5-13-2011

Metformin and/or Exercise Training Affect Metabolic Health in Men and Women with Prediabetes

Steven K. Malin
smalin@kin.umass.edu

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METFORMIN AND/OR EXERCISE TRAINING AFFECT METABOLIC HEALTH IN MEN AND WOMEN WITH PREDIABETES

A DISSERTATION PRESENTED

by

STEVEN K. MALIN

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

MAY 2011

Department of Kinesiology
METFORMIN AND/OR EXERCISE TRAINING AFFECT METABOLIC HEALTH IN MEN AND WOMEN WITH PREDIABETES

A DISSERTATION PRESENTED

by

STEVEN K. MALIN

Approved as to style and content by:

________________________________________________
Barry Braun, Chair

________________________________________________
Stuart Chipkin, Member

________________________________________________
Patty Freedson, Member

________________________________________________
Alayne Ronnenberg, Member

________________________________________________
Patty Freedson, Department Chairperson
Department of Kinesiology
DEDICATION

I dedicate this project to my wife and children. Thank you for your endless love, support, and patience throughout these last several years. You make life worthwhile and I am blessed to have you in my life.
ACKNOWLEDGEMENTS

This dissertation project would not be possible without the help of several individuals. Thanks to Dr. Patty Freedson for always reminding me that asking the most basic question can often lead to a deeper understanding. I am grateful to Dr. Alayne Ronnenberg for her support and advice on this project. Thank you Dr. Stuart Chipkin for reminding me that there is more to life than just muscle. My time in the Energy Metabolism lab would not have been the same if it were not for you.

I don’t really have the words to express how thankful I am to my mentor, Dr. Barry Braun. Despite your hectic and very stressful schedule, you always found time for me. I don’t know many individuals that would be so understanding and give me the freedom to get things done the way I saw fit. Most of all I appreciate you always being there for me when I needed you the most. I will truly miss being in the Energy Metabolism Laboratory.

Many thanks also to my incredible present and past colleagues in the Energy Metabolism Laboratory. I am truly indebted to Kirsten, Rob and Rich for being willing to do whatever was needed to help complete this project. I also thank Carrie Sharoff, Todd Hagobian and Brooke Stephens for challenging me to be a better scientist. Lastly, I thank my other friends in the Department of Kinesiology for their endless support and untiring enthusiasm during the last several years.
ABSTRACT

METFORMIN AND/OR EXERCISE TRAINING AFFECT METABOLIC HEALTH IN MEN AND WOMEN WITH PREDIABETES

MAY 2011

STEVEN K. MALIN, B.S., KING’S COLLEGE
M.S., UNIVERSITY OF DELAWARE
Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST
DIRECTED BY: PROFESSOR BARRY BRAUN

Prediabetes is defined by elevated blood glucose concentrations not high enough to meet criteria for type 2 diabetes. Exercise or metformin, a common “anti-diabetes” medication, may attenuate the progression from prediabetes to type 2 diabetes by improving insulin sensitivity and cardio-metabolic health. Because each treatment has its primary action in different tissues, combining exercise (muscle) with metformin (liver) may further enhance insulin sensitivity and cardio-metabolic health. **Purpose:** To determine the efficacy of combining exercise training with metformin on insulin sensitivity and cardio-metabolic health in men and women with prediabetes. We hypothesized that the combined treatment would improve insulin sensitivity and cardio-metabolic health more than either treatment alone.

**Methods:** Thirty-two men and women with prediabetes were placed in placebo (P), metformin (M), exercise training and placebo (EP), or exercise training and metformin (EM) groups. Pill distribution was double-blind, and the groups were well-matched for age, weight, and fitness. There were no baseline differences in any characteristic. Subjects were provided P or 2000mg/d of M for 12 weeks and EM and EP underwent a progressive training protocol. Insulin sensitivity was measured 28-30hr post-exercise with a euglycemic hyperinsulinemic clamp. Traditional cardio-metabolic measures were also collected in the
fasted state (e.g. blood pressure, blood lipids and inflammation). Group means were compared using a 2-way repeated measures analysis of variance. **Results:** Relative to baseline, all 3 interventions increased insulin sensitivity ($p < 0.05$), however, EP increased insulin sensitivity approximately 25-30% more than either EM or M. Compared to control, EP and M both lowered systolic blood pressure and C-reactive protein ($p < 0.05$, $p = 0.06$) and these reductions were approximately 15% more than EM. Each treatment raised HDL ($p < 0.05$). Enhanced insulin sensitivity was associated with increased non-oxidative glucose metabolism (i.e. glucose storage) ($r = 0.85$; $p < 0.01$). **Conclusions:** Despite more weight loss (4 kg), metformin blunted, rather than accentuated the effects of training on enhancing insulin sensitivity and lowering systolic blood pressure and inflammation. Given that metformin and physical activity are widely recommended treatments for prediabetes, it is important to better understand the mechanisms and ramifications of the combined treatment.
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CHAPTER 1
INTRODUCTION

In the United States (U.S.), approximately 24 million individuals have type 2 diabetes. The mortality and medical costs associated with type 2 diabetes make this disease an important public health concern and have prompted much research to determine effective interventions that prevent and/or delay the onset of type 2 diabetes. In 2002, the U.S. Diabetes Prevention Program (DPP) demonstrated that low-fat diet and regular physical activity, or treatment with metformin, a common anti-diabetes drug, could prevent and/or delay the progression from prediabetes to type 2 diabetes. Individuals with prediabetes are at high risk for type 2 diabetes because their blood glucose concentrations are elevated but not high enough to meet criteria for type 2 diabetes. Approximately 57 million individuals in the U.S. have prediabetes and it’s likely that the incidence will increase if daily living habits are maintained (e.g. sedentary and positive energy balance). Current recommendations suggest prescribing both exercise and metformin to prevent and/or delay the onset of developing type 2 diabetes in some individuals with prediabetes. To date however, but no previous study had directly assessed the efficacy of this treatment in men and women with prediabetes. Optimizing treatment for prediabetes requires a better understanding of how interventions, such as exercise and metformin, improve metabolic health. Metabolic health is defined as the status of metabolic variables (e.g. fasting blood glucose levels, insulin sensitivity, etc.) that are predictive of type 2 diabetes. Thus, targeting men and women with prediabetes is of utmost importance for reducing the incidence of type 2 diabetes.
Purpose:

The overall purpose of this study was to understand the effect of combining exercise with metformin on metabolic health in men and women with prediabetes. Although the cause for prediabetes remains unknown, an important component includes insulin resistance and disturbances in substrate metabolism. Specifically, disturbances in ability to utilize carbohydrate and fat during the fasted, fed and exercise state is associated with insulin resistance. Therefore, the specific aims of this proposal were:

1. To evaluate the effect of exercise training with metformin, compared to either treatment alone, on whole-body insulin sensitivity and substrate metabolism in 32 individuals (16 men and 16 women) with prediabetes.

The outcomes included:

a. Whole-body insulin sensitivity (i.e. rate of glucose disposal divided by steady-state plasma insulin).

b. Hepatic insulin sensitivity (i.e. decreased rates of glucose appearance during insulin stimulation).

c. Adipose tissue insulin sensitivity (i.e. suppression of non-esterified free fatty acids during insulin stimulation).

d. Basal hepatic glucose output (i.e. rate of glucose appearance in the fasted state).

e. Non-oxidative glucose disposal (i.e. reflects glucose storage as glycogen) compared to glucose oxidation during insulin stimulation.

f. The magnitude of the shift from whole-body fat to carbohydrate oxidation during the fasted to insulin stimulated state (i.e. metabolic flexibility).

Hypotheses: It was expected that exercise training with metformin would enhance whole-body insulin sensitivity more than either treatment alone. Exercise training with placebo was
expected to increase whole-body insulin sensitivity more than metformin, while metformin would have enhanced whole-body insulin sensitivity more than the control (i.e. placebo). Enhanced insulin sensitivity was expected to be associated with increased non-oxidative glucose metabolism. Exercise training with metformin was expected to reduce rates of glucose appearance and lower non-esterified free fatty acid concentrations compared to either treatment alone. Exercise training with placebo was expected to have little impact on hepatic and adipose tissue insulin sensitivity, but metformin was expected to improve hepatic and possibly adipose tissue insulin sensitivity. Exercise training with metformin was expected to reduce basal hepatic glucose output (HGO) more than either treatment alone. Exercise training with placebo and metformin were expected to reduce HGO similarly more than placebo. Combining exercise with metformin was also expected to enhance metabolic flexibility (i.e. magnitude of the shift from primarily fat oxidation during the fasted state to primarily carbohydrate oxidation under insulin stimulation) compared to either group alone (a summary of these expected results may be found on page 44).

2. To evaluate the effect of exercise training with metformin, compared to exercise training with placebo on exercise substrate metabolism in 16 individuals (8 men and 8 women) with prediabetes.

The outcome measures included:

a. Whole-body carbohydrate and fat oxidation (i.e. respiratory exchange ratio (RER), rate of carbohydrate and fat oxidation, and the relative percentage of energy from carbohydrate and fat).

b. Glucose flux (i.e. rates of glucose appearance and disposal)

c. Blood metabolites and hormone: non-esterified free fatty acids, glycerol, glucose, lactate, and insulin concentrations.
**Hypotheses**: Exercise training with metformin was expected to raise fat oxidation during exercise compared to exercise training with placebo. The effect of metformin to elevated fat oxidation was expected to be related to increased non-esterified fatty acid and glycerol concentrations and decreased plasma insulin. Exercise training with metformin was also expected to cause greater decreases in the rate of glucose appearance, disposal, and estimated muscle glycogen utilization compared to exercise training and placebo (a summary of these expected results may be found on page 46).

**Significance**

Many health professionals are targeting people with prediabetes to reduce the incidence of type 2 diabetes. The American Diabetes Association currently recommends combining metformin with exercise in some men and women with prediabetes (86). Exercise and metformin each improve insulin sensitivity and the capacity to utilize fat for energy. Because each treatment targets a different tissue, it is possible that the combined treatment would improve metabolic health more than either treatment alone. Therefore, the goal of this study was twofold: first, to determine the effect of exercise training with metformin on insulin sensitivity; second, to determine the effect of metformin on exercise substrate metabolism in these same individuals. Information from this study will provide a better metabolic understanding of how exercise and metformin interact to potentially reduce diabetes risk, allow future studies to more specifically target mechanisms that affect insulin sensitivity, and ultimately design better treatments that lower risk for type 2 diabetes.
CHAPTER 2
LITERATURE REVIEW

Introduction

Exercise training and metformin each affect blood glucose regulation through changes in insulin sensitivity and substrate metabolism in individuals with prediabetes. Prediabetes is defined by impaired fasting blood glucose concentrations and/or impaired glucose tolerance. Although blood glucose is affected by distinct tissues in the fasted (e.g. brain and kidney) and insulin stimulated state (e.g. digestive tract, liver, muscle and adipose tissue), blood glucose homeostasis is in general a balance between endogenous glucose production and glucose uptake. Endogenous glucose production consists of hepatic glucose output with small contributions from the kidney (approximately 15-20%). Blood glucose uptake is mainly a result of insulin and occurs in peripheral tissues, like skeletal muscle (approximately 80%). An inability of insulin to mediate glucose regulation in tissues, such the liver and skeletal muscle, is referred to as insulin resistance (see Figure 2.1). Insulin resistance is a hallmark characteristic of type 2 diabetes. Exercise enhances skeletal muscle insulin stimulated glucose uptake, whereas metformin reduces hepatic glucose output. Thus, combining exercise with metformin may enhance blood glucose regulation, compared to either treatment alone, because each therapy

Figure 2.1. Metabolic characteristics associated with insulin resistance. Exercise or metformin improves insulin sensitivity.
targets a different tissue. Improvements in carbohydrate and fat metabolism may contribute to the improvements in insulin sensitivity with exercise and metformin. Therefore, the overall purpose of this literature review was to discuss the effects of exercise training and metformin on metabolic health. Metabolic health was defined as the status of metabolic variables that are predictive of type 2 diabetes. Given that sedentary behavior is such an important component of metabolic health, this literature review starts by discussing the importance of replacing sedentary behavior with physical activity. Then the independent effects of exercise training and metformin on insulin sensitivity and substrate metabolism were discussed. Finally, this literature review concluded with a discussion on the current body of literature pertaining to the combined effect of exercise with metformin.

**Sedentary behavior and metabolic health.**

Matthews et al (79) reported that Americans, regardless of age and/or ethnicity, spend approximately 8 hours a day in sedentary behavior (e.g. sitting, reclining, lying, etc). Sedentary behavior (defined as <1.5 METS or lack of whole body movement) has been linked to several adverse metabolic health conditions, including heart disease and diabetes (42). Helmerhorst et al. (46) showed that fasting insulin concentrations, which were used as a surrogate for insulin resistance, were positively associated with time spent in sedentary behavior, independent of time spent in moderate to vigorous activity. Preventing sedentary behavior has the potential to maintain or improve metabolic health. It’s been shown that breaking up prolonged periods of sitting, by reducing time spent in uninterrupted sedentary behavior, has the potential to prevent weight gain and improve metabolic health (42). Further, increased breaks in sedentary behavior are also beneficially associated with a lower waist circumference, body mass index (BMI), and 2-hour blood glucose concentration (42). Thus, preventing/minimizing sedentary behavior is a viable strategy for maintaining metabolic health.
**Physical activity and metabolic health**

Lifestyle modification, consisting of increased physical activity, prevents and/or delays the progression from prediabetes to type 2 diabetes (35, 70, 88, 94, 116). Physical activity is defined as any bodily movement (Metabolic equivalent: (MET) between 1.5 and 3.0) that substantially increases energy expenditure above resting conditions. Exercise is defined as *structured* activity (MET values >3.0) with purposes of caloric expenditure for health and/or performance. This distinction is important because changes in physical activity, independent of exercise, may alter the blood glucose regulation and metabolic health.

Time spent in light to vigorous activity (30, 43) promotes metabolic health. Physical activity reduces blood glucose concentrations in response to a carbohydrate load (31, 44) and lowers fasting glucose, insulin, and triglycerides in sedentary adults with a family history of type 2 diabetes (32) or the metabolic syndrome (106). In other studies, total physical activity (44) or time spent in light and moderate-vigorous activity (43) did not reduce fasting blood glucose concentrations. These findings suggest that physical activity may be more effective at regulating post-prandial glucose concentrations compared to fasting blood glucose concentrations. Balkau et al (4) observed an association between total physical activity, independent of light, moderate or vigorous activity, and improved whole-body insulin sensitivity measured by the hyperinsulinemic-euglycemic clamp in normal weight men and women. These findings indicate that the improved responses to carbohydrate loads (31, 44) are often attributable to enhanced insulin sensitivity. Together, these findings may have important clinical and public health implications as most Americans spend greater than 50% of their time in sedentary behavior; substituting sedentary behavior with activity may improve metabolic health.

**Exercise and insulin sensitivity**

Despite the importance of physical activity on metabolic health, major health organizations, such as the American Diabetes Association, recommend both aerobic and
resistance exercise for metabolic health. Aerobic (25, 49, 66, 98) and resistance exercise (83, 109) are well known to improve metabolic health since physically trained individuals have enhanced insulin sensitivity compared to their weight-matched untrained counterparts (47, 80).

Single bouts of aerobic exercise improve insulin sensitivity (5, 27, 28), but this effect is transient (approximately 24-hours) (45, 81, 103). Therefore, to maintain this enhanced insulin sensitivity, it is necessary to perform repeated bouts of exercise (i.e. exercise train). Holloszy et al (49) demonstrated that exercise training for 12 months (5-day/week at 70% VO₂max) not only improved insulin sensitivity, but also reversed prediabetes. Skeletal muscle accounts for approximately 80% of the improved insulin sensitivity (26), although there are contributions from the liver and adipose tissue (97). Skeletal muscle is therefore the major tissue causing the 20-30% increase in whole-body insulin sensitivity seen after most aerobic training programs (69).

Most exercise training interventions have been aerobic based and consist of individuals training 3-5 days per week at a moderate intensity exercise (e.g. >60% VO₂max) for a total of approximately 200 minutes (see Table 2.1). Although public health recommendations suggest

<table>
<thead>
<tr>
<th>Subject Population</th>
<th>Exercise Dose</th>
<th>Hours Measured Post-exercise</th>
<th>Δ in Whole-body Insulin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Houmard et al 2004</td>
<td>Overweight/obese 6mo 12mi 40-55% VO₂peak 170min, 12mi 65-85% VO₂peak 110min, 22mi 65-85% VO₂peak 170min</td>
<td>24 hr</td>
<td>↑ 80% 170min conditions ↑ 35% 110min</td>
</tr>
<tr>
<td>Gan et al 2003</td>
<td>Overweight and obese men 10 wk; 4-5d/wk for 40min at 55-70% VO₂peak</td>
<td>24-36 hr</td>
<td>↑ 16%</td>
</tr>
<tr>
<td>DeFronzo et al 1987</td>
<td>Obese men insulin resistant 6 wk; 4-5d/wk at 65% VO₂peak for 60min on cycle or treadmill</td>
<td>48-72hr</td>
<td>↑ 31% peripheral</td>
</tr>
<tr>
<td>Christ-Roberts et al 2004</td>
<td>Overweight with and without T2D 8wk 3-4d/wk for 20-45min at 60-75% VO₂peak on cycle</td>
<td>24 hr</td>
<td>↑ 14% overweight ↑ 27% T2D</td>
</tr>
<tr>
<td>Hughes et al 1993</td>
<td>IGT men and women 12wk 50-75% HR reserve for 55min/d 4d/wk</td>
<td>96 hr</td>
<td>↑ 11%</td>
</tr>
<tr>
<td>Solomon et al 2008</td>
<td>Overweight elderly IGT men and women 12wk walking/cycling 5d/wk for 60min at 75% VO₂peak</td>
<td>48 hr</td>
<td>↑ 60%</td>
</tr>
<tr>
<td>Bruce et al 2004</td>
<td>T2D men 8 wk cycling 3d/wk 60min at 70% VO₂peak</td>
<td>36-48hr</td>
<td>↑ 30%</td>
</tr>
<tr>
<td>Segal et al 1991</td>
<td>Lean, obese IR, T2D men 12 wk 70min 4d/wk at ventilatory threshold (~50% VO₂peak). Treadmill</td>
<td>72 – 120 hr</td>
<td>No change</td>
</tr>
</tbody>
</table>
that individuals exercise 30 minutes per day on most days of the week, the optimal exercise prescription for metabolic health remains unclear. Houmard et al (55) demonstrated that 170-minutes of exercise per week, (i.e. volume), independent of exercise intensity (approximately 45% vs. 70% VO₂max), improved insulin sensitivity compared to 115 minutes of exercise in overweight insulin resistant individuals. Hughes et al (56) also demonstrated that there were no differences in insulin sensitivity between moderate (50% VO₂max) or high (75% VO₂max) intensity training conducted for a total of 220 minutes a week in older overweight individuals with prediabetes. These findings suggest that time spent exercising is an important for insulin sensitivity than intensity; however, they do not indicate how exercise improves insulin sensitivity.

The mechanism by which exercise affects insulin sensitivity remains unclear. Cross-sectional studies in healthy lean trained individuals have demonstrated relationships between insulin signaling proteins (e.g. IRS-1 and PI3K) and insulin sensitivity (54, 68). However, studies conducted in individuals with insulin resistance (112) and type 2 diabetes (18) do not support such relationships. Thus, in clinically relevant populations (i.e. obese, insulin resistant individuals), it is thought that exercise training enhances insulin sensitivity through alternative mechanisms. Some of these alternative mechanisms include: weight/fat loss and increased skeletal muscle oxidative capacity.

Exercise may contribute to weight loss, and reductions in body weight may contribute to enhancing insulin sensitivity (101, 110). When energy balance is maintained during aerobic training, minimal improvements in insulin sensitivity are observed (101, 104). Segal et al (104). Segal et al (104) showed that maintaining energy balance while training had no effect on enhancing whole-body insulin sensitivity in lean or obese men with or without type 2 diabetes despite 27% increases in cardio-respiratory fitness. Compared to weight loss, when energy expended during exercise is fed back to maintain body weight, obese men (101) and women (102) do not improve insulin sensitivity. Weight loss enhances whole-body insulin sensitivity, in part, by decreasing amounts of body fat and increasing GLUT-4 protein and/or translocation.
There are training studies however that show improvements in insulin sensitivity independent of weight loss (14, 56). One possible explanation for these conflicting results may be due to decreases in total body fat and/or visceral fat.

Elevated body fat, particularly visceral fat, is associated with insulin resistance. Training, independent of weight loss, reduced visceral fat in obese individuals (73). Hughes et al (56) demonstrated that aerobic training at either 50% or 75% VO2max without weight loss also decreased waist to hip ratio, which is indicative of reduced visceral fat. This latter finding was significantly related to improved insulin sensitivity (r = 0.70; p < 0.05). Gan et al (38) measured visceral fat through use of magnetic resonance imaging (MRI) and observed reductions in visceral adipose tissue after 10 weeks of aerobic training. Reductions in visceral fat were significantly associated with improvements in insulin sensitivity (r = -0.54; p < 0.05). Decreasing visceral fat may lead to reductions in circulating non-esterified fatty acid (NEFA) concentrations that are delivered to the liver and periphery. Elevated NEFA concentrations contribute to elevated hepatic glucose output and reductions in skeletal muscle glucose uptake (64). Thus, treatments that reduce body fat may contribute to improving insulin sensitivity. However, other factors, independent of weight/fat loss, may promote skeletal muscle glucose uptake.

Aerobic training promotes changes in intracellular fat metabolites that, at least partially, mediate improvements in insulin sensitivity. Kim et al (65) demonstrated that 12 weeks of aerobic training reduced intramuscular triglycerides (IMTG) in individuals with prediabetes. Reduced IMTG concentrations were inversely related to GLUT-4 protein concentrations. This is potentially important because increased expression of GLUT-4 protein is related to enhanced glucose uptake (18). Bruce et al (13) examined the effect of exercise training on IMTG and insulin sensitivity in individuals with type 2 diabetes. Aerobic training in individuals with type 2 diabetes reduced IMTG concentrations to lean control concentrations, but this reduction in IMTG failed to normalize insulin sensitivity. This finding suggests that IMTG concentrations likely serve as a marker of insulin resistance and not a direct role in mediating insulin sensitivity.
For example, trained athletes have similar IMTG concentrations as individuals with type 2 diabetes, yet trained athletes have higher insulin sensitivity (39). This phenomena is likely explained by the observation that trained individuals have an increased capacity to oxidize fat (39). Lower fat oxidation may not only increase IMTG concentrations, but it may also increase fatty acid intermediates (e.g. DAG and ceramides) that directly impair insulin sensitivity (64).

Individuals with insulin resistance are characterized by reduced oxidative capacity and elevated fat uptake compared to their insulin sensitive counterparts (63, 64, 107). Bruce et al (15) indicated that oxidative capacity was the strongest predictor of insulin sensitivity, compared to skeletal muscle lipid status, across a wide range of metabolic phenotypes (e.g. lean healthy to type 2 diabetes). More recently, it was demonstrated in animals that exercise training enhanced the capacity of skeletal muscle to oxidize excess fat and reverse high fat feeding induced insulin resistance. It’s thought that reduced oxidative capacity under states of excess fat intake leads to decoupling between β-oxidation and the Krebs cycle, and this mismatch between metabolic pathways increases the production of “incomplete” lipid species. These incomplete lipid species have been associated with impaired insulin signaling (71). Therefore, it seems likely that the training induced improvements in insulin sensitivity are partially mediated through to the ability to balance fat storage and fat oxidation.

Despite exercises clinical benefit, long-term adherence is often poor. The exact reason for this is unclear, but it is often attributed to feelings of tiredness and a lack of self-motivation (113). Resistance exercise may be an attractive alternative to aerobic exercise because it can be done in shorter periods of time and it enhances muscle strength. Improvements in strength may lead to improved physical function in individuals with low cardio-respiratory fitness (17, 62) and subsequently lead to greater compliance with aerobic based programs. Unfortunately, there little is known regarding the effects of resistance training on enhancing insulin sensitivity.

Resistance exercise enhances insulin sensitivity in individuals along the continuum of insulin resistance to type 2 diabetes (see Table 2.2; 51, 52, 60, 82, 83). Miller et al (82) first
demonstrated the beneficial effects of resistance training on blood insulin responses to a carbohydrate load in healthy individuals. Ishi et al (60) showed that resistance training enhanced insulin sensitivity by 48% when scaled to fat-free mass, independent of VO$_2$max.

**Table 2.2 Effect of resistance exercise training on insulin sensitivity**

<table>
<thead>
<tr>
<th>Study Population</th>
<th>Exercise training</th>
<th>Hours measured post-exercise</th>
<th>▲ in Whole-body Insulin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eriksson et al 1998</td>
<td>Pre-diabetes</td>
<td>3d/wk: 3 x 8-10 repetitions at 50% 1 RM for 10 weeks compared to 3d/wk at 60% HRmax for 1hr per session</td>
<td>96 hour</td>
</tr>
<tr>
<td>Holten et al 2004</td>
<td>Lean T2D</td>
<td>6wk; 3/dwk one legged exercise. 1-2wk = 10 repetitions 50% 1RM; 3-6wk = 10-12 repetitions at 80% 1 RM;</td>
<td>16 hr</td>
</tr>
<tr>
<td>Ishi et al 1998</td>
<td>Nonobese T2D</td>
<td>4-6wk; 5d/wk 2 x 10-20 repetitions of 9 exercises</td>
<td>48 hr</td>
</tr>
<tr>
<td>Miller et al 1984</td>
<td>Healthy males</td>
<td>10wk; 3d/wk; 3 x 8 repetitions: no intensity reported</td>
<td>48hr</td>
</tr>
<tr>
<td>Miller et al 1994</td>
<td>Elderly Insulin resistant men</td>
<td>3d/wk; 3-4 repetitions at 90% 1RM and 15 repetitions; 16wk</td>
<td>22-24hr</td>
</tr>
</tbody>
</table>

or body composition in individuals with type 2 diabetes. Eriksson et al (34) measured insulin sensitivity by using the hyperinsulinemic-euglycemic clamp and showed that circuit-resistance training increased insulin sensitivity by 23% in overweight men with prediabetes. Miller et al (82) also determined the effectiveness of resistance training on insulin sensitivity in insulin resistant men and observed a 24% improvement in insulin sensitivity when normalized to fat-free mass. Scaling insulin sensitivity to fat-free mass (i.e. skeletal muscle), was important because it suggested that skeletal muscle adaptations, not skeletal muscle mass per se, were responsible for the enhanced insulin sensitivity. Holten et al (51) later demonstrated that 6 weeks of one-legged resistance training enhanced insulin sensitivity in individuals with type 2 diabetes and was, in part, due to increased insulin signaling proteins: GLUT-4, insulin receptor, and Akt protein. There were no changes in oxidative enzymes concentrations (citrate synthase or β-HAD), suggesting that resistance training enhances insulin sensitivity through increasing insulin signaling proteins.

Given that major health organizations recommend performing both aerobic and resistance exercise, it is surprising so few studies have investigated the effect of combining
such exercises on metabolic health. Wallace et al (121) compared the effects of aerobic and resistance training to aerobic training only on fasting insulin concentrations (used as a surrogate for insulin sensitivity) in individuals with insulin resistance. Aerobic and resistance training lowered fasting insulin (8µU/ml vs. 3µU/ml) and glucose concentrations (11.1mg/dl vs. 6mg/dl) more than aerobic training. Aerobic and resistance training improved triglycerides, blood pressure and high-density lipoproteins, compared to aerobic training only. Cuff et al (22) also observed greater improvements in insulin sensitivity after performing 16 weeks of aerobic and strength training compared to aerobic exercise only in postmenopausal women with type 2 diabetes. A limitation, however, with the aforementioned studies is that individuals performing both modes of exercise usually exercised 30 minutes longer each day. Consequently, the improvements in metabolic health may be a result of increased energy expenditure and/or greater weight loss and not the combination of aerobic and resistance exercise itself. Despite this limitation, these data demonstrate that combining exercise modalities are favorable for insulin sensitivity. To date, however, no study has been published characterizing the efficacy of aerobic and resistance training on insulin sensitivity in individuals with prediabetes.

In summary, aerobic and resistance exercise is known to improve insulin sensitivity in individuals at risk for type 2 diabetes. This suggests that exercise is a vital treatment for improving the ability to clear blood glucose in individuals with prediabetes. Under insulin stimulated states, skeletal muscle clears approximately 70-90% of the blood glucose (69). Although this highlights the importance of skeletal muscle in response to insulin, it suggests that other tissues are responsible for the remaining 20-30% improvement in insulin sensitivity. Perhaps adding a therapy that targets liver tissue, thereby reducing endogenous blood glucose production, may accentuate improvements in insulin sensitivity.
**Metformin and hepatic glucose metabolism**

The U.S. Diabetes Prevention Program (DPP) demonstrated that metformin effectively prevents and/or delays the onset of type 2 diabetes in individuals with prediabetes (70). Metformin is an orally administered drug and is routinely used to lower blood glucose concentrations in individuals with type 2 diabetes (67).

Metformin reduces fasting blood glucose concentrations approximately 30% in individuals with type 2 diabetes by, in large part, reducing hepatic glucose output (HGO) (23, 58, 111). Although the exact mechanism by which metformin reduces HGO is unclear, it appears related to reductions in gluconeogenesis (58).

Metformin likely reduces gluconeogenesis through two possible mechanisms by either decreasing gluconeogenic precursors and/or enhancing hepatic insulin sensitivity. Metformin had been reported to lower lactate uptake and decrease the conversion of alanine to pyruvate in animal models (67). Consistent with decreased lactate uptake, elevated fasting blood lactate concentrations have been reported in some (58) but not all (23, 111) studies. Metformin also lowers non-esterified fatty acid (NEFA) concentrations by 10-30% at rest and during insulin stimulation (67). Since NEFAs act as a gluconeogenic stimulators through increasing the availability of acetyl-CoA, lower NEFA concentrations could also contribute to reducing HGO (67).

Alternatively, metformin may enhance hepatic insulin sensitivity (i.e. decreased rates of glucose appearance during insulin stimulation) and reduce post-prandial (24, 33) and day-long glucose concentrations (1, 123). Wu et al (123) showed that metformin lowered blood glucose concentrations by 25% over the course of the day (from 0800 to 1600 hours) in individuals with type 2 diabetes. There were no differences in insulin stimulated glucose uptake, suggesting that the majority of the blood glucose improvement was a result of HGO suppression. Lower NEFA concentrations are one possible explanation for how metformin enhanced hepatic insulin sensitivity. Perriello et al (92) showed that metformin suppressed NEFA concentrations by 17%
and lowered lipid oxidation 25% during a glucose clamp in individuals with type 2 diabetes. Reduced NEFA concentrations during the glucose clamp were significantly associated with HGO suppression (r = 0.70; p <0.001) but not increased glucose uptake. Riccio et al (96) also showed that 4 weeks of metformin treatment lowered fasting plasma NEFA turnover rate and NEFA oxidation by 27 and 24%, respectively, in individuals with type 2 diabetes.

The mechanism by which metformin enhances hepatic insulin sensitivity is unclear. Zhou et al (124) demonstrated that metformin increased hepatic fat oxidation and reduced fat synthesis in isolated hepatocytes. This suggests that metformin may decrease hepatic fat storage and remove the deleterious effects of hepatic fat metabolites on insulin resistance. However, Tiikkainen et al (114) demonstrated that 16 weeks of metformin treatment did not affect hepatic fat content in individuals with type 2 diabetes, despite enhanced hepatic insulin sensitivity. A reasonable explanation for the differing results in hepatic fat content is likely because the dosage used by Tiikkainen et al (114) was considerably lower, although more clinically relevant, than the pharmacological dose used previously (124).

Independent of hepatic fat content, it’s possible that changes in fat turnover could play a role in ameliorating hepatic insulin resistance (39). Cleasby et al (19) demonstrated that metformin opposed the development of lipid-induced insulin resistance in rat liver tissue through activation of AMPK. AMPK is associated with increased phosphorylation of acetyl-CoA carboxylase (ACC), which is related to decreasing malonyl-CoA synthesis and increasing the entry of fat into the mitochondria for oxidation. Thus, perhaps improving the flux of fatty acids is more important than decreasing hepatic fat content for enhancing hepatic insulin sensitivity.

**Metformin and whole-body insulin sensitivity**

Metformin lowers insulin concentrations at rest (24, 85) and during carbohydrate loads (24, 33), suggesting improved insulin sensitivity. Metformin improves whole-body insulin sensitivity in some (24, 29, 74, 96), but not all studies (105, 111, 123). Differences between
these studies may be related to pre-treatment body weight and fasting blood glucose concentrations. For example, the most favorable effects on insulin sensitivity are often observed in individuals with excess body weight and elevated blood glucose concentrations (3).

Dorella et al (29) showed that 4-weeks of metformin enhanced whole-body insulin sensitivity by approximately 30% in overweight-insulin resistant hypertensive individuals. Lehtovirta et al (74) demonstrated that 6 months of metformin treatment also enhanced insulin sensitivity by 20% in impaired glucose tolerant individuals.

A possible mechanism by which metformin enhances insulin sensitivity is by causing weight loss. Metformin decreases body weight by approximately 1-5kg (24, 78, 85), of which 90% is body fat (111). Tiikkainen et al (114) showed that metformin decreased subcutaneous body fat in individuals with type 2 diabetes with average waist-to-hip ratios of 0.98 (obesity defined as: >0.95 for men and >0.86 for women). Pasquali et al (90) demonstrated that metformin significantly reduced total body fat and visceral fat in women with polycystic ovarian syndrome (PCOS). Weight and/or fat loss has been shown to increase GLUT 4 protein expression (85) and/or its translocation to the cell membrane under insulin stimulated conditions in skeletal muscle (57). Consistent with enhanced insulin sensitivity, metformin has been shown to increase non-oxidative metabolism (85) thereby increasing glycogen storage in skeletal muscle (2, 85) and hepatic tissue (114).

Metformin may also enhance insulin sensitivity because it reduces skeletal muscle fat content. Collier et al (21) demonstrated that metformin limited muscle lipid storage in rat skeletal muscle and improved the coupling between fat uptake and oxidation by reducing FAT/CD36 transporter. Reduced FAT/CD36 transporter could potentially lead to decreased fat uptake in skeletal muscle and decrease the synthesis of DAG and ceramide concentrations that are associated with insulin resistance (108). Thus, improvements in skeletal muscle fat oxidation and/or storage may contribute to the improved insulin sensitivity with metformin treatment.
In summary, metformin lowers blood glucose concentrations by primarily reducing hepatic glucose output, improving hepatic insulin sensitivity, and to a lesser extent, stimulating insulin mediated skeletal muscle glucose uptake. The mechanism by which metformin improves hepatic glucose metabolism is unclear, but may be due to weight loss and/or fat metabolism. Given the beneficial effects metformin have on the liver, combining metformin with exercise training may accentuate metabolic health in individuals with prediabetes.

**Role of substrate metabolism on insulin sensitivity.**

The mechanism underlying the development of insulin resistance has yet to be fully elucidated. However, it is clear that disturbances in carbohydrate and fat metabolism are involved (16, 64). Individuals with high insulin sensitivity are characterized as metabolically flexible and able to switch from predominately fat utilization in the fasted state to chiefly carbohydrate utilization during the insulin-stimulated state. Metabolic “inflexibility” is a characteristic found in individuals with insulin resistance. Insulin resistant individuals utilize less fat for energy during the fasted state and do not switch to carbohydrate use under insulin-stimulated states, compared to insulin sensitive individuals (64). Elevated fat oxidation under insulin-stimulated conditions suggests that insulin does not suppress adipose tissue lipolysis. High rates of lipolysis under insulin stimulated conditions would lead to increased concentrations of NEFAs and impair HGO suppression and glucose uptake (64). Exercise may overcome this “inflexibility” by increasing the capacity to oxidize fat during and after bouts of exercise (110, 119). Thus, interventions that increase fat utilization may enhance insulin sensitivity by: reducing lipid species (14, 39) and/or improving “complete’ fat oxidation (71).

**Exercise and substrate metabolism.**

Endurance training increases the reliance on fat and decreases the dependence on carbohydrate utilization during rest (99, 115) and exercise (12, 20, 37, 59). Few studies however
have characterized the effect of exercise training on carbohydrate and fat oxidation in overweight and/or obese individuals. Exercise training effects on fat utilization in overweight and/or obese individuals is summarized in Table 2.3.

When individuals exercise at 65% of pre-training VO$_2$max (i.e. ~55% post-training VO$_2$max), lower RER values (indicating elevated fat use) are observed in obese individuals (13). Bruce et al (14) demonstrated that exercise training increased skeletal muscle mitochondrial fat oxidation 120% and was related to enhanced insulin sensitivity. Muscle biopsies demonstrated that elevated mitochondrial fat oxidation was related to reductions in diacylglycerol (DAG) and saturated DAG fat species. These observations suggest that improved fat oxidation may, at least partially, be related to enhanced insulin sensitivity. Venables et al (120) compared interval training to a novel training protocol eliciting Fatmax on fat oxidation and insulin sensitivity in obese men. These men exercised at their Fatmax (i.e. approximately 45% VO$_2$max) for 30-60 minutes over 4 weeks. After a 1 month wash-out period, these same participants underwent interval training at ± 20% of their Fatmax for 4 weeks. Training at the Fatmax, enhanced fat oxidation 44%, compared to interval training. Elevated fat oxidation was modestly related to reductions in insulin concentrations during a carbohydrate load ($r = -0.44; p < 0.05$). This finding suggests a possible relationship between fat utilization during exercise and insulin sensitivity.

Table 2.3. Exercise training on substrate use in overweight individuals.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Exercise Training</th>
<th>ΔWhole-body substrate use during exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruce et al 2004</td>
<td>Type 2 diabetes</td>
<td>5d/wk for 60min @ 65% VO$_2$max for 8 weeks</td>
<td>† Fat Use</td>
</tr>
<tr>
<td>Venables et al 2008</td>
<td>Hyperinsulinemic Obese men</td>
<td>5d/wk for 30-60min @ 45% VO$_2$max for 4 weeks</td>
<td>† Fat Use</td>
</tr>
<tr>
<td>Dumortier et al 2003</td>
<td>Metabolic syndrome</td>
<td>3d/wk for 40min @ 45% VO$_2$max for 8 weeks</td>
<td>† Fat Use</td>
</tr>
<tr>
<td>Van Aggel-Leijssen et al 2001</td>
<td>Overweight men</td>
<td>3d/wk for 60min @ 40%VO$_2$max for 12weeks</td>
<td>† Fat Use</td>
</tr>
<tr>
<td>Bruce et al 2006</td>
<td>Obese men</td>
<td>5d/wk for 60min @ 65% VO$_2$max for 8 weeks</td>
<td>† Fat Use</td>
</tr>
</tbody>
</table>
The healthful effects of exercise, however, are not limited to increased energy expenditure and fat oxidation during exercise, but are extended into the recovery period when insulin sensitivity in increased.

Following aerobic or resistance exercise, carbohydrate oxidation is reduced and fat oxidation is elevated in lean (6, 7, 11, 72, 93) and obese (77) individuals. Elevated fat oxidation in the fasted-state favors metabolic flexibility (64) and insulin stimulated glucose uptake (8, 48, 53). Exercise training improves fasting fat oxidation in obese individuals with (110) or without prediabetes (40). Goodpaster et al (40) demonstrated that obese men and women without type 2 diabetes shifted fasting fat utilization from 38% to 52% and enhanced insulin sensitivity by approximately 50%. The strongest predictor of insulin sensitivity was elevated fasted fat oxidation ($r = -0.62; p < 0.05$) (40). Thus, exercise training improves the capacity to utilize fat and enhances insulin sensitivity.

In summary, exercise training increases fat oxidation during and after exercise in overweight individuals. Given that disturbances in fat metabolism have a role in insulin resistance, elevated fat oxidation during or after exercise may be a mechanism by which exercise training causes favorable changes in insulin sensitivity (110, 119). Adding a therapy that accentuates fat oxidation in the fasted state may lead to greater improvements in metabolic flexibility and metabolic health.

**Metformin and substrate metabolism.**

Although some studies report that metformin increases non-oxidative glucose disposal (23, 24, 85, 111), others report elevated carbohydrate oxidation (29, 74, 92, 96). It is not entirely clear why there are such discrepancies in the literature. One possibility is due to weight loss. Metformin promotes weight loss, which is both associated with elevated carbohydrate oxidation (87). Short-term studies without weight loss however show that metformin raises carbohydrate
oxidation in the fasted state. This suggests that metformin elevates carbohydrate oxidation independent of weight loss.

Alternatively, metformin may enhances carbohydrate utilization because it lowers fasting NEFA concentrations (29, 92, 96). It has been suggested that lower NEFA concentrations during insulin stimulation may favor glucose uptake because of reduced inhibition on key insulin signaling proteins (64). Perhaps elevated carbohydrate oxidation during insulin stimulation was also a result of lower NEFA and explains the enhanced insulin sensitivity in individuals with prediabetes (74). Nevertheless, elevated carbohydrate oxidation during insulin stimulation would be expected to enhance metabolic flexibility (64) and favor enhanced insulin sensitivity.

Long-term studies with metformin however show no change in fat oxidation during the fasted or insulin stimulated state when NEFA concentrations do not change (23, 24, 85, 111). Musi et al (85) demonstrated no change in carbohydrate or fat oxidation after 10 weeks of metformin treatment; however, there were significant improvements in non-oxidative carbohydrate metabolism and skeletal muscle glycogen concentrations. A possible explanation for the discrepancy between studies on carbohydrate oxidation is that standardized meals were provided in some (23, 85, 111), but not all studies (29, 74). Perhaps individuals consumed extra carbohydrate on the day(s) prior to the glucose clamp, which increased glycogen stores and shifted glucose uptake towards oxidation.

In summary, metformin lowers blood glucose concentrations by primarily decreasing glucose production, and to a lesser extent, elevating whole-body insulin sensitivity. The effect of metformin on non-oxidative versus oxidative glucose metabolism remains unclear; however, in studies where metformin lowers NEFA concentrations there is a compensatory rise in carbohydrate oxidation during fasting and insulin stimulated state.
**Metformin added to exercise.**

Since exercise primarily targets skeletal muscle and metformin predominately affects the liver, combining these two treatments may accentuate effects on substrate metabolism and insulin sensitivity (86). Few studies have assessed the combination of metformin and exercise on metabolic health or fitness parameters (e.g. VO$_2$max, substrate utilization, etc), and most studies to date have been conducted in lean healthy individuals (10, 41, 61).

In a group of recreationally active individuals, metformin reduced VO$_2$max and heart rate max by approximately 3% (10), but metformin was reported to have little effect on submaximal exercise VO$_2$, RER, or heart rate. This later observation suggests that minor decreases in maximal capacities have minimal effect on submaximal exercise physiology. However, decreases in VO$_2$max would be expected to increase the relative intensity of exercise when performed at the same absolute workload. If metformin increases the relative intensity of exercise and acts to stimulate glucose uptake (85), then it’s likely that metformin would elevate carbohydrate oxidation during exercise. Malin et al (76) however showed that metformin enhanced whole-body fat oxidation (see Figure 2.2; pilot study published chapter 5) during exercise across a range of exercise intensities (~30 to 70% Wpeak) by approximately 25% in recreationally active individuals. These data are contrary to what may be expected given the effect of metformin to alter fitness and/or increase glucose uptake. The mechanism by which metformin raised fat oxidation is unclear, but may be related to either the direct effect of metformin to enhance fat utilization or the indirect effects of limited carbohydrate availability.

![Figure 2.4. Metformin raises fat oxidation during exercise in healthy individuals.](image)
Metformin has been shown to activate AMPK (85). AMPK is associated with facilitating entry of non-esterified fatty acids into the mitochondria for subsequent fat oxidation (75). Metformin has also been reported to decrease blood insulin concentrations during exercise in healthy individuals. Lower insulin concentrations were associated with elevated circulating non-esterified fatty acid concentrations during exercise. Elevated NEFA concentrations would be expected to substrate available for potential oxidation (41). However, metformin also acts to reduce hepatic glucose production, which could lower the availability of plasma glucose and constrain carbohydrate oxidation (95). Use of glucose isotopes during exercise would partially address this latter mechanism and provide further characterization of substrate oxidation during exercise.

To date, the only published study characterizing the combined effect of exercise and metformin on insulin sensitivity in humans is from a study in overweight sedentary insulin resistant individuals following a single bout of exercise (105). AMPK has been suggested to be an important regulator of post-exercise whole-body insulin sensitivity (75). Because exercise and metformin have been shown to independently activate AMPK in skeletal muscle (84, 85), it was hypothesized that combining metformin with exercise, compared to exercise alone, would produce additive effects on AMPK in skeletal muscle and, at least partially, contribute to enhanced whole-body insulin sensitivity. Metformin or placebo was provided to all participants for 2-3 weeks at 2000mg/d. After treatment, individuals exercised for approximately 30 minutes at 65% and 10 minutes at 85% VO₂peak. Insulin sensitivity was measured approximately 4 hours post-exercise by the hyperinsulinemic-euglycemic clamp. The results indicated that exercise alone enhanced whole-body insulin sensitivity by 54% compared to baseline, but metformin blunted the exercise effect. Although these preliminary findings suggest that combining metformin with exercise may not be additive, they could have important ramifications for the treatment of prediabetes. Individuals with prediabetes are recommended to perform repeated bouts of exercise to improve metabolic health. From a practical standpoint, it is
important to assess the effect of exercise training and metformin on insulin sensitivity in individuals with prediabetes to better understand if exercise and metformin combined is a useful treatment option.

The Indian Diabetes Prevention Program (IDPP) recently tested the efficacy of combining lifestyle modification (i.e. exercise and low-fat diet) with metformin on the incidence of type 2 diabetes in individuals with prediabetes over 3 years compared to lifestyle modification, metformin or placebo (94). All treatments reduced the incidence of type 2 diabetes by 40% compared to placebo. This suggests that the combined treatment is not better than either treatment alone. However, individuals in the IDDP were typically overweight but not obese, highly active, and ate a relatively low-fat diet. Thus, the generalization of these data maybe limited to this population. The only study to characterize the effects of training with metformin to date was conducted in high fat fed obese Zucker rats (108). For 8 weeks, rats performed treadmill exercise and were provided metformin treatment. Exercise training with metformin and exercise training alone increased insulin stimulated *skeletal muscle* glucose uptake similarly, but both exercise conditions were better than metformin alone. These findings suggest that combining exercise training and metformin is not better than exercise training alone. However fasting blood glucose concentrations were slightly lower with the combined treatment compared to either exercise training or metformin alone. Because assessing skeletal muscle insulin sensitivity does not incorporate the impact of the intervention on other key tissues (e.g. liver), it remains possible that *whole-body* insulin sensitivity would be improved when exercise and metformin are combined. Moreover, this previous work (108) only tested type I muscle fiber (i.e. soleus) glucose uptake. Given that type II muscle fibers are an important depot for glucose storage, it’s possible that studying whole muscle may provide different results.

To date, no study has directly assessed the combined effects of exercise training with metformin on whole-body insulin sensitivity or substrate metabolism in the clinically relevant population of men and women with prediabetes. From a clinical perspective, understanding how
the combined treatment affects metabolic health may potentially improve the management of 
blood glucose concentrations. From a public health perspective, understanding which treatment 
plan is best at enhancing metabolic health may ultimately lead to greater reductions in the 
incidence of type 2 diabetes.

**Conclusion.**

Exercise is a cornerstone therapy for the prevention of type 2 diabetes. Determining the 
impact of exercise and/or metformin on insulin sensitivity and substrate metabolism is important 
to understanding optimal treatments for metabolic health. It is possible that exercise-induced 
adaptations in skeletal muscle, combined with metformin-mediated improvements in liver, may 
produce additive effects on whole-body insulin sensitivity and substrate metabolism. 
Collectively, the current literature supports that:

1. Exercise training increases insulin sensitivity in a variety of populations, including 
   individuals with prediabetes, by primarily targeting insulin-stimulated skeletal muscle 
   glucose uptake.
2. Metformin improves blood glucose homeostasis by reducing hepatic glucose output, 
   enhancing hepatic insulin sensitivity and to a lesser extent peripheral insulin sensitivity.
3. Exercise training enhances whole-body fat oxidation in both the fasted and exercise 
   state and this may favor high insulin sensitivity.
4. Metformin stimulates glucose uptake in skeletal muscle, enhances non-oxidative and 
   oxidative glucose metabolism during insulin stimulation, and enhances fat oxidation 
   during exercise.
5. Exercise and metformin have different effects on skeletal muscle and liver that increase 
   the propensity of improving whole-body insulin sensitivity and altering substrate 
   metabolism in individuals with prediabetes.
CHAPTER 3

METHODS

Overview of study design

The overall goal of this study was to determine the effect of combining exercise training with metformin on metabolic health (AIM 1) and substrate metabolism during exercise (AIM 2) compared to either exercise training or metformin alone. To test aim 1, 32 individuals with prediabetes were tested in 1 of 4 conditions over 12 weeks:

1) Placebo (P)
2) Metformin (M)
3) Exercise training + placebo (EP)
4) Exercise training + metformin (EM)

Sixteen individuals from the exercise conditions were tested in aim 2. Pills were distributed in a double-blind manner. An overview of the study design is provided in Figure 3.1.

Figure 3.1. Overview of Study Design
To address Aim 1 a through f (effect of adding metformin to exercise training on whole-body insulin sensitivity and substrate metabolism), insulin-mediated glucose disposal, hepatic glucose output and suppression of non-esterified fatty acids were assessed using the hyperinsulinemic-euglycemic clamp technique (i.e. glucose clamp) combined with glucose stable isotope tracers. Whole-body carbohydrate and fat oxidation were also measured during the glucose clamp by indirect calorimetry at baseline and after the intervention with P, M, EP or EM.

To address Aim 2 a through c (effect of adding metformin to exercise training on substrate oxidation during exercise), rates of glucose appearance and disposal were assessed using the glucose stable isotope dilution method and whole-body carbohydrate and fat metabolism was measured via indirect calorimetry at baseline and after approximately 10 weeks of EP and EM.

**Subjects**

Overweight to obese (body mass index = 25-45 kg/m²), sedentary (<60 minutes of moderate activity per week as measured by questionnaire) men and women between the ages of 25 to 60 were recruited. All potential participants performed an oral glucose tolerance test (OGTT) to ensure they had prediabetes (i.e. impaired glucose tolerance in the absence or presence of impaired fasting glucose concentrations), but were in otherwise good health. All subjects were weight stable for at least 3 months (< 5% body weight change) prior to enrollment in the study. Subjects with family history of type 2 diabetes were included. Subjects were excluded if they use tobacco products, had cardiovascular disease or type 2 diabetes or took dietary supplements/medications known to affect substrate metabolism and exercise capacity (e.g. chromium, niacin, ephedrine).
**Screening**

Subjects underwent a 2-hour Oral Glucose Tolerance test (OGTT) after reading and signing the informed consent documents approved by the University of Massachusetts institutional review board. After a 5-hour minimum fast, individuals had their fasting blood sample was taken from a forearm vein. Subjects consumed 75 grams of glucose and blood samples were collected 1 and 2 hours post-carbohydrate load. Whole-blood glucose was analyzed for determination of blood glucose concentrations. Individuals with elevated fasting glucose concentrations (5.5-6.9mmol/L) and/or elevated 2-hour glucose concentrations (7.8-11.1mmol/L) were included in the study. Participants with blood glucose values higher than 6.9mmol/L (fasted) or 11.1 mmol/L (2-hour) were recommended to seek medical counsel. However, if those same subjects had repeated fasting blood glucose concentrations <6.9mmol/L (indicating they do not have type 2 diabetes) they were enrolled into the study.

**Preliminary testing**

Peak oxygen consumption (VO2peak), i.e. cardio-respiratory fitness, was determined using a continuous progressive exercise test on a cycle ergometer (SensorMedics 800, Yorba Linda, CA). After a 5-minute warm-up, the workload on the cycle ergometer was increased by approximately 30 W every 2 minutes until the subject was unable to maintain a pedal cadence of 60 rpm. During the test, respiratory gases (VO2 and VCO2) were collected by indirect calorimetry using open-circuit spirometry (ParvoMedics Truamax 2400, Consentius Technologies, Sandy, UT). Heart rate was monitored throughout the test (Polar, Inc., Lake Success, NY). VO2peak and Work peak (Wpeak) were defined as the highest value obtained during the test. This test was considered valid if at least 3 of the following criteria are met: a plateau in VO2 (< 150 mL/min) between 2 exercise stages, respiratory exchange ratio (RER) > 1.1, heart rate within 15 beats per minute of age predicted heart rate peak (HRpeak; 220-age), or if the participant voluntarily stops the test. Resting metabolic rate (RMR) was assessed in the
supine position for 30-minutes via indirect calorimetry using open spirometry after an overnight fast. Only the last 2 minutes were used to assess resting energy expenditure and an activity factor of 1.4 was used to estimate total daily energy needed. Body composition was measured using dual-x-ray absorptiometry (DEXA; Lunar Prodigy, Madison, WI) for determination of body fat percentage and fat-free mass (FFM).

**Euglycemic hyperinsulinemic clamp and stable isotope tracer infusion**

Insulin sensitivity (AIM 1) was measured before and after the intervention. Subjects were provided food (55% carbohydrate, 30% fat, and 15% protein) 24 hours prior to testing to standardize diet. After an overnight fast (i.e. 8-12 hours), subjects reported to the laboratory and an indwelling catheter was placed in a superficial vein of each forearm for continuous infusion of glucose stable isotope solution ([6,6-2H glucose]) and venous blood sampling. Baseline blood samples were collected. A priming bolus of 200 mg 6,6-2H glucose was given followed by a 90 minute infusion of 6,6-2H glucose at a rate of 3.0 mg/min as delivered by peristaltic infusion pump (Harvard Apparatus Pump 22, Holliston, MA). Blood samples were collected at 0, 75 and 90 minutes. Breath samples were collected between 80-90 minutes. The last 2 minutes was used for estimation of substrate oxidation. The infusate was then changed to a primed (250 mU/m²·min) constant infusion (80 mU/m²·min) of insulin diluted in saline containing 4% (v/v) of the subject’s own serum. A variable infusion of a glucose and isotope solution (20% glucose + 2% 6,6-2H) was adjusted every 5 minutes to maintain plasma glucose at approximately 5mmol/L over 120 minutes. Blood samples were collected for glucose analysis every 5 minutes, and insulin, isotopic enrichment, and non-esterified fatty acid measures were collected at minutes 15, 60, 75, 90, 105, and 120. Breath samples were also collected between minutes 105-120 of the clamp for determination of whole-body substrate oxidation.
Submaximal Exercise protocol

Specific to aim 2, substrate metabolism during exercise was determined before and after 10 weeks training with or without metformin. Changes in estrogen and progesterone during the female menstrual phase may impact substrate utilization during exercise (9). Women were tested between 5-10 days post-menses and tested after training in the same midfollicular phase; i.e., women were tested after approximately 10 weeks of training because we were timing measures of exercise substrate metabolism around the midfollicular phase.

Subjects reported to the laboratory following a 10-12 hour fast and indwelling catheters were placed in a superficial vein of each forearm for continuous infusion of glucose stable isotope solution ([6,6-2H glucose]) and venous blood sampling. Baseline blood samples were collected and a priming bolus of 200 mg 6,6-2H glucose was given followed by a 90 minute infusion of 6,6-2H glucose at a rate of 3.0 mg/min delivered by peristaltic infusion pump. Blood samples were collected at baseline, 75 and 90 minutes. Subjects were positioned on the cycle ergometry and baseline respiratory gases were collected for 8 minutes. Subjects warmed up for 5 minutes on the cycle ergometer at 25 W. Subjects cycled at 60% of the pre-training VO₂peak for 45 minutes before and after intervention. Two-minute recovery periods at minutes 15 and 25 were provided to ensure exercise compliance. Breath and blood samples were collected at 15, 25, 35, and 45 minutes. Respiratory gases were collected during the first 15 minutes to ensure steady-state conditions had been reached, and subjects continued to wear the one-way mouthpiece with nose clip for 7 minutes prior to each respective time point. Respiratory gases were averaged during the last 2 minutes at each time point for substrate oxidation analysis. Substrate oxidation analysis included the respiratory exchange ratio (RER), total rate of carbohydrate and fat oxidation, and relative percentage of energy from carbohydrate and fat. Blood glucose, lactate, non-esterified fatty acid, glycerol, insulin, rating of perceived exertion (RPE), and heart rate were also collected during the last 2 minutes of each exercise time point.
Subjects were asked to record dietary intake 24-hour prior to exercise testing and were instructed to replicate this diet prior to post-training exercise testing. Dietary compliance was monitored by subjects recording any changes in their diet prior to post-testing. In addition, subjects were instructed to avoid strenuous activity 24-36 hours prior to testing and all subjects were tested at a similar time of day.

**One Repetition Maximum**

To determine the effects of the exercise training on strength, 1 repetition maximum (1-RM) was assessed in all subjects exercising by performing a progressive resistance exercise test until no more weight could be lifted. Subjects were given 5 attempts to reach maximum lifts for: chest press, latissimus pulldown, leg press, bicep curl, triceps pushdown, and upright rows. Weight lifted was considered maximum if proper technique could not be maintained through the lift or the subject voluntarily informed the staff that they could not lift the weight.

**Metformin protocol**

Subjects were screened for the presence of any underlying contraindications (e.g. respiratory disease, heart failure, renal and hepatic disease) by questionnaire. They were advised about the particular risk for lactic acidosis from alcohol consumption while on metformin. Subjects were also advised about possible side effects of metformin including, diarrhea, flatulence, bloating, nausea, metallic tastes and lethargy. Subjects were instructed to take metformin with food in order to minimize side effects. Subjects started treatment with placebo or metformin, at 500 mg/d and increased each week by 500 mg/d until a dose of 2000 mg/d was reached by week 4. Subjects were instructed to take metformin or placebo once in the morning and once in the evening. Subjects remained on this dose for 8 weeks prior to post-testing. If subjects missed a dose, they were instructed to take the next dose at the respective time. They were asked to turn in any pills not taken.
Exercise Training

Detailed dietary, anthropometric and habitual activity data were collected before (PRE), during (MID) and after (POST) the 12 week aerobic and resistance training protocol. Subjects exercised 3 days a week for approximately 60-75 minutes per exercise session (total 4 hours/wk: ~400 kcal per session). Table 3.1 shows the progression of aerobic exercises. On the first and third day of each week, subjects performed aerobic and resistance exercise, while on the second day of the week only aerobic exercise was performed. Cycling was the primary mode of aerobic exercise and subjects cycled on the first and third day of each week.

Table 3.1. Progression of aerobic exercise training

<table>
<thead>
<tr>
<th>Week</th>
<th>Duration (min/d)</th>
<th>Intensity % of Maximum Heart Rate</th>
<th>Frequency (d/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>6 to 8</td>
<td>45</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>10 to 12</td>
<td>45</td>
<td>70</td>
<td>3</td>
</tr>
</tbody>
</table>

On the second day, subjects were able to choose any aerobic exercise (e.g. walking, rowing, stair master, etc) as long as it was at the appropriate intensity/duration. Prior to exercise, all participants warmed-up on a cycle ergometer for 5 minutes. During aerobic training, subjects exercised at 70% of their pre-training heart rate peak for 45 minutes. Subjects wore a heart rate monitor throughout the exercise so heart rate could be maintained at the proper intensity. The last training session was approximately 28 hours before the hyperinsulnemic-euglycemic clamp and conducted on the cycle ergometer (approximate energy expenditure = 250-350 kcal) at 70-75% of pre-training HRpeak for 45 minutes.

Resistance exercise was performed at 70% of the subject’s 1-RM. Weights were increased approximately 5% when 2 sets of 12 repetitions could be lifted while maintaining
proper form. Table 3.2 shows the progression for resistance exercises. The resistance training program targeted all major muscle groups and included: the chest press, latissimus pull down, leg press, bicep curl, triceps pushdown, and shoulder raise. In addition all subjects performed calf-raises and abdominal crunches to ensure a whole-body resistance work out.

**Table 3.2. Progression of resistance training.**

<table>
<thead>
<tr>
<th>Week</th>
<th>Sets (n)</th>
<th>Intensity % of 1 Repetition Maximum</th>
<th>Repetitions (n)</th>
<th>Frequency (d/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>60</td>
<td>8 to 12</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>60</td>
<td>8 to 12</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>60</td>
<td>8 to 12</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>70</td>
<td>8 to 12</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>70</td>
<td>8 to 12</td>
<td>2</td>
</tr>
<tr>
<td>6 to 8</td>
<td>2</td>
<td>70</td>
<td>8 to 12</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>70</td>
<td>8 to 12</td>
<td>2</td>
</tr>
<tr>
<td>10 to 12</td>
<td>2</td>
<td>70</td>
<td>8 to 12</td>
<td>2</td>
</tr>
</tbody>
</table>

**Blood sample collection**

Blood samples were collected in 3 mL syringes, transferred to vacutainers, spun at 3000 rpm and aliquoted to cryotubes for storage at -80°C. Samples for analysis of isotopic enrichment, glucose and lactate were transferred to vacutainers containing sodium fluoride (to inhibit glycolysis). Samples for analysis of insulin and non-esterified fatty acid s were also collected in vacutainers containing EDTA (an anticoagulant).

**Blood sample analysis**

Plasma glucose and lactate concentrations were determined enzymatically using a glucose/lactate analyzer (GL5 Analyzer, Analox Instruments, Lunenberg, MA). Insulin was measured by radioimmunoassay (Millipore, St. Charles, MO). Non-esterified fatty acid s were measured by enzymatic colorimetry (Wako Chemicals, Richmond, VA). Glycerol was measured by spectrophotometry (Sigma Aldrich, St. Louis, MO). Glucose isotopic enrichment was measured by high performance liquid chromatography and mass spectrometry (HPLC-MS).
Stored samples were removed from the -80°C freezer and thawed to room temperature. In separate microcentrifuge tubes, 300 µL of sample was added into 970 µL of chilled acetone, vortexed and chilled in the freezer for 10 minutes. After chilling, the samples were centrifuged at 17,000 g for 2 minutes. The supernatant was collected using a 1 mL plastic syringe, and filtered through a 4 mm, 0.45 µm polyethersulfone, syringe filter into an HPLC vial containing a glass insert. The samples were capped and placed in the autosampler compartment for immediate analysis. A 10 µL sample was injected and separated on a Shodex Asahipak NH2P-50 4E Analytical column, (4.6 X 250 mm, Thompson Instrument Co.) installed on an Agilent 1100 series HPLC equipped with a mass spectrometer detector (Bruker). Selected ion monitoring was used to compare the abundance of the unlabelled fragment with that of the enriched isotope (Chemstation Software). After correcting for background enrichment, the abundance of the dideuterated isotope (m/z = 205) was expressed as percentage of total glucose species (m/z = 203+204+205).

**Calculations**

Whole-body insulin sensitivity was the primary outcome measure. Insulin sensitivity was defined as the rate of blood glucose disposal (Rd) per unit plasma insulin concentration (steady-state plasma insulin: SSPI). Whole-body insulin sensitivity can be calculated during the glucose clamp in two ways:

1) The rate of blood glucose disposal was calculated as the average exogenous glucose infusion rate (GIR) during the final 30 minutes of the clamp per SSPI (GIR/SSPI). This method assumes that endogenous hepatic glucose production is zero or negligible, an assumption that may not be valid in individuals with prediabetes. Since we have a direct measurement of endogenous glucose production via glucose isotope tracers, we can make more accurate measures of total blood glucose uptake.
2) Using isotope-derived glucose flux rates per SSPI: Glucose rates of appearance (Ra) and rates of disposal (Rd) was calculated using the non-steady state equations (122).

\[ \text{Glucose Ra (mg/min)} = \frac{F-V[(C1 + C2) / 2] [IE2-IE1] / (t2-t1)]}{[IE2 + IE1] / 2} \]

\[ \text{Glucose Rd (mg/min)} = Ra - V[(C2-C1) / (t2-t1)]. \]

Where F is the isotope infusion rate, IE1 and IE2 are enrichments of plasma glucose with isotope label at time t1 and t2, C1 and C2 are plasma glucose concentrations, V is the estimated volume of distribution for glucose (180 mL/kg).

To calculate whole-body insulin sensitivity, the Rd was scaled to the steady-state insulin concentrations (SSPI). The Rd/SSPI was calculated using measures from the last 30 minutes of the clamp at minutes 90, 105, and 120. Non-oxidative glucose disposal (NOGD) is usually assumed to be reasonably equivalent to glucose storage of muscle glycogen. Expired breath samples were collected using indirect calorimetry and used to calculate carbohydrate oxidation (72). NOGD and carbohydrate oxidation was calculated during the last 30 minutes of the clamp:

3. NOGD (mg/min) = Rd – CHO oxidation rate.

To calculate hepatic insulin sensitivity, the suppression of basal hepatic glucose production (HGO_{basal}) during the last 30 minutes of the clamp was calculated as:

4. 1 – (HGO_{inf}/HGO_{basal}) * 100.

HGO_{basal} is the Ra in the basal state and is defined by the average of 75 and 90min. HGO during the infusion (HGO_{inf}) is calculated as: (steady state Ra) – (glucose infusion rate).

The percent suppression of non-esterified fatty acids (NEFA) during insulin stimulation is defined as suppression of circulating NEFA during the last 30 minutes of the clamp compared to basal values and was calculated as:

5. 1 – NEFA_{clamp}/NEFA_{basal} * 100.
Calculations a and b were also used for the submaximal exercise test to determine glucose flux (i.e. Ra and Rd). In addition, several calculations were used to determine carbohydrate and fat utilization (calculations 4-7). Although there are limitations with the use of indirect calorimetry as an estimate of substrate oxidation, it has been validated by Romijn et al (100) using the $^{13}$C/$^{12}$C ratio technique and concluded that indirect calorimetry could be used to accurately determine carbohydrate and fat oxidation at exercise intensities up to 85% VO$_2$peak. Thus, energy derived from carbohydrate and fat was determined as (72):

4. RER = VCO$_2$/VO$_2$

5. Percent of energy from carbohydrate (CHO) and fat

\[
\text{% Energy CHO} = \frac{[\text{RER} - 0.71]}{0.29} \times 100
\]

\[
\text{% Energy Fat} = 100 - \frac{[\text{RER} - 0.71]}{0.29} \times 100
\]

6. Rate of total CHO and fat oxidation (91).

CHO oxidation rate (g/min) = 1.6946 VO$_2$ – 1.7012 VCO$_2$

Fat oxidation rate (g/min) = 4.5850 VCO$_2$ – 3.2255 VO$_2$

7. An estimate of muscle glycogen utilization (EMGU) was determined by:

\[
\text{EMGU (mg/min)} = \text{Total CHO oxidation rate} - \text{blood glucose Rd}
\]

Calculation 7 is based on the assumption that 100% of blood glucose taken up from the blood is oxidized. This assumption is unlikely to be true, i.e. the percent of Rd oxidized is probably 70-90% (36). The calculation thus underestimates glycogen use and is best described as minimal muscle glycogen utilization.

**Statistics**

The sample size was estimated to provide sufficient statistical power to detect whether the combination of exercise and metformin would elicit larger changes compared to either treatment alone. The most metabolically and clinically relevant outcome measure on which to base the calculation was whole-body insulin sensitivity. Based on the literature, it was
anticipated that insulin sensitivity would increase approximately 30% with a standard deviation of 20% with exercise training. Metformin alone is expected to raise whole-body insulin sensitivity approximately 15% with a standard deviation of 10%. Based on these estimates for metformin versus exercise training and metformin (effect size = 0.69), power was set at 80% and an alpha level set to 0.05. Power analysis indicated that 8 subjects per group would be needed to detect differences between conditions.

Baseline characteristics (e.g. body weight, BMI, body fat %, VO₂peak, etc) were measured with a one-way analysis of variance (ANOVA) for AIM 1 and AIM2. Statistical differences in baseline variables (e.g. body weight, VO₂peak, insulin sensitivity, etc.) were treated as covariates in order to determine the independent effects of the exercise and/or metformin intervention. For AIM 1, group means of: whole-body insulin sensitivity, hepatic insulin sensitivity, adipose insulin sensitivity, fasting blood glucose, insulin, non-esterified fatty acid s, non-oxidative glucose disposal, and substrate oxidation (i.e. carbohydrate and fat oxidation) before and during the glucose clamp were compared at baseline and after the intervention with a two-way (time by condition) repeated measures analysis of variance (ANOVA). Tukey’s post-hoc analysis was used to detect group mean differences when there is a significant interaction. Pearson’s product-moment correlation coefficient would be used to examine the relationship between VO₂peak, adipose insulin sensitivity, body weight, and non-oxidative glucose metabolism and whole-body insulin sensitivity. Correlation analysis were done comparing NEFA and lactate concentrations and hepatic glucose output.

For AIM 2, group means of: RER, whole-body carbohydrate and fat oxidation, relative percentage of energy from carbohydrate and fat use, blood glucose, lactate, insulin, non-esterified fatty acid s and glycerol were compared at baseline and after the intervention with a three-way (group by time by condition) repeated measures ANOVA. Tukey’s post-hoc analysis was used to detect group mean differences when there was a significant 3-way interaction. Pearson’s product-moment correlation coefficient was used to examine the relationship between
VO_{2}\text{peak}, NEFA, glycerol, rates of glucose appearance, estimated muscle glycogen utilization and fat oxidation during exercise.

**Expected Results & Interpretations**

There are several hypotheses to be tested by AIM 1, and the outcome measures, compared to placebo, are outlined in the below table (Table 3.3).

**Table 3.3. Predicted results for outcomes in AIM 1.**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Metformin</th>
<th>Exercise Training and Placebo</th>
<th>Exercise Training and Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-body insulin sensitivity</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Hepatic insulin sensitivity</td>
<td>↑</td>
<td>Slight ↑</td>
<td>Slight ↑</td>
</tr>
<tr>
<td>Adipose insulin sensitivity</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Fasted hepatic glucose output</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose and insulin</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Fasting NEFA</td>
<td>Slight ↓</td>
<td>Slight ↓</td>
<td>Slight ↓</td>
</tr>
<tr>
<td>Fasting Fat oxidation</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Insulin stimulated CHO oxidation</td>
<td>↑</td>
<td>↔</td>
<td>Slight ↑</td>
</tr>
<tr>
<td>Non-oxidative CHO metabolism</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Metabolic flexibility</td>
<td>↔</td>
<td>↑</td>
<td>Slight ↑</td>
</tr>
</tbody>
</table>

Note: All data are compared to placebo results; carbohydrate (CHO), non-esterified free fatty acid (NEFA).

In general, exercise training and metformin was expected to produce additive effects on metabolic health. However, not all outcomes are expected to have additive effects. The predicted results are discussed below

1. Combining exercise and metformin was expected to have additive effects on whole-body insulin sensitivity, compared to either treatment alone. Combining exercise and metformin was expected to enhance insulin sensitivity because each therapy has different effects on liver, skeletal muscle, and to a lesser extent, adipose tissue insulin sensitivity. Exercise primarily enhances skeletal muscle glucose uptake by increasing
non-oxidative glucose metabolism. Metformin has secondary effects on skeletal muscle glucose uptake and non-oxidative glucose. As a result, it is expected that non-oxidative glucose metabolism would be related the enhanced whole-body insulin sensitivity with the combined treatment. Moreover, fasting blood glucose and insulin concentrations are both expected to be lower, compared to either treatment alone. Lower concentrations of glucose and insulin are expected not only because of enhanced insulin sensitivity, but also larger reductions in the rate of glucose appearance.

2. Combining exercise training and metformin may be as effective as exercise training or metformin alone on hepatic and adipose insulin sensitivity, fasting concentrations of NEFA and insulin-stimulated carbohydrate oxidation. Exercise training enhances hepatic and adipose insulin sensitivity in some studies; however, these effects are often negligible or of smaller magnitude when compared to skeletal muscle insulin sensitivity. Metformin primarily effects hepatic insulin sensitivity, but has less of an effect on adipose insulin sensitivity. Improvements in hepatic and adipose insulin sensitivity are often related to changes in NEFA concentrations. The evidence is mixed as to the effects of each treatment on lowering NEFA concentrations. Thus, combining exercise with metformin may produce similar effects, compared to each treatment alone, on hepatic and adipose tissue insulin sensitivity.

3. Combining exercise training and metformin may produce opposing effects on fasting fat oxidation and metabolic flexibility, compared to exercise training with placebo. Exercise training typically elevates fasting fat oxidation, while metformin lowers fat oxidation. Because metformin reduces fat oxidation and produces a compensatory rise in fasting carbohydrate oxidation, elevated carbohydrate use was expected to decrease metabolic flexibility. During insulin stimulation, exercise training generally does not alter
carbohydrate oxidation, but rather increases non-oxidative glucose disposal. Metformin has mixed results on increasing non-oxidative glucose disposal and raising insulin-stimulated carbohydrate oxidation. As a result, combining exercise and metformin may either decrease metabolic flexibility or not effect it at all.

There are several hypothesis to be tested by AIM 2, and the outcome measures are outlined in the below table (Table 3.4).

Table 3.4. Predicted results for outcomes in AIM 2.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Exercise training and Placebo</th>
<th>Exercise training and Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory exchange ratio (RER)</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Percentage of energy from fat</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Glucose</td>
<td>↔</td>
<td>Slight ↓</td>
</tr>
<tr>
<td>Lactate</td>
<td>↓</td>
<td>Slight ↓</td>
</tr>
<tr>
<td>Insulin</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>NEFA</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Glycerol</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Estimated Muscle Glycogen Use</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Rate of Glucose Appearance</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Rate of Glucose Disposal</td>
<td>↓</td>
<td>↓↓</td>
</tr>
</tbody>
</table>

Note: Exercise training with or without metformin results are compared to baseline.

In general, exercise training and metformin was expected to produce additive effects on fat oxidation during exercise. However, not all outcomes were expected to have additive effects.

The predicted results are discussed below:

1. Combining exercise training with metformin, compared to exercise training with placebo, was expected to have additive effects on enhancing whole-body fat oxidation. Training enhances fat oxidation during exercise by, in part, reducing the rate of glucose appearance and increasing skeletal muscle oxidative capacity. Adding metformin to
training is expected to enhance exercise fat oxidation because metformin constrains hepatic glucose output and enhances oxidative enzymes. If metformin constrains hepatic glucose output (i.e. decrease rate of glucose appearance), then lower blood glucose concentrations would be expected. Lower rates of glucose appearance would also be expected to decrease glucose disposal thereby favoring elevated rates of fat oxidation. Increases in oxidative capacity would also favor elevated fat oxidation, which would reduce skeletal muscle glycogen use. Decreased glucose flux would also lower insulin concentrations, which would favor increased concentrations of NEFA and glycerol for elevated fat oxidation.

2. Combining exercise training with metformin, compared to exercise training with placebo, might increase glucose flux and carbohydrate oxidation during exercise. Elevated carbohydrate oxidation would be contrary to the original hypothesis, but it is a plausible alternative outcome based on the effect of metformin to stimulate glucose uptake and warrants recognition. Training generally lowers the rate of glucose appearance, the rate of glucose disposal and estimated muscle glycogen use during exercise. Metformin however has the potential to stimulate skeletal muscle glucose uptake during exercise and raise muscle glycogen concentrations in the rested state. Exercise increases skeletal muscle glucose uptake, compared to rest, and adding metformin may accentuate this effect during exercise training. Because metformin increases resting concentrations of muscle glycogen, and muscle glycogen concentrations are positively related to the rate of glycogen use during exercise, it was also possible that metformin may cause greater skeletal muscle glycogen utilization during exercise. If metformin enhances glucose flux, it was possible that the combined treatment would not enhance fat oxidation during exercise and conceivably favor carbohydrate oxidation.
Confounding factors

1. Reductions in energy intake and/or changes in macronutrient composition (e.g. high carbohydrate to low carbohydrate diet) may lead to weight loss. Changes in these dietary habits may lead to weight loss and are thus potential confounding factors. By design, dietary changes were not rigidly controlled during the course of the study and weight loss or gain was allowed to fluctuate. Metformin tends to cause minor weight loss and preventing this by having subjects maintain energy balance throughout the study would reduce the clinical utility of the findings (89). Instead, weight will be recorded biweekly throughout the 12 week protocol. Statistically, weight loss was used as a co- variate to account for the potential confounding effects of weight loss on insulin sensitivity.

2. Daily habitual physical activity may be a potential confounding factor. By design, habitual physical activity was not rigidly controlled during the course of the study and activity levels were allowed to fluctuate. The effects of exercise training on habitual physical activity patterns is mixed. Some studies show that exercise training causes individuals to decrease habitual activity levels, whereas others show it has no effect (50). Individuals were encouraged to maintain their normal activity levels at the beginning of the study. Habitual physical activity was recorded by use of a pedometer at the beginning, middle and end of the study to document if any changes in habitual physical activity occur. Statistically, habitual activity was used as a co-variate to account for the potential confounding effects of weight loss on insulin sensitivity.

3. Prediabetes pathology may be a potential confounding factor. By design, individuals with impaired glucose tolerance (IGT) were enrolled into the study in the presence or absence of impaired fasting glucose tolerance (IFG). As a result, some individuals may be enrolled with IGT only or IFG+IGT. Individuals with IGT are primarily characterized as having peripheral insulin resistance. Individuals with IFG+IGT are characterized by
hepatic and peripheral insulin resistance. Given that exercise targets skeletal muscle, while metformin targets the liver, it is possible that the combined treatment may affect prediabetes pathologies differently.

4. Subjects were not blinded to exercise training and this may be a potential confounding factor. By design, exercise training was not blinded (i.e. placebo and metformin conditions were not provided activity protocols) because the primary objective was to determine if exercise training with metformin was better than either treatment alone. If metformin was provided with light activity protocols, then it would not address the overall question in this study. Many individuals with prediabetes may take metformin without exercise/activity. Thus, having metformin and placebo perform light activity protocols could reduce the clinical utility of the findings as.
References


CHAPTER 4

METFORMIN WITH EXERCISE TRAINING & INSULIN SENSITIVITY

Title: Independent and combined effects of exercise training and metformin on insulin sensitivity in individuals with prediabetes.

Authors: Steven K. Malin, Robert Gerber, Stuart R. Chipkin, and Barry Braun

Energy Metabolism Laboratory, Department of Kinesiology, University of Massachusetts, Amherst, MA 01003

Supported by NIH 5 R56 DK081038

Running Head: Combining Exercise and Metformin on Insulin sensitivity

Correspondence to:
Barry Braun, PhD
Dept. of Kinesiology
107 Totman Building
University of Massachusetts
Amherst, MA 01003
Phone: (413) 577-0146
Fax: (413) 545-2906
Email: bbraun@kin.umass.edu
Abstract

Physical activity or metformin contribute to slowing the progression from prediabetes to type 2 diabetes. Combining the two treatments may have more potent effects on insulin sensitivity because each targets a different tissue. **Purpose:** To evaluate the effects of exercise training plus metformin on insulin sensitivity in men and women with prediabetes, compared to each treatment alone. **Methods:** Thirty-two men and women with prediabetes were assigned to: placebo (P), metformin (M), exercise training with placebo (EP), or exercise training with metformin (EM). P and 2000mg/d of M were distributed in a double-blind fashion and half of the subjects underwent exercise training. Insulin sensitivity was measured by the euglycemic hyperinsulinemic (80 mU/m2/min) clamp enriched with [6,6-²H] glucose. **Results:** All interventions enhanced insulin sensitivity (p < 0.05) independent of weight loss. EP enhanced insulin sensitivity 25-30% more than either EM or M. Higher insulin sensitivity was strongly correlated with increased glucose storage (r = 0.85; p < 0.01). **Conclusions:** Twelve weeks of exercise training or metformin enhances insulin sensitivity in individuals with prediabetes, but there was no additive effect of combining treatments. Higher insulin sensitivity was mainly explainable by increased glucose storage. Subtle differences among condition means suggest that adding metformin blunts the full effect of exercise training.

**KEY WORDS:** Impaired glucose tolerance, type 2 diabetes, insulin resistance, exercise
**Introduction**

Approximately 79 million individuals in the U.S. have prediabetes and are at high risk to develop type 2 diabetes (1, 17). Exercise lowers diabetes risk by, in large part, enhancing skeletal muscle insulin sensitivity (9, 11, 13, 14, 16). Metformin decreases diabetes risk through alternative mechanisms; it mainly reduces hepatic glucose output (7, 8, 15).

Because exercise or metformin primarily targets different tissues, it is possible that combing the two treatments would have more potent effects on insulin sensitivity (20). There are few studies testing the efficacy of combining lifestyle modification with metformin (4, 6, 18, 22). Despite some showing that the combined treatment promotes 2-5kg more weight loss than the lifestyle modification alone (4, 18), there is little (4, 6) or no further (26) improvement in insulin sensitivity. However, the direct effects of supervised exercise training plus metformin on insulin sensitivity (via euglycemic clamp) remains unclear because exercise was previously based on self-reports (4, 6, 18, 22) and insulin resistance was estimated from fasting and/or post-carbohydrate load measures (4, 6, 22). Therefore, the purpose of this study was to determine the effect of combining exercise training with metformin on insulin sensitivity in individuals with prediabetes, compared to either treatment alone.

**Methods**

*Overview:* In a double-blind, placebo-controlled study design, 32 men and women with impaired glucose tolerance (IGT) were enrolled in this study. Prior to testing, individuals were assigned to either: placebo (P), metformin (M), exercise training with placebo (EP) or exercise training with metformin (EM) for the determination of these treatments effects on insulin sensitivity.

*Subjects:* Subjects were non-smoking, weight stable (<5% weight change over last 3mo), free of cardiovascular disease or diabetes, and did not take dietary supplements (e.g. chromium, niacin) or medications (sulfonylureas, acarbose, etc) that are likely to affect insulin sensitivity.
Subjects were excluded if they had any contraindications to metformin (e.g. respiratory disease, heart failure, renal and hepatic disease). Subject characteristics are outlined in Table 4.1. Prior to testing, all subjects were verbally briefed about the study and signed informed consent documents approved by the Institutional Review Board at the University of Massachusetts Amherst.

*OGTT Screening:* An oral glucose tolerance test (OGTT) was used to determine if subjects had prediabetes. After a minimum 5-hour fast, blood samples were taken from a forearm vein. Subjects consumed 75 grams of glucose and blood samples were collected 2 hours later. All subjects had impaired glucose tolerance (IGT; 2-hour glucose concentrations between 7.8-11.1mmol/L or 140-199mg/dl). Subjects with IGT who had fasting glucose concentrations between 5.5-6.9mmol/L (100-125mg/dl) were also included.

*Metformin or Placebo protocol:* Pills were administered to the subjects and they were instructed to take metformin or placebo with food in order to minimize potential side effects. Subjects started treatment with 500 mg/d of metformin. The dose was increased 500 mg/d each week until a clinical dose of 2000 mg/d was reached by week 4. Subjects remained at this dose for the last 8 weeks of the 12 week protocol.

*Preliminary testing:* Peak oxygen consumption (VO$_2$peak) was determined using a continuous progressive exercise test on a cycle ergometer (SensorMedics 800, Yorba Linda, CA). VO$_2$peak was defined as the highest value obtained during the test (19). One repetition max (1-RM) tests were conducted for the: chest press, latissimus pull down, leg press, bicep curl, triceps pushdown, and upright rows. 1-RM was defined as the highest weight lifted with proper technique through the full range of motion. Dual-x-ray absorptiometry (DEXA; Lunar Prodigy, Madison, WI) was used for determination of body fat, central fat (i.e. from the last floating rib to the top of the iliac crest divided by total body fat mass) and fat-free mass (FFM) (5).

*Euglycemic hyperinsulinemic clamp:* Subjects were provided food (55% carbohydrate, 30% fat, and 15% protein) 24 hours prior to pre- and post-testing. After an overnight fast, subjects
reported to the laboratory and indwelling catheters were placed in superficial veins of a forearm for the collection of baseline blood samples. A priming bolus of 200 mg 6,6-$^2$H glucose was given followed by a 90 minute infusion of 6,6-$^2$H glucose at a rate of 3.0 mg/min by peristaltic infusion pump (Harvard Apparatus Pump 22, Holliston, MA). Blood samples were collected at 75 and 90 minutes. Expired breath samples were collected between 80-90 minutes, with the last 2 minutes used to estimate substrate oxidation. A primed (250 mU/m$^2$·min) constant infusion (80 mU/m$^2$·min) of insulin diluted in saline containing 4% (v/v) the subject’s own serum was started. After 20 minutes of insulin infusion, a 20% glucose solution containing 2% 6,6-$^2$H was infused at a variable rate to maintain plasma glucose at 5mM for the remaining 100 minutes. Blood samples were collected for glucose analysis every 5 minutes, and for the measurement of insulin, isotopic enrichment of glucose, and non-esterified fatty acids (NEFA) at minutes 75, 90, 105, and 120. Expired breath samples (VO$_2$ and VCO$_2$L/min) were collected between minutes 110-120 of the clamp for determination of insulin-stimulated substrate oxidation. Twenty-eight to thirty hours before post-intervention measurements, subjects assigned to the training conditions performed a standardized exercise bout. Exercise was conducted on a cycle ergometer at 75% of pre-training HRpeak for 45 minutes. Based on heart rate, exercise intensity was not different between EP and EM (EP = 125.4 ± 4.7 vs. EM = 122.2 ± 4.6; p = 0.64).

*Exercise Training*: Exercise was supervised 3-days a week for 60-75 minutes per session (total 3.5 hours/wk: ~400 kcal per session). Subjects performed aerobic and resistance exercise on the first and third day of each week. To minimize muscle soreness, only aerobic training was performed on the second day. Participants warmed-up on a cycle ergometer for 5 minutes, followed by cycling at 70% of their pre-training HRpeak for 45 minutes. Resistance exercise was performed at 70% of the subject’s 1-RM. Weight was increased approximately 5% when 2 sets of 12 repetitions could be lifted with proper form. Resistance training targeted all major muscle conditions and included: the chest press, latissimus pull down, leg press, bicep curl, triceps pushdown, shoulder raise, calf raises, and abdominal crunches.
Blood sample collection: Blood samples were collected in 3 mL syringes, transferred to vacutainers, spun at 3000 rpm, and plasma was aliquoted to cryotubes for storage at -80°C. Samples for the analysis of glucose isotopic enrichment, glucose and lactate were transferred to vacutainers containing sodium fluoride to inhibit glycolysis. Plasma samples for the analysis of insulin and NEFA were collected in vacutainers containing the anticoagulant EDTA.

Analysis of metabolites and hormone: Plasma glucose and lactate concentrations were determined enzymatically using a glucose/lactate analyzer (GL5 Analyzer, Analox Instruments, Lunenberg, MA). Plasma insulin concentrations were measured by radioimmunoassay (Millipore, St. Charles, MO). Plasma NEFA concentrations were measured by enzymatic colorimetry (Wako Chemicals, Richmond, VA). Glucose isotopic enrichment was measured by high performance liquid chromatography and mass spectrometry as previously described (25).

Calculations: Standard equations were used to determine glucose rates of appearance (Ra) and disappearance (Rd) (27). Insulin sensitivity was defined as the Rd per unit plasma insulin (I) during the final 30 minutes of the clamp. Basal hepatic glucose production, i.e. Ra, was averaged during minutes 75 and 90 of the resting isotope infusion. Endogenous hepatic glucose production (HGP) during the clamp was defined as the difference between HGPclump and the exogenous glucose infusion rate. The suppression of HGP was defined as [1-(HGPclump/HGPfast)*100%]) and it was used to provide an estimate of hepatic insulin sensitivity. Insulin stimulated suppression of NEFA was defined as: [1-(NEFACLAMP/NEFAFAST)*100%]. Carbohydrate oxidation was determined by indirect calorimetry using standard equations (21). Non-oxidative glucose disposal (NOGD) was calculated during the final 30 minutes of the clamp (NOGD (mg/min) = Rd – rate of carbohydrate oxidation).

Statistical Analysis: Condition means were compared using the R statistical software package (version 2.4.0, The R foundation, Vienna, Austria, 2006). Mean differences among conditions in baseline characteristics were assessed with a one-way analysis of variance (ANOVA). There was no statistical difference in any baseline outcome variable. Outcomes were assessed using
a two-way (condition by test) repeated measures ANOVA. Baseline insulin sensitivity, weight loss, and changes in VO$_2$peak were included as covariates to independently test the effects of each treatment. Using the changes in cardiorespiratory fitness or weight loss as covariates did not did not alter the treatment effects on insulin sensitivity. When there was a significant interaction, Tukey’s post-hoc analysis was used to determine differences between conditions and paired t-tests were used to compare within condition means. Pearson’s correlation coefficient was used to examine relationships. Significant differences were accepted as $\alpha \leq 0.05$.

**Results**

**Anthropometrics and Cardio-respiratory fitness.** Metformin (M) and exercise training plus metformin (EM) reduced body weight by approximately 4kg compared to placebo (P) ($p < 0.05$) and exercise training plus placebo (EP) ($p = 0.07$; Table 4.2). Although both exercise conditions reduced body fat ($p < 0.01$) and central fat ($p = 0.056$), only EP increased fat free mass compared to P ($p < 0.02$; Table 4.2). Both exercise conditions increased VO$_2$peak ($p < 0.05$; Table 4.2).

**Fasting Hormone, Metabolites and Substrate use.** Although M, EP, and EM lowered plasma insulin concentrations 13-25% (effect of time: $p < 0.05$), only the exercise conditions lowered c-peptide concentrations compared to baseline ($p < 0.05$; Table 4.3). Plasma glucose concentrations and fasting carbohydrate oxidation did not change after any treatment (Table 4.3).

**Peripheral Insulin Sensitivity.** M, EP and EM enhanced insulin sensitivity ($p < 0.05$; Figure 4.1) relative to baseline. EP enhanced insulin sensitivity 25-30% more than EM and M. All 3 treatments increased non-oxidative glucose disposal (NOGD; $p < 0.05$; Figure 4.2) relative to baseline. EP increased NOGD 60-70% more than EM and M. There was no effect of treatment on insulin stimulated carbohydrate oxidation.
**Hepatic glucose output and insulin sensitivity.** Fasting hepatic glucose output (HGP\textsubscript{fast}) did not change after any treatment (p = 0.2; Table 4.3). Hepatic insulin sensitivity, defined as suppression of HGP\textsubscript{fast} during the clamp, was also unaffected (Table 4.5).

**Non-esterified Fatty Acids (NEFA).** EP decreased fasting NEFA concentrations, but EM increased NEFA concentrations (p < 0.02; Table 4.3). Exercise and/or metformin had no effect on insulin stimulated suppression of NEFA (Table 4.3).

**Correlations:** Increased maximal oxygen consumption (r = 0.57; p < 0.05), NOGD (r = 0.85; p < 0.01) and weight loss (r = -0.42, p < 0.05) were directly correlated with enhanced insulin sensitivity. Higher fasting NEFA concentrations were correlated with smaller improvements in insulin sensitivity (r = -0.42; p < 0.05). (see S.A.1, S.A.2, S.A.3, and S.A.4).

**Discussion**

Contrary to our original hypothesis, adding metformin partially blunted the effects of exercise training on insulin sensitivity in this group of men and women with prediabetes.

Combining lifestyle modification with metformin has been shown to have both additive and non-additive effects. Ateback et al (4) reported that lifestyle modification plus metformin reduced body mass, fasting hyperinsulinemia and 2-hour plasma insulin concentrations in obese adolescents compared to lifestyle modification alone. Love-Osbourne et al (18) demonstrated that lifestyle modification plus metformin caused more weight loss than lifestyle modification alone, and weight loss was correlated to lower 2-hour blood glucose concentrations. In other studies, combining metformin with lifestyle modification caused little additional health benefits (6, 22, 25). The Indian Diabetes Prevention Program (IDPP) showed that low dose metformin (e.g. 500mg/d), lifestyle modification, and the combined treatment equally improved insulin sensitivity and reduced the progression from prediabetes to type 2 diabetes (26). Our findings are consistent with the previous 3 studies (6, 22, 25) indicating that
the combination of exercise and metformin does not enhance insulin sensitivity more than either treatment alone.

Metformin did not add to the effects of training on insulin sensitivity and there are several potential reasons. Metformin may have affected the peripheral adaptations to training that contributed to enhanced insulin sensitivity. Sharoff et al. (25) demonstrated that short-term metformin treatment blunted AMPK activation following a single bout of exercise. If metformin attenuated AMPK throughout training in our study, then GLUT4, hexokinase, and glycogen synthesis adaptations may have been minimized (12). Without direct measurements of cellular mediators or muscle glycogen concentrations, it is not possible to know the role of these factors on insulin sensitivity. However, cardiorespiratory fitness and strength were not statistically different between exercise conditions, indicating that fitness differences are unlikely to explain the current findings.

Weight loss enhances insulin sensitivity (23, 24) Although EM and M promoted approximately 4kg more weight loss than EP, training alone enhanced insulin sensitivity the most. Both exercise conditions enhanced insulin sensitivity more than metformin alone and this was paralleled by similar reductions in body fat, which is strongly related to insulin sensitivity (10). Thus, similar reductions in body fat may explain why training, with or without metformin, enhanced insulin sensitivity more than metformin alone. However, similar reductions in body fat between exercise conditions, suggest other mechanisms may explain how metformin blunts the full effects of training.

Elevated non-esterified fatty acid (NEFA) concentrations impair insulin sensitivity. We previously showed that combining metformin with a single bout of exercise raised NEFA concentrations during the clamp and was associated with the blunted improvement in insulin sensitivity (25). In this study, exercise training plus metformin raised fasting NEFA concentrations, compared to training alone. Although this was moderately associated with attenuated insulin sensitivity, there were no differences in NEFA concentrations during the
clamp (data not shown). Thus, elevated NEFA concentrations are unlikely to be the primary mechanism explaining the attenuated improvements in insulin sensitivity when combining metformin with training. More sophisticated measures using lipid isotopes and/or muscle biopsies would be needed to substantiate the role of fat metabolism on insulin sensitivity after combining metformin and training.

We originally hypothesized that combining exercise with metformin could have additive effects on insulin sensitivity because exercise influences skeletal muscle, while metformin affects the liver. In this study, M or EM had no effect on fasting hepatic glucose production (HGP) or hepatic insulin sensitivity. The insulin concentrations during the clamp did not fully suppress HGP (~85%), suggesting that our subjects had hepatic insulin resistance. It’s possible that hepatic insulin sensitivity varied within our subjects and limited our ability to detect statistical differences. Baseline hepatic insulin sensitivity, defined as the suppression of HGP_{fasting} or the product of HGP_{fasting} and fasting insulin concentrations (3; data not shown), did not correlate with enhanced peripheral insulin sensitivity. Thus, variations in hepatic insulin resistance are unlikely to explain why metformin was ineffective at lowering HGP, or why adding metformin to training blunted improvements in insulin sensitivity.

Although not quite statically significant, the 25-30% difference in insulin sensitivity between training and M or EM (see Figure 4.3) may be physiologically meaningful. First, our sample size may have been too small to detect a difference of that magnitude. A post-hoc power analysis indicated that 16 individuals per condition would have been needed for 80% power to detect a statistical difference at an alpha of 0.05. Second, prediabetes pathology may have increased inter-individual variability between conditions (2). Prediabetes is defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both. We included individuals with IGT or IFG + IGT because they are insulin resistant (2). The response to all 3 interventions was accentuated in the individuals with fasting hyperglycemia, i.e. those with IGT + IFG. (see Figure 4.3). Based on these results, it would be fruitful to study the effects of
exercise and/or metformin on subgroups of prediabetes to better understand the effects of fasting hyperglycemia (see S.A.4 and S.A.5).

Summary: Exercise training, independent weight loss, effectively enhanced insulin sensitivity in individuals with prediabetes. Adding metformin to training did not accentuate improvements in insulin sensitivity, and it may have blunted the full effects of training. We caution that our results not be interpreted to indicate that combining exercise with metformin is not a useful therapeutic strategy to improve metabolic health. Further work is required to identify the effects of combining metformin with training on cardiovascular risk factors as well as insulin sensitivity in different subsets of prediabetes.

Acknowledgments

S.K.M, S.R.C, and B.B. contributed to the study design and data collection. R.G. researched data and contributed to data analysis. S.K.M was primarily responsible for data analysis and statistical integrity. S.K.M wrote the manuscript and S.R.C and B.B. reviewed/edited the manuscript. The authors would like to thank Kirsten Granados and Richard Viskochil for technical assistance and helpful discussion. We also thank John Staudenmeyer, PhD for statistical consulting and the dedicated undergraduate research assistants, trainers and participants for their time and effort.

This research was supported by NIH 5 R56 DK081038.
References

1. CDC Diabetes Fact Sheet. 2011.


Figure Captions

Figure 4.1. Insulin Sensitivity across all conditions. *Compared to baseline (p < 0.05).
^Compared to placebo (p < 0.05). Values are mean ± standard error of the mean.
Figure 4.2. Non-oxidative glucose disposal across all conditions. †Significant effect of test (p < 0.05). *Compared to baseline (p < 0.05). ^Compared to placebo (p < 0.05). Values are mean ± standard error of the mean.
Figure 4.3. Prediabetes classification and response to each condition. P consisted of 4 IFG+IGT and 4 IGT. M consisted of 3 IFG+IGT and 5 IGT. EP and EM consisted of 5 IFG+IGT and 3 IGT. Values are reported as mean change.
Table Captions

Table 4.1. Subject Characteristics. No significant differences were observed between conditions. Values are mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>M</th>
<th>EP</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>49.8 ± 10.9</td>
<td>45.0 ± 7.5</td>
<td>45.4 ± 8.0</td>
<td>49.1 ± 6.6</td>
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<tr>
<td>Height (cm)</td>
<td>168.1 ± 8.5</td>
<td>165.9 ± 7.1</td>
<td>173 ± 4.3</td>
<td>168.1 ± 8.5</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>34.0 ± 6.3</td>
<td>33.9 ± 5.2</td>
<td>33.5 ± 4.1</td>
<td>31.2 ± 5.3</td>
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<tr>
<td>Fasting Glucose (mM)</td>
<td>5.8 ± 0.6</td>
<td>5.6 ± 0.6</td>
<td>5.4 ± 0.4</td>
<td>5.5 ± 0.8</td>
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<td>2-hour Glucose (mM)</td>
<td>9.4 ± 1.3</td>
<td>9.3 ± 1.5</td>
<td>10.2 ± 1.0</td>
<td>9.5 ± 1.7</td>
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</table>
Table 4.2. Body weight, cardiorespiratory fitness and strength. †Significant effect of test (p < 0.05). *Compared to baseline (p < 0.05). ^Compared to placebo (p < 0.05). #Compared to metformin (p < 0.05). Strength refers to the sum of chest press, leg press and latissimus pulldown. Values are mean ± standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>M</th>
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<tr>
<td>Body weight (kg)</td>
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<tr>
<td>Pre</td>
<td>93.5 ± 6.0</td>
<td>101.5 ± 5.8</td>
<td>95.5 ± 5.1</td>
<td>94.1 ± 6.5</td>
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<tr>
<td>Post</td>
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<td>97.4 ± 5.7^</td>
<td>95.0 ± 5.4</td>
<td>89.9 ± 5.6^</td>
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<tr>
<td>Body Fat % †</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre</td>
<td>45.9 ± 3.1</td>
<td>41.4 ± 2.5</td>
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<td>41.4 ± 2.7</td>
<td>40.6 ± 2.3</td>
<td>38.7 ± 2.6</td>
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<td>Central fat %</td>
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<tr>
<td>Pre</td>
<td>50.1 ± 0.8</td>
<td>43.4 ± 2.5</td>
<td>45.9 ± 2.3</td>
<td>46.1 ± 2.6</td>
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<td>43.3 ± 2.3</td>
<td>44.4 ± 1.9</td>
<td>44.7 ± 2.5</td>
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<tr>
<td>Fat-free mass (kg)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>47.7 ± 2.8</td>
<td>56.6 ± 2.3</td>
<td>52.9 ± 3.7</td>
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<tr>
<td>Post</td>
<td>48.8 ± 3.1</td>
<td>54.9 ± 2.5^</td>
<td>54.9 ± 2.5#</td>
<td>52.4 ± 3.9</td>
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<tr>
<td>VO₂ peak (ml/kg/min)</td>
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<tr>
<td>Pre</td>
<td>21.5 ± 2.3</td>
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<td>25.5 ± 2.5</td>
<td>27.3 ± 1.8</td>
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<td>Post</td>
<td>21.2 ± 2.2</td>
<td>25.7 ± 2.6</td>
<td>29.9 ± 2.5#^</td>
<td>30.0 ± 2.3*</td>
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<td>Strength (kg) †</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pre</td>
<td>-</td>
<td>-</td>
<td>253.7 ± 17.3</td>
<td>223.9 ± 13.9</td>
</tr>
<tr>
<td>Post</td>
<td>-</td>
<td>-</td>
<td>286.9 ± 21.2</td>
<td>294.8 ± 15.1</td>
</tr>
</tbody>
</table>
Table 4.3. Fasting hormone, metabolite and substrate use values. Non-esterified free fatty acids (NEFA). * Significant compared to exercise training plus metformin (p < 0.05).
†Significant effect to test (p < 0.05). Values are mean ± standard error of the mean.

<table>
<thead>
<tr>
<th>Fasting</th>
<th>P</th>
<th>M</th>
<th>EP</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.3 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td>5.3 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Post</td>
<td>5.3 ± 0.1</td>
<td>5.1 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.74 ± 0.2</td>
<td>0.76 ± 0.2</td>
<td>0.67 ± 0.1</td>
<td>0.71 ± 0.1</td>
</tr>
<tr>
<td>Post</td>
<td>0.88 ± 0.4</td>
<td>1.46 ± 0.3</td>
<td>0.70 ± 0.1</td>
<td>0.73 ± 0.1</td>
</tr>
<tr>
<td>Insulin (pM)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>120.8 ± 24.9</td>
<td>144.4 ± 24.2</td>
<td>83.1 ± 11.2</td>
<td>92.9 ± 21.4</td>
</tr>
<tr>
<td>Post</td>
<td>129.5 ± 29.4</td>
<td>100.8 ± 18.9</td>
<td>73.1 ± 9.0</td>
<td>75.8 ± 14.3</td>
</tr>
<tr>
<td>C-peptide (nM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.87 ± 0.15</td>
<td>1.34 ± 0.16</td>
<td>1.00 ± 0.13</td>
<td>0.95 ± 0.08</td>
</tr>
<tr>
<td>Post</td>
<td>0.97 ± 0.14</td>
<td>1.15 ± 0.15</td>
<td>0.86 ± 0.13*</td>
<td>0.69 ± 0.06*^</td>
</tr>
<tr>
<td>NEFA (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.65 ± 0.05</td>
<td>0.56 ± 0.03</td>
<td>0.64 ± 0.04</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>Post</td>
<td>0.63 ± 0.03</td>
<td>0.53 ± 0.05</td>
<td>0.54 ± 0.05#</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>CHO oxidation (mg/kg-FFM/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.16 ± 0.28</td>
<td>1.36 ± 0.20</td>
<td>1.97 ± 0.25</td>
<td>1.92 ± 0.74</td>
</tr>
<tr>
<td>Post</td>
<td>2.06 ± 0.22</td>
<td>1.82 ± 0.28</td>
<td>2.06 ± 0.16</td>
<td>1.16 ± 0.49</td>
</tr>
</tbody>
</table>
Table 4.4. Clamp hormone and metabolites. Non-esterified fatty acids (NEFA) suppression (Supp). †Significant effect of test (p < 0.05).

<table>
<thead>
<tr>
<th>Clamp</th>
<th>P</th>
<th>M</th>
<th>EP</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin† (pM)</strong></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Pre</td>
<td>1462.8 ± 26.7</td>
<td>1570.0 ± 32.2</td>
<td>1444.4 ± 38.3</td>
<td>1319.3 ± 33.8</td>
</tr>
<tr>
<td>Post</td>
<td>1450.9 ± 21.9</td>
<td>1255.8 ± 42.3</td>
<td>1265.7 ± 18.7</td>
<td>1151.7 ± 22.6</td>
</tr>
<tr>
<td><strong>Glucose (mM)</strong></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Pre</td>
<td>5.0 ± 0.0</td>
<td>5.0 ± 0.0</td>
<td>5.1 ± 0.0</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>Post</td>
<td>4.9 ± 0.0</td>
<td>4.9 ± 0.1</td>
<td>5.0 ± 0.0</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Lactate (mM)</strong></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Pre</td>
<td>0.78 ± 0.1</td>
<td>0.52 ± 0.1</td>
<td>0.53 ± 0.1</td>
<td>0.77 ± 0.1</td>
</tr>
<tr>
<td>Post</td>
<td>0.57 ± 0.1</td>
<td>0.72 ± 0.2</td>
<td>0.80 ± 0.2</td>
<td>0.78 ± 0.1</td>
</tr>
<tr>
<td><strong>NEFA Supp (%)</strong></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Pre</td>
<td>56.4 ± 4.4</td>
<td>47.6 ± 3.6</td>
<td>51.8 ± 2.5</td>
<td>46.9 ± 2.3</td>
</tr>
<tr>
<td>Post</td>
<td>59.1 ± 3.7</td>
<td>40.7 ± 6.8</td>
<td>42.5 ± 2.5</td>
<td>51.2 ± 4.9</td>
</tr>
</tbody>
</table>
Table 4.5. Total Glucose infusion rate (GIRT). Rate of glucose disposal (Rd) and appearance (Ra). Residual Ra refers to total glucose infusion rate minus Ra during the clamp. Hepatic insulin sensitivity (HIS). Values are mean ± standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>M</th>
<th>EP</th>
<th>EM</th>
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</thead>
<tbody>
<tr>
<td><strong>Fasting Ra (mg/kg-FFM/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.92 ± 0.32</td>
<td>6.13 ± 1.14</td>
<td>5.30 ± 0.21</td>
<td>5.58 ± 0.58</td>
</tr>
<tr>
<td>Post</td>
<td>7.89 ± 1.58</td>
<td>6.30 ± 0.75</td>
<td>6.01 ± 0.63</td>
<td>5.75 ± 0.73</td>
</tr>
<tr>
<td><strong>Clamp Rd (mg/kg-FFM/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>13.0 ± 2.4</td>
<td>7.9 ± 1.5</td>
<td>9.2 ± 1.1</td>
<td>11.1 ± 2.3</td>
</tr>
<tr>
<td>Post</td>
<td>10.9 ± 1.3</td>
<td>9.0 ± 1.9</td>
<td>14.1 ± 1.8</td>
<td>11.4 ± 1.5</td>
</tr>
<tr>
<td><strong>Clamp GIR (mg/kg-FFM/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>11.89 ± 2.05</td>
<td>9.37 ± 1.40</td>
<td>9.15 ± 1.23</td>
<td>10.38 ± 1.72</td>
</tr>
<tr>
<td>Post</td>
<td>10.09 ± 1.51</td>
<td>9.89 ± 1.25</td>
<td>12.92 ± 1.84</td>
<td>12.21 ± 0.96</td>
</tr>
<tr>
<td><strong>CHO oxidation (mg/kg-FFM/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.75 ± 0.63</td>
<td>3.07 ± 0.60</td>
<td>3.73 ± 1.09</td>
<td>3.78 ± 0.83</td>
</tr>
<tr>
<td>Post</td>
<td>4.00 ± 0.63</td>
<td>2.87 ± 0.35</td>
<td>3.13 ± 0.88</td>
<td>3.89 ± 0.52</td>
</tr>
<tr>
<td><strong>Clamp Ra (mg/kg-FFM/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>12.97 ± 2.44</td>
<td>7.94 ± 1.52</td>
<td>9.20 ± 1.05</td>
<td>11.08 ± 2.29</td>
</tr>
<tr>
<td>Post</td>
<td>10.89 ± 1.26</td>
<td>9.01 ± 1.88</td>
<td>14.12 ± 1.76</td>
<td>11.42 ± 1.46</td>
</tr>
<tr>
<td><strong>Clamp Residual Ra (mg/kg-FFM/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.41 ± 0.58</td>
<td>0.36 ± 0.23</td>
<td>0.54 ± 0.22</td>
<td>1.84 ± 1.35</td>
</tr>
<tr>
<td>Post</td>
<td>1.62 ± 0.94</td>
<td>0.59 ± 0.38</td>
<td>1.61 ± 0.61</td>
<td>0.85 ± 0.56</td>
</tr>
<tr>
<td><strong>HIS (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>77.8 ± 9.6</td>
<td>91.6 ± 4.9</td>
<td>90.7 ± 3.6</td>
<td>74.1 ± 12.6</td>
</tr>
<tr>
<td>Post</td>
<td>86.3 ± 5.2</td>
<td>89.5 ± 6.1</td>
<td>74.1 ± 9.4</td>
<td>89.2 ± 7.2</td>
</tr>
</tbody>
</table>
CHAPTER 5
PILOT WORK FOR AIM 2

Published in International Journal of Sports Nutrition and Exercise Metabolism

Title: Exercise and post-exercise substrate oxidation are altered by metformin treatment.

Authors: Steven K. Malin, Brooke R. Stephens, Carrie G. Sharoff, Todd A. Hagobian, Stuart R. Chipkin, Barry Braun

Energy Metabolism Laboratory, Department of Kinesiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA 01003

Supported by American Diabetes Association grant 7-04-JF-10

Running Head: Substrate oxidation and Metformin

Correspondence to:
Barry Braun, PhD
Dept. of Kinesiology
107 Totman Building
University of Massachusetts
Amherst, MA 01003
Phone: (413) 577-0146
Fax: (413) 545-2906
Email: bbraun@kin.umass.edu
Abstract

Exercise and metformin may prevent or delay type 2 diabetes by, in part, raising the capacity for fat oxidation. Whether the addition of metformin has additive effects on fat oxidation during and after exercise is unknown. Therefore, the purpose of this study was to evaluate the effect of metformin on substrate oxidation during and after exercise. Using a double-blind, counter-balanced cross-over design, substrate oxidation was assessed by indirect calorimetry in 15 individuals taking metformin (2000 mg/d) and placebo for 8-10d. Measurements were made during cycle exercise at 5 submaximal cycle workloads, starting at 30% work peak (Wpeak) and increasing by 10% every 8min to 70% Wpeak. Substrate oxidation was also measured for 50’ post-exercise. Differences between conditions were assessed using analysis of variance with repeated measures and values reported here are means ± SE. During exercise, fat oxidation (0.19 ± 0.03 vs. 0.15 ± 0.01g/min); p < 0.01) and percentage of energy from fat (32 ± 3 vs. 28 ± 3%; p < 0.01) were higher with metformin compared to placebo. Post-exercise, metformin slightly lowered fat oxidation (0.12 ± 0.02 to 0.10 ± 0.02g/min; p < 0.01) compared to placebo. There was an inverse relationship between post-exercise fat oxidation and the rate of fat oxidation during exercise (r = -0.68; p < 0.05). In healthy individuals, metformin has opposing actions on fat oxidation during and after exercise. Whether the same effects are evident in insulin resistant individuals remains to be determined.

KEY WORDS: Prediabetes, type 2 diabetes, exercise metabolism, biguanide
Introduction

Exercise or metformin, a common anti-diabetes drug, oppose insulin resistance and the development of type 2 diabetes (Knowler et al., 2002; Orchard et al., 2005). One of the mechanisms underlying their efficacy may be increasing the capacity for fat oxidation. There are strong data showing inverse relationships between resting fat oxidation and insulin resistance (Goodpaster, Katsiaras, & Kelley, 2003; Horowitz, 2003; Kelley & Mandarino, 2000; Winder, 2000).

During low to moderate intensity exercise, the absolute rate of fat oxidation is elevated from resting values (Achten, Gleeson, & Jeukendrup, 2002; Braun, Sharoff, Chipkin, & Beaudoin, 2004; Brooks & Mercier, 1994; Perez-Martin et al., 2001; Romijn et al., 1993; Dumortier et al., 2003). Prior exercise also raises resting fat oxidation for up to 24 hours (Broeder et al., 1991; Henderson et al., 2007; Horton, Pagliassotti, Hobbs, & Hill, 1998; Kuo, Fattor, Henderson, & Brooks, 2005; Phelain, Reinke, Harris, & Melby, 1997). Fat oxidation during and after exercise is elevated by increasing triacylglycerol mobilization, fatty acid uptake into tissues, and lipid oxidation (Jeukendrup, 2002).

Metformin is a biguanide compound used to oppose hyperglycemia in people with prediabetes or diabetes (American Diabetes Association, 2007; Nathan et al., 2007). Metformin decreases resting hepatic glucose production (Zhou et al., 2001) and may elevate whole-body and hepatic fat oxidation (Long & Zierath, 2006; Zhou et al., 2001). Because the mechanism for altering fat mobilization, tissue uptake and utilization may not be identical for exercise and metformin (Long & Zierath, 2006), adding metformin to exercise could accentuate fat oxidation and potentially increase the effects of exercise to oppose insulin resistance. To date, how adding metformin to exercise impacts fat oxidation has not been reported.

Therefore, the purpose of the present study was to assess the effect of metformin on fat oxidation during and after exercise in non-obese recreationally active individuals. Healthy subjects were selected to isolate the independent actions of metformin from potentially
confounding effects of mitochondrial dysfunction (Kelley & Mandarino, 2000), insulin resistance (Braun et al., 2004) and/or pharmacological interaction on substrate oxidation. We hypothesized that metformin treatment, compared to a placebo condition, would increase fat oxidation during and after exercise.

**Methods**

**Overall Study Design:** The effect of metformin on substrate oxidation was tested using a double-blind, counter-balanced cross-over design in which men and women served as their own controls. Subjects were treated with metformin or placebo for 8-10 days before performing a submaximal exercise test. Subjects were treated with the alternative treatment for 8-10 days to allow adequate washout of the previous treatment. During and after submaximal exercise, substrate oxidation was measured by indirect calorimetry using open-circuit spirometry.

**Subjects:** Healthy recreationally active (> 3d/wk of either aerobic or resistance training) men (n=7) and women (n=8) participated in this study. Subjects were free from cardiovascular or metabolic disease, were nonsmokers, and were not taking any dietary supplements (e.g. chromium, niacin, ephedrine, etc.) or medications known to impact substrate oxidation. Subject characteristics are outlined in Table 5.1. We did not strictly control for menstrual cycle phase, but based on questionnaires detailing the date of last menses, half of the women were in their follicular phase of the cycle when on metformin. No noticeable differences in substrate use occurred between these groups of women. Most well controlled studies using stable isotope tracers show that the effects of menstrual cycle phase on substrate oxidation during exercise are subtle or non-existent (Braun & Horton, 2001). Prior to testing, all subjects gave informed consent document approved by the Institutional Review Board at the University of Massachusetts, Amherst. A total of 15 subjects participated in the entire study. A subgroup of 10 subjects (the final 10 subjects recruited for the study) also participated in the measurement of blood lactate and post-exercise substrate oxidation.
**Metformin and Placebo Condition:** Subjects were treated with either metformin or placebo for 8-10 days (Braun et al., 2008). They began treatment on day 1 with 500mg and the dose was increased by 500mg per day up to the standard clinical dose of 2000mg (usually occurred after 4 days). Subjects maintained treatment at 2000mg for 4-5 consecutive days prior to each exercise test (see below). Subjects were instructed to take metformin or placebo with food in order to minimize potential side effects. Although it was designed to be a double-blind protocol, the presence of metallic taste in the mouth and/or gastrointestinal symptoms caused several subjects (these side effects were listed in the informed consent document as per IRB requirements) and the investigators to suspect which treatment was being given in a few cases. Despite this limitation, few subjects were certain which treatment they had been given and several were convinced they had metformin when they actually had the placebo.

**Test of peak work capacity:** Subjects performed a continuous progressive exercise test on a cycle ergometer (SensorMedics 800, Yorba Linda, CA) to determine peak work capacity (Wpeak) and oxygen consumption (VO₂peak) after 7 days of treatment with metformin or placebo. After a 5-minute warm-up, the workload on the cycle ergometer was increased by 30W every 2 minutes until the subject was unable to maintain a pedal cadence of 60rpm. During the test, respiratory gases (VO₂ and VCO₂) were collected by indirect calorimetry using open-circuit spirometry (ParvoMedics TrueMax 2400, Consentius Technologies, Sandy, UT). Heart rate was monitored throughout the test (Polar, Inc., Lake Success, NY). Wpeak and VO₂peak were defined as the highest values obtained during the test. Three subjects obtained a higher Wpeak during placebo treatment. Submaximal exercise intensities were scaled to a relative percentage of their new peak value to minimize the impact of exercise intensity on substrate use. Each test was considered valid if at least 3 of the following criteria were met: a plateau in VO₂ (< 150ml), respiratory exchange ratio (RER) > 1.1, heart rate within 15 beats per minute of age predicted peak heart rate (220-age) or the subject voluntarily stopped the test (Braun et al., 2008).
**Submaximal Exercise and Post-exercise Tests:** Following the test of Wpeak subjects were instructed to avoid structured exercise for 24 hours. The following morning, subjects took their last dose or metformin or placebo and reported to the laboratory in the post-absorptive state (10-14 hour fast). Baseline respiratory gases were collected for 8 minutes. Subjects warmed up for 5 minutes on the cycle ergometer at 25 Watts. Cycle ergometry was then performed at 5 submaximal workloads, starting at 30% Wpeak and increasing by 10% every 8 minutes to a final workload of 70% Wpeak. Eight-minute exercise stages were selected to make certain steady-state conditions were reached for accurate measures of substrate use (Bordenave et al., 2007). To ensure that submaximal exercise occurred at the same absolute intensity across conditions, the intensity was scaled to peak workload, rather than peak VO2, because our laboratory has shown that metformin treatment can reduce VO2peak (Braun et al., 2008). Subjects selected a preferred pedal cadence during the initial submaximal test, but were required maintain a pedal cadence of 50rpm. Subjects repeated their preferred pedal cadence during the second submaximal test. Two-minute active recovery periods at 25W followed each submaximal workload to minimize the impact each exercise stage would potentially have on ensuing measures of substrate use. Respiratory gases were collected during the last 4 minutes, of each 8-minute exercise workload. The last 2 minutes were used to calculate substrate oxidation to ensure achievement of steady-state conditions. Whole blood (30µl), rating of perceived exertion (RPE), and heart rate were collected during the last 2 minutes of each exercise workload.

Upon completion of exercise, subjects were moved to a semi-recumbent position and respiratory gases were collected continuously for 50 minutes using a ventilated hood system to measure substrate oxidation at 20, 30, 40, 50 and 60 minutes after exercise. Respiratory gases were averaged for the last 2 minutes at each time point for substrate oxidation analysis. Substrate oxidation analysis included the respiratory exchange ratio (RER), total rate of carbohydrate and fat oxidation, and relative percentage of energy from carbohydrate and fat. When RER exceeded 1.0 during exercise, which occurred 7 times with metformin and 6 times
with placebo (all at the final workload) RER was assumed to equal 1.0. Subjects were instructed to maintain their normal diet and activity regimen throughout the study and all subjects were tested at a similar time of day.

**Blood Analysis:** Whole blood lactate concentrations were collected in 30μl capillary tubes and were determined enzymatically using a glucose-lactate analyzer (GL5 Analyzer, Analox Instruments, Lunenbergh, MA).

**Calculations:** Although there are limitations to the use of indirect calorimetry to assess substrate oxidation, it has been validated using the $^{13}$C/$^{12}$C ratio technique and the authors (Romijn, Coyle, Hibbert, & Wolfe, 1992) concluded that indirect calorimetry could be used to accurately determine carbohydrate and fat oxidation at exercise intensities up to 85% VO$_{2\text{max}}$. Thus, energy derived from carbohydrate and fat were determined as (Kuo et al., 2005):

1. RER = VCO$_2$/VO$_2$

2. Percent of energy from carbohydrate (CHO) and fat
   
   $\% \text{ Energy CHO} = \frac{[\text{RER} - 0.71]}{0.29} \times 100$
   
   $\% \text{ Energy Fat} = 100 - \frac{[\text{RER} - 0.71]}{0.29} \times 100$

3. Rate of total CHO and fat oxidation were calculated (Peronnet & Massicotte, 1991).

   $\text{CHO oxidation rate (g/min) = 1.6946 VO}_2 - 1.7012\text{VCO}_2$
   
   $\text{Fat oxidation rate (g/min) = 4.5850 VCO}_2 - 3.2255 \text{VO}_2$

**Statistical Analysis:** Data was analyzed using the R statistical software package (version 2.4.0, The R foundation, Vienna, Austria, 2006). Paired t-Tests were used to detect differences between conditions for anthropometric variables, heart rate peak, VO$_2$peak and Wpeak. A 2-factor (condition x intensity or time) repeated measures analysis of variance (ANOVA) was used to determine differences for RER, relative percentage of energy from carbohydrate and fat, rate of total carbohydrate and fat oxidation, lactate, heart rate, submaximal oxygen consumption, energy expenditure, and RPE. Tukey's post hoc analysis was performed when significant
interactions were observed. Pearson’s correlation was used to assess relationships when possible. Significant differences were accepted as $\alpha < 0.05$.

**Results**

*Exercise Substrate Oxidation.* During submaximal exercise there was no statistical difference between conditions for oxygen consumption, energy expenditure, or heart rate (Table 5.2), but as expected outcome variables did increase with increasing exercise intensity ($p < 0.05$). Overall, metformin, compared to placebo, significantly lowered RER values across exercise intensities ($p < 0.03$; Figure 5.1). Based on those values, the relative percentage of energy derived from fat ($p < 0.01$; Table 5.3) and rate of total fat oxidation was increased across exercise intensities with metformin treatment compared to placebo ($p < 0.01$; Figure 5.2a and 5.2b). There was no interaction between treatment and exercise intensity ($p = \text{ns}$), indicating that the effect of increasing exercise intensity on the balance between carbohydrate and fat oxidation was not different with metformin or placebo.

*Blood Lactate and RPE During Exercise.* Metformin significantly increased blood lactate concentrations ($p < 0.05$; Figure 5.3) and RPE values ($p < 0.05$; Table 5.2) compared to placebo at all exercise intensities. Both metformin and placebo had similar patterns of blood lactate and RPE values as exercise intensity increased ($p < 0.05$). There was no interaction between the treatment and exercise intensity ($p = \text{ns}$), indicating that blood lactate responded similarly to increasing exercise intensity regardless of the treatment. Considering both blood lactate and RPE increased significantly, we observed significant correlations between the change ($\Delta$) in blood lactate and the $\Delta$ in RPE ($r = 0.74$; $p < 0.05$), indicating that higher blood lactate during exercise was associated with higher perceptions of effort.

*Post-exercise Substrate Oxidation.* Post-exercise oxygen consumption and energy expenditure was not different between metformin and placebo conditions (data not shown) at any time point. Post-exercise RER values were higher with metformin treatment compared to placebo ($p <$
0.03; Figure 5.1) and accordingly, metformin slightly lowered the relative percentage of energy derived from fat (p < 0.03; Table 5.4) and rate of total fat oxidation (0.10 ± 0.02 vs. 0.12 ± 0.02; p < 0.01). There was a significant inverse correlation between the Δ in rate of total fat oxidation during exercise and the Δ in rate of total fat oxidation post-exercise (r = -0.68; p < 0.05), indicating that higher fat oxidation during exercise was associated with lower fat oxidation post-exercise.

**Discussion**

*Summary of Results.* This study demonstrates that metformin alters substrate oxidation during and after exercise in healthy recreationally active individuals. The main findings were that, compared to placebo, metformin 1.) raised reliance on fat as a fuel source during exercise, 2.) increased blood lactate concentrations and rating of perceived exertion (RPE) during exercise, and 3.) lowered fat oxidation after exercise.

*Effects of Metformin on Exercise Substrate Oxidation.* Treatment with metformin elevated the amount of fat oxidized both in absolute (grams of fat) and relative terms (percentage of energy derived from fat). Since metformin had no impact on oxygen consumption or energy expenditure during exercise, differences in relative exercise intensity cannot explain the elevated fat oxidation. As seen in Figure 5.2a and 5.2b, the reciprocal changes in the oxidation of carbohydrate and fat can be visually represented using the crossover concept originally described by Brooks and Mercier (Brooks & Mercier, 1994). The addition of metformin visually shifted the crossover point from approximately 50% (placebo) to 55% Wpeak indicating increased reliance on the use of fat at the same exercise (absolute or relative) intensity. Whether the effect of metformin to increase fat oxidation during a single exercise bout would be maintained, accentuated or attenuated with regular exercise training needs to be determined.

Whether the increased use of fat with metformin treatment is a direct result of stimulating fat utilization or an indirect effect of increasing fat availability cannot be determined using the
current study design. Metformin activates AMP-activated protein kinase (AMPK), which raises the capacity for fat oxidation by facilitating entry of fatty acids into the mitochondria (Long & Zierath, 2006; Merrill, Kurth, Hardie, & Winder, 1997) for oxidation. Metformin also raises circulating fatty acid concentrations (Gudat, Convent, & Heinemann, 1997) increasing lipid availability. Lastly, by reducing hepatic glucose production, metformin could lower the availability of plasma glucose and constrain carbohydrate oxidation with a compensatory rise in the oxidation of fat (Randle, 1998). More sophisticated studies using palmitate and glucose tracers would be necessary to evaluate the mechanism underlying the shift in fat use.

*Metformin Increases Blood Lactate Concentrations.* Typically, a rise in circulating lactate concentration is directly related to the greater use of carbohydrate that parallels increasing exercise intensity. Paradoxically, metformin increased blood lactate concentrations at rest and at all exercise intensities despite a lower reliance on carbohydrate compared with placebo. These data suggest that a shift to greater reliance on non-oxidative carbohydrate metabolism and/or less clearance of lactate. Without lactate tracers, we cannot distinguish between the two explanations, but the well-characterized effect of metformin to decrease hepatic glucose production is consistent with lower lactate clearance (Owen, Doran, & Halestrap, 2000). Our results contrast with those of Gudat et al (Gudat et al., 1997), who reported that metformin did not increase lactate concentrations during exercise at a single exercise intensity (200 watts) in healthy subjects. A possible explanation for this discrepancy is the inter-individual variability introduced when exercise is performed at a single absolute intensity. Because there are variations in fitness, muscle power, body size, etc. there is a wide range of relative exercise intensities that cause considerable variability in the blood lactate response. In the current study, individuals exercised at the same relative intensity to minimize variability and increase the likelihood of detecting true differences among conditions.

*Metformin and Rating of Perceived Exertion (RPE).* Consistent with the elevated circulating lactate concentrations, RPE was slightly higher during exercise with metformin treatment
compared to placebo. The greatest difference in RPE occurred at 40% Wpeak, which is an exercise intensity that falls within the low to moderate intensity range typically recommended for previously sedentary individuals in the early stages of exercise programs. If exercise at the same absolute intensity “feels” harder with metformin, individuals may be less likely to comply with exercise programs. If this difference in RPE turns out to be a common side-effect of metformin treatment, practitioners may need to adjust exercise prescription.

*Effects of Metformin on Post-exercise Substrate Oxidation.* In contrast with the effect to elevate fat oxidation during exercise, metformin increased carbohydrate oxidation after exercise. The difference was most evident immediately after exercise and was diminished as time went on. The increase in carbohydrate oxidation could be a direct effect of metformin to raise glucose use or an indirect consequence of the elevated fat oxidation during exercise. Consistent with the latter explanation, there was a strong inverse relationship between fat use during exercise and fat use after exercise. Although this association may not be causal, it is consistent with the literature. For example, compared to eucaloric high intensity exercise, low intensity exercise (higher contribution of fat to total energy expenditure), reduces post-exercise fat oxidation (Kuo et al., 2005; Phelain et al., 1997). Henderson and colleagues (Henderson et al., 2007) showed that compared to men, women oxidized more fat during exercise but less fat after exercise. The consensus among these researchers is that big elevations in fat oxidation after hard exercise result from high rates of endogenous carbohydrate use during exercise and a consequent directing of carbohydrate to muscle glycogen replenishment rather than oxidation (Bielinski, Schutz, & Jequier, 1985; Kiens & Richter, 1998; Kimber, Heigenhauser, Spriet, & Dyck, 2003). It is possible that metformin treatment, by lowering carbohydrate use during exercise, reduces the stimulus to direct glucose toward storage after exercise. Although resting fat oxidation may be lower after exercise with metformin, the increased reliance on carbohydrate could be useful in managing hyperglycemia for individuals with established diabetes.

*Limitations.* There are several limitations to our study that could affect interpretation of the data.
First, the experimental treatment was performed in healthy, recreationally active individuals. This was done to minimize the impact of insulin resistance (Braun et al., 2004), mitochondrial dysfunction (Kelley & Mandarino, 2000; Kelley, He, Menshikova, & Ritov, 2002; Simoneau, Veerkamp, Turcotte, & Kelley, 1999) and/or potential interaction with other common medications. Whether metformin impacts substrate oxidation in individuals with pre-diabetes or diabetes remains to be determined. Second, because measuring substrate oxidation with indirect calorimetry may be less accurate at high exercise intensity, we did not determine substrate oxidation rates at exercise intensities above 70% Wpeak (Romijn et al., 1992). Fat oxidation rates may be underestimated during high intensity exercise because RER increases in response to non-metabolic CO₂ production. Furthermore, following high intensity exercise, alterations in sodium bicarbonate pools and retention of CO₂ may overestimate fat oxidation.

Third, the relatively short treatment period used in the current study precludes direct extrapolation of these data to the effects of long-term (months/years) treatment with metformin. Fourth, the lack of rigid dietary control during the study means that we are not aware of potential changes in dietary macronutrients that could affect substrate use. However, since we used a cross-over study design in which the order of conditions was balanced and the subjects were blinded to the condition, it is unlikely that subjects significantly altered their dietary patterns only when they taking metformin. Fifth, we found no difference in the effect of metformin to increase fat oxidation in the follicular or luteal phase. We acknowledge however with our small sample size and without more sophisticated measures of fat or carbohydrate metabolism we cannot completely rule out the role menstrual cycle phase in response to metformin. Sixth, because we used a graded exercise test to assess substrate oxidation, we cannot pinpoint the effects of metformin at any single exercise intensity in the absence of confounding effects of the preceding intensities. In addition, we cannot discern whether the effects on post-exercise metabolism were related to the net effect of the entire series of workloads or attributable to just the final workload (70% Wpeak). Lastly, 60 minutes of post-exercise measurement may not be
sufficient to fully characterize the effects of metformin on post-exercise metabolism. The difference between the treatments narrowed considerably in the last 20 minutes and may have been negligible or even reversed after 2 or 3 hours.

Conclusion. Metformin increased the rate of fat oxidation, blood lactate concentrations and ratings of perceived exertion (RPE) during submaximal exercise. In contrast, following exercise, metformin slightly reduced fat oxidation and that change was strongly correlated with increased fat oxidation during exercise. If these results are reproducible in people with pre-diabetes or diabetes, they may be useful to understand how the combination of metformin and exercise can be used to oppose insulin resistance and, potentially, to prevent/delay the onset of Type-2 diabetes. A deeper understanding of the mechanisms underlying these results will require further research using more invasive techniques.

Acknowledgments

The authors would like to thank Rebecca Hasson, MS for helpful discussion, and Kirsten Granados for technical assistance. We also extend our appreciation to the dedicated participants for their time and effort.

This research was supported by American Diabetes Association grant 7-04-JF-10.
References


Figure Captions

Figure 5.1. RER at each submaximal workload and for 60 minutes after exercise.

*Significant effect of condition (p < 0.03). #Significant effect of intensity (p < 0.05). Values are mean ± standard error of the mean.
Figure 5.2a and 5.2b. Rate of fat oxidation at each submaximal workload with placebo (a) and metformin (b). Significant effect of condition ($p < 0.01$). Significant effect of intensity ($p < 0.05$). Values are mean ± standard error of the mean.

**Figure 5.2a.**

**Figure 5.2b.**
Figure 5.3. Blood lactate at each submaximal exercise workload. *Significant effect of condition (p < 0.05). #Significant effect of intensity (p < 0.05).
Table Captions

Table 5.1. Subject Characteristics. Women n = 8 and Men n = 7. Values are mean ± standard deviation. No significant differences were observed between conditions. Bpm = beats per minute

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>25.0 ± 4.4</td>
<td>25.0 ± 4.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>69.2 ± 12.7</td>
<td>68.9 ± 12.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 2.7</td>
<td>22.8 ± 2.7</td>
</tr>
<tr>
<td>Heart rate peak (bpm)</td>
<td>184.7 ± 8.3</td>
<td>181.8 ± 10.1</td>
</tr>
<tr>
<td>VO₂ peak (L/min)</td>
<td>3.2 ± 0.7</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>VO₂ peak (ml/kg/min)</td>
<td>47.6 ± 6.8</td>
<td>46.9 ± 6.8</td>
</tr>
<tr>
<td>Work peak (Watts)</td>
<td>241.3 ± 54.3</td>
<td>235.7 ± 55.3</td>
</tr>
</tbody>
</table>
Table 5.2. Values are reported as mean ± standard error of the mean. *Significant main effect of condition; p < 0.05. #Significant effect of intensity (p < 0.05). RPE= rating of perceived exertion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Rest</th>
<th>30% Wpeak</th>
<th>40% Wpeak</th>
<th>50% Wpeak</th>
<th>60% Wpeak</th>
<th>70% Wpeak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm) #</td>
<td>Placebo</td>
<td>74 ± 2.4</td>
<td>108 ± 2.0</td>
<td>126 ± 2.4</td>
<td>143 ± 2.8</td>
<td>156 ± 2.1</td>
<td>169 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>75 ± 2.3</td>
<td>110 ± 2.9</td>
<td>124 ± 2.4</td>
<td>140 ± 3.1</td>
<td>154 ± 3.3</td>
<td>168 ± 3.3</td>
</tr>
<tr>
<td>Oxygen consumption (ml/kg/min) #</td>
<td>Placebo</td>
<td>4.20 ± 0.1</td>
<td>18.4 ± 0.7</td>
<td>22.5 ± 0.8</td>
<td>28.3 ± 1.5</td>
<td>31.7 ± 0.8</td>
<td>36.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>4.11 ± 0.2</td>
<td>18.4 ± 0.7</td>
<td>22.9 ± 0.8</td>
<td>26.7 ± 1.1</td>
<td>31.4 ± 1.2</td>
<td>36.6 ± 1.4</td>
</tr>
<tr>
<td>Energy Expenditure (kcal/min) #</td>
<td>Placebo</td>
<td>1.8 ± 0.4</td>
<td>6.3 ± 0.4</td>
<td>7.7 ± 0.5</td>
<td>9.3 ± 0.5</td>
<td>10.9 ± 0.6</td>
<td>12.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>1.5 ± 0.1</td>
<td>6.3 ± 0.4</td>
<td>7.8 ± 0.5</td>
<td>9.6 ± 0.5</td>
<td>10.8 ± 0.7</td>
<td>12.9 ± 0.8</td>
</tr>
<tr>
<td>RPE #</td>
<td>Placebo</td>
<td>-</td>
<td>9.3 ± 0.4</td>
<td>11.1 ± 0.4</td>
<td>12.8 ± 0.3</td>
<td>14.6 ± 0.4</td>
<td>16.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>-</td>
<td>9.4 ± 0.3</td>
<td>11.7 ± 0.2</td>
<td>13.3 ± 0.3</td>
<td>14.7 ± 0.3</td>
<td>17.3 ± 0.3</td>
</tr>
</tbody>
</table>
Table 5.3. Relative percentage of energy across all submaximal workload. *Significant effect for condition (p < 0.01). #Significant effect of intensity (p < 0.05). Values are mean ± standard error of the mean.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Rest</th>
<th>30% Wpeak</th>
<th>40% Wpeak</th>
<th>50% Wpeak</th>
<th>60% Wpeak</th>
<th>70% Wpeak</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO *#</td>
<td>Placebo</td>
<td>41.1 ± 3.1</td>
<td>60.7 ± 2.7</td>
<td>69.4 ± 2.7</td>
<td>78.3 ± 3.2</td>
<td>87.4 ± 2.6</td>
<td>97.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>39.7 ± 3.3</td>
<td>54.7 ± 2.5</td>
<td>63 ± 2.4</td>
<td>74.3 ± 3.3</td>
<td>83.0 ± 3.2</td>
<td>92.4 ± 2.4</td>
</tr>
<tr>
<td>Fat *#</td>
<td>Placebo</td>
<td>58.9 ± 3.1</td>
<td>39.3 ± 2.7</td>
<td>30.6 ± 2.7</td>
<td>21.7 ± 3.2</td>
<td>12.6 ± 2.6</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>60.3 ± 3.3</td>
<td>45.3 ± 2.5</td>
<td>37 ± 0.8</td>
<td>25.7 ± 3.3</td>
<td>17.0 ± 3.2</td>
<td>7.6 ± 2.4</td>
</tr>
</tbody>
</table>
Table 5.4. Percentage of energy expenditure attributable to carbohydrate and fat oxidation at each time point after exercise. *Significant effect of condition (p < 0.03).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>20min</th>
<th>30min</th>
<th>40min</th>
<th>50min</th>
<th>60min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO *</td>
<td>Placebo</td>
<td>7.4 ± 2.6</td>
<td>3.7 ± 1.6</td>
<td>6.2 ± 2.4</td>
<td>11.6 ± 3.7</td>
<td>21.9 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>16.1 ± 5.2</td>
<td>15.3 ± 5.5</td>
<td>21.9 ± 5.2</td>
<td>19.2 ± 6.7</td>
<td>13.6 ± 3.7</td>
</tr>
<tr>
<td>Fat *</td>
<td>Placebo</td>
<td>92.6 ± 2.6</td>
<td>96.3 ± 1.6</td>
<td>93.8 ± 2.4</td>
<td>88.4 ± 3.7</td>
<td>78.1 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>83.9 ± 5.2</td>
<td>84.7 ± 5.5</td>
<td>78.1 ± 5.2</td>
<td>80.1 ± 6.7</td>
<td>86.4 ± 3.7</td>
</tr>
</tbody>
</table>
CHAPTER 6
METFORMIN WITH EXERCISE TRAINING & SUBSTRATE USE

Title: Effects of combining metformin and exercise training on fat utilization during exercise in adults with impaired glucose tolerance

Authors: Steven K. Malin and Barry Braun

Energy Metabolism Laboratory, Department of Kinesiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA 01003

Supported by NIH 5 R56 DK081038 & the ACSM Doctoral Student Foundation Grant S17100000000113

Running Head: Exercise substrate utilization and metformin

Correspondence to:
Barry Braun, PhD
Dept. of Kinesiology
107 Totman Building
University of Massachusetts
Amherst, MA 01003
Phone: (413) 577-0146
Fax: (413) 545-2906
Email: bbraun@kin.umass.edu
Abstract

Exercise training increases fat utilization during exercise and we previously showed that metformin raised fat utilization during exercise in healthy individuals. It is unknown if metformin raises fat utilization further after training in individuals prescribed this agent. **Purpose:** To determine the effect of combining metformin with exercise training on substrate utilization during exercise in individuals with impaired glucose tolerance (IGT). **Methods:** Using a double-blind design, 16 men and women with IGT underwent exercise training plus metformin (2000 mg/d; EM) or exercise training plus placebo (EP). Substrate utilization during exercise at 60% of pre-training VO$_2$peak was assessed before and after 10 weeks of endurance and resistance training by indirect calorimetry and the isotope dilution method. The pre and post-training group means were compared across conditions using analysis of variance with repeated measures. **Results:** Fat utilization increased ($p < 0.001$) and muscle glycogen decreased ($p < 0.001$) after EM and EP. Blood glucose utilization increased after EM compared to EP ($p < 0.04$). **Conclusion:** Metformin did not enhance the effects of training on fat utilization during exercise. Training, with or without metformin, had similar effects on fat and glycogen utilization, but only metformin increased the reliance on blood glucose during exercise after training. These findings suggest that training with metformin has subtle effects on substrate utilization during exercise in individuals with impaired glucose tolerance.

**KEY WORDS:** Prediabetes, type 2 diabetes, substrate metabolism, resistance training
**Introduction**

Individuals with impaired glucose tolerance (IGT) are at high risk for type 2 diabetes (1, 2, 23). Lifestyle modification, which consists of low-fat diet and increased physical activity, opposes the progression from IGT to type 2 diabetes (16). To prevent this transition, the American Diabetes Association (ADA) strongly recommends habitual exercise. Metformin, a commonly prescribed oral medication that reduces hyperglycemia, also delays the transition to type 2 diabetes. As a result, the ADA recommends that physicians consider metformin along with lifestyle changes for people with IGT (20). But, we know very little about the effects on combining exercise with metformin in individuals with IGT.

Although the exact mechanism by which exercise improves insulin sensitivity is unclear, enhancing fat utilization may be one potential mechanism because it is associated with reduced insulin resistance (12, 27, 28). Endurance training increases fat utilization during exercise in lean healthy individuals (10, 11, 15). We previously demonstrated that metformin increased fat utilization during exercise in healthy, recreationally active individuals (17). It is possible that metformin elevated fat utilization by constraining hepatic glucose output and lowering the availability of blood glucose for subsequent oxidation (8, 14). Whether metformin affects fat utilization or hepatic glucose output during exercise in individuals with IGT after training has yet to be investigated. Therefore, the primary purpose of this study was to determine the effects of combining exercise training and metformin on exercise fat utilization in men and women with IGT. We hypothesized that exercise training with metformin would increase fat utilization during exercise more than exercise training alone. Since the primary mechanism of action for metformin is to lower hepatic glucose output, we also hypothesized that elevated fat utilization would be paralleled by reduced hepatic glucose output.
Methods

Study overview: Using a double-blind design, 16 men and women were assigned to either exercise training plus metformin (EM) or exercise training plus placebo (EP) for 10 weeks. Subjects were non-smoking, weight stable (<5% weight change over last 3mo), free of cardiovascular disease or type 2 diabetes, and did not take dietary supplements or medications that affect substrate metabolism (e.g. chromium, niacin, ephedrine). Subjects were excluded if they had any contraindications to metformin (e.g. respiratory disease, heart failure, renal and hepatic disease). Women were tested between 5-10 days post-menses and tested after training in the same midfollicular phase; i.e., women were tested after approximately 10 weeks of training because we timed measures of exercise substrate metabolism around the midfollicular phase. As a result, the precise duration of training varied slightly between women depending on their menstrual cycle. Prior to testing, all subjects were verbally briefed about the study and signed informed consent documents approved by the Institutional Review Board at the University of Massachusetts Amherst.

OGTT Screening: An oral glucose tolerance test (OGTT) was used to determine if subjects had impaired glucose tolerance. After a minimum 5-hour fast, blood samples were taken from a forearm vein. Subjects consumed 75 grams of glucose and blood samples were collected 2 hours later. All subjects had impaired glucose tolerance (IGT; 2-hour glucose concentrations between 7.8-11.1mmol/L or 140-199mg/dl). Subjects with IGT who had fasting glucose concentrations between 5.5-6.9mmol/L (100-125mg/dl) were also included.

Preliminary testing: Peak oxygen consumption (VO₂peak) was assessed by performing a continuous progressive exercise test on a cycle ergometer (SensorMedics 800, Yorba Linda, CA). After a 5-minute warm-up, the workload was increased by approximately 30W every 2 minutes until the subject was unable to maintain a pedal cadence of 60 rpm. During the test, respiratory gases (VO₂ and VCO₂L/min) were measured by indirect calorimetry (ParvoMedics Truemax 2400, Consentius Technologies, Sandy, UT). Heart rate was also monitored
throughout the test (Polar, Inc., Lake Success, NY). VO₂peak was defined as the highest values obtained during the test using standard criteria (19). One repetition max (1-RM) tests were also conducted for the: chest press, latissimus pull down, leg press, bicep curl, triceps pushdown, and upright rows. 1-RM was defined as the highest weight lifted with proper technique through the full range of motion. Body composition was measured using dual-x-ray absorptiometry (DEXA; Lunar Prodigy, Madison, WI) for the determination of body fat and fat-free mass (FFM). These data were previously presented (see Chapter 4), but are reported in the results for clarity. *Metformin or Placebo protocol:* Pills were administered to the subjects and they were instructed to take metformin or placebo with food in order to minimize potential side effects. Subjects started treatment with 500 mg/d of metformin. The dose was increased 500 mg/d each week until a clinical dose of 2000 mg/d was reached by week 4. Subjects remained at this dose for the last 6 weeks of the 10 week protocol. *Exercise Training:* Exercise was supervised 3-days a week for 60-75 minutes per session (total 3.5 hours/wk: ~400 kcal per session). Subjects performed aerobic and resistance exercise on the first and third day of each week. To minimize muscle soreness, only aerobic training was performed on the second day. Participants warmed-up on a cycle ergometer for 5 minutes, followed by cycling at 70% of their pre-training HRpeak for 45 minutes. Resistance exercise was performed at 70% of the subject’s 1-RM. Weight was increased approximately 5% when 2 sets of 12 repetitions could be lifted with proper form. Resistance training targeted all major muscle conditions and included: the chest press, latissimus pull down, leg press, bicep curl, triceps pushdown, shoulder raise, calf raises, and abdominal crunches. *Submaximal Exercise protocol:* At baseline and after 10 weeks of training, subjects reported to the laboratory after a 10-12 hour fast. Indwelling catheters were placed in a superficial vein of each forearm for continuous infusion of glucose stable isotope ([6,6-2H glucose]) and blood sampling. After baseline blood samples were collected, a primed (bolus of 200 mg 6,6-2H glucose) continuous infusion of 6,6-2H glucose was delivered for 90 minutes at 3.0 mg/min by
peristaltic infusion pump. Blood samples were collected at 75 and 90 minutes. Following the last blood sample, subjects were moved to a cycle ergometer and expired respiratory gases were collected for 8 minutes. Subjects warmed up on the cycle ergometer at 25 W for 5 minutes. Exercise was performed for 45 minutes at 60% of the pre-training VO₂peak before and after training. Two-minute break periods were provided at 15 and 25 minutes to facilitate completion of exercise. Breath and blood samples were collected at 15, 25, 35, and 45 minutes. Expired gases were collected during the first 15 minutes to ensure steady-state conditions were reached, and expired gases were collected for 7 minutes prior to each time point. During the last 2 minutes at each time point, expired gases were used to calculate substrate utilization. Substrate utilization analysis included: the respiratory exchange ratio (RER), rate of total carbohydrate and fat oxidation, and percent of total energy expenditure derived from carbohydrate or fat (see calculations below). Blood concentrations of glucose, lactate, non-esterified free fatty acids (NEFA), glycerol, and insulin, the rating of perceived exertion (RPE) and heart rate were also collected during the last 2 minutes of each time point. Subjects recorded dietary intake 24-hour prior to baseline testing and replicated this diet prior to post-training testing. There was no statistical difference in energy or macronutrient intake between conditions (data not shown). Subjects refrained from exercise 24-36 hours prior to testing and all subjects were tested at a similar time of day.

Blood sample collection: Blood samples were collected in 3 mL syringes, transferred to vacutainers, spun at 3000 rpm and aliquoted to cryotubes for storage at -80°C. Samples for analysis of glucose isotopic enrichment, glucose and lactate were transferred to vacutainers containing sodium fluoride. Samples for analysis of insulin, glycerol and non-esterified fatty acids (NEFA) were collected in vacutainers containing the anticogulant EDTA.

Analysis of metabolites and hormone: Plasma glucose and lactate concentrations were determined enzymatically using a glucose/lactate analyzer (GL5 Analyzer, Analox Instruments, Lunenberg, MA). Plasma insulin was measured by radioimmunoassay (Millipore, St. Charles,
MO). NEFA and glycerol were measured by enzymatic colorimetry (Wako Chemicals, Richmond, VA). Glucose isotopic enrichment was measured by high performance liquid chromatography and mass spectrometry (HPLC-MS) as previously described (25).

Calculations: Standard equations were used to assess rates of glucose disposal (Rd) and glucose rates of appearance (Ra) (29).

\[
\text{Glucose Ra (mg/min) = } \frac{F-V[(C1 + C2) / 2] [IE2-IE1] / (t2-t1)}{[IE2 + IE1] / 2}
\]

\[
\text{Glucose Rd (mg/min) = Ra – V[C2-C1] / (t2-t1)].
\]

Where F is the isotope infusion rate, IE1 and IE2 are enrichments of plasma glucose with isotope label at time t1 and t2, C1 and C2 are plasma glucose concentrations, V is the estimated volume of distribution for glucose (180 mL/kg).

Rates of carbohydrate and fat oxidation were estimated from VO₂, VCO₂ and RER using standard equations (21). Estimated muscle glycogen utilization (EMGU) was calculated as:

\[
\text{EMGU (mg/min) = total rate of carbohydrate oxidation – blood glucose Rd.}
\]

This estimate is based on the assumption that 100% of blood glucose taken up from the blood is oxidized. This assumption is unlikely to be true, i.e. the percent of Rd oxidized is probably 70-90% (10). The calculation thus underestimates glycogen use and is best described as minimal muscle glycogen utilization.

Statistical Analysis: Group means were analyzed using the R statistical software package (version 2.4.0, The R foundation, Vienna, Austria, 2006). Unpaired t-tests were used to compare group means among subject characteristics and post-training % of VO₂ peak. Baseline group means were assessed by a one-way analysis of variance (ANOVA) and there were no statistical differences between conditions for any outcome. Group means of: VO₂ peak, work peak, heart rate peak, and body mass were compared at baseline and after the intervention with a two-way (group by test) repeated measures ANOVA. Group means of: Ra, Rd, EMGU, RER, carbohydrate and fat oxidation, percentage of energy from blood glucose, muscle glycogen and
fat utilization, as well as metabolites and hormones were compared at baseline and after the intervention with a three-way (group by time by test) repeated measures ANOVA. Tukey’s post-hoc analysis was used to detect group mean differences when there was a significant 3-way interaction. Significant differences were accepted as $\alpha \leq 0.05$.

**Results**

*Subject Characteristics.* Exercise training plus metformin (EM) and training plus placebo (EP) conditions were similar for sex (EM and EP = 3F/5M), age (EM = 49.1 ± 6.6 vs. EP = 45.4 ± 8.0yr; $p = 0.32$) and 2-hour glucose concentrations (EP = 9.46 ± 1.70 vs. EP = 10.23 ± 1.02mM; $p = 0.28$). Both training groups increased VO$_2$peak; $p < 0.01$; Table 6.2). Body mass was reduced in EM compared to EP ($p <0.05$; Table 6.2). Fasting metabolic characteristics were similar between groups after training (Table 6.3).

*Exercise Characteristics.* Submaximal exercise oxygen consumption, expressed relative to body weight was not altered during exercise after training in either EM or EP. Metformin blunted the reduction in the pre-training VO$_2$peak percentage during exercise after training, although this was not statistically significant (EM = 54.5 ± 1.2 vs. EP = 50.8 ± 2.8%; $p = 0.11$). However, heart rate and RPE during exercise were lower after training in both groups ($p < 0.05$; Table 6.4).

*Exercise Substrate Oxidation.* RER was lowered after training by both EM and EP ($p < 0.05$; Figure 6.1a and 6.1b). Based on RER values, the total rate of fat oxidation increased ($p < 0.01$; data not shown) and the total rate of carbohydrate oxidation decreased ($p < 0.01$; data not shown) during exercise after training in both groups.

*Exercise Glucose Flux.* Plasma isotopic enrichment was not different during exercise in either EM or EP after training (data not shown). Glucose Ra (Figure 6.2a and 6.2b) and Rd (Figure 6.2c and 6.2d), were also not different after training in EM or EP (group by test effect; $p = 0.11$).
Relative Percentage of Energy Expenditure. The contribution of fat utilization to total energy expenditure increased (p < 0.001) and estimated muscle glycogen utilization decreased (p < 0.001; Figure 6.3) during exercise after training in both EM and EP. The percent of total energy expenditure attributable to blood glucose oxidation during exercise increased after training in EM compared to EP (group by test effect: p < 0.04 Figure 6.3).

Blood Metabolites and Hormone During Exercise. Although neither training group effected plasma glucose concentrations during exercise, plasma lactate concentrations decreased similarly during exercise after training in both EM and EP (p < 0.05; Table 6.4). The training alone condition had higher plasma non-esterified fatty acid (NEFA) concentrations at baseline compared to the training plus metformin condition (p < 0.01; Table 6.4). NEFA concentrations were elevated across the exercise time-points after training in EM compared to EP (group by test effect: p < 0.03). Plasma insulin concentrations were lower across the exercise time-points after training in the EM compared to EP (p < 0.01; Table 6.4).

Discussion
Overview: Metformin might enhance the capacity for fat oxidation. As a result, we hypothesized in the current study that metformin would accentuate the effects of training on fat utilization during exercise in individuals with impaired glucose intolerance (IGT). Contrary to our hypothesis, metformin did not accentuate the effects of training on fat utilization during exercise in these men and women with IGT. Combining metformin with exercise training increased fat utilization and decreased muscle glycogen oxidation to the same extent as training alone.

Effect of exercise training & metformin on fat utilization. Endurance training increases fat utilization during exercise at the same absolute workload as pre-training in lean healthy (4, 9, 15) and overweight insulin resistant individuals (6, 7). We originally demonstrated that metformin raised fat oxidation during an acute bout of exercise in healthy individuals (17). This suggested that metformin had the potential to raise fat oxidation during exercise after training.
Although it’s hard to directly compare our previous work to the present study because of differences in subject population (i.e. healthy vs. IGT), administration of metformin (1 week vs. 10 weeks), and exercise protocols (i.e. acute ramped progressive exercise test vs. a single submaximal test), our current findings suggest that metformin does not accentuate the effects of training on fat utilization during exercise when performed at the same absolute workload.

The lack of elevated fat utilization with the combined condition was somewhat surprising, given that elevated plasma non-esterified fatty acids (NEFA) concentrations have been associated with increased fat utilization (22). However, we are not the first to show that metformin raises NEFA concentrations during exercise (13). From our work and others, it is not possible to know if NEFA concentrations increased during exercise as a result of elevated lipolysis, reduced re-esterification, or decreased uptake in the liver and/or skeletal muscle. More sophisticated measures, such as palmitate and glycerol tracers, would be needed to determine the effect of metformin on lipid flux during exercise. However, plasma glycerol concentrations might be considered a crude measure of lipolysis (24). In our study, we found that they did not change after training with metformin, suggesting that metformin did not increase lipolysis. Alternative mechanisms may therefore explain the effects of the combined condition on elevated NEFA concentrations during exercise. Smith et al (26) showed that metformin reduced skeletal muscle FATCD/36 transporter concentrations, suggesting that metformin may reduce NEFA uptake after training. Thus, metformin might have subtle effects on lipid metabolism during exercise after training.

Effect of exercise training & metformin on carbohydrate utilization. Endurance training reduces total carbohydrate oxidation during exercise when performed at the same absolute workload as pre-training in lean healthy men and women (5, 10, 11). In our study, training, with or without metformin, decreased total carbohydrate utilization during exercise in these men and women with IGT. Since blood glucose utilization did not decrease after training, the reduction in total carbohydrate oxidation is explained by reduced reliance on glycogen. Decreased glycogen
utilization after training was similar between training conditions. Thus, metformin does not alter the glycogen sparing effect of training in individuals with IGT.

Endurance training decreases glucose flux (i.e. Ra and Rd) during exercise when performed at the same absolute workload as pre-training in healthy individuals (4). However, Minsink et al (18) demonstrated that 12 months of lifestyle modification, including and aerobic and resistance exercise, had no effect on reducing glucose flux during exercise in individuals with IGT. Consistent with this finding, we found that 10 weeks of training, with or without metformin, had no effects on lowering hepatic glucose output or glucose uptake during exercise in individuals with IGT. Together, these findings suggest that training may have different effects on glucose flux during exercise in individuals with IGT compared to normal glucose tolerance.

Effect of exercise training & metformin on maximal oxygen consumption. Relative to baseline, training plus metformin increased VO₂peak (scaled to body weight) by approximately 10% whereas training alone improved VO₂peak by 20%. These findings are consistent with our earlier work (3) and indicate that metformin may partially blunt the effects of training on cardio-respiratory fitness in individuals with IGT. Health professionals should be cognizant of this potential “side effect” because it could have important implications for designing exercise programs. If the blunting effects of metformin on maximal oxygen consumption were significant, submaximal exercise effort could be affected.

Effect of exercise training & metformin on Rating of Perceived Exertion (RPE). Relative to baseline, training decreases RPE during submaximal exercise at the same absolute intensity. We showed that metformin increased RPE during exercise in healthy men and women (17). Despite subtle differences in maximal and submaximal oxygen consumption (i.e. expressed as % VO₂peak) between conditions in the current study, metformin did not blunt the effects of training on lowering RPE. It’s unclear why metformin increased RPE in healthy individuals but not in these men and women with IGT. Differences in study design (i.e. cross-over vs. prospective cross-sectional) complicate the interpretation. Thus, our data indicates that training
with metformin does not affect perceived exertion during exercise and exercise should not “feel” harder.

Limitations. There are some limitations to this study that may affect the interpretation of our data. First, we tested individuals after training at the same pre-training absolute workload. Our findings may not be applicable to situations where individuals are tested at the same relative exercise intensity after training. Second, the inclusion of individuals with IGT in the presence of impaired fasting glucose (IFG) may have affected the response to training. After exercise training, fat utilization during exercise increased more in individuals with IGT than those with IFG+IGT. This observation suggests that individuals of different prediabetes classifications (e.g. IFG vs. IGT vs. IFG+IGT) may increase the capacity for fat utilization differently following exercise training (see S.B.1 and S.B.2). Thus, it would be beneficial for future work to determine the impact exercise has on substrate utilization in individuals based on the classification of prediabetes.

Conclusion. Endurance and resistance training, with or without metformin, increased fat and lowered glycogen utilization during exercise in men and women with impaired glucose tolerance. Metformin did not accentuate the effects of training on fat utilization during exercise. However, changes in non-esterified fatty acid concentrations suggest that metformin has some effects on lipid metabolism during exercise. Similar training effects on fat oxidation during exercise after training, with and without metformin, suggest that alternative mechanisms are likely to account for differences in insulin sensitivity. More invasive measures are required to understand the mechanism by which metformin and exercise interact to affect insulin sensitivity (Chapter 4), as this will provide better insight into the potential effect of the combined treatment on preventing/delaying the onset of type 2 diabetes.
Acknowledgments: S.K.M and B.B. contributed to the study design and data collection. S.K.M was primarily responsible for data analysis and statistical integrity. S.K.M wrote the manuscript and B.B. reviewed/edited the manuscript. The authors would like to thank Kirsten Granados, Robert Gerber, and Richard Viskochil for technical assistance and helpful discussion. We also thank Stuart R. Chipkin, MD, for medical consult and John Staudenmeyer, PhD, for statistical consulting. In addition, we thank the dedicated undergraduate research assistants, trainers and participants for their time and effort.

This research was supported by NIH 5 R56 DK081038 and the American College of Sports Medicine Doctoral Student Foundation Grant S17100000000113.
References


26. Smith AC, KL Mullen, KA Junkin, J Nickerson, A Chabowski, A Bonen, DJ Dyck. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of


Figure Captions

Figure 6.1a and 6.1b. RER values during exercise before and after training with metformin (a) and placebo (b). *Significant effect of test (p < 0.03). #Significant effect of time (p < 0.05). Values are mean ± standard error of the mean.
Figure 6.2a, 6.2b, 6.2c, and 6.2d. Rate of glucose appearance before and after training with metformin (a) and placebo. Rate of glucose disposal before and after training with metformin (c) and placebo (d). There was no statistical difference between conditions before or after training. Values are mean ± standard error of the mean.
Figure 6.3. Relative Percentage of energy from blood glucose (Glc), estimated muscle glycogen use (EMGU) and fat. Values are mean ± standard error of the mean.
Table Captions

Table 6.1. Anthropometric and Fitness Characteristics. Rating of perceived exertion (RPE). *Significant effect of test; p < 0.05. ^Significant group by test interaction (p < 0.05).

Values are reported as means ± standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>EM</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.2 ± 1.9</td>
<td>31.8 ± 1.6^</td>
<td>33.5 ± 1.5</td>
<td>33.3 ± 1.6</td>
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<tr>
<td>Body Fat (kg)</td>
<td>38.5 ± 4.0</td>
<td>35.3 ± 3.4^</td>
<td>40.8 ± 3.1</td>
<td>38.4 ± 2.9^</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>56.6 ± 2.6</td>
<td>58.4 ± 2.5^</td>
<td>55.4 ± 2.2</td>
<td>57.3 ± 2.3^</td>
</tr>
<tr>
<td>VO₂ peak (ml/kg/min)</td>
<td>27.3 ± 1.8</td>
<td>30.0 ± 2.3^</td>
<td>25.5 ± 2.5</td>
<td>29.9 ± 2.5^</td>
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<tr>
<td>HR peak (bpm)</td>
<td>166 ± 7</td>
<td>165 ± 4</td>
<td>170 ± 6</td>
<td>167 ± 6</td>
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<tr>
<td>Work peak (watts)</td>
<td>155 ± 12</td>
<td>187.5 ± 15.3^</td>
<td>163 ± 20</td>
<td>197 ± 23^</td>
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Table 6.2. Metabolic Fasting Characteristics. No significant effect of training for any outcome. Values are reported as means ± standard error of the mean.

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<td>Baseline</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>5.91 ± 0.23</td>
<td>5.60 ± 0.28</td>
<td>5.38 ± 0.17</td>
<td>5.21 ± 0.26</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>NEFA (mM)</td>
<td>0.50 ± 0.06</td>
<td>0.48 ± 0.05</td>
<td>0.59 ± 0.06</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Glycerol (mM)</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.12 ± 0.03</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Insulin (pM)</td>
<td>91.5 ± 15.7</td>
<td>75.7 ± 10.9</td>
<td>89.0 ± 7.9</td>
<td>75.5 ± 7.4</td>
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</table>
Table 6.3. Submaximal Exercise Characteristics. *Significant effect of test; p < 0.05.

Values are reported as means ± standard error of the mean.

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<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td><strong>Baseline</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Heart rate (bpm)</td>
<td>75 ± 1</td>
<td>75 ± 1</td>
<td>72 ± 3</td>
<td>82 ± 3</td>
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<tr>
<td>Oxygen cost (ml/kg/min)</td>
<td>2.7 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>3.1 ± 0.3</td>
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<tr>
<td><strong>Exercise</strong></td>
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<tr>
<td>Heart rate (bpm)</td>
<td>128 ± 6</td>
<td>118 ± 4*</td>
<td>138 ± 7</td>
<td>122 ± 5*</td>
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<tr>
<td>RPE</td>
<td>14 ± 1</td>
<td>12 ± 1*</td>
<td>14 ± 1</td>
<td>11 ± 1*</td>
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<tr>
<td>Oxygen cost (ml/kg/min)</td>
<td>17.0 ± 1.1</td>
<td>16.2 ± 1.2</td>
<td>15.6 ± 1.6</td>
<td>15.2 ± 1.7</td>
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<td>Pre-training VO2peak (%)</td>
<td>61.1 ± 0.4</td>
<td>58.6 ± 1.9</td>
<td>61.6 ± 0.4</td>
<td>59.7 ± 1.9</td>
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Table 6.4. Submaximal metabolites and hormone characteristics. *Significant effect of test; p < 0.05. †Significant group by test interaction (p < 0.05). # Significant effect of time (p < 0.05). ^Significant group by test interaction (p < 0.05). Values are reported as means ± standard error of the mean.

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<td>Test 15min</td>
<td>25min</td>
<td>35min</td>
<td>45min</td>
<td>15min</td>
<td>25min</td>
<td>35min</td>
<td>45min</td>
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<tr>
<td>Glucose</td>
<td>Pre 5.74 ± 0.29</td>
<td>5.58 ± 0.39</td>
<td>5.49 ± 0.39</td>
<td>5.42 ± 0.37</td>
<td>5.60 ± 0.22</td>
<td>5.25 ± 0.21</td>
<td>5.28 ± 0.30</td>
<td>5.10 ± 0.33</td>
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<tr>
<td>(mM)</td>
<td>Post 5.65 ± 0.24</td>
<td>5.49 ± 0.31</td>
<td>5.51 ± 0.31</td>
<td>5.57 ± 0.37</td>
<td>5.40 ± 0.40</td>
<td>5.17 ± 0.25</td>
<td>5.32 ± 0.21</td>
<td>5.23 ± 0.22</td>
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<tr>
<td>Lactate</td>
<td>Pre 2.7 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.7 ± 0.3</td>
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<td>2.3 ± 0.3</td>
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</tr>
<tr>
<td>(mM)</td>
<td>Post 1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.3 ± 0.1</td>
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<td>1.4 ± 0.2</td>
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<tr>
<td>NEFA$^#$</td>
<td>Pre 0.36 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>0.41 ± 0.03</td>
<td>0.49 ± 0.06</td>
<td>0.59 ± 0.04</td>
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<td>0.61 ± 0.05</td>
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<tr>
<td>(mM)</td>
<td>Post 0.39 ± 0.04</td>
<td>0.42 ± 0.04</td>
<td>0.48 ± 0.04</td>
<td>0.49 ± 0.07</td>
<td>0.54 ± 0.03</td>
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<td>0.59 ± 0.05</td>
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<tr>
<td>Glycerol</td>
<td>Pre 0.31 ± 0.05</td>
<td>0.35 ± 0.06</td>
<td>0.34 ± 0.04</td>
<td>0.40 ± 0.05</td>
<td>0.25 ± 0.06</td>
<td>0.28 ± 0.09</td>
<td>0.38 ± 0.12</td>
<td>0.38 ± 0.05</td>
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<tr>
<td>(mM)</td>
<td>Post 0.31 ± 0.06</td>
<td>0.35 ± 0.03</td>
<td>0.40 ± 0.04</td>
<td>0.45 ± 0.06</td>
<td>0.27 ± 0.07</td>
<td>0.28 ± 0.05</td>
<td>0.28 ± 0.12</td>
<td>0.34 ± 0.07</td>
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<tr>
<td>Insulin</td>
<td>Pre 76.3 ± 7.5</td>
<td>72.1 ± 10.3</td>
<td>70.1 ± 11.3</td>
<td>71.2 ± 8.4</td>
<td>63.2 ± 7.4</td>
<td>52.7 ± 5.0</td>
<td>48.6 ± 4.4</td>
<td>43.0 ± 3.6</td>
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<tr>
<td>(pM)</td>
<td>Post 61.0 ± 9.3</td>
<td>65.6 ± 11.1</td>
<td>71.2 ± 10.0</td>
<td>57.7 ± 7.1</td>
<td>56.0 ± 5.5</td>
<td>63.0 ± 6.2</td>
<td>55.3 ± 4.9</td>
<td>50.6 ± 5.1</td>
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CHAPTER 7

METFORMIN WITH EXERCISE TRAINING & CARDIOVASCULAR RISK

**Title:** Effects of exercise training and/or metformin for lowering cardiovascular risk factors in individuals with impaired glucose tolerance.

**Authors:** Steven K. Malin\(^1\), Joy Nightingale\(^1\), Sung-eun Choi\(^2\) Stuart R. Chipkin\(^1\), and Barry Braun\(^1\)

\(^1\) Energy Metabolism Laboratory, Department of Kinesiology, University of Massachusetts, Amherst, MA 01003. \(^2\) Department of Family, Nutrition, and Exercise Sciences, Queens College, Flushing, NY.

Supported by NIH 5 R56 DK081038

**Running Head:** Exercise plus metformin on CVD risk factors

**Correspondence to:**
Barry Braun, PhD
Dept. of Kinesiology
107 Totman Building
University of Massachusetts
Amherst, MA 01003
Phone: (413) 577-0146
Fax: (413) 545-2906
Email: bbraun@kin.umass.edu
Abstract

Individuals with impaired glucose tolerance (IGT) are at elevated risk for cardiovascular disease. Although regular exercise or metformin may reduce cardiovascular disease (CVD) risk, the efficacy of combining treatments has not been investigated. **Purpose:** To determine the effects of exercise training plus metformin on reducing the level of CVD risk factors in individuals with IGT compared to each treatment alone. **Methods:** Subjects (n = 32) were assigned to either: placebo (P), metformin (M), exercise training plus placebo (EP), or exercise training plus metformin (EM). In a double-blind design, P or 2000mg/d of M were administered over 12 weeks and half were trained 3 days/week for approximately 60 minutes/session (70% heart rate peak and 70% 1-RM max). CVD risk factors included: body weight, blood pressure, blood lipids and C-reactive protein (CRP). **Results:** M and EM decreased body weight compared to P and EP (p <0.05), while all treatments lowered waist circumference (time effect: p < 0.05). M and EP lowered systolic blood pressure (p < 0.05), diastolic blood pressure (p < 0.05), and CRP (M: p = 0.06; EP: p < 0.05) compared to P. All treatments increased HDL (p< 0.05; EM: p = 0.06) compared to P and lowered triacylglycerols (time effect: p < 0.05). **Conclusion:** Metformin, with or without training, reduced body weight compared to training alone and all 3 treatments lowered WC and TAG and raised HDL. Combining metformin with training did not have additive effects on reducing CVD risk factors. Metformin may blunt the full effects of training on specific CVD risk factors in people with IGT.

KEY WORDS: Prediabetes, Metabolic Syndrome, Diabetes, Obesity, Hypertension
Introduction

Individuals with impaired glucose tolerance (IGT) are at elevated risk for cardiovascular disease (CVD) (5, 8, 20, 33, 46) and approximately half of these individuals have the metabolic syndrome (43). CVD risk is largely explained by insulin resistance and excess body weight (24, 50). Treatments that enhance insulin sensitivity or lower body weight may lower CVD risk in individuals with IGT.

Exercise reduces CVD risk by enhancing insulin sensitivity (12, 19, 28, 29) and lowering blood lipids (e.g. Triacylglycerol, low-density lipoproteins, etc), blood pressure, and C-reactive protein (3, 18, 26). Metformin lowers CVD risk by improving insulin sensitivity (36, 42) and reducing hyperglycemia and body weight (10, 11). It has been suggested that individuals with IGT and at least 1 CVD risk factor (e.g. hypertension, triacylglycerol, low HDL, hyperglycemia, etc) be considered for metformin treatment while participating in a regular exercise program (47). However, no study has determined the efficacy of combining metformin with exercise training on CVD risk factors in individuals with IGT. We previously showed that metformin blunted the effects of training on insulin sensitivity in individuals with IGT (Chapter 4). Whether the combined treatment opposes the reduction in the level of CVD risk factors remains unclear. Therefore, the purpose of this study was to determine the effects of combining metformin plus exercise training on reducing the levels of CVD risk factors in men and women with IGT, compared to either treatment alone. Given the blunting effects of metformin on training induced improvements in insulin sensitivity, we hypothesized that the combined treatment would oppose the reduction in the levels of CVD risk factors compared to either treatment alone.

Methods

Overview: In a double-blind, placebo-controlled study design, 32 men and women with impaired glucose tolerance (IGT) were enrolled in this study. Cardiovascular disease (CVD)
risk factors were defined as: body weight, blood pressure, blood lipids (triacylglycerol, total cholesterol, high density and low density lipoproteins), and C-reactive protein. Prior to testing, individuals were assigned to either: placebo (P), metformin (M), exercise training plus placebo (EP) or exercise training plus metformin (EM).

*Subjects:* Subjects (characteristics in Table 7.1) were non-smoking, weight stable (<5% weight change over last 3mo) and free of CVD or type 2 diabetes. Individuals on blood pressure (M; n = 1; EP; n = 1; EM; n = 3) or cholesterol (M; n = 1; EP; n = 1; EM; n = 1) medication were enrolled and asked to continue treatment throughout the study. Subjects were excluded from the study if they had any underlying contraindications to metformin (e.g. respiratory disease, heart failure, renal and hepatic disease). All subjects were verbally briefed about the study and signed informed consent documents approved by the Institutional Review Board at the University of Massachusetts Amherst.

*OGTT Screening:* An oral glucose tolerance test (OGTT) was used to determine if subjects had impaired glucose tolerance. After a minimum 5-hour fast, blood samples were taken from a forearm vein. Subjects consumed 75 grams of glucose and blood samples were collected 2 hours later. All subjects had impaired glucose tolerance (IGT; 2-hour glucose concentrations between 7.8-11.1mmol/L or 140-199mg/dl). Subjects with IGT who had fasting glucose concentrations between 5.5-6.9mmol/L (100-125mg/dl) were also included. *Metabolic syndrome:* Subjects meeting the ATP III criteria were also considered to have the metabolic syndrome (23). There were similar numbers of individuals with the metabolic syndrome in each condition (M = 6; EP = 7 and EM = 6).

*Preliminary testing:* Time to exhaustion was used to characterize cardio-respiratory fitness using a continuous progressive exercise test on a cycle ergometer (SensorMedics 800, Yorba Linda, CA) (41). One repetition max (1-RM) tests were performed in subjects assigned to EM and EP. Subjects performed a progressive resistance exercise test for the chest press, latissimus pull down, leg press, bicep curl, triceps pushdown, and upright rows.
Weight lifted was considered maximum if proper technique could be maintained throughout the full range of motion.

Metformin or Placebo protocol: Pills were administered to the subjects and they were instructed to take metformin or placebo with food in order to minimize potential side effects. Subjects started treatment with 500 mg/d of metformin. The dose was increased 500 mg/d each week until a clinical dose of 2000 mg/d was reached by week 4. Subjects remained at this dose for the last 8 weeks of the 12 week protocol.

Exercise Training: The training protocol was previously described (see Chapter 4), but in short, subjects exercised 3 days/week for 60-75 minute per session (approximately 190 minutes/week total). Subjects cycled for 45 minutes at 70% of their pre-training heart rate peak 3 days/week, and performed whole-body resistance exercise at 70% of the subject’s 1-RM 2 days/week. Pedometers (Omron HJ112) were provided to all subjects at week 0, week 6 and week 12 to characterize habitual ambulation. Pedometers were worn around the waistband for 7 consecutive days and the data were averaged for analysis.

Body Weight & Waist Circumference: Body weight was recorded without shoes to the nearest 0.1 kg on a calibrated scale. Body weight was recorded in the morning fasted for the first and last measurement. Additional measurements were taken bi-weekly at consistent times of day for each subject. Waist circumference was measured with a plastic tape measure to the nearest 0.25 cm approximately 2 cm above the umbilicus in the standing position. Bi-weekly waist circumference measures were taken at the same time as body weight. Food intake was assessed using 3-day food journals. Subjects selected 3 days (one regular and 2 irregular days) at week 0 and kept the same 3 days through the entire study. Food journals were analyzed (by the same investigator S.C.) using a commercially available software program (Fitday, El Segundo, California). The 3-day food journal was averaged to provide an estimated of caloric intake at week 0, 6, and 12.
**CVD risk factor collection:** Subjects were provided food (55% carbohydrate, 30% fat, and 15% protein) 24 hours prior to pre- and post-testing. After an overnight fast, subjects reported to the laboratory. Systolic (SBP) and diastolic (DBP) blood pressure were determined following a minimum 5-10 minute quiet sitting period and measurements were standardized to the left arm using an automated system (Mark of fitness, Inc., Shrewsbury, NJ). After these measures, indwelling catheters were placed in a superficial vein of a forearm and baseline blood samples were collected in 3 mL syringes. Plasma samples for the analysis of glucose were transferred to vacutainers containing sodium fluoride to inhibit glycolysis. Plasma samples for the analysis of total cholesterol (TC), high-density lipoproteins (HDL), and triacylglycerol (TAG) were collected in vacutainers containing the anticoagulant EDTA. Serum samples for the analysis of C-reactive protein (CRP) were collected in vacutainers containing the serum separator SST.

**Plasma analysis:** Plasma glucose, TC and HDL concentrations were determined enzymatically (GL5 Analyzer, Analox Instruments, Lunenberg, MA). TAG concentrations were measured by colorimetric assay (Sigma Alrich, St. Louis, MO). CRP concentrations were measured using high-sensitivity ELISA kit (Diagnostic Systems Laboratory, Webster, TX).

**Calculations:** Mean arterial pressure (MAP) was calculated as: MAP = 2/3(DBP) + (1/3SBP) (40). The cardiac risk ratio was calculated as total cholesterol divided by HDL (32). Low density lipoprotein (LDL) concentrations were estimated using the Friedwald equation (LDL = TC – HDL – (TG* 0.2) (21).

**Statistical Analysis:** Data were analyzed using the R statistical software package (version 2.4.0, The R foundation, Vienna, Austria, 2006). Baseline subject characteristics were measured between conditions by a one-way analysis of variance (ANOVA). There was no statistical difference in any baseline value except for DBP. Using baseline DBP as a co- variate did not affect the response to the treatments. All other condition means were
analyzed before and after the intervention with a 2-way (condition by test) repeated measures ANOVA. Because baseline CRP concentrations were not normally distributed, the data were log transformed for statistical analysis. When there was a significant condition by test interaction, Tukey’s Post hoc analysis was used to detect between conditions differences and paired t-tests were used to compare within condition differences. Paired-t-tests were used to determine changes within the treatment conditions for the metabolic syndrome Z-score and ATP III score as described previously (30). Sex specific Z-scores were used to describe the differences in ATP III criteria between men and women. The equations used were: Z-score = [(40-HDL)/10.7] + [(TAG-150/88.5)] + [(FPG-100)/11.9] + [(WC-102)/14.3] + [(MAP-100)/8.8] for men, and Z-score = [(50-HDL)/11.0] + [(TAG-150/88.5)] + [(FPG-100)/11.9] + [(WC-88)/13.6] + [(MAP-100)/8.8] for women. McNemar’s test was used to assess the prevalence of the metabolic syndrome (i.e. yes/no) after each treatment. Pearson’s correlation was used to examine relationships between weight loss, cardio-respiratory fitness, CVD risk factors and insulin sensitivity (Chapter 4). Significant differences were accepted as $\alpha \leq 0.05$.

**Results**

*Fitness and strength characteristics.* Exercise training plus metformin (EM) and exercise training plus placebo (EP) increased time to exhaustion by approximately 15-20% compared to placebo (P; $p < 0.05$; P: pre = 10.3 ± 1.1 vs. post = 10.3 ± 1.1; M: pre = 12.0 ± 0.7 vs. post = 11.0 ± 0.6; EP: pre = 13.4 ± 1.2 vs. post = 15.1 ± 1.2; EM: pre = 13.0 ± 0.9 vs. post = 16.3 ± 1.3). EP and EM both increased leg strength (EP: pre = 205.0 ± 11.8 vs. post = 241.4 ± 12.8; EM: pre = 214.0 ± 18.2 vs. post = 247.1 ± 18.8; time effect: $p < 0.05$), but EM increased chest strength more than EP (EP: pre = 112.5 ± 10.1 vs. post = 126.5 ± 10.7; EM: pre = 91.3 ± 7.5 vs. post = 130.0 ± 8.3; $p < 0.05$).
**Weight loss & Central Obesity.** Metformin (M) and EM decreased body weight more than P and EP (p < 0.05; Figure 7.1). All treatments decreased waist circumference by 2-3% throughout the intervention (time effect: p < 0.01; Figure 7.2).

**Blood pressure and C-reactive protein (CRP).** Compared to baseline, SBP and DBP increased with placebo (P; p < 0.05). M and EP lowered SBP (p < 0.01), but all 3 treatments lowered DBP compared to P (p < 0.05; Table 7.2). Given the changes in blood pressure, M and EP decreased MAP with compared to P (p < 0.05; Table 7.2). EP and M lowered CRP by approximately 20% compared to P (EP: p < 0.05 and M: p = 0.06 respectively; Table 7.4).

**Fasting Hyperglycemia:** Plasma glucose concentrations were previously reported (see Chapter 4), but are shown here as condition mean differences (i.e. change between pre and post measures). Exercise training and/or metformin had no effect on fasting glucose concentrations (P = -0.1 ± 0.2mM; M = -0.2 ± 0.3mM; EP = 0.0 ± 0.3mM; EM = -0.4 ± 0.2mM).

**Triacylglycerol (TAG) and Cholesterol:** Exercise and/or metformin lowered TAG by approximately 13% (time effect: p < 0.05; Table 7.3) but had subtle effects on total cholesterol (time effect: p = 0.08) or LDL. All 3 treatments increased HDL concentrations by 8-13% compared to P (M or EP; p < 0.05 and EM; p = 0.06; Table 7.3). Each treatment also improved the cardiac risk ratio (p < 0.05; Table 7.3).

**Metabolic syndrome:** Exercise training and/or metformin reduced the metabolic syndrome Z-score compared to baseline (EP; p = 0.07 and EM or M; p < 0.05). Although each treatment reversed the metabolic syndrome (M = 6 to 1; EP = 7 to 4; EM 6 to 3; p < 0.05), only EP and M reduced ATP III score (p < 0.05) compared to baseline (Table 7.4).

**Correlation Analysis:** Reductions in TAG concentrations were associated with enhanced insulin sensitivity (r = -0.60; p < 0.05). Decreased CRP concentrations were associated with reductions in systolic blood pressure (r = 0.46; p < 0.05).
Discussion

Overview. Our findings indicate that metformin may blunt the full effects of training on lowering the cardiovascular disease risk factors, systolic blood pressure and C-reactive protein, in individuals with impaired glucose tolerance (IGT).

Effects of exercise and/or metformin on weight & central obesity. Although the effects of training on weight loss are mixed (7, 14, 31), metformin commonly promotes weight loss upwards of 5kg (see review (13)). Some, (2, 9, 38) but not all (45), show greater weight loss when metformin is added to lifestyle modification. In this study, exercise training plus metformin promoted more weight loss than training, but not metformin. Previously we showed that the weight loss with metformin was comprised of both fat and fat-free mass, whereas training plus metformin was only body fat (Chapter 4). Maintenance of muscle mass may be important for continued weight loss success (27, 39). The exact mechanism by which metformin reduces body weight remains unclear, but it is either related to increased energy expenditure or reduced caloric intake. Ambulation and resting metabolic rate did not change after any treatment (see S.C.1 and S.C.2), suggesting that metformin did not increase energy expenditure. Although metformin, with or without exercise, non-significantly lowered caloric intake, we observed a significant correlation between weight loss and reduced caloric intake (see S.C.3 and S.C.4). Thus, these observations suggest that reductions in caloric intake may explain the effects of metformin on weight loss (35, 44, 53).

Effects of exercise and/or metformin on blood pressure and C-reactive Protein. Although exercise is known to lower blood pressure (3), the effects of metformin are mixed (15, 22, 34, 36). Few have characterized the combined treatments effects on blood pressure (1, 16). We found that metformin blunted the effects of exercise on lowering systolic, but not diastolic, blood pressure in individuals with IGT. It is difficult to directly compare these previous studies (1, 16) to our own because of differences in the subjects underlying
pathophysiology, exercise protocols, dietary manipulation, and interaction of other medications. Because we observed no associations between weight loss, reductions in central obesity, cardio-respiratory fitness, or insulin sensitivity and systolic blood pressure, our findings suggest that alternative mechanisms explain the blunting effects of the combined treatment on lowering systolic blood pressure.

C-reactive protein (CRP), a marker of vascular inflammation, has been associated with elevated blood pressure in individuals with IGT (24). Consistent with previous work (6, 25, 26, 49), we demonstrated that metformin or exercise training alone reduced CRP concentrations. However, combining treatments had no effect on CRP concentrations. We observed a significant correlation between CRP and systolic blood pressure, suggesting that reduced inflammation was associated with lower systolic blood pressure. It is unclear how the combined treatment blunted reductions in CRP concentrations or systolic blood pressure, but it may be related to nitric oxide synthesis (51), which altered vascular reactivity. Whether reduced AMPK activation in tissues other than skeletal muscle, such as the endothelium, is responsible for altered nitric oxide production and accounts for the blunting effects of the combined treatment on systolic blood pressure warrants further investigation (37, 48).

Effects of exercise and/or metformin on triacylglycerol. Exercise or metformin are known to lower triacylglycerol concentrations (18). Most have characterized the effects of lifestyle modification and metformin on triacylglycerol (1, 2, 9). Andrealis et al (1) indicated that combining metformin with lifestyle modification had similar effects to lifestyle modification alone on lowering TAG in individuals with IGT. In contrast, metformin with lifestyle modification reduced TAG concentrations more than lifestyle modification alone in overweight insulin resistant adolescents (2, 9). In the current study, exercise training and/or metformin lowered TAG concentrations, which were associated with enhanced insulin sensitivity ($r = -0.60; p < 0.05$ (see S.C.5). The obese insulin resistant adolescents may
have responded better to metformin with lifestyle modification than adults with IGT because they were more insulin sensitive based on glucose tolerance. Severe insulin resistance could result in higher TAG concentrations through persistent inhibition of lipoprotein lipase. Thus, our results suggest that exercise and/or metformin may reduce the delivery of fatty acids to skeletal muscle and improve insulin sensitivity (33).

Effects of exercise and/or metformin on cholesterol. Although exercise or metformin raise HDL concentrations (15, 17, 42), the effectiveness of each treatment on lowering total cholesterol and LDL is mixed (15, 17, 36, 52). Few have characterized the effects of exercise plus metformin on total cholesterol, HDL, or LDL. Andrealis et al (1) reported that adding metformin to lifestyle modification further reduced total cholesterol and LDL concentrations in individuals with IGT. Driscoll et al (16) demonstrated that the exercise training plus metformin had no effect on total cholesterol, LDL or HDL compared to metformin alone in HIV infected patients. In this study, we demonstrated that exercise and/or metformin raised HDL concentrations and non-significantly lowered total cholesterol and LDL. Elevated HDL concentrations are consistent with our current reductions in TAG concentrations (33). A possible reason we did not detect statistically significant reductions in total cholesterol or LDL is because of inter-subject variability between conditions. Only 3 subjects in each treatment condition had total cholesterol concentrations above the CVD risk factor threshold of 5.2mM (i.e. 200 mg/dl). We observed a relationship between elevated baseline concentrations and the response to exercise and/or metformin, suggesting that individuals with elevated cholesterol concentrations responded “better” to the exercise and/or metformin than individuals with cholesterol values within normal range (4). Nonetheless, exercise and/or metformin improved the cardiac risk ratio (i.e. total cholesterol:HDL), suggesting that these treatments may reduce certain aspects of CVD risk in individuals with IGT (32).
Effects of exercise and/or metformin on the metabolic syndrome: Although lifestyle modification or metformin reduces the incidence of the metabolic syndrome (43), few have assessed the combined treatments effect on lowering metabolic syndrome risk factors (1). Andreadis et al (1) demonstrated that adding metformin to lifestyle modification reduced the incidence of the metabolic syndrome more than lifestyle modification alone. We showed that exercise training and/or metformin reduced the metabolic syndrome Z-score, indicating that all treatments lowered the severity of the metabolic syndrome. In line with this, all treatments reduced the incidence of the metabolic syndrome. However, our findings indicated that only exercise training or metformin reduced the ATP III score, implying that these treatments reversed more metabolic syndrome risk factors than the combined treatment. The combined treatments blunting effect on reversing the ATP III score was primarily explained by blunting reductions in systolic blood pressure (data not shown). Thus, our findings suggest that combining metformin with exercise may oppose the reversal of some metabolic syndrome risk factors in individuals with IGT and potentially alter the incidence of the metabolic syndrome in individuals with IGT.

Limitations. Some aspects to this study may limit our interpretation of the data. First, pedometers were used to assess habitual physical activity. It is possible that activity intensity increased or individuals stood/took more breaks in sedentary behavior, which increased energy expenditure and contributed to the weight loss seen with metformin. Second, error with food recording or quantification might have limited our ability to detect statistical significance. Since subjects selected the days they recorded food intake, their overall eating patterns may have been affected. Lastly, we used an aerobic and resistance training program combined with 2000mg/d of metformin. Thus, our findings may not be applicable to different exercise modes/intensities combined with other doses of metformin.

Conclusion. Metformin and/or exercise training lowered blood pressure and triacylglycerol as well as raised HDL concentrations in individuals with impaired glucose tolerance. The
novel observation in this study is that combining metformin with exercise training did not promote additive effects on any cardiovascular disease risk factor measured in this study. Metformin may blunt the full effects of exercise training on lowering systolic blood pressure and C-reactive protein. If these findings are reproducible, then these data may have adverse clinical consequences for lowering cardiovascular disease risk in individuals with IGT and/or the metabolic syndrome.

Acknowledgments
S.K.M, S.R.C, and B.B. contributed to the study design and data collection. S.K.M was primarily responsible for data analysis and statistical integrity. J.N. contributed to data entry and data analysis. S.C. was solely responsible for food data analysis. S.K.M wrote the manuscript and S.R.C and B.B. reviewed/edited the manuscript. The authors would like to thank Kirsten Granados, Richard Viskochil, and Robert Gerber for technical assistance and helpful discussion. We thank John Staudenmeyer, PhD for statistical support. We extend our appreciation to the dedicated participants for their time and effort. Lastly we thank the hard work of all the undergraduate research assistants for help on this project. This research was supported by NIH 5 R56 DK081038 Grant.
References


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on inflammation and coagulation in participants with impaired glucose tolerance. *Diabetes.* 2005; 54(5): 1566-72.


Figure Captions

Figure 7.1. Change in body weight over the 12 week protocol. ^Significant effect of M and EM compared to P (p < 0.05). Significant effect of M(*) and EM(‡) compared to baseline (p < 0.05). Values are reported as mean change.
Figure 7.2. Change in waist circumference over the 12 week intervention. *Significant effect of time (p < 0.05). Values are reported as mean change.
Table Captions

Table 7.1. Subject Characteristics. Beat per minute (bpm). No significant differences were observed between conditions. Values are mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>M</th>
<th>EP</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>6F/2M</td>
<td>4F/4M</td>
<td>5F/3M</td>
<td>5F/3M</td>
</tr>
<tr>
<td><strong>Age (year)</strong></td>
<td>49.8 ± 10.9</td>
<td>45.0 ± 7.5</td>
<td>45.4 ± 8.0</td>
<td>49.1 ± 6.6</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>34.0 ± 6.3</td>
<td>33.9 ± 5.2</td>
<td>33.5 ± 4.1</td>
<td>31.2 ± 5.3</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>93.5 ± 6.0</td>
<td>101.5 ± 5.8</td>
<td>95.5 ± 5.1</td>
<td>94.1 ± 6.5</td>
</tr>
<tr>
<td><strong>VO₂ peak (ml/kg-ffm/min)</strong></td>
<td>41.2 ± 8.2</td>
<td>43.4 ± 10.3</td>
<td>45.7 ± 9.4</td>
<td>48.2 ± 4.9</td>
</tr>
<tr>
<td><strong>Fasting Glucose (mM)</strong></td>
<td>5.8 ± 0.6</td>
<td>5.6 ± 0.6</td>
<td>5.4 ± 0.4</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td><strong>2-hour Glucose (mM)</strong></td>
<td>9.4 ± 1.3</td>
<td>9.3 ± 1.5</td>
<td>10.2 ± 1.0</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td><strong>Steps per day</strong></td>
<td>4984 ± 2104</td>
<td>5028 ± 2047</td>
<td>5871 ± 1912</td>
<td>6444 ± 1529</td>
</tr>
</tbody>
</table>
Table 7.2. SBP (systolic blood pressure), DBP (diastolic blood pressure), and MAP (mean arterial pressure). *Significant compared to baseline; p < 0.05. ^ Compared to P (p < 0.05). Values are means ± standard error of the mean.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Test</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong></td>
<td>Pre</td>
<td>126.1 ± 3.1</td>
<td>76.8 ± 2.8</td>
<td>92.3 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>134.5 ± 5.4*</td>
<td>82.9 ± 2.3*</td>
<td>99.1 ± 1.2</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td>Pre</td>
<td>134.0 ± 3.8</td>
<td>82.9 ± 2.9</td>
<td>98.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>123.9 ± 2.7**^</td>
<td>78.9 ± 1.7^</td>
<td>92.9 ± 0.6^</td>
</tr>
<tr>
<td><strong>EP</strong></td>
<td>Pre</td>
<td>136.8 ± 2.4</td>
<td>88.1 ± 2.1</td>
<td>103.3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>128.0 ± 3.9^</td>
<td>81.4 ± 2.0^*</td>
<td>95.9 ± 1.0**^</td>
</tr>
<tr>
<td><strong>EM</strong></td>
<td>Pre</td>
<td>126.8 ± 5.6</td>
<td>82.1 ± 2.9</td>
<td>96.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>126.3 ± 5.1</td>
<td>78.5 ± 2.5^</td>
<td>93.3 ± 1.2</td>
</tr>
</tbody>
</table>
Table 7.3. TC (total cholesterol), LDL (low-density lipoprotein), HDL, (high density lipoprotein), TAG (triacylglycerol), CRP (C-reactive protein), CRR (cardiac risk ratio). *Significant compared to baseline; \( p < 0.05 \). *Significant effect of test; \( p < 0.05 \). ^Compared to P (\( p < 0.05 \)). 5Compared to P (\( p = 0.06 \)). Values are means ± standard error of the mean.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Test</th>
<th>TC (mM)</th>
<th>LDL (mM)</th>
<th>HDL (mM)</th>
<th>CRR</th>
<th>TAG (mM)</th>
<th>CRP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Pre</td>
<td>4.4 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>90.2 ± 20.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>4.5 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>3.4 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>114.3 ± 33.2</td>
</tr>
<tr>
<td>M</td>
<td>Pre</td>
<td>4.8 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>1.5 ± 0.1</td>
<td>3.3 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>100.4 ± 19.3</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>4.5 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>1.7 ± 0.1*</td>
<td>2.7 ± 0.2^</td>
<td>1.9 ± 0.3</td>
<td>94.5 ± 30.2^</td>
</tr>
<tr>
<td>EP</td>
<td>Pre</td>
<td>4.9 ± 0.3</td>
<td>2.1 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>72.0 ± 19.9</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>4.7 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>1.7 ± 0.1^</td>
<td>2.8 ± 0.2*</td>
<td>2.1 ± 0.3</td>
<td>55.7 ± 22.0^*</td>
</tr>
<tr>
<td>EM</td>
<td>Pre</td>
<td>4.9 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>1.5 ± 0.1</td>
<td>3.2 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>64.0 ± 17.1</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>4.5 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>1.7 ± 0.1*^</td>
<td>2.8 ± 0.3**^</td>
<td>1.6 ± 0.3</td>
<td>58.5 ± 15.2</td>
</tr>
</tbody>
</table>
Table 7.4. Effect of each condition on metabolic syndrome Z-Score and ATP III score.
*Pre to post observation was statistically different from baseline; p < 0.05. $p = 0.07.$

Values are mean ± standard error of the mean.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Test</th>
<th>Z-score</th>
<th>ATP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Pre</td>
<td>1.40 ± 0.53</td>
<td>3.50 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>-1.56 ± 0.22$^*$</td>
<td>2.00 ± 0.12$^*$</td>
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<tr>
<td>EP</td>
<td>Pre</td>
<td>0.52 ± 0.36</td>
<td>3.57 ± 0.08</td>
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<tr>
<td></td>
<td>Post</td>
<td>-1.04 ± 0.41$^*$</td>
<td>2.28 ± 0.20$^*$</td>
</tr>
<tr>
<td>EM</td>
<td>Pre</td>
<td>0.68 ± 0.42</td>
<td>3.16 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>-1.00 ± 0.43$^*$</td>
<td>2.50 ± 0.20</td>
</tr>
</tbody>
</table>
CHAPTER 8
REVIEWS AND IMPLICATIONS

Title: Combining exercise with metformin: is it optimal for reducing diabetes risk?

Authors: Steven K. Malin, Stuart R. Chipkin and Barry Braun

Energy Metabolism Laboratory, Department of Kinesiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA 01003

Supported by NIH 5 R56 DK081038

Running Head: Combining exercise and metformin

Correspondence to:
Barry Braun, PhD
Dept. of Kinesiology
107 Totman Building
University of Massachusetts
Amherst, MA 01003
Phone: (413) 577-0146
Fax: (413) 545-2906
Email: bbraun@kin.umass.edu
Abstract

Exercise or metformin is suggested to reduce the risk for developing type 2 diabetes. Combining these treatments may confer greater benefits on insulin sensitivity and cardiovascular disease risk as well as impact exercise capacity. In this article we highlight the recent progress in the literature on this topic because understanding the effects of combining exercise and metformin may provide better insight to preventing/delaying type 2 diabetes.

KEY WORDS: Obesity, type 2 diabetes, insulin resistance, metabolic syndrome
Introduction

In the United States (U.S.), approximately 25 million individuals have type 2 diabetes (1). The mortality and medical costs associated with type 2 diabetes makes this disease an important public health concern. One approach to reduce the incidence of type 2 diabetes is to specifically target individuals at risk (e.g. insulin resistant, metabolic syndrome, prediabetes). Although the exact cause for type 2 diabetes remains unknown, a key component underlying this disease is insulin resistance (see below). Thus, identifying treatments that enhance insulin sensitivity may be advantageous for reducing the incidence of diabetes.

Several large clinical trials have shown the beneficial effects of lifestyle modification (i.e. low-fat diet and increased physical activity) on delaying/preventing type 2 diabetes (7, 37, 50)). However, few have determined the independent effects of exercise on diabetes prevention (49). Because exercise tolerance might be impaired in individuals at risk for type 2 diabetes, pharmacological interventions may also be helpful to promote the prevention of diabetes. Metformin, a common anti-diabetes medication, has been shown to reduce the incidence of type 2 diabetes and is suggested to be combined with regular exercise (46). There are few studies however characterizing the efficacy of combining exercise plus metformin on metabolic health (8, 18, 24, 32, 43, 54). If the interaction between exercise and metformin are additive, then combining these treatments deserves to be encouraged. However, if one treatment attenuates the other, combining treatments would be counterproductive. Our recent work showed that metformin attenuated the effects of exercise on metabolic health in individuals with insulin resistance, suggesting to us that exercise alone may be “better” than the combined treatment (54) (Chapter 4 and 7). This review article will focus on recent data centered on the efficacy of combining exercise with metformin on insulin sensitivity, cardiovascular disease risk, and exercise capacity in individuals at risk for diabetes.
**Insulin resistance affects blood glucose regulation.**

Insulin resistance is a key component in the development of hyperglycemia and type 2 diabetes (51). Although blood glucose concentrations are affected by distinct tissues in the fasted (e.g. brain and kidney) and insulin stimulated state (e.g. digestive tract, liver, muscle and adipose tissue), blood glucose homeostasis is in general a balance between endogenous glucose production and glucose uptake (Figure 8.1). Endogenous glucose production consists of hepatic glucose production with small contributions from the kidney (approximately 15-25%) (3). Glucose uptake into peripheral tissues, such as skeletal muscle (approximately 80%), is primarily a result of insulin. The inability of insulin to suppress glucose production and/or increase glucose uptake in the aforementioned tissues is referred to as insulin resistance. Insulin resistance often leads to metabolic abnormalities that include, but are not limited to, dyslipidemia, hypertension, and hyperglycemia (51). As a result, insulin resistance not only increases risk for type 2 diabetes but also for cardiovascular disease (19). Thus, identifying treatments that enhance insulin sensitivity is critical to the prevention of type 2 diabetes and cardiovascular disease.

**Exercise or Metformin affects Insulin Sensitivity**

It has been well established that exercise enhances insulin sensitivity in individuals across the glucose tolerance continuum (i.e. normal glucose tolerant to type 2 diabetes) (see reviews: (26, 30)) and contributes to the lowering blood glucose concentrations (27). Metformin is an orally administered drug that is routinely used to lower blood glucose concentrations in individuals with type 2 diabetes. More recently, metformin has been considered a useful agent in those with prediabetes (52). Although metformin improves whole-body insulin sensitivity in some (16, 40), but not all studies (54, 56, 59), it's mechanism of action is different than exercise. The primary tissue affected by metformin is
the liver (see review: (35)), whereas exercise largely improves skeletal muscle glucose uptake (36).

**Does combining exercise with metformin have additive effects on insulin sensitivity?**

Because each treatment targets a different tissue (i.e. skeletal muscle vs. liver), we hypothesized that combining treatments would have an additive effect on insulin sensitivity. Recently, our lab determined the effect of combining exercise plus metformin on whole-body insulin sensitivity in overweight, sedentary, insulin resistant individuals following a single bout of exercise (54). Metformin or placebo was provided to all participants for 2-3 weeks at 2000mg/d, after which individuals exercised for approximately 30 minutes at 65% and 10 minutes at 85% VO$_2$peak. Insulin sensitivity was measured approximately 4 hours post-exercise by the hyperinsulinemic-euglycemic clamp. The results indicated that exercise alone enhanced whole-body insulin sensitivity by 54% compared to baseline, but when metformin was added to exercise there was no change in insulin sensitivity (Figure 8.2). Because 5-adenosine monophosphate kinase (AMPK) has been suggested to be an important regulator of post-exercise insulin sensitivity (20, 44, 45), we also assessed the combined treatments effect on AMPK in skeletal muscle. Metformin blunted the effects of exercise on skeletal muscle AMPK activation, suggesting that combining exercise and metformin may attenuate the action of cellular mediators to enhance insulin sensitivity. The clinical impact of these findings (54) however remained unclear. Although individuals with insulin resistance are at risk for type 2 diabetes, individuals with prediabetes are of greater concern because of the increased rates of progression to type 2 diabetes (37). In addition, repeated bouts of exercise are recommended to reduce diabetes risk (15). Therefore, from a practical standpoint, it was important to assess the effect of training plus metformin on metabolic health in individuals with prediabetes.
Our lab designed a study to determine the effects of combining exercise training and metformin on insulin sensitivity in men and women with prediabetes (Chapter 4). For 12 weeks, individuals with prediabetes were assigned to 1 of 4 groups: placebo, metformin, exercise training plus placebo, or exercise training plus metformin. All individuals received either metformin at 2000mg/d or placebo, while half participated in a progressive aerobic and resistance training program at 70% of their heart rate peak and 1-repetition max. Whole-body insulin sensitivity was measured approximately 28-30 hours post-exercise by the hyperinsulinemic euglycemic clamp. The results indicated that metformin or exercise training enhanced whole-body insulin sensitivity by approximately 55% and 90% compared to baseline, respectively. Adding metformin to exercise training enhanced whole-body insulin sensitivity by only 65% compared to baseline (Figure 8.3), implying that metformin blunted the improvement in insulin sensitivity after training.

Although not quite statistically significant, exercise training plus placebo enhanced insulin sensitivity by approximately 30% more than either metformin, with or without training (Chapter 4). We may not have detected statistical differences between groups because of the inter-subject variability introduced by including different subsets within the prediabetes category. Prediabetes includes impaired glucose tolerance (IGT), impaired fasting glucose (IFG) concentrations, or both (i.e. IFG+IGT). Peripheral insulin resistance (e.g. skeletal muscle) is a primary defect in individuals with IGT, whereas hepatic and peripheral insulin resistance characterizes individuals with IFG+IGT (2). Individuals with IFG+IGT might be expected to respond more favorably to exercise and/or metformin than individuals with IGT because metformin primarily affects the liver and exercise largely affects skeletal muscle. Subgroup analysis indicated that all treatments accentuated insulin sensitivity in individuals with IFG+IGT compared to IGT alone (Chapter 4). These findings are in line with current recommendations suggesting many Americans with IFG+IGT be considered for metformin while exercising (52). Regardless, our findings indicate that the combined treatment blunted
the improvement in insulin sensitivity from exercise in people with IGT, in the absence or presence fasting hyperglycemia (i.e. IFG + IGT). This suggests that exercise plus metformin may impair the optimal benefit of exercise on insulin sensitivity in individuals with IGT.

Our results are not the first to show that the combined treatment does not have additive effects on insulin sensitivity (25, 33, 50, 55). The direct comparison between studies is complicated by differences in the subject population (e.g. animal vs. human or prediabetes vs. type 2 diabetes), measurement of insulin sensitivity (e.g. surrogate or clamp), and exercise protocols (e.g. recommended vs. supervised). However, the consistency of the findings across methods, protocols, and subjects strengthens the conclusion that metformin does not add but opposes the effects of exercise on insulin sensitivity (54) (Chapter 4).

Although we did not design our training studies (Chapter 4 and 7) to determine the cellular mechanism by which metformin and/or exercise affected insulin sensitivity, but the combined treatments blunting effect on insulin sensitivity may be related to altered peripheral adaptations. We previously showed in human skeletal muscle that short-term metformin treatment attenuated AMPK activation (54). If this effect persisted throughout exercise training, then it is possible that the attenuated AMPK minimized adaptations in skeletal muscle (e.g. GLUT4, hexokinase, glycogen synthase, etc) favoring glucose uptake (28). It is unclear why metformin would blunt the activation of AMPK, but it may be related to the antioxidant properties of metformin. Ristow et al (53) demonstrated that antioxidants blunted the exercise training effect on enhancing insulin sensitivity in overweight men. Attenuated insulin sensitivity was associated with bunted mitochondrial adaptations (e.g. PGC1-alpha, citrate synthase, etc). Since metformin might reduce reactive oxygen species (ROS) (6), it is tempting to speculate in our study (Chapter 4) that metformin reduced ROS production and minimized AMPK activation when combined with exercise training. Further
work is required to elucidate the cellular mechanisms by which metformin acts on skeletal muscle adaptations to better understand its effects on whole-body insulin sensitivity.

**Does combining exercise with metformin have additive effects on cardiovascular risk?**

Insulin resistance is associated with several cardiovascular disease (CVD) risk factors, including: hyperglycemia (51), hypertension (13), dyslipidemia (38) and inflammation (19). Because metformin attenuated the effects of exercise on enhancing insulin sensitivity, we thought it would be relevant to determine the combined treatments effect on hyperglycemia, blood pressure, dyslipidemia, and inflammation in individuals with prediabetes (Chapter 7).

After 12 weeks, metformin, exercise training, or the combined treatment had no effect on fasting glucose concentrations (Chapter 4). Although metformin would be expected to lower fasting glucose concentrations (29), a likely reason why we did not detect decreased glucose concentrations is because most of our subjects had fasting normoglycemia (i.e. IGT only). Subgroup analysis indicated that only metformin, with or without training, lowered fasting glucose concentrations in individuals with IFG+IGT (Figure 8.4). This suggests that metformin is mostly effective at lowering fasting glucose concentrations when they are elevated (39). Thus, if the primary outcome is management of blood glucose concentrations, then our findings indicate that metformin, with or without exercise, may be most appropriate choice for individuals with mild fasting hyperglycemia (i.e. 100-125mg/dl; IFG+IGT).

We found that metformin or exercise training lowered systolic blood pressure and C-reactive protein (CRP) by approximately 7% and 25%, respectively, in men and women with prediabetes. The combined treatment blunted reductions on systolic blood pressure and CRP by 0% and 8%, respectively (Chapter 7). Others have found that combining metformin
and lifestyle modification (i.e. low-fat diet/increase physical activity) has little or no further improvement in blood pressure (4, 5, 14). In contrast to our findings, Driscoll et al (17) demonstrated that the combination of exercise training with metformin for 12 weeks lowered systolic blood pressure compared to metformin alone in HIV infected patients. CRP was not assessed in the HIV infected patients so it is not possible to know if differences in vascular inflammation account for the differences in systolic blood pressure between studies. We found a moderate correlation between CRP and systolic blood pressure (Chapter 7), implying that reduced inflammation was associated with lower systolic blood pressure. Reductions in systolic blood pressure and CRP were not associated with changes in insulin sensitivity, suggesting that the blunting effect of metformin on reducing CRP and systolic blood pressure was independent of improved insulin sensitivity. It is possible that CRP affected nitric oxide production, which affected vascular reactivity (58). Thus, the blunting effect of exercise and metformin on CRP and systolic blood pressure may be, to some extent, mediated by decreased nitric oxide production. Whether reduced AMPK activation in tissues other than skeletal muscle, such as the endothelium, is responsible for altered nitric oxide production remains to be seen (41, 54). Further work is required to elucidate the mechanism by which combining metformin with exercise affects blood pressure and CRP concentrations in individuals with insulin resistance since this has potential implications for hypertension and cardiovascular health.

We found that metformin and/or exercise training lowered triacylglycerol (TAG) and raised HDL (Chapter 7), suggesting that all three treatments have some effects at improving blood lipids. Our findings are consistent with previous work (4) that showed the combination of metformin with lifestyle modification had similar effects on lowering TAG in individuals with prediabetes. In contrast, metformin with lifestyle modification reduced TAG concentrations more than lifestyle modification alone in overweight insulin resistant adolescents (5, 14). Potentially important differences in age, insulin resistance, and exercise
protocols may have affected the response to the intervention (5, 14, Chapter 4 and 7). These findings together, however, indicated that combining metformin with exercise training effectively lowers TAG, which may contribute to the reduction in insulin resistance and diabetes risk (Chapter 7).

Although lifestyle modification or metformin reduced the incidence of the metabolic syndrome (47), few have assessed the combined treatments effect on lowering metabolic syndrome risk factors (4). Andreadis et al (4) demonstrated that adding metformin to lifestyle modification reduced the incidence of the metabolic syndrome more than lifestyle modification alone. However, our findings indicate that combining metformin and exercise does not promote greater cardiovascular health compared to either treatment alone. In fact, combining treatments may have blunting effects. In subgroup analysis, we stratified individuals according to criteria from the National Cholesterol Education Program ATP III for the metabolic syndrome (22). Our results indicated that metformin reversed the metabolic syndrome in 5 of 6 individuals, while both training, with or without metformin, reserved the metabolic syndrome in 3 of the 6 and 7 individuals respectively (Figure 8.5a). The combined treatments blunting effect on reversing the ATP III risk factors were primarily explained by blunting reductions in systolic blood pressure (Figure 8.5b, 8.5c, 8.5.d). Thus, our findings indicate that the combined treatment is not “better” at reversing the metabolic syndrome and that exercise may blunt the effects of metformin. Future work is needed to determine the clinical ramifications of combining metformin and exercise on affecting the incidence of cardiovascular disease in individuals with the metabolic syndrome.

**Does metformin affect exercise capacity?**

Affecting the ability to exercise can have a beneficial or detrimental impact on the metabolic response to exercise. Metformin has been shown to increase skeletal muscle oxidative enzyme concentrations (57), suggesting an increased ability to consume oxygen
and produce energy. Although improving skeletal muscle oxidative capacity may lead to improved exercise tolerance, metformin has been reported to inhibit complex I in the mitochondria (12, 48). Alterations in the ability of the mitochondria to utilize oxygen may detrimentally affect cardio-respiratory fitness and exercise capacity. Since metformin may potentially affect energy metabolism, we were interested in determining the effect of metformin on exercise capacity.

We conducted a 7-10 day study to determine the effects of metformin on exercise capacity in a group of recreationally active men and women (8). Recreationally active men and women were selected to minimize the confounding effects of mitochondrial dysfunction in individuals with insulin resistance (34). We found that short-term administration of metformin at 2000mg/d reduced VO$_2$peak, heart rate peak and exercise duration by approximately 3-5%. This was in contrast to previous work (32) showing no effect of a single dose of metformin at 1000mg/d on maximal oxygen consumption in healthy men. A potentially important difference between studies is that we provided metformin for approximately 1 week at a higher dose (1000 vs. 2000mg/d), which more effectively lowers glucose concentrations (21). Thus, our findings may be more representative of the individuals chronically exposed to metformin. However, the physiologic relevance of these data is limited. If the effect of metformin on attenuating VO$_2$peak persisted over a prolonged period time and were replicated in a clinical population, then health professionals would potentially need to adjust exercise prescriptions to account for the increased relative exercise intensity.

Malin et al (see Chapter 4) recently compared the effects of combining metformin plus exercise training compared to either treatment alone on cardio-respiratory fitness in individuals with prediabetes. Although training, with or without metformin, improved VO$_2$peak, training with metformin increased VO$_2$peak by approximately 10% whereas training alone improved VO$_2$peak by 20%. Thus, metformin non-significantly blunted the
effects of training on increasing VO$_2$peak compared to training alone. Training, with or without metformin, however similarly increased exercise duration and workload peak compared to baseline. Metformin alone had no effect on any maximal cardio-respiratory fitness outcome. These findings indicate that metformin has subtle effects on cardio-respiratory fitness after training in individuals with prediabetes and was consistent with our earlier work (9). However, it is worth noting that metformin may improve exercise capacity in other clinical populations (31). Taken together, metformin may have a small negative effect on maximal oxygen consumption in individuals with prediabetes. Health professionals should be cognizant of this potential “side effect” because it could have important implications for designing exercise programs. If the blunting effects of metformin on oxygen consumption were significant, evaluation of effort and energy metabolism during submaximal exercise would be important.

**Does metformin affect submaximal exercise metabolism or perception of effort?**

The effects of exercise training on substrate metabolism and perception of effort have been well documented (see review: (10)). In short, endurance training reduces the perception effort and increases the reliance on fat by sparing muscle glycogen utilization during exercise at the same pre-training absolute workload.

Given the potential effects of metformin on VO$_2$peak (8), a potentially important effect of decreased cardio-respiratory fitness when performed at the same absolute workload is an increase in the relative intensity of exercise. Exercise at a higher intensity would be expected to shift fuel metabolism towards carbohydrate utilization and increase the perception of effort. As a result, we designed a study to test the effect of metformin on exercise substrate utilization (42). Metformin was administered for 7-10 days to recreationally active men and women. Individuals exercised on a cycle ergometer between 30-70% of their work peak (Wpeak). Importantly, men and women exercised at the same
absolute and relative intensity to determine the independent effects of metformin. Using indirect calorimetry, we found that metformin enhanced whole-body fat oxidation (see Chapter 5) during exercise by approximately 25% across the range of exercise intensities (~30 to 70% Wpeak). Metformin also increased blood lactate concentrations across the range of exercise intensities and this correlated with an increased perception of effort. Increased fat oxidation was contrary to our original hypothesis (8). From these data, we were not able to discern whether metformin restrained carbohydrate metabolism (e.g. decreased hepatic glucose output and blood glucose concentrations) thereby indirectly elevating fat oxidation or directly enhanced fat oxidation. Our findings were however consistent with previous work showing that metformin reduced the “anti-lipolytic environment” and favored fat oxidation (23). Whether metformin added to the effects of training on fat oxidation during exercise in a clinically relevant population was of significant interest. Given that elevated fat oxidation has been suggested to be a potential mechanism for increasing insulin sensitivity (11), the potential for the combined treatment to enhance fat oxidation and affect insulin sensitivity was intriguing.

We determined the effects of adding metformin to a 10-week training program on substrate metabolism in individuals with prediabetes (Chapter 6). Men and women exercised on a cycle ergometer at 60% of their pre-training VO₂peak, and we used indirect calorimetry and the isotope dilution method to determine fat oxidation and glucose flux during exercise. Although training, with or without metformin, lowered heart rate and the perception of effort, metformin tended to attenuate the reduction in submaximal oxygen consumption (i.e. 55% vs. 50% VO₂peak; p = 0.11). However, we found that training, independent of metformin, enhanced fat oxidation during exercise. This was somewhat surprising given that metformin increased plasma non-esterified fatty acids (NEFA) and decreased plasma insulin concentrations, suggesting that metformin enhanced lipolysis. However, we used plasma glycerol concentrations as a crude surrogate of lipolysis and
demonstrated that training, with or without metformin, had no effect on glycerol concentrations. This suggested that lipolysis was not enhanced. A possible explanation for this increase in NEFA concentration is that metformin reduced NEFA clearance. Smith et al (55) demonstrated that metformin decreased skeletal muscle FATCD36 transporter in rodents. Thus, in this study, reduced skeletal muscle clearance of NEFA may have explained why metformin increased circulating NEFA concentrations during exercise without subsequently enhancing fat oxidation. Future work using palmitate and glycerol tracers would be required to determine the effect of metformin on lipid metabolism during and after exercise. Using lipid stable isotopes may also clarify the negative associations between elevated blood lipids (i.e. NEFA) and insulin sensitivity in our studies ((54) Malin chapter 4). If combining metformin with training detrimentally affects insulin sensitivity through alterations in lipid metabolism, then other treatments (i.e. diet/ pharmacology) may be needed to correct for the effects of metformin on fat metabolism and insulin sensitivity. Collectively, the literature suggests that metformin alters submaximal exercise energy metabolism, with little to no effect on the perception of effort during exercise. Thus, there seems to be little translational effect of metformin on maximal exercise to submaximal exercise tolerance when performed at the same absolute workload. However, the current work is limited in characterizing the different combinations of metformin and exercise. Future research looking at the effects of metformin on different exercise intensities/modes could provide greater insight into this potential interaction.

**Clinical implications/ramifications of combining exercise with metformin**

Although the Indian Diabetes Prevention Program demonstrated that lifestyle modification plus metformin reduced the incidence of type 2 diabetes to a similar extent as lifestyle modification or metformin alone, it does not provide insight into the efficacy of exercise on diabetes prevention (50). Exercise enhances insulin sensitivity and contributes
improved glycemic control (27). Whether combining metformin with exercise adds to the prevention type 2 diabetes is less clear. To date, there are no large-randomized clinical trials that determine the effectiveness of combining metformin with exercise on delaying/preventing the onset of type 2 diabetes. Thus, the question becomes, should clinicians prescribe metformin and have individuals exercise? Our findings indicate that metformin blunted the effects of exercise on insulin sensitivity, regardless of the basis for prediabetes classification (i.e. IGT vs. IFG+IGT) (Chapter 4). The combined treatment also blunted reductions in systolic blood pressure and CRP concentrations in individuals with IGT (Chapter 7). Moreover, we found that only metformin or exercise training reversed risk factors for the metabolic syndrome. If our findings are replicated in the “real world”, then there would be potentially important clinical ramifications. If metformin blunts the effects of exercise on enhancing insulin sensitivity, then more individuals treated with metformin plus exercise may develop type 2 diabetes than those participating in exercise alone. Individuals treated with metformin plus exercise might not also derive the same cardio-protective effects on systolic blood pressure and develop more cardiovascular abnormalities compared to exercise training alone. Our findings also highlight the importance of tailoring exercise treatment to individuals based on their respective pathology. Since exercise training accentuated insulin sensitivity in individuals with IFG+IGT more then individuals with IGT, it suggests that these individuals may derive more metabolic benefit from exercise alone. Future work should look to characterize the effects of exercise training on insulin sensitivity across the prediabetes spectrum (i.e. IFG vs. IGT vs. IFG+IGT), as this will improve our overall understanding of the role exercise has in preventing/delaying type 2 diabetes. Based on our findings, we suggest that exercise be considered a key part of the first line therapies for improving insulin sensitivity and cardiovascular health (e.g. blood pressure, TAG, etc) in insulin resistant individuals. If exercise training is ineffective at improving blood glucose regulation in these individuals, then pharmacological agents, like metformin, may be
considered as an additional therapy to exercise. Future work should be cognizant of other drug-exercise-drug interactions. We suggest others consider investigating the effects of multiple drug combinations and exercise to see if these treatments have no effect, add, or oppose changes in metabolic health outcomes.

**Conclusions**

Insulin resistance is a key component in the progression from normal glucose tolerance to impaired glucose tolerance to frank type 2 diabetes. Insulin resistance is associated with dyslipidemia, hypertension, and inflammation, all of which increase risk for cardiovascular disease. Because exercise or metformin improve insulin sensitivity, they should be considered for preventing/delaying cardiovascular disease and type 2 diabetes. Although our work demonstrates the effectiveness of exercise training for improving insulin sensitivity and lowering cardiovascular disease risk factors, we find that metformin blunts the full effects of exercise on insulin sensitivity, systolic blood pressure, and C-reactive protein in individuals with insulin resistance. Together, these observations suggest that exercise alone may provide more benefit to individuals at risk for type 2 diabetes than either treatment alone. However, we caution that our data should not be over interpreted to mean that individuals should not take metformin and exercise. If the question is, should individuals be treated with exercise plus metformin or metformin alone, then our data supports the combined treatment because of its effects on insulin sensitivity and cardio-respiratory fitness compared to metformin alone. Thus, from a public health perspective, co-prescribing treatments is likely to have “better” effects than metformin alone. However, metformin may be the more desirable treatment depending on the outcome of interest. If the primary outcome is hyperglycemia, then our findings indicate that metformin, with or without training, is better for lowering blood glucose concentrations in people with fasting hyperglycemia (i.e. IFG+IGT) compared to insulin resistant, normoglycemic individuals (e.g. IGT). From a basic
science perspective, it is also important that we understand how these two treatments work together on reducing diabetes risk. More studies are needed (e.g. animal and in vitro) to describe the cellular mechanism by which metformin and exercise interact to affect insulin sensitivity and cardiovascular health. Overall, our findings suggest that choosing the most appropriate treatment is likely to depend on the persons risk factor (e.g. hyperglycemia, blood pressure, etc) or underlying pathology (e.g. IGT or IFG+IGT or metabolic syndrome). Ultimately, further research in the aforementioned areas will help determine which treatment plan is best for preventing/delaying the progression to type 2 diabetes.

**Acknowledgments**

S.K.M wrote the manuscript and S.R.C and B.B. reviewed/edited the manuscript. The authors would like to thank Carrie Sharoff, Ph.D., Todd Hagobian, Ph.D., Kirsten Granados, Richard Viskochil, Jennifer Rivero, and Robert Gerber for technical assistance and helpful discussion. We extend our appreciation to all the dedicated participants and undergraduate research assistants for help on this project.

This research was supported by NIH 5 R56 DK081038 Grant.
References

1. CDC Diabetes Fact Sheet. 2011.


Figure Captions.

Figure 8.1. Blood glucose regulation: the role of liver and skeletal muscle insulin resistance.

Normoglycemia

Glucose Production Exceeds Uptake

Impaired Glucose Uptake Relative to Production

Glucose Uptake and Production are Impaired
Figure 8.2. Effects of metformin, exercise or exercise plus metformin on insulin sensitivity in insulin resistant individuals. Open bars, baseline; hatched bar, exercise only; black bars, metformin; gray bar, exercise + metformin. *Significantly different from placebo baseline (p < 0.0001). Rd/I, rate of glucose uptake per unit insulin. Values are mean ± standard error of the mean (54).
Figure 8.3. Insulin Sensitivity across all conditions. Significant effect of test (p < 0.05).

*Compared to baseline (p < 0.05). ^Compared to placebo (p < 0.05). Rd/I, rate of glucose uptake per unit insulin. Values are mean ± standard error of the mean.
Figure 8.4. Change (i.e. difference of pre and post measures) in fasting plasma glucose (FPG) in response to each respective condition. Note that metformin, with or without training, lowered FPG more than EP.
Figure 8.5a. Effect of exercise training with placebo (EP), exercise training with metformin (EM) and metformin (M) on reversing the metabolic syndrome in individuals with IGT. * Significantly different compared to pre-test (p < 0.05).
Figure 8.5b. Effect of exercise with metformin on risk factors related to the metabolic syndrome. Note triacylglycerol (TAG) and fasting blood glucose (FBG) are reduced.
Figure 8.5c. Effect of exercise with placebo on risk factors related to the metabolic syndrome. Note systolic and diastolic blood pressure (SBP and DBP), triacylglycerol (TAG), and HDL are reduced.
Figure 8.5d. Effect of metformin on risk factors related to the metabolic syndrome. Note systolic and diastolic blood pressure (SBP and DBP), triacylglycerol (TAG), and FBG are reduced.
Supplement A.4.1. The change (difference of pre and post measures) in insulin sensitivity (i.e. rate of glucose disposal (Rd)/plasma insulin (I) concentrations) correlated with the change in non-oxidative glucose disposal (NOGD).

Supplement A.2. The change (difference of pre and post measures) in cardio-respiratory fitness (i.e. VO2peak) correlated with the change in insulin sensitivity (i.e. rate of glucose disposal (Rd)/plasma insulin (I) concentrations).
Supplement A.3. The change (i.e. difference of pre and post measures) in body weight correlated with the change in insulin sensitivity (i.e. rate of glucose disposal (Rd)/plasma insulin (I) concentrations).

![Graph showing correlation between change in body weight and change in Rd/I ratio](image1)

Supplement A.4. Change (i.e. difference of pre and post measures) in fasting plasma non-esterified fatty acids (NEFA) correlated with insulin sensitivity (i.e. rate of glucose disposal (Rd)/plasma insulin (I) concentrations).

![Graph showing correlation between change in NEFA and change in Rd/I ratio](image2)
Supplement A.5. Change (i.e. difference of pre and post measures) in fasting plasma glucose (FPG) in response to each respective condition. Data expressed as group mean. Note that metformin (M) and exercise training with metformin (EM) lowers FPG more than exercise training with placebo (EP) in individuals IFG+IGT compared to IGT.

Supplement A.6. Change (i.e. difference of pre and post measures) in fasting plasma insulin (FPI) concentrations in response to each respective condition. Data expressed as group mean. Note that insulin concentrations tend to decrease more after each respective treatment in individuals with IFG+IGT compared to IGT.
Supplement B.1. Effects of exercise training on substrate utilization (expressed as the respiratory exchange ratio (RER)) in individuals with impaired glucose tolerance (IGT). Data expressed as group mean ± SEM (n = 4). Note that exercise training lowered RER values more in individuals with IGT than individuals with IFG+IGT.

Supplement B.2. Effects of exercise training on substrate utilization (expressed as the respiratory exchange ratio (RER)) in individuals with impaired fasting glucose (IFG) concentrations plus impaired glucose tolerance (IFG+IGT). Data expressed as group mean ± SEM (n = 4).
Supplement C.1. Effects of placebo (P), metformin (M), exercise training with placebo (EP) and exercise training with metformin (EM) on ambulation in individuals with prediabetes. Data expressed as group mean ± SEM.

Supplement C.2. Effects of placebo (P), metformin (M), exercise training with placebo (EP) and exercise training with metformin (EM) on resting metabolic rate (RMR) in individuals with prediabetes. Data expressed as group mean ± SEM.
Supplement C.3. Effects of placebo (P), metformin (M), exercise training with placebo (EP) and exercise training with metformin (EM) on caloric intake in individuals with prediabetes. Data expressed as group mean ± SEM.

Supplement C.4. The change (i.e. difference of pre and post measures) in caloric intake correlated with the change in body weight.
Supplement C.5. The change (i.e. difference of pre and post measures) in triacylglycerol (TAG) correlated with the change in insulin sensitivity (i.e. rate of glucose disposal (Rd)/plasma insulin (I) concentrations).