AN ABSTRACT FOR THE THESIS OF

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Daniel P. Williams

The presence of an increased accumulation of intraabdominal fat (IAF) has been linked to dyslipidemia, hyperinsulinemia, and hyperglycemia, which precede the development of type 2 diabetes and coronary artery disease (CAD). It has been shown that IAF begins depostition during childhood. Human studies suggest that regular endurance exercise, that does not necessessarily produce an increase in aerobic capacity, can effectively reduce IAF accumulation during these early years. In contrast to human research, studies using animal models of human disease typically employ extremely large volumes of exercise with the intent to maximize aerobic capacity. The present study examined whether half the amount of endurance training, that was previously reported to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle in rats, would reduce the growth-related accumulation of IAF in growing female rats. Twenty-two 4-week-old female Sprague-Dawley rats were randomly assigned to a running experimental or a non-running control group. The runners exercised on a treadmill

5 days/week for 60 min/day at a speed of 27m/min and up a 15% grade for 10 weeks. After 10 weeks, the parametrial, omental and mesenteric IAF depots and the heart were excised and weighed. Compared with non-runners, the runners had a significantly lower mean parametrial fat mass (2.22 g vs. 3.13 g, $p = 0.05$) and a higher mean heart weight (0.97 g vs. 0.90 g, $p = 0.05$) at the end of 10 weeks. In addition, the lower mean parametrial fat mass in the runners vs. the non-runners (2.19 g vs. 3.19 g, $p = 0.02$) remained significant even after adjusting for the greater heart weights of the runners. One-half the amount of exercise, that was previously reported to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle in rats, yielded an 8% greater heart weight and a 29% lower parametrial IAF mass, on average, in growing female rats. In addition, the effects of treadmill running on reducing parametrial fat accumulation were independent of the effects of running on increasing heart weight. Thus, future studies examining the effects of exercise on IAF and other health-related metabolic outcomes in rats may consider using lower-dose endurance training protocols that are not designed to maximize improvements in aerobic capacity.

Reduced Intraabdominal Fat after Lower-Dose Treadmill Training in Growing Female Rats

by

Lynne Catherine David

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APPROVED:

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Major Professor, representing Human Performance

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Chair of Department of Exercise and Sport Science

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Dean of Graduate School

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Lynne Catherine David, Author

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CONTRIBUTION OF AUTHORS

Dr. Daniel Williams contributed with the overall study design, analysis of the data, and writing of the manuscript. Dr. Jeffrey Widrick and Dr. Philip Brownell advised with rat procedures and surgical technique. Dr. Barbara Smith advised with animal care and equipment.

TABLE OF CONTENTS

LIST OF FIGURES

REDUCED INTRAABDOMINAL FAT AFTER LOWER-DOSE TREADMILL TRAINING IN GROWING FEMALE RATS

CHAPTER 1: INTRODUCTION

Childhood obesity is on the rise with 20-30% of today's youth considered overweight or obese.[1,2,3] The incidence of obesity-related childhood diseases, especially type 2 diabetes, is also increasing.[2,3] Although childhood obesity itself is problematic, the primary cause for concern is the tracking of childhood obesity into adolescence and adulthood.[4,5,6] Obese adults, who were overweight as children, tend to have increased rates of diabetes, coronary artery disease (CAD), atherosclerosis, hip fracture and gout,[5,7,8] resulting from their long-term obesity as compared to obese adults who were not overweight as children.[5] Preventing or successfully treating childhood obesity may reduce the incidence and severity of adult obesity and its numerous pathological, metabolic complications.

Central obesity, or more specifically, a greater accumulation of intraabdominal fat (IAF), has been shown to be particularly troublesome in promoting the dyslipidemia, hyperinsulinemia, and hyperglycemia which precede the development of type 2 diabetes and CAD. Although these metabolic complications frequently remain asymptomatic and undetected during childhood, an increased and unhealthy accumulation of IAF deposition has been documented in children as early as 7 and 8 years of age.[9,10,11] The accumulation of excess IAF in early life may also contribute to the premature development of type 2 diabetes and CAD in later life.

Several mechanisms have been proposed to explain the physiological link between excess IAF accumulation and the increased risks of developing type 2 diabetes and CAD.[8,9,12] The IAF has a high metabolic turnover due to its characteristic and simultaneous elevations in lipogenesis and lipolysis. Relative to fat cells in other regions of the body, the IAF cells are especially sensitive to the lipogenic actions of cortisol and the lipolytic actions of the catecholamines. For instance, the IAF cells have a particularly high density of cytoplasmic glucocorticoid receptors.[13] Cortisol, in turn, stimulates lipogenesis with regional specificity for IAF cells by increasing lipoprotein lipase (LPL) synthesis and activity.[14] In turn, LPL is tethered to the endothelial surface of the fat cell where it hydrolyzes the triglyceride core of circulating lipoproteins, thereby facilitating the cellular uptake of non-esterified fatty acids (NEFAs), which are re-esterified to triglycerides in fat cells during lipogenic fat cell storage. Moreover, the site-specific increase in lipolytic sensitivity to catecholamines in IAF cells may be explained by their increased numbers of stimulatory beta-3 adrenergic receptors and their decreased numbers of inhibitory alpha-2 adrenergic receptors. Taken together, the shift in the distribution of adrenergic receptor subtypes in IAF cells results in a greater production of the stimulatory intracellular second messenger, cyclic AMP, in response to a given amount of circulating catecholamines.[15] Greater intracellular cyclic AMP levels in IAF cells, in turn, result in greater activation of the enzyme, hormone-sensitive lipase, which activates lipolysis by hydrolyzing stored triglycerides into NEFAs and glycerol that enter the hepatic-portal circulation that drains IAF tissue.

There are a number of potential metabolic consequences to the liver and the systemic circulation that are associated with the lipolysis of IAF. For instance, an excess accumulation of IAF with a high lipolytic activity results in an over-abundant delivery of circulating NEFAs and glycerol to the liver. The over-abundance of NEFAs in the hepatic-portal circulation supplies the liver with an excess of fatty acid substrate for excess triglyceride-rich lipoprotein production, especially very low-density lipoprotein (VLDL) production. Thus, the lipolysis of excess IAF stores accounts for the abdominal obesity-related increases in circulating triglyceride levels. In addition, the increased delivery of NEFAs to the liver also interferes, by poorly understood mechanisms, with the hepatic clearance of circulating insulin levels.[16] The reduction in hepatic insulin clearance, in turn, extends the plasma half-life of insulin, thereby exacerbating the hyperinsulinemic response to abdominal obesity-related increases in insulin resistance. The increased delivery of glycerol to the liver results in an over-abundance of gluconeogenic substrate for hepatic glucose production that further exacerbates abdominal obesity-related increases in hyperglycemia. Therefore, the lipolysis of excess IAF stores contribute to the hypertriglyceridemia, hyperinsulinemia, and hyperglycemia that increase the risk for type 2 diabetes and CAD.[16]

More recently, excess IAF has been mechanistically implicated in the development of the ever-expanding, insulin resistant metabolic syndrome that underlies type 2 diabetes and CAD.[12] Today, excess fat tissue is increasingly recognized as an over-active endocrine organ, which secretes an excess of pro-inflammatory cytokines (i.e., interleukin-6) that chronically over-stimulate an acute phase hepatic response. The

chronic, obesity-related acute phase response has been advanced as a new component of the insulin resistant metabolic syndrome,[17] which amplifies vascular inflammation and increases the likelihood of blood clot formation, thereby increasing CAD risk.[18] The chronic, obesity-related acute phase response may also contribute to the gradual deterioration of pancreatic beta-cell responsiveness, which signals the transition from insulin resistance to diabetes.[18]

Normally, increases in circulating insulin levels have an inhibitory effect on fat cell lipolysis. However, IAF cells seem to be especially resistant to the antilipolytic actions of insulin. Thus, hyperinsulinemia occurs concomitantly with elevated circulating NEFA levels in individuals with excess IAF.[12] Insulin also stimulates lipogenesis, yet there is no clear explanation, at present, for why large fat cells, especially in the abdominal region, may become resistant to the antilipolytic but not to the lipogenic effects of insulin. Unfortunately, most studies, to date, have focused on the metabolic actions of insulin in skeletal muscle rather than fat tissue because skeletal muscle is the largest organ in the body for glucose disposal. Thus, there is a relative paucity of studies examining the effects of obesity treatments on insulin action in fat tissue. In fact, the narrow focus, to date, on the effects of obesity treatments, like endurance exercise, on insulin action in skeletal muscle have contributed to the development of endurance training protocols that are primarily designed to alter skeletal muscle rather than fat tissue.

Endurance training is an effective way to reduce body fatness, and some studies suggest that endurance training may even preferentially reduce IAF over other the other fat depots.[19] However, most studies using animal models of human health have used high-

dose endurance training aimed at maximizing increases in skeletal muscle aerobic capacity.[20] For many years, researchers have assumed that large gains in aerobic capacity are a prerequisite for deriving substantial health benefits from regular exercise. Therefore, at present, there is a dearth of research using smaller doses of exercise aimed, specifically, at reducing the unhealthy IAF depot. Perhaps, a smaller dose of endurance training may be capable of reducing IAF accumulation in young, growing animals. If so, researchers may be more apt to consider using smaller doses of endurance training in future studies. Thus, the long-term goal of the present study is to provide an impetus that may eventually help to better characterize the metabolic response to more realistic and lower doses of endurance training in animal models of human health. In turn, we may eventually gain more appropriate mechanistic insight into the metabolic responses to more comparable amounts of endurance activity performed by humans.

CHAPTER 2

REDUCED INTRAABDOMINAL FAT AFTER LOWER-DOSE TREADMILL TRAINING IN GROWING FEMALE RATS

Lynne C. David, Daniel P. Williams, Jeffrey J. Widrick, and Barbara L. Smith

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Abstract

Human studies suggest that the health benefits of regular endurance exercise may be attained without maximizing improvements in aerobic capacity. Thus, we examined whether half the amount of endurance training, that was previously reported to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle in rats, would reduce the growth-related accumulation of intraabdominal fat (IAF) in growing female rats. Twenty-two 4-week-old female Sprague-Dawley rats were randomly assigned to a running experimental or a non-running control group. The runners exercised on a treadmill 5 days/week for 60 min/day at a speed of 27m/min and up a 15% grade for 10 weeks. After 10 weeks, the parametrial, omental and mesenteric IAF depots and the heart were excised and weighed. Compared with non-runners, the runners had a significantly lower mean parametrial fat mass (2.22 g vs. 3.13 g, $p = 0.05$) and a higher mean heart weight (0.97 g vs. 0.90 g, $p = 0.05$) at the end of 10 weeks. In addition, the lower mean parametrial fat mass in the runners vs. the non-runners (2.19 g vs. 3.19 g, $p = 0.02$) remained significant even after adjusting for the greater heart weights of the runners. One-half the amount of exercise, that was previously reported to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle in rats, yielded an 8% greater heart weight and a 29% lower parametrial IAF mass, on average, in growing female rats. Furthermore, the effects of treadmill running on reducing parametrial fat accumulation were independent of the effects of running on increasing heart weight. Thus, future studies examining the effects of exercise on intraabdominal fat and other

health-related metabolic outcomes in rats may consider using lower-dose endurance training protocols that are not designed to maximize improvements in aerobic capacity.

Introduction

The prevalence of obesity in children and adolescents is currently on the rise with 20-30% of today's youth defined as obese.[1,2,3,21] Intraabdominal fat (IAF), which begins accumulating in early childhood,[1,9,10,11] is of particular concern. In brief, IAF is in close anatomical proximity to the liver. The lipolysis of excess IAF, in turn, has been linked to adverse increases in hepatic glucose production and hepatic very low-density lipoprotein (VLDL) production that contribute to the hyperglycemia and hypertriglyceridemia that accompany insulin resistance and frequently precede the development of type 2 diabetes.[16] Furthermore, type 2 diabetes is a stronger risk factor for coronary artery disease (CAD) in women than in men.[22] Because obesity tracks strongly from adolescence to adulthood,[4,5,6] adolescent girls with increased IAF accumulation are likely to become women with abdominal obesity who have an increased risk for developing type 2 diabetes and CAD. Thus, preventing obesity and excess intraabdominal fatness through healthy lifestyle modification during growth and development in girls may be a particularly safe and effective strategy for reducing CAD risk in adult women.

It is difficult to evaluate the effects of lifestyle modification on the accumulation of intraabdominal fat in growing children and developing adolescents. Anthropometric estimates of body fatness are practical for widespread application, but they are incapable of differentiating between subcutaneous and IAF depots.[5,11,23] Magnetic resonance imaging (MRI) and computed tomography (CT) offer accurate estimates of IAF areas. However, MRI and CT are costly imaging techniques. In addition, the repeated exposure of growing children to the ionizing radiation of CT could be harmful to their development.

By contrast to human subjects, rodent models offer the opportunity to directly assess the effects of endurance training on IAF accumulation during growth without relying on indirect anthropometric methods or costly imaging techniques.

Although endurance training reduces IAF mass in rats, most of the studies, to date, have relied on larger male animals. Two reports suggest that endurance training may also reduce IAF mass in smaller female rats.[24,25] However, the studies of young female rats used high-dose endurance training protocols that included 5 or more kilometers per day of wheel running[24] or 3 hours per day of swimming[25] to achieve significant reductions in IAF accumulation. Most endurance training protocols for animals are designed to maximize cardiac hypertrophy and skeletal muscle aerobic capacity[20] and are therefore based on fitness rather than health benefits. High-dose endurance training protocols also offer the advantage of maximizing the treatment effects of exercise while appropriately minimizing unnecessary animal sacrifices. However, human studies suggest that the health benefits of regular endurance exercise may be attained without maximizing improvements in aerobic capacity.[26,27] Thus, there is a substantial gap between the lower doses of endurance exercise recommended for improving human health[28] and the considerably higher doses of endurance exercise that are routinely used to study animal models of human health.[20,25,29,30,31,32,33,34] In fact, we are aware of no reports of the potential preventive effects of lower-dose endurance training on the accumulation of IAF in growing female rats.

The primary aim of the present study was to determine whether half the amount of endurance training, that was previously reported to induce cardiac hypertrophy and

approximately double the aerobic capacity of skeletal muscle in rats,[20] would reduce the growth-related accumulation of IAF in immature female rats. The secondary aim was to determine whether any endurance training-related reductions in IAF accumulation would be independent of any endurance training-related increases in heart weight. For the secondary aim, heart weight was used as a marker of one cardiovascular component of aerobic capacity.

Methods

Animals.

Female Sprague-Dawley rats, approximately 4 weeks old and weighing 140-160g were housed individually in wire-bottom cages where temperature (21°C ± 2°C) and lighting (12:12 hour light/dark cycle: light cycle 3:00 - 15:00) were controlled. Chow and tap water were provided ad libitum. Food intake and body weight were monitored weekly. Twenty-two rats were randomly assigned to a treadmill running experimental group or a non-running control group. Based on our preliminary experiments with treadmill running, we anticipated some attrition in our treadmill running group due to the potential for injuries to occur during training. Thus, we used a modification of the Efron procedure[35] to force a balanced experiment in anticipation of expected attrition the treadmill running group without introducing selection bias. As a result, 59% (n=13) of the animals were assigned to treadmill running, whereas 41% (n=9) of the animals were assigned to a non-running control. Our training protocol actually proved to be more efficacious than expected, as none of the 13 runners sustained an injury that limited its ability to run. The study protocol was reviewed and approved by the University Institutional Animal Care and Use Committee.

Exercise Intervention.

Treadmill running was selected over in-cage wheel running because it provides a more quantifiable and reproducible exercise session duration and intensity. The advantage of in-cage wheel running is its voluntary nature, which likely avoids the psychological

stress associated with motorized treadmill running. However, the main disadvantage of in-cage wheel running is the inability to control for the distance or intensity of running. Because we were interested in quantifying the effects of a specific dose of endurance training on IAF growth and development, we selected treadmill running. The present endurance training program was modified from a treadmill protocol that induced cardiac hypertrophy and approximately doubled the aerobic capacity (i.e., citrate synthase activity) of skeletal muscle in rats.[20] We cut the original dose of endurance training[20] in half by replicating the training frequency (5 days/week) and intensity (27 m/min up a 15% grade), while cutting the daily exercise duration in half (from 2h/day to 1 h/day) for 10 weeks. After quarantine and 1-day acclimation to new housing arrangements, rats assigned to running began training on a Seanhope Scientific SAT 2000 treadmill. Training occurred between 15:00 and 23:00 hours during the animals' more active dark cycle. The endurance training protocol was gradually phased-in beginning with 2 weeks of acclimation to treadmill running.[31,36] By the end of the second week of training, the rats were running the complete endurance protocol described above, which was maintained for 10 complete weeks. To ensure that no differences in study outcomes could be ascribed to the extra handling of the treadmill running rats, study technicians handled each non-running control rat for 5 min/day for 5 days/week at the same time of night that the treadmill running rats were training.[37]

Tissue Excision and Weighing.

At the conclusion of the 10-week training protocol, all rats were sacrificed by carbon dioxide inhalation. After sacrifice, every rat was dissected, so that the IAF depots and the heart could be excised and weighed. All of the fat pads attached to the wall of the abdominal cavity and viscera were excised and weighed in anatomically distinct depots. Omental fat was defined as all of the fat attached to the lesser and greater curvatures of the stomach.[38] Mesenteric fat was defined as all of the fat attached to the mesentery, including the mesentery itself.[38] Parametrial fat was defined as all of the fat attached to the uterus and bordered by the peritoneal walls, kidneys and perineum.[38] The heart was excised by sectioning the aorta artery and the vena cava veins at the attachment to the pericardial sac. The excised IAF depots and hearts were individually weighed on a calibrated analytical balance scale (American Scientific).

Statistical Analysis.

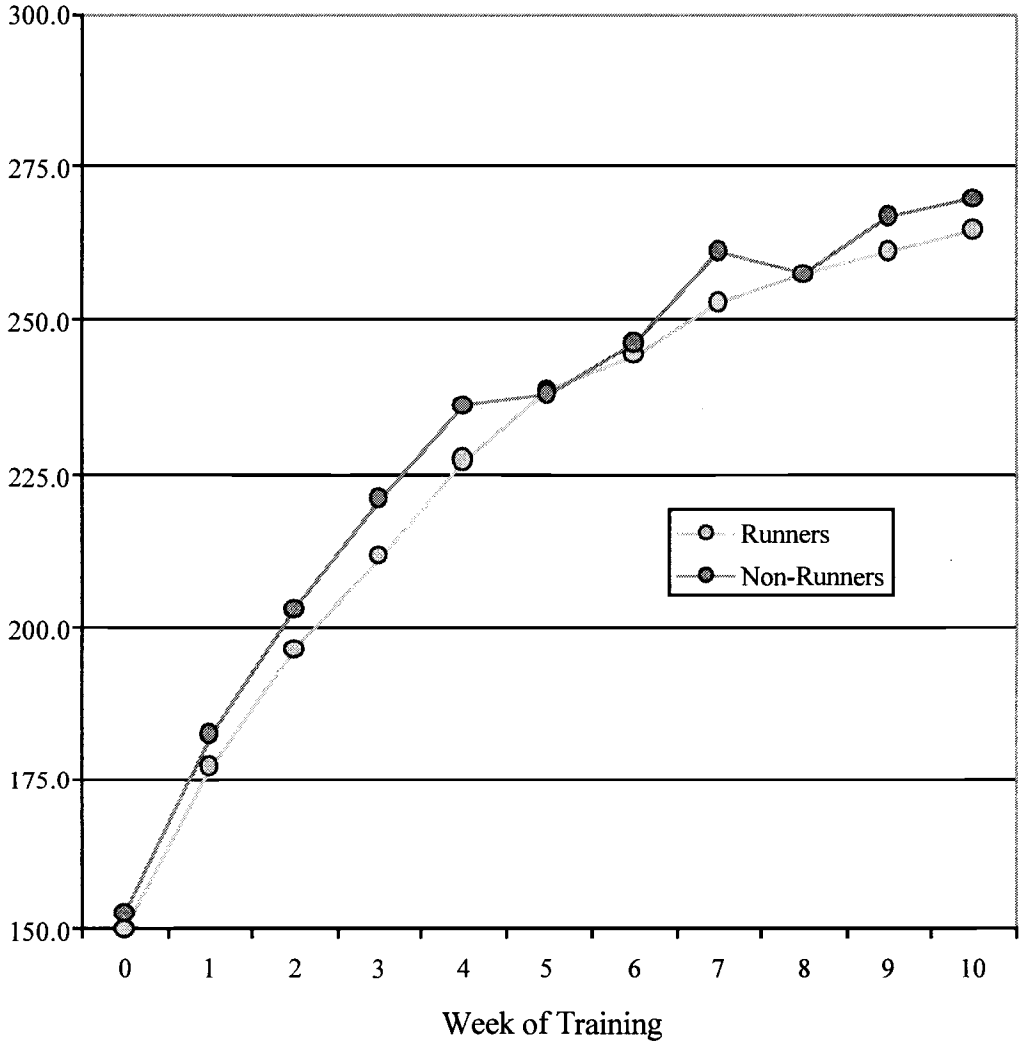
To determine whether there were significant main effects of growth and running and significant interactive effects of growth by running on body weight and food intake, a mixed factor repeated measures analysis of variance was used. To determine whether there were any mean differences in the final IAF masses or the final heart weight between the runners and the non-runners, two-tailed independent t-tests with unequal variances (to account for the unequal sample sizes) were used. To determine whether any of the running-related mean differences in IAF masses, if observed, were independent of running-related differences in heart weight, an analysis of covariance was used. Statistical

hypothesis tests with probability values at or below 0.05 were considered statistically significant. Statistical analyses were completed using SPSS (SPSS Inc.) software.

Results

The 10-week changes in body weight in running vs. non-running control animals is shown in Figure 1.

Figure 1: *Body Weight Growth of Runners and Non-runners*



There was a significant growth-related gain in mean body weight in all 22 animals from 151 g at week 1 to 267 g at week 10 ($P < 0.001$). However, there was no difference

in the gain in mean body weight over 10 weeks between the runners (+77%) and the non-runners (+76%, P = 0.799). A similar trend was observed for food intake. For instance, there was a significant increase in mean food intake in all 22 animals from 62 g/week during week 1 to 120 g/week during week 10 (P < 0.001). However, there was no difference in the increase in mean food intake over 10 weeks between the runners (+89%) and non-runners (+103%, P = 0.338).

The differences in final IAF masses and heart weights between the runners and the non-runners at the end of 10 weeks are shown in Table below.

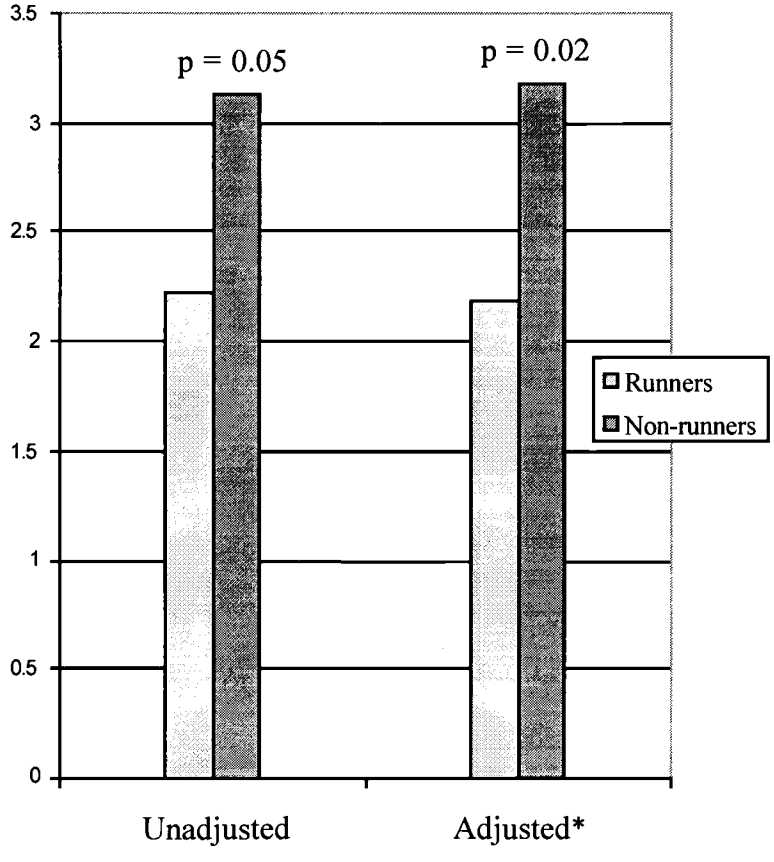
Tissue Weights of Runners and Non-runners

Tissue	Runners Mean ± SD	Non-runners Mean ± SD	p-value	% Mean Difference
Heart, g	0.97 ± 0.12	0.90 ± 0.09	0.05	8%
Parametrial Fat mass, g	2.23 ± 0.46	3.13 ± 1.16	0.05	29%
Mesenteric Fat mass, g	1.40 ± 0.41	1.51 ± 0.47	0.59	7%
Omental Fat mass, g	0.32 ± 0.04	0.35 ± 0.07	0.39	10%

The final mean heart weight was 7.8% higher and the final mean parametrial fat mass was 29.1% lower in the runners vs. the non-runners (p = 0.05). By contrast, there was no difference in the final mean mesenteric or omental fat masses between the runners and non-runners (P ≥ 0.392). Because the final mean heart weight was approximately 8% higher in runners vs. non-runners, we wanted to quantify the extent to which the 29% lower parametrial fat mass was dependent upon the effect of running on increasing heart

weight. Thus, with heart weight held constant, the adjusted mean parametrial fat mass was 31.3% lower in the runners vs. the non-runners ($p = 0.02$, see Figure 2).

Figure 2: *Parametrial Fat Mass*



* Adjusted for training-related increase in heart mass

Discussion

The present study shows that half the amount of endurance training, that was previously shown to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle in rats,[20] is capable of producing an 8% greater heart weight and a 29% lower accumulation of IAF in the parametrial depot in growing female rats. In addition, the effects of treadmill running on reducing parametrial fat accumulation were independent of the effects of running on increasing heart weight. To our knowledge, the present study is the first to report that a considerably lower dose of endurance training, relative to the most commonly used doses,[20,25,29,30,31,32,33,34] may be used to effectively reduce IAF accumulation in young, growing rats. Our finding is significant because it may help to narrow the gap between the realistic doses of exercise recommended to improve human health[28] and the considerably higher doses of exercise that are commonly used in experimental animal models of human health.[20,25,29,30,31,32,33,34] From a purely practical perspective, lower dose endurance training also reduces the animals' exposure to potentially injurious treadmill exercise, and it reduces technician time and costs.

Our finding that 10 weeks of lower dose treadmill training yielded a significant 29% lower accumulation of parametrial fat yet nonsignificant 7-9% lower accumulations of mesenteric and omental fat raises two potential explanations. One explanation is the law of the initial value.[39] In other words, the greatest potential to detect endurance training-related reductions in IAF accumulation may reside in the parametrial depot simply because it is larger than either the mesenteric or omental depots in young female rats (Table 1). We are aware of no previous studies reporting the effects of endurance

training on the relatively smaller mesenteric and omental fat depots, so it is not known whether higher doses of endurance training may be capable of reducing IAF accumulation in these smaller depots in growing female rats. Another potential explanation for the greater endurance training-related reduction in the parametrial fat depot surrounding the uterus is that exercise-related reductions in IAF accumulation may be gender-specific.[38] For instance, high-doses of in-cage wheel running[24] and swimming[25] have been reported to reduce parametrial fat accumulation in female rats, whereas high doses of in-cage wheel running,[40] swimming,[30] and treadmill running[41] have been reported to reduce epididymal fat accumulation in male rats.

The present study questions whether high-dose endurance training may be necessary for detecting significant reductions in IAF accumulation in growing animals. In fact, the lower accumulation of parametrial fat in the runners was statistically independent of their greater heart weights, which suggests that endurance training-related reductions in IAF accumulation may be dissociated, to some extent, from the cardiovascular adaptations to training. Furthermore, the present dose of 60 minutes per day of treadmill running covered a distance of 1.6 kilometers per day. By comparison, prior reports of endurance training-related reductions in parametrial fat accumulation included 5 or more kilometers per day of in-cage wheel running[24] or 3 hours per day of swimming.[25] Thus, the present dose of endurance training is considerably closer to the 40 minutes per day of exercise, which was recently reported to reduce the mean MRI-derived estimates of IAF accumulation over 4 months in trained (+0.5%) vs. untrained (+8.1%, $P = 0.02$) obese children aged 7-11 years.[23]

There are a number of study limitations and assumptions to consider. First, by confining our study to female animals, we were unable to examine potential gender differences in endurance training-related reductions in IAF accumulation. Second, by confining our study to only one dose of endurance training, we could not define the lowest possible threshold of endurance training that may be effective for reducing IAF accumulation in growing rats. Third, it is not known whether the present 29% reduction in IAF accumulation would be accompanied by potential endurance training-related improvements in insulin action in fat tissue. Finally, we assumed that endurance training-related differences in cardiac hypertrophy reflect training-related increases in oxygen delivery and aerobic capacity. Although cardiac hypertrophy is typically accompanied by increases in skeletal muscle or whole body aerobic capacity,[42,43,44] endurance training-related increases in aerobic capacity have also been reported in the absence of cardiac hypertrophy in rats.[45]

In conclusion, the present study shows that one-half the amount of endurance training, that was previously reported to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle, resulted in an 8% greater heart weight and a 29% lower accumulation of parametrial fat mass, on average, in growing female rats. Lower-dose endurance training protocols based on health-related outcomes, like IAF accumulation, may help to close the substantial gap between the lower doses of endurance activity recommended to improve human health[28] and the considerably higher doses of endurance training used to study animal models of human health.[20,25,29,30,31,32,33,34]

CHAPTER 3: CONCLUSION

The children of today are much less active than the children growing up in prior decades, and there are secular trends of an increasing prevalence of childhood and adolescent obesity and obesity-related diseases.[2,3,21] There is an emerging consensus in the adult human literature that one must engage in approximately 30 minutes per day of moderate-intensity physical activity on a nearly daily basis to improve health.[28] By contrast, animal research uses much larger doses of endurance training, typically 2 hours or more of high-intensity aerobic exercise on a nearly daily basis, with the intent of *maximizing* increases in aerobic capacity. Thus, animal models of human health greatly exaggerate the doses of endurance exercise prescribed to, and more importantly, adopted by most humans. As a result, physiological differences between mammalian species is not the only factor currently limiting the extrapolation of the potential metabolic health benefits of endurance training from animal studies to humans.

Adherence to a specific endurance training protocol can be controlled far better in animals than in humans. Moreover, IAF accumulation cannot be directly assessed in humans. Thus, animal models may be a particularly useful way to gain important insight regarding the effects of more reasonable and lower doses of endurance training on IAF accumulation during growth and development. In the present study, a smaller dose of endurance training effectively reduced IAF accumulation in growing female rats. The exercise-related reduction in IAF accumulation was also isolated to the parametrial depot surrounding the uterus. Moreover, the exercise-related reduction in IAF accumulation

was independent of the effect of exercise on inducing cardiac hypertrophy. Taken together, the main findings suggest that endurance training-related reductions in IAF accumulation may be gender-specific and may *not* require large increases in aerobic capacity.

The current study is a highly specific animal study that could be used as a "stepping-stone" for several future studies. For instance, future studies examining the effects of 2-3 different lower-dose endurance training protocols in both male and female animals would help to determine whether the present reduction in IAF accumulation is dose-dependent and gender-specific. In addition, isocaloric endurance training protocols should be compared across several different exercise intensities to determine whether more moderate intensities of exercise may be as effective or more effective than the relatively high-intensity protocol used herein. The present observation of a modest exercise-induced cardiac hypertrophy is suggestive of a cardiac adaptation to endurance training that is consistent with an increase in oxygen delivery and aerobic capacity. However, the future assessment of actual whole body aerobic capacity or skeletal muscle oxidative enzyme activity would help in determining the extent to which endurance training-related reductions in IAF accumulation may be associated with or dissociated from endurance training-related increases in aerobic capacity.

In conclusion, one-half the amount of endurance training, that was previously reported to induce cardiac hypertrophy and approximately double the aerobic capacity of skeletal muscle, resulted in an 8% greater heart weight and a 29% lower accumulation of parametrial fat mass, on average, in growing female rats. Future studies aimed at

elucidating the mechanisms whereby endurance exercise may reduce IAF accumulation should compare the insulin-mediated stimulation of lipogenesis and the insulin-mediated inhibition of lipolysis in IAF cells isolated from endurance trained vs. untrained control animals.

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APPENDICES

Appendix A

LAR-ID# _____
APPROVAL DATE _____

INSTITUTIONAL ANIMAL CARE AND USE
ANIMAL USE FORM
form must be typewritten

Investigator Daniel P. Williams Dept. Health & Human Performance Phone 737-5922 E-mail Dan.Williams@orst.edu
Co-investigator Dept. Phone E-mail

Grant or Proposal Title Effects of Regular Endurance Exercise on Visceral Abdominal Fat Accumulation and Insulin Resistance in Female Rats

Granting Agency IACUC

Proposed starting date March 1998 Proposed finish date September 1998

Animal species required (common names): Sprague-Dawley Rats

Number of animals required: First year: 63 Second year: Third year:

Is this a field study involving wild animals? ☐ Yes ☒ No If 'Yes', please go directly to section II.

I. Special Husbandry requirements: Do your animals have any special needs to be addressed by the animal care staff? ☐ Yes ☒ No If no special husbandry required, animals will be cared for according to LAR's standard operating procedures for that species.

a. Temperature: (°F): 69 F Hours light/dark: light: 3AM-3PM
b. Caging Type: wire frame Filter tops? ☐ Yes ☒ No Autoclaved? ☐ Yes ☒ No
Hanging wire? ☒ Yes ☐ No
3. Bedding Type: Not Cedar Autoclaved? ☐ Yes ☒ No
4. Type of water (tap, sterile, distilled, deionized): Tap Water
5. Diet and feeding requirements: Powered feed / measureed 2x's per week
6. Hazards LAR staff should be aware of (chemical carcinogens, radioactive materials, toxicants, etc):

none

7. Other instructions for LAR staff:

Rats weighed by LAR staff 2x's per week

h. Instructions for Care of Sick Animals:
☒ Call Investigator
☐ LAR staff to treat
☐ Euthanize by LAR staff

Instructions for Disposal of Dead Animals
☒ Call Investigator
☐ Necropsy (at PI's expense unless previously arranged with LAR)
☐ Bag and place in cooler for investigator
☐ Bag for disposal

Who will provide veterinary care for your animals (if other than LAR)?_____

II. Objectives of proposed research (Summarize in space provided).

The objective is to analyze risk factors for non-insulin dependent diabetes mellitus (NIDDM) and coronary artery disease (CAD) in sedentary and endurance-exercised rats. The specific risk factors to be assessed are mesenteric, omental and perineal fat depots and glucose, insulin and free-fatty acid blood concentrations.

1. Briefly explain the significance of this research in language that a non-scientist can understand.

The accumulation of upper abdominal fat has been highly correlated with the incidence of NIDDM and CAD. Since this fat depot is highly sensitive to physical activity levels, the differences in fat accumulation and corresponding blood constituents, after an oral glucose challenge, from sedentary to exercised rats will help to elucidate some of the mechanisms by which exercise reduces risk for NIDDM and CAD.

2. Does this proposal use:

8. Surgery:
☐ Yes ☒ No

9. Anesthesia, analgesia or tranquilizers:
☐ Yes ☒ No

10. Radioactive isotopes in the animal:
☐ Yes ☒ No

11. Infectious agents:
☐ Yes ☒ No

12. Chemical carcinogens
☐ Yes ☒ No

13. Recombinant DNA
☐ Yes ☒ No

If any of the above are answered yes, please include details (*i.e.*, detailed description of surgical procedure; medication's generic name, dose, route and frequency of administration) in the section V. titled Detailed Animal Experimental Procedures.

III. The following questions pertain only to field studies using wild animals.

1. Field Study Location_____

2. Live capture and release? ☐ Yes ☐ No Non-survival collection? ☐ Yes ☐ No

14. Method of capture __
_____
_____
_____

15. Method of euthanasia for non-survival collection_____

16. What are expected injury/mortality rates?

17. What precautions will be used to minimize injury and/or mortality?

18. In the event of injury or illness necessitating euthanasia, what method will be used?

19. What precautions will be taken to reduce capture of non-target species?

20. Where will captured animals be released? _____

3. Describe marking procedures to be used:

4. If a telemetry unit is to be attached, please describe weight of unit, location and method of attachment to the animal, and procedure(s) for removal at the conclusion of the study

IV. Justification: Federal guidelines and the Public Health Service Assurance Agreement filed by Oregon State University require a justification for all vertebrate animals used in research, testing or teaching. Please answer the following questions:

1. Why are animals used rather than an alternative method? (Video, cell culture, microbiological, assay, etc.)

Animals are necessary with this study in order to examine the complex systemic effects of the exercise intervention. A model or cell culture wouldn't be capable of simulating this type of physiological response. This study is intended to complement studies conducted with humans by examing dissectable tissues and by exacting experimental controls that are easier to accomplish in rats.

5. Indicate the sources you have used to determine that alternatives to painful procedures contained in this proposal are not available **or state that there are no painful procedures other than minor pain (such as from injections or blood sampling) in this proposal.** Include sources checked or databases used (*i.e.*, The Animal Welfare Information Center).

There are no painful procedures in this study proposal, however, there will be some slight discomfort to the rat when aspirating blood from the tail vein. This location is most commonly used in current research and does not require anesthesia.

3. Why did you choose this particular species? Could a smaller vertebrate or nonvertebrate be used?

The rat is commonly used in the literature and is the smallest species used for this type of exercise intervention. A non-vertebre would not be applicable because of the physiological differences. It's also important that current references and a means for data comparison are available. Finally, the exercise apparatus available for use with this study is designed for rats.

6. State your rationale for choosing the number of animals listed on Page 1. How did you determine that these numbers of animals are necessary to achieve your experimental objectives? Be specific. **The product of the number of animals per group and the number of groups must equal the number of animals listed on Page 1.**

The original proposal requested 32 rats plus 6 experimental rats, sufficient for detecting a statistically significant exercise effect. Due to changes in the original proposal, 25 additional rats were purchased to provide larger sample sizes and procedure practice.

5. Does this research duplicate previous research work done by you or others? If so, please justify the necessity of this project.

No.

V. Detailed Animal Experimental Procedures. Please describe in detail how each animal group will be treated. Describe each procedure (especially surgical procedures) to which any animal in any group may be subjected. Include a list of physical, chemical, or biological agents (name, dose, route, frequency) that may be administered. If the proposal covers more than one year, be specific for each year. Use additional pages if necessary.

One half of the rats will undergo motorized treadmill training, running 5 days per week for 10 weeks, 90 minutes per session. The other half of the rats will remain sedentary.

At the end of 10 weeks, all rats will be fasted overnight for 10 hours. After this time, 0.7mL of blood will be aspirated from a tail vein. A glucose solution will then be orally administered via gavage after which successive blood samples of 0.7mL each will be taken 30, 60 and 90 minutes after the glucose ingestion. Animals will then be euthanized in a CO2 chamber. Abdomens will be opened and the omental, the mesenteric, and the perineal fat will be excised and weighed. Next, the gastrocnemius and soleus muscles will be excised, weighed and frozen in liquid nitrogen for later analysis of fiber differences. Blood will be centrifuged and serum separated and frozen at -70 C for later analysis of free-fatty acid, insulin and glucose concentrations.

VI. Does this study involve survival surgery? ☐ Yes ☒ No If 'No', please skip to section VII.

21. Will surgery be performed in the laboratory or field? Please name building/room number or field site:

b. Will more than one survival surgery be performed on an animal? ☐ Yes ☐ No If 'Yes',please justify. (Survival surgical procedure pertains to any surgical procedure, including biopsies, where an animal is allowed to recover from anesthesia, regardless of the length of the survival period.)

22. Surgeon's name, experience with species and procedures to be performed:

23. Describe pre-operative preparation of the animals:

1. Food restriction (hours):
2. Water restriction (hours):

24. Sterile techniques that will be used (circle all that apply):

1. Sterile instruments
2. Sterile gloves
3. Cap and mask
4. Sterile gown
5. Sterile operating area
6. Clipping hair or plucking feathers
7. Skin preparation with betadine or similar product
8. Practices to maintain sterility of instruments during surgery

25. Will post-operative analgesics be used? ☐ Yes☐ No If 'No', please justify. If 'Yes', please provide drug name, dose, frequency and route of administration.

26. How often will animals be monitored during recovery?

VII. Will these animals be used for antibody production? ☐ Yes ☒ No

a. Polyclonal or monoclonal antibodies?

b. What type(s) of antigen will be used?

c. What adjuvant will be used for the initial injection?

d. What adjuvant will be used for the subsequent injections?

e. Injection procedures:

What route for injections?

What anatomical location and volume will be injected at each site?

How frequently will be injections be given?

f. Polyclonal antibody blood collection procedures:

Who will collect blood?

From what anatomical location?

How frequently will blood be collected?

What Volume?

g. Monoclonal antibody production:

How often will the animals be assessed for abdominal distention?

How often will they be tapped?

h. Will animals be sedated or anesthetized for blood draws or tapping?

Drug(s) used:

Dose (mg/kg) and route of adminstration:

VIII. Is death an end point in your experimental procedure? ☒ Yes ☐ No In other words, must the

animals die as a result of your procedure(s) or treatment(s)? If yes, explain why it is not possible to euthanize the animals at an earlier point in the study before the onset of any pain or suffering. If you can euthanize the animals at an earlier point, describe the clinical signs that will indicate that an animal should be euthanized.

Ten weeks of training are necessary to observe an exercise effect before rats are euthanized.

a. What will be done with any animals not euthanized at the conclusion of the project?

Any animals not euthanized at conclusion of the study will be given to a Masters committee member, Jeff Widrick, for later muscle studies.

b. Method of euthanasia. Please be specific as to agent, dose and route of administration.

A tank will be primed for 7 minutes with CO_2. After priming, the rat will be placed in the tank and will remain inside for 5 minutes or until euthanasia is accomplished

27. How will carcasses be disposed of?

Carcasses will be placed in zip-lock bags and deposited in the Animal Resource Lab outside freezer for later incineration.

Daniel P. Williams

Signature - Principal Investigator

8/29/98

Date

++

COMMITTEE USE ONLY

Approved ____________

Disapproved _________

Hold for further information __________

Date ________________

Signature ____________________________

Date ________________________________

Next Approval Date: ____________________

IACUC Action __________________________

Signature ____________________________

Appendix B

Raw Data: Tissue Weights, g

Activity	Heart	Parametrial Fat	Mesenteric Fat	Omental Fat
0	0.84	2.90	1.51	0.26
0	0.88	3.05	1.32	0.32
0	0.87	2.39	1.33	0.28
0	0.94	4.41	2.29	0.50
0	0.92	3.81	1.84	0.45
0	0.88	5.07	1.99	0.40
0	0.95	1.86	0.86	0.25
0	0.88	1.50	0.91	0.26
0	0.93	3.18	1.50	0.44
1	1.08	2.13	1.04	0.29
1	1.01	2.90	1.75	0.32
1	0.97	2.71	2.26	0.41
1	1.03	1.60	0.71	0.28
1	0.77	2.43	1.07	0.41
1	1.21	2.75	1.54	0.39
1	0.95	2.19	1.51	0.30
1	0.95	2.05	1.70	0.36
1	1.00	2.03	1.32	0.26
1	0.90	1.63	1.03	0.19
1	1.10	2.67	1.60	0.40
1	0.82	2.34	1.55	0.31
1	0.86	1.50	1.11	0.20

Appendix C

Raw Data: Weekly Body Weight Measurements, g

Activity	0	1	2	3	4	5	6	7	8	9	10
0	141	175	208	210	231	224	236	260	256	255	265
0	150	188	201	213	244	238	246	247	258	264	265
0	150	175	198	209	223	230	234	243	245	248	253
0	155	180	208	236	242	250	260	278	272	283	285
0	157	190	201	225	248	247	257	280	263	280	283
0	162	190	218	233	246	245	252	259	269	278	280
0	153	185	213	234	237	241	251	275	262	273	274
0	150	169	180	203	208	215	220	230	231	243	239
0	156	188	200	227	243	250	260	278	260	278	283
1	158	178	202	212	235	240	255	251	268	279	266
1	158	198	206	225	236	242	256	258	268	270	276
1	140	165	191	199	214	245	237	246	253	253	256
1	147	183	203	218	240	245	255	259	268	269	278
1	138	158	170	191	195	200	209	214	217	224	231
1	150	183	203	222	244	268	265	264	266	269	274
1	142	164	185	199	211	225	230	238	243	248	251
1	142	168	203	222	238	258	259	267	274	269	278
1	155	175	200	202	213	228	232	244	258	253	258
1	154	187	198	222	239	240	257	279	270	275	282
1	162	184	207	232	252	253	261	282	269	282	289
1	155	182	195	210	222	240	241	253	266	267	264
1	150	175	186	197	212	216	217	229	228	236	238

Appendix D

Raw Data: Weekly Food Consumption, g

Activity	0	1	2	3	4	5	6	7	8	9	10
0	56	140	109	116	120	109	120	136	107	100	114
0	62	126	76	121	132	87	112	113	114	115	110
0	59	144	119	n/a	124	126	120	125	112	130	113
0	62	169	127	135	96	118	137	116	106	128	122
0	58	148	85	132	123	121	143	119	122	128	136
0	60	140	113	116	120	111	124	132	118	130	125
0	64	131	115	115	108	113	127	147	90	120	123
0	58	129	99	128	117	116	124	112	108	111	109
0	63	139	101	127	131	141	152	123	111	126	133
1	58	128	96	113	122	120	120	112	142	134	109
1	64	141	106	135	133	128	77	132	127	123	122
1	101	126	125	103	132	152	118	116	119	121	118
1	59	137	97	124	175	139	167	125	121	158	127
1	51	122	92	112	101	108	111	106	112	116	98
1	63	131	71	134	143	147	122	124	133	118	118
1	56	172	107	136	122	137	132	123	134	118	129
1	75	137	121	119	138	128	133	123	131	125	127
1	50	107	105	127	121	132	163	156	124	100	119
1	n/a	124	103	146	122	132	142	122	115	132	128
1	64	132	110	139	125	132	144	106	123	131	135
1	56	130	89	122	126	132	110	125	140	103	116
1	57	110	100	144	117	125	160	117	113	110	107