ALINEA

NUTRITION

APRIL 2023

www.alineanutrition.com

TABLE OF

CONTENTS

Ferraz-Bannitz R, Beraldo RA, Peluso AA, Dall M, Babaei P, Foglietti RC, Martins LM, Gomes PM, Marchini JS, Suen VMM, de Freitas LCC, Navegantes LC, Pretti MAM, Boroni M, Treebak JT, Mori MA, Foss MC, Foss-Freitas MC. Dietary Protein Restriction Improves Metabolic Dysfunction in Patients with Metabolic Syndrome in a Randomized, Controlled Trial. Nutrients. 2022 Jun 28;14(13):2670.

What We Know, Think We Know, or Are Starting to Know

There is a long lineage of research dating back almost a century that has shown that calorie restriction results in increased lifespan in a variety of organisms, from insects to rodents to monkeys [1,2]. However, an observation in this research area was that in organisms such as flies and rodents, restriction of dietary amino acids [i.e., proteins] could also result in lifespan extension [2].

This opened a question: did protein restriction explain the benefits of calorie restriction? Research in mice has in fact suggested that the metabolic benefits of calorie restriction can be achieved *without* severe restrictions of total energy intake by adopting a low-protein, high-carbohydrate diet [3]. In particular, a low protein-carbohydrate ratio [i.e., lowering protein with increasing carbohydrate] improved metabolic outcomes to the same extent as calorie restriction [3].

Now, Dear Reader, you will have no doubt already been asking an astute question here – how are flies, monkeys, and mice, relevant to humans? Of course, animal models are merely experimental and not necessarily translational, unless such effects have also been shown in humans. Observationally, one traditional diet and culture associated with longevity are the Okinawans, local to the island of Okinawa off the south coast of Japan, for whom the traditional diet consists of ~10% protein and 85% carbohydrate [4].

However, the Okinawan diet is also characterised by long-term relatively lower energy intakes, again making it impossible to determine whether the macronutrient balance is a relevant factor above what total energy intakes may explain [4].

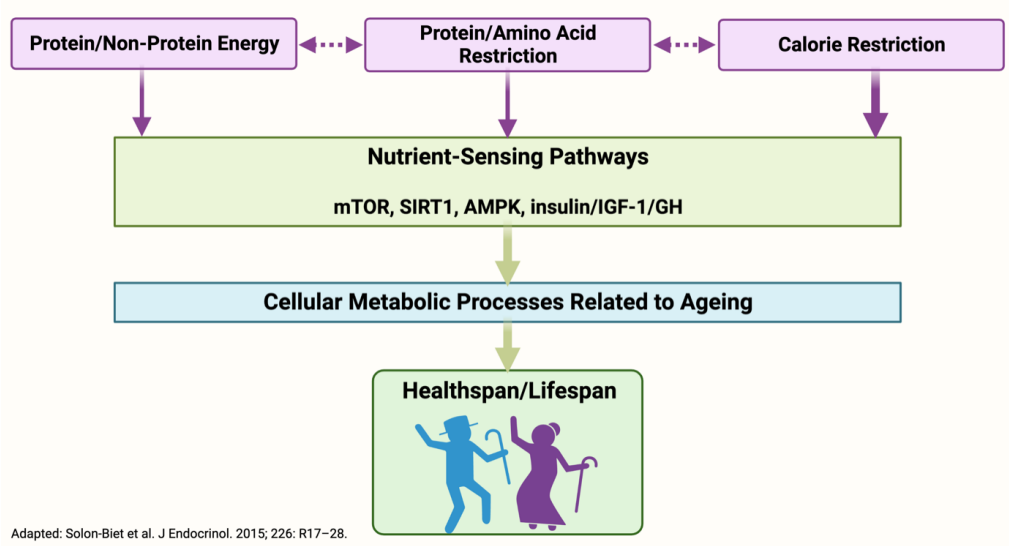


***Figure** illustrating the proposed processes linking dietary factors to healthspan/ lifespan.*

Nevertheless, these lines of evidence have ultimately generated a hypothesis, largely supported by rodent models, that the ratio of protein to non-protein energy intake may be more influential on metabolic health than total energy intake alone [(5)]. The present study investigated whether protein restriction could result in improvements in metabolic markers similar to calorie restriction.

The Study

21 patients in São Paulo, Brazil, completed a 27-day inpatient study where they were resident in a clinical research facility for the duration of the study. To be included, participants were required to have a diagnosis of type-2 diabetes [T2D], hypertension, and dyslipidaemia.

Prior to study period, participants were required to consume a diet of ~2,000kcal/d with a macronutrient content of 50% carbohydrate, 30% fat, and 20% protein [examples of diets were provided to participants for this period]. On entry to the in-patient study period, participants were randomised to one of two dietary interventions:

- **Calorie Restriction [CR]**: a 25% energy deficit continuing with the macronutrient content of 50% carbohydrate, 30% fat, and 20% protein.
- **Protein Restriction [PR]**: an isocaloric [i.e., energy balance] diet with a 50% restriction on protein [i.e., 0.8g per kg bodyweight vs. 1.5g/kg]. Thus, the macronutrient content was 60% carbohydrate, 30%, and 10% protein.

11 participants consumed the CR diet and 10 consumed the PR diet, both for 27-days. Diets were prepared by the researchers at their facility.

The primary outcomes were changes in HbA1c and insulin sensitivity. Secondary outcomes included changes in blood lipids, fasting glucose and insulin, and C-reactive protein [CRP]. Exploratory outcomes included changes in blood pressure, body composition, and resting energy expenditure.

Results: Results: All participants randomised to each diet completed the full 27-day inpatient study. Average age of participants was 49yrs and 51yrs on the CR and PR groups, respectively. 7 of 11 and 5 of 10 participants on the CR and PR diets, respectively, were female. Participants had a T2D for ~8-9yrs. Energy intake on the CR diet averaged ~1,400kcal/d, while energy intake on the PR diet averaged 2,011kcal/d.

Primary Outcomes – HbA1c and Insulin Sensitivity: HbA1c decreased by 1.6% and 0.85% on the CR and PR diets, respectively. Insulin sensitivity increased by 62.3% and 93.5% on the CR and PR diets, respectively [discussed further under ***Relevance***, below].

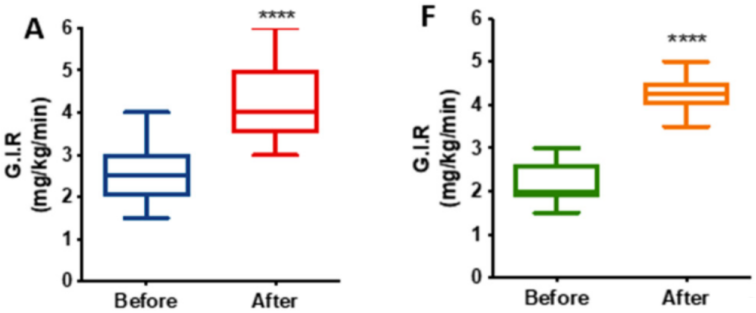


Figure *from the paper illustrating the change in "G.I.R" or Glucose Infusion Rate, representing glucose uptake in tissues relative to infusion of glucose into the body, i.e., tissue sensitivity to insulin, in the CR group [left] and PR group [right]. <4.9mg/kg/min represents insulin resistance.*

Secondary Outcomes – Blood Lipids, Fasting Glucose/Insulin, and CRP*:** LDL-cholesterol decreased by 1.4mmol/L [54mg/dL] and 1.2mmol/L [46mg/dL] in the CR and PR groups, respectively [discussed further under ***Relevance, below]. Triglycerides decreased by 1.5mmol/L [132mg/dL] and 0.9mmol/L [79mg/dL] in the CR and PR groups, respectively.

Fasting glucose decreased by 8.3mmol/L [149mg/dL] and 7.0mmol/L [126mg/dL] in CR and PR groups, respectively. There were no significant changes in fasting insulin levels.

CRP decreased from 9.2mg/dL to 3.9mg/dL in the CR group, and decreased from 8.5mg/dL to 2.6mg/dL in the PR group.

***Exploratory Outcomes – Blood Pressure, Body Composition, and Energy Expenditure*:** Average blood pressure decreased [systolic/diastolic] by 46.4/57.3mmHg in the CR group, while in the PR group average blood pressure decreased by 44.6/58.5mmHg.

Total weight loss during the study was 6.9kg and 5.4kg in the CR and PR groups, respectively. As there was no change in fat-free mass, this was almost entirely explained by decreases in fat mass of 11.5% and 9.9% in the CR and PR groups, respectively.

Energy expenditure decreased by 6% in the CR group, while in the PR group there were no significant differences in energy expenditure [discussed further under ***Key Characteristic***, below].

The Critical Breakdown

Pros: The study hypothesis was clearly stated. The intervention was highly controlled with participants randomised to diets and residing as inpatients for the duration of the study. Study diets were prepared by the research team at their facility, and study diets were prepared with similar foods thus differing only in total energy and macronutrient composition. Participants were not informed of the composition of diets. Energy expenditure and insulin sensitivity were determined using "gold standard" methods. For such a highly controlled inpatient study, a near month duration is quite good and on a par with the duration of Kevin Hall's metabolic studies.

Cons: The method of randomisation was not stated. No data on diet is presented, which given the enormous magnitude of changes, is a big omission; we know nothing about composition beyond basic energy and macronutrients. There is also no detail on participant medication use, which given the risk profile of participants, is a big omission.

The reporting of the study results flashes as an amber light. No baseline values for participants are provided for the primary and secondary outcomes. In fact, no raw data is presented for insulin sensitivity measures, which as one of two primary outcomes is sloppy, if not unacceptable. Raw data and baseline values are similarly unreported for almost all secondary outcomes, e.g., we do not know what the actual baseline levels of total cholesterol, LDL-C, or triglycerides are. Changes are reported either as the absolute change or as percentages, giving little context to the meaning of the magnitude of change for many outcome measures. Fasting glucose – which was stated to be a secondary outcome – is reported as a primary outcome with weight loss, which was an exploratory outcome. The results section leads with the exploratory outcomes, and the primary outcomes are only reported in the fourth and fifth subsections of the results.

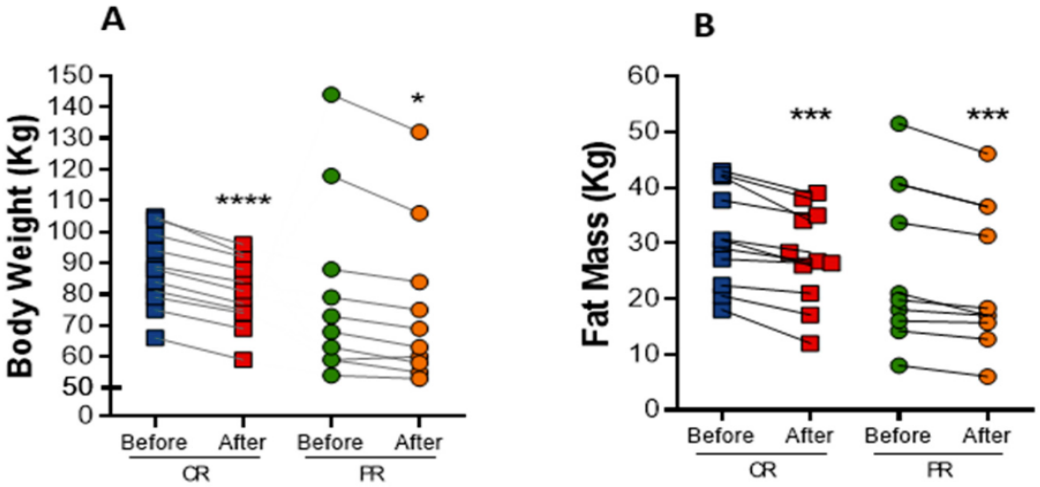
The sample size was quite small, and given the enormous magnitudes of change observed for almost all outcome variables, these findings should be taken with a fistful of salt.

Key Characteristic

Let's remind ourselves of the importance of a clearly stated hypothesis for the conduct of good science. This is one thing the study stated clearly, stating that they *"tested the hypothesis that isocaloric dietary protein restriction is sufficient to confer the beneficial effects of CR by reversing metabolic dysfunction..."*

But did they in fact test this hypothesis? If we are taking the data as a whole, there is an argument that the participants in the PR group may have been in some degree of negative energy balance. With such a small study, they are prone to the influence of large changes in outcomes in only a few participants.

For example, let's take the average weight loss in the PR group of 5.4kg; this is a rate of weight loss of nearly 1.5kg per week. And the study has not presented us with any raw data for weight. But they have plotted bodyweight change and kilograms in decrease of fat mass using paired-sample graphs, which means that we can visualise the before-after data for each individual participant [see **figure** below].



And we can see here, if we look at the **left graph** first, is that the magnitude of change appears to have been strongly influencing by two participants in the PR group [the green-orange pairings, at the top-right of that graph]. And we can see this is similar for the decrease in fat mass in the **right graph**. We can also see that the magnitude of change in many of the other participants is far more modest, or in some cases appears to be no change at all.

Again, recall the hypothesis is that protein restriction without the need for energy restriction confers benefits. The authors state in their discussion section: *"Given that neither energy intake nor total energy expenditure is affected by PR...and given that individuals in the PR group had an isocaloric diet...it is not clear how these individuals lost weight."*

Turns out there is a well-known medical aphorism for this; *"when you hear hoofbeats, first think of horses rather than zebras."* Which leads us to our ***Interesting Finding***...

Interesting Finding

The phrase *"when you hear hoofbeats, first think of horses rather than zebras"* is intended to convey that we first look to the most likely explanation, the one that requires the least assumptions [also known as parsimony]. Let's think about the components of energy balance considered in this study. The first is that the authors state they individualised energy intake to participants based on their "basal energy expenditure" measure. Except the authors did not measure "basal" energy expenditure, which requires very strict conditions to be considered "basal"; they measured resting energy expenditure for 30mins in an outpatient setting. There is no further detail provided as to the condition of the participants [i.e., fed or fasted, time of day, etc.], which would influence a measure of resting energy expenditure.

Secondly, it is customary to multiply any baseline resting energy expenditure measure by a physical activity multiplier [(6,7)], even if participants are sedentary as in the present study, which is particularly important if maintaining weight in participants in desired. No detail is provided in the paper regarding any physical activity multiplier applied to the measure of resting energy expenditure.

And the present study didn't find *any* measure of energy expenditure altered in the PR group, whether resting energy expenditure or carbohydrate and fat oxidation. So, if energy balance is a function of energy expenditure and energy intake, and one side of this equation hasn't changed at all, what do we think likely explains the findings?

Given that our current state of knowledge in nutrition is that energy balance is the fundamental determinant of changes in body mass [(8,9)], the most parsimonious explanation for some of the observed effects is that the investigators underestimated the energy requirements of participants, resulting in a degree of negative energy balance in at least some participants.

Relevance

The present study appears to be one of the first to attempt to test that hypothesis in humans that protein restriction, without the need for calorie restriction, may lead to improvements in metabolic outcomes that are independent of energy intake. In terms of its overall methodological quality, the study falls short of this task, and the shoddy reporting of the data leaves us with some further amber lights over what we can take away from this paper.

In particular, it is important to have some caution regarding the enormous differences in metabolic outcomes observed in the study due to it small sample size, lack of information about the diets, and the very high-risk profile of participants with long-standing diagnoses of T2D, hypertension, and dyslipidaemia. The participants appear to have been quite sick, and having a very controlled diet fed to them in an inpatient environment for one month may have resulted in some profound improvements. Given the absence of data on medication use, it is also possible that medication use, or lack thereof, may have influenced the magnitude of change.

Bear in mind that the 'Portfolio Diet', designed to maximise cholesterol reduction, has been shown to lead to an average LDL-C decrease of 0.61mmol/L [25mg/dL] over 1yr [(10)];

the present study showed decreases of 1.2–1.4mmol/L [46–54mg/dL] in just 27-days. Bear in mind the average blood pressure reduction on the DASH diet, which we covered in a previous Deepdive, is 3.9/2.1mmHg in individuals with hypertension; the present study showed decreases of up to 45-55mmHg, although the strong effects of calorie restriction on blood pressure are well established [11].

Further bear in mind that similar considerations apply to the seemingly profound improvements in insulin sensitivity when expressed as a percentage. However, recall that a glucose disposal rate of <4.9mg/kg/min represents insulin resistance [12]; although the present study didn't present the raw data for this primary outcome, looking at the figure in the paper we can see that the participants went from highly insulin resistant to borderline insulin resistant.

In terms of the stated hypothesis, we also need to disentangle the interactive effects of nutrients and calories. The PR group in the present study had a protein intake of 0.8g/kg/bw, right on the general recommendation for protein adequacy [13]. However, the study was aiming for conditions of energy balance, and while it appears this wasn't achieved in all participants, the reality is that protein requirements are not independent of total energy intake, as energy intake itself may preserve nitrogen balance [13]. Thus, the final point to bear in mind is that if this hypothesis is being taken from animal models, those models use protein restrictions of a *minimum* of 50%, up to 85% [2].

In the future this hypothesis is going to have to be refined in terms of whether it is *insufficient* protein intake, rather than a generic definition of "low protein" but still adequate protein, in relation to energy balance, that may or may not translate the findings from rodents to humans.

Application to Practice

And so the search for the magic bullet continues; the animal models overall still point more towards an overall effect of calories than protein [2]. And the present study just has far too many gaps and missing data to be considered a serious test of the protein restriction hypothesis in humans.

If this study has truly demonstrated weight loss and enormous reductions in numerous metabolic outcomes, without any degree of energy restriction, it has turned our current knowledge base on its head. The fact that it was published in *Nutrients*, rather than *Cell Metabolism* or *The American Journal of Clinical Nutrition*, probably says a lot about the data.

One observational study found some associations between higher protein intakes and cancer risk, but in the 50-65yrs old age group, not in those >66yrs of age [14]. However, this was based on cross-sectional dietary data from the U.S. National Health and Nutrition Examination Survey [NHANES], and is not a reliable dietary assessment method to use to investigate prospective outcomes over time [15].

In sum, at this point the evidence for purposeful protein restriction is unsupported in humans. The fact that the present study still had participants consuming at least adequate amounts of protein doesn't really provide any rigorous test of what is observed in the rodent models. Continue with being a human, for now...

References

1. Rattan SIS. Hormesis in aging. Ageing Res Rev. 2008 Jan;7(1):63–78.
2. Speakman JR, Mitchell SE, Mazidi M. Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. Exp Gerontol. 2016 Dec;86:28–38.
3. Solon-Biet SM, Mitchell SJ, Coogan SCP, Cogger VC, Gokarn R, McMahon AC, et al. Dietary Protein to Carbohydrate Ratio and Caloric Restriction: Comparing Metabolic Outcomes in Mice. Cell Rep. 2015 Jun;11(10):1529–34.
4. Willcox BJ, Willcox DC, Todoriki H., Fujiyoshi A, Yano K, He Q, et al. Caloric Restriction, the Traditional Okinawan Diet, and Healthy Aging: The Diet of the World's Longest-Lived People and Its Potential Impact on Morbidity and Life Span. Ann N Y Acad Sci. 2007 Oct 1;1114(1):434–55.
5. Le Couteur DG, Solon-Biet S, Wahl D, Cogger VC, Willcox BJ, Willcox DC, et al. New Horizons: Dietary protein, ageing and the Okinawan ratio. Age Ageing. 2016 Jul;45(4):443–7.
6. Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. World Rev Nutr Diet. 1992;70:68–131.
7. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr. 1990 Feb 1;51(2):241–7.
8. Schoeller DA, Buchholz AC. Energetics of obesity and weight control: Does diet composition matter? J Am Diet Assoc. 2005;105(5 SUPPL.):24–8.
9. Hall KD, Guo J. Obesity Energetics: Body Weight Regulation and the Effects of Diet Composition. Gastroenterology. 2017;152(7):1718–27.
10. Jenkins DJ, Kendall CW, Faulkner DA, Nguyen T, Kemp T, Marchie A, et al. Assessment of the longer-term effects of a dietary portfolio of cholesterol-lowering foods in hypercholesterolemia1–3. Am J Clin Nutr. 2006 Mar;83(3):582–91.
11. Nicoll R, Henein M. Caloric Restriction and Its Effect on Blood Pressure, Heart Rate Variability and Arterial Stiffness and Dilatation: A Review of the Evidence. Int J Mol Sci. 2018 Mar 7;19(3):751.
12. Tam CS, Xie W, Johnson WD, Cefalu WT, Redman LM, Ravussin E. Defining Insulin Resistance From Hyperinsulinemic-Euglycemic Clamps. Diabetes Care. 2012 Jul 1;35(7):1605–10.
13. FAO Expert Consultation. Dietary protein quality evaluation in human nutrition. Rome; 2013.
14. Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, et al. Low Protein Intake Is Associated with a Major Reduction in IGF-1, Cancer, and Overall Mortality in the 65 and Younger but Not Older Population. Cell Metab. 2014 Mar;19(3):407–17.
15. Willet WC. Nutritional epidemiology. 3rd ed. New York: Oxford University Press; 2013.