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Research Article

Metabolic Health Outcomes Following Nine Months of Mild Caloric Restriction in Male Rats Adhering To a Western or Vegan Diet

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Abstract

Background: Both diet composition and total calorie intake can influence body mass and measures of metabolic health.

Methods: We assigned male Long-Evans rats (N=28) to either a high-fat Western Diet (WD) or a moderate fat, purified Vegan Diet (VD) for 12 months, the first 3 of which food was provided ad libitum and the latter 9 in which food was restricted to approximately 90% of animals' daily needs. Half of the animals in each diet group were assigned to be exercise trained (+E) by running on a treadmill three days per week and the other half served as sedentary controls. Body mass was monitored daily and reported at three month intervals; plasma indicators of metabolic health were determined at the conclusion of the 12-month period.

Results: After three months of ad libitum feeding, body mass was higher for the WD compared to the VD (549g vs 484g; p<0.00001; pooled data for both exercise groups), and slightly less for the exercise-trained animals as compared to the sedentary animals (494g vs 539g; p=0.006; pooled data for both diet groups). Following 9 months of mild caloric restriction, body mass decreased approximately 15% for all groups, except for the WD+E group, for which the decrease was approximately 8%. Oxidative stress biomarkers, as measured by advanced oxidation protein products (p<0.0001) and malondialdehyde (p=0.05), were lower for the VD groups as compared to the WD groups. Triglycerides (p<0.0001) and cholesterol (p=0.0006) were also lower for the VD groups as compared to the WD groups, but glucose was not (p=0.09).

Conclusion: While mild caloric restriction results in a similar percent decrease in body weight in animals adhering to both a WD and VD, the improved metabolic health of animals following the VD, as indicated by lower plasma biomarkers of oxidative stress and blood lipids, highlights the fact that consuming a purified diet yields benefits that extend beyond mere weight loss.

Keywords: Caloric restriction; Cholesterol; Dietary restriction; Fasting; Oxidative stress; Triglycerides; Vegan body mass

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Introduction

Aside from caloric restriction, manipulating diet composition by adjusting macronutrient ratios and types can have a profound influence on overall health outcomes [1-4]. One form of manipulation involves the removal of animal-derived foods, and in the strictest sense includes veganism. This pattern of eating eliminates all animal products and has been reported to yield favorable outcomes related to cardio-metabolic health, including reductions in body mass [5-7]. Unlike caloric restriction, which typically calls for a significant reduction in daily dietary energy needs, veganism places no limitation on calories and allows for ad labium food intake.

We have studied a very stringent form of vegan-based dieting over the past several years, with reductions of body mass, blood lipids and measures of oxidative stress in as little as three weeks [8-12]. This approach, referred to as the "Daniel Fast" plan, allows for ad libitum food intake but places firm restrictions on the type of food that is allowed, with choices primarily limited to fruits, vegetables, whole grains, legumes, nuts, seeds and plant-based oils. No alcohol, sweeteners or refined foods are allowed resulting in carbohydrate sources that are complex with low glycemic indices. By default, this plan has an abundance of dietary fiber and plant-derived fatty acids, and relatively high concentrations of antioxidants.

While altering the diet composition in a way similar to that described above can be useful as a health promotion strategy, the most commonly utilized approach is to simply reduce calorie intake the classic "hypocaloric" dieting. While reducing calories may be achieved using a variety of different diet plans (e.g., simple caloric restriction, intermittent or alternate day fasting), the majority of plans do in fact result in a lower total calorie intake, leading to a reduction in body weight. However, while weight loss is often achieved by many dieters, what is often not considered in particular, outside of the context of a controlled research study or a clinical case is how diet composition may impact other measures of health; that is, those that may not be "visible" through a simple assessment of body mass.

The present study sought to determine the influence of mild, chronic caloric restriction in male rats. Outcome variables included body mass but also biochemical measures of health that are important but often not considered by those adopting a weight loss diet. We hypothesized that intake of a purified diet would yield results that were more favorable as compared to a Western Diet, in particular with regards to the biochemical outcomes. Moreover, we included thrice weekly treadmill walking/running in the model to determine the potential additive influence of exercise on the outcome measures.

Methods

Animals and housing

Male Long-Evans rats (N=28) were purchased from Harlan Laboratories, Inc. (Indianapolis, IN) at the age of 3-4 weeks. Rats were individually housed in a climate controlled room (21ºC) with a 12:12-h light-dark cycle. They were initially fed a standard rat chow (Harlan 1018) with ad libitum water, and then transitioned to the assigned diet after two weeks. During this period the rats were familiarized with the treadmill on three separate days. All experimental procedures were approved by The University of Memphis *Institutional Animal Care and Use Committee.*

Diet group assignment

The rats were randomly assigned to one of four intervention groups: Western Diet with exercise (WD+E; n=7); Western Diet without exercise (WD; n=7); Vegan Diet with exercise (VD+E; n=7); Vegan Diet without exercise (VD; n=7). Both diets (provided in pellet form) were purchased from Research Diets, Inc. (New Brunswick, NJ). The WD (D12079B) was formulated to mimic a typical human WD, containing 17% protein, 43% carbohydrates (sucrose and corn) and 40% fat (butter and corn oil), whereas the VD included 15% protein, 60% carbohydrates, and 25% fat. The nutrient composition of both diets has been presented previously [13]. The types and quantities of macronutrient sources in the VD were based on the diet plans of the human subjects in our prior studies using a vegan diet [8,10,11]. The VD was rich in inulin+cellulose and flax oil, contributing to the carbohydrate and lipid content, respectively. The dietary intervention period was 12 months in duration. During months 1-3, food was provided ad libitum, while during months 4-12, the rats were weighed daily and provided a quantity of food thought to maintain them at approximately 90% of their free-feeding body mass measured just prior to food restriction. Water was provided ad libitum throughout the 12-month period.

Treadmill exercise

Animals in the exercise groups performed walking/running exercise on a level motorized treadmill three days per week, with a progressive increase in speed and duration. Specifically, the animals began training at 20 $m \cdot min^{-1}$ for 15 $min \cdot day^{-1}$ (week 1), progressed to 25 $m \cdot min^{-1}$ for 30 $min \cdot day^{-1}$ (week 2) and 25 $m \cdot min^{-1}$ for 35 $min \cdot day^{-1}$ (weeks 3-52).

Outcome measures

Body mass was measured daily using a Mettler Toledo PG2002-S balance equipped with dynamic weighing. At the end of the 12-month intervention, rats were euthanized via CO_2 inhalation and blood was collected from the inferior vena cava into vacutainer tubes containing EDTA. Plasma was separated and aliquots stored at -70ºC for analysis of metabolic health biomarkers. Advanced Oxidation Protein Products (AOPP) was measured using the methods described by the reagent manufacturer (Cell Biolabs, Inc.San Diego, CA; product #: STA-318). Malondialdehyde (MDA) was analyzed following the procedures of Jentzsch et al., (1996) using reagents purchased from Northwest Life Science Specialties (Vancouver, WA; product #: NWK-MDA01). Triglycerides (TAG), total cholesterol, and glucose were analyzed following standard enzymatic procedures as described by the reagent manufacturer (Thermo Electron Clinical Chemistry). All samples were analyzed in duplicate.

Statistical analysis

All plasma variables were analyzed using a one-way Analysis of Variance (ANOVA). Contrasts were used to compare individual groups with regards to bloodborne variables and body mass. All analyses were performed using JMP statistical software (SAS Institute; Cary, NC). Statistical significance was set at $p \leq 0.05$. All data are expressed as the mean ± SD.

Results

Following the initial three months of ad libitum feeding, body mass was higher for animals in the WD groups as compared to the VD groups, and slightly less for the exercise-trained animals as compared to the sedentary animals. Following the 9-month period of mild caloric restriction, body mass decreased in all groups, with an approximate 15% decrease for the WD, VD and VD+E groups, and an approximate 8% decrease for the WD+E group (Table 1). After the 12-month intervention, pooled data for both VD groups were lower compared to WD groups for advanced oxidation protein products ($p<0.0001$) and malondialdehyde ($p=0.05$), and for triglycerides ($p<0.0001$) and cholesterol ($p=0.0006$), with a trend for lower glucose ($p=0.09$). Biochemical data for all groups and the noted differences are displayed in table 2.

	Western Diet + Exercise	Western Diet	Vegan Diet + Exercise	Vegan Diet
Month 0 (Baseline)	187±9	187±12	193±8	185±14
Month 3	519±28	579±37	471±47	498±36
Month 6	487±28	515±22	425±26	432±22
Month 9	478±23	516±27	414±26	403±76
Month 12	475±24*†	490±22*§	398±27*	412±19*

Table 1: Body mass (g) of male rats assigned to two different diets with and without exercise for 12 months.

Values are mean ± SD.

* Significant difference between months 3 and 12 for WD+E ($p=0.03$), WD ($p<0.0001$), VD+E ($p=0.0001$) and VD ($p<0.0001$)

† Significant difference at 12 months between WD+E and VD+E ($p=0.0002$); WD+E and VD ($p=0.002$)

§ Significant difference at 12 months between WD and VD+E ($p<0.0001$); WD and VD ($p<0.0001$)

Discussion

To our knowledge, this is the first study to determine the combined influences of dietary composition and exercise during a period of caloric restriction on outcomes specific to metabolic health. There are several key findings from this study. Consumption of an ad libitum WD leads to greater weight gain as compared to a VD. Exercise reduces body mass gain during the ad libitum period, and particularly for the WD. Despite a similar percentage weight loss for the WD and VD groups during the period of caloric restriction, plasma lipids and indicators of oxidative stress were significantly lower for the VD. During the period of caloric restriction, exercise had little impact on plasma markers of oxidative stress and lipids, with the exception of AOPP and triglyceride levels with the WD.

	Western Diet + Exercise	Western Diet	Vegan Diet + Exercise	Vegan Diet
AOPP ($\mu mol \cdot L^{-1}$)	114±73	151±71	22±14	22±11
Malondialdehyde ($\mu mol \cdot L^{-1}$)	4.0±2.0	3.5±2.6	1.8±0.7	1.8±0.1
Triglyceride ($mg \cdot dL^{-1}$)	90±44	151±62	32±9	37±19
Cholesterol ($mg \cdot dL^{-1}$)	94±14	90±21	63±8	68±12
Glucose ($mg \cdot dL^{-1}$)	121±34	127±22	97±17	107±17

Table 2: Biochemical data of male rats assigned to two different diets with and without exercise for 12 months.

Values are mean ± SD.
AOPP (Advanced Oxidation Protein Products): Significant difference between WD+E and VD+E (p=0.002) and VD (p=0.002); between WD and VD+E (p<0.0001) and VD (p<0.001)
Malondialdehyde: Significant difference between WD+E and VD+E (p=0.03) and VD (p=0.03)
Triglyceride: Significant difference between WD+E and VD+E (p=0.01) and VD (p=0.02); between WD and VD+E (p<0.0001) and VD (p<0.001)
Cholesterol: Significant difference between WD+E and VD+E (p=0.0005) and VD (p=0.002); between WD and VD+E (p=0.001) and VD (p=0.006)

The lower weight gains during the period of ad libitum feeding support observations that vegetarian diets are associated with better weight maintenance [14]. Although the amount of food consumed by each animal was not measured during the period of ad libitum feeding, we believe that the volume was similar, as rats have been reported to consume relatively equal amounts of food, despite differences in caloric density [15].

Interestingly, regardless of the type of food consumed, when animals were subjected to a mild caloric restriction, all experienced a decrease in body mass of approximately 15%, except for the WD+E group, for which the reduction was approximately 8%. The lower percent loss could have been due to the fact that the exercise in the WD group attenuated the weight gain (i.e., weight gain in the WD group without exercise was far greater) and there was less weight to lose in the WD+E group. From a practical perspective, it appears that regardless of food composition, body mass can be reduced following a period of mild caloric restriction. This should be viewed as encouraging, in particular for those individuals who may not have the desire to alter what they eat but may be capable of reducing the quantity of food consumed.

As alluded to above, engaging in thrice weekly aerobic exercise resulted in a reduction in the amount of body weight gained during the initial three months of ad libitum feeding, which was expected based on prior knowledge of exercise as a therapeutic tool [16]. We anticipated that the exercise groups would have a lower overall body mass due to the increased caloric expenditure from the exercise bouts. For example, animals that did not exercise and were assigned to the WD gained 18% more weight, while those who did not exercise and were assigned to the VD gained 13% more weight. Exercise results in energy expenditure and is a commonly used modality to aid overall cardio-metabolic health [17]. Multiple studies support the role of exercise as an adjunct to diet therapy for purposes of weight management [18]. Interestingly and as shown in table 1, similar findings have been noted in a recent animal study, in which rats placed on an 8% caloric restriction, high fat diet experienced similar weight reduction with or without exercise [19].

As mentioned above, the percentage weight loss for the WD and VD groups was similar during the restriction period. However, despite this, oxidative stress biomarkers and blood lipids were significantly lower in animals adhering to the VD. Often, individuals engage in a dietary program with the goal of losing weight and becoming "healthier". In a non-clinical/research setting, the former is easy to determine; individuals can simply stand on a scale. The latter is much more complex but often is determined by the lay person simply based on how they feel. Our data show that despite a similar degree of weight loss during the nine-month restriction period, outcomes related to metabolic health are very different depending on the type of food that is consumed.

For example, protein oxidation, as measured by AOPP, was approximately 6-7 folds higher for the WD groups as compared to the VD groups, while MDA values were approximately 2 fold higher. Findings of lower oxidative stress in vegetarians have been noted previously [20]. Oxidative stress is associated with cardiovascular and neurodegenerative disease, as well as most other human diseases and minimizing the overall oxidative burden is generally viewed as a favorable health outcome [21,22]. Due to the fact that body mass (obesity) has been associated with oxidative stress, it is possible that the elevated values in relation to animals consuming the WD are a function of their higher absolute body mass [23]. That is, despite the fact that the WD and VD animals experienced a similar overall percent decrease in body mass during the restriction period, the absolute body mass of the WD animals at the conclusion of the 12-month intervention remained approximately 20% higher as compared to the VD.

While this difference in body mass may have contributed to our findings, an alternative hypothesis is that the diet composition was responsible for the differing oxidative stress values. Indeed, high intake of saturated fat and simple sugar as found in the WD, is associated with increased production of reactive oxygen species and related negative outcomes [24,25]. Moreover, flax oil and dietary fiber two components of the VD have been reported to provide favorable effects with regards to oxidative stress [26,27]. In the same way as for oxidative stress variables, both triglycerides and cholesterol were lower for the VD following the restriction period, with non-statistically significant lowering for glucose. These results are logical, as reducing intake of saturated fat, cholesterol and simple sugar is often met with a lowering in blood triglycerides and cholesterol.

Finally, as with the percentage of weight lost during the restriction period, exercise had little impact on oxidative stress and lipid levels; the exception being a lowering of triglyceride levels when adhering to the WD. Physical activity has been reported to result in lower levels of oxidative stress, with mixed findings for blood lipids [28]. It is possible that the impact of the diet restriction overwhelmed any effect of the exercise, to the extent that it was not observed in our sample of animals. Alternatively, the exercise in the present study may have been of too low of an intensity or volume to alter oxidative stress or cholesterol. Future work is needed to replicate our study and determine the independent and combined role of aerobic exercise, perhaps of higher volume and intensity, on measures of oxidative stress and blood lipids when animals/individuals adhere to a Western diet or purified vegan diet.

Conclusion

To our knowledge, this is the first study to investigate the impact of both dietary restriction and exercise, inclusive of ad libitum feeding and caloric restriction, on measures of metabolic health. The findings suggest that macronutrient composition, and not simply calorie intake, has an influence on the degree of oxidative stress, as well as blood lipids. These findings underscore the value of consuming a purified diet if the main objective is not simply to lose weight but to become healthy overall from a metabolic perspective.

Conflict of Interest

The authors declare no conflicts of interest related to this work.

Author Contributions

RJB was responsible for the study design, biochemical analyses, statistical analyses and manuscript preparation. JMS and TAG were responsible for animal training, data collection, database management, and assistance with the study design and manuscript editing. MB was responsible for manuscript preparation. All authors read and approved of the manuscript.

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