RESEARCH PLAN

1. Specific Aims

The overall goal of the Longer Life Foundation (LLF) Longevity Research Program (LRP) is to conduct and stimulate new leading-edge research that supports the LLF's mission to "identify factors that either predict the mortality and morbidity of selected populations or influence improvements in longevity, health, and wellness". The major goals of the LLF-LRP during the current 3-year funding period were to: i) establish a new translational science collaboration to study the effect of high dietary protein intake on monocyte/macrophage metabolic function and atherogenesis and ii) generate preliminary data for additional grants to conduct studies that evaluate the pathophysiological factors involved in cardiometabolic diseases. We have demonstrated considerable success in accomplishing these goals as evidenced by the number of publications and new grant applications that have resulted from these research activities (see section 4. *Progress Report*). The goal during the next funding cycle is to continue to evaluate to effect of high protein intake on factors involved in the pathogenesis of atherosclerosis and to strengthen existing and establish new collaborations. We will conduct in vitro experiments in cultured cell systems and leverage the resources of two NIH-funded clinical trials that focus on the effects of high protein intake on glucose metabolism (R01 DK121560) and monocyte macrophage function (R01 HL159461); the ancillary studies supported by the LLF-LRP will specifically focus on platelet and endothelial cell function.

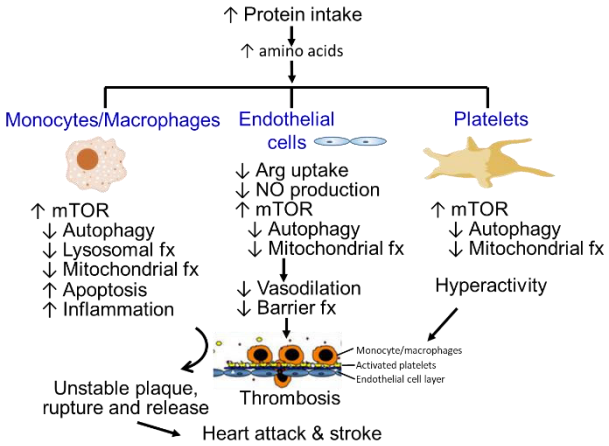

Figure 1. Graphic illustration of the working hypothesis.

Figure 1 summarizes the overall working hypothesis. The following Specific Aims will be addressed in this proposal:

Specific Aim 1. To evaluate the effect of high protein intake and amino acids on mTORC1 signaling to autophagy/mitophagy in platelets.

Specific Aim 2. To evaluate the effect of high protein intake and amino acids on endothelial cell biology.

We hypothesize that high protein intake inhibits autophagy/mitophagy in platelets and endothelial cells because of leucine-mediated mTORC1 activation. In addition, we hypothesize that amino acids inhibit arginine uptake and subsequent nitric oxide production by endothelial cells.

Specific Aim 3. To strengthen existing and establish new collaborations.

2. Relevance of the Project to the Mission of the Longer Life Foundation

Our proposal represents a paradigm shift in how a Western-type diet affects vascular health. Traditionally, the increased risk of atherosclerosis is attributed solely to the high saturated fat, cholesterol, and refined carbohydrate intake (1). Accordingly, dietary approaches to reduce cardiovascular risk focus on reducing the intake of these nutrients, whereas high protein intake is recommended and has become popular. However, our published (2) and additional preliminary data suggest high protein intake could have detrimental health consequences. The studies we propose in this application will enhance our understanding of the cellular and molecular mechanisms responsible for the pro-atherogenic effect of high protein intake and will focus on both proteins from animal and plant sources.

3. Background and Significance

LLF-LRP at Washington University (WU). The overall goal of the LLF-LRP is to conduct and stimulate new leading-edge research that supports the LLF's mission to "identify factors that either predict the mortality and morbidity of selected populations or influence improvements in longevity, health, and wellness". The primary focus of the research supported by the LLF-LRP since its inception in 2008, has been on cardiometabolic diseases, specifically type 2 diabetes (T2D) and atherosclerotic vascular disease. Under the leadership of Drs. John Holloszy and Luigi Fontana (until 2019), the LLF-LRP has evaluated the effects of chronic calorie restriction on risk factors for T2D, heart attacks, and stroke (3). These cardiometabolic diseases are the leading cause of death accounting for >25% of all deaths, and more than 80% of heart attacks and strokes are of the ischemic type due to atherosclerosis (4-6). Chronic calorie restriction was found to be protective and the cardiovascular biological age of late middle-aged adults who have practiced calorie restriction for most of their adult lives was estimated to be about 20 years less than their chronological age would suggest (7-11). In 2019, Dr. Bettina Mittendorfer, who has been a past recipient of a LLF pilot and feasibility grant, assumed leadership of the LLF-LRP and the LLF-LRP's primary research focus evolved to evaluating the interaction between dietary protein intake and

cardiometabolic function. In addition, it has become a major goal of the LLF-LRP to stimulate new collaborations among investigators with expertise in different disciplines to allow them to pursue new research directions and/or more comprehensive, translational research projects, and to assist young investigators interested in longevity research obtain funding for their career development to help them establish their scientific independence.

Dietary protein intake recommendation and trends. The recommended daily intake of protein to maintain nitrogen balance is 0.8 g/kg/d, which corresponds to about 11% of total energy requirements (12). Many people try to increase their protein intake because of the belief that dietary protein improves health and lean body and muscle mass (13-15). Accordingly, daily protein intake has been steadily increasing for the last several decades (16, 17). The majority (about two-thirds) of total protein in Western-type diets is derived from animal proteins, with only a relatively minor contribution of protein from plant sources (18-23). The increased demand for dietary protein has led to an increase in marketing of protein and also an increase in commercial food products that are enriched with proteins from a variety of sources, including both animal and plant sources (24). On average, people in Western societies consume about a third more protein (1.1 g/kg/d) than the recommended daily intake, and about one quarter of the population consumes even more than twice the recommended daily intake (i.e. more than 1.6 g/kg/d or 22% of daily energy as protein) (18, 25-28). Median protein intakes at lunch and dinner are 25 g and 35 g of protein, respectively (16, 29, 30).

Effect of high dietary protein intake on cardiometabolic function. Although amino acids from dietary protein are important building blocks for muscle and other proteins in the body, consuming protein in excess of the recommended daily intake does not increase muscle or total lean body mass, muscle strength, and overall physical function (28, 31, 32), but may have adverse cardiometabolic consequences. The results from studies conducted in animals (33-35) and several population studies (20, 32, 36-41) demonstrate that high protein intake is associated with an increased risk for T2D, cardiovascular diseases, and mortality. The risk is generally greater with high animal protein than high plant protein consumption (20, 32, 36-41). The mechanisms responsible for the link between high protein intake and cardiometabolic diseases and for the difference in cardiometabolic disease risk associated with high animal protein compared with high plant protein intake are not well understood. A major goal of Dr. Mittendorfer's research program over the past decade has been to evaluate the effect of high protein intake on the factors involved in causing prediabetes and T2D (42-44). In 2019, she received an NIH-funded R01 award (DK121560) to conduct a randomized clinical trial to evaluate the effects of high animal and high plant protein intake on insulin sensitivity and β-cell function, which are the key factors involved in the pathogenesis of prediabetes and T2D. To gain insight into the effect of high protein intake on the pathogenesis of atherosclerosis, Dr. Mittendorfer established a new translational science collaboration with Dr. Babak Razani and leveraged the resources of this clinical trial to study the effect of dietary protein intake on the pathophysiological factors involved in atherosclerosis. Dr. Razani is a basic science physician investigator in the Division of Cardiology at WU with expertise in the autophagy-lysosome system and macrophage biology. The data generated from these collaborative studies so far demonstrate that high protein intake drives atherosclerosis and atherosclerotic plaque complexity in apoE$^{-/-}$ mice by inhibiting autophagy in macrophages through activation of mTORC1 (2). Autophagy is a cytoprotective process, whereby intracellular contents (e.g., lipids and lipoproteins) and organelles (e.g., mitochondria) are broken down within lysosomes (45, 46). In macrophages, autophagy serves to protect against lipid accumulation, proinflammatory activation, and progression into atherosclerotic foam cells (47, 48). These data established a mechanistic link between high dietary protein intake and atherosclerotic cardiovascular disease risk and served as preliminary data for a new multi-PI (Mittendorfer/Razani) NIH R01 application (HL159461) that was recently funded to gain deeper insights into the cellular mechanisms involved in the alterations of macrophage biology associated with high protein intake and to evaluate the effects of dietary proteins from animal and plant sources on monocyte/macrophage mTORC1 signaling and atherogenesis.

Platelet hyperactivity and endothelial dysfunction (defined as reduced expression and release of vasoactive molecules, most importantly nitric oxide, and increased production of thrombomodulin, thromboxane, and reactive oxygen species that increase adhesion and aggregation of platelets and recruitment of monocytes) are also involved in atherosclerotic vascular disease (49-51). Autophagy/mitophagy has been implicated in regulating both platelet and endothelial cell function (52-54). In addition, it is possible that high protein intake reduces transport of arginine, the precursor for nitric oxide synthesis, across the endothelial cell layer due to competitive transporter inhibition (55-57) by the increase in plasma amino acid concentration. Excess amino acids can also promote arginine export from endothelial cells in exchange for neutral amino acid uptake (55-57); thus creating an arginine deficit that could limit nitric oxide production. During the next LLF-LRP funding cycle, we will test the hypothesis that high protein intake activates mTORC1 signaling to autophagy, specifically mitophagy, in platelets and endothelial cells and this effect is mediated by select amino acids, most prominently leucine. We will also evaluate the effect of amino acids on arginine uptake and nitric oxide production in endothelial cells. This new research direction will benefit from the expertise and resources of the Cellular and Molecular Biology Core of the WU Nutrition and Obesity Research Center, which is directed by Dr. Nada Abumrad, who is an expert in endothelial cell biology (58-63).

4. Progress Report

We conducted a coordinated series of in vitro experiments with human monocyte-derived macrophages, studies in mouse models of atherosclerosis, and monocytes isolated from study participants who consumed standard and high-protein meals that demonstrated: i) high protein intake drives atherosclerotic plaque size and complexity in apoE$^{-/-}$ mice by inhibiting autophagy in macrophages (2); ii) high protein intake activates mTORC1 signaling in circulating monocytes, the precursors of atherosclerotic plaque macrophages, in people (Figure 2); iii) leucine is the primary amino acid that initiates mTORC1 signaling and downstream sequelae in macrophages (Figure 3); iv) the activation of mTORC1 signaling in macrophages requires a concentration of leucine (>100 µM) (Figure 3) that is reached in plasma after consuming about 25 grams of protein (168 ± 12 µM vs 117 ± 11 µM at 1 h after the high-protein meal and the standard meal in the study described in Figure 2); v) protein intake in excess of the human equivalent of ~20% of energy requirements drives atherosclerosis in apoE$^{-/-}$ mice (Figure 4). These data served as preliminary data for an NIH R01 application (HL159461; Mittendorfer/Razani, MPI) that was recently funded to evaluate the effects of dietary proteins

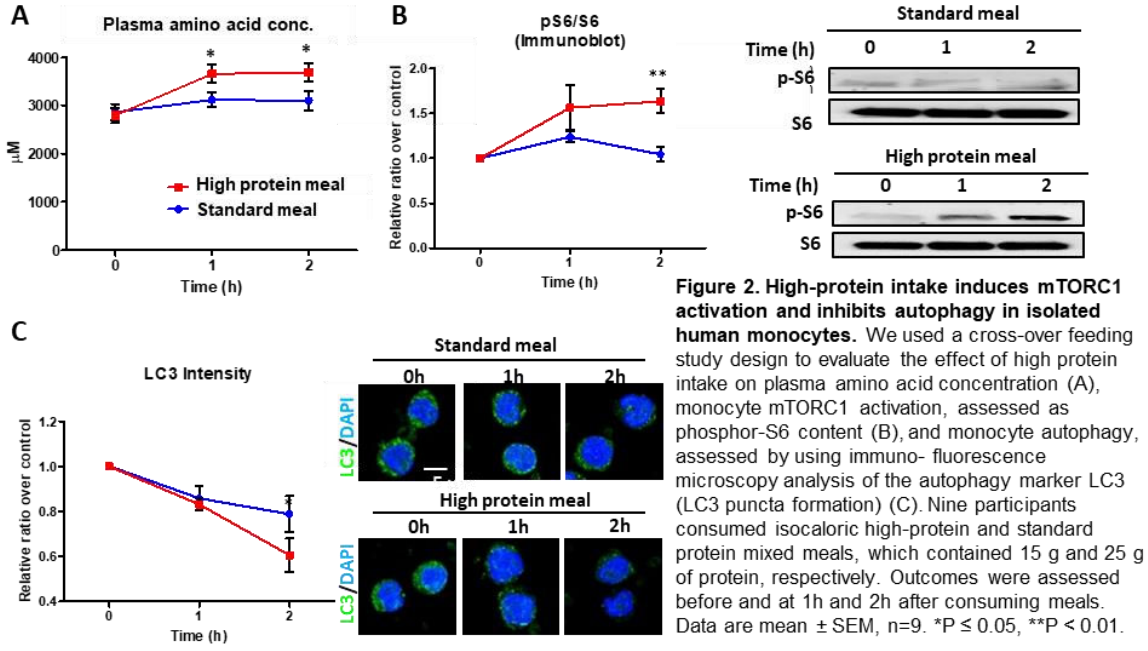

Figure 2. High-protein intake induces mTORC1 activation and inhibits autophagy in isolated human monocytes. We used a cross-over feeding study design to evaluate the effect of high protein intake on plasma amino acid concentration (A), monocyte mTORC1 activation, assessed as phosphor-S6 content (B), and monocyte autophagy, assessed by using immuno- fluorescence microscopy analysis of the autophagy marker LC3 (LC3 puncta formation) (C). Nine participants consumed isocaloric high-protein and standard protein mixed meals, which contained 15 g and 25 g of protein, respectively. Outcomes were assessed before and at 1h and 2h after consuming meals. Data are mean ± SEM, n=9. *P ≤ 0.05, **P < 0.01.

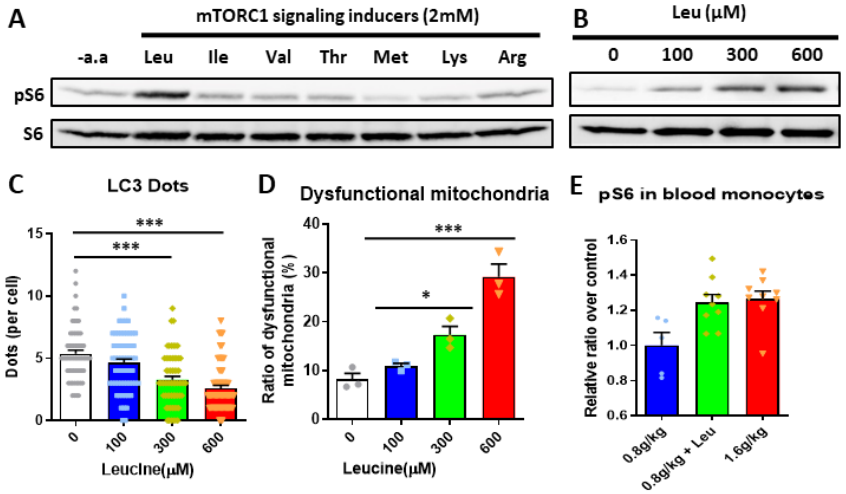

Figure 3. *Panel A.* We evaluated the effect of 21 amino acids on mTORC1 activation, assessed as S6 phosphorylation by using Western blot analysis, in human monocyte-derived macrophages, and found leucine, but no other amino acid increased mTORC1 activity (shown are a select few amino acids only). *Panels B-D.* Furthermore, we found the effect of leucine on mTORC1 activation (B) and downstream effects on autophagy, assessed as LC3 puncta as in Figure 1 (C) and specifically mitophagy, assessed as the contribution of dysfunctional to total mitochondria (D) is dose-dependent within the postprandial range of leucine concentrations. *Panel E.* We assessed mTORC1 activation (phospho-S6 content) in blood monocytes from mice gavaged with 0.8 g/kg protein (n=5), 1.6 g/kg protein (n=9), and 0.8 g/kg protein plus enough leucine to make up the difference in the leucine content of the 0.8g/kg and 1.6g/kg protein (n=9), and found the addition of leucine to was sufficient to recapitulate the high protein induced increase in mTOR activation. Data in C-E are mean±SEM. *P≤0.05, **P<0.01.

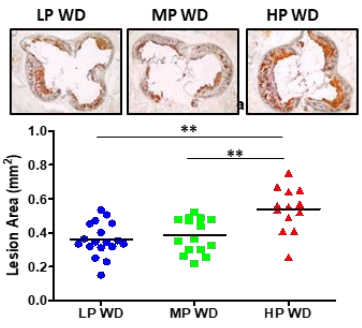

Figure 4. Atherosclerotic plaque burden in Oil Red O–stained aortic root sections from ApoE$^{-/-}$ mice that were fed a low-protein (LP), medium-protein (MP), or high-protein (HP) Western diet (WD) for 8 weeks. Top: representative roots; bottom: total lesion areas. **P < 0.01.

from animal and plant sources on monocyte/macrophage mTORC1 signaling and autophagy, because animal proteins generally contain more leucine than plant proteins (64, 65). The clinical trial proposed in this grant will be leveraged to conduct the studies in human subjects proposed in Specific Aims 1 and 2 in this LLF-LRP application. The LLF-LRP has also provided support for additional preliminary studies for Specific Aim 2 (see Figure 5) and for additional ancillary studies/analyses that helped generate preliminary data for new projects that focus on the mission of the LLF.

The research supported by the LLF-LRP has resulted in 12 publications (5 original research papers, 7 review articles and invited perspectives), and 2 funded grant applications, totaling $2,444,00 in direct cost. Several additional papers and 3 grant applications are currently pending. Following is a complete list of published and pending papers and a list of awarded and pending grant applications that that benefitted from the LLF-LRP.

Publications, including impact factor (IF)

1. B Mittendorfer, S Klein, L Fontana. Dietary protein: is more really better? Nat Rev Endocrinol. 16(1):59-66, 2020. **IF 43.3**
2. X Zhang, I Sergin, TD Evans, SJ Jeong, A Rodriguez-Velez, D Kapoor, S Chen, E Song, KB Holloway, JR Crowley, S Epelman, CC Weihl, A Diwan, D Fan, B Mittendorfer, NO Stitziel, JD Schilling, IJ Lodhi, B Razani. High-protein diets increase cardiovascular risk by activating macrophage mTOR to suppress mitophagy. Nat Metab. 2:110-125, 2020. **IF 13.5**
3. A Fappi, B Mittendorfer. Dietary protein intake and obesity-associated cardiometabolic dysfunction. Curr Opin Clin Nutr Metab Care. 23:380-386, 2020. **IF 3.6**
4. HE Koh, S van Vliet, T Pietka, GA Meyer, B Razani, R Laforest, RJ Gropler, Mittendorfer B. Subcutaneous adipose tissue metabolic function and insulin sensitivity in people with obesity. Diabetes. 70:2225-2236, 2021. **IF 9.5**
5. HE Koh C Cao, B. Mittendorfer. Insulin clearance in obesity and type 2 diabetes. Int J Mol Sci. 23:596, 2022. **IF 5.9**
6. B Mittendorfer, BW Patterson, GI Smith GI, M Yoshino, S Klein. Beta-cell function and plasma insulin clearance in people with obesity and different glycemic status. J Clin Invest. 132(3):e154068, 2022. **IF 14.8**
7. HE Koh, S van Vliet, C Cao, BW Patterson, DN Reeds, R Laforest, RJ Gropler, YS Ju, B Mittendorfer. Effect of obstructive sleep apnea on glucose metabolism. Eur J Endocrinol. 186:457-467, 2022. **IF 6.7**
8. JW Beals and B. Mittendorfer. The secret to a long "musclespan" is a little hard work. J Physiol. In Press. **IF 5.2**
9. HE Koh, BW Patterson, DN Reeds, B Mittendorfer**.** Insulin sensitivity and kinetics in African American and White people with obesity: insights from different study protocols. Obesity. 30:655-665, 2022. **IF 5.0**
10. F Magkos, B Mittendorfer. Type 2 diabetes therapeutics: weight loss and other strategies. Curr Opin Clin Nutr. In Press. **IF 3.6**
11. C Cao, HE Koh, BW Patterson, DN Reeds, R Laforest, RJ Gropler, B Mittendorfer. Increased plasma fatty acid clearance, not fatty acid concentration, is associated with muscle insulin resistance in people with obesity. Metabolism. In Press. **IF 8.7**
12. F Magkos, B Mittendorfer. Evolution of the diagnostic value of "the sugar of the blood": hitting the sweet spot to identify alterations in glucose dynamics. Physiol Rev. In Press. **IF 37.3**

Publications currently undergoing peer-review

1. X Zhang, D Kapoor, SJ Jeong, J Stitham, A Fappi, I Sergin, E Yousif, A Rodriguez-Velez, YS Yeh, A Park, S Epelman, JD Schilling, M Sardiello, A Diwan, J Cho, NO Stitziel, IJ Lodhi IJ, B. Mittendorfer, B Razani. Identification of a leucine-mediated threshold effect governing macrophage mTOR signaling and cardiovascular risk. Nat Metab, pending.
2. C Cao, HE Koh, DN Reeds, BW Patterson, B. Mittendorfer. Reliability of commonly used indices to evaluate beta-cell function. Obesity. Pending, minor revisions.
3. X Zhang, TD Evans, S Chen, I Sergin, J Stitham, SJ Jeong, A Rodriguez-Velez, YS Yeh, A Park, IH Jung, A Diwan, JD Schilling, S Epelman, J Cho, IJ Lodhi, B Mittendorfer, B Razani. Loss of macrophage-specific mTORC2 accelerates atherosclerosis via FOXO1-mediated IL-1β secretion. Circ Res, pending
4. B Mittendorfer, B. Patterson, D Haire-Joshu, A. Cahill, RI Stein, S Klein. Insulin sensitivity and beta-cell function during early and late pregnancy in women with gestational diabetes mellitus.

Funded grant applications

1. Mittendorfer/Razani (MPI), R01 HL159461, NIH. "Dissecting the impact of dietary protein on macrophage mTOR signalling and atherosclerosis", 04/01/22-03/31/27, $2,385,000
2. Lai (PI), Mittendorfer/Razani (collaborators), Midwest Stone Institute, "Protein-mediated nephrolithiasis", 05/01/22-04/30/23, $60,000

Pending grant applications

1. Mittendorfer/Razani/Schilling (MPI), R01 DK131188, Harnessing macrophage lysosomal lipid metabolism in obesity-associated diseases, 07/01/22 - 06/30/27, $2,499,160; 11th percentile with "intent to fund" notice from NIH
2. Lodhi (PI), Mittendorfer (Co-I), NIH R01, Branched-chain fatty acid metabolism and the regulation of energy balance, 01/01/23-12/31/27; pending peer-review
3. Ippolito (PI), Mittendofer (Co-I); Siteman Cancer Center, Research Development Award, Leucine metabolism and glioblastoma therapy. pending peer-review.

5. Research Design and Methods

AIM 1: *To evaluate the effect of high protein intake and amino acids on mTORC1 signaling to autophagy in platelets*

This aim will leverage resources of the randomized, double-blind, cross over study that is part of R01 HL159461 (Mittendorfer/Razani, MPI) to evaluate the effects of dietary proteins from animal and plant sources on monocyte/macrophage mTORC1 signaling and autophagy. The blood samples collected from the participants in this study will be used to isolate both monocytes (for the purpose of the experiments proposed in the R01 application) and to isolate platelets (for the purpose of this LLF-LRP application) to evaluate the effect of high protein ingestion on mTORC1 signaling and downstream sequelae in platelets and to assess whether this response depends on the type (animal vs plant) of protein and specific amino acids. We hypothesize that high protein meals, compared with the standard protein meal, will increase mTORC1 signaling and downstream effects, including inhibition of autophagy/mitophagy, increased production of reactive oxygen and proinflammatory cytokines, activation of pro-apoptotic pathways, and platelet activation (assessed as selectin, thrombomodulin expression and TXA2 production). We also hypothesize that these effects will be greater after the high animal than the high plant protein meal and greater after the leucine-supplemented high plant protein meal than the high plant protein meal alone. Moreover, we hypothesize that these effects are predominantly caused by leucine, not other amino acids, at concentrations observed postprandially after high-protein but not standard protein meal ingestion, which helps explain the difference in the platelet response after animal- compared with plant protein ingestion because plant protein ingestion results in lower postprandial plasma leucine concentration than animal protein.

We will study 24 middle-aged men (n=12) and postmenopausal women (n=12) who are overweight and moderately obese on four separate occasions in a randomized, double-blind, cross over experiment. Participants will consume one of four isocaloric, liquid mixed meals that differ in the amount and source of protein: i) standard protein meal, ii) high animal protein meal, iii) high plant protein meal, and iv) high plant protein meal to which leucine has been added to match the leucine content of the high animal protein meal. Blood samples to isolate monocytes/platelets and to determine amino acid, glucose and insulin concentrations will be obtained before ingesting the meal and at 1 h, 2 h, and 3 h after consuming the meal. The meals will be prepared by the metabolic kitchen in the Clinical and Translational Research Unit. The standard protein meal will contain 15 grams (11% of energy) of protein (half from animal and half from plant sources), 55% as carbohydrates, and 34% as fat; the high protein meals will contain 25 grams (22% of energy) of energy, 44% as carbohydrates and 34% as fat. The additional protein will be either from animal sources (mix of milk, chicken, and egg protein isolates) or plant sources (mix of soy, rice, and pea protein isolates). We chose these sources of protein because they are common in the diet, are frequently used in protein-enriched food products, and vary considerably in their leucine content (65-67). Both the protein content and overall macronutrient composition of the standard protein meal are consistent with the recommended daily protein intake; the protein contents of the high protein meals is consistent with habitual protein intakes in the general population (Background section). In experiments akin to those we have previously used in monocytes/macrophages (2), with minor modification to optimize assays for platelets (53), we will conduct studies in platelets isolated from blood samples collected before and after meal ingestion in people and in cultured platelets to evaluate whether high protein intake and amino acids activate mTORC1 signaling, suppress autophagy/mitophagy pathways, increase apoptotic pathways, reactive oxygen and proinflammatory cytokine production, platelet propensity toward apoptosis, and platelet activation (assessed as P-selectin expression, thrombomodulin and thromboxane production and platelet spreading when adhered on fibrinogen-coated surfaces) and aggregation (53, 68, 69). We will also determine whether these responses differ depending on type of protein (animal vs plant) and the amount and type of amino acids.

AIM 2: *To evaluate the effect of high protein intake and amino acids on endothelial cell biology*

We have generated preliminary data (Figure 5) that suggest: i) high protein intake impairs endothelial function (assessed by using the reactive hyperemia index (70)) and ii) arginine transport into endothelial cells is inhibited by high amino acid concentrations, presumably through competitive inhibition of arginine transport (55-57). During the next funding cycle, we will conduct in vitro experiments in human umbilical vein endothelial cells (HUVECs) akin to those in our previous (2) and ongoing studies in monocytes/macrophages and those described in Specific Aim 1 for platelets, to assess the effects of amino acids on mTORC1 signaling and autophagy/mitophagy, and apoptotic pathways. In addition, we will evaluate the effects of high amounts of amino acids (total and specific amino acids) on arginine uptake and nitric oxide production. Lastly, we will evaluate endothelial function (assessed as reactive hyperemia index) in participants who are enrolled in the newly funded clinical trial (R01 HL159461, MPI: Mittendorfer/Razani) that will evaluate the effects of dietary proteins from animal and plant sources on monocyte/macrophage biology, and will also collect endothelial cells before and after meal ingestion from these participants to evaluate the effect of high protein intake from animal and plant sources on gene expression and mTOR signaling.

Statistical analysis

Analysis of variance will be used to evaluate the effect of different treatments and meals on the study outcomes. Statistical analysis will be conducted in collaboration with the statistical support staff in the Center for Human Nutrition, who will ensure appropriate statistical procedures and adjustments for potential confounding variables and multiple comparisons are used.

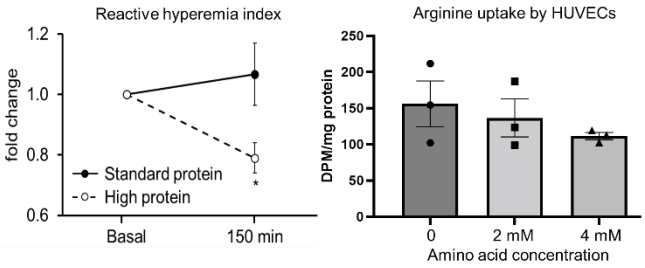

Figure 5. Effect of high protein intake and amino acids on endothelial function and arginine uptake by endothelial cells. *Left*: Change in the reactive hyperemia index after consuming a standard meal, containing 15 g of protein, and a high protein meal, containing 25 g of protein (n=9). *p<0.05. *Right*: Radiolabeled arginine uptake by human umbilical vein endothelial cells (HUVECs) in the absence and presence of incremental amounts of amino acids in the medium (n=3 each). Data are mean ± SEM.

Statistical power

The primary outcome in the studies we propose will be the phospho-S6 content in platelets and endothelial cells. In our completed and ongoing studies (see 4. *Progress Report*), we found that phospho-S6 content in monocytes after ingesting a high protein meal that contained 25 g of protein was 2.8 ± 1.2 (mean ± SD) times greater than after ingesting a standard meal that contained 15 g of protein. Furthermore, in pilot studies in mice, we found that the increase in monocyte phospho-S6 content is twice as great after gavage with an animal protein-rich than a plant protein-rich liquid meal. Using these data and assuming a probability of Type I error of <5%, 80% power, and two-sided testing, we estimate that 6 participants are needed to detect a 2-fold difference in monocyte phospho-S6 after consuming the different meals and 24 participants are needed to detect a 0.7-fold difference.

AIM 3: To strengthen existing and establish new collaborations

A major goal of the LLF-LRP is to stimulate new and strengthen existing collaborations among investigators with expertise in different disciplines to allow them to pursue new research directions and/or more comprehensive, translational research projects. The LLF-LRP will also assist young investigators interested in aging research obtain funding for their career development or path towards independence. These goals are accomplished by proactively engaging with investigators with similar research interests and complementary expertise, providing access to sample repositories for ancillary analyses, and sharing preliminary data. The chain of events that stimulated the highly productive collaboration between Drs. Bettina Mittendorfer and Babak Razani serves as a perfect example for the feasibility and effectiveness of this approach. Dr. Mittendorfer, is a clinical translational metabolism investigator in the Division of Geriatrics and Nutritional Science in the Department of Medicine. She has been conducting clinical trials to evaluate the effects of acute protein ingestion and increased protein intake during diet induced weight loss on mTOR signaling, muscle protein synthesis, and insulin sensitivity in people with obesity. Dr. Razani is a basic science physician investigator in the Cardiovascular Division in the Department of Medicine. He has been studying the effect of mTOR signaling on atherosclerotic plaque development and stability in cultured cell systems and mice. When Dr. Razani presented his data in a Cardiovascular Research Center seminar, Dr. Mittendorfer became interested in his techniques for her own research. The two met to review their shared interests and developed the hypothesis that protein overconsumption alters monocyte/macrophage signaling in ways that impairs vascular health and causes adipose tissue dysfunction and insulin resistance, thereby increasing the risk for developing T2D. They developed the protocols to obtain new outcome measures needed to test this hypothesis and with support from the LFL-LRP began to collect the preliminary data presented in this application.

6. Plans for Obtaining Additional Extramural Funding

As demonstrated in section 4. *Progress Report*, we have a strong track record of obtaining extramural funding to stimulate new research and to expand the research conducted with support from the LLF-LRP. During the next funding cycle, we plan to submit an NIH R01 (or VA MERIT) award application to test the hypothesis that reducing protein intake in people with obesity and prediabetes who consume high amounts of protein (>1.5 times the recommended daily intake) will improve cardiometabolic function even in the absence of weight loss. We also plan to apply for an NIH U19 award (PAR-19-374, Complex Multi-Component Projects in Aging Research) to evaluate new putative therapeutic strategies to improve cardiometabolic and physical function in older adults. This will include new collaboration with Dr. Gary Patti in the Department of Chemistry at WU, who is an expert in metabolomics, and Dr. Gretchen Meyer in the Physical Therapy Program at WU, who is an expert in skeletal muscle biomedical engineering. In addition, we expect that the data collected from the research supported by the LLF-LRP will provide the preliminary data for career-development awards from the American Heart Association (AHA) and the American Diabetes Association (ADA) for Drs. Xiangyu Zhang and Alan Fappi, and postdoctoral fellowships (NIH T32, AHA, ADA).

7. Literature cited

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