintain optimal membrane fluidity, an element particularly relevant for the functionality of nerve membranes and myelin sheath.

Natural sources richest in alpha-lipoic acid

In nature, the richest sources of alpha-lipoic acid are animal tissues with high metabolic activity (red meat, heart, liver, kidney) that are not consumed often.

Among the plant sources there are, in descending order: spinach, broccoli, tomatoes, peas, Brussels sprouts, salads, rice.

Doses, method of use and collateral effects

The most widely used daily acid-alpha-lipoic dosages are those of 300-600 mg, for a period of at least three weeks. In particular 600 mg / day is the dose to be taken when the symptoms are acute; 300 mg / day is instead a suitable dose for the maintenance phase. The chemical characteristics of Acido-Alpha-Lipoic allow an easy intestinal absorption, at the level of the small intestine, and an excellent bio distribution, also spread to the central nervous system, for the ability to cross the blood-brain barrier.

Some studies have documented that, given orally or intravenously at 600-1200 mg / day, the ALA promotes the reduction of symptoms of diabetic neuropathy (tingling and numbness in the legs, burning pain, stun sensation) in the bow 3-5 weeks of integration.

Liver. Berkson et al. were the pioneers using alpha lipoic acid to treat Amanita mushroom poisoning. The mushroom damages the liver and blocks the liver glutathione activity, leading to death. Alpha lipoic acid restores the liver glutathione levels and promotes recovery.

Burning Mouth Syndrome. A pathology that affects about 1.3 million Americans. There is evidence of the efficacy of alpha lipoic acid supplements can reducie the symptoms of this painful condition, as reported in a clinical study on 42 women and 18 men, supplemented with 200 mg of alpha lipoic acid or placebo three times a day for two months.. Nearly all of the patients on alpha lipoic acid showed improvement compared to placebo, and 75 percent benefited substantially, with some experiencing complete recovery.

Weight Loss. Alpha lipoic acid has potential benefits in appetite control and weight loss, according to animal and human studies. In a four-week study of 360 obese men and women, those who took 1,800 mg of lipoic acid (a very large amount) lost more weight than those who took placebos.

The use of acid-alpha-lipoic acid is contraindicated in cases of hypersensitivity to the active substance, as well as during pregnancy and breastfeeding.

The simultaneous administration of group vitamins is recommended because alpha lipoic acid may cause a loss. It is also noted that a malodorous urine (as for asparagus) during the intake of alpha lipoic acid.

Previous studies have shown that ALA improves insulin sensitivity and repairs impaired glucose tolerance; it also involved the administration of ALA weight reduction and reduction of food intake in animal models. When ALA is given to rats, glucose-stimulated insulin is increased throughout the body and in the muscular skeleton. The main objective of our study was to investigate whether ALA can improve insulin resistance and glucose metabolism in NAFLD mice induced by high-fat diet. The results of the present study demonstrated that ALA has improved insulin sensitivity and reduced tolerance to glucose and that ALA has a particularly good ability to improve insulin signaling pathways. Mice treated with ALA had a reduced body weight, liver weight, blood glucose level, water intake, BMI and abdominal circumference compared to the HFD mice feeder. NAFLD is currently considered the major liver manifestation of insulin resistance and metabolic disorder. Chronic overnutrition and excess energy accumulation in hepatocytes trigger signals that generate insulin resistance. Furthermore, the deleterious effect of glucose accumulation is aggravated by the deterioration of NAFLD. In the present study, the main aim was to investigate whether ALA played a key role in glucose metabolism in HFD induced NAFLD mice. Several studies have been performed with the aim of demonstrating the role of ALA in glucose metabolism. ALA supplementation showed, in mice, a better body composition, glucose and insulin tolerance. Furthermore, ALA has significantly mediated glycolysis, gluconeogenesis and glycogen in HFD induced NAFLD livers. New evidence show that ALA could improve glucose metabolism by modulating key molecules such as ChREBP and GSK3β. Metformin is used in the treatment of type 2 diabetes and has been studied for other diseases in which insulin resistance may be an important factor, such as NAFLD. In this study metformin was used as a positive control drug during the experiment. We found that metformin-treated group showed improvement in whole body glucose levels and impaired intolerance tests.

These results suggest that there is no meaning between the metformin-treated group and the ALA administered group. However, changes in blood glucose levels showed that treatment with metformin was more conducive to reducing glucose concentrations than was treatment with ALA, especially at 19-24 weeks. Furthermore, the intraperitoneal insulin tolerance test suggested that ALA treatment improves insulin-stimulated glucose lowering to 1 hour after injection compared to metformin treatment. Taken together, our study showed that metformin could be effective for reducing blood sugar and promoting glucose uptake and utilization and for which ALA could be effective to improve insulin sensitivity and trigger insulin signaling pathways. The target molecule of Akt is a protein kinase that drives glucose metabolism through the mediation of insulin signaling. When insulin resistance arises, insulin signaling is suppressed. Our results showed that ALA was able to activate Akt phosphorylation. Studies have reported that there are many transcription factors involved in glucose metabolism, such as ChREBP and GSK3. ChREBP is a basic transcription factor that plays a fundamental role in glycolysis and in fatty acid synthesis. Previous studies have confirmed that ChREBP induces GCK and PK in hepatocytes. In mice fed with HFD, we found that ALA increased the nuclear protein expression of ChREBP and significantly increased the mRNA of GCK and PK levels. ChREBP deficiency triggers reduced glucose tolerance and insulin resistance in C57BL / 6J mice, while ChREBP knockdown improves liver steatosis and insulin resistance in ob / ob mice. GSK3 and GSK3β have a great effect on glycogen metabolism. Excess GSK3 expression induces glycogen synthase (GS) phosphorylation and prevents glycogen production. Our results indicated that ALA significantly increased levels of GS mRNA and liver glycogen production. ALA also significantly lowered PEPCK and G6Pase expression of mRNA and mRNA of citrate synthase increased (CS) expression in livers of mice fed with HFD; therefore, ALA reduced hepatic glucose production (HGP) and accelerated hepatic glycemic output. The current study has shown that ALA improves hepatic insulin sensitivity and prevents the development of NAFLD; in addition, ALA improves glucose metabolism by modulating the insulin signaling pathway. Overall, ALA facilitates the liver's ability to produce glucose and glucose utilization and may be effective in reducing whole body glucose catabolism in mice fed with HFD.

Alpha-Lipoic Acid and Diabetic Neuropathy

Diabetic neuropathy is defined by the signs and symptoms of peripheral nerve dysfunction in diabetic patients. Diabetic neuropathy includes a number of different syndromes, depending on the classes of nerve fibers involved.

At least 25% of diabetic patients are affected by distal symmetric polyneuropathy, which is a major public health problem, as it is responsible for considerable morbidity and mortality. This is a major contributing factor for diabetic foot ulcer, osteoarthopathy, osteomyelitis, and lower limb amputation. The latter is fifteen times higher in diabetic patients than in the general population. Neuropathic pain affects approximately 16% of diabetic patients. This subjective symptom impairs quality of life and sleeping as it usually gets worse at night. It is often the major complaint that motivates patients to seek health care. However, treatment of painful diabetic symmetric polyneuropathy is still a challenge for the physician. Antidepressants (SSRIs and tricyclic), opioids (e.g. controlled-release oxycodone), and older anticonvulsants (e.g. carbamazepine) all seem to alleviate pain, but have several adverse side effects.

Newer anticonvulsants such as gabapentin and pregabalin have a high affinity binding to α2-δ subunit of voltage-activated calcium channels. They combat painful diabetic neuropathy, partly via calcium channel modulation in the pathogenesis of diabetic neuropathy. Hyperglycemia-induced oxidative stress induces programmed cell death of nerves, which contributes to the pathology of diabetic neuropathy.

Treatment of diabetic neuropathy based on normoglycemia is generally accepted as the first approach towards preventing diabetic neuropathy. Treatment with alpha-lipoic acid increases reduced glutathione (GSH) in vivo and in vitro. GSH is an important endogenous antioxidant. Together with lipoic acid it seems to play a major role in redoxdependent mechanisms of various cellular targets. Alpha-lipoic acid is a powerful lipophilic free radical scavenger of peripheral nerve both in vitro and in vivo. As diabetes has been associated with increased

production and/or decreased clearance of ROS, oxidative stress has been suggested to contribute to defective nerve blood supply and endoneurial oxidative damage. The increased availability of glucose in diabetes induces enhanced production of AGEs. This process is defined as auto-oxidative glycosylation and is considered the major cause of increased ROS production among diabetic subjects. The increased availability of glucose leads to glycation of antioxidant enzymes. Therefore, the process of glucose auto-oxidation might be responsible for enhanced ROS production and for decreased availability or activity of antioxidant enzymes. Diabetic patients with neuropathy treated with alpha-lipoic acid 600 mg i.v. daily for three weeks, have reduced pain, paresthesias, and numbness. According to a recent meta-analysis comprising 1,258 patients, the same treatment ameliorated neuropathic symptoms and deficits after three weeks. Acute infusion of alpha-lipoic acid improved nitric oxide-mediated endotheliumdependent vasodilation in diabetic patients, and improved microcirculation in patients with diabetic polyneuropathy.

Oral treatment with alpha-lipoic acid for five weeks improved neuropathic symptoms and deficits in 187 patients with diabetic symmetrical polyneuropathy. This is an encouraging finding as deficits are major risk factors in the development of neuropathic foot ulcer. An oral dose of 600 mg once daily seems to provide the optimum risk-to-benefit ratio in the SYDNEY 2 trial. In the ISLAND Study, 300 mg of alpha-lipoic acid was applied as monotherapy and in combination with 150 mg imbesartan daily. There was a significant increase in endothelium-dependent flow-mediated vasodilation of the brachial artery, by 44% and 75% respectively, compared with placebo treatment after four weeks. This effect was accompanied by reductions in plasma levels of interleukin-6 and plasminogen activator-1, suggesting that alpha-lipoic acid may improve endothelial dysfunction via anti-inflammatory and antithrombotic mechanisms. These anti-inflammatory and antithrombotic properties have previously been observed in streptozotocin-diabetic rats by significant decreases in fibrinogen factor VII and von Willebrand factor (vWF) after treatment with alpha-lipoic acid.

Alpha-lipoic acid has been shown to down-regulate the expression of cell-adhesion molecules ICAM-1 and VCAM-1 in a dose-dependent manner. These observations might be of preventive and/or therapeutic benefit in arteriosclerosis and other inflammatory disorders. Clinical and postmarketing surveillance studies have revealed a highly favorable safety profile of the drug. Nevertheless, further studies are necessary to assess the neurophysiological and clinical properties of alpha-lipoic acid.

Alpha lipoic acid and sport

Many of the aforementioned alpha lipoic acid cellular activities could be successfully used in sports. In fact, recently introduced in the sports field is the evaluation of the effects of oxidative stress on the performance and on the state of health of the athlete.

According to various experts, in fact, the increased production of oxygen free radicals during physical activity, especially if prolonged over time or characterized by high intensity, could determine: alterations of the extracellular matrix, significantly increasing the risk of injury; the onset of cellular lesions in the muscle fibers responsible for lengthening recovery times; activation of catabolic cellular pathways, responsible for a gradual decline in lean mass; alterations in normal cell singalling processes, undermining muscle recovery and adaptation to physical exercise; an alteration of the fibro-cellular microenvironment, reducing the contractile capacity and consequently compromising the performance. With this in mind, therefore, controlling the production and the effects of these oxygen free radicals could result in an improvement of the athlete's performing abilities as well as an improvement in his health status. Consequently, as noted in different studies, adequate supplementation with alpha lipoic acid could be useful in: protect muscle cells from oxidative insult, ensuring a better contractile yield during exercise; reduce the onset of central fatigue and peripheral muscle fatigue, thus allowing a lengthening of the operating times; to reduce the risk of accidents; facilitate muscle recovery, also promoting the molecular action of insulin, essential in post-training phases in the optimization of recovery and in particular of protein synthesis rate.

In most of the studies the protective activities of alpha lipoic acid are already carried out through the daily intake of 600 mg, a quantity significantly higher than that obtainable through the diet. In order to op-

timize the bioavailability of this nutrient, it is recommended to take it on an empty stomach, one hour before or two hours after a meal. In numerous studies, the antioxidant activity in the sport of alpha lipoic acid has been enhanced by the concomitant use of other antioxidants such as Coenzyme Q10.

Safety of use

Generally, the appropriate use of alpha lipoic acid has proved to be safe and well tolerated. Undesirable effects would be observed following the assumption of high concentrations of alpha lipoic acid.

In this case rash, urticaria, pruritus, nausea, vomiting, diarrhea and vertigo would have been the most frequently described adverse reactions. In certain cases, allergic reactions have also been observed. The use of alpha lipoic acid without medical advice is contraindicated in cases of renal or hepatic disease, cardiovascular disease and / or hypertension, during pregnancy, lactation and under 12 years.

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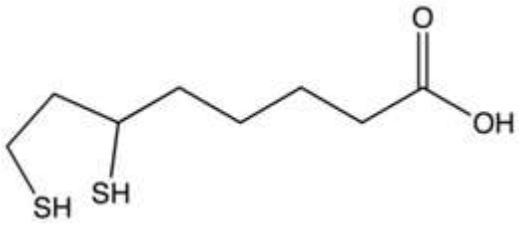
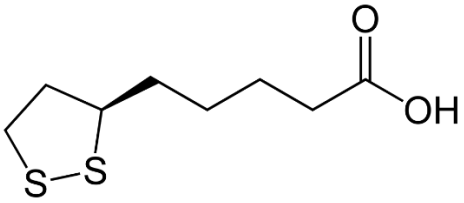
Figure 1. DHLA and LA





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KETOGENESIS AS PREVENTIVE THERAPY FOR AGING IN HEALTHY INDIVIDUAL AND INNOVATIVE TREATMENTS FOR OXIDATIVE STRESS

Folloni Stefania.[1]; Giugliano Gian Mario[1]; Iorio Eugenio Luigi[1,2]

[1]Post Graduate University Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.
Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy
[2]International Observatory on Oxidative stress, Salerno Italy

Email address: nutriketo@unisa.it

Abstract

The ketogenic diet (KD) is a low-carbohydrate and high-fat diet that induces the condition of ketosis in which ketone bodies are formed, including the known beta-hydroxybutyrate. As shown previously, ketogenesis has been able to modulate redox balance and protect from oxidative stress. Recent literature provided evidence regarding its ability to prevent oxidative damage in healthy people without any history of chronic degenerative diseases and proposed diagnostic tests to evaluate the oxidation-reductive state and possible food supplements combined to the diet. This review focuses on the ketogenic diet as a preventive anti-aging medicine.

Keywords: *ketogenic diet (KD), oxidative stress, preventive medicine, anti-aging*

Introduction

Aging is a progressive loss of physiological integrity leading to death [1]. To extend and improve the quality of life, it is important to understand which biomarkers and biological events regulate this process and how their alteration can lead to the onset of degenerative diseases [2].

Genomic and epigenetic alterations, shortening of telomeres, loss of proteostasis, metabolic alterations, cellular senescence, altered intracellular communication and the exhaustion of stem cells: these are considered distinctive signs of the aging process [1].

According to the theory of free radical related aging, mitochondrial dysfunction has been identified as potential cause: uncontrolled production of reactive oxygen species (ROS), reduced biogenesis and chronic inflammation with consequent oxidative damage of macromolecules, nuclear and mitochondrial DNA [3].

Pro-inflammatory cytokines that accumulate during oxidative stress may result in greater migration of macrophages into peripheral tissues and infiltration into pancreatic, liver and adipose cells, interfering with the metabolic pathways and increasing the progression of insulin resistance (IR) [4].

In recent decades, research has identified some genetic mechanisms that can delay aging from nematodes to mice. In particular, it has been observed that the mutation of the anomalous gene of the daf-2 / IGF -1 receptor of the insulin-like growth signal (IIS) has led to an increase in life span in *C. elegans.* In some models it was observed that this increase could be duplicated in a state of ketogenesis with a caloric restriction diet, which lowered glucose and insulin levels through the inhibition of some inflammatory pathways and the activation of some transcriptional regulators that are associated with longevity for their antioxidant action, including the FOXO regulator, the NAD^+ / NADH ratio, the Nrf2 regulator, the sirtuin family, AMP-activated protein kinase and the mechanistic target of the rampamycin mTOR [5].

In this study we propose the anti-inflammatory action of the ketogenic diet as a nutritional therapy able to extend the duration of life in humans and that restores the correct mitochondrial function, preventing a series of metabolic, cardiovascular and neurological diseases associated with oxidative damage [6].

Methods

We studied some previous articles published on Pubmed bibliographic server searching for keywords "ketogenic diet", "oxidative stress", "mythocondrial disfunction", "longevity", "ageing" alone and correlated with key-congiunctions "and" "or" and "not". We searched both on Pubmed servers and Antiaging Journals for studies regarding "diagnostic tests for oxidative stress" and we choose those regarding studies on healthy people and animals, with genetic metabolic disorders, cardiovascular risk factors, light neuronal and inflammatory disorders, or those regarding people who practice high intensity physical activity or being in contact with pollutants, who were given a ketogenic diet as a therapy for their high oxidative stress levels. clinical practice shows us that these are considered factors that predispose patients to the chronicity of oxidative stress and increase the risk of insulin resistance and cognitive decline up to the final stage: age -related diseases.

The populations were tested for inflammatory damage (in particular total cholesterol, HDL, LDL, triglycerides, fasting glucose , inflammatory cytokines, ketonemia in the blood) and the body mass index (Kg / m2), lean mass (kg), fat mass (%), body weight (kg) in order to evaluate visceral fat and select overweight patients and animals. In the studies, some methods have been proposed to better understand the degree of oxidative stress and the well-being conditions of the populations under examination in order to design therapeutic strategies for individuals. Among the most reliable approaches we chose clinical Redoxomics [7] [8], which allows us to test the oxidative state of a biological sample. Cellular biology discoveries have shown that mitochondria are the main endogenous source of ROS (reactive oxygen species) that lead to cellular senescence, so we added paramagnetic resonance spectroscopy (EPR or ESR) as another technique to measure oxidative damage [9].We proposed d-ROMs test since it can measures the total oxidant capacity and BAP test which measures the total antioxidants capacity from a blood sample [10][11].

In order to evaluate a patient's antioxidant capacity we recognised other techniques: ferric reduction

capacity test (FRAP), the new OXY-adsorbent spectrophotometric test, which are quick, cheap and simple to perform [12] and the malondialdehyde test (MDA), a marker that allows us to evaluate the status of lipid peroxidation and an eventual oxidative distress condition [13].

Once all the clinical information was collected with the support of the listed analysis, we supposed that the ketogenic diet, due to its high percentage of free fatty acids and the very low carbohydrate content (VLCD), could minimize oxidative damage, thanks to the therapeutic potential offered by beta -hydroxybutyrate (BHB) as a treatment for various pathological conditions, including insuline-resistance, hypoxia and free radical damage [14]. Several studies confirm its usefulness in the treatment of epilepsy not responsive to drugs in young people and adults [15]. Recently, new horizons are opening up for Alzheimer's disease, where ketogenesis seems to improve memory through the inhibition of certain inflammatory pathways such as histone deacetylase, NLRP3 inflammation and modulation of NAD^+ / NADH ratio (oxidised and reduced forms), the activation of the nuclear factor Nrf2 and the promotion of the electron transport chain for the expression of decoupling proteins [16]. In this review we analyzed ketogenesis as both short and long-term nutritional therapy for the maintenance of good health, excluding pharmacological therapies. Hypothetically, after ketogenic therapy, it will be necessary to repeat the tests described to observe any improvements and, based on the responses of individuals and the results of diagnostic tests, we propose to follow the same diet and, if necessary, to integrate the ketogenic diet with supplements based on polyunsaturated fatty acids and phytotherapic extracts which support the antioxidant activity. Alternatives or good prosecution for KD therapies are the Mediterranean diet, as confirmed by many previous and current studies, thanks to its acid based foods monounsaturated, polyunsaturated and medium-chain fats drastically reduces the incidence of chronic diseases and is used as a long-term nutritional therapy in all populations, improving their health and longevity [17].

Results

From the literature we reported some data comparing the effects of ketone bodies form a ketogenic diet compared to other diets with a higher percentage of carbohydrates.

A study on longevity of mice was conducted in 2017: the male mice were treated with, a Ketogenic Diet (89% kcal of fat), a Low Carbohydrate Diet (70% kcal of fat) and a control diet (65% of carbohydrates) and a series of physical tests were conducted to study strength, speed, coordination and resistance, meanwhile also behavioral tests assessed memory retention after 1 and 14 months of dietary intervention. The results were evaluated on mice at 13 and 26 months of age respectively.
Mice treated with ketogenic diet survived more than the control group, the memory was preserved more in the old mice fed with ketogenic diet than those belonging to other groups. From physical tests it was observed in mice fed with ketogenic diet a greater resistance to fall from the hanging thread and a greater force of adherence to the anterior limb and a greater speed.

Metabolic changes were analyzed with a panel of serum biomarkers and mice with KD had reduced glucose tolerance but improved insulin tolerance compared to other groups.

Moreover, after a month of ketogenic diet or low carbohydrate diet , the inhibition of histone deacetylase (HDAC) given by KB (ketone bodies) increased acetyl-Lys levels in mice with KD, the tumor suppressor p53 acetylated protein and the histone 3 to Lys9 (H3K9) and with the acetylation of the latter, there was an up-regulation of superoxide dismutase of manganese and of the gene expression of FOXO3A.

The levels and activation status of the target mechanistic signaling pathway of rapamycin mTOR complex 1 (mTORC1) were also examined. After a month of intervention with LCD and KD in mice, the phosphorylated levels of mTOR were not detected; on the other hand, a lowering of the phosphorylated binding protein (4E-BP1) and of the phosphorylated s6 ribosomal protein (rpS6) was observed, which may lead to a reduction in mTORC1 signaling. In contrast, an increase in DNA transcription-inducible DNA 4 (DDIT4) protein levels was defined in mice treated with KD as a negative regulator of mTORC1 [18].
Another study on animals showed the hepato-protective action of ketogenesis against lipotoxicity: the an-

imals were evaluated for the increase of lipid peroxidation (LPO) and of hepatic reactive oxygen species for the PPAR-alpha receptor deficiency, responsible for mitochondrial control and peroxisomal beta-oxidation.

Ten-week wild-type female mice and mice deficient in PPAR-alfa were studied. Wild-type mice and PPAR -alfa deficient mice were fed on high doses of fish oil (FO) and sunflower oil (SO) and were treated for 4-12 days, in order to assess the patho-physiological role of PPAR-alfa in the liver response with high oxidative stress. Their blood samples were evaluated on oxidative stress, mRNA analysis, metabolite analysis. High doses of FO caused an increase in oxidative stress, acute liver failure, coma and death in mice deficient in PPAR-alfa after 12 days of treatment, while mice deficient in PPAR-alfa and wild-type mice treated with sunflower oil (SO) maintained the conditions of well-being. The study demonstrates that the reconstitution of PPAR alfa with the use of AAV8 in mice deficient in PPAR alfa protected against lipotoxicity and LPO induced by FO and has been shown to restore beta-oxidation and ketone body levels. After the treatment with FO the antioxidant action of the ketone ester was evaluated with the ip injection with sodium beta-hydroxybutyrate in wild-type mice and in mice deficient in PPAR-alfa. Through the expression of the genes involved in the antioxidant response, such as Foxoa2 and metallothionein (Mt2), the liver ROS levels neutralized oxidant activity, and the injection of beta-hydroxybutyrates saved 90% of the mice deficient in PPAR-alfa from the development of acute liver failure and death after 12 days of treatment with FO [19]. Mices showing genetic deficiencies in pyruvate dehydrogenase (PDHc) and in the type 1 glucose transporter (GLUT-1DS) can use KD as a valid therapy, since it increases the supply of mitochondrial energy. Therefore, careful clinical laboratory monitoring is important in patients with inherited metabolic disorders who choose to follow KD for its marked catabolic action [20].

On the contrary, it seems that ketogenesis has correlations donating oxidative damage in young mice lacking in selenium, where oxidative lesions could be gradually increased [21].

Other series of problems have occurred in studies of animals with type 1 diabetes (T1D), where there is evidence in a 2015 study that hyperchetonemia in T1D rats caused infiltration of macrophages in the liver and increased biomarkers (NOX4) and oxidative stress associated with reduced glutathione levels (GSH) and the catalytic subunit of glutamate-cysteine ligase (GCLC) compared to type 2 and control diabetic rats [22]. Other studies show us the therapeutic potential of beta-hydroxybutyrate in various neurodegenerative disorders or neurodevelopment.

In a recent study (August 2018) it has been analyzed how ketone bodies can prevent DNA damage: 22 male Sprague-Dawley rats aged 11-14 weeks were taken and fed a diet high in carbohydrates (CD) and a ketogenic (KD) diet with a ratio of 6: 1 (fat: proteins + carbohydrates) for 2 days or 3 weeks. After the dietary treatment the activity of NAD^+/ NADH ratio was analyzed, the hippocampal levels of the sirtuins, the PARP-1 proteins that function as a sensor of DNA damage, and the oxidative damage of DNA were measured by measuring the levels of marker 8 -OHdG (8-hydroxy-2'deoxyguanosine) and an increase of oxidized form NAD^+ was observed, proportionally increasing the NAD^+/ NADH ratio, in rats treated for 2 days and 3 weeks with KD.

The increase of the NAD^+ cofactor was correlated with the activity of deacetylation of the sirtuin nuclear enzymes and the expression of Sirt 1 mRNA, as they showed a marked increase after only 2 days of dietary treatment in KD mice compared to the CD group and then decreased after 3 weeks. On the contrary, the 8-OHdG biomarker and the PARP-1 protein levels were drastically reduced after 2 days and 3 weeks in rats treated with ketogenic diet compared to the control diet group [23].

Furthermore, the activation of the Sirt 1 enzyme and the inducible hypoxia-1-factor alpha (HIF-1alpha) mediated by ketogenesis may probably promote neuronal macrophage through the reduction of mTORC1 activity, the elimination of damaged mitochondria which up -regulate superoxide dismutase and the elimination of protein aggregates, preventing a series of neurodegenerative disorders [24].
A recent study [25] shows a cross-comparison between the energetic metabolism of glucose and

acetoacetate and the metabolism of ketones in the brains of men.

Specifically, 24 cognitively healthy adults (CTL), 20 adults with mild cognitive impairment (MCI) and 19 adults at the beginning of Alzheimer's disease (AD) were all involved with similar ages and underwent a PET and MRI protocol double tracer. PET was used to evaluate the absorption rate and cerebral glucose metabolism and acetoacetate. T1-magnetic resonance was used to evaluate Brain volume and Cortical thickness.

The results describe that a ketogenic diet increases the availability of energy for the brain and could delay further cognitive decline by compensating glucose deficiency in adult MCI and AD [25].
Obesity and overweight are conditions considered to be associated with the risk of developing cardiovascular deseases and can accelerate the aging process [26][27].

A study conducted in 2015, took 377 patients aged between 30 and 69 years across Italy to evaluate the preventive and therapeutic efficacy of the ketogenic diet on sick patients and on subjects with cardiovascular risk factors. Among these, 21,1% of subjects had hypertension, 10% suffered from impaired fasting glucose and 3,2% were in prevention for cardiovascular disease. All the subjects enrolled were subjected to a general medical examination and to the evaluation of the haemodynamic and anthropometric parameters. They were subjected to a VLCK diet containing high biological formulations and a protein content of 1,2 / 1,5 g / kg of ideal body weight, followed by a slow food re-education.

At the end of the study all the pre-established objectives were achieved: a significant reduction in body weight, waist, fat mass from baseline to 4 weeks and from 4 weeks to 12 weeks was observed. No change was observed of these parameters from 12 weeks to 12 months [26].

Endothelial dysfunction is another risk factor for cardiovascular disease, often generated by cellular oxidative imbalance. This oxidative challenge can be counteracted by activation of the transcription factor Nrf2, which in the event of stress, the Nrf2-Keap1 complex is interrupted and the Nrf2 translocates into the nucleus, binds to the ARE residue by activating the transcription of the target genes.

In a recent review of an in-vitro model of vascular endothelium, the metabolic responses of HMEC-1 human endothelial cells to KB exposure and ketone bodies were evaluated. Testing toxicity of KB, HMEC-1 endothelial cells were treated with various concentrations of beta-hydroxybutyrate and acetoacetate at different times: 24, 48, 72 hours. 4 mM concentrations of beta-hydroxybutyrate and 1 mM of acetoacetate did not show important results on cell viability for up to 48 hours. DNA damage was assessed in integrated cells or not with KB at different hours: 2, 24, 48 hours and after subjected to a secondary oxidative insult with H_2O_2. The gene expression of Nrf2 was evaluated on control cells and in those treated with KB at different times: 2, 6, 14, 24 hours. Activation of the Nrf2 pathway was investigated through the evaluation of the gene expression of HO-1. Cell exposure to KB showed a moderate genotoxic effect and an increase in DNA oxidative damage. Cells treated with KB and subsequently with H_2O_2 showed a decrease in DNA damage compared to oxidized control cells, thanks to the activation of Nrf2. In cells treated with KB, they detected an increase in Nrf2 gene expression after 2 hours and a higher OH-1 expression compared to control cells. The results show that the oxidative stress induced by KB activates the transcription of genes involved in the antioxidant defense system [28].

The effects of ketogenesis in young healthy athletes of Taekwondo are interesting: some of them were subjected to a ketogenic diet and after 3 weeks of treatment there was an increase in the markers with antioxidant capacity (HDL) in the KD group and an increase in LDH and MDA in the NKD group (not ketogenic diet) [29].

Another study conducted on 6 overweight divers of average age 55 years it shows that a short term KD can prevent mitochondrial and CNS damage caused by the respiration of Enriched Air Nitrox (EAN) and implement anti-inflammatory mechanisms during the diving-sessions.

Each test session concerned a dive in which a light intensity underwater-exercise was performed; subsequently, blood and urine samples were taken from the divers for immunological tests and determination of the inflammatory status of each sub: (CTL: control), (EAN: breathe EAN), (K-EAN: in ketosis and

breathe EAN). The divers were recommended daily foods associated with special tisanoreica proteins and dietary fiber.

After 7 days of ketogenic diets all divers had a weight loss. There was a significant decrease in the levels of 8-isoPGF2alpha and 8-OH-dG (lipid perossidation and DNA damage) after immersion in K-EAN ketosis subjects: -5% compared to CTL and subjects with EAN. Furthermore, there was a reduction in inflammation in K-EAN subjects: TNF-alpha: -17% and IL-6: -51% compared to other groups [30].

Some studies propose that a Mediterranean ketogenic diet associated with supplements will bring additional benefits on healthy patients and cardiovascular risk factors. A 2011's study was conducted on Italian council' employees, which were assigned a ketogenic diet with phytoextracts [31], another similar study was repeated in 2015, in which 34 overweight male subjects were analyzed, aged between the ages of 30 and 65, leading a sedentary lifestyle.

Another randomized controlled trial in which patients followed a ketogenic diet protocol with or without omega 3 integration. 19 subjects were selected for a ketogenic diet without omega 3 (KD) and 19 other subjects for ketogenic diet with omega 3 (KDO3). Subjects consumed a multivitamin supplement tablet and subjects belonging to the KDO3 group also ingested 2 omega 3 capsules.

The Mediterranean ketogenic diet was associated with some specific herbal extracts and each participant was given a menu containing permitted and forbidden foods.

The subjects of both groups (KD and KDO3) showed an important reduction in body mass index and fat mass. Both groups also showed a significant reduction in total cholesterol, glucose and LDL cholesterol. Insulin and triaciglycerol decreased in both groups but to a greater extent in the KDO3 group. In KDO3 there was a significant reduction in inflammatory cytokines (IL-1beta and IL-6 and TNF-alpha) [32].

Discussion

The results obtained showed us the important role of beta-hydroxybutyrate in various aging diseases and in the treatment of oxidative di-stress and how a shift from glycolysis to beta-oxidation may increase in some way the lifespan. First of all, important caution should be used in both human and animal models with selenium deficiency and type 1 diabetes, in which this type of diet should be excluded, since studies show that the increase in the concentrations of ketone bodies in the blood compromise more the conditions of affected animals, resulting in systemic damage. Unlike them, on the other hand, studies show the protective and preventive action of beta-hydroxybutyrate in healthy animals and humans with greater oxidative risk, thanks to the high energy that the ketonic ester provides from liver to the various peripheral tissues and because of the stimulation of mechanisms with antioxidant activity that defend the organism from external oxidative insults. Therefore, after medical examination, it may be considered appropriate to invite this category of individuals to undergo more frequently diagnostic tests, to estimate their health conditions based on the status of lipid peroxidation, levels of circulating reactive species, antioxidant activity enzyme levels. After that, the nutrition professional could start with the prescription of a ketogenic diet in subjects with high oxidative imbalance to prevent the initial inflammation from becoming a pathological state, and then advise to continue feeding with a Mediterranean ketogenic diet to those at risk and assign a Mediterranean ketogenic diet with phytoextracts and supplements in healthy subjects. Obviously the nutrition comes along with increasing adequate lifestyles and behaviors that help reduce oxidative stress and delay aging (such as mild fisical activities, non-sedentary lifestyles etc). Although the mechanisms that implement ketogenesis healthy aging are not yet confirmed, the results obtained in this work suggest that it is worthwhile to continue to invest time to deepen the effects of ketogenesis on healthy populations and conduct more studies on them in the future.

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NATURAL PRODUCTS WITH HYPOCHOLESTEROLEMIC ACTIVITY: AN OVERVIEW

Andrea Muratore; Domenico Bellomo, Viviana Losanno; Massimo Ulderico De Martino

Post Graduate Universitary Course Master in Diete e terapie nutrizionali chetogeniche: integratori e nutraceutici.

Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

Email address: nutriketo@unisa.it

Abstract

Hypercholesterolemia is a recognized risk factor for atherosclerotic disease. Supplements market is growing progressively, they are very useful to counter a wide spectrum of pathologies, including cardiovascular diseases also due to high blood cholesterol levels.

Supplements are effective but the attention is focused on side effects caused by their intake, and to new approaches less harmful. This article reviews compounds of natural origin which are capable of lowering plasma levels of cholesterol. Among natural products with hypocholesterolemic activity are vitamin, plant extracts with drainage activity, natural statins.

Keywords: *cholesterol; supplements; statin; drainage*

Introduction

The quality of supplements in pharmacy gets better each year thanks to the ability of industry to produce and innovate products with a high quality profile whose value in primary prevention is recognized by doctors and pharmacists who advise them to their patient. Supplements market is constantly growing, they have a great utility in preventing the onset of diseases, and are also used as an adjunct in certain treatments. There is a great need to inform public opinion about the world of supplements, to expand the conceptual universe still strongly anchored to the function of re-integration, recovery and reinforcement, to clearly transfer the functions and methods of use of the products.

Supplements are a valuable tool to pursue the welfare of the public community, thanks to their ability to act on a wide spectrum of diseases or treatments.

Among several pathological conditions that can be countered by the use of specific supplements, hypercholesterolemia, especially a higher level of low density lipoproteins (LDL), is one of the major risk factors for atherosclerosis and cardiovascular diseases.

Cardiovascular disease

Cardiovascular disease is the leading cause of mortality in industrialized countries, including Europe for which it is reported a particularly high incidence in the northern, central and eastern regions, and lower incidence in the countries of the Mediterranean region. (1)

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. Cholesterol is a waxy, fat-like substance that is produced in the body and obtained from animals foods(particularly egg yolks, meat, poultry, fish, and dairy products). The body needs this substance to build cell membranes, make certain hormones, and produce compounds that aid in fat digestion. A high cholesterol blood concentration, however, increases a person's risk of developing heart disease like coronary heart disease: this condition occurs when excess cholesterol in the bloodstream is deposited in the walls of blood vessels, particularly in the arteries that supply blood to the heart (coronary arteries). The abnormal buildup of cholesterol forms clumps (plaque) that narrow and harden artery walls. As the clumps get bigger, they can clog the arteries and restrict the flow of blood to the heart. The plaque formation in the coronary arteries causes a form of chest pain called angina and greatly increases a person's risk of having a heart attack.(2)

Statins

Statins are among the most effective drugs to reduce total cholesterolemia and LDL, they are largely used in clinics in the treatment of patients with cardiovascular diseases for their effect on lowering circulating cholesterol.

Statins inhibit biosynthesis of mevalonate, a rate-limiting step of cholesterol synthesis, by inhibiting HMG-CoA reductase, an important enzyme that intervenes in one of the first stages, catalyzing the reduction of the enzyme HMG-CoA to mevalonate. (3). There are significant side effects due to the use of statins.

Muscle symptoms are the most significant side-effects of statin therapy; statin-associated muscle symptoms represent an important barrier to maintain long-term persistence to statin treatment. These symptoms reduce the quality of life and rare complications may extend to rhabdomyolysis. The molecular pathology of muscle symptoms is heterogeneous. (4)

Nutritional supplements

Red yeast rice

Over the statins, the use of supplements to lower cholesterol levels is widespread.

The main supplement used to counteract high levels of cholesterol in the blood is the red yeast rice.

Red yeast rice (RYR) is a chinese herbal supplement produced by fermenting white rice with the yeast, mainly *Monascus purpureus* or red yeast, which during its fermentative activity produces a group of substances called monacolins.

Chinese people have used red yeast rice to flavor, color, and preserve foods and as a traditional medicine for many years.

It has been used as an alternative to statin therapy in treating patients with a moderate hypercholesterolemia.

RYR contains a variety of monacolins, which inhibit HMG CoA reductase, the rate-limiting step in

cholesterol synthesis. The Monacolin present in RYR can be a valid alternative to statins for people who do not want to take prescription medicines or who cannot for the intolerance to myalgia due to standard therapy with statins. RYR also contains in small quantities phytosterols, isoflavones and monounsaturated fats that can contribute to the beneficial effects on cholesterol.

It is possible that some people react better to a natural product, thanks to the balance of the substances contained in the plant complex, rather than to the statins.

Among the monacolinas that we find inside the RYR there is also monacolina K, which is chemically identic to lovastatina. For this reason it is not recommended to take statins and RYR at the same time, because high levels of monacolina K could cause side effects like myalgia. (5)

There are several studies that show various benefits of red yeast rice to achieve a reduction in cholesterol levels, that is an important parameter for every individual and especially in obeses because obesity is a common cause of hyperlipidemia.

An important study conducted in 2015 tried to investigate the effects of RyR on obesity and hyperlipidemia.

Mice were randomly separated into five groups: the control group with a normal diet, the high-fat diet (HFD) group fed a HFD without any treatment, and HFD-fed groups supplemented with RYR.

Body weight was recorded twice and food intake three times for each week. The study continued for 28 weeks. Liver and fat pads were surgically removed and weighed. The levels of lipid parameters, liver enzymes, and leptin levels were measured. The HFD feeding resulted in obesity, which was associated with increases in liver weight, body weight, fat pad weight, liver enzymes, and plasma leptin levels with the development of hyperlipidemia. RYR prevented weight gain and fat pad weight in mice fed a HFD. RYR alleviated blood lipid parameters, liver enzymes, and leptin levels. These findings suggest that RYR has therapeutic potential in treating obesity and hyperlipidemia. (6)

In addition to the effect on cholesterol levels there are many benefits and properties of red yeast rice: it contains mineral salts, especially magnesum and phosphorus, and that's why it is recommended for menopause or premenstrual syndrome, because it works against stress and tension.

Red yeast rice is full of fiber, it is useful for the regularization of intestinal motility and for improving digestion.

It counteracts the activity of free radicals and protects the skin from UV rays, so it is an excellent antioxidant.

It performs purifying action, improving the respiratory problems, helping to reduce the risk of obesity, and prevents the risk of heart disease by lowering cholesterol, as we have seen for the presence of monacolin K.

The effectiveness of the product is not the same for everyone because it depends on the amount of monacolin K (7).

In America, for example, The US Food and Drug Administration, the regulator of drugs and food, does not allow companies to put the power of the product on the labels of supplements, so there is no way for the consumer to know how much monacolin K find in the red rice yeast they are taking. Without knowing the concentration of monacolina K taken, there is no certainty of taking that food supplement to achieve an effective lowering of cholesterol.

There are also numerous side effects concerning the intake of red yeast rice. The main effect, like in statins, is muscle symptoms and myopathy. The use of RYR can also involve some gastrointestinal disorders, and headache is quite common for who take it for long periods. RYR also contains citrinin, a fungal toxin that represents a toxic element for the proper functioning of the kidneys. It has highly toxic, mutagenic, teratogenic and carcinogenic properties. This toxin should be present in very small quantities within our body, otherwise we may risk the malfunction of our kidney activity. Among the side effects of RYR, the increase in transaminases, which could compromise the proper functioning of our liver, must certainly be reported.

Another contraindication is the possibility of experiencing joint pains, those who already suffer from arthritis and similar, should not approach the consumption of RYR. It seems also that pregnant women should not consume fermented red rice, which could give problems to future mothers but also to the unborn child.

An important negative effect of RYR is that it involves the lowering of ubiquinone levels, a particular anti-oxidant molecule, which is very important in our body. It is for this reason that usually, in order to avoid metabolic imbalances, RYR is used in association with coenzyme Q10 supplements, capable of balancing the decompensation (7).

Coenzyme Q10

Coenzyme Q10, also called ubiquinone, is a powerful antioxidant found in almost every cell in the body. Its main role is to convert food into energy. It is found, in particular, in cell membranes and in mitochondria, organelles inside cells, which are what we could define cellular energy plants.

One of the functions of coenzyme Q10, probably the basic function, is the intervening in the chemical reactions inside these organelles, which allow to recover the energy contained in food and accumulate it in adenosine triphosphate molecules (ATP), so that it is kept ready to be used.

Coenzyme Q10, also called vitamin Q, is produced by the body, but its concentration is naturally reduced with aging, as well as in the case of poor quality diet or real malnutrition and in the presence of some chronic diseases, like diseases of the cardiovascular system, neurodegenerative diseases such as Parkinson's, diabetes, tumors. Coenzyme Q10 is a fundamental element that can help in the management of certain diseases, especially cardiac diseases like heart attack, thanks to its central role in the production of energy within cells.

Several clinical studies conducted on a small number of subjects suggest that coenzyme Q10 could reduce blood pressure in those suffering from arterial hypertension. The benefits, however, could take from 4 to 12 weeks to occur.

In addition to heart and cardiovascular preventive action, vitamin Q seems valuable for migraine sufferers. Some studies, in fact, showed that in many subjects with a chronic migraine are found lower than normal levels of this molecule: it is hypothesized, therefore, that its deficiency, leading to a reduction in energy production by cells, is one of the factors that trigger the cascade of reactions that lead to painful symptoms (8).

The intake of food supplements based on coenzyme Q10 is generally indicated also to counteract fatigue, asthenia, reduce the sensation of fatigue and promote muscle well-being and, by virtue of its antioxidant role, to counteract the so-called oxidative stress induced by free radicals.

Coenzyme Q10 appears to be safe and does not cause any particular side effects, apart from occasional stomach upset (such as nausea and abdominal pain) (8).

Bergamot extract

Considering all these contraindications, it would be useful to look for a new approach in order to combat hyperlipidemia: bergamot extract stands out among the various alternatives.

In the Calabrian bergamot, there are high concentrations of polyphenols, substances with a high antioxidant power. It has now been clearly demonstrated that the polyphenol complex present in the juice shows a high capacity to promote the metabolism of fats and sugars. Bergamot juice was traditionally recognized by the local population as a valuable ally against cholesterol. The scientific confirmation of the multiple benefits of bergamot juice not only to maintain optimal blood sugar levels, but also for its hypolipidemic and hypoglycemic activity is recent. Bergamot differs from other citrus fruits not only for the composition of its antioxidant flavonoids, but also thanks to the high presence of such compounds. Polyphenols such as naringin and neoexperin help maintain healthy levels of blood sugar and improve many aspects of the metabolic syndrome.

All these aspects are demonstrated by various studies. In a 2016 study wereincluded 80 subjects (42 men and 38 women, mean age: 55 ± 13 years) with moderate hypercholesterolemia [eg. With plasma concentrations of LDL cholesterol between 160 and 190 mg / dl (between 4.1 and 4.9 mmol / l)]. An extract derived from bergamot (Bergavit R) was administered at a fixed daily dose (150 mg of flavonoids, with 16% of neoeriocitrin, 47% of neohesperidin and 37% of naringin) for 6 months. Lipoprotein subfractions were evaluated by gel electrophoresis. With this methodology, the subclasses of low-density lipoprotein (LDL) are distributed in seven bands. After six months, the extract of bergamot reduced total cholesterol, triglycerides, and LDL-cholesterol, while HDL-cholesterol increased. In conclusion Bergamot juice extract supplementation significantly reduced plasma lipids and improved the lipoprotein profile, and that's why it may represent a safe, alternative

therapeutic approach, especially in subjects suffering from statin intolerance, that may contribute to a diminished risk for atherosclerosis. These findings shed a new light on the potential use of bergamot-extract supplements in the prevention and/or reduction of overall cardiometabolic risk. (9)

Drainages

Another interesting type of supplements is the drainage class.

The supplement acts within the body purifying, encouraging diuresis and helping to dispose of excess fluids that cause water retention, swelling, venous insufficiency and cellulitis. Generally these draining products are natural because they are characterized by one or more officinal herbs, known for their ability to perform a recognized diuretic and purifying action.

Among the main plants that favor drainage we find the *Ananas sativus*,*Betulla alba* and *Centella*.

Ananas sativus has a decongestant action thanks to the presence of bromeline, as well as being rich in mineral salts and vitamins. Its stem is famous in the fight against cellulite and water retention, but also having a remarkable diuretic action, thanks to organic acids, it stimulates the elimination of excess fluids accumulated in the subcutaneous tissue favoring diuresis.

Betulla alba has a purifying and diuretic effect. It is also one of the elective remedies in the treatment of cellulite, because it helps the elimination and disappearance of lymph-connective nodules characteristic of this skin imperfection.

Centella is used to combat cellulite, it also allows the structure and the tonicity of the vessel walls to be preserved thanks to its phlebotonic activity. In fact, the *Centella*, strengthening and elasticizing the wall of blood vessels, promotes proper peripheral circulation. Several studies have shown important health benefits like antidiabetic, wound-healing, antimicrobial, memory-enhancing, antioxidant and neuroprotecting activities. (10)

Specific supplements to facilitate this kind of function can have different formulations such as: draining drops, draining and deflating tablets and draining drinks.

In particular, the drinking drains can have liquid formula and be marketed in the form of real drinks to be consumed during the day, or be produced in powder form to be dissolved in water to obtain an infusion.

The strongest draining acts on the lymphatic system through which the body manages the balance of liquids. In fact, the accumulation of liquids is one of the main causes of edema formation, cellulite, water retention and orange peel skin.

The lymphatic system is a system parallel to the cardiovascular system. It drains all corners of the human body thus preventing excess fluids from being cumulative. The drainage of the lymph happens thanks to the action of contraction and relaxation of all the muscles of the human body. These muscles form a kind of pump along all the lymphatic vessels. The lymph nodes, filtering systems necessary for the defense against pathogens and the site of the immune response are part of this system.

The lymph nodes filter and purify the lymph through the production of antibodies by lymphocytes and they eliminate bacteria and viruses through macrophages, which are located within the lymph node. As peripheral lymphoid organs, they have the role of allowing the development of both humoral and cell-mediated immune responses thanks to their organization that favors the interactions between T lymphocytes, B lymphocytes, APC and other cells involved in the process. Immunological memory is also generated in the lymph nodes.

In the human body there are certain areas (such as neck, roots of the limbs, retroperitoneal spaces of the abdomen, pelvis and mediastinum) rich in lymph nodes due to their particular position with respect to the drained territories.

The symptoms of a slowed circulatory system, which does not effectively perform its draining action, may be differents. Often, water retention, edema on feet, ankles and the eye area (bags and dark circles) are observed. Equally, difficulty in maintaining the upright position can often occur. As well as pain and sense of heaviness in the legs, especially at night or after being very much on their feet. It is very common to have a non-functioning lymphatic system and in fact it is one of the main causes of illness, because the immune system does not reach where it should and the waste and toxins are not expelled from the body.(11)

Effective drainage products aim to perform specific functions and are classified as follows:

- Anti-Cellulite Draining: these supplements are not only purifying and detox, but above all draining liquids that have stagnated, counteracting water retention and cellulite.
- Slimming action draining: some products contain fibers that improve intestinal transit or other ingredients that give a boost to the metabolism. In general, as mentioned, the most powerful draining systems allow the elimination of excess fluids, also favoring weight loss.

Conclusion

It is important to remember that supplements cannot and should not be considered as substitutes for a healthy and complete nutrition. A diet rich in fiber, vitamins, minerals and completed with the intake of at least a liter and a half of water a day, is necessary to ensure hydration and reduce water retention. It is also very important to limit the consumption of salt and glutamate, as they promote the stagnation of liquids.

These products promote the disposal of toxins, drain liquids and promote diuresis. So they can be a valuable ally if you are following a weight loss diet because the loss of excess fluids also leads to weight loss. It is understood that these products are effective to combat water retention, characterized by an accumulation of fluid in the tissues, but alone are not enough to lose weight. To be able to lose weight it is important to follow a proper diet and perform regular physical activity. The best drainage supplement is therefore the one that can perform an effective detox action, which promotes diuresis and has an anticellulite and water retention effect.

Even if it is natural products based on plant extracts it is however important to use them in the correct way and taking into account the possible contraindications. These supplements are not recommended during pregnancy and lactation, they should be taken carefully following the indications and methods of recruitment. People suffering from hypertension and cardiovascular disorders, kidney problems or constipation should always seek the advice of their doctor before using drainage, also for allergic people because the supplements may contain plant extracts.

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