Effect of Dietary Intervention with a High Intake of Vitamin K on Insulin, Osteocalcin, Leptin, Vitamin K Serum Levels and Cardiovascular Risk in Young Adults With and Without Overweight or Obesity

February 2021

STUDY PROTOCOL

Overweight and obesity are public health problems worldwide and in Mexico, both related to the increase in weight and body fat that are risk factors for the development of chronic degenerative diseases such as type 2 diabetes mellitus (DM2) and cardiovascular diseases (CVD). The World Health Organization (WHO) defines obesity as the excessive accumulation of fat that can harm health and is diagnosed when the Body Mass Index (BMI) is greater than or equal to 30 kg/m^2.
In Mexico, according to figures from the National Health and Nutrition Survey (ENSANUT), at the national level, 75.2% of adults are overweight or obese (39.1% overweight and 36.1% obese), with respect to chronic degenerative diseases there is a prevalence of 10.3 % for diabetes, 18.4% for hypertension and it has been reported that 19.5% of the population has elevated cholesterol and triglycerides.

Correct diet and a healthy lifestyle are factors that help to avoid overweight and obesity; However, the current diet of the adult Mexican population is not adequate, because there is low consumption of the recommended food groups. Fruits and vegetables are healthy foods that less than 50% of the country's population consume daily. Regarding other recommended food groups, eggs are consumed by 29.9% of the population, dairy products by 47%. and unprocessed meats by 64.6%. Which is inadequate, due to the vitamins and minerals that these food groups provide.

Vitamins are considered micronutrients and they play an essential role in metabolism. Vitamins are classified according to their nature as fat-soluble or water-soluble. Water-soluble vitamins include vitamin B1, B1, B2, B3, B5, B6, B7, B9, B12 and C, while fat-soluble vitamins are vitamins A, E, D and K. Vitamin K (VK) has two main structural forms, which are phylloquinone (K1) which is found naturally in plant foods, mainly in green leafy vegetables, and menaquinone (K2) which is produced in the intestine and is found in some fermented foods. VK acts as a cofactor for the carboxylation of glutamic acid residues in proteins involved in blood coagulation and bone metabolism, facilitating calcium uptake through the carboxylation of the protein osteocalcin. This last protein, osteocalcin in its carboxylated form (OCc), serves as an indicator of the level of VK, the more OCc exists in the blood it is considered that there is a good level of VK, the osteocalcin that is not carboxylated thanks to the VK, circulates in the blood and is known as undercarboxylated osteocalcin (OCuc). VK requirements are 120mcg/day for men and 90mcg/day for women; Although VK is fat-soluble, the body stores small amounts that are quickly depleted without regular daily ingestion.

Various studies mention that the high intake of VK has positive effects on insulin sensitivity and cardiovascular risk; likewise, VK deficiency is negatively associated with the same factors. Yoshida et al. suggest that the increase of VK intake to through diet can reduce the progression of insulin resistance. The mechanism through which VK improves insulin sensitivity is unknown. In animals, OCuc has been shown to stimulate beta cell proliferation and increase insulin secretion; However, Yoshida et al. found that in subjects receiving VK supplements the OCuc levels were lower and the insulin resistance was reduced, compared to the control group without supplements. This suggests

that VK may intervene in glucose metabolism through other mechanisms not fully described, such as suppression of inflammation. Also, it has been reported that VK improves insulin sensitivity in peripheral tissues without intervening in insulin resistance. Rasekhi et al. analized the effects of administering 100 mcg of VK through supplements on glucose metabolism in premenopausal prediabetic women. They found that in the group with VK ingestion, OCc levels increased and OCuc levels decreased and insulin sensitivity showed to improve. There were no alterations in total osteocalcin or in the HOMA-IR index. Which suggests that VK could improve insulin action in peripheral tissues.

Dietary supplementation with VK has been associated with an increase in insulin sensitivity and improvement in glycemic status. Yoshida et al. studied vitamin K supplementation for 36 months in men and women, administering 500 mcg/day of VK, 600 mg of calcium in the form of calcium carbonate and 10 mcg of vitamin D. They found that VK in supplements produces a protective effect on the progression of insulin resistance; Hussein et al. showed in animal models with type 2 diabetes the effect on glycemic status after the administration of VK supplements for 8 weeks. Those rats type showed a decrease in fasting serum glucose, HbA1c and insulin resistance, in addition to improving insulin sensitivity. When comparing the control group with the diabetes group without supplements, total OC levels were lower in the diabetes group; however, there was a dose-dependent relationship and the group with VK supplements showed to increase OC levels. In Mexico, the numbers of obesity and overweight increase considerably, the dietary factor has high relevance in the generation of these conditions, currently, studies on vitamin supplementation have been carried out, however in our country there is no evidence of the effect of dietary intervention with a high intake of VK on people with metabolic disorders such as overweight and obesity, so carrying out this study may contribute to explaining the participation of a high intake of VK on the improvement and prevention of overweight and obesity, in addition to evaluating the possibility to improve metabolic and bone health from a diet calculated in the Mexican population.

Work hypothesis

Dietary intervention with a high intake of vitamin K of 500mcg for 6 weeks, increases VK and OC serum levels, decreases insulin and leptin levels and reduces cardiovascular risk in young adults, with and without overweight or obesity.

Aims

General aim

To evaluate the effect of dietary intervention with a high intake of vitamin K of 500mcg on serum levels of vitamin K, insulin, osteocalcin, leptin and on cardiovascular risk in young adults with and without overweight or obesity.

Specific aims

- Design a dietary intervention with a 500mcg of vitamin K

- Compare serum levels of vitamin K, insulin, osteocalcin, leptin and cardiovascular risk markers before and after the intervention with high vitamin K intake in subjects with and without overweigh or obesity.
- Analyze the relation between vitamin K serum levels and body composition characteristics, metabolic markers and insulin, osteocalcin, leptin and cardiovascular risk percentage in subjects with and without overweigh or obesity.

Ethical considerations:

The study will adhere to the regulations established by the Mexican government in the regulations of the general health law regarding health research.
It will be carried out in accordance with the ethical standards and principles for medical research on human subjects established in the Declaration of Helsinki in Helsinki, Finland in 1964, updated in 2008 in Seoul, South Korea.
The subjects who will participate in the study will voluntarily sign the informed consent letter. The project aims to contribute to the study of metabolic diseases in adults and young people with the aim of finding strategies for prevention or use as early dietary treatment and the consideration of foods with a high supply of vitamin K. The participants in the study will be assigned an identification key to guarantee the reliability of their clinical data. If they decide to withdraw from the study, the clinical history and biological samples associated with the individual will be deleted. All individuals who participate in the study will benefit from obtaining and explaining their clinical data, information about the cardiovascular risk they have at the time of the intervention and after it, in addition to being provided with nutritional care and intervention by professionals of nutrition, all this without costs.

Biosafety aspects:

This is an investigation with minimal risk, the laboratory has the necessary infrastructure and materials for the management of RPBI according to the Official Mexican STANDARD NOM-087-ECOL-SSA1-2002, Environmental protection - Environmental health - Biological hazardous waste - infectious - Classification and handling specifications. Waste from the use of reagents for ELISA and wet chemistry will be discarded according to the manufacturer's criteria. The reagents will be stored according to what is stipulated in the manufacturer's sheets and according to the provisions of NOM-054-SEMARNAT1993 and NOM-052-SEMARNAT-2005, no toxic, radioactive or explosive reagents will be worked with. The implementation of the project will be subject to what is described in the General Health Law on health research, DOF reform 04-02-2014 Art. 13 to 17. The main researcher has been trained by the CUCS Biosafety Committee.

METHODOLOGY

Technical methods and instruments for collection, recording, processing and analysis:

- Peripheral blood samples will be taken by venipuncture using the Vacutainer® system.
- The serum variables of glucose, triglycerides and lipids will be obtained by means of colorimetric enzymatic reagents. Vitamin K and insulin will be obtained through ELISA Kits.
- Anthropometric measurements will be carried out using a TANITA brand bioimpedance scale, SECA brand measuring tape and Slim Guide brand plycometers. All measurements will be taken according to ISAK.
- Blood pressure will be measured with an OMRON brand baumanometer.
- Patient data will be recorded in a log and in an Excel database. For the statistical processing of the data, the Excel, SPSS Statistics version 20.0 and GraphPad Prism 8 programs will be used.

Research organization:

The study will be carried out at the University of Guadalajara, Department of Molecular Biology and Genomics, Biochemistry Research Laboratory of the University Center for Health Sciences.

1. Once the patients are included, an initial evaluation will be carried out that consists of:
- Evaluation of nutritional status: Dietary analysis by 24-hour recall, anthropometric analysis (with bioimpedance, measurement of skin folds and circumferences), clinical history.
- Blood sample collection: determination of glucose and serum lipids, vitamin K, insulin and osteocalcin.
- Blood pressure measurement.
- Dietary intervention: Explanation of the study and dietary indications.

2. Dietary intervention with a high intake of vitamin K for 6 weeks
- Weekly monitoring of dietary adherence
- Nutritional advice: resolve doubts, evaluation of dietary adherence.

3. Final evaluation
- Evaluation of nutritional status: anthropometric analysis.
- Blood sample collection: determination of glucose and serum lipids, vitamin K, insulin, osteocalcin and leptin.
- Blood pressure measurement.
- Final evaluation of dietary adherence through a survey.

4. Data analysis, statistics.

5. Results report

Statistical analysis:

The statistical analysis will be carried out with the statistical package SPSS version 20, GraphPad Prism 9 and Excel 2011. Initially, the Kolmogorov-Smirnov test will be applied to all variables to verify if they followed a normal distribution model. Those variables that followed a normal distribution will be analyzed using parametric tests; Otherwise, non-parametric statistics will be applied.

Descriptive Analysis: mean, standard deviation, standard error of the mean, minimum, maximum and range. Inferential Analysis: Parametric tests (Student's t for independent samples), non-parametric tests (Kruskal Wallis). Comparative analysis: Chi square. Correlations: Spearman for non-parametric and Pearson for parametric tests.

Probability values less than 0.05 will be considered significant.