Longer Life Foundation Longevity Research Program
Dietary protein and cardiovascular health (PI: Bettina Mittendorfer)
Year 1 Progress Report

Abstract

The goal of the Longer Life Foundation (LLF) Longevity Research Program (LRP) at Washington University is to conduct research that supports the LLF's mission to identify factors that: i) assist in predicting mortality and morbidity of selected populations and ii) improve people's health and longevity. Studies supported by the LRP over the last decade found chronic calorie restriction protects against the cardiometabolic abnormalities associated with obesity. The results from studies conducted in animals and our own preliminary data suggest that part of the beneficial cardiometabolic effect of calorie restriction is due to the concomitant reduction in protein intake. The goal of this 3-year LRP project is to evaluate the effect of dietary protein intake on cardiovascular health in people and to determine the physiological and cellular mechanisms involved. This project will involve a new translational collaboration among several clinical and basic science investigators and will also leverage the resources of a newly initiated, NIH-funded (R01 DK121560; PI: Mittendorfer) randomized clinical trial that will evaluate the effect of high protein intake from different sources (animal and plant) on several key metabolic functions involved in the pathogenesis of type 2 diabetes. We hypothesize that high protein intake will increase the blood concentration of the proatherogenic secondary gut metabolite TMAO, which is derived from animal protein-rich foods, increase platelet aggregation, stimulate proatherogenic metabolic functions in blood monocytes, which are the precursors for atherosclerotic plaque, and impair vascular function (assessed as endothelial function and vascular compliance). We also hypothesize that the adverse effects of high protein intake will be greater with high protein intake from animal foods (e.g. meats and dairy), which are rich in the TMAO precursor carnitine, than plant foods. The results from the proposed studies will increase our understanding of the influence of dietary protein on healthy (disease-free) aging and could identify novel biomarkers of cardiometabolic health. This issue has become particularly important because high protein intake and consumption of protein-fortified foods is now a popular trend.

Lay Summary

Cardiovascular diseases, including heart attack and stroke, are the leading causes of death. Diet is an important determinant of heart health and risk of death. In the proposed project, we will evaluate the effect of high protein intake on the factors involved that cause cardiovascular diseases. We hypothesize that eating too much protein increases the risk for heart attack and stroke.

Introduction - Overview of the study

The goal of this 3-year LRP project is to evaluate the effect of dietary protein intake on cardiovascular health in people in people and to determine the physiological and cellular mechanisms involved. This project will involve a new translational collaboration among several clinical and basic science investigators and will also leverage the resources of a newly initiated, NIH-funded (R01 DK121560; PI: Mittendorfer) randomized clinical trial that will evaluate the effect of high protein intake from different sources (animal and plant) on several key metabolic functions involved in the pathogenesis of type 2 diabetes. We hypothesize that high protein intake will increase the blood concentration of the proatherogenic secondary gut metabolite TMAO, which is derived from animal protein-rich foods, increase platelet aggregation, stimulate proatherogenic metabolic functions in blood monocytes, which are the precursors for atherosclerotic plaque, and impair vascular function (assessed as endothelial function and vascular compliance). We also hypothesize that the adverse effects of high protein intake will be greater with high protein intake from animal foods (e.g. meats and dairy), which are rich in the TMAO precursor carnitine, than plant foods. The results from the proposed studies will increase our understanding of the influence of dietary protein on healthy (disease-free) aging and could identify novel biomarkers of cardiometabolic health. This issue has become particularly important because high protein intake and consumption of protein-fortified foods is now a popular trend.

Results

We evaluated the effect of high protein intake on the pathogenesis of atherosclerosis by detailed phenotyping of genetically engineered mouse models and assessing the effects of amino acids on isolated macrophages (Zhang et al 2020). We found that increasing the protein content of a high fat diet from 15% of total energy to 45% of total energy increased atherosclerotic lesion size in atherosclerosis-prone ApoE$^{-/-}$ mice.

Moreover, high protein intake increased apoptosis and necrotic core formation in atherosclerotic plaques; these features are characteristics of complex/unstable plaques that are most likely to rupture. The adverse effects of high protein intake on atherosclerotic plaques were mediated by the effects of amino acids on mTORC1 signaling, because amino acids (in particular leucine) stimulated mTORC1 signaling in isolated macrophages and macrophage deletion of the critical mTORC1 component, Raptor, reduced atherosclerosis and prevented the development of atherosclerosis in mice fed a high protein diet. These studies have contributed essential preliminary data for a collaborative (Razani/Mittendorfer) grant application to the NIH (R01 HL148197 Dissecting the Impact of Dietary Protein on Macrophage mTOR Signaling and Atherosclerosis; 09/01/20 - 08/31/25, $ 373,711) to further evaluate the effects of high protein intake on vascular health (both in people with atherogenic dyslipidemia and mechanistically in vitro and in vivo in mice).

We have also started to evaluate the effects of high protein intake and amino acids on circulating monocyte/macrophage and endothelial function in people. Fourteen participants (9 women, 5 men; 47 ± 4 years) completed two mixed meal metabolic tests each: a standard protein meal, containing 15 g of protein, and a high protein meal, containing 25 g of protein from either predominantly animal (n=7) or plant (n=7) sources. Preliminary results from these studies suggest the adverse effects of high protein intake on vascular health observed in vitro and in vivo in animal models are also present in people. Ingesting the high protein, compared to the standard protein meal, raised serum leucine concentration and increased phosphorylation of the mTORC1 target S6 in circulating monocytes. Furthermore, the high- but not the standard protein meal reduced the reactive hyperemia index, a measure of endothelial function that correlates with disease progression and predicts cardiovascular events. The adverse effect of high protein intake was observed after consuming meals with both high protein content from animal and plant sources (Figure 1).

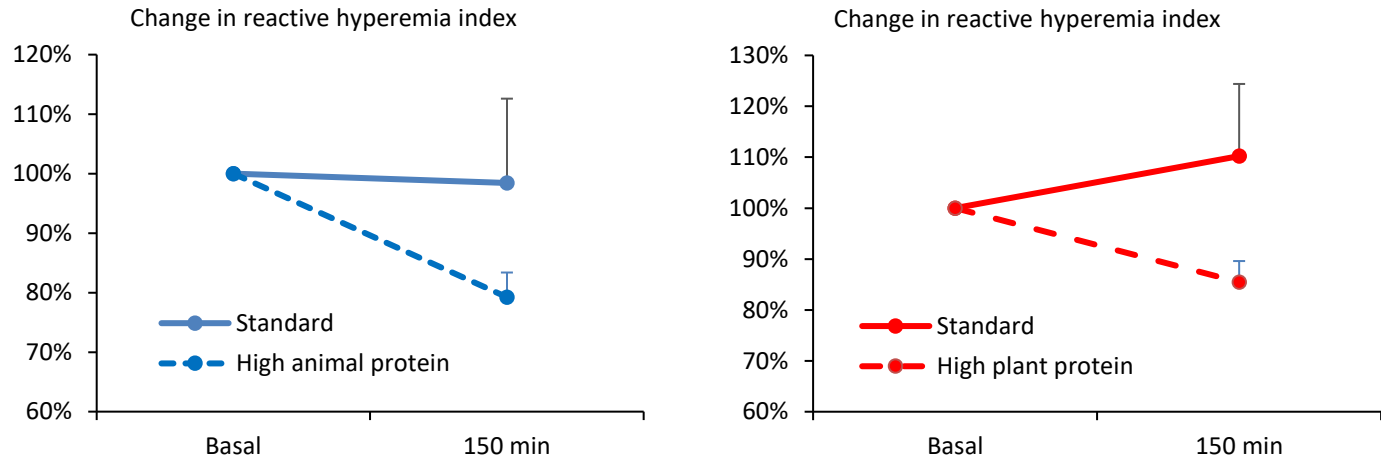

Figure 1. Change in reactive hyperemia index after consuming a standard-protein or a high protein meal, containing additional protein from either predominantly animal (left) or plant (right) sources.

Plans

During the next year, we will continue to perform metabolic testing to evaluate the acute and chronic (12-week diet intervention) effects of high protein intake from animal and plant sources on metabolic and cardiovascular function, including assays in endothelial cells harvested from study participants, process study samples, and analyze data. We also plan to submit an R01 application in October 2020 to evaluate the effect of weight loss induced with a standard-protein calorie-reduced vs a high-protein calorie-reduced diet on cardiovascular function in people with type 2 diabetes.

Grant applications resulting from this work

Submitted (March 2020): NIH R01 HL148197 Dissecting the Impact of Dietary Protein on Macrophage mTOR Signaling and Atherosclerosis; 09/01/20 - 08/31/25; $373,711 (annual direct).

Planned (anticipated submission October 2020): NIH R01 to evaluate the effect of weight loss induced with a standard-protein calorie-reduced vs a high-protein calorie-reduced diet on cardiovascular function in people with type 2 diabetes.

References cited

Zhang X, Sergin I, Evans TD, Jeong SJ, Rodriguez-Velez A, Kapoor D, Chen S, Song E, Holloway KB, Crowley JR, Epelman S, Weihl CC, Diwan A, Fan D, Mittendorfer B, Stitziel NO, Schilling JD, Lodhi IJ, Razani B. High-protein diets increase cardiovascular risk by activating macrophage mTOR to suppress mitophagy. *Nat Metab*. 2(1):110-125, 2020.