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Influence of Dietary Modification With and Without Exercise on Physical Performance in Male Rats

JohnHenry Michael Schriefer

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JohnHenry M. Schriefer, BS

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Abstract

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This study examined if specific dietary plan, with and without exercise, can positively impact physical performance in male rats. The present research examined three aspects of physical performance in relation to the Daniel Fast diet (DF) compared to a Western diet (WD). Long-Evans rats (n=56, aged 3-4 weeks) were on a 12 week diet and exercise intervention. Results demonstrated that treadmill run time to exhaustion increased in both exercise groups, with a greater increase in the DF+E group. Wire hang and slant board performance decreased from pre to post in all groups at a similar rate. Body weight gain was greater in WD vs. all groups. Body composition demonstrated a lower fat mass in DF groups vs. WD groups. Improved performance in DF compared to WD for treadmill run may be due to the favorable composition of the DF diet (lower glycemic carbohydrate; poly-unsaturated fatty acids) and the resultant lower fat mass.

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Background

The Daniel Fast (DF), a biblically-inspired fasting protocol, is a form of dietary restriction that does not limit the amount of food that can be eaten, but rather places a restriction on the type of food that is allowed (Bloomer et al., 2010; Bloomer, Kabir, Trepanowski, Canale, & Farney, 2011; Trepanowski & Bloomer, 2010; Trepanowski, Canale, Marshall, Kabir, & Bloomer, 2011). It is very similar to a vegan diet, where the foods consumed are mostly fruits, vegetables, whole grains, legumes, nuts, seeds, and oil. However, this plan is much more stringent, in that no sweeteners or refined foods are allowed. Therefore, carbohydrate sources are primarily low glycemic and complex in nature (e.g., whole grains), with only few exceptions that offer exceptional nutritional value (e.g., fruits). The diet plan also includes an abundance of fiber. Therefore, individuals appear much more satiated on the eating plan as compared to typical dietary plans. Finally, the plan includes a relatively high quantity of healthy plant-derived fatty acids. By following the DF regimen, individuals are infused with a wealth of healthful nutrients, and report a continuous "feel" of energy throughout the day (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski, Kabir, Alleman, & Bloomer, 2012).

Previous findings with the DF have included a significant reduction in systolic and diastolic blood pressure, total and LDL-cholesterol, LDL-HDL ratio, blood oxidative stress biomarkers, blood glucose, blood insulin, HOMA-IR, and C- reactive protein (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012). The dietary plan has also been shown to increase blood nitrate/nitrite (a biomarker of nitric oxide), which may have implications for physical performance, as well as antioxidant capacity (Anderson, 2000; Powers & Jackson, 2008). The research to date has focused exclusively on human health outcomes and not physical performance (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012). That said, certain outcomes such as increased blood nitrate/nitrite and decreased oxidative stress may have implications for enhanced performance, as has been suggested previously. In controlled laboratory studies starting in 2009, we received multiple anecdotal reports of favorable changes in vitality, vigor, and mood (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012). These variables—and more specifically how they relate to objective physical performance measures—remain to be investigated in a controlled study.

Today in the United States the majority of the population consumes a Western diet (WD) consisting of mostly processed high glycemic foods, often high in simple sugar and saturated fat. Overconsumption of processed, sugar-rich, and fatty foods, coupled with a lack of physical activity, has sparked a trend of obesity and overweight status at an unprecedented rate (Cordain et al., 2005). In fact, current data indicate that within the United States approximately 34.5% of adults are obese (Ogden, Carroll, Kit, & Flegal, 2012). Excess body fat can be detrimental to physical performance for a variety of reasons, including but not limited to the need to move excess "non-functional" weight, increased shortness of breath, and low back pain (Deforche et al., 2003; Hedley et al., 2004). Following a dietary model such as the DF could drastically reduce the incident rate of obesity due to the low-calorie and nutrient dense nature of the foods consumed (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012).

Diets with a high ratio of carbohydrate to saturated fat have been reported to favorably impact performance (Hammer, Barrier, Roundy, Bradford, & Fisher, 1989). Specifically, high carbohydrate, low fat diets may aid endurance capacity

(Chryssanthopoulos, Williams, Nowitz, Kotsiopoulou, & Vleck, 2002; Foskett, Williams, Boobis, & Tsintzas, 2008), as high carbohydrate diets can increase muscle glycogen stores leading to enhanced endurance performance (Jacobs & Sherman, 1999). Moreover, diets high in fat content (saturated fat in particular) have been reported to decrease power output and endurance performance (Fleming et al., 2003a; Jacobs & Sherman, 1999). In addition to endurance performance, evidence supports improved performance during high intensity and short duration exercise bouts when individuals consume a diet high in carbohydrates and moderate or low in fat—similar to what is observed when following a DF regimen (Foskett et al., 2008; Maughan et al., 1997a). Considering the typical WD which contains approximately 40% fat, a large percentage of which is saturated fat, the DF dietary plan may prove favorable for overall physical performance.

In addition to dietary intake, regular exercise training is known to improve physical performance (Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005; McClenton, Brown, Coburn, & Kersey, 2008), with novice trainees experiencing the most benefit. Using young animals, improvements in both aerobic and anaerobic exercise performance have been noted following regular treadmill exercise (Eliakim, Moromisato, Moromisato, & Cooper, 1997). It is possible that the combination of regular exercise training and dietary intake in accordance with the DF regimen may improve physical performance beyond what is observed for either exercise or diet alone. The present study sought to determine the independent and combined impact of dietary intake and exercise on physical performance in male rats.

Methods

Overview of Experimental Design

Male Long-Evans rats (n=56, aged 3-4 weeks) were purchased from Harlan Laboratories, Inc. (Indianapolis, IN). Upon arrival to the facility, all rats were individually housed at the animal care center on The University of Memphis main campus. Animals were initially maintained on a standard 12:12-h light-dark cycle. Animals had a two week acclimation to the animal care facility. Acclimation included familiarization to the cage, to the assigned diet (using a combination of the assigned diet and standard rat chow, in various proportions), and to the handlers. In addition, animals underwent familiarization to the treadmill, wire hang, and slant board test protocol (described below). For familiarization to the performance testing, animals underwent three trials of the slant board and wire hang tests. They were also exposed to the treadmill on three separate days (i.e., walking on the treadmill for 5 minutes at 15-20 m/min). The animals were gradually transitioned to a 12:12-h light-dark cycle (3:00am/3:00pm) during this two-week time period.

Although our prior work with the DF has exclusively involved human subjects, animals were used in the present design for the following reasons. First, using rats provided us with the ability to maintain control of all key variables known to impact physical performance (i.e., sleep, type and volume of food, volume of exercise, and intensity of exercise, health, genetics, enviroment). While recommendations can be made to humans to abide by our guidelines, these variables cannot be controlled with precision, as can be done in rats (Copp, Davis, Poole, & Musch, 2009; Koch, Meredith, Fraker, Metting, & Britton, 1998; Lee, Bruce, Spriet, & Hawley, 2001). Second, it was our belief that using rats would reduce variability in response in performance measures as compared to human subjects. Follow-up work using human subjects may be warranted.

Physical Performance Assessments

Animals were tested after the two week acclimation period. These baseline assessments were conducted on three different days. The order was as follows: body weight and slant board test for sensorimotor control; wire hang test for muscular strength/endurance; treadmill run test to exhaustion for cardiovascular endurance. The post intervention testing began during the week following the twelve week intervention (one test per day as detailed above, in an attempt to avoid fatigue).

Sensorimotor control assessment (slant board test):

This testing protocol is based on the design of Murphy et al. (Murphy, Rick, Milgram, & Ivy, 1995) with the exception of changing the degree of inclination. The animal was placed 10cm from the top on a slanted Plexiglas board which started at an angle of 30º. The animal needed to remain stationary for five seconds without sliding backwards in order to receive a "pass." If the animal passed this initial degree angle test, the incline was raised two degrees. The animal had three attempts at each angle, with five minutes of rest after a failed attempt. If the animal failed to pass the starting angle of 30°, the angle was decreased two degrees until the animal passed. The degree of incline was continually raised until the animal failed. The greatest angle passed was recorded, as previously described (Murphy et al., 1995).

Muscular strength/endurance assessment (Wire hanging test):

The animal's forelimbs were placed on a horizontal wire approximately 2 mm thick, suspended approximately 50 cm over a padded surface. The animal was suspended until it could no longer hold on. Total hang time was recorded in seconds (Bowenkamp, Lapchak, Hoffer, & Bickford, 1996; Shukitt-Hale, Mouzakis, & Joseph, 1998).

Cardiovascular endurance assessment (treadmill run test):

Animals began the test by running on a treadmill at a speed of 20 m/min without incline for 15 minutes. Speed increased by 5 m/min every 15 minutes to a maximum speed of 35m/min. When the animal was unable or unwilling to maintain pace with the treadmill belt, the test was terminated. Fatigue was detected when the animal started to lower its hindquarters and raise its snout, resulting in significantly altered gait—to the point of not being able to remain on the treadmill. When this degree of fatigue was noted and the animal had difficulty remaining on the treadmill belt, the animal was taken off the treadmill. Similar procedures have been used previously (Copp et al., 2009; Koch et al., 1998; Lightfoot et al., 2007). Run time was recorded to the nearest second.

Anthropometric Tests

All animals underwent a Dual Energy X-ray Absorptiometry (DXA) exam following the intervention—one week following the treadmill test. This was done to determine body composition of the animals. No DXA exam was performed pre intervention. Animals were anesthetized for approximately 10 minutes (using isoflurane) to allow for the scan to be performed.

Dietary and Exercise Intervention

Following baseline assessments the animals were randomly assigned to one of four intervention groups: Western Diet with exercise (WD+E; n=14); Western Diet without exercise (WD; n=14); Daniel Fast with exercise (DF+E; n=14); Daniel Fast without exercise (DF; n=14). Both diets (provided in pellet form) were purchased from

Research Diets, Inc. (New Brunswick, NJ). The WD is a standard product produced to mimic a typical human WD, containing 17% protein, 43% Carbohydrates, and 40% Fat. A custom rat chow was used to replicate the “cleanliness” of the DF plan, including a macronutrient breakdown of approximately 15% protein, 60% Carbohydrates, and 25% Fat. The specific nutrient breakdown of each plan is provided in table 1. The dietary intervention period was twelve weeks in duration. In all groups, the animals were allowed to feed ad libitum. Water was also allowed ad libitum. Weekly body weights were measured and recorded. In addition to the two different dietary regimens, animals were assigned to either exercise or no exercise. Animals in the no exercise group were placed on the treadmill daily for a period of 5 minutes, simply to allow for familiarization to the apparatus. Animals in the exercise groups performed endurance exercise on a motorized treadmill three days per week for the 12 week intervention. The speed and duration was progressively increased. Specifically, the animals began training at 20 m/min for 15 min/day (week 1), progressed to 25 m/min for 30 min/day (week 2), and 25 m/min for 35 min/day (weeks 3-12). Progression of this sort is typical in animal training studies (Jin et al., 2000).

Statistical Analysis

The physical performance results and body mass were analyzed using a four (condition) by two (time) repeated measures analysis of variance (RMANOVA) for variables tested. Tukey post hoc tests and single degree of freedom contrasts were used to determine post-hoc significance. The anthropometric results were analyzed using a one way ANOVA. Statistical significance was set at $p \leq 0.05$. All data are expressed as the mean ± *SEM*.

Results

A total of 55 animals completed all aspects of this study. One animal in the WD+E group died during week two of the intervention—approximately 30 minutes following the exercise training session. Upon examination by the University veterinarian, it was noted that the animal's abdomen was filled with blood, with a suspected aneurism or tear in liver. All other animals successfully completed the intervention.

Results from treadmill run time for all remaining animals are presented in Table 1. It should be noted that the post intervention wire hang and slant board tests were performed but data were highly inconsistent, with all animals performing very poorly as compared to pre intervention testing (e.g., 3-5 second hang-time for wire hang, compared to 60 second hang time at pre intervention). Therefore, data are not shown for these two tests.

Results for treadmill run time to exhaustion displayed a group effect ((WD+E & DF+E) > (W & DF)) ($p < 0.0001$) and time effect (Post > Pre) ($p = 0.0005$); as well as a group x time interaction effect ($p < 0.0001$). The treadmill run time to exhaustion was increased in both exercise groups, demonstrated by a change in percent improvement from pre to post intervention in the WD+E (51% ↑) and the DF+E (99% ↑) groups, with a greater increase noted in the DF+E group ($p = 0.02$) when using a single degree of freedom contrast.

Several differences were noted for anthropometric measurements. For example, body weight displayed a group effect (WD > all other groups) ($p < 0.0001$) and a time effect: pre to post (WD+E 177%↑, WD 205%↑, DF+E 148%↑, DF 168%↑) ($p < 0.0001$); as well as a group x time interaction effect ($p < 0.0001$). Body weight gain was greater in

WD vs. all other groups ($p < 0.05$). There was no significant difference between DF groups and WD+E for body weight gain ($p > 0.05$).

A group effect was noted for mean fat mass ($p < 0.0001$), with DF groups lower than WD groups; WD+E was lower than WD ($p < 0.05$). A group effect was noted for body fat percentage ($p < 0.0001$), with DF groups lower than WD groups; WD+E was lower than WD ($p < 0.05$). There was no significant difference in lean mass between groups ($p = 0.14$). Anthropometric data for all animals are presented in Table 2.

Discussion

This was the first study to our knowledge which investigated the physical performance aspects related to the Daniel Fast diet, with and without exercise training. The main findings from this study are as follows: 1) treadmill run time to exhaustion increased in both exercise groups, with a greater increase in the DF+E group compared to the WD+E, 2) the wire hang and slant board performance tests decreased from pre to post intervention in all groups at a similar rate and should not be used in future similar studies involving rats across a similar span of growth, 3) body weight gain was greater in WD vs. all other groups; no difference was noted between DF groups and WD+E, and 4) body composition analysis demonstrated a lower fat mass and percent body fat in DF groups vs. WD groups, with no difference in lean mass.

Several studies have previously reported that exercise training will increase endurance performance time (Dolinsky et al., 2012; Helge et al., 1998; Huang et al., 2008; Lee et al., 2001; Mazzeo & Horvath, 1986). It was our initial hypothesis that the exercise training groups would perform better than the sedentary groups, and that the DF+E group would perform better than the WD+E group. Our results support this hypothesis and are in agreement with other studies indicating that a diet high in fat would result in a lesser degree of performance gain (Helge, Richter, & Kiens, 1996; Murray et al., 2009).

Some studies have noted that dietary intake does not play a role in endurance performance (Helge et al., 1998; Helge, Wulff, & Kiens, 1998). However, differences in study design between these studies and the present work could contribute to the discrepancies in findings. For example, the length of the diet intervention could influence outcomes (e.g., 4 weeks compared to the 12 week intervention in the present study). Other studies have shown that a high fat diet produced favorable results in physical performance as compared to a high carbohydrate diet, opposing our findings (Lee et al., 2001; Miller, Bryce, & Conlee, 1984). For instance, in the study done by Lee et al. 2001, investigators used a diet that consisted either of high fat (78.1% Fat; 21.9% Protein; 0% Carbohydrate) or high carbohydrate (16% Fat; 20% Protein; 64% Carbohydrate), with greater performance noted in the high fat group. The animals trained at four days per week at either a low intensity (8 m/min for 125 minutes) or maximal voluntary running speed (28 m/min for 36 minutes). In our study, all animals in the exercise groups ran at 25 m/min for 35 minutes, which is similar to the maximal voluntary running speed group that had showed an increase in performance on the high fat diet in the work of Lee and coworkers. Also of note, animals in the Lee et al. study were assessed at two training speeds (16 m/min and 28m/min) for the entire test duration but with an increase of incline every 30 minutes of five degrees. Our animals were exposed to increasing speed but a zero percent grade. Differences in the testing protocol, as well as in the type of each macronutrient, may have been responsible for discrepancy in findings.

It is our thought that the WD diet may impair endurance performance because of the combination of high fat content and a higher proportion of saturated fat, which can lead to increased body mass (adipose tissue in particular). This extra mass has been shown to result in an increase in oxygen consumption and perceived exertion during exercise (Ekkekakis & Lind, 2006; Norman et al., 2005). High fat diets have also been implicated in impaired glucose metabolism within the skeletal muscle (Tanaka et al., 2007), which is important for muscle oxidation during exercise (Wahren, Felig, Ahlborg, & Jorfeldt, 1971). When examining dietary profiles, it is noted that diets with high levels of saturated and monounsaturated fat, along with lower levels of polyunsaturated fatty acids, yield poorer performance in both animal models and humans (Murray et al., 2009; Rowlands & Hopkins, 2002). The WD used in the present investigation was indeed comprised of high levels of saturated and monounsaturated fat, and lower polyunsaturated fatty acids. On the other hand, the DF diet was comprised of low levels of saturated and monounsaturated fat and high levels of polyunsaturated fat.

Animals in the DF groups demonstrated better run times than animals in the WD groups, possibly due to differences in fat amount and form, as well as carbohydrate amount and form. Related to the latter, diets inclusive of high carbohydrate intake have exhibited increased time to exhaustion (Chryssanthopoulos et al., 2002; Jacobs & Sherman, 1999). The increased availability of carbohydrate delays complete oxidation of muscle glycogen, resulting in prolonged exercise bouts (Coyle, Coggan, Hemmert, & Ivy, 1986; Foskett et al., 2008). When focusing on the type of carbohydrate, it can be observed that DF diet contains a majority of complex carbohydrates of low glycemic index. This type of carbohydrate has been reported to enhance physical performance when ingested prior to exercise (DeMARCO, Sucher, Cisar, & Butterfield, 1999; Thomas DE, Brotherhood JR, Brand JC, 1991). Thomas et al. 1991 demonstrated that subjects ingesting a carbohydrate meal of low glycemic index increased endurance time significantly compare to the carbohydrate meal of high glycemic index. Similarly, Demarco et al. 1999 noted that subjects fed a meal of low glycemic index foods significantly outperformed the subjects fed a meal of moderately high glycemic index foods. Indeed, both the quantity and quality of the macronutrients may influence physical performance outcomes.

While data for the treadmill performance testing are "clean" and capable of being replicated, the same is not true for the other two performance tests. Our results in wire hang time were drastically lower compared to the test results in the study done by Shukitt-Hale et al. 1998. This may be due to both the age and size of the animals (e.g., our animals were initially tested when very young and light; post intervention animal weight was nearly 4-5 fold higher than pre intervention weight). It is also possible that differences could be attributed to the animal strain (Biesiadecki, Brand, Metting, Koch, & Britton, 1998; Shukitt-Hale et al., 1998).

Regarding the slant board test, data were much closer to pre intervention testing but a decrease was still noted. Murphy et al.1995 that showed an increase in degree of angle with age, until around six months of age, which is double the age at which we concluded testing (Murphy et al., 1995). As stated above for the wire hang test, it is possible that the rapid weight gain of animals could be responsible for the lack of improvement in slant board performance. Moreover, the lack of specific "slant board" training could be a factor. That said, it should be noted that familiarization sessions for both the wire hang and slant board tests were conducted before both the pre- and post intervention testing sessions. Based on our experience, these two tests should not be used in future studies with similar training protocols, with similar time frames of testing in terms of animal age and weight, due to the poor test results.

The body weight gain was the same across all groups, with the exception of the sedentary WD group—which was greater than all others. The difference in weight gain in the WD sedentary group can be accounted for by a lack of physical activity (Ekelund, Brage, Besson, Sharp, & Wareham, 2008), in addition to the high percentage of fat in the diet (Milagro, Campión, & Martínez, 2006).

Considering body weight, one must account for body composition as well. Our data shows that the WD groups gained more body fat as compared to the DF groups. Research has shown that the DF diet has the ability to lower body mass, with some impact on body fat content (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012). The lower body fat may be somewhat responsible for the noted longer run times to exhaustion in the DF+E group. Research has shown that lower body fat values correlates with faster running speeds (Coleman & Lasky, 1992; Silvestre, West, Maresh, & Kraemer, 2006), which can relate to the animals being able to keep up with the higher speeds in the later part of the endurance test. Lower body fat values have been correlated to improved anaerobic power (Kim, Cho, Jung, & Yoon, 2011). It has been demonstrated that a body with less fat mass and more lean mass will require less energy expenditure and be capable of performing at a higher level (Stöggl, Enqvist, Müller, & Holmberg, 2010).

Conclusion

To our knowledge, this is the first study to assess the physical performance aspects related to the Daniel Fast diet with and without exercise training. Our results indicate the DF diet favorably impacts exercise performance, as compared to a WD, when coupled with regular exercise training. The increased performance may be attributed to the macronutrient mix (low glycemic carbohydrate, high polyunsaturated fat), coupled with the drastically lower body fat levels, as compared to the WD animals. Based on our results, further investigation of the effects of a DF diet on physical performance using human subjects is warranted.

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Tables

Table 1. Specific nutrient breakdown

Nutrient	Western Diet		Daniel Fast	
	gm%	kcal%	gm%	kcal%
Protein	20	17	15	15
Carbohydrate	50	43	58	59
Fat	21	40	11	25
Fiber	5	0	13	1
Total		100		100
kcal/gm	4.7		3.9	
Casein	195	780	0	0
Soy Protein	0	0	170	680
DL-Methionine	3	12	3	12
Corn Starch	50	200	0	0
Corn Starch-Hi Maize 260 (70 % Amylose and 30% Amylopectin)	0	0	533.5	2134
Maltodextrin 10	100	400	150	600
Sucrose	341	1364	0	0
Cellulose, BW200	50	0	100	0
Inulin	0	0	50	50
Milk Fat, Anhydrous	200	1800	0	0
Corn Oil	10	90	0	0
Flaxseed Oil	0	0	130	1170
Ethoxyquin	0.04	0	0.04	0

Mineral Mix S1001	35	0	35	0
Calcium Carbonate	4	0	4	0
Vitamin Mix V1001	10	40	10	40
Choline Carbonate	2	0	2	0
Ascorbic Acid Phosphate, 33% active	0	0	.41	0
Cholesterol	1.5	0	0	0
Total	**1001.54**	**4686**	**1187.95**	**4686**
Saturated g/kg	122.6		7.8	
Monunsaturated g/kg	60.2		19.7	
Polyunsaturated g/kg	13.5		77.7	
Cholesterol mg/kg	2048		0	
Saturated % Fat	62.4		7.4	
Monunsaturated %Fat	30.7		18.7	
Polyunsaturated %Fat	6.9		73.9	
Ascorbic Acid mg/kg	0		114	

Table 2. Treadmill run time (min) to exhaustion of male rats assigned to two different diets with and without exercise

	Western Diet + Exercise	Western Diet	Daniel Fast Diet + Exercise	Daniel Fast Diet
Pre Intervention	35.5±3.5	28.72±3.3	29.31±2.8	33.57±4.1
Post Intervention	48.3±1.9	24.39±1.5	52.88±1.9	28.84±1.1

Values are *mean±SEM*.
A group effect noted for treadmill run time to exhaustion ($p < 0.0001$).
A time effect noted for treadmill run time to exhaustion ($p = 0.0005$).
A group by time interaction effect noted for treadmill run time to exhaustion ($p < 0.0001$).

Table 3. Anthropometric data of male rats assigned to two different diets with and without exercise

	Western Diet + Exercise	Western Diet	Daniel Fast Diet + Exercise	Daniel Fast Diet
Body Mass (g) *Pre Intervention*	186.5±3.3	187.0±4.5	192.6±2.7	185±4.8
Body Mass (g) *Post Intervention*	516.8±10.7	571.1±14.7	478.7±11.3	496.8±13.5
Fat Mass (g) *Post Intervention*	161.6±8.0	195.5±8.4	100.73±7.4	124.45±9.8
Lean Mass (g) *Post Intervention*	366.0±9.2	386.8±6.7	391.4±8.8	376.5±7.8
% Fat *Post Intervention*	30.6±1.3	33.5±1.0	20.3±1.3	24.6±1.4

Values are *mean±SEM*.
A group effect noted for body mass ($p < 0.0001$).
A time effect noted for body mass ($p < 0.0001$).
A group by time interaction effect noted for body mass ($p < 0.0001$).
A group effect noted for fat mass ($p < 0.0001$).
A group effect noted for % fat ($p < 0.0001$).
No other statistically significant effects noted ($p > 0.05$).

Extended Literature Review

Introduction

It is well described that regular exercise training has the ability to improve physical performance (Burgomaster et al., 2005; McClenton et al., 2008), with adaptations generally being much greater for novice trainees (Burgomaster et al., 2005; McClenton et al., 2008). Specifically, exercise has been reported to affect both strength and endurance of the muscular system, as well as develop endurance specific to the cardiovascular system. It is known that a relationship exist between training volume and intensity, in such a way that higher volumes and intensities generally result in greater improvements in performance variables (up to a point, after which time there may be diminishing returns).

While muscle strength is noted to increase most by engaging in low volume and high intensity loading, muscular endurance is noted to increase most by longer duration exercise bouts with repetitive loading at low to moderate intensity (Campos et al., 2002). Endurance training (such as running) can produce adaptations such as mitochondrial changes and biochemical changes in the skeletal muscle in as little as one week following the onset of training (Green et al., 1992), with the major adaptation related to skeletal muscle substrate metabolism (Gibala et al., 2006; Holloszy & Coyle, 1984). This adaptation is attributed to improved respiratory control sensitivity from increase mitochondrial density (Gibala et al., 2006; Holloszy & Coyle, 1984). These adaptations can improve exercise capacity or the ability to sustain a submaximal workload for a longer duration (Gibala et al., 2006; Holloszy & Coyle, 1984).

Dietary intake also plays a major role in influencing physical performance, with both macronutrients and micronutrients having an impact (Maughan et al., 1997b). Multiple forms of dietary restriction have been studied, including dietary restriction (i.e., eliminating one or more nutrients from the diet). Specific forms of dietary restrictions have been reported to enhance health in both animals and humans; however we are unaware of studies seeking to determine the influence of dietary restriction on physical performance.

Common forms of dietary restriction include protein restriction, single amino acid restriction (e.g., methionine), and animal product restriction (e.g., vegan dietary plans). An example of the vegan dietary plan is the Daniel fast (DF), which is a popular fast practiced by Christians and derived from the Biblical story of Daniel (1:8-14 NIV). In this text the prophet Daniel resolved not to defile himself with the royal food and wine and requested permission to consume nothing but vegetables (pulse) and water for 10 days. Later in the same book (Daniel 10:2-3 NIV), Daniel again followed a 21 day period of fasting, during which time he ate no choice food (meat or wine). "Based on these two passages, a modern day DF involves ad libitum intake of specific foods, but the food choices are restricted to fruits, vegetables, whole grains, legumes, nuts, seeds, and oil." (Trepanowski & Bloomer, 2010).

Quite simply, the DF is a dietary program in which certain foods are restricted but the amount of food is not. This is in contrast to caloric restriction, where calories are typically reduced by 20-40% of daily requirements (Trepanowski et al., 2011). With both dietary and caloric restriction plans, life span may be extended, possibly by attenuation in oxidative damage (Sohal, 2002). Moreover, improved cardiovascular health due to reduced levels of triglycerides, phospholipid, blood pressure, heart rate, LDL-cholesterol and total cholesterol have been linked to caloric restriction (Koubova & Guarente, 2003; Sohal & Weindruch, 1996; Trepanowski et al., 2011).

In the United States most individuals consume a Western diet consisting of mostly processed/packaged foods, often high in simple sugars, saturated fats and cholesterol. The incident rate of obesity and overweight status has been on the rise in recent years and may be explained largely by adherence to a poor diet and a lack of physical exercise (Cordain et al., 2005). Excess body fat can be detrimental to physical performance (Cureton & Sparling, 1980; Deforche et al., 2003) for a variety of reasons, including but not limited to the need to move excess "non-functional" weight, increased shortness of breath, and low back pain (Hedley et al., 2004). Following a dietary model such as the DF could aid people from reaching levels of obesity or overweight status due to the low-calorie and nutrient dense nature of the foods consumed (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012).

Effects of Dietary Manipulations of Macronutrients on Physical Performance

It is well accepted that dietary intake plays a major role in influencing physical performance. The manipulations of macronutrients have displayed effects on performance. For example, diets with a high contribution of carbohydrate and a low contribution of saturated fat have been reported to favorably impact performance. Specifically, high carbohydrate and low fat meals may aid endurance capacity (Chryssanthopoulos et al., 2002), as high carbohydrate diets can increase muscle glycogen stores leading to enhanced endurance performance (Jacobs & Sherman, 1999). Moreover, diets high in fat content have been reported to decrease power output and endurance performance (Fleming et al., 2003a; Jacobs & Sherman, 1999). In addition to endurance performance, research has shown high intensity and short duration exercise bouts can be improved by consuming a diet high in carbohydrates and moderate or low in fat, similar to what is observed when following a DF regimen (Foskett et al., 2008; Maughan et al., 1997a).

Acute (three to four days prior to exercise) consumption of a diet rich in carbohydrates has been shown to increase exercise capacity during high intensity exercise, and to enhance endurance performance for a brief duration (Erlenbusch, Haub, Munoz, MacConnie, & Stillwell, 2005; Maughan et al., 1997b; McCleave et al., 2011). Conversely, low carbohydrate and moderate protein consumption has yielded increased time to exhaustion but reduced endurance capacity for a brief duration (Erlenbusch et al., 2005; Maughan et al., 1997b; McCleave et al., 2011). A two-day restriction of carbohydrates has exhibited reduced muscle glycogen, which in turn resulted in reduced iso-inertial strength (LEVERITT & Abernethy, 1999). Intake of whey protein prior to strength training has demonstrated improvements in training adaptations and has had varying effects on body composition (Kerksick et al., 2006). When examining high fat feedings with moderate protein intake, peak power during Wingate testing was reported to decrease (Fleming et al., 2003b). It is believed that high fat feedings result in decreased glycogen levels, mild acidosis and decreased buffering capacity, which result in decreased power output. High fat feeding has revealed a depletion of glycogen stores forcing the increase of the fat oxidation process which resulted in small decreases in peak power and endurance (Fleming et al., 2003b; Lambert et al., 2001). This type of diet also exhibited reduced phosphorylation potential which shortens exercise capacity. Several weeks of high fat feedings prior to an event, coupled with a carbohydrate loading period immediately prior to the event, improves cycle time trial performance (Fleming et al., 2003b; Lambert et al., 2001).

Manipulations of macronutrients post exercise have also displayed influences on recovery and performance. When ingesting a carbohydrate plus protein supplement post exercise, it has shown an increased rate of muscle glycogen replenishment compared with a carbohydrate alone resulting in faster recovery time (Ivy et al., 2002)—which may possibly influence subsequent performance bouts. Related to this topic, consuming a carbohydrate plus protein (Chocolate milk) post-exercise is more effective at improving subsequent exercises than carbohydrates only (Ferguson-Stegall et al., 2011). Ingesting carbohydrate with protein has displayed beneficial effects for health, hydration, and muscle soreness, which in turn could lead to improved performance during subsequent exercise bouts (Beelen, Burke, Gibaia, & Van Loon, Luc J. C., 2010; Flakoll, Judy, Flinn, Carr, & Flinn, 2004; Luden, Saunders, & Todd, 2007).

In addition to the actual percentage of macronutrients contained within the diet, the manipulation of nutrient type can influence exercise performance. Specifically, the consumption of lower glycemic foods prior to performance has been noted to aid endurance exercise (Siu & Wong, 2004). Additionally, the inclusion of unsaturated fatty acids such as those in flax seed has been shown to aid performance by being energy dense and easily digested for quick energy use (Sabaté, 2003).

Effects of Dietary Manipulations of Micronutrients on Health and Physical Performance

Micronutrients are necessary components found in foods that can have a profound impact on health and physical performance. Ingestion of micronutrients known to have antioxidant potential helps to decrease the incidence of oxidative damage and viral infection (Bloomer, 2008; Evans & Halliwell, 2001). For example, children with adequate ingestion of micronutrients have exhibited optimal function of the immune system, physical growth, and neuromotor development (Singh, 2004). In the typical American diet, half of the population fails to meet the recommended daily allowance for several micronutrients (Cordain et al., 2005), possibly due to the increased reliance on processed and packaged foods which typically do not contain an abundance of micronutrients.

Over time, negative health implications and vitamin deficiency diseases can emerge if micronutrient intake is marginal. Some more notable findings from this lack of micronutrient intake are low serum concentrations of vitamins, carotenoids and HDL cholesterol (Cordain et al., 2005; Kant, 2000). Athletes with mineral deficiencies have shown impaired physical performance (Lukaski, 2004), possibly due to a loss of micronutrients from sweat, urine and feces (Fogelholm, 1999; Lukaski, 1995; Lukaski, 2000; Maughan, 1999).

Specific Dietary Restrictions (Protein, Methionine)

Dietary restriction of protein may produce beneficial effects with regard to human health. For example, protein restriction in humans suffering from renal insufficiency has displayed a slower rate of decline in renal function (Klahr et al., 1994). Protein restriction appears to lead to a decrease in mitochondrial reactive oxygen species (ROS) production, which can decrease oxidative damage to mitochondrial DNA and nuclear DNA in the liver (Oliveira et al., 2004; Sanz, Caro, & Barja, 2004).

Specific amino acid restriction has also been studied. Methionine is the most studied amino acid in relation to dietary restriction. Eliminating methionine from the diet of obese adults with metabolic syndrome has resulted in decreased body weight and adiposity, in addition to improved insulin sensitivity (Plaisance et al., 2011). Results from rat studies with methionine restriction have shown increased maximum life span, inhibited growth hormone leading to actual delay in aging process, decreased mitochondrial ROS generation, and decreased oxidative damage to mitochondrial DNA and proteins (Richie JP Jr FAU - Leutzinger,,Y. et al., 0125; Sanz et al., 2006).

Overall Dietary Restrictions (Vegan Diet, Daniel Fast)

A vegan diet is known as an overall dietary restriction approach, in which individuals consume vegetables, fruits, grains, and legumes, with the avoidance of all animal products. Vegan dietary intake has been noted in the range of 15% protein, 60-75% carbohydrate, and 10-25% fat. Such a dietary pattern, in particular when attention is paid to the quality of the food choices, can result in significant weight loss, improvement in glucose tolerance, insulin sensitivity, and reductions in plasma lipid concentrations (Barnard et al., 2006; Barnard, Scialli, Turner-McGrievy, Lanou, & Glass, 2005; Barone et al., 1991; Fontana, Klein, & Holloszy, 2006; Jenkins et al., 2003). Favorable lipid levels are observed when the diet is low in saturated fat and cholesterol, while high in dietary fiber (Barnard et al., 2006; Barnard et al., 2005; Barone et al., 1991; Fontana et al., 2006; Jenkins et al., 2003). Rheumatoid arthritis patients following a vegan diet have been noted to exhibit decreased C-reactive protein levels and significant decreases in IgG anti-gliadin and IgG anti-β-lactoglobulin levels (Craig, 2009; Hafström et al., 2001; Sabaté, 2003).

The Daniel fast is a form of vegan diet that is quite stringent. That is, the foods consumed are restricted to fruits, vegetables, whole grains, nuts, seeds, and oils, with the complete avoidance of preservatives, additives, sweeteners, flavorings, caffeine, and alcohol (Dwyer, 1988; Key, Appleby, & Rosell, 2006; Trepanowski & Bloomer, 2010). Specific to lab findings, significant reductions have been noted in systolic and diastolic blood pressure, total and LDL-cholesterol, LDL-HDL ratio, blood oxidative stress biomarkers, blood glucose, blood insulin, HOMA-IR, and C- reactive protein (Bloomer et al., 2010; Bloomer et al., 2011; Trepanowski et al., 2012). The dietary plan has also been shown to increase blood nitrate/nitrite (a measure of nitric oxide; which may have implications for physical performance) and antioxidant capacity. These findings are specific to human subjects, with no data currently available related to physical performance. That said, certain outcomes such as increased blood nitrate/nitrite and decreased oxidative stress may have implications for enhanced performance, as has been suggested in the extant literature.

Conclusion

Both regular exercise training and dietary intake can favorably influence exercise performance. The Daniel Fast is a form of vegan fasting that has demonstrated effects on human health. It is possible that such a dietary regimen may favorably influence exercise performance when followed chronically—possibly due to the high concentration of low glycemic carbohydrate and essential fatty acids, as compared to the Western diet which consists largely of saturated fat, simple sugar, and processed nutrients. Comparing the two dietary approaches may provide more information pertaining to the role of nutritional intake on physical performance. If results are promising using animals, future study may be conducted using human subjects in a larger-scale controlled study to test the impact of the DF diet regimen on human physical performance.

THE UNIVERSITY OF MEMPHIS

IACUC PROTOCOL ACTION FORM

To:	Rick Bloomer
From	Institutional Animal Care and Use Committee
Subject	Animal Research Protocol
Date	9-25-13

The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No. Dietary modification in rats (0734)

☒ Your proposal is approved for the following period:

From: September 25, 2013 To: September 24, 2016

☐ Your protocol is not approved for the following reasons (see attached memo).

☐ Your protocol is renewed without changes for the following period:

From: To:

☐ Your protocol is renewed with the changes described in your IACUC Animal Research Protocol Revision Memorandum dated ____ for the following period:

From: ____ To: ____

☐ Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Revision Memorandum dated ____.

Prof. Guy Mittleman, Chair of the IACUC

Dr. Karyl Buddington, University Veterinarian
And Director of the Animal Care Facilities