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Examination of metabolism in diabetic offspring

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EXAMINATION OF METABOLISM IN DIABETIC OFFSPRING

A Dissertation

Submitted to Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

In

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by
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ABSTRACT

The purpose of this study was to categorize aberrant metabolic function in diabetic offspring (FH+). This study examined metabolic flexibility (MF), and changes in fasting blood-glucose concentrations and markers of lipotoxicity with resistance training in college age FH+ and FH-. Results are significant at $p = 0.05$. MF testing indicate no baseline differences in RMR, VO_2 , REE, fat or CHO use were noted between T2D, FH+, or FH-. Passive stretching caused increased metabolism overall, however the T2D group temporarily displayed increased CHO use during passive stretching, which quickly returned to pre-stretched levels during recovery as compared with FH-. Both T2D and FH+ display impaired MF as compared with FH- via indirect calorimetry as noted by the change in RER. With training, changes in glucose: lactate ratios were no different between groups, but increased immediately after exercise with training, and decreased at five and ten minutes post-exercise with training. Lastly, there were no differences between FH- and FH+ in pre-training strength, BMI, or in markers measured in plasma before or after exercise. Strength increased from pre to post training similarly. However, changes in NEFA and insulin were noted in weight loss subjects vs non-weight loss. Negative correlations exist between weight loss and: TG, NEFA, insulin and HOMA, and strength, and positive correlations with blood glucose AUC.

Though there are differences metabolic flexibility and recovery kinetics between groups with and without a family history of diabetes, this study does not reveal any such differences in glucoregulatory function, or markers of lipotoxicity. Resistance training did not affect FH+ differently than FH-, however there were differences in these markers when groups were re-categorized by weight loss. We were unable to isolate specific factors likely to contribute to the development of IR or T2D within the confines of the current study. However, further research,

such as lipid tracers and MRI studies are needed to determine factors leading to more aberrant metabolic function in order to better understand what factors lead to the development of IR and T2D.

CHAPTER 1: INTRODUCTION

Diabetes is a metabolic disorder characterized by reduced ability to maintain healthy blood-glucose levels. Type-2 diabetes (T2D) is the fastest growing worldwide health pandemic [1, 2], caused in large part by an imbalance of over-feeding and physical inactivity (positive energy balance) leading to obesity [3]. The key deficiency in T2D is the inability to maintain proper blood-glucose levels, and an important aspect of blood-glucose regulation is insulin's ability to sufficiently stimulate glucose transport into the muscle cells. This is done via insulin-stimulated glucose transport (GLUT4) protein translocation from within the cell to the cell membrane [2, 4, 5]. Though there are several important glucose transporters that play a role, GLUT4 is the only insulin-stimulated glucose transporter. In healthy conditions, insulin stimulates a cascade of events within the muscle cell leading to the translocation of GLUT4 from within the cell to the membrane, allowing it to transport glucose from the blood to within the muscle. If this process is interrupted, blood-glucose levels increase.

Preceding elevated blood-glucose is increased insulin production in early stages, followed by decreased production in more advanced diabetes when the pancreatic islet cells become damaged as a result of advanced diabetes [2, 6]. Several metabolic factors including lipotoxicity and oxidative stress have been identified as major contributors leading to this condition. This reduced ability of insulin to stimulate GLUT4 translocation is called insulin resistance (IR), which ultimately results in the decline of glucoregulatory function in diabetes. The combination of increased lipid and low oxidative capacity are key features in the development of muscular IR [7-9]. Smaller and fewer mitochondria, in combination with reduced PGC1- α (transcriptional factor for mitochondrial biogenesis) in muscle cells translate to lower ATP synthesis in diabetics and first-degree relatives [10] leading to reduced capacity of insulin to properly dispose of blood-

glucose. Thus, mitochondrial dysfunction and metabolic inflexibility (defined as the impaired capacity to increase fat oxidation upon increased fatty acid availability or to switch between fat and glucose as the primary fuel source after a meal [11]) are considered major aspects of lipid accumulation, and IR [12]. This reduction in metabolic flexibility further exacerbates the initial lipotoxicity. Consequently, the development and progression of IR by lipotoxicity-related factors has been the primary culprit in the development of T2D, and hence is the focus of much research.

Despite extensive research in the field of IR, the progression of this epidemic still increases. Current research has focused on improving glucose regulation and reducing IR in T2D. However, in order to prevent T2D from developing, mechanisms responsible for the initial development of IR must be revealed. Recent investigations of the possible initial causes of IR have indicated that factors from lipotoxicity may be responsible for the development of oxidative stress and inflammation- all common co-morbidities in T2D. Therefore, a logical next step in studying T2D is to focus on lipotoxicity and the development of IR at its earliest stages.

Lipotoxicity refers to an overabundance of fat in blood or other tissues leading to pathologic conditions, such as mitochondrial dysfunction and metabolic inflexibility in diabetes [13]. With obesity comes an overabundance of stored adipose tissue in visceral and subcutaneous storage depots. Increased triglyceride storage in these cells leads to an increased production of non-esterified fatty acids (NEFA) which aggravates insulin resistance in skeletal muscle [14] and are often associated with increased intramyocellular triglyceride (IMCT) storage. The underlying mechanisms whereby lipotoxicity causes decreased insulin sensitivity are unclear but appear to involve elevated circulating (triglycerides, NEFA) and stored (IMCT) lipids acting directly on the insulin-signaling pathway, and involve stimulation by mitochondrial dysfunction- both

leading to serine phosphorylation on the insulin receptor substrates 1 and 2 (IRS1-2) of GLUT4 protein.

Elevated IMCT have long been associated with IR and oxidative stress [15]. However, recent evidence suggests it is not the mere presence of IMCT that causes IR, but more likely that IMCT are either a symptom of the mitochondria's inability to oxidize fat at the pace at which it becomes available (fat supply outpaces oxidation leading to a positive energy balance) [16], or excess reactive oxygen species (ROS) produced by the mitochondria due to mitochondrial dysfunction and/or elevated β -oxidation interact with the neighboring IMCT to cause oxidative damage to the lipid (peroxidation). This process leads to increased IR [17] and an accumulation of isoprostanes, like 4-HNE. Additionally, adipose tissue itself is known to go beyond fat storage and serve as an endocrine organ producing substances that cause serine phosphorylation of IRS1-2, like interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), leptin and resistin, any of which can also lead to insulin resistance.

Besides IMCT and adipokines leading to IR, circulating fat has been shown to be elevated in obesity and IR states [18], and may be partially responsible for the development of IR. One possibility is that the mitochondria are unable to maintain high enough levels of β -oxidation in the presence of increased lipid concentrations due to decreased physical activity, which leads to increased NEFA [3]. Recent data suggest that elevated levels of circulating NEFA, in combination with reduced metabolic activity may be major contributors to both IR and agents antagonistic to mitochondrial function, such as oxidative stress and inflammation, both of which contribute to progression of diabetes [3]. NEFA have been shown to lead to increased production of reactive lipids such as diacylglycerol (DAG), and results in the activation of protein kinase c (PKC), which in turn activates the serine/threonine phosphorylation of IRS1-2 [13]. When the

serine/threonine ends of IRS 1-2 are phosphorylated, the tyrosine end is inhibited from being phosphorylated [19], which is essential for insulin-stimulated GLUT4 translocation.

Furthermore, an abundance of NEFA availability leads to increased mitochondrial selection of lipid as substrate, competing with glycogen. Combined with decreased metabolism, this increased lipid oxidation leads to increased production of ROS, and incomplete breakdown of lipid in the mitochondria (acylcarnitine build-up).

In addition to the direct impact of circulating fat has on insulin resistance, elevated lipids lead to increased fat oxidation and a subsequent accumulation of acetyl-CoA leading to increased acylcarnitine levels. Even chain acylcarnitines containing up to 20 carbons accumulate in response to incomplete fatty acid β -oxidation. In contrast, amino acid catabolism produces odd chain-length species like C3 and C5–acylcarnitines, although C4– acylcarnitines can be derived from either amino acid or fatty acid metabolism. Acetyl (C2)–carnitines are derived from carbohydrate catabolism and from the ultimate product of β -oxidation, acetyl-CoA [20].

This rise in acetyl-CoA can exceed cellular energy requirements, and as a competitive inhibitor of pyruvate dehydrogenase, effectively reduce the pyruvate dehydrogenase complex, and glucose metabolism [11]. Furthermore, the subsequent increase in glucose-6-phosphate accumulation within the cell leads to hexokinase inhibition, which results in a negative feedback reducing glucose uptake. This reduction in glucose metabolism leads to less glucose uptake into muscle cells, and a subsequent rise in blood-glucose. In addition, there is also a build-up of malonyl-CoA seen in T2D [21-23]. These elevated concentrations of malonyl-CoA inhibit palmitoyltransferase-1, the rate limiting step in the transport of long-chain fatty acids into the mitochondria [21]. This leads to a build-up of acylcarnitines in the muscle cell, causing lipid peroxidation of the mitochondria and increasing mitochondrial dysfunction [24].

To further complicate the diabetic profile, a build-up of Acetyl-CoA inhibits β -oxidation. However, with the surplus of lipid available to metabolize, the Co-A on Acyl-CoA interacts with carnitine so that the fat molecule can be transported into the mitochondria for β -oxidation. With increased β -oxidation also comes more ROS being produced, which can lead to mitochondrial damage and dysfunction. The combination of increased acylcarnitine production and ROS, and reduced β -oxidation lead to a further build-up of acylcarnitines, thus perpetuating a cycle.

In addition to the above complications caused by elevated acetyl-CoA, there is also an inherent proton leak from the mitochondria that is associated with beta-oxidation [25]. This proton leak reacts with molecular oxygen to form superoxide (SO), which is an extremely potent free-radical and the first ROS molecule formed in the oxidative process. From there, SO can react with several protective molecules like superoxide dismutase (SOD) or iron to form the hydroxyl radical (OH \cdot), or the less reactive hydrogen peroxide (H₂O₂). These radicals are further neutralized by subsequent endogenous antioxidants like glutathione peroxidase (GPx) or Catalase (CAT). When ROS production is no longer effectively neutralized by endogenous antioxidants (like SOD, GPx, or CAT), a state called *oxidative stress* exists. Oxidative stress can lead to cellular damage (oxidative damage) causing increased IR, or the production of c-Jun Kinase (JNK) which can either inhibit glucose transport by phosphorylating the serine/threonine ends of the IRS1-2, or cause further mitochondrial dysfunction [25]. Oxidative damage can be quantified from either protein (protein carbonyl) or lipid (8-isoprostane, 4-NHE).

It is well-established that exercise improves glucose tolerance. Endurance training specifically has been shown to improve insulin sensitivity by up-regulating GLUT4 expression, improve nitric oxide-mediated skeletal muscle blood flow [26], reduce hepatic glucose output [27], and normalize blood-lipids [28]. Resistance training on the other hand not only has similar

consecutive exercise effects as aerobic, but also is associated with significant skeletal muscle mass increases, thereby improving whole-body glucoregulatory function [29]. Regardless of exercise type, total energy expenditure has been shown to be the biggest determinant of exercise-induced changes in glucose homeostasis [30-32]. Given these considerations, perhaps the best exercise option for improving glucoregulatory function is to incorporate both aerobic and resistance training to burn more calories, such as a circuit training regime designed to increase strength, burn calories, and keep heart rate elevated.

- Conclusion

In light of the increasing prevalence of T2D, it is important for research to focus not only on treatment of diabetes but also prevention and halting its progression at its earliest stages.

Interestingly, IR individuals with a family history of diabetes (FH+) may be an ideal group in which to study the initial development of IR because they have been shown to be at approximately 40% higher risk for developing T2D than those with no family history (FH-) [33, 34], and have impaired hexokinase II activity similar to T2D [19]. The benefit of studying IR in this population is that they have not yet developed many of the confounding factors that may contribute to the development of T2D like glucotoxicity or obesity, yet FH+ display a 50% reduction in the rate of insulin-stimulated whole-body glucose metabolism due to decreased muscle glycogen syntheses rates [33], indicating that the pathogenesis of IR can be examined at its earliest time points [35]. In addition, there is a lack of understanding concerning the pathologic connection shared with T2D that pre-disposes them to be more likely to develop this disease in the future. Recent data suggests that impaired metabolic flexibility (MF) similarly affects FH+ and T2D, indicating that lipotoxicity may be an early contributing factor for the development of IR in the FH+ population [3, 15].

In exploring the metabolic profile of the FH+ population, a multi-factor approach is needed to establish a more accurate understanding of what pathologic link this group shares with T2D. For example, with IR often comes mitochondrial dysfunction leading to metabolic inflexibility. However, this has yet to be established in the FH+ group without documented IR existing. It also may be possible to alter glucoregulatory function of prostaglandins via acute aspirin ingestion in this population. It is possible that lipid-induced development of chronic inflammation leads to IR, and inhibition via NSAIDs could help reverse this. Furthermore, exercise interventions have been shown to successfully decrease IR and aid in glucoregulatory function in T2D. However, such an intervention may also alter glucoregulatory function in apparently healthy FH+.

Chapter 1 introduced the topic of T2D and lipotoxicity. Several characteristics of IR are highlighted as seen in T2D, and FH+ are introduced as a possible early IR population for further study. In Chapter 2, metabolic flexibility will be compared between FH-, FH+ and T2D. Chapter 3 will examine whether acute prostaglandin inhibition affects OGTT by using 975 mg of aspirin. In chapter 4, the effects of seven weeks of resistance training on fasting blood-glucose in controls and FH+ will be examined. Finally, chapter 5 will include an in-depth examination of biochemical markers of lipotoxicity in both controls and FH+, and if resistance training can affect these markers.

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CHAPTER 2: DEVIATIONS IN METABOLIC FUNCTION IN HEALTHY DIABETIC OFFSPRING: DEVIATIONS IN METABOLIC FLEXIBILITY

Introduction

Type-2 diabetes (T2D) is a pandemic disease [36] characterized by insulin resistance (IR), which develops one to two decades before the onset of type-2 diabetes (T2D), [34, 37] and is the best predictor for future development of this disease [2, 37]. Since IR is related to several co-morbidities like elevated lipid levels, loss of metabolic flexibility (MF) and impaired glucoregulatory function, much attention has been given to studying these co-morbidities.

IR subjects not only display elevated fasting β -oxidation, but also impaired MF, which is characterized by impaired ability to switch to carbohydrate as substrate from the fasted-to-fed transition [16]. Several factors are likely to contribute to the development of this condition, including decreased hexokinase activity and peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1) expression, which are both seen in T2D [38]. In addition, disturbances of free-fatty acid/nonesterified fatty acid (FFA/NEFA) metabolism, elevated basal rate of lipolysis, and impaired suppression of plasma NEFA concentration by insulin, are also associated with IR and reduced MF [39-41]. Although there is a strong indication that these factors are related to MF, with so many co-morbidities and additional confounding factors (like obesity and impaired glucoregulatory function) often present in T2D patients, it is difficult to distinguish which factor may be the initial trigger for developing IR.

Interestingly, IR individuals with a positive family history of diabetes (FH+) may be an ideal group in which to study the initial development of diabetes and its earliest co-morbidities for several reasons. These individuals have been shown to be at approximately 40% higher risk for developing T2D than those with no family history (FH-) [33, 34], and have impaired hexokinase II activity and PGC1 expression similar to T2D [19, 38] which may lead to decreased expression

of nuclear respiratory factor 1 (NRF-1) [38]. The benefit of studying IR in this population is that these individuals often have not yet developed many of the confounding factors that may contribute to the development of T2D, like glucotoxicity or obesity, yet they display a 50% reduction in the rate of insulin-stimulated whole-body glucose metabolism due to decreased muscle glycogen synthesis rates [33]. This suggests that the pathogenesis of T2D in the FH+ population can be examined at its earliest time points [35]. To date, much of the research on FH+ has been in sedentary, IR FH+ only, making it difficult to isolate the pathologic connection shared between FH+ and T2D leading to the development of IR and diabetes [42, 43]. Furthermore, Galgani suggests that assessment of MF at rest is insufficient to detect deficiencies in fat metabolism, as the demand for fat oxidation at rest is minimal [44]

Therefore, the purpose of the current study was to determine whether a decline of MF exists in a young, active FH+ population with normal glucoregulatory function with traditional glucose loading, and with separate metabolic stimulus, and to compare the MF of active FH- with that of active FH+ and T2D. Data from the present study could potentially aid in development of effective screening tools and interventions to prevent T2D progression in the FH+ population.

It was hypothesized that that FH- are highly metabolically flexible as measured by changes in RER after an oral glucose load. Furthermore, FH+ and T2D, populations shown to have impaired hexokinase and PGC-1 [19, 38], are likely to display increasing metabolic inflexibility with glucose load. In addition, passive stretching was expected to illicit a more substantial shift in substrate use in FH- as compared with FH+ and T2D due to the increased metabolic demands from mild exercise [44] than that of traditional MF testing via glucose loading.

Methods

- Subjects.

Thirty-seven subjects were recruited from the Baton Rouge area using local advertisements and word of mouth to participate in one morning of fasting metabolic testing. FH+ was categorized as being a 1st or 2nd degree relative of a person with T2D. The subject population consisted of 10 healthy, active controls with no family history (FH-) of diabetes, 16 FH+, and 11 T2D subjects. Age, gender, BMI, and activity were matched between FH+ and FH-. The purpose, potential risks and benefits of participation in the study were fully explained to each participant before written consent was obtained prior to testing. The study was approved by the Ethical Committee of Louisiana State University (LSU office of IRB). All FH+ and FH- subjects had normal fasting blood-glucose prior to testing using a hand-held finger-prick glucose meter. Additional inclusion criteria for FH+ and FH- groups were no overt disease, including but not limited to cardiovascular disease, hypertension, impaired glucose tolerance (IGT) kidney or renal disease, are physically active, blood-glucose concentrations under 10 mmol/L (180 mg/dL) an hour after glucose loading, BMI between 18.5-24.9 kg per m², and not taking prescription medication other than oral contraceptives. T2D subjects had no BMI exclusions, and were allowed to take prescribed glucose-lowering medication. A physical activity questionnaire was completed prior to participation in the study to determine eligibility. Subject characteristics are summarized in Table 2.1.

- Research Design.

The study consisted of a single session with three components: 1) assessment of resting metabolic rate (RMR), 2) assessment of response to a low-intensity metabolic stimulus (passive

Table 2.1 Subject characteristics: Age (years), Height (cm), Weight (cm), and Body Surface Area (BSA). Mean \pm SE.

	FH+	FH-	T2D	P
Age (years)	24 \pm 1.1	23.1 \pm 2.0	*37.7 \pm 6.1	0.01
Height (cm)	170.5 \pm 2.9	173.8 \pm 1.9	170.0 \pm 3.3	NS
Weight (kg)	70.6 \pm 3.2	76.0 \pm 3.2	*88.4 \pm 6.8	.037
BSA	1.81 \pm .06	1.90 \pm .05	1.98 \pm .08	NS

stretching), and 3) assessment of physiologic responses to an oral glucose load while under a metabolic canopy, including MF.

- RMR Assessment.

Participants reported to the lab to begin the study at 7:45 in the morning after a ten-hour fast. Participant's height and weight were measured and they were asked to fill out an activity questionnaire; then at 8:00 AM, they were placed in the supine position for thirty minutes prior to RMR testing. During this time, fasting blood-glucose were collected via finger prick and measured using an AccuChek CompactPlus glucose monitor (Indianapolis, IN), a precise and reliable means of assessing blood-glucose [45]. After the initial thirty minute rest period, a plastic canopy connected to a metabolic system was placed over the subject's head to collect and analyze resting expired gases for 50 minutes. This time period included a 20-minute stabilization period followed by 30 minutes of data collection with continual monitoring to ensure wakefulness. For one subject, an extra ten minutes of stabilization time was required to re-calibrate the metabolic cart. Figure 2.1 shows a timeline for the test morning. Expired gas was analyzed using a Moxus Max-II Metabolic System (AEI Metabolic Systems, version 2.7.01, Naperville, IL) which was calibrated by a trained technician per manufacturer's instructions using two gas mixtures with known O₂ and CO₂ composition before each test. RMR values were monitored, and after twenty minutes of stable RMR data were collected, the subsequent thirty minutes of RMR were recorded, averaged, and used for analysis as fasting oxygen kinetics at rest (Figure 2.1)

- Metabolic Response to Passive Stretch.

At 9:22 AM, immediately following the initial RMR, another blood-glucose assessment was conducted via finger prick, and twenty minutes of passive stretching was performed while the

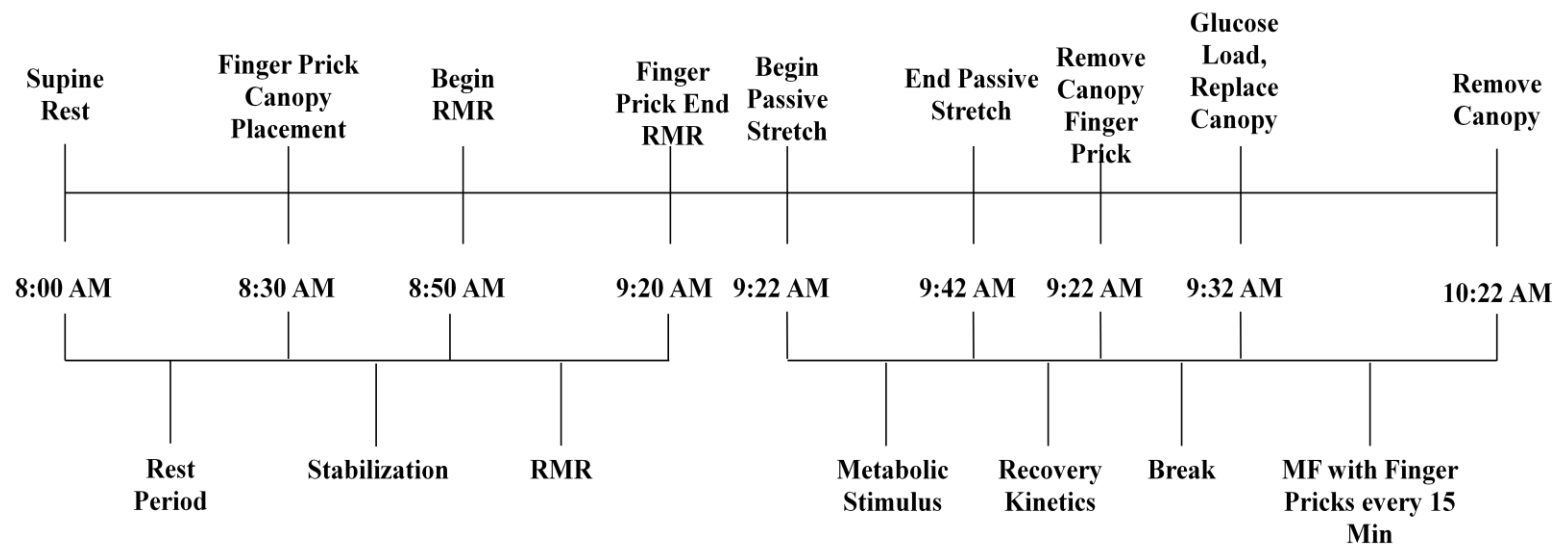


Figure 2.1 Timeline of study test morning.

participants were resting under the metabolic canopy. Passive stretching was subsequently used as a mild metabolic stimulus, and was conducted by a lab technician while gases were collected under the RMR canopy to determine substrate utilization. Each participant indicated attainment of appropriate muscular tension by lifting one finger. When the appropriate tension was reached as indicated by the participants, the stretch was held for twenty seconds, and then repeated after a ten second break. Muscles stretched included gastrocnemius, hamstring, quadriceps, hip abductors, gluteal muscles, pectorals major and minor, and forearms. RMR data were collected up to five minutes post-stretching to measure oxygen recovery kinetics as oxygen and substrate selection changes during recovery, and then post-recovery determination of blood glucose was again conducted via finger prick and glucometer analysis. Since the metabolic stimulus from passive stretching was considered constant for the entire 20-minutes, peak stretching values consisted of average values during the last five minutes of passive stretching. Post-stretching recovery values consisted of the last minute of the five-minute recovery period (Figure 2.1).

- Metabolic Flexibility Assessment.

Following stretching and recovery, participants were given a ten minute break before beginning the MF testing using an oral glucose dose rather than insulin clamp as suggested by previous studies [43, 46, 47]. While still fasting, each participant consumed a solution of 50g glucose dissolved in 10oz of water. Each glucose beverage was consumed within two minutes, and then the participants went back to the supine position with the metabolic canopy over their heads for another 60 minutes with continual monitoring to ensure wakefulness. Blood-glucose was analyzed via finger prick and glucometer every fifteen minutes for 1-hour to determine blood-glucose responses to the oral glucose load, and expired gas was continuously measured for 60-

minutes similar to studies examining postprandial hyperglycemia, a well-recognized risk-factor of T2D [48, 49].

- Analyses.

RMR analysis was computed in part by Moxus software, including VO_2 (ml/kg/min), VCO_2 (ml/kg/min), RER, VEO_2 (l/ml), VECO_2 (l/ml), as well as VO_2 and VCO_2 adjusted for $\text{BSA}(\text{ml}/\text{m}^2)$. Additional determinations of resting metabolic rate (RMR) were calculated with consideration of protein metabolism using the abbreviated formulas of Weir [50]. Fat (g), Fat (kcal) and CHO (kcal) were calculated using equations from additional reviews, and MF were calculated in Microsoft Excel 2007 with the following equations:

$$\text{RMR} = (3.9 \times \text{VO}_2 \text{ L/min}) + (1.1 \times \text{VCO}_2 \text{ L/min}) [50]$$

$$\text{Fat g} = (1.67 \times \text{VO}_2 \text{ L/kg}) - 1.67 \times \text{VCO}_2 \text{ L/kg} [51]$$

$$\text{Fat kcal} = 9 \times (\text{Fat g}), [51]$$

$$\text{CHO kcal} = \text{REE} - \text{Fat kcal}$$

$$\text{CHO kcal} = 4.58 \text{ VO}_2 \text{ L/kg} - 3.23 \text{ VCO}_2 \text{ L/kg} [51]$$

$$\text{MF} = (\text{RER stimulus}) - (\text{RER rest}) [52]$$

Where stimulus was average RER every 30 seconds during the hour following glucose loading.

All statistics were calculated using SPSS 15 (Somers, NY). Subject characteristics were analyzed using 1-way ANOVA. Variables such as pre stretch RMR, blood-glucose concentration after glucose loading, or MF area under the curve (AUC) differences between groups were calculated using a 1-way ANOVA with Tukey post-hoc comparisons. Changes pre to post-stretching, or pre to post blood-glucose concentrations were analyzed using repeated measures with LSD post-hoc comparisons to determine magnitude of change differences between groups.

Pearson product moment correlation was used to determine relationship between variables. All comparisons were considered significant at the alpha level $p = 0.05$.

Results

T2D were characterized by higher weight, BMI, and age than FH+ or FH-, and had significantly higher fasting blood-glucose than the other groups (85.1 ± 1.8 , 91.2 ± 2.9 , and 108.9 ± 7.3 mg/dl FH-, FH+, T2D mean \pm SE, respectively). No significant differences in resting metabolism were noted between any of the groups (Table 2.2).

Relative VO_2 significantly increased with stretching in FH-, FH+, and T2D (12.3 ± 2.2 , 11.8 ± 2.4 , and 18.3 ± 2.3 % respectively, $p < 0.001$) with no significant differences between groups ($p = 0.83$). CHO use significantly increased overall with stretching ($p = 0.01$) with a trend of more increased CHO use for FH- to FH+ and T2D (0.034 ± 0.01 , 0.127 ± 0.01 , 0.331 ± 0.01 kcal/min respectively; $p = 0.17$) (Figure 2.2). \ However, a rapid return of CHO use from peak stretching to the last minute of the 5-minute recovery was noted overall (0.29 ± 0.01 , 0.10 ± 0.01 , -0.29 ± 0.02 , FH-, FH+, T2D respectively, $p = 0.002$) with significantly greater change in T2D than FH- ($p = 0.029$). FH+ was not significantly different than either T2D ($p = 0.11$), or FH- ($p = 0.39$) (Figure 2.3).

Additionally, blood-glucose dropped from pre to post stretching overall ($p = .03$) with significantly greater blood-glucose decline in T2D than both other groups ($p = .001$). Increases in overall fat use with stretching were non-significant ($p = .29$). Changes in RER with stretching or recovery were not significantly different between groups ($p = .16$). Pre-stretching, peak-stretching, and post-recovery values are shown in Table 2.2 (above).

Blood-glucose concentrations were 30% higher for T2D than FH- or FH+ (Figure 2.4) within one hour of glucose-load. Resting energy expenditure (REE) and relative VO_2 remained constant

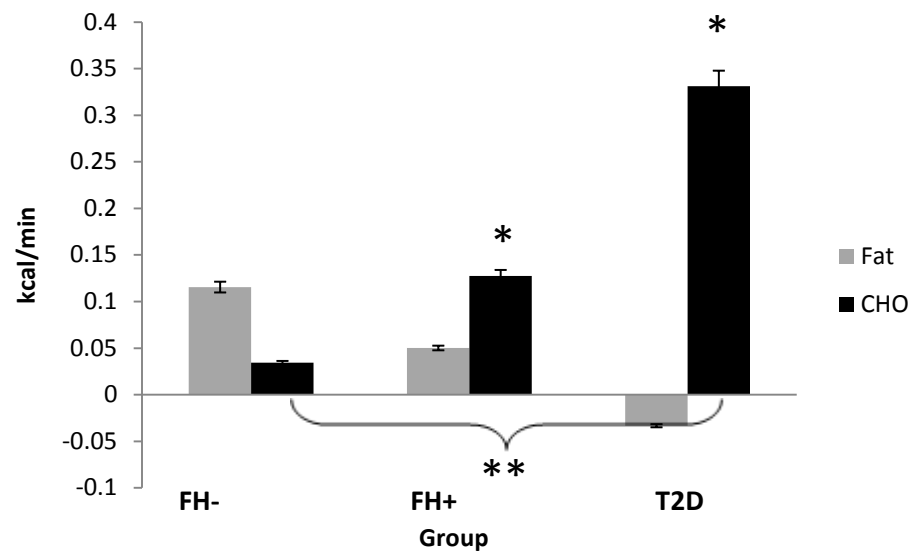
Table 2.2 Mean \pm SE for fasting: Relative VO₂, Respiratory exchange ratio (RER), Blood-glucose (BG), carbohydrates (CHO) and fat used at rest for FH-, FH+, and T2D.

	Pre-Stretching			Peak Stretching			Post-Recovery		
	FH-	FH+	T2D	FH-	FH+	T2D	FH-	FH+	T2D
VO ₂ (ml/kg/min)	3.17 \pm .29	2.89 \pm .16	2.68 \pm .32	3.56 \pm .22	3.23 \pm .24	3.17 \pm .3	3.45 \pm .23	3.24 \pm .13	2.49 \pm .44
RER	.78 \pm .02	.78 \pm .02	.76 \pm .02 ^{b c} 108.9 \pm	.78 \pm .01	.79 \pm .01	.79 \pm .03	.8 \pm .01	.82 \pm .02	.73 \pm .05
BG (mg/dl)	85.1 \pm 1.8	91.2 \pm 2.9	7.3	-	-	-	^a 82.3 \pm 2.8	^a 84.2 \pm 1.6	^{abc} 102.3 \pm 5.1
CHO (kcal/min)	.30 \pm .07	.31 \pm .06	.26 \pm .07	^a .33 \pm .05	^a .44 \pm .07	^a .59 \pm .19	.62 \pm .14	.54 \pm .06	^b .30 \pm .08
Fat (kcal/min)	.75 \pm .08	.72 \pm .07	.79 \pm .08	.86 \pm .11	.79 \pm .09	.75 \pm .10	^a .70 \pm .04	^a .67 \pm .06	^a .63 \pm .10

^a Significant from previous measurement at 0.05

^b Change significantly different than FH- at 0.05

^c Change significantly different than FH+ at 0.05



* Significant change from pre to peak

** Change significantly greater than other groups ($p = .001$)

Figure 2.2 Change in substrate use from rest to peak passive stretching showing significant increase in CHO use in overall group, with more significant changes in T2D than FH- or FH+ ($p = .001$). Mean \pm SE.

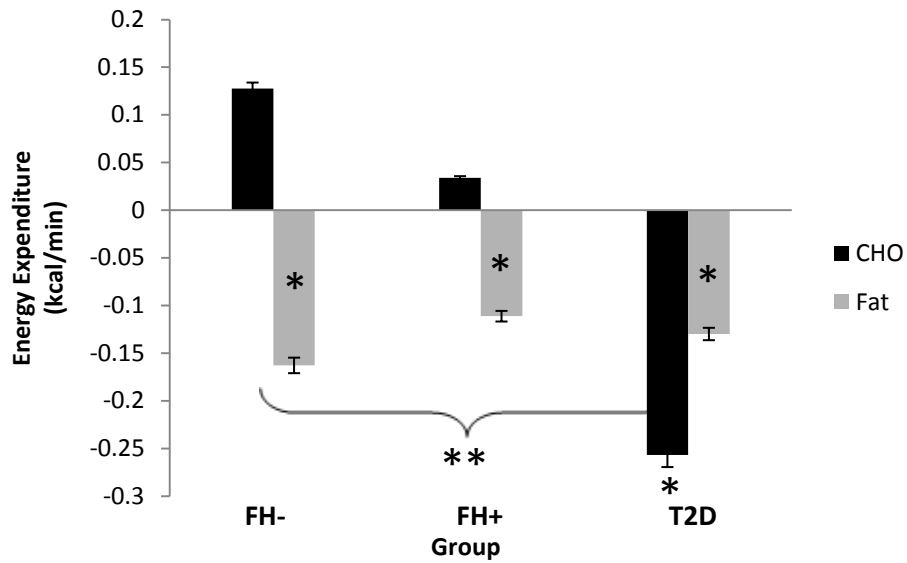


Figure 2.3 Changes in Fat and CHO kcal/min from peak stretching to recovery. Significant drop in Fat use from peak stretch to recovery, and significantly less CHO use in T2D than FH-. * Significant change from pre to post ($p = .01$); **Change significantly greater than other groups ($p = .001$). Mean \pm SE.

throughout the 1-hour post glucose loading. However, changes in kcal/min of both fat and CHO differed significantly between T2D and FH- from rest to peak passive stretching and subsequent recovery (Figures 2.2 and 2.3). Also, MF AUC was significantly higher in FH- than either FH+ or T2D, with no differences between FH+ and T2D (Figure 2.5).

Discussion

No baseline differences in RMR, VO_2 , REE, fat or CHO use were noted between T2D, FH+, or FH-. As expected, fasting blood-glucose was significantly higher in T2D than other groups with no difference in CHO oxidation. Contrary to previous studies [53], fasting whole-body fat oxidation was not significantly higher at rest in T2D than FH- in this study. In support of the secondary hypotheses, FH+ displayed impaired substrate shifts between that of FH- and T2D as noted by the lack of significant differences between FH+ and either of the other two groups. Passive stretching caused increased metabolism overall, however the T2D group temporarily displayed increased CHO use during passive stretching, which quickly returned to pre-stretched levels during recovery as compared with FH-. The overall hypothesis of progressively increased metabolic inflexibility in FH+ and T2D was substantiated by the data. FH+ and T2D displayed similar impairments in MF as compared with FH- via indirect calorimetry as noted by the change in RER. Lastly, 60-minute blood glucose concentrations AUC for T2D were significantly higher than FH+ or FH-, which is similar to previous findings of postprandial hyperglycemia [54] indicating high postprandial hyperglycemia in T2D with mixed-meal and oral glucose load.

- **Stretching**

Passive stretching significantly increased VO_2 and RER which is consistent with previous data indicating stretching not only increases metabolism, but also CHO use [27]. For example, studies have shown that a passively stretched *in vitro* muscle exhibited increased heat production

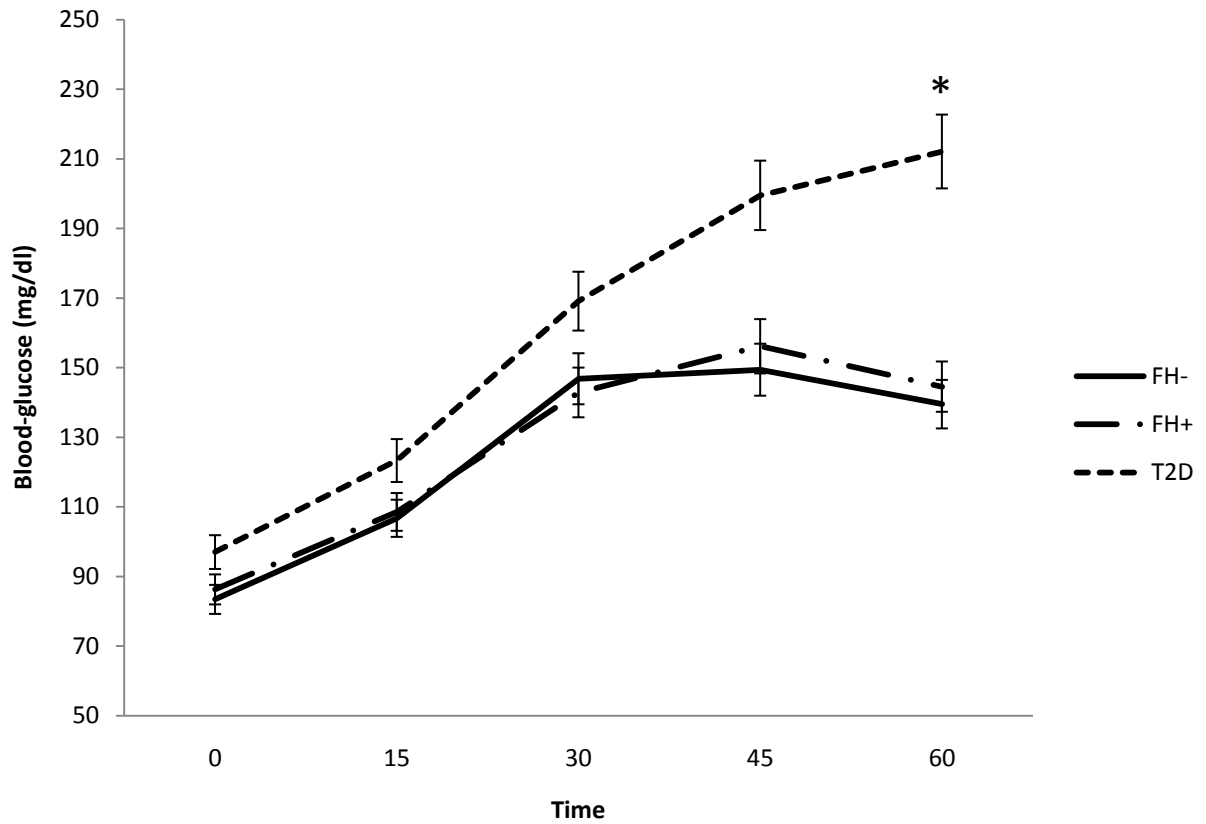


Figure 2.4 1-hour blood-glucose concentrations post glucose-loading indicate significantly worse glucoregulatory function in T2D than FH+ or FH- ($p = .002$). Mean \pm SE.

*Significantly different than FH+ and T2D.

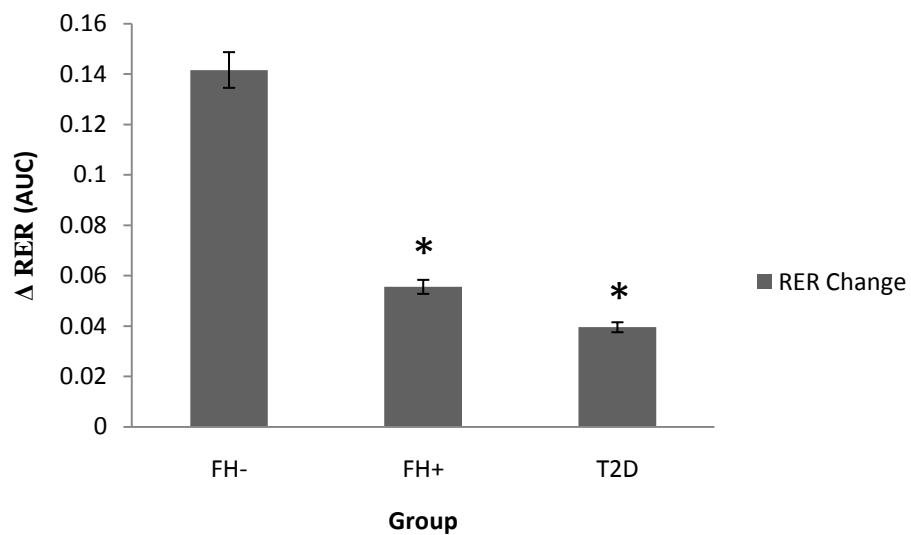


Figure 2.5 Mean \pm SE for Metabolic Flexibility: (RER during glucose load) – (RER Fasting) Area under the curve. *Significantly different than FH- (P = .002).

[55] and increased oxygen consumption [56] [57] similar to our findings. In other related studies, passive stretching increased glycogen breakdown [58], increased lactic acid production [57], and decreased phosphocreatine concentrations [59]

Since increased metabolic activity is related to increased activation of the AMPK GLUT 4 activation pathway [60] [61] [62], it is plausible that the increased metabolic activity accompanying passive muscle stretching could activate GLUT 4 in the stretched muscles [63] which would help explain increased CHO use we exhibited with stretching in the present study (Figure 2.3).

Although there was a significant increase in CHO use with stretching, group differences were not found. It should be noted that in previous studies, similar substrate utilization was observed between T2D and controls while T2D displayed significantly greater NEFA turnover [64-66]. Similar to previous findings in T2D [53, 64, 67-69], blood glucose declined significantly after passive stretching during the recovery period, however, the reductions were greater in T2D than FH- or FH+, likely because of the higher starting point ($p = 0.001$). Interestingly, unlike the previous studies, the metabolic stimulus used in the present study (passive stretching), stimulated metabolism increases to a much less degree, indicating it may not be the magnitude of energy expenditure that leads to reduced plasma glucose as much as the mere presence of a mechanical stimulus. This is in accordance with previous findings of Hansen [70]. However, though blood-glucose appears to decrease more in T2D and FH+ than FH- (-2.8, -6.0, -6.6 FH-, FH+, T2D respectively), no significant difference was reached with the absolute change from pre-stretching to post-recovery between groups ($p = 0.75$). The fact that blood-glucose was lower across all groups with stretching supports the idea that passive stretching activates non-insulin induced

GLUT4 translocation which would support previous findings [53] indicating plasma glucose declines with exercise in T2D at the same time as plasma insulin. Though our methods do not specifically isolate glycogen use/turnover, it is interesting to note the increased CHO use occurs simultaneously with reduction in blood-glucose, further supporting the contention of Morino and Shulman [35] that glycogen turnover is affected by glucose transport. However, it was not possible within the constraints of this study to determine a timeline for reduced blood-glucose vs. increased CHO use with stretching, and thus it is also not possible to establish which one leads to the other. However, in the current study, there was a significant correlation between decreased blood-glucose and increased CHO use from pre to post-stretching overall (.385, $p = .027$), showing a direct relationship between the two.

CHO use from peak stretch to recovery revealed a significantly greater drop in the T2D group than FH- similar to Boon's findings [53], but no significance between FH+ and either FH- or T2D, indicating 1) though stretching causes up-regulation of CHO use and decreased blood-glucose concentration, greater CHO use from passive stretching are short-lived during recovery for T2D subjects, and 2) FH+ subjects fell in between FH- and T2D for increased CHO use and decreased blood-glucose changes. This indicates that though the FH+ subjects displayed no overt signs of IR, and as young, non-obese, active individuals, they may be an ideal group to study the earliest steps towards pathologic metabolic complications leading to IR. When considering Boon's findings, the similarities in post-stretching fat and CHO oxidation between T2D and FH+ may indicate increased plasma NEFA oxidation in a young, active FH+ population. However, further research is needed for verification.

Our results of a single bout of passive stretching indicate reductions in blood-glucose levels in T2D similar to other studies [71]. What is interesting to note in the present study is the more

significant reduction of blood-glucose in the T2D group than FH-. Granted, the T2D had a significantly higher fasting blood-glucose, which would indicate that greater reductions are more likely. However, we also noted a larger drop in CHO use with this population than FH- or FH+ indicating that without a metabolic stimulus, T2D subjects are not as effective at using CHO after stretching. These data suggest that stretching may be able to increase MF somewhat in adverse populations; however, since the metabolic stimulus was so low, it is likely that a more intense stimulation (e.g. walking) may increase post-exercise metabolic demands and thus prolong the beneficial effects of increased metabolism. Although it has been established that moderate to high exercise intensity aids glucoregulatory function [18, 70] the current data suggests that even the least aggressive kind of exercise (passive stretching) may still have some benefit. However, future work is needed to examine a possible dose-response to exercise: first, to determine if the increased CHO use is related to the metabolic stimulus, and second, to determine if the increased CHO use is further related to decreased blood-glucose. Future studies are needed to better isolate the minimum dose (time, intensity, kcal, etc) needed to increase post-exercise CHO use, and determine if oxygen kinetics and substrate selection are linked with improved glucoregulatory function.

Though FH- displays significantly different CHO use than T2D with stretching, no differences in increased CHO use were noted between FH+ and either the FH- or T2D group with passive stretching. This possibly suggests that though the FH+ group is young, active, and not overweight, their metabolic function may function somewhere inbetween healthy FH- and T2D, and thus would be an ideal population to study the earliest progression of IR and metabolic dysfunction.

- Metabolic Flexibility

Though IR was not assessed, the declining blood-glucose concentrations after glucose loading revealed that FH- and FH+ groups had similar blood-glucose responses, and both were significantly better than the T2D group. Given the age, BMI, activity level, and blood-glucose responses of the FH+ group, it is unlikely that they suffered from insulin resistance.

It was expected that the T2D group would have more impaired glucoregulatory function and metabolic flexibility than FH-, especially given the differences in age, weight, and diabetes status. Furthermore, recent studies have indicated that 1st degree FH+ with IR also display impaired MF. However, in the current study, 1st and 2nd degree FH+ displayed impaired MF independent of IR. In addition, this group was not only age, BMI, and activity-matched with the FH- group, but unlike previous studies [43], both groups were highly physically active (having spent more than 5 hours per week in vigorous activity) as determined by an exercise questionnaire. Thus, metabolic inflexibility in this group is a novel finding (Figure 2.6) given the level of intensity and duration of physical activity for both groups. The degree of MF impairment was not only significantly worse than FH-, but actually matched that of the T2D group. This suggests that independent of favorable blood-glucose concentration responses to an oral glucose load, or ideal fasting blood-glucose levels, FH+ and T2D are less able to effectively shift substrate use after ingesting a glucose load than FH-. Previous studies that indicate that reduced MF is likely due to declining disposal rate [72], or high fasting blood-glucose concentrations [44], though in the current study stretching induced a reduction of blood-glucose concentration, and both FH- and FH+ had similar fasting blood-glucose concentration. This would suggest that other factors may affect MF, including genetics [73].

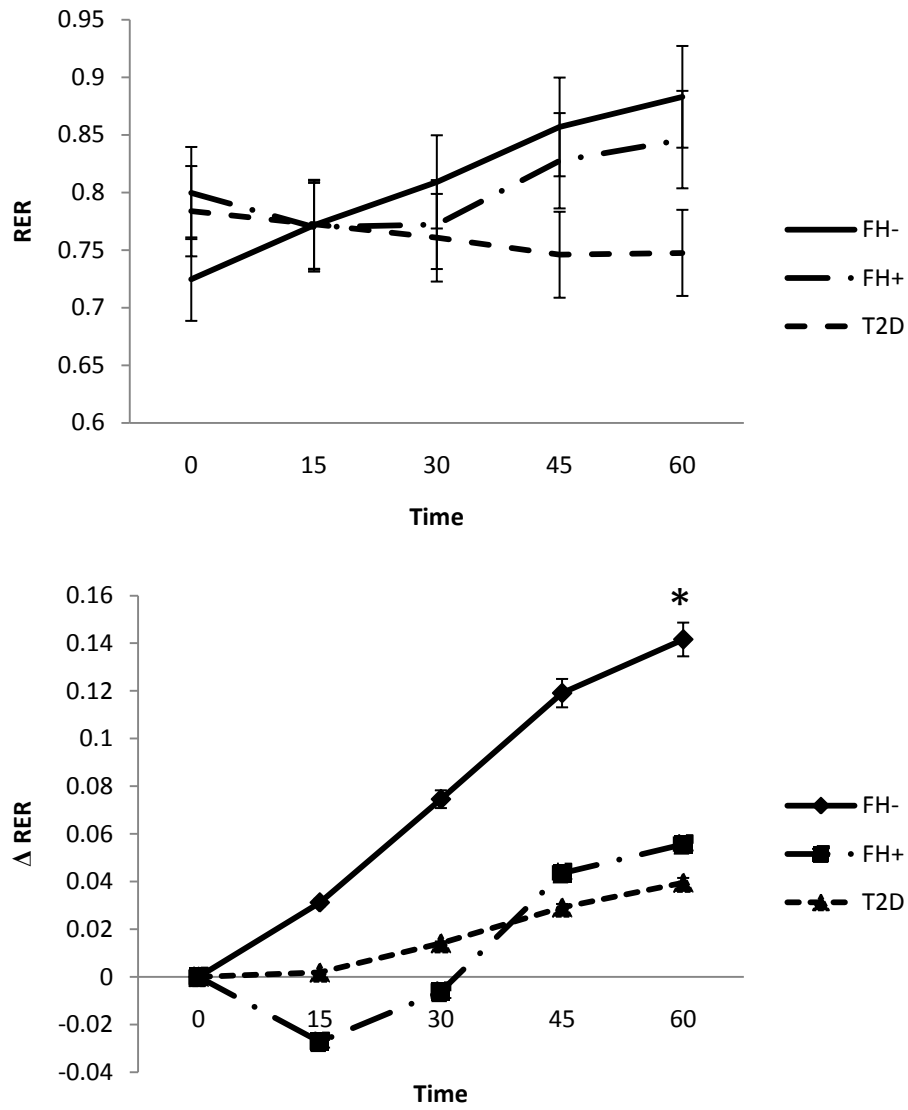


Figure 2.6 Mean \pm SE for RER (Top) and change in RER (metabolic flexibility) (Bottom) up to 1-hour post glucose load. *Significantly different than FH+ and T2D, $p = 0.05$.

Factors that may contribute to the reduced MF in the FH+ group may include impaired fat oxidation in the mitochondria, together with triglyceride fluxes from liver and intestine leading to a build-up of IMCT and/or acylcarnitines [3, 11, 15, 44]. CD-36 expression is also often up-regulated in IR, promoting increased fatty acid transport leading to competition with CHO as substrate [11]. However, combined with the data illustrating no differences between groups in fat oxidation in the 1-hour test, it seems unlikely that fatty acid uptake is a culprit in the reduction of MF. However, it may be possible to employ the measure of CHO utilization after an oral glucose load, or during and after exercise as a possible early indicator in the development of IR in at-risk populations. It has also been shown that FH+ individuals have impaired hexokinase-II activity as compared with controls [19]. This reduction could lead to decreased glyconeogenesis, thereby reducing the cell's glycogen stores and its ability to utilize CHO as substrate, and further limit glucose-uptake.

The T2D group had a significantly greater decrease in fat oxidation with glucose stimulus than FH-, with FH+ having no significant differences between either FH- or T2D. This indicates that T2D are less able to shift from fat oxidation than FH-. This could be due to competitive inhibition that fat oxidation elicits on glycolysis [11, 19, 44], the supply of fat outpacing energy needs leading to partial breakdown of fat [3, 11], a build-up of lipid intermediates such as diacylglycerol (DAG), or JNK [19], or effects of hormones such as leptin or resistin which are produced by fat cells [74, 75]. However, with no such measurements performed in this study, further research is needed to be able to specifically identify such factors inhibiting cellular shifts in substrate utilization.

Though this study lacks the experimental design needed to elucidate alterations in use of muscle-specific substrates, i.e. use of tracers or magnetic resonance spectroscopy, it does

illustrate that MF can be measured in as little as one hour using standard indirect calorimetry methods. Also, finger-prick blood-glucose data are not considered as accurate as running clinical chemistry analysis of glucose on plasma. However, recent studies indicate that such testing can be a precise and reliable means of assessing blood-glucose [45], especially with the practice of duplicate testing used in the present study. Thus, the use of the Accu-check Compact Plus appears to be a valid tool for blood-glucose measurements in this study. In addition, though there was no control diet given in the present study, participants were in an 11-hour fasted state prior to the glucose-load and MF assessment, which began over an hour after arrival. It has also been reported that regulation of RQ/RER is the most important reason for administering a control diet for MF testing [44]. Recent studies indicate in order for food quotient (FQ) to become equal to respiratory quotient (RQ), it could take up to one week of regulated diet [44]. Therefore, the importance of dietary regulation is to regulate FQ long enough so FQ equalizes with RQ in all groups. Since some studies report higher fasting RQ/RER in T2D than controls, calculations of reduced MF are skewed by a higher starting RQ [44]. However, in this study there were no significant differences in fasting RER between any groups, making it less critical to control diet. Lastly, the T2D group was significantly older than either the FH- or FH+ groups. However, the purpose for including a T2D group for to establish a point of reference for advanced diabetes. As such, FH+ displaying similar metabolic inflexibility to T2D, even with significant age differences, is further evidence that having a family history of T2D is an important factor in aberrant metabolic function.

The current study also included a large population of subjects with three strongly defined populations. In addition, FH- and FH+ were matched for age, height, weight and activity level,

and in spite of the closely matched groups, FH+ and T2D still exhibited reduced MF relative to FH-.

Conclusion

Passive stretching leads to a short-lived increased CHO use in T2D, FH+, and FH- populations with significant reductions in blood-glucose, especially in T2D. Fat use with stretching remains similar throughout each group in spite of previously reported fat oxidation differences in T2D [19, 44, 53].

An oral dose of glucose leads to increased CHO use in all groups, with significantly higher CHO use in FH- than either FH+ or T2D. Furthermore, change in RER with oral glucose (metabolic flexibility) is far greater in FH- than either FH+ or T2D, which exhibit equally reduced MF as compared to FH-. Therefore, FH+ either with no IR, or low-level IR may be an ideal group to study mechanisms that could potentially lead to the development of IR.

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CHAPTER 3: FASTING BLOOD-GLUCOSE CHANGES IN DIABETIC OFFSPRING AND CONTROLS WITH SEVEN WEEKS OF RESISTANCE TRAINING

Introduction

Type-2 diabetes (T2D) is a pandemic disease [1] characterized by insulin resistance (IR), which develops one to two decades before the onset of type-2 diabetes (T2D) [2, 3]. According to WHO criteria, T2D is defined as having fasting plasma glucose equal or above 7.0 mm/L (126 mg/dL), or if 2 hours post 75 g oral glucose tolerance test (OGTT) plasma glucose concentration rises equal or above 11.1 mmol/L (200 mg/dL) [4]. Exercise is the only treatment strategy that consistently results in improved whole body and skeletal muscle oxidative capacity [5], however there is debate over what kinds of exercise are most beneficial in treatment of T2D [6].

Interestingly, IR in persons with a family history of T2D (FH+) increases the likelihood of developing T2D by 40% [2, 7], and is the best predictor for developing this disease in the future for this population [3, 8]. Recent studies have indicated that FH+ with IR exhibit impaired metabolic flexibility (MF) and/or impaired mitochondrial activity prior to developing T2D [9-11]. Interestingly, some studies also show impaired MF in FH+ without having IR ([12], Russell et al., unpublished data). Recent studies suggest that impaired MF contributes to increasing obesity and IR [13, 14]. Furthermore, fasting blood glucose and glucose disposal rate are hypothesized to be among the most influential factors for impaired MF [15, 16].

Exercise intervention has been shown to enhance insulin sensitivity associated with a reduction in whole body fasting respiratory quotient (RQ) in obese insulin-resistant individuals [17] and improved MF in T2D [18]. A very recent study has shown that aerobic and resistance exercise has similar lowering effects on fasting blood-glucose and improvements in insulin sensitivity, yet combination of the two has an additive effect [19]. However, to our knowledge there have been no studies examining the relationship between long-term resistance training

intensity and fasting blood glucose in FH+ using traditional multi-set and fast-paced circuit training.

The preference of exercise modality for possibly delaying or preventing the development of T2D in this at-risk population has not been examined. Furthermore, it is unclear whether risk factors such as fasting blood glucose can be altered in a young, apparently healthy FH+ population with different forms of resistance training. Since impaired MF is directly related to fasting blood glucose [15], and has been observed in young, active FH+ population (Russell et al., unpublished data), it is important to further examine whether or not specific exercise programs are more helpful than others at reducing this risk factor.

Therefore, the present study compared the clinical benefits of traditional resistance training focused solely on strength gains vs. fast-paced resistance circuit training designed to maintain highly elevated heart rates in healthy young people with (FH+) and without (FH-) a family history of T2D. Changes in estimated maximal strength in bench press, squat, and dead-lift were used to assess effectiveness of training modality. Fasting blood glucose concentrations were assessed before and three days after intervention in both groups. It was expected that strength progression would be similar for both modes of training, and that circuit training would result in more significant reductions in fasting blood glucose concentrations. Finally, it was expected that FH- would have greater reductions in fasting blood glucose than FH+.

Methods

- **Subjects.**

14 FH- and 9 FH+ subjects were recruited from the Baton Rouge area using local advertisements and word of mouth to participate in a 7-week study examining the effects of resistance training on fasting blood glucose. FH+ was categorized as being a 1st or 2nd degree

relative of a person with T2D. The subject population consisted of fourteen healthy, active controls with no family history (FH-) of diabetes, and nine healthy active FH+. Age, gender, BMI, and activity were matched between FH+ and FH-. The purpose, potential risks and benefits of participation in the study were fully explained to each participant before written consent was obtained prior to testing. The study was approved by the Ethical Committee of Louisiana State University (LSU office of IRB). All FH+ and FH- subjects had normal fasting blood-glucose prior to testing using a hand-held finger-prick glucose meter. Additional inclusion criteria for FH+ and FH- groups were no overt disease, physically active, blood-glucose concentrations under ten mmol/L (180 mg/dL) an hour after glucose loading, BMI between 18.5-24.9 kg per m², and not taking prescription medication other than oral contraceptives. A physical activity questionnaire was completed prior to participation in the study. Subject characteristics were summarized in Table 3.1.

- Research Design.

The study consisted of three main parts. 1) a pre-training test day including fasting and post-exercise blood glucose followed by determination of calculated maximal strength on bench press, squats, and dead-lift, 2) 7-weeks of resistance training using either traditional multi-set resistance training or fast-paced single-set circuit training, and 3) a post-training test day including fasting and post-exercise blood glucose and re-evaluation of calculated maximal strength. Participants were instructed not to perform strenuous exercise, nor consume alcohol or caffeine at least two days prior to either test day.

- Blood-Glucose.

Table 3.1 Subject characteristics: Age (years), Height (cm), Weight (kg), and Body Mass Index (BMI). Mean \pm SE.

	FH+	FH-	P
Age (years)	23.0 \pm 1.1	24.1 \pm 2.0	NS
Height (cm)	170.5 \pm 2.9	173.8 \pm 1.9	NS
Weight (kg)	70.6 \pm 3.2	73.0 \pm 3.2	NS
BMI	24.3 \pm .06	24.2 \pm .05	NS

After a ten-hour fast, blood glucose was measured using an AccuChek CompactPlus glucose monitor (Indianapolis, IN), a precise and reliable means of assessing blood-glucose [20]. Blood-glucose measurements were taken after ten minutes of rest, and then each participant was given a 110 kcal breakfast snack bar containing 3g fat, 110mg sodium, 24g carbohydrate, and 2g protein. Blood glucose was re-evaluated immediately after (time 0), and 10 minutes after (time 10) both test day workouts. Participants were asked to record their diet the day prior to the first test day, and repeat the same diet on the day preceding the post-training test day. Blood lactate was also measured at the same time as glucose, using a lactate monitor (Lactate Pro, Quesnel, BC).

- Exercise Training.

Two kinds of training modalities were utilized. Each modality included exercising five days per week, with resistance training on Monday and Friday, core body work on Tuesday and Thursday, and explosive interval training on Wednesday (Figure 3.1). Eight participants performed 7 weeks of traditional, multi-set resistance training featuring two sets of resistance exercise for each muscle group, the second set going to complete muscular fatigue of: squat, bench-press, lateral pull down/seated row, shoulder press, push-up, bicep curl, tricept extension, and dead-lift exercises. Training progression was continuously monitored and load adjusted for all participants in both exercise groups to ensure that each participant increased the resistance load with increasing strength, enabling all participants to continue to work out at 65-85% of 1RM for each workout. Sixteen participants performed 7-weeks of fast-paced circuit resistance training featuring one set to complete muscular fatigue of the same exercises. One participant from the circuit group was excluded for non-compliance. Both groups had similar ratios for gender and family history. Both groups began training on the same day an hour apart, and performed the same exercises. Each group was encouraged to reach muscular fatigue within 8-12

repetitions at 65-85% of calculated 1-repetition maximum (1RM). If more repetitions were required to achieve complete muscular fatigue, weight was increased on the subsequent workout.

- Analyses.

Strength assessment was conducted via a modified Epley formula [21]:

$$1RM = \left[\left(\frac{r}{30} \right) + 1 \right] \cdot w$$

Where r = repetitions, w = weight lifted. After a thorough warm-up, each person attempted one set of bench press, squats, and dead-lift to complete muscular fatigue, then used the formula above to estimate 1RM. Blood glucose and lactate concentrations were assessed via finger prick as described above.

All statistics were calculated using SPSS 17 (Somers, NY). Subject characteristics were analyzed using comparison of means. Variables such as fasting blood-glucose and lactate differences between groups were calculated using a 1-way ANOVA. Pre to post-training group changes in blood-glucose and lactate concentrations were analyzed using repeated measures ANOVA, factoring family history. Pearson product moment correlations were used to determine relationship between variables. All comparisons were considered significant at the alpha level $p \leq 0.05$.

Results

Prior to training, there were no differences in blood glucose or calculated maximal strength between FH+ and FH-. No differences in strength increases or fasting blood glucose concentrations were noted between the two different training modes. Strength increased from pre to post training similarly in both groups (Table 3.2), while fasting blood glucose concentrations were significantly lower after training overall, with a strong trend towards FH- having the greater

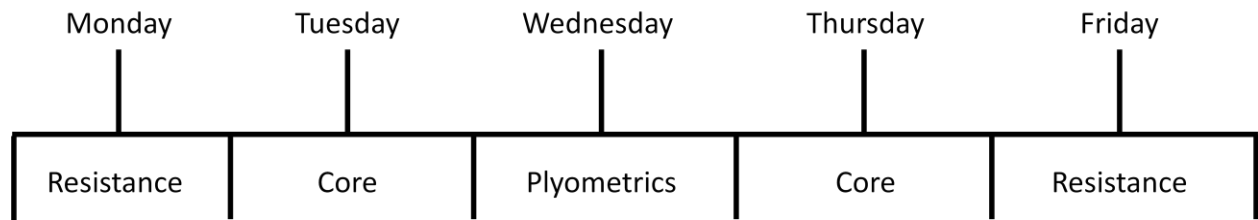


Figure 3.1 Illustration of workout schedule for each week in both exercise modalities.

Table 3.2 Training-induced changes in strength and fasting glucose. Values represent mean \pm SE.
P = overall group changes from pre to post training.

	FH+	FH-	P
Strength	1430 \pm 70.3	168.7 \pm 128.2	0.0001
% Strength	56.8 \pm 46.9	42.4 \pm 32.9	0.0001
Fasting BG	-3.6 \pm 7.5	-4.8 \pm 6.5	0.015

decrease ($p = 0.17$) (Figure 3.2). There was also a correlation between percent strength gains and decreased blood glucose concentrations from pre to post training ($r = -0.515$, $p = 0.046$) (Table 3.2). Fasting glucose: lactate ratio was not different from pre to post training. However, from pre to post training, glucose: lactate ratio was higher immediately after exercise, and lower five and ten minutes after exercise (Table 3.3).

Discussion

There were no differences between FH- and FH+ in pre-training strength, BMI, or blood glucose or lactate before or after exercise. The hypothesis that both modes of training would illicit the same strength gains was supported, however, the subjects also yielded the same fasting glucose and lactate responses with time. Therefore, data were combined from both modes of training to increase statistical power for glucose and lactate changes with training in FH+ and FH-. Fasting blood-glucose dropped in FH- and FH+ alike with training. Furthermore, changes in glucose: lactate ratios were no different between groups, but increased immediately after exercise with training, and decreased at five and ten minutes post-exercise with training.

- **Strength**

With both modes of strength training yielding the same strength gains over 7 weeks, the two modes were combined for greater strength in statistical analysis for glucose and lactate. A high-volume load for strength training was utilized to increase potential exercise benefits, specifically since previous research indicates that 8 weeks of low-intensity, low-volume resistance is not sufficient to decrease metabolic risk factors in T2D [22]. Strength increased on average nearly 57% with training overall. Resistance training is associated with not only increased strength as seen in this study, but also with increased muscle mass and improved whole-body glucose disposal rate [23]. Furthermore, a recent study indicates that similar resistance-type circuit



Figure 3.2. Fasting blood glucose concentrations before and after 7 week resistance training. Mean \pm SE

Table 3.3 Changes from pre to post training in Glucose: Lactate ratio. Values are mean \pm SE.

	Pre-Training	Post-Training	P
0 Glu Lac Ratio	13.5 \pm 1.1	9.46 \pm 0.8	0.034
5 Glu Lac Ratio	27.1 \pm 3.5	14.8 \pm 1.1	0.001
10 Glu Lac Ratio	32.2 \pm 3.6	27.9 \pm 3.8	0.003

training can decrease metabolic risk factors in young overweight males [24]. The increased strength seen in this study would indicate that the stimulus used for resistance training was sufficient to elicit a physiologic adaptation. However, lean muscle mass was not evaluated in this study.

- Fasting Blood Glucose

Fasting blood glucose decreased with training (Figure 3.2). Though there was a trend of FH+ not decreasing as much as FH- ($p = 0.17$), no significant differences were noted in fasting blood glucose with training. This lack of significance between groups may be due to a small sample population, or insufficient length of training duration. However, it is also possible that since fasting blood glucose in both groups was already well within normal range, it was less likely to observe significant differences in reduced fasting glucose than if this population had higher pre-training values. The overall decline in blood glucose concentrations indicates tighter glucoregulatory control with resistance training. This may be due to greater glucose uptake in muscle as previously indicated [23]. However reduced hepatic glucose release cannot be ruled out since we did not measure the glucose source specifically. Therefore it was not possible to distinguish between appearance and removal rates, and thus have only described what is acutely visible in the blood at specific time points. Nonetheless, whether a fasting decrease was from reduced appearance or increased removal, a tighter blood glucose regulation exists with training further supporting previous studies indicating that this type of training reduces insulin resistance [24, 25], independent of body mass changes.

- Blood Lactate

In the current study, there were no changes in fasting lactate, however sharp increases in lactate in both groups were observed immediately after exercise with seven weeks of training.

This acute post-exercise lactate increase was attenuated significantly after training, along with increased estimated 1-RM, indicate a significantly greater absolute workload was achieved after seven weeks of training without increasing the relative workload. With training, there were reductions in glucose: lactate ratio immediately post-exercise, and at five and ten-minutes post-exercise. Though blood lactate was higher immediately after exercise with training, there were no differences in blood lactate at five and ten minutes after exercise from pre to post training. This indicates a greater rate of lactate removal with training (Figure 3.3). This greater decrease in blood lactate concentrations may be an indication of greater oxidative enzyme capacity with training, as lactate is removed by either oxidative muscle fibers adjacent to lactate-producing glycolytic fibers or by liver [26, 27].

Simoneau and Kelley [28] showed that an increased ratio of glycolytic to oxidative enzymes contributes to insulin resistance in skeletal muscle of patients with type 2 diabetes. Granted, the current study was not designed to monitor lactate removal, nor to determine if the greater decrease within ten-minutes of passive recovery was due to less lactate appearance, greater rate of removal, or a combination of both. Since a large amount of lactate comes from glycogenolysis [29], it is reasonable to assume that exercise training does not affect production of post-exercise lactate in passive recovery to a great extent. Therefore, it is likely that lactate removal is occurring at a faster rate after training. When considering that exercise up-regulates mitochondrial biogenesis transcription factors like PGC-1 α [30], and the various ways in which lactate can be removed, including by oxidative muscle fibers [27], it is plausible to expect that resistance training in the current study elicited an up-regulation of PGC-1 α leading to increased oxidative muscle fibers and higher mitochondrial content, leading to decreases in both fasting blood glucose and 10 minute post-exercise blood lactate concentrations.

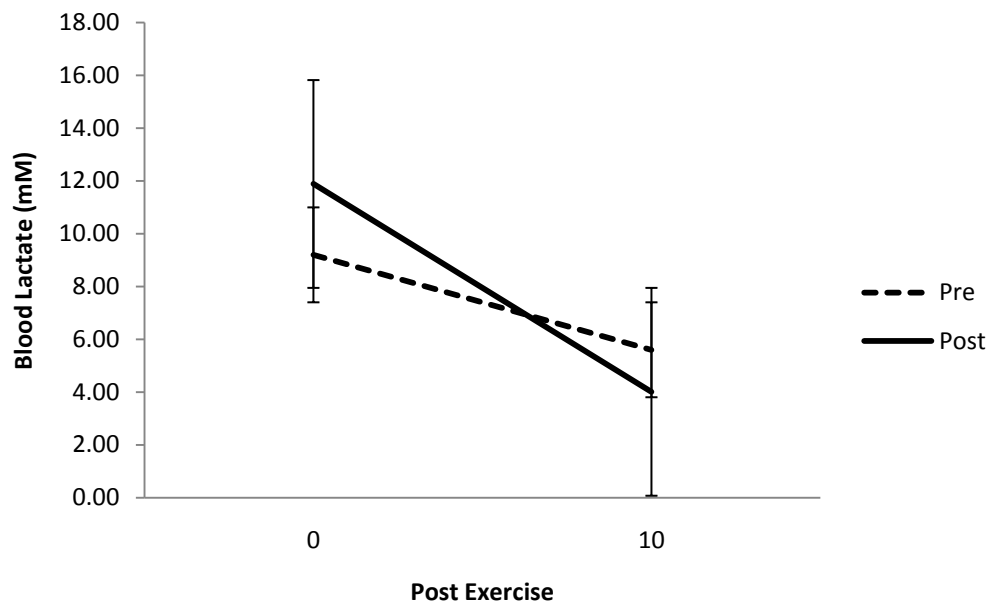


Figure 3.3 Changes in blood lactate concentrations at 0 and 10 minutes after acute exercise, before (Pre) and after (post) resistance training. Mean \pm SE.

- Glucose: Lactate Ratio

As expected, there was no change in glucose: lactate ratio at rest with training. However, with elevated lactate immediately post-exercise, combined with reductions in glucose: lactate ratio after exercise with training indicate a greater lactate production with the greater absolute workload and faster lactate removal with passive recovery. Though post-exercise blood glucose concentrations were 104.4 ± 3.5 mg/dl (mean \pm SE) after training, the glucose: lactate ratio was significantly reduced at 0, 5 and 10 minutes post exercise with training, with no differences between FH+ and FH-. Since the workout itself started with the largest muscle groups and maintained a constant intensity for the entire 10 minutes to completion, then ending with dead-lifts, it is reasonable to believe that the blood lactate concentrations increased early and remained high throughout the workout until a possible peak with dead-lift at the end, allowing lactate measurements at time 0 to represent lactate at their highest concentrations. This was evidenced by the reduction of lactate, and the glucose: lactate ratio at all time points. It is important to note that the ten-minute recovery was passive. It is possible that both glucose and lactate may have dropped more in this recovery period had it been active. However, in an effort to normalize recovery somewhat between people with different muscle mass, passive recovery was chosen. It is interesting to note that even with passive recovery, with training, this ratio at five and ten-minutes post exercise was well below fasting levels. This may indicate that the recovering muscles and liver, etc may be using a greater ratio of blood glucose to lactate for recovery with training, especially since absolute lactate production post-exercise with training increased.

- Conclusions

High-intensity resistance circuit training and traditional multi-set resistance training are equally effective in increasing strength in moderately active young adults. Furthermore, fasting

blood glucose is significantly reduced with seven weeks of resistance training, with a trend of less reductions in FH+ than FH- ($p = 0.17$) similar to metformin use in obese adolescents [31]. Further research is needed to determine if longer duration training increases this significance, and also to determine if resistance training can affect risk factors for the development of T2D in this young FH+ population other than fasting blood glucose, for example, blood lipid profiles.

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CHAPTER 4: LIPOTOXICITY AND EARLY INSULIN RESISTANCE: EFFECTS OF EXERCISE TRAINING ON LIPOTOXICITY MEASURES IN 1ST AND 2ND DEGREE OFFSPRING OF DIABETICS

Introduction

Type-2 diabetes (T2D) is a pandemic disease [1] characterized by insulin resistance (IR), which develops one to two decades before the onset of type-2 diabetes (T2D), [2, 3] and is the best predictor for future development of this disease [3, 4]. Since IR is related to several co-morbidities like elevated lipid levels, loss of metabolic flexibility (MF) and impaired glucoregulatory function, much attention has been given to studying these co-morbidities. IR subjects not only display elevated fasting β -oxidation, but also impaired MF, which is characterized by impaired ability to switch to carbohydrate as substrate from the fasted-to-fed transition (Russell, et al., unpublished data, [5]). Several factors are likely to contribute to the development of this condition, including decreased hexokinase activity and peroxisome proliferator-activated receptor gamma coactivator 1 α 1 (PGC1) expression, which are both seen in T2D [6]. One prominent theory suggests that muscle insulin resistance arises from impaired mitochondrial uptake and oxidation of fatty acids [7]. In this process, disturbances of free-fatty acid/nonesterified fatty acid (FFA/NEFA) metabolism, elevated basal rate of lipolysis, and impaired suppression of plasma NEFA concentration by insulin, are also associated with IR and reduced MF [8-10]. Although there is a strong indication that these factors are related to mitochondrial dysfunction and impaired MF, with so many co-morbidities and additional confounding factors (like obesity and impaired glucoregulatory function) often present in T2D patients, it is difficult to distinguish which factor may be the initial trigger for developing IR.

Interestingly, IR individuals with a positive family history of diabetes (FH+) may be an ideal group in which to study the initial development of diabetes and its earliest co-morbidities for

several reasons. These individuals have been shown to be at approximately 40% higher risk for developing T2D than those with no family history (FH-) [2, 11], and have impaired hexokinase II activity and PCG1 α 1 expression similar to T2D [12] [6] which may lead to decreased expression of Nuclear respiratory factor-1 (NRF-1), which is a key transcription factor involved in regulating mitochondrial DNA transcription [6]. In addition, previously unpublished work from our lab indicate they also exhibit impaired MF similar to T2D, yet have similar glucoregulatory function as those with no family history. The benefit of studying IR in this population is that these individuals often have not yet developed many of the confounding factors that may contribute to the development of T2D, like glucotoxicity or obesity, yet they display a 50% reduction in the rate of insulin-stimulated whole-body glucose metabolism due to decreased muscle glycogen syntheses rates [11]. This suggests that the pathogenesis of T2D in the FH+ population can be examined at its earliest time points [7]. To date, much of the research on FH+ has been in IR FH+ only, making it difficult to isolate what pathologic connection is shared between FH+ and T2D leading to the development of IR, which makes FH+ more likely to develop diabetes [13, 14]. However, previously unpublished data from our lab shows that FH+ population not only has impaired MF similar to T2D, but also may have a less favorable glucoregulatory improvement in response to resistance training. However, it is not clear what metabolic factors may be involved in the impaired MF of FH+, though recent studies would suggest problems with lipid metabolism being a likely culprit [8-10].

Therefore, the purpose of the current study was to determine whether differences in markers of lipotoxicity and glucoregulatory function exist between young, active FH+ and FH- populations, and to compare possible changes in response to resistance training. Data from the present study could potentially aid in development of effective screening tools and interventions

to prevent T2D progression in the FH+ population. It was hypothesized that markers of lipotoxicity and cardiovascular disease would be elevated in FH+ as compared with FH- prior to training, and that these markers will decrease less in FH+ than FH- with resistance training.

Methods

- **Subjects.**

Twenty-three subjects were recruited from the Louisiana State University student population to participate in 6.5 weeks of resistance training, and one morning of fasting blood testing before and after training. FH+ was categorized as being a 1st or 2nd degree relative of a person with T2D. The subject population consisted of 10 healthy, active controls with no family history (FH-) of diabetes, and 13 FH+ subjects. Age, gender, BMI, and activity were matched between both groups. The purpose, potential risks and benefits of participation in the study were fully explained to each participant before written consent was obtained prior to testing. The study was approved by the Ethical Committee of Louisiana State University (LSU office of IRB). All FH+ and FH- subjects had normal fasting blood-glucose prior to testing using a hand-held finger-prick glucose meter (AccuChek CompactPlus, Indianapolis, IN). Additional inclusion criteria for FH+ and FH- groups were no overt disease, capable of physical activity, blood-glucose concentrations under 10 mmol/L (180 mg/dL) an hour after glucose loading, and not taking prescription medication other than oral contraceptives. A physical activity questionnaire was completed prior to participation in the study. Subject characteristics are summarized in Table 4.1.

- **Research Design.**

Subjects were tested prior to, and 3 days after training. The testing consisted of two components: 1) assessment of blood glucose concentration responses to an oral glucose load, and 2) fasting blood samples drawn and centrifuged to separate and freeze the plasma until further

Table 4.1 Pre-training values for FH- and FH+. Age (years), weight (kg), BMI, fasting blood glucose (BG, mg/dl), Tryglyceride (mg/dl), NEFA (mM/L), Insulin (mmol/L), Homeostatic model assessment (HOMA) and acylcarnitine (C18-2). Values are mean \pm standard error.

	FH+	FH-	P
Age	23.0 \pm 1.1	24.1 \pm 2.0	NS
Weight	76.7 \pm 5.3	64.8 \pm 4.9	NS
BMI	26.7 \pm 1.7	23.5 \pm 1.2	NS
Fasting BG	86.2 \pm 2.6	82.7 \pm 3.2	NS
OGTT AUC	125.9 \pm 5.4	125.7 \pm 5.3	NS
Triglyceride	107.4 \pm 7.8	128.7 \pm 12.0	NS
NEFA	384.5 \pm 64.9	441.1 \pm 37.0	NS
Insulin	10.8 \pm 3.2	7.5 \pm 2.4	NS
HOMA	2.3 \pm 0.7	1.5 \pm 0.4	NS
C18-2	0.03 \pm 0.0	0.04 \pm 0.0	0.04

analysis. The training component of the study consisted of 6 ½ weeks of fast-paced circuit-type resistance training as used in previous studies (Russell et al., unpublished data). This method was shown to be as effective as traditional multiple set resistance training for increasing strength and reducing fasting blood glucose concentrations (Russell et al., unpublished data).

- Glucoregulatory Assessment.

While still fasting, each participant consumed a solution of 50g glucose dissolved in 10oz of water. Each glucose beverage was consumed within two minutes while resting. Finger prick capillary glucose was determined via a glucometer (AccuChek CompactPlus, Indianapolis, IN), a precise and reliable means of assessing blood-glucose [15]. Finger prick and glucose measurements were determined every twenty minutes for 1-hour to determine blood-glucose responses to the oral glucose load, similar to studies examining postprandial hyperglycemia, a well-recognized risk-factor of T2D [16, 17]. Previous data from our lab indicates that a 60 minute modified oral glucose tolerance test (OGTT) is sensitive enough to differentiate glucoregulatory function between FH+ and T2D (Russell et al., unpublished data). Furthermore, a 60-minute OGTT has been shown to better determine glucoregulatory impairment in pre IGT patients [18].

- Fasting Blood Samples.

Fasting blood samples were drawn using both EDTA and heparinized vacutainers by a licensed phlebotomist prior to administration of the oral glucose beverage, and were centrifuged at 4 degrees C for ten minutes at 3500 RPM. The plasma was pipetted and quickly frozen at -80 degrees C and stored until analysis. Triglycerides (Teco Diagnostics, Anaheim, CA) and non-esterified fatty acids (Roche Applied Science, Indianapolis, IN) were determined spectrophotometrically according to kit manufacturer's instructions. Insulin was assessed using

an enzyme immunoassay kit from ALPCO Diagnostics (Salem, NH) according to manufacturer's instructions.

Acylcarnitine profiles were measured in collaboration with Dr. Indu Kheterpal, Director of the Proteomics and Metabolomics Core Facility at Pennington Biomedical Research Center.

For mass spectrometric analysis of acylcarnitines, 50 µL of blood plasma/serum was supplemented with a mixture of deuterium labeled acylcarnitines (Cambridge Isotope Labs.) as internal standards. Samples were de-proteinized with acetonitrile, and the supernatant was dried under nitrogen at 50 °C. The residues were incubated with 3M Butanol-acetylchloride at 50 °C for 15 min to form butyl ester derivatives of the acylcarnitines. The reagent was evaporated under nitrogen and samples were resuspended in 150 µL of acetonitrile/water/formic acid (80/20/0.1). An electrospray ionization (ESI) tandem quadrupole mass spectrometer (XEVO TQMS, Waters, Corp., Milford, MA) equipped with an Acquity ultra performance liquid chromatography system (Waters Corp., Milford, MA) was used for acylcarnitine measurement. Samples were directly injected into the ESI source of the MS and acylcarnitine profiles were generated using precursor ion scan (m/z 85) function of the MS. Ratio of each acylcarnitine to its assigned internal standard was used to determine concentration of each analyte.

- Strength Assessment and Training.

Strength assessment was done via a modified Epley formula [19]:

$$1RM = \left[\left(\frac{r}{30} \right) + 1 \right] \cdot w$$

Where r = repetitions, w = weight lifted. After a throughout warm-up, each person attempted one set of bench press, squats, and dead-lift to complete muscular fatigue, then used the formula above to estimate 1RM. Participants exercised five days per week, with resistance training on Monday and Friday, core body work on Tuesday and Thursday, and explosive interval training

on Wednesday (Figure 4.1). Eight participants performed 6.5-weeks of fast-paced circuit-style resistance training consisting of one set to complete muscular fatigue of: squats, bench-press, lateral pull-down/seated row, shoulder press, push-ups, bicep curls, tricep extension, and dead-lifts. Each participant was encouraged to reach muscular fatigue within 8-12 repetitions at 65-85% of calculated one repetition max (1RM). If more repetitions were required to achieve complete muscular fatigue, weight was increased on the subsequent workout.

- **Statistical Analyses.**

All statistics were calculated using SAS 9.2 (Somers, NY). Subject characteristics were analyzed using comparison of means. Principal components analysis was used as a means of data-reduction and clustering relevant acylcarnitines and to correlate levels of specific AcylCN species with metabolic parameters. Pre-training variables such as blood-glucose concentration after glucose loading, strength, BMI, or lipotoxicity markers between untrained groups, were calculated using one-way ANOVA. Changes with training between groups were analyzed using repeated measures ANOVAs.

Results

- **Pre-Training.**

No pre-training differences existed between FH- and FH+ in estimated strength, glucoregulatory function as per 60-minute modified OGTT, fasting blood-glucose, body weight, TG, NEFA, insulin or Homeostatic Model Assessment (HOMA) (Table 4.1). Most acylcarnitines were not different between groups prior to training, except for C18-2 $p = 0.042$ which was lower in FH+ ($p = 0.04$). FH+ display lower levels of the long-chain acylcarnitine, C18-2 as compared with FH-.

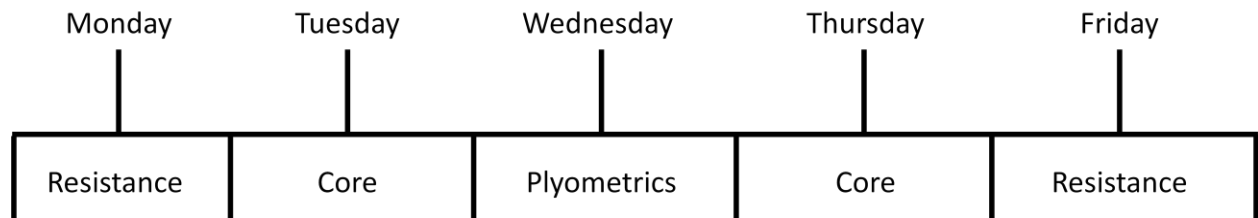


Figure 4.1 Illustration of workout schedule for each week in both exercise modalities.

- Training.

Training progression, measured as estimated strength increases in bench press, squat and dead lift, were the same in both groups. There were overall changes with training in body weight, BMI and strength ($p < 0.05$). A trend in greater weight loss was noted in FH+ ($p = 0.065$), with BMI being significantly lower in FH+ with training.

There was also a significant decrease in fasting plasma NEFA with training. However, there was no significant difference in NEFA change between groups with training (Figure 4.2). Principal components revealed several acylcarnitines are primarily responsible for the variation in metabolic data (Table 4.2), with two short-chain acylcarnitines, C3:1 and C4-OH, changing differently with training between FH- and FH+. When groups were separated according to weight loss (WL+) or no weight loss (WL-), there was a significant increase in plasma insulin with the WL+ group over WL- with training.

Discussion

Neither of the hypotheses was supported as there were no differences between FH- and FH+ pre-training strength, BMI, or in markers measured in plasma before or after exercise. Strength increased from pre to post training similarly. However, changes in NEFA and insulin were noted in non-weight loss weight loss subjects vs. (Figure 4.3). Negative correlations were observed between weight loss and: TG, NEFA, insulin and HOMA, and strength, and positive correlations were found between weight loss and blood glucose AUC.

- Glucoregulatory Function.

Although changes in blood glucose concentrations for fasting, 60-minutes post glucose beverage consumption, or with area-under-the-curve (AUC) for the 60-minutes of observation

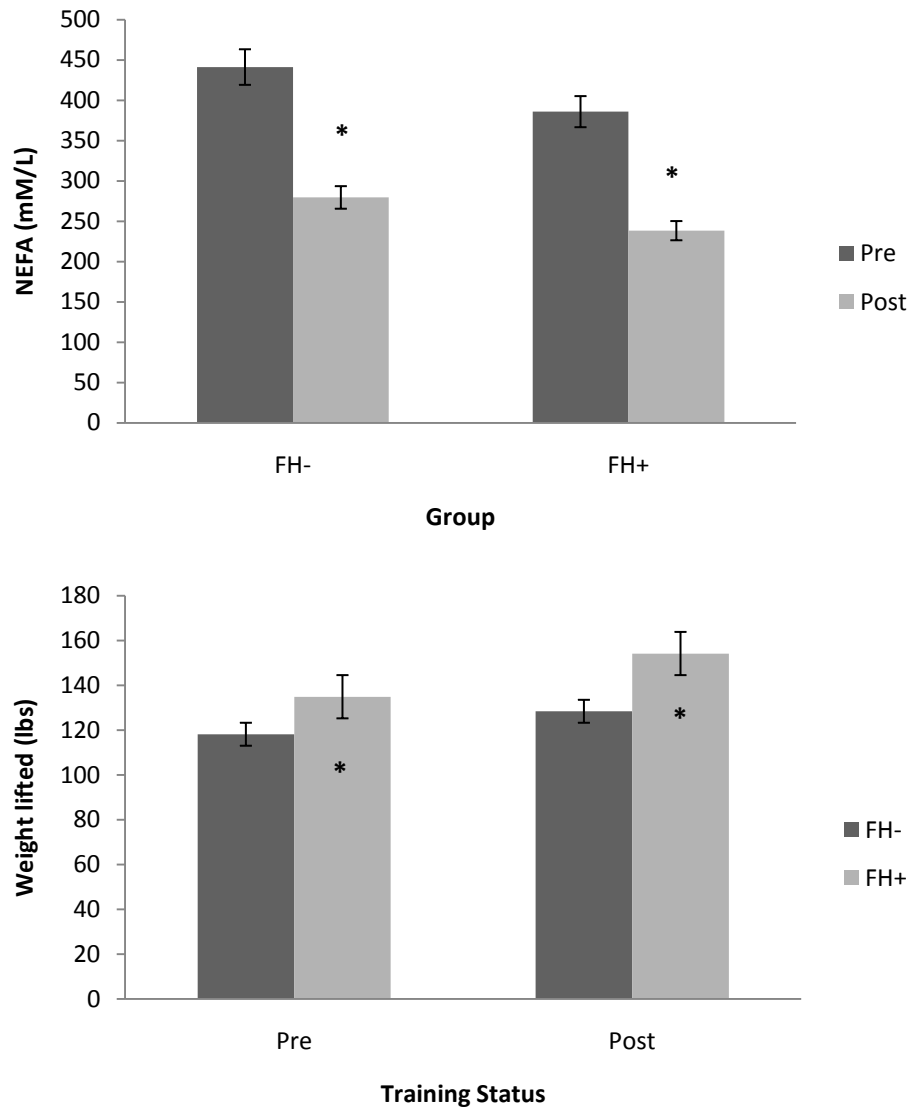


Figure 4.2 Mean \pm SE. Changes with training (NEFA –Top, and strength- Bottom) in FH- and FH+. $p = 0.05$

Table 4.2 Rotated factor pattern from principal components analysis of acylcarnitines showing important correlations with metabolic profile. Significant correlations are bold.

	Component		
	4	5	6
CN	0.62	-0.02	-0.03
C3	0.68	-0.04	-0.04
C4	0.72	-0.08	0.05
C5	0.68	0.9	0.11
C6-OH	0.56	-0.28	0.33
C8-1	-0.08	-0.02	0.84
C10-3	0.11	0.06	0.8
C10-2	0.26	0.12	0.59
C18/C12:1- DC	0.18	0.76	0.08
C20:4	0.64	0.01	0.09
C20/C14:1- DC	0.18	0.83	-0.02
C14-DC	0.06	0.52	0.15
C22/C16:1- DC	-0.34	0.77	-0.1
C18-DC	-0.17	0.57	0.21

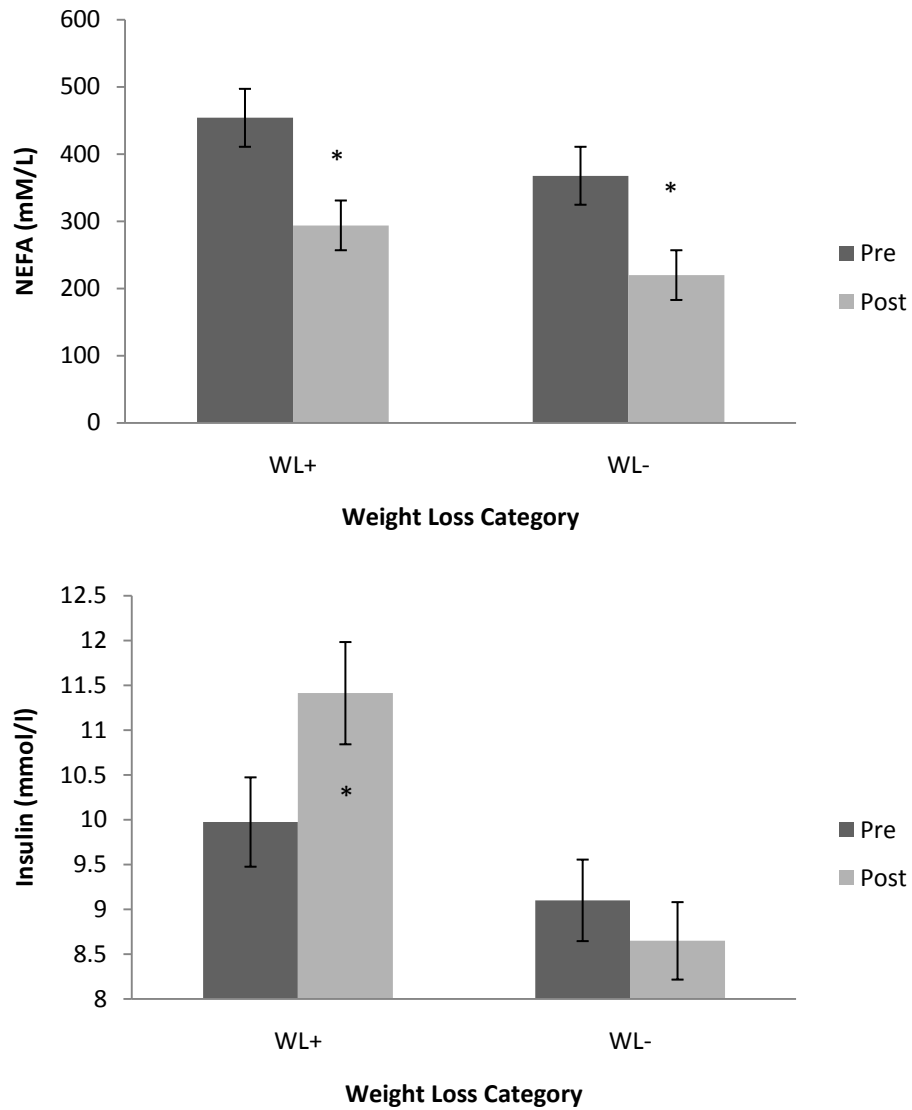


Figure 4.3 Mean \pm SE. Plasma NEFA (mM/L) and insulin (mmol/L) changes before (Pre) and after (Post) resistance training with and without training-induced weight loss. $p = 0.05$

*Significant change from pre to post training.

function increased (as per decreased glucose AUC) from pre to post training with weight loss. However, when examining the group that lost weight by itself using a paired T-test, there was a significant improvement in blood glucose AUC with training. However, no significant differences in glucoregulatory function existed between FH- and FH+ before or after training. This would imply that body weight and caloric balance are more important for overall glucoregulatory function than a family history of T2D.

- Non-Esterified Fatty Acid.

NEFA is produced from lipolysis of stored triglycerides in adipocytes which delivers NEFAs to the plasma, from where they are taken up by the metabolizing tissues, mainly skeletal muscle, heart and liver [20]. In the current study, there was a significant reduction in NEFA concentrations overall from pre to post training ($p = 0.008$) with no differences between FH- and FH+. This may indicate a reduced reliance on fasting FA metabolism with resistance training, and thus diminished lipolysis, an increase in insulin to enhance liponeogenesis and reduce NEFA production, or enhanced NEFA uptake by muscle or liver. There were also no differences in pre-training NEFA concentrations between FH- and FH+. However, a correlation exists between NEFA and blood glucose AUC, indicating that NEFA may affect glucoregulatory function, and the early development of IR. Since insulin is known to stimulate liponeogenesis [20], it should also reduce overall NEFA appearance in plasma. However, no correlations were noted between changes in insulin and NEFA concentrations in either group, though insulin was increased post-training in the weight loss group. This increase in insulin may help explain the overall decrease in NEFA production with training, however the plasma NEFA decrease was universal across all groups where increased insulin was limited to weight loss only, thereby implying that resistance training may lead to more efficient FA metabolism (removal) in the fasting state.

- Insulin and HOMA.

There were no differences in insulin concentrations or HOMA between FH- and FH+ prior to training. Likewise, there were no significant changes in the overall group with training for either insulin concentrations or HOMA, but when FH+ group was analyzed separately, there were significant increases in insulin and HOMA with training. However, these increases were accounted for when factoring in weight loss. Contrary to initial speculation, losing weight is attributed to increased insulin and HOMA in the current study. This is likely due to the lack of dietary control, meaning that all weight loss came from increased energy costs associated with exercise; and since the post-testing did not occur until 3.5 days after the last exercise bout, the energy balance had begun to shift back towards weight gain. Being in a positive energy balance causes an increase in insulin and HOMA to initiate liponeogenesis and subsequent weight gain. This change was independent of decreased glucoregulatory function however, which might be attributed to the increased lean muscle mass often associated with resistance training [21], but not likely to the acute affects of exercise since over three days had passed between exercise and testing.

- Triglyceride.

In order to examine the chronic, rather than acute effects of resistance training, blood samples were collected from the participants three days after the final exercise session. Triglycerides did not differ between groups either before or with training. This appears to contradict aerobic studies showing an improvement in these parameters [22], however, previous resistance training studies have yielded variable results [21, 22]. These differences are likely due to large variations in participant populations, training volume, study duration, modality, and intensity. For the current study, high-intensity circuit training in a young, healthy population did

not elicit any changes overall in plasma TG or insulin. It should be noted that plasma NEFA concentrations significantly decreased post-training while TG did not, indicating that fatty acid transport into muscle cells is up-regulated as a result of resistance training while lypolysis may not be up-regulated.

- Acylcarnitine.

Acylcarnitines are metabolic byproducts of incomplete substrate degradation formed from their respective acyl-CoA intermediates by a family of carnitine acyltransferases that reside principally within mitochondria. Most even chain species reflect incomplete fatty acid oxidation, and odd chain species from amino acid catabolism, whereas acetylcarnitine is derived from acetyl-CoA, the universal degradation product of all metabolic substrates [5, 23, 24].

In the current study, there was only one acylcarnitine that was different between groups before training, C18-2, and seven after training. However, very little is known about C18-2 and any associations with aberrant lipid metabolism. However, virtually no differences in acylcarnitine levels were displayed between groups either before or after training. This would imply that there is not yet a breakdown in lipid metabolism or acylcarnitine transport into the mitochondrial matrix in healthy active FH+ as compared with FH-.

Interestingly, C3-1 and C4-OH, which have been shown to be elevated in T2D [25], decreased more in FH+ than FH- with training. C3-1 is derived from amino acid catabolism, and C4-OH from incomplete fatty acid oxidation. Elevated C4-OH in particular has been well-characterized in the literature as being associated with Medium/Short-chain hydroxyacyl-CoA dehydrogenase (M/SCHAD) deficiency, a fatty acid oxidation disorder characterized by increased metabolic demands on fat oxidation [26]. Though fat oxidation was not specifically assessed, the decreased C3-1 and C4-OH with training moves both groups further from levels

seen in T2D. With normal clinical values ranging between 0 and 0.4 [27], any reduction can be seen as improvement since there is no lower limit, and is evidence of properly-functioning SCHAD enzymes used for fat oxidation. Thus, in both FH- and FH+, similar quantities of NEFAs are readily activated into AcylCN that can either pass into the mitochondria *via* carnitine AcylCN translocase or can exit the cell for other disposal. Inside the cell, the AcylCN is de-esterified back to acyl-CoA and enters the β -oxidation spiral [25]. However, given the decrease in circulating NEFA with training, it would be expected to see more of a reduction in acylcarnitines associated with the NEFA decrease.

Given the data indicating impaired metabolic flexibility in FH+, future research is needed to determine if acylcarnitine levels decrease with inhibited NEFA production via hyper-insulinemic clamp metabolic flexibility studies. With the added insulin suppressing NEFA production, there should be decreased acylcarnitine production displayed in the plasma in metabolically flexible individuals as compared with T2D or FH+.

- Limitations.

As previously mentioned, the post-exercise intervention blood samples were collected three days after the completion of the study which impacted plasma TG and insulin levels. In addition, due to limited resources, diet was not controlled or measured for this study, however all fasting was done after an overnight fast, and all participants were instructed to maintain a similar diet throughout training, and prior to both test days.

- Conclusion.

Though there are differences between groups with and without a family history of diabetes (Russell et al., unpublished data) [2, 11, 12], this study does not reveal any such differences in glucoregulatory function, or markers of lipotoxicity. Resistance training did not affect FH+

differently than FH-, however there were differences in these markers when groups were re-categorized by weight loss. We were unable to isolate specific factors likely to contribute to the development of IR or T2D within the confines of the current study. However, further research, such as lipid tracers and MRI studies are needed to determine factors leading to more aberrant metabolic function in order to better understand what factors lead to the development of IR and T2D.

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CHAPTER 5: GENERAL DISCUSSION

Type-2 diabetes (T2D) is a pandemic disease [1] characterized by insulin resistance (IR), which develops one to two decades before the onset of type-2 diabetes (T2D), [2, 3] and is the best predictor for future development of this disease [3, 4]. Since IR is related to several co-morbidities like elevated lipid levels, loss of metabolic flexibility (MF) and impaired glucoregulatory function, much attention has been given to studying these co-morbidities. IR subjects not only display elevated fasting β -oxidation, but also impaired MF, which is characterized by impaired ability to switch to carbohydrate as substrate from the fasted-to-fed transition [5], elevated mitochondrial reactive oxygen species (ROS) production [6, 7], and markers of lipotoxicity [8, 9] which in turn lead to even more enhanced ROS production [6, 10]. In addition, disturbances of free-fatty acid/nonesterified fatty acid (FFA/NEFA) metabolism, elevated basal rate of lipolysis, and impaired suppression of plasma NEFA concentration by insulin, are also associated with IR and reduced MF [11-13].

Interestingly, IR individuals with a positive family history of diabetes (FH+) may be an ideal group in which to study the initial development of diabetes and its earliest co-morbidities for several reasons. These individuals have been shown to be at approximately 40% higher risk for developing T2D than those with no family history (FH-) [2, 14], and have impaired hexokinase II activity and PCG1 expression similar to T2D [15, 16] which may lead to decreased expression of NRF-1 [16]. This suggests that the pathogenesis of T2D in the FH+ population can be examined at its earliest time points [9]. To date, much of the research on FH+ has been in IR FH+ only, making it difficult to isolate the pathologic connection is shared between FH+ and T2D leading to the development of IR, that makes FH+ more likely to develop diabetes [17, 18].

Therefore, the purpose of the current document was to categorize aberrant metabolic characteristics in healthy, active FH+ independent of IR to better understand which factors in this population are different from age and activity matched FH- which may precede the development of IR.

Key Results

In order to better understand the development of IR and T2D, it is important to isolate the earliest mechanisms that may lead to IR. Therefore, healthy, active FH+ were used as a model of earliest IR. However, many studies have examined FH+ with IR, and compared them to FH- without IR. For contextual purposes, it is important not only to understand how FH+ are similar to T2D, but also how they may differ from matched FH- as well. Therefore, **Chapter 2, experiment 1** examined metabolic characteristics, including metabolic flexibility (MF), fasting metabolism and substrate selection with a metabolic stimulus in T2D, FH- and FH+. Passive stretching led to a short-lived increased CHO use in T2D, FH+, and FH- populations with significant reductions in blood-glucose, especially in T2D. Fat use with stretching remained similar throughout each group in contrast to previously reported fat oxidation differences in T2D [15, 19, 20]. An oral dose of glucose led to increased CHO use in all groups, with significantly higher CHO use in FH- than either FH+ or T2D. Furthermore, change in RER with oral glucose (MF) was far greater in FH- than either FH+ or T2D, which exhibit equally reduced MF as compared to FH-. Since this experiment indicated a reduction of MF in FH+, and since fasting blood glucose is a major contributor to impaired MF [21], FH+ either with no IR, or low-level IR may be an ideal group to study mechanisms that could potentially lead to the development of IR. **Chapter 3, experiment 2** examined the relationship between resistance training and fasting blood glucose in FH+ and matched FH-. It was noted that fasting blood glucose was significantly

reduced with seven weeks of resistance training, with a trend of less reductions in FH+ than FH- ($p = 0.17$). Given the duration of the study, it is likely that by increasing the training duration from seven weeks to several months, the differences in fasting blood glucose decreases would have been significant if the observed pattern remained constant. Furthermore, if this kind of exercise is maintained over several years, results may show significant clinical differences between the two groups. Although the pre-training blood glucose values were not above normal, this may be an indication that FH+ has slightly diminished glucoregulatory capacity than FH-. However, impaired glucoregulatory function is the result of a breakdown in one or more of the various metabolic processes. Therefore, to better understand why FH+ seems to be metabolically different than FH-, **Chapter 4, experiment 3** examined glucoregulatory function, and fasting markers of lipotoxicity before and after resistance training. Though there are differences between groups with and without a family history of diabetes (Russell et al., unpublished data) [2, 14, 15], this study did not reveal any such differences in glucoregulatory function, or markers of lipotoxicity. Resistance training did not affect FH+ differently than FH-, however there were differences in these markers when groups were re-categorized by weight loss. It was not possible to illustrate specific factors likely to contribute to the development of IR or T2D within the confines of the current study.

Limitations

The biggest limiting factors for the above studies were, 1) limited subject pool, and more importantly, 2) lack of resources for scientific analysis. Finger-prick glucose monitoring, while simple, fast, inexpensive, and reproducible [22], is not the most accurate means of testing blood-glucose. Furthermore, the oral glucose tolerance tests (OGTT) performed in these experiments were conducted for one-hour rather than two. The reason for this is that decreases in blood-

glucose concentrations were noted within 60 minutes after consuming the glucose beverage, and had already dropped below 180 mg/dl, the standard for hyperglycemia. Furthermore, plasma acylcarnitines are a snapshot of lipid and amino acid breakdown, but do not give a complete picture. Therefore, they were examined in the context of circulating triglycerides, NEFA, and insulin as well. However, without muscle biopsies, it is not clear if the levels of metabolites remained unchanged with training because: 1) training did not affect lipid metabolism, 2) either increased or decreased production was matched by decreased/increased removal/metabolism.

Future Research

The above experiments have mainly focused on simple and inexpensive methods of testing. However, it is likely that with more advanced technology, aberrant metabolic pathways leading to IR may be easier to establish. For example, passive stretching yielded increased CHO use as determined by indirect calorimetry, and decreases in blood glucose concentrations. However, the increased CHO use stopped with the cessation of passive stretching. Therefore additional testing using various isocaloric exercise intensities may alter recovery kinetics in T2D so that CHO is used for a longer time period post-exercise, further enhancing glucoregulatory control. Though chapter 2 illustrated impaired MF in FH-, further investigation on substrate switching from rest to exercise and recovery using could aid in our understanding of MF since the current trend of MF testing is under resting conditions in which demand for fat metabolism at rest is very low. In addition, post-exercise MRI may indicate what and how substrates are used after a bout of exercise in different diseased populations as compared to FH+.

Also, differences between plasma and muscle acylcarnitines, in conjunction with carnitine quantification, a fatty acid transporter, may help elucidate possible breakdowns in lipid metabolism, especially taken in the context of lipid availability, i.e., triglycerides, NEFA, and

intramyocellular triglycerides (IMCT). Additionally, further research, such as lipid tracers and MRI studies are needed to determine factors leading to more aberrant metabolic function in order to better understand what factors lead to the development of IR and T2D. Taken together, lipid tracers should be used to label specific dietary fats so they can be traced via MRI as: triglycerides → adipose tissue → NEFA → IMCT → acylcarnitines. Such a study incorporating T2D, healthy active FH+ and matched FH- can illuminate the current understanding of how breakdown in lipid metabolism occurs and will aid in uncovering how increased lipid metabolism contributes to IR.

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APPENDIX I: LITERATURE REVIEW

Influences of Exercise on Oxidative Stress in Resting Metabolism in People with DMII: A literature Review

A Review of Literature for General Examination for the degree of
Doctor of Philosophy in the Department of Kinesiology

Submitted to the Graduate Faculty of the
Louisiana State University and Agricultural and Mechanical College
in partial fulfillment of the requirements of the general examination
for the degree of Doctor of Philosophy

in

The Department of Kinesiology

by

Ryan Russell

B.S. Marquette University, 2001

Introduction:

- Overview of Social and Economic implications from Diabetes

Diabetes is a major cause of morbidity and mortality in the United States [1] resulting in substantial human and economic costs [1], [2]. The U.S. Census indicates that the incidence of diagnosed diabetes in the United States has increased rapidly, and that the major predictive cause of diabetes is obesity [3]. According to the CDC, approximately 20.8 million people were suffering with diabetes in 2005, and of those, 90-95% had non-insulin dependent diabetes mellitus (type II diabetes, or DMII) (http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf). The National Health Interview Survey indicates that 85% of all Americans with diabetes require either oral medication or insulin infusion to maintain optimal health [4].

Heart disease and stroke account for 65% of deaths in people with diabetes. This is approximately four times higher in adults with diabetes. Other secondary health problems associated with diabetes are hypertension, blindness, kidney disease, nervous system damage, amputations, dental disease and other metabolic problems (http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf), leading to increased morbidity and health care cost.

The cost of health care in the United States is an estimated \$1.3 trillion (National Institute of Health Sciences). The cost of treating a person with chronic disease or disability is substantially higher than a healthy person the same age. For instance, in 2004 a 50-year old man recently diagnosed with diabetes has an annual health care cost of \$4174 higher than a 50-year old without diabetes [5]. Each year, the cost of treating a person with diabetes increases by as much as \$140 over the cost of somebody who is aging without diabetes or its complications [5]. That is

a total increased health care cost of up to \$868,192,000,000.00 each year for the 20.8 million diabetic people that over and above their expected treatment cost if they did not have diabetes. That number increases by \$55-\$140 each year, for each of the 20.8 million people. This is an increase of between \$114 million and \$400 million in health care costs each year just with the incidence of diabetes.

Government spending on health care (Medicare/Medicaid) makes up most of the cost of people diagnosed with DMII (National Institute of Health Sciences). Even if only half of all people with DMII are on government insurance, there is still as much as a \$1,456,000,000.00 increase in health care spending each year being by the Federal Government on a condition that is often preventable, and highly treatable with proper diet and exercise [6]. Reducing the prevalence of DMII would not only increase the quality of life for millions of people, but also help the economy by reducing government spending on healthcare. As previously stated, exercise and diet are effective tools in prevention and treatment of DMII, but the exact mechanism through which they work is uncertain.

- Purpose

That being said, the purpose of the following review is to provide an in-depth review of the influence of exercise training on oxidative stress and how it affects insulin sensitivity in resting metabolism in persons with DMII.

- Oxidative stress: a potential mechanism

A current theory about the progression of DMII is increased oxidative stress [7]. Both pro-oxidants (reactive oxygen species (ROS), e.g. hydrogen peroxide (H_2O_2), superoxide (OH^\cdot), and peroxynitrite ($ONOO^\cdot$)) and antioxidants [e.g. superoxide dismutase (SOD), catalase (CAT),

glutathione (GSH), and glutathione peroxidase (GPx)] exist naturally in the body. When the antioxidant system cannot change ROS to H₂O₂ and then to water as quickly as ROS is produced, the shift in pro and antioxidant balance is termed *oxidative stress* [8]. When this shift becomes large enough to cause damage to either protein or fat, it is termed *oxidative damage* [8]. In diabetics and pre-diabetics, there are higher levels of oxidative stress and oxidative damage after an overnight fast, indicating that oxidative stress may be a possible cause of insulin insensitivity leading to DMII [9].

At rest, a healthy person's metabolism uses mostly fat as an energy source. However, fat, glycogen, and creatine phosphate are used at all times in various ratios. In order to maintain a healthy blood glucose level, insulin is needed to transport glucose into the cell for metabolism. With DMII, not only are tissues less sensitive to insulin making glucose transport more difficult, but there is also an increase in both lipid peroxidation and oxidation of proteins, which may lead to increased production of pro-oxidants [9]. Ingestion of a high fat meal increases oxidative stress in diabetics, and leads to further insulin insensitivity (Anderson, A.J., 2009 (ahead of print)). With the link between resting metabolism and oxidative stress and the likelihood that oxidative stress leads to insulin insensitivity, it is important to be able to detect and quantify metabolism and pro and antioxidants in vivo.

- Measurement tools

Oxidative stress measurements can be done on any one of several mediums (muscle, fat, blood, or plasma). Traditionally when metabolism is in question, we see pro and antioxidants measured in muscle since muscle controls the majority of one's metabolism. Muscle biopsies are a standard technique used to measure muscle antioxidants. A typical muscle biopsy sample and handling procedure has been outlined by [9]. A muscle sample is taken by percutaneous needle

biopsy from the muscle being examined, using local anesthetic to numb skin and subcutaneous fascia. Muscle specimens (50–100 mg) are then frozen immediately in liquid N₂ and stored at –70°C. When all samples are taken from all participants, the frozen muscle is powdered under liquid N₂ and aliquots transferred to tubes containing various buffers for the analysis of the different antioxidant markers. Commercially made kits for assays of pro and antioxidants, and markers of oxidative damage are available from Millipore, Cayman Chemical, SABiosciences, and BDBiosciences to name a few.

Adipose tissue also has metabolic properties of its own, and can be used for measuring oxidative stress in adipose tissue. Samples are taken using similar techniques used for muscle biopsies. However the metabolic activity of adipose tissue is very low compared to muscle, and studies in this field are relatively new.

Blood and plasma are also used to determine oxidative stress and damage, especially when cardiovascular disease is a concern. These techniques are much less invasive, and require a simple blood draw and freezing of the blood sample until analysis. Commercial enzyme-linked immunosorbent assay (ELISA) kits are readily available for multiple measures of oxidative stress and oxidative damage in blood, and range in price from \$250 to over \$600. Blood assays will be the focus of this dissertation as they are less invasive and less expensive.

Metabolic rate can be readily measured through two widely used non-invasive techniques, direct and indirect calorimetry. Direct calorimetry requires a special room with extremely sensitive equipment used to determine minute changes in temperature from oxygen being utilized by the person in the room. It is very expensive and not many labs have this capability. The simplest and cheapest way to measure metabolism is through indirect-calorimetry. Indirect

calorimetry measures the gas expelled from a person at rest to determine the change in composition from expelled air and ambient (inspired) air. By examining differences between ambient oxygen, and expelled oxygen, we can calculate oxygen consumption (V_{O_2}), carbon dioxide production (V_{CO_2}), respiratory quotient (RQ) or respiratory exchange ratio (RER), and resting energy expenditure (REE). For example, to calculate REE, we can use:

$$REE = \left[n \frac{\text{kcal}}{\text{L O}_2} \right] [\dot{V}_{O_2}] \text{ where } n \text{ is the estimated amount of kcal used to burn one liter of oxygen.}$$

Actual values of n are listed in the table below:

Caloric Equivalents for Oxygen and Foodstuff Contributions for various Nonprotein Respiratory Exchange Ratios

RER/RQ	Kcal/liter O₂	Fat %	Carb %
1.00	5.047	0	100
0.98	5.022	6	94
0.96	4.997	12	88
0.94	4.973	19	81
0.92	4.948	26	74
0.90	4.928	32	68
0.88	4.900	38	62
0.86	4.875	47	53

0.84	4.850	53	47
0.82	4.825	62	38
0.80	4.801	68	32
0.78	4.776	74	26
0.76	4.752	81	19
0.74	4.727	88	12
0.72	4.702	94	6
0.70	4.686	100	0

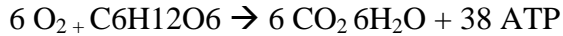
V_{O_2} and V_{CO_2} are measured via indirect calorimetry, and REE is calculated automatically continuously by computer. This provides a relatively inexpensive, minute-by-minute, accurate estimation of REE.

Also, we can use RER to determine the ratio of different substrates used to see how much glucose is being utilized vs. lipid. For example, an RER of 0.7 would indicate that fat is primarily being used where an RER of 1.00 would indicate glucose is the primary substrate as illustrated by the following equation:

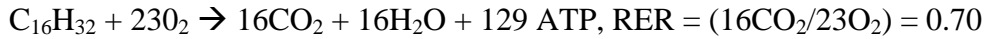
To calculate the RER, we use:

$$RER = (V_{CO_2} / V_{O_2})$$

If CHO is completely oxidized to CO_2 and water, then the relationship is as follows:



If lipid is completely oxidized to CO_2 and water, then the relationship is as follows:



If RER goes above 1.00, it is typically due to increased H^+ production from a shift in the bicarbonate buffer system. Excess H^+ is expired and causes this shift in RER. Before these additional H^+ ions are expired, they can react with oxygen creating SO , or OH^- and shift the ratio of pro and antioxidants to favor oxidative stress.

- Treatments for Diabetes

Diabetes is a chronic disease that requires care in treatment and alterations of lifestyle. Eighty-five percent of Americans with diabetes are either taking oral medication or using insulin (National Institute of Health Sciences). However these are not the preferred methods for diabetes control. The American Diabetes Association recommends a strict diet with specific units of measure combined with regular exercise. Only when these are not effective for completely regulating an individual's diabetes do we see the need for oral medications or insulin.

- Outline for Literature Review

The current review of literature will be organized in the following way. First, there will be an overview of diabetes, glucose transport, insulin resistance, and some etiologies leading to the disease. Second, there will be an overview of the research on oxidative stress in healthy individuals' resting metabolism. It will consist of the role of insulin, the effects of insulin sensitivity on substrate utilization, the effects of both of these on oxidative stress, including

metabolic bi-products and consequences, the role of SOD and catalase as antioxidants, and the effects of oxidative stress on resting metabolism.

After a complete explanation of oxidative stress in resting metabolism in healthy individuals, oxidative stress in resting metabolism in DMII will be discussed. This section will be organized similarly to the previous section, except special focus on issues related to DMII will be the main focus, including reduction in insulin sensitivity, reduced glucose uptake, difficulty with beta-oxidation, and increased lipid peroxidation. An overview of measurement tools for both short and long term glucose monitoring will be discussed. Lastly, consequences of altered oxidative stress and insulin sensitivity in person with DMII will be discussed, including both vascular and metabolic.

The fifth chapter of this review will deal with the influences of physical activity on fasting oxidative stress in DMII. Previous studies covered will include cross-sectional designs in which active and inactive diabetics are tested, and intervention studies in which physically inactive diabetics are tested, exercise trained, and then re-tested. One of the measures of importance is insulin sensitivity that increases with increased physical activity, which would also include improved glucose clearance via enhanced GLUT-4, insulin-insulin receptor binding, and post-receptor signaling. Secondary to that is the improved triglyceride clearance via a reduced chylomicron-triglyceride half-life enhanced lipoprotein lipase activity, and very importantly, increased antioxidant up-regulation and effectiveness. Some of these antioxidants include SOD, catalase, and glutathione and glutathione peroxidase. Other benefits that will be examined will be enhanced short-term glucose regulation, increased oxidative stress/damage protection, and possible substrate utilization shifts. One way of measuring this shift in substrate utilization is via RMR and indirect calorimetry as displayed via RER. With this enhanced short-term glucose

regulation and possible shift in substrate utilization, we also will examine the long-term glucose regulation as measured by HbA1C.

Overview of insulin resistance and metabolic pathway functions in diabetes

- What is type-2 Diabetes?

T2D is the most common metabolic disease in the world, and it is rapidly increasing its numbers [10]. Although all of the likely causes of this disease are unknown, insulin resistance is an early symptom and a leading predictor of the development of T2D. It is characterized by reduced insulin-stimulated glucose uptake and later by decreased glucose-stimulated insulin secretion (GSIS) from pancreatic beta cells [10-12]. In order to understand T2D and insulin resistance more fully, it is first important to first understand how glucose is transported, especially into skeletal muscle.

Glucose Transport

Glucose is used as a major energy source in most cells of the body. As it is a large molecule, transportation of glucose is required for it to get into the cells. There are three class-one glucose transport proteins (GLUTs) in the human body: GLUT1, GLUT 3, and GLUT 4. These are considered high affinity glucose transporters. Of these, a brief description of GLUT 1, 3, and 4 will follow, along with a more in-depth description of GLUT 4 which is insulin-dependent for activation.

GLUT 1 is found in most cells, but the highest concentrations are found in erythrocytes, kidney, colon, and cells of blood-tissue barriers (like blood-brain barriers) [13] and has a low K_m (1-2mM)¹for glucose. GLUT 1 exists as a homotetramer with interaction between two GLUT 1 dimers, and within each dimer, one subunit has an exofacial site exposed while the other has a

¹ K_m is the substrate concentration that gives half maximal velocity of an enzymatic reaction. The higher the K_m the lower the affinity.

cytoplasmic sugar binding site accessible [14]. GLUT 1 is considered primarily responsible for non-insulin dependent basal glucose transport [15].

GLUT3 receptors are found mainly in neurons, placenta, and testes and have a low K_m for glucose. The most significant function of this transporter is glucose transport into neurons [16]. This transporter, like GLUT1, does not require insulin. The specific function in the brain of this transport protein is to provide glucose to the neurons of the brain, as opposed to GLUT1, which transports glucose through the blood brain barrier [17].

GLUT4 receptors are of more interest for this review because they are located in skeletal and cardiac muscle, have a low K_m for glucose, and are the only glucose transporters activated by insulin. Unlike the other transport proteins discussed, GLUT4 proteins are sequestered under basal conditions into specialized storage vesicles within the cell's interior. GLUT4 receptors translocate between the cell membrane where they are able to transport glucose into the cell, and their intracellular storage compartments with increases and decreases in insulin respectively [18]. A more complete discussion of GLUT4 receptor translocation is in order. However, since it is activated by insulin, we must first understand the role of insulin in order to understand GLUT4 translocation.

- Role of Insulin

Insulin is secreted by pancreatic β cells and performs several important functions ranging from glucagon regulation to glucose transport via activation of GLUT4. Activation of insulin receptors on the cell membrane is the first step in insulin-stimulated GLUT4 translocation and insulin-stimulated glucose uptake.

In order for insulin to affect GLUT4 translocation, insulin binds to the transmembrane insulin receptor activating a series of protein cascades and triggers a large increase in the rate of GLUT4

vesicle exocytosis and a small decrease in the rate of internalization by endocytosis. This insulin-mediated increase in exocytosis is the major step for GLUT4 translocation [18]. When the insulin receptor is (1) activated by insulin, it (2) triggers tyrosine phosphorylation of the IRS family of proteins which (3) engage the SH2 domain of the PI3-kinase. The PI3-kinase (PI3K) then (4) produces PIP3 which (5) stimulates kinase activity of PDK and (5) reacts with PKB to (6) stimulate the production of a more efficient (unknown) substrate. The PDK created in the first step of the two step (5) processes, then (6) stimulates kinase activity of PDK which then (7) phosphorylates the lambda and zeta family members of the PCK family of proteins. These two PCK proteins then (8) induce GLUT4 translocation to the cell membrane for (9) transport of glucose into the cytoplasm [18]. A schematic of this that follows was adapted from one by Watson, et al in 2001 [18].

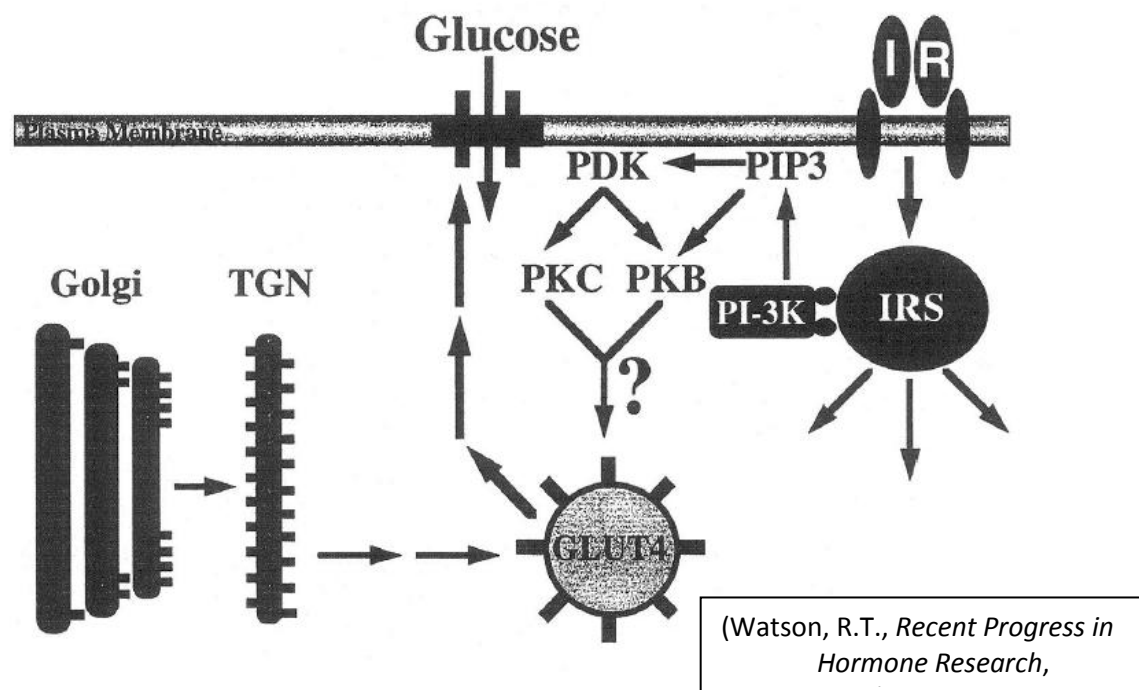


FIG. 1, Schematic model for the role of PI 3-kinase in insulin-stimulated GLUT4 translocation. Following GLUT4 protein synthesis in the endoplasmic reticulum and processing through the Golgi apparatus and the trans-Golgi network (TGN), the GLUT4 protein is localized to both tubulovesicular bodies and small cytosolic vesicles scattered throughout the cytoplasm. Activation of the insulin receptor induces the tyrosine phosphorylation of the IRS family of protein substrates that, in turn, engages the SH2 domains of the p85 PI 3-kinase regulatory subunit. The activation and/or localization of the PI 3-kinase generates the formation of phosphatidylinositol(3,4,5)trisphosphate

(PIP3). PIP3 stimulates the kinase activity of phosphoinositide-dependent protein kinase (PDK) and interacts with protein kinase B (PKB) to make it a more-efficient substrate. In addition, PDK can phosphorylate and activate the atypical protein kinase C (PKC) family members, zeta and lambda.

In diabetes, there is a significant decrease in the ability of insulin to stimulate the translocation of GLUT4 to the cell membrane. This decreased sensitivity to insulin is a major cause in the increases in both fasting and postprandial blood glucose. While there are likely several causes for this decreased sensitivity that we know of, none are completely understood.

Factors affecting insulin sensitivity

Since diabetes is not caused by any one single problem, several different conditions have been linked with the onset of diabetes. While a mutation in the insulin receptor gene has been identified as a likely candidate for the onset of type I diabetes [19], recent evidence suggests that oxidative stress [20] is a more likely pathogenic mechanism that leads to beta cell degradation in T2D. In addition, defects in insulin signal transduction, gene expression, muscle glycogen synthesis, and accumulation of intramyocellular triglycerides (IMCT) are also associated as potential mediators of insulin resistance [11, 21-25].

While there are several mechanisms that lead to the development of T2D, there are also several ways to help counteract the decrease in insulin sensitivity. For example, weight loss (specifically a decrease of abdominal adiposity) and reduced abdominal adiposity [26] have been linked to increased insulin sensitivity. Similarly, aerobic fitness (VO_{2max}) has a strong negative association with insulin sensitivity. However, with a high level of aerobic fitness we also see increases in intramyocellular triglyceride (IMCT) content. Elevated IMCT is also highly associated with insulin *resistance* (IR) if a high VO_{2max} is not also present [27, 28]. The exact mechanisms that seem to aid in insulin sensitivity with increased IMCT are not yet known. However, the changes in insulin sensitivity have been shown to affect metabolic energy sources, and vice versa.

- Energy sources

Insulin resistance and diabetes are associated with a shift in metabolic substrate use, including decreased glycogen utilization, and high lipolytic rates in obese individuals. Using contemporary stable isotope methods, Boon (2007) found that whole body fat oxidation rates were significantly higher in long-standing T2D patients vs. age, weight, and oxidative capacity matched controls [29]. These changes were attributed to the increase in nonesterified fatty acid (NEFA) circulating through the bloodstream. This is contradictory to other studies that indicate similar whole-body fat oxidation between T2D patients and control groups [30-35]. Unlike these studies, Boon reported that despite elevated plasma glucose appearance rates, the total carbohydrate oxidation rates in T2D were reduced as compared with controls [29].

The differences between Boon's study and the previous studies mentioned were the use of stable isotope methods and muscle biopsies as compared with isotope tracer and forearm balance techniques in the previous studies. Also, the long term T2D patients in this study differed from the previous studies in that these individuals no longer exhibit compensatory hyperinsulinemia. With the lack of the body's ability to compensate for being insulin resistant by producing vast amounts of insulin, the mitochondria may be able to adapt to using FFA as an alternate energy source. This could explain the differences between Boon's results in 2007 indicating elevated whole-body fat oxidative and the previous studies that show no changes for fat oxidation in diabetics compared to controls.

The uptake and subsequent metabolic fate of excessive fatty acids in skeletal muscle of persons with advanced T2D may be responsible for fatty acid-induced changes in muscle insulin action [36]. The higher uptake of fatty acids than their subsequent metabolism results in fatty acid intermediates in the cell, for example, ceramide, diacylglyceride (DAG), and long chain

fatty acyl CoA. These intermediates increase insulin resistance through pro-inflammatory/stress pathways [36] and increased serine-kinase activation [37].

However, there is an overall lack of agreement on disturbances in substrate metabolism in people with T2D. For example, basal metabolism of whole body lipids in T2D has been reported to be both elevated [29] and similar to [29, 31, 32, 34, 35, 38] healthy lean controls. Two key factors causing discrepancies here could be different stages of progression of the disease, and differences in body fat content in the different studies' participants.

It is difficult to know whether the resistance to insulin causes a shift in substrate utilization in T2D, or the shift leads to the development of insulin resistance and T2D. However, we do see a genetic link in the shift of fasting substrate utilization with the decrease in insulin sensitivity. Both diabetic and pre-diabetic muscles have reduced oxidative phosphorylation gene expression [39]. Several genes regulating lipid transport and metabolism are severely decreased in T2D and pre-diabetes, including 3-hydroxyacyl CoA dehydrogenase which catalyzes the oxidation of L-3-hydroxyacyl CoA by NAD^+ in beta oxidation, mitochondrial 3,2-transenoyl-CoA isomerase (which transforms 3-cis and 3-trans intermediates to 2-trans-enoyl-CoA compounds in unsaturated fatty-acid beta oxidation), and 2,4-dienoyl CoA reductase 1 (also involved in unsaturated fatty-acid beta oxidation) [39]. In addition, Patti et al. [39] also found that expression of several glycolytic and tricarboxylic acid cycle genes were significantly decreased in these populations, including glucose phosphate isomerase (catalyzes the conversion of glucose-6-phosphate into fructose 6-phosphate), fructose 1,6, biphosphatase 2 (which opposes phosphofructokinase in gluconeogenesis), pyruvate kinase (catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, yielding pyruvate and ATP), pyruvate dehydrogenase A1 (catalyzes conversion of pyruvate to Acetyl CoA), α -ketoglutarate

dehydrogenase (in the citric acid cycle, it catalyzes conversion of α -ketoglutarate to succinyl CoA), and succinate dehydrogenase B (important in both the citric acid cycle, and the electron transport chain). However, Patti et al. [Patti, 2003 #97] had a relatively low number of subjects and used microarray data which sometimes gives false positive tests. These factors may confound the results of the study.

In addition to the findings of Patti et al. [Patti, 2003 #97], other non-genetic studies also illustrate that T2D and pre-diabetes manifest decreased glucose oxidation and storage, and reduced activity of tricarboxylic acid cycle, β -oxidation, and electron transport enzymes [38]. For example, pro-inflammatory cytokines such as TNF α , which act through the serine/threonine phosphorylation of insulin signaling pathways, have also been associated with reduced insulin sensitivity [40]. Causes for increases in pro-inflammatory cytokines are numerous, and beyond the scope of this review. Though metabolic energy sources can be regulated by many mechanisms, the availability of substrate is a major factor in both substrate utilization and mechanisms responsible for the onset of insulin resistance.

- Metabolic substrate availability on insulin resistance

With the increase of NEFA and other circulating long-chain FFA seen with diabetes come the risk of elevated lipotoxicity and insulin resistance. Lipotoxicity refers to the process leading to end-organ damage or dysfunction following excess exposure to fatty acids [41]. The associated insulin resistance that comes with elevated NEFA is likely to be caused by impaired glucose transport at the level of the plasma membrane by deficiencies in insulin activation of IRS-1 associated PI-3-k activity in skeletal muscle [42]. This reduction of insulin activation of IRS-1 is thought to be due to a block in insulin receptor tyrosine phosphorylation of IRS-1 in GLUT4 translocation [37]. In addition to NEFA affecting insulin sensitivity at the level of the

insulin receptor, accumulation of such lipids as NEFA and DAG may also lead to activation of nPKC which, in turn, also reduces insulin-stimulated GLUT4 translocation [41], however the mechanisms of which are not completely understood.

In addition to circulating long-chain fatty acids seen in T2D, intermyocellular triglycerides (IMCT) are also sharply elevated. While this is a beneficial adaptation in endurance athletes, it is detrimental to the function of the mitochondria in T2D. The overabundance of IMCT seen in diabetes has been linked with increased ROS-induced peroxidation, which in turn causes damage to the mitochondria [43], making IMCT use less likely to occur and perpetuating the cycle of increased NEFA and IMCT, reduced insulin resistance, elevated ROS and increased concentration of fatty acyl CoA's, DAG, and ceramides. This may be responsible for the reduced expression of mitochondrial electron transport system genes seen in T2D which causes NADH accumulation in beta cells, thereby halting the tricarboxylic acid cycle on one hand, and on the other hand, facilitating anaerobic glucose metabolism [44]. This may be related to the changes in the Futile cycle (glycerolipid/free fatty acid, or GL/FFA cycle) from decreased expression of GyK in adipocytes. This leads to a shift from the oxidative futile cycle to anaerobic futile cycle (insulin-independent) which creates pre-cursors for gluconeogenesis in the liver, resulting in an increase in blood glucose. It is possible that the increases in IMCT seen in diabetes would NOT occur if ROS did not cause mitochondrial dysfunction, and vice versa.

Not only is there problems with elevated IMCT in T2D, but there is also decreased glycogen loading into skeletal muscle. Shulman et al. [45] found that muscle glycogen synthesis was reduced in diabetics by as much as 50%, and that this is a major contributor for insulin resistance under hyperinsulinemic and hyperglycemic conditions. Using ^{13}C and ^{31}P MRS, Morino et al. [42] argued that the rate of insulin-stimulated muscle glycogen synthesis was due to a reduction

of glucose transport into the muscle, and therefore suggest that decreased glucose conversion to glycogen slows glucose transport, and is responsible for increased insulin resistance.

Contradicting this theory is the work of Cline et al. In their study, rats treated with a glycogen kinase-3 inhibitor (for more activated glycogen synthase) still experienced fat-induced insulin resistance in skeletal muscle [46]. This would indicate that there are likely several confounding mechanisms that lead to the development of insulin resistance. Therefore, multiple approaches in studying the causes of insulin resistance are needed so that better and more individualized treatments can be developed.

- Treatments of insulin resistance

Increasing insulin sensitivity is a key for controlling T2D. Weight loss, medication, diet and exercise have been shown to decrease insulin resistance in T2D. Studies have shown that weight loss, or even a change in dietary composition not only improves insulin sensitivity, but also lowers blood triglycerides and ROS as well [47]. It is not clear which is affected first, the triglycerides or the ROS. Nonetheless, dietary regulation is the first step required to regulate blood glucose and improve insulin sensitivity.

Often when diet and weight loss are not enough to regulate blood glucose, medication is also given. For example, there are available medications (e.g. thiazolidinediones, TZDs) that act as ligands for nuclear receptor peroxisome proliferator-activated receptor (PPAR)- γ , markedly induce adipocyte glycerol kinase (GyK) gene expression and subsequently lowering free fatty acid (FFA) and helping to control insulin sensitivity. Also, Ca^{++} release from muscle contraction during exercise reduces PGC-1 α production, which aids in insulin sensitivity and decreased incidence of diabetes [48]. A combination of medication, diet/weight loss, and

exercise are often prescribed in different combinations to better control insulin resistance and subsequent blood glucose levels.

Separate from diet and weight loss, exercise has also been shown to improve several markers in diabetics, however combining dietary regulation with regular exercise has been shown to be most effective. For example, Roberts et al. [47] shows that an unrestricted diet of low-fat and high fiber in combination with exercise significantly lowers ROS, blood pressure, cholesterol, and vastly improve insulin sensitivity. A more in-depth discussion on the effects of exercise on resting oxidative stress and insulin resistance will be given in a later chapter.

- Measurement Tools

With such long-term problems as peripheral neuropathy, blindness, amputation, and death, there is a need to closely monitor glucose levels in diabetes. Luckily, at-home testing is readily available and easy to use. For example, Roche Accu-Chek, Prodigy Voice and One-Touch make some of the more common glucose monitors that can be purchased for approximately \$15-\$100. Each requires a small amount of blood for analysis. A lancing device is used for finger and forearm pricks. Glucose monitors and lancets use disposable test strips and needles respectively. Typically, results are available in just a few seconds. These devices will indicate current blood glucose levels, and are helpful in that they enable people with T2D to make adjustments (e.g. like eating or using insulin) to better control their blood glucose levels.

A better indication of blood glucose management can be determined by using a *long-term* glucose recall test called hemoglobin a-1c (HbA1c). HbA1c is a test that measures the amount of glycated hemoglobin in the blood. Glycated hemoglobin is a substance in red blood cells formed when blood sugar (glucose) attaches to hemoglobin. In essence, it is a three month glucose recall

test and is very important in determining how well each person is able to control his/her blood glucose over the course of two to three months.

In addition to testing for blood glucose levels, diagnostic tests are important in determining the likelihood that a person will develop T2D, or has insulin resistance. Diagnostic testing for T2D is typically conducted by performing either an insulin sensitivity test, or a glucose tolerance test. Insulin sensitivity testing utilizes simultaneous intravenous insulin and glucose infusion in set doses. The insulin is administered immediately prior to glucose infusion in a small, set dose. Glucose is then dripped intravenously with careful watch over the quantity. The basis of this test is to determine how much glucose has to be administered in order to overcome the previously infused insulin and raise the blood glucose to a pre-determined cut-off level. This directly measures how sensitive each person is to insulin. However, this is a lengthy, expensive, and potentially dangerous test that requires a careful supervision of a physician.

An easier, safer and less expensive diagnostic test is the oral glucose tolerance test (OGTT or GTT). For this test, the patient has their blood glucose measured in a fasted state using a simple glucose meter mentioned above. Then a pre-measured GT beverage (fruit punch) is given and consumed within 2 minutes. Glucose is checked at regular intervals for up to three hours. The cutoff for blood glucose levels to indicate gestational T2D are: 180mg/dL after 1-hour, 155mg/dL after 2-hours, and 140 mg/dL at 3-hours.

Aside from insulin and glucose testing, resting metabolism can easily be measured via direct or indirect calorimetry. Direct calorimetry involves being in a closed-in room where the slightest changes in temperature can be accurately measured. The increase in heat production can be calculated as the amount of oxygen used to burn a certain number of calories. This is a very expensive and time-consuming method. A less expensive and fast alternative is indirect

calorimetry. With indirect calorimetry, the fasting person maintains a lying position with a vented hood over his/her face. The hood is connected to a metabolic gas analysis cart which measures the difference between the [oxygen and CO₂] breathed in and exhaled, calculating amount of O₂ consumed and CO₂ produced. With this measurement, respiratory exchange ratio (RER: the ratio of metabolic substrates burned; fat vs. sugar) and energy expenditure (EE) can be calculated. Energy expenditure (EE), or resting metabolic rate (RMR) can be calculated with this method. Lower RMR indicates burning less energy at rest, and a lower RER indicates that more fat is being burned as compared with glycogen. For example, a RER close to 0.7 indicates almost all fat is being utilized, but the closer to 1.0 RER is, the more CHO is burned.

- Summary

In T2D, we see decreases in insulin-stimulated glucose uptake leading to possible decreases in GSIS. The initial etiology of T2D is not completely understood. This is likely because there is not a single causative factor leading to the development of T2D, but more likely there are several. Of these possible causes, genetics most certainly cannot be ruled out. However, other possible causes are likely such as poor diet and exercise habits. These factors often lead to obesity, which has been linked with impaired whole-body beta-oxidation, increased NEFA, and insulin resistance. Lipotoxicity, inflammation, and oxidative stress have all been suggested as mechanisms that are part of a deleterious cycle of mitochondrial dysfunction leading to increased oxidative stress, which in turn has been linked with increased lipotoxicity and inflammation- both of which can lead to increased ROS production and insulin resistance. In fact, since there are several likely causes of T2D, it may be beneficial to think of T2D not as a disease so much as a common syndrome consisting of similar symptoms stemming from multiple causes. With this

concept, it is easier to understand the need for different approaches in discovering the cause and solution to the world's fastest growing epidemic.

Overview of Oxidative stress in Resting Metabolism in Healthy Individuals

- Pro- and Antioxidant Overview

Pro-oxidants and antioxidants are formed as a normal product of aerobic metabolism. However, when production rates of pro-oxidants are elevated, they may exceed the ability of body's antioxidant defense system to neutralize the oxidants as they are formed. When this happens, the shift towards pro-oxidants may result in cellular damage. This damage is called oxidative stress [49].

Of the pro-oxidants, some of the most abundant are reactive oxygen species (ROS) and reactive nitrogen species (RNS). However, the focus of this review is on ROS. The major ROS oxidants are ultimately reduced to water through a series of intermediate steps, each of which forms an oxidant. The oxidant chain is as follows: superoxide \rightarrow hydrogen peroxide \rightarrow hydroxyl radical \rightarrow water. If the ROS is not neutralized to form water, oxidative damage occurs.

The superoxide anion (O_2^-) is the primary ROS molecule, and is formed through metabolic processes using oxygen. This superoxide production mostly occurs inside the mitochondria when the mitochondrial electron transport chain leaks small numbers of electrons to oxygen prematurely [50, 51]. It is highly reactive with an extremely short half-life of a fraction of a second and can react with different organelles inside the cell causing oxidative damage, or with other molecules and metals to form secondary ROS molecules [52]. However, the specific half-life of any ROS molecule is dependent upon the concentration of the opposing antioxidants present. The reduction of superoxide by biological antioxidant superoxide dismutase (SOD) forms a less reactive oxidant, hydrogen peroxide (H_2O_2). Since this oxidant is less reactive (it has

a half-life of several seconds) and permeable to the cell membrane, it is therefore able to travel to more distant locations causing oxidative damage away from the site of production. Since H_2O_2 is lipid permeable, it not only can cause intracellular protein and DNA damage, but can also initiate fatty acid peroxidation [53]. However, H_2O_2 can also be reduced further to form yet another radical, the hydroxyl radical ($\cdot\text{OH}$). $\cdot\text{OH}$ is highly reactive with an extremely short half-life of a fraction of a second [54] and is produced when hydrogen peroxide is reduced by intracellular iron ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$) [55].

These oxidants are neutralized by enzymatic antioxidants produced in the body, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [49]. With each step, superoxide is reduced one step closer to water. However, each step until then yields a potentially dangerous oxidant capable of causing cellular damage both at the site of origin, and further downstream. Since ROS is naturally formed from the metabolic processes, certain factors of metabolism affect ROS production, and vice versa.

- Effects of oxidative stress \leftrightarrow resting metabolism

Resting metabolism in healthy individuals can utilize glucose and glycogen more readily through the citric acid cycle because they are sensitive to insulin. During the citric acid cycle, NAD accepts a H^+ ion to form NADH which then donates a H^+ to the electron transport chain where oxygen is the final acceptor, creating H_2O . From start to finish, glycolysis, intermediate step and the Krebs Cycle generate: Glycolysis (2 pyruvate, 2ATP, 2 NADH), intermediate step (x2) (2acetyl CoA, 2 CO_2 , 2 NADH), Krebs Cycle (4 CO_2 , 2 ATP, 6NADH, 2FADH+H). As glucose and glycogen are metabolized, depleted stores are replaced by the breakdown of fat (lipolysis) [56]. The result of this efficient resting metabolism is a small production of ROS via the electron transport chain. However, if the system becomes less sensitive to insulin, as in

disease states like diabetes, metabolism relies more heavily on fat metabolism instead of glucose, resulting in a higher concentration of IMCT, increased ROS production, elevated markers of inflammation, higher blood-glucose levels, and further insulin insensitivity.

The low production of ROS in *healthy* individuals can serve several functions. As a product of normal cellular metabolism, ROS can actually aid the body's defense against infection and in the induction of a mitogenic response [55]. However, with mitochondrial dysfunction, the harmful effects ROS overproduction can lead to oxidative stress as discussed previously. While ROS production is not normally thought to be high enough to induce harmful levels of oxidative stress for most healthy individuals, it does exist in limited quantity at rest and can potentially lead to oxidative stress, especially when metabolic circumstances change to favor ROS production.

Elevated ROS production and pathologic levels of oxidative stress are typically present when proper mitochondrial function is disrupted [57]. The delicate balance between beneficial and deleterious effects of ROS is termed *redox regulation* [55]. Maintaining this redox regulation balance can aid in many needed biological processes, including immune function. However, shifting away from redox regulation towards increased ROS production and oxidative stress can lead to many pathologic conditions, including T2D, cancer, cardiovascular disease and aging [55].

Some of the conditions that increase resting ROS production include cigarette smoking [58], improper micro and macro nutrient intake [49, 59], stress [60] and gender [59]. With smoking or overeating, chronic stress is placed on the metabolic system causing damage. Unlike exercise, this stress is often relentless, never allowing the body to adapt and overcome these stressors. Diet and exercise have also been shown to significantly reduce plasma oxidative stress and

cholesterol [47]. This study [47] also shows that increased exercise and a diet of reduced fat and increased fiber affected not only resting oxidative stress, but also reduce blood pressure and cholesterol, and increase insulin sensitivity. Thus, the contribution of a healthy diet and regular exercise are important for maintaining not only typical health markers such as blood pressure and cholesterol, but also healthy redox regulation.

- Oxidative Damage

With high enough concentrations, ROS can play a key role in mediation of damage to cellular structures, nucleic acids, lipids, and proteins [61]. The hydroxyl radical reacts with all DNA components, causing damage to both the purine and pyrimidine bases and the deoxyribose backbone. Oxidative damage to DNA causes permanent modification of genetic material and represents the first step towards mutagenesis, carcinogenesis, and ageing [55].

ROS can also react with polyunsaturated fatty acid residues of phospholipids leading to lipid peroxidation, with malondialdehyde (MDA) being the final product of this peroxidation [61-64]. MDA is thought to be both mutagenic and carcinogenic [52, 55]. In addition to mutagenic MDA production, lipid peroxidation can also lead to 4-hydroxy-2-nonenal (HNE) which is weakly mutagenic, but is a major toxic product of lipid peroxidation. Furthermore, increases in lipid peroxidation have long been associated with decreased function of glucose-6-phosphatase [65] which may lead to increased hyperlipidemia [13], which as previously mentioned is one of many pre-cursors to oxidative stress and insulin resistance. Increased lipid peroxidation has also been implicated in the promotion and progression of carcinogenesis and the development of atherosclerosis and increased inflammation [66, 67].

Since proteins are a major component of most biological systems, they also make up a highly concentrated target for oxidative damage. Increased protein oxidation has long been associated

with aging and age-related diseases, along with decreased function of many biological enzymes [68]. Of the different sub-units that make up proteins, the cysteine and methionine residues of proteins are most susceptible to oxidation by ROS [69]. Cysteine residue oxidation leads to the formation of disulphides between protein thiol groups (-SH) and low molecular weight thiols, for example, GSH (S-glutathiolation). These are formed when amino acids that form protein are broken apart, and can be measured as a marker of protein peroxidation. Some of these free amino groups that result from ROS oxidation of protein can react with carbohydrate to form advanced glycation end products (AGEs) [70]. Most AGEs are unstable, reactive compounds that can cause further molecular damage as previously discussed. Furthermore, oxidation of a protein induces inactivation of that molecule from its original function and also leads to protein backbone fragmentation.

- Role of SOD, Catalase and GPX

The different ROS molecules react at different rates and can cause various kinds of cellular damage both near and far from the site of production if left undeterred. That is why understanding and regulation of ROS is vitally important. Keeping ROS in a healthy balance requires the constant influence of other biological processes. These processes require the help of the antioxidant system.

The three major antioxidants responsible for keeping ROS in check and regulating oxidative balance are glutathione peroxidase (GPx), SOD, and catalase CAT. GPx and CAT have many functions that protect against oxidative stress. Among them are the ability to scavenge the hydroxyl radical directly, and detoxifying H_2O_2 and lipid peroxides [55]. SOD acts to neutralize ROS (O_2^- , H_2O_2 , OH^-) [71]. It scavenges superoxide and reduces it to H_2O_2 so that GPx and CAT can further reduce them to water [71]. This is a relatively efficient method in most healthy

individual. However, in cases involving smoking, stress, high-intensity exercise and a high-fat meal, OH^\cdot is often produced faster than SOD can work, leading to increased oxidative damage. OH^\cdot is an extremely dangerous oxidant that can react with almost anything in a biological system, especially DNA [72], therefore its reduction to H_2O_2 is vital.

H_2O_2 is a dangerous radical because it has a long half-life (seconds) and can pass through the mitochondrial membrane and inflict intra-cellular damage away from the site of formation [55]. However, H_2O_2 is often effectively broken down by CAT and GPx to form H_2O . In this respect, a long half-life is a hidden benefit in that it can remain stable long enough for CAT and GPx to reduce it further. However, inability to efficiently neutralize oxidants leads to oxidative damage, which in apparently healthy individuals has been linked to endothelial dysfunction [60] and the onset of diabetes [73]. The overall effect of the antioxidant system, SOD, GTx, and CAT are to reduce oxidative stress to form water, and maintain normal biologic levels of ROS and help prevent oxidative stress-induced deleterious conditions such as cancer, cardiovascular dysfunction, and insulin resistance as seen in diabetes.

- Measurement Tools for Oxidative Stress

There are several ways in which to measure oxidative stress. Traditionally we look for markers of damage. For example, Thiobarbituric Acid Reactive Substances (TBARS), DNA adducts, N-nitroso compounds, Malondialdehyde (MDA), and 1-hydroxypyrene are a few. More recently, it has become possible to measure ROS directly in RBCs through a spin trap method. These measurements typically give higher values than damage markers, most likely because not all the ROS measured in the spin trap causes oxidative damage. Oxidative stress occurs on the cellular level, so evidence of oxidative stress and damage can be seen in muscle, adipose tissue, and blood. Performing muscle or fat biopsies is an extremely invasive procedure that requires

expertise; therefore measurements from blood are most often done using commercially available ELISA kits. Since O_2^- can either oxidize or reduce, assays have been designed to either oxidize compounds like epinephrine or tiron, or to reduce tetranitro methane or cytochrome c [71].

At rest, healthy individuals utilize oxidative pathways to breakdown both glucose and fat to generate energy, ending with the electron transport chain. With the effects of insulin, there is ample ability to use glycogen for this process [49], though beta-oxidation is used most at rest.

Resting metabolism can use different substrates for energy, including triglyceride or glucose. Burning triglyceride is thought to be preferred. However, to be able to use triglycerides, certain proteins are required, including carnitine, and Uncoupling protein 3 (UCP3). Carnitine is used as a transport (which transports long chain FFA like 18 C steric acid, the most commonly stored length TG) for fat into the mitochondria (carnitine transferase) to be oxidized to produce energy. UCP3 for example, is another member of the mitochondrial carrier superfamily. Based upon its high homology with UCP1, UCP3 has been suggested to play important roles in regulating energy expenditure, body weight and thermoregulation [60].

Typically, fat is the primary source for energy at rest; however there are reasons that CHO would be used more in resting metabolism. For example, if a person is in a state of positive energy balance (consuming more calories than he/she burns), there is a shift to utilize more CHO and store more fat. Also, in certain disease states breakdown of fatty acid is inhibited, and CHO is the main sources for aerobic metabolism at rest.

- Summary

In summary, healthy individuals typically have normal levels of insulin production, and sensitivity. This helps upload and utilize glucose as part of a normal healthy metabolism. ROS and other pro-oxidants are formed from aerobic metabolism, and serve both positive and negative functions in the body. Antioxidants are vital to the removal of oxidizing factors such as

ROS (O_2^- , OH^- and H_2O_2) and helping maintain a healthy oxidative balance in the body, called *redox regulation*. When pro-oxidants are more abundant than antioxidants can handle, the balance shifts towards the formation of pro-oxidants. We call this shift *oxidative stress*.

Oxidative stress is kept at low levels by a healthy antioxidant system including, but not limited to, SOD, glutathione peroxidase and catalase. Several factors act to increase levels of ROS production in healthy individuals, including smoking, improper diet, obesity and lack of physical activity. When adverse factors come into play, such as oxidative stress, insulin sensitivity decreases leading to chronic health problems, including cardiovascular disease and diabetes.

Overview of type-2 diabetes, including oxidative stress in resting metabolism and its effect on insulin resistance

Since diabetes is a metabolic disorder, great effort has gone into isolating the mechanism(s) responsible for the development of diabetes. Many studies have shown increased oxidative stress in association with insulin resistance (IR) and T2D. In 2003, Brownlee developed a unifying hypothesis that overproduction of hyperglycemia-induced mitochondrial superoxide uncouples protein 2, which eventually leads to IR [74]. With diabetes, we not only see increased oxidative stress with hyperglycemia, but also from significantly elevated levels of NEFAs and other long-chain circulating fatty acids. This elevation of long-chain fatty acids can lead to a reduced proton gradient in the inner mitochondrial space leading to mitochondrial dysfunction and impaired oxidative capacity, matrix swelling and enhanced production of ROS [41]. Since ROS production can be caused by several different pathways, it is important to consider the development of T2D as a result of one of several potential metabolic processes gone astray

including high mitochondrial fatty acid oxidation, endoplasmic reticulum (ER) stress or elevated TNF α . Any of these can also activate inflammatory pathways (JNK-AP1, and I κ K β -NF κ B) leading to increased cellular inflammation, which in turn leads to increased ROS production [75]. This section is an attempt to reconcile the various ways that ROS production at rest leads to IR in T2D.

- Role of oxidative stress \leftrightarrow resting metabolism

Oxidative stress is produced mostly from aerobic metabolism in the electron transport chain. However, when looking for specific ways in which it is produced, there are several. For example, Complex II in the electron transport chain produces ROS in diabetes, but the question is why? Also, high blood-glucose levels have been strongly associated with oxidative stress, but is it the high levels of glucose that leads to ROS production and mitochondrial dysfunction, or the other way around? In addition to high blood-glucose, high circulating FFA is also associated with elevated oxidative stress. Similar questions arise: is it an increase in IMCT that leads to oxidative stress, the increased FFA, or is there a metabolic problem prohibiting the efficient use of FFA that makes these levels appear so high that could be to blame? Associated issues with ROS production may affect metabolism, and therefore also should be considered. For example, NADPH in circulating neutrophils produce ROS leading to potential oxidative stress. These and other metabolism related problems will be discussed in this section.

- Complex II production of ROS

It has been suggested that the main location for the production of ROS leading to oxidative stress is the Complex II proteins within the electron transport chain of the mitochondria in diabetes. Complex II is one of 5 protein complexes used as a shuttle to move H $^+$ from NADH to

molecular oxygen to form water [76, 77]. Investigating this, Yamagishi et al, (2001) used a complex 2 inhibitor (TTFA) along with an uncoupler of oxidative phosphorylation, and saw a significant decrease in SO production. However, blaming Complex II proteins from this research is highly speculative because the series of projects were done *in vitro*, thus lacking the natural endogenous antioxidant capacity. Also, though claims of SO production were made, it is extremely difficult to directly measure SO because its $\frac{1}{2}$ life is 10^{-9} s. Even so, the research does strongly suggest that Complex II is an area in diabetes where there is either higher ROS production, or lower antioxidant capacity.

- Glucose toxicity

ROS formation and IR are also associated with high levels of blood glucose over an extended period of time. This is called glucotoxicity. In some cases, glucose auto-oxidizes, and in others, it reacts with H_2O_2 , iron, or copper to form a dangerous hydroxyl radical [78]. Glucotoxicity-induced ROS production can be detrimental to insulin-producing islet cells in the pancreas because of the inherent lack of endogenous antioxidant production/availability found in pancreatic islet cells. As a result, insulin production is decreased due to diminished beta cell gene expression [79-81]. In addition, glucotoxicity has also been shown to diminish glucokinase gene expression [82]. While these studies measured specific actions specifically due to high glucose, they were *in vitro* and limited to acute exposure to high glucose. As such, the actual findings of many of these studies were on glucose *desensitization* rather than *toxicity*, which would develop after long-term exposure. Robertson et al. [79] exposed cultured HIT-T15 cells and animal models to long-term glucose elevation. This exposure showed a strong relationship with beta cell dysfunction in both animal models and *in vitro*, and was reversed when animals were treated with glucose-lowering factors that contain antioxidants (aminoguanidine, phlorizin, and N-

acetylcysteine- which lower glucose) as well as troglitazone and phlorizin which lower triglycerides and have antioxidant capacity. Thus, based on the findings of Robertson et al. [79] using both *in vitro* and animal models, it is reasonable to conclude that both glucotoxicity and ROS were involved with beta cell dysfunction..

Robertson's idea to conduct research on high chronic levels of glucose leading to ROS production and oxidative stress was not novel. In fact, it was preceded by several studies examining the effects of glucotoxicity on pancreatic islet cells. Due to their inherent low antioxidant capacity, islet cells are very susceptible to oxidative damage, including glucose auto-oxidation [83-86]. The study by Wolff and Dean [86] showed that ROS-generating glucose auto-oxidation leads to islet damage and decreased insulin production. Addition of GPx protected the islet cells from oxidative damage *in vitro* with high glucose addition [87, 88]. Both the Ihara et al. [88] and Tanaka et al. [87] specifically tested the addition of GPx on islet oxidative damage, then inhibited GPx and found that damage increased again. However, the Tanaka et al. [87] study was with cultured cells *in vitro*, which lack the complexity of human defenses against oxidative stress under non-physiologic conditions. Ihara et al. [88] used an animal model which, while under physiologic conditions, is unlike in humans in that the animals were engineered to display a particular trait through ONE mechanism. Thus, it is likely that the results from knock-out mice not be exactly duplicated in a human model. Also, these studies tested ROS (which is extremely difficult to directly measure as the half-life is 10^{-9} s) as a result of glucose toxicity, a subsequent symptom in the development of diabetes. Furthermore, the threshold for glucose levels to actually cause glucose toxicity is not currently known, so extremely high levels of glucose were administered and were likely not of a physiologic level.

More recently, studies have supported the Tanaka et al. (1999) and Ihara et al. (1999) findings that pancreatic beta cells are more susceptible to oxidative damage via glucotoxicity because of low enzymatic antioxidants (SOD, CAT, GPx) [89, 90]. These researchers work also suggest that ROS generated from hyperglycemia activates several deleterious pathways, including advanced glucose end-products (AGE), receptors for AGE (RAGE), PKC, nuclear factor κ B (NF- κ B), and nitrogen-activated protein kinase (MAP). Reduced insulin sensitivity and production was reversed in both *in vivo* and *in vitro* with the addition of antioxidants, illustrating that IR can be blunted by reducing the harmful oxidizing effects of ROS. Beta-cell function was blunted with the addition of H₂O₂ *in vivo*, and was reversed with the addition of vitamins E and C and lipoic acid. However, antioxidant interventions *in vivo* had a low sample size and were administered for a short duration. In addition, the *in vitro* methods used extremely high levels of antioxidants not found in that concentration naturally in the body. Also, these antioxidants were not target specific, or more accurately, not made near the site of ROS production, so *in vivo* they could react with numerous molecules throughout the body; thus the results *in vivo* are not in full agreement with these data.

Since pancreatic mRNA damage from glucotoxicity has been suggested as a likely cause for declining insulin secretion later in diabetes, it is important to find ways to prevent or reverse this process. There have been studies to find mechanisms of protection that may help prevent this from happening. In one study [91], researchers subjected rat islet cells to oxidative stress and found that JNK, P38, and protein kinase C was activated. This activation occurred before any decrease of insulin gene expression was noted. Over-expression of dominant-negative c-Jun NH₂-terminal kinase (DN-JNK) was shown to protect PDX-1 insulin promoting factor and insulin gene mRNA from oxidative damage [91]. These findings however, do not distinguish

from decreased ROS production or a decrease in apoptosis. Also, since the study design was based on cell transplants and had no quantitative measurement of apoptosis, they were not able to distinguish between cellular protection from ROS over-production and a decrease in apoptosis.

Hyperglycemia may also lead to the upregulation of angiotensin II, a neurohumoral agent that stimulates superoxide generation by the mitochondria [92]. This follows along with Brownlee's unifying hypothesis of diabetes, and also compliments the work of [93, 94]. These researchers found that hyperglycemia leads to reductions of endogenous antioxidant activity via suppressed taurine transporter. Unfortunately, neither Salceda [93] nor Stevens et al. [94] were able to discover the mechanism of how taurine affected antioxidant activity, or its overall effects on redox-regulation.

- Lipotoxicity

The idea that an elevation in circulating FFA can lead to oxidative damage and insulin resistance is a relatively new idea. There have been strikingly few studies that specifically examine the effects of lipotoxicity on oxidative stress in diabetes. However, Carpentier wrote a review article in 2008 about postprandial oxidative stress [41], and in it he briefly discussed the effects of lipotoxicity. In theory, the elevated FFA leads to an increase in oxidative stress and subsequent IR. The following is a review of the few studies focused on this concept.

Elevated FFA has been related to increases in IMCT, and both increased FFA and IMCT have been linked to IR and oxidative stress through changes in metabolic function. For years, evidence has indicated that increased oxidation of IMCT leads to elevated levels of intracellular acetyl CoA and citrate which in turn inhibits pyruvate dehydrogenase and phosphofructokinase [95]. These enzymes are crucial to glucose utilization, and their inhibition could lead to increased

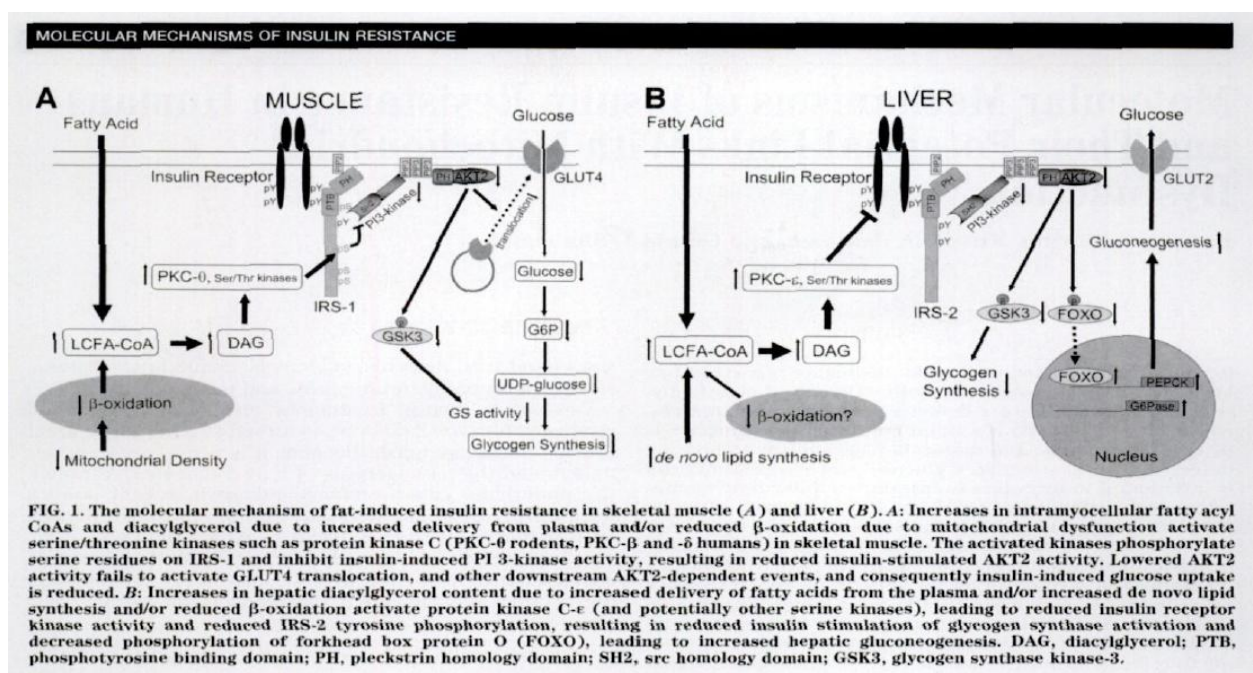
intracellular glucose making insulin-stimulated glucose uptake even less likely to occur.

However, more recently, Rosca et al. [96] described a cascade of events recognized to be one of the most important pathways leading to the development of T2D. They suggested that overproduction of ROS led to oxidative damage, which caused stress-related defects in oxidative phosphorylation machinery and subsequent mitochondrial oxidation led to excess accumulation of intracellular triglyceride in muscle (IMCT) and liver. This build-up of IMCT led to subsequent insulin resistance [96]. Rosca et al. [96-98] showed specific pathways where oxidized proteins (MGO) attach to mitochondria to form AGE, which inhibited renal mitochondrial function. However, this study ignored damage leading to MGO elevation and placed focus on a downstream mechanism instead. It would have been beneficial to probe the factors leading to the MGO elevation associated with increases in IMCT. Also, the greatest source for metabolic activity in the body is skeletal muscle, and the Rosca et al. [96] study focused on renal protein and mitochondria in rats.

Aside from Rosca et al., [96], there have been studies that implied elevated FFA was one likely cause in the development of IR, using advanced magnetic resonance spectroscopy (MRS) technology [42]. MRS studies have shown glycogen synthesis and not hexokinase (previously thought to be responsible for IR) was responsible for IR under hyperinsulinemic conditions. With a decline in glycogen synthesis in diabetes, there was less glycogen available for glycolysis to create energy in resting metabolism. Further support for decreased glycogen synthesis leading to IR was that mice with over-expression of hexokinase were not protected from IR when a glycogen synthase kinase-3 inhibitor was given [99]. Morino et al. [42] described the deleterious process of how the serine kinase cascade affected insulin sensitivity (figure 1) as three processes: 1) lower insulin-stimulated IRS-2 tyrosine phosphorylation, 2) lower IRS-2-associated PI-3-k

activity, and 3) lower AKT2 activity of the GLUT4 translocation process [42, 99]. This cascade initiated a series of events deleterious to insulin sensitivity including a reduction of insulin-stimulation of glycogen synthase activity resulting in lower hepatic glucose uptake. Also, the reduced activity of AKT2 resulted in decreased phosphorylation of forkhead box protein O (FOXO) making it permeable to the nucleus so it could activate transcription of gluconeogenesis' rate controlling enzymes (phosphoenolpyruvate carboxykinase, glucose-6-phosphate phosphatase). As a consequence, the increased gluconeogenesis further exacerbated hepatic IR and thereby caused an increase in fasting hyperglycemia [99-101]. Having the ability to accurately measure such things in real-time with MRS can lead to exceptional work. However these tests require the participant to lie perfectly still in uncomfortable positions for extended periods of time, and any movement can blur the image being saved. Also, human analysis of MRS can lead to subjective interpretation of data.

Figure 1



Not only has elevation of FFA in both rat and human diabetics abolished insulin activation of IRS-1-associated PI 3-kinase activity required for GLUT4 translocation, but also in DOS. Recent indications were that IMCT metabolites, not just FFA, increased IR via a serine kinase which phosphorylates serine on IRS-1, and this seems to have had a negative effect on insulin sensitivity [42, 57]. If there was a weakness to be found in the Morino et al. [42, 57] theory and findings, it was that rodent models were used (fat-fed and knock-out) which demonstrated very limited ways that IR progresses. Also, serine-kinase seemed to be a secondary mechanism, possibly second to FA-CoA or DAG (which activates PKC isoforms). Finally, the question of whether or not it is increased IMCT that leads to IR, or decreased use of IMCT still remains. Also, the accumulation byproducts from the altered metabolism is possibly to blame. Additionally, very little has mentioned of the relationships between lipotoxicity and oxidative stress, which are also likely culprits in this scenario.

Previous to Morino's work, Segall, et al. [102] also looked into the idea that elevated FFA led to IR. This study suggested that prolonged β -cell exposure to FFA caused decreased mitochondrial membrane potential and increased uncoupling proteins, which led to opening of K^+ sensitive ATP channels and impairment of glucose stimulated insulin secretion [102]. Although this *in vitro* study clearly indicated that β -cell mitochondrial membrane potential was disrupted by fat, *in vitro* studies lack do not account for complex intra-and intercellular responses that occur *in vivo*, and also only look at one aspect at a time. It is much more likely that not one single aspect leads to IR and diabetes, and as such, future studies should focus on a balance of beneficial and deleterious factors affecting metabolism, including pro and antioxidants, gluco- and lipotoxicity, and exercise.

- ROS from NADPH

NADPH oxidase is a known source for ROS production in vasculature and kidney in diabetes. NADPH is found in neutrophils that produce ROS to aid in nonspecific host defense. They are, however, mostly dormant until stimulated by cytokines, high glucose, or hyperlipidemia [103, 104]. The Li and associates (2003) findings indicated that upregulation of neutrophilic NADPH ROS production may have led to oxidative stress via increased angiotensin II labeling. This stress was blunted by type 1 receptor antagonists and PKC (used in GLUT4 translocation) inhibitors, implicating this family of kinases in the regulation of hyperglycemia-induced NADPH oxidase activity. However, much of the Li and associates (2003) conclusions were extrapolations based on findings from other studies, and this production of ROS was not necessarily diabetes specific. This production has also been found in healthy individuals, and is likely to aid in more than just non-specific immune functions. Further research is needed to determine if the H_2O_2 production in neutrophils might also play a role in stimulating the body's endogenous antioxidant response to an imminent increase in ROS production.

- Additional ROS Sources

Elevated ROS not only affects kidney and mitochondria as discussed above, but also several other structures including the endoplasmic reticulum (ER). In order for the ER to protect itself, protein folding in the ER occurs and is termed UPR. UPR has been associated with oxidative stress, excess energy-substrate utilization and Ca^{++} loading to name a few [105, 106]. This folding process (UPR) is a protective mechanism that occurs in order to alleviate ER stress. However, this process to alleviate ER stress is also associated with increases in ROS and inflammatory pathways [107-111]. Unfortunately, it was not clearly established if ER stress

leads to ROS production, or vice versa. Herein potentially lies yet another cycle of ROS generation likely caused first by increases in NEFA and further exacerbated by ER stress and UPR for ER protection.

- ROS and IR

As seen from the previous studies, ROS, oxidative stress, and metabolism are strongly related to IR. However, since there is such strong evidence on the effects of oxidative stress and IR, further exploration of this relationship is warranted.

Insulin resistance is seen not only in muscle, liver and kidney, but also in adipocytes as a result of increased ROS production. A series of studies illustrated several steps in which ROS affects IR negatively. For example, [75, 112] showed that adipocytes became insulin resistant with exposure to glucocorticoids or tumor necrosis factor alpha (TNF α) *in vitro*. Also, ROS interfered with insulin signaling at various levels and were able to inhibit the translocation of GLUT4 in the plasma membrane [113]. Glucocorticoids decreased inflammation and helped regulate glucose metabolism by acting through a nuclear hormone receptor. TNF α stimulated inflammatory agents and signals via a cell-surface cytokine receptor. In the above studies, both TNF α and glucocorticoids were shown to stimulate JNK [a member of the MAPK (mitogen-activated protein kinase) superfamily], which helped mediate ROS. Also, adipocytes were treated with TNF α and dexamethasone in order to induce insulin resistance in mice. Thirty-four up-regulated genes were common to both treatments, and 18% of those were ROS related. Gene set enrichment analysis (GSEA) indicated that ROS-related genes were the highest scoring for both treatments, and the ONLY high-scoring set common to both treatments. DCF (oxidized dye) was more oxidized with treatments, and oxidation occurred before IR. Protein carbonyl elevation was

also noted, which may have helped explain the development of IR via oxidative damage. After treatment, IR was then reduced in two different ways: 1) by washing out the agents used (TNF α and glucocorticoids), and by reduction of ROS with addition of antioxidants NAC and MnTBAP. By seeing a decrease in IR with the specific reduction of ROS, it was not unreasonable to assume that ROS had a causal role in the development of IR. Also, when TZD and MnTBAP were given to leptin deficient ob/ob mice, it resulted in a dose-dependant decrease in oxidative stress and IR. In spite of the strong evidence that this studies presented, the results were limited to *in vitro* experiments and observations made in mice engineered to display IR from a specific pathway, rather than from an unknown number of mechanisms like in humans. Also, IR was estimated using OGTT and not directly measured. This actually did not show IR as much as it did a reduction of glucose tolerance- a downstream outcome from IR.

Traditionally, T2D and IR have largely been associated with obesity and hyperglycemia. However, IR develops before a decline in glucose regulation, so it is important to focus on *early* stages of IR and what may cause this. There have been several studies that looked at early IR as it was related to oxidative stress, and evidence indicated that elevated ROS was a precursor to hyperglycemia, especially in obese subjects [90]. Further research into this phenomenon indicated that elevated free-fatty acid (FFA) was a likely cause [114-118]. These studies showed that FFA inhibited insulin-stimulated glucose transport. Furthermore, FFA and their metabolites interfered with transcription factors for lipid and carbohydrate metabolism by elevating the intramitochondrial acetyl CoA/CoA and NADH/NAD⁺ ratios with subsequent inactivation of pyruvate dehydrogenase. This inactivation then caused citrate concentrations to increase, which led to inhibition of phosphofructokinase and subsequent increases in intracellular G-6-P concentration, which may have inhibited hexokinase activity resulting in increased intracellular

glucose concentrations and reductions in muscle glucose uptake. This was based on a theoretical construct developed from correlational studies with quantification of FFA and oxidized glutathione and MDA, which has since been shown to be an unreliable and dirty measurement of lipid oxidation. It was unclear whether FFA increases oxidative stress, or impairs endogenous antioxidant capacity because specific mechanisms have not been fully recognized. It is entirely possible that inflammation intermediates could be the mechanistic trigger for FFA and ROS development.

- Oxidative Damage

Elevated ROS causes damage, and that damage can have large negative effects, such as DNA mutation, IR, metabolic dysfunction, and diabetes to name a few. However, the loss of function of the damaged cell may not be the only problem associated with oxidative stress. The markers of damage may also have further deleterious effects on human physiology other than simply a *marker* of immediate damage from ROS.

Various consequences of oxidative stress in diabetic subjects also involve accumulation of damage markers from either lipid or protein. Common sites of lipid peroxidation are cellular membranes. As such, damage is typically done with a slightly more stable oxidants, like H_2O_2 , which are more permeable to the cell membrane and can travel from the site of production. Recently, accumulation of 4-hydroxy-nonenal (4-HNE) has become a premier marker of lipid peroxidation. However the role of 4-HNE in diabetes is not yet clear, but their presence are clear indications of oxidative damage [119]. Traverso et al. [119] were able to show that certain isoprostanes (4-HNE, MDA) specifically from liver were the direct result of damage from ROS in diabetic rats as compared to control. Although the *in vivo* methods clearly indicated that

isoprostanes were elevated with increased ROS, it was unclear whether the isoprostanes increase was due to increased in production, or decreased removal. More importantly it could be determined if an increase in ROS production led to this isoprostane increase, or a reduction in antioxidant quantity or activity. Furthermore, in the 8-weeks that the rats were engineered, the experimental rats were not able to gain as much weight as the control rats. Body weight differences may have interfered with metabolic activity and confound the results of this experiment.

Other markers of oxidative stress which can help determine protein peroxidation are advanced oxidation protein products, sulphhydryl groups, or uncoupling proteins and protein carbonyls. Since these proteins are damaged inside the cells (DNA, organelles, etc) damage is either occurring from fast-reacting SO and hydroxyl, or from more stable oxidants that can pass through the cell membrane, like H₂O₂. These types of markers have been measured easily in both plasma and urine, but greater sensitivity has been seen in plasma [120]. Negative effects of uncouple protein-2 (UCP2) activity, protein carbonyls, and other markers of protein oxidation have been shown to increase SO formation in T2D [51]. These markers were negative regulators of insulin secretion in isolated mitochondria and intact cells, and were up-regulated in ob/ob and diabetic mice. Unfortunately these studies that indicated the specific functions of such damaged proteins were done in knockout mice, which lack the complexity of the natural state of T2D. Furthermore, when further studies were done *in vivo*, unnaturally high SO levels were used to achieve this protein damage. Hence, it is not completely clear how these damage markers may affect the progression of IR and diabetes, or if it was just that damage occurs that is the sole detriment.

- Antioxidant Activity

There is a relationship between elevated ROS levels and oxidative damage *in vitro*, in animals, and in humans. It has also been shown that increases antioxidant levels helped protect cells from oxidative damage and helped reduce ROS levels. However, much focus has been placed on *increased* ROS levels as the cause of the oxidative damage. It is not beyond reason that a decrease in antioxidant concentration or activity may be at least partially responsible for the ROS elevation and oxidative damage. With so much focus on oxidative damage and ROS production, relatively little is known about the possible changes in endogenous antioxidant function as a possible cause for the increased oxidative damage, rather than increased ROS production. It has been shown that reducing L-arginine via alloxan treatment in rats reduced GSH, SOD, and CAT activity in rats while also increasing MDA (a common marker of lipid peroxidation) levels [121]. Interestingly, L-arginine is a pre-cursor to nitric oxide (NO) which can potentially be a powerful oxidant in addition to a vasodilator. It has been shown that NO was reduced in diabetic subjects, and was related to a decline in gastric emptying and reduced NO availability [122]. Hence, this potential potent oxidant also seemed to serve a purpose in the function and regulation not only as a vasodilator, but of antioxidant activity as well. However, these findings showed the ill-effects of alloxan rather than specifically showing the affects of L-arginine reductions in rats while ignoring the possible side-effects of drug administration. Also, supplemental L-arginine administration affected pro and antioxidant status rather than normal physiologic levels of endogenous L-arginine *in vivo*. Studies should be conducted to better determine optimal physiologic levels of L-arginine rather than supplementing with large doses.

Antioxidant intervention may help aid the endogenous antioxidant defenses against ROS and oxidative stress. However, with so many kinds of antioxidant supplements, it is difficult to know

exactly what effect individual antioxidants may have, and in what concentration they may be beneficial. Antioxidant supplementation has been shown to aid in overall antioxidant activity in T2D as related to ROS production. More specifically, high doses of grape seed extract for four weeks has been shown to lower inflammation markers and glycaemia, a marker of oxidative stress, in people with T2D [123]. This strongly implied that the deleterious oxidizing effects of ROS were lowered with antioxidants. However it is not safe to assume that oral administration of high-doses would act the same way as endogenous antioxidants. Also, there was no target specified nor was there a direct measure of ROS. Therefore, this study showed a strong association of oral antioxidant intervention and a decline in one marker of oxidation. It is not clear if this was because the grape seed extract acts to neutralize ROS, prevent its formation, or assist in the function of endogenous antioxidants. Also, these results are controversial because they are not uniformly supported by other studies performing antioxidant interventions.

One study that did help support these findings showed that short term antioxidant supplementation helped moderately improve IR [124]. However, as with many other studies looking at antioxidant supplementation, Vincent et al. [124] did not directly measure ROS production, or oxidative damage, nor were there any mechanisms shown that affected IR. In addition, since this was a human study, little control over confounding factors like diet and activity were in place. Even though, considering both this and the Kar et al. [123] study, it is likely that antioxidants can improve insulin sensitivity by reducing oxidative damage. More studies need to be conducted examining the balance of pro and antioxidants, and how shifts in this balance may lead to oxidative stress and IR.

- Summary

Oxidative stress, related defects in oxidative phosphorylation machinery, and mitochondrial beta-oxidation lead to excess accumulation of intracellular triglycerides in muscle and liver, and to subsequent insulin resistance [96]. In addition, beta-oxidation of long-chain FFA provides energy for cardiac and skeletal muscle, however increased FFA is also associated with lipotoxicity and muscular IR and abnormal beta cell function at least ten years prior to the diagnosis of T2D, and are also increased by oxidative stress [55]. Metabolic byproducts IMCT including fatty acyl CoAs, diacylglycerol (DAG), or ceramides are all thought to be detrimental to the sensitivity of insulin [37, 117, 125]. In addition to lipotoxicity, ROS and IR become elevated through increased chronic levels of blood-glucose, leading to glucotoxicity [96].

There is very little evidence indicating how and why elevated ROS is produced in diabetes, or how it acts to increase IR. We know that changes in metabolic function (via hyperglycemia or hyperlipidemia) can adversely affect the body's ability to handle increased ROS production leading to oxidative stress. However it is unclear whether this is due to increased ROS production or decreased level and/or activity of endogenous antioxidants.

In reality, the increases in ROS and oxidative damage are closely related to circulating cytokines and long-chain fatty acid. What we do NOT know is what the initial cause of this deleterious cycle is. One novel idea is an increase in ROS production leads to mitochondrial damage. This damage then triggers the body to protect itself against further oxidative damage by down-regulating mitochondrial DNA expression to reduce metabolism, thereby reducing the production of ROS. However, this theoretical protective measure is not without consequence. The decreased mitochondrial activity (or mitochondrial dysfunction) inhibits the muscle from

utilizing the IMCT causing a back-up of both circulating long-chain fatty acid and glucose in the blood and in the electron transport chain, leading to proton leaking and further oxidative damage [126]. These in turn further inhibit the cell's ability to respond to insulin, leading to T2D.

- Future Research

Given the current literature and its lack of *in vivo* human studies, future studies should include aspects of diabetes like chronic blood-glucose and FFA regulation and how they may be related to ROS and antioxidant concentrations, and how that balance affects oxidative damage markers and IR. For example, higher HbA1c is due to poor glucose regulation, which may lead to glucose toxicity and glucose auto-oxidation causing increased ROS, higher islet apoptotic rates, and reduced insulin secretion. These can easily be estimated using an at-home HbA1c test, measuring pro and antioxidants in plasma as well as damage marker, and then determining if a shift in the pro and antioxidant balance that leads to oxidative stress is related to a decline in OGTT.

Influences of physical activity on oxidative stress in resting diabetes metabolism

- Acute Exercise and Oxidative Stress

Acute exercise increases oxidative stress in diabetics and healthy individuals. Specifically, diabetes induces oxidative stress and impairs myocardial HSP60 expression in rats, thereby increasing oxidative damage [127]. With acute exercise, we see sharp increases in ROS production and subsequent decreases in antioxidants, theoretically to offset the increase in ROS.

- FFA Lipolysis

As previously indicated, studies show that increased insulin sensitivity and improved fatty-acid oxidation are related to lower oxidative stress at rest. Triglyceride clearance is improved

with exercise, leading to lower oxidative stress and improved insulin sensitivity in several ways. First, exercise stimulates increases in endogenous antioxidant enzyme activity. Second, it improves blood glucose clearance via enhanced GLUT 4 translocation and protein content, as well as enhanced insulin-insulin receptor binding and post-receptor signaling. Third, exercise also improves blood triglyceride clearance via a reduction in chylomicron-triglyceride half-life and enhanced lipoprotein lipase activity [128], [129].

What remains unclear however is if it is the oxidative stress that leads to decreased fatty acid oxidation, or vice versa. Indications are that some of these discrepancies could be due to the different stages of the disease. For example, some preliminary studies indicated that early stage T2D patients displayed impaired lipolysis and beta oxidation over controls [30, 32, 130, 131]. In what seems like contradictory findings, a more recent study examining long-term diagnosed T2D patients showed that fasting whole-body fat oxidation rates are elevated in long-term diagnosed T2D patients as compared to recently diagnosed T2D patients from his previous studies [29]. In this study, increased fat oxidation is attributed to greater plasma FEFA appearance rates, elevated plasma FEFA availability, and increased plasma NEFA disappearance and oxidation rates. Since insulin inhibits adipose tissue lipolysis, long-term diabetics that lack hyperinsulinaemia do not display this inhibition, and thus have increased fat oxidation. Furthermore, hyperglycaemia in long-standing T2D patients were associated with increased plasma glucose appearance and disappearance rates, but were not accompanied by elevated carbohydrate oxidation rates. The moderate intensity exercise program showed improved plasma glucose disposal [29]. This study however, only showed the effects of acute, low-intensity aerobic exercise on sedentary diabetics. Another potential problem with this study was the passive recovery, which allowed metabolic byproducts to stagnate in the muscle because of

decreased muscle-pump action forcing blood to wash them away. Furthermore, researchers attempted to equalize exercise work-loads, *relative* workloads were 63% and 54% for T2D and control groups respectively. It is possible that the changes in this study may have been due to a higher relative work-load in the T2D group.

- Antioxidant Activity

In addition to improved FFA lipolysis, it has been well established that as little as a single bout of aerobic exercise can improve insulin sensitivity. However, a direct link between exercise, oxidative stress, and improved insulin sensitivity has not yet been clearly established. One possible explanation is that though exercise has been shown to increase ROS production, a possible simultaneous stimulation of endogenous antioxidants may help combat the ROS production and have a compensatory effect on IR above the aforementioned increases in lipolysis [132, 133].

The development of oxidative stress comes about from either, 1) an increase in the production of ROS or NOS, or 2) decreased availability/effectiveness of endogenous antioxidants to combat the oxidative damage. The latter has gained increasing attention in recent years. Studies showed that a single bout of aerobic exercise can increase SOD activity in T2D and not in control groups [134]. GPx was also significantly increased in T2D groups, but not as much as Mn-SOD, but was increased more in T2D patients with diabetic complications following a bout of exercise. Unfortunately, the authors of this study failed to indicate how long after exercise the blood draws were taken. It is entirely possible that if too much time had elapsed, a more effective circulatory system in healthy controls subjects may have been able to wash away SOD and GPx markers and filter them through the liver faster than the T2D group. In

addition, this was a single bout of aerobic exercise, which we know to also increase ROS measures as well. However the authors did not examine ROS markers, and thus cannot indicate with any authority if an increase in ROS stimulated antioxidant production, or if it was another mechanism of mechanical contraction.

- Chronic Exercise and Oxidative Stress

More importantly than how the body reacts to a single bout of exercise is how well it adapts to the introduction of a continued exercise training protocol to help counter the effects of oxidative stress and IR in T2D. In exercise intervention studies, we see improvements in diabetes control and lower oxidative stress. One such study showed that exercise had beneficial effects on glycaemic control, insulin resistance, cardiovascular risk, oxidative stress-defense parameters in overweight and obese type 2 diabetics [135]. More specifically, after three and six months of aerobic exercise training, plasma MDA significantly decreased and CAT significantly increased in T2D males. In his study, Lazarevic followed a group of sedentary diabetic males in an aerobic exercise protocol for six months. Since aerobic exercise has been shown to reduce IR acutely, without a follow-up test visit several days after ending training, it was not possible to determine if aerobic exercise training had an effect on antioxidants and oxidative stress, or if it was just the short-lived effects of an acute bout of aerobic exercise. In his report, there were also no explanations as to how these adaptations came about. Further explanation is needed on what happens to elicit such changes.

- FFA Lipolysis

Recently, we have seen exercise described as not only a model for oxidative stress, but also as an important tool in diabetes management [136]. Exercise training aids in redistributing body

fat and lipid balance, but mechanisms are yet to be defined as to how this affects IR and oxidative stress. In a recent review, Muoio suggested that one possibility was that exercise training leads to a shift from white to red (oxidative) muscle fibers, leading to possible increases in fat oxidation [126].

There is a well-established link between oxidative stress and the ability to efficiently metabolize fats. However, since the data on the effects of exercise training on fasting oxidative stress is lacking, some attention should be paid to its effects on fat metabolism. As with acute exercise, conflicting findings on the effects of exercise training on FFA lipolysis. For example, there have been studies that indicate aerobic training increases lipolytic rate and insulin sensitivity [137], though some have suggested these adaptations are reversed in as little as four days [138]. These indications are not consistent, however. These findings may have been influenced by postprandial blood sampling, and showed no changes in lipolytic rate after an overnight fast without weight loss [36]. One possible explanation for the rapid decline from the benefits of exercise is that in these studies, they were strictly aerobic in nature, which does not lead itself to muscle mass gains. These possible changes in fatty acid metabolism, though short-lived, may have contributed to lower oxidative stress at rest within a few days of exercise.

Supporting the idea that exercise training enhances lipid metabolism was a recent study by Bordenave. In this study, people with T2D were exercise trained twice a week for ten weeks on cycle ergometers at a level that was optimal for lipid oxidation ($\text{LIPOX}_{\text{max}}$) twice a week on a cycle ergometer. What was found was increased lipid oxidation during exercise after training and improved mitochondrial respiration as measured by pyruvate and citrate synthase activity in muscle biopsies [139]. Circulating NEFA was not seen to decline, indicating that the increased fat oxidation was primarily from IMCT. However, there were no significant changes seen in

blood-glucose, glycated hemoglobin, or glucose oxidation. Given that the intervention was only ten weeks, a significant reduction in HbA1c would not be expected, as it measures the preceding 12 weeks of glycation. A longer training intervention, followed by a six month follow-up may have shown significant decreases in HbA1c levels. Also, the training stimulus may not have been adequate in that there was a lack of progression throughout the ten weeks of aerobic training.

- Antioxidant Activity and Exercise

It is clear that exercise has impacts on oxidative stress and insulin resistance, but very little has been offered to explain how this takes place. With diabetes, we see elevated oxidative stress and damage, mostly attributed to increased ROS production. However, exercise also increases ROS, yet also seems to have a lowering effect on oxidative damage. One possible explanation is that exercise helps boost the body's endogenous antioxidant defense system. Recent evidence suggested that aerobic exercise training increased antioxidant capacity in diabetic rats [140]. This increased antioxidant capacity (specifically thioredoxin-1, or TRX-1 in the brain) led to an enhanced ability to offset ROS and NOS and possible reduction in oxidative stress. However, with animal studies, the effects may not translate to human models. Another aspect not examined was how training enhanced antioxidant status. One possible explanation is that endurance training may have helped up-regulate antioxidant mRNA and decrease lipid peroxidation. Further illustrating how exercise helps the body protect itself via enhanced antioxidant capacity was a study by Gul and Laaksonen. In his study, Gul showed that after eight weeks of aerobic treadmill exercise with 10% incline, rats exhibited increased aerobic endurance, upregulated GPx and decreased TBARS in both muscle and kidney [141]. In addition, there was better control over hyperglycemia in T2D rats after training. It is unclear if the reduction in hyperglycemia was a direct result of decreased oxidative damage (TBARS) or increased GPx

regulation, or even if the up-regulation of GPx was responsible for the decrease in oxidative damage. However, it is likely that a better-trained antioxidant system was better able to handle ROS production, and thereby reduce oxidative damage and aid in glucose regulation.

Not only does exercises seem to boost endogenous antioxidants, but aerobic exercise training can have a reducing effect on the accumulation of oxidative stress markers [142]. In his study, Ristow showed that in as little as four weeks of aerobic and circuit training, significant reductions in ROS measures and TBARS were seen as well as increases in antioxidants and glucose infusion rates via insulin clamp. His implication was that the increases in ROS production from exercise had a beneficial effect on insulin sensitivity and glucose clearance. However, with the increased antioxidant levels at rest from this study, it is more likely that exercise produced ROS production challenged the antioxidant system to improve, causing a shift in the endogenous antioxidant defense status at rest [132, 133], which may have been partially responsible for the reduction TBARS and IR. For example, regular exercise has long been shown to strengthen antioxidant defenses in non-diabetic animal models and may have been responsible for decreased resting and acute exercise-induced oxidative stress as a result [132, 133, 143-146]. In addition to his unorthodox conclusions, there was also no mention of plasma volume increases due to exercise training. Some of these adaptations seen may have been affected by the increased plasma volume often associated with chronic exercise training or from decreased plasma volume from an acute bout of exercise. Also, TBARS may not have been the ideal marker of damage, especially with exercise training which utilizes protein (muscle) so extensively.

Several studies have been conducted on the effects of exercise on oxidative stress, however most of them have examined the effects of acute exercise rather than chronic. Even with some of

the exercise training studies performed, it has been difficult to separate out the physiologic changes may be from acute vs. chronic exercise. However, Chang et al. [147] has shown the effects of both acute and chronic aerobic exercise on oxidative stress markers. In this eight week aerobic treadmill study, these researchers showed significantly improved antioxidant defense function of Mn-SOD, GPx, and GSH in obese Zucker rats [147]. In this study, to account for acute vs. chronic exercise effects, rats were killed 48 hours after last exercise session thus reducing the acute effects. No change in fasting glucose was seen between trained and untrained rats, however Mn-SOD activity was significantly increased in trained vs. un-trained rats. Also, impaired GPx and GSH in pre-trained obese rats significantly improved with training. Chang also noted increased mRNA levels of both Mn-SOD, and GPx. It is quite interesting to see that the up-regulated antioxidant system remained that way 48 hours post exercise, however with no markers of damage taken or measures of IR, it is unclear if these antioxidant changes had any diminishing effects on oxidative damage or IR.

The balance between pro and antioxidants is important to maintain proper cellular health. We have seen that exercise (both acute and chronic) has positive effects on endogenous antioxidant status [142, 147], however little is known about the effects of antioxidant supplementation on oxidative stress with exercise in T2D. In the exercise training study by Ristow mentioned earlier, he also examined the effects of supplementation with oral antioxidants (vitamin C and E) after exercise training. Though it would seem that more is better, the positive changes seen from his exercise intervention (decreased TBARS and ROS, and increased glucose infusion rates) were nullified with oral antioxidant supplementation. The explanation he offered was that the ROS produced from exercise had beneficial effects on glucose tolerance. However, it is likely that oral supplementation of antioxidants (as with amino acids) may have inhibited/limited the body's

ability to produce endogenous antioxidants on its own. These oral antioxidants may actually blunt the beneficial effects of SOD, CAT, or GPx. However, since his study did not measure levels of these endogenous antioxidants, any speculation on this cannot be verified.

- Summary

Acute and chronic exercise has been shown to decrease oxidative stress by increasing endogenous antioxidant production and activity leading to an overall decrease in oxidative damage. These adaptations may precede the reduced IR that also accompanies exercise. The decreased IR may be due to lower blood-glucose from insulin-independent glucose transport, or from possible enhanced, or complete, metabolism of lipid, which in turn can also decrease fasting oxidative stress. There seems to be no starting point in the deleterious chain that leads to IR and diabetes, however aerobic exercise seems to intervene on several levels to help better regulate glucose and fat metabolism, and lower IR and oxidative stress.

- Future Considerations

Little is known of the effects of exercise interventions on fasting oxidative stress as it relates to lipid and glucose metabolism and insulin sensitivity. Even less is known of the effects of resistance training on oxidative stress or glucose tolerance and IR. Future research is needed to determine if continuous resistance training can aid in restoring redox balance in diabetes and pre diabetes. There have been several studies that have shown increases in glucose tolerance and reductions in insulin resistance with aerobic training, however these effects are quickly lost after as little as three days without exercise. One possible explanation is that aerobic training does not increase muscle mass, making any possible changes seen based on long-term recovery kinetics rather than longer-lasting metabolic adaptations. It is possible that the increased muscle mass that

accompanies resistance training may allow such beneficial adaptations to remain longer after cessation of training.

Offspring of diabetics have been targeted as a highly likely group to develop T2D if glucose tolerance is low. It is critical to be able to identify and target specific mechanisms which would reduce development of T2D. With the effects of aerobic training on glucose tolerance being short-lived, perhaps incorporating resistance into an intervention will yield longer-lasting results. Since both glucotoxicity and lipotoxicity lead to diabetes, any exercise interventions should include measures of glycated hemoglobin and circulating NEFA, and determine whether resistance training has an effect on these measures as well as resting glucose and fat oxidation and oxidative stress.

Summary

T2D has been identified principally as a metabolic disease. As such, research has focused in large part on metabolic mechanisms that affect IR and glucose uptake. Among these have been glucose toxicity, lipotoxicity, inflammation and oxidative stress. Oxidative stress is of particular interest in the study of T2D because it seems to be related to almost every mechanism of T2D development.

For example, oxidative stress, along with related defects in oxidative phosphorylation machinery, and mitochondrial beta-oxidation lead to excess accumulation of IMCT in muscle and liver, and to subsequent insulin resistance [96]. However, the scope of much of the existing research has been too limited to get a clear picture of what is really happening to the altered metabolism of diabetics. Elevated IMCT- whose metabolic byproducts, including fatty acyl CoAs, diacylglycerol (DAG), or ceramides are all thought to be detrimental to the sensitivity of

insulin in diabetics has been blamed for IR [37, 117, 125], yet highly-trained aerobic athletes also show increased IMCT, but do NOT exhibit the oxidative stress or insulin resistance.

This leads to the supposition that it is not the retention of IMCT that causes these problems, but perhaps a problem with their use. In addition, beta-oxidation of long-chain FFA provides energy for cardiac and skeletal muscle. However, increased FFA is also associated with lipotoxicity and muscular IR and abnormal beta cell function at least ten years prior to the diagnosis of T2D, and are also increased by oxidative stress [55]. In the early stages of T2D, higher than normal insulin levels have an inhibitory effect on lipolysis, but as the disease progresses, the drop in insulin results in beta oxidation no longer being suppressed [114, 125]. What is evident from existing research is not what mechanisms cause the development of IR, or even what causes oxidative stress to increase so much as it reveals a lack of understanding of the diabetic model in its complexity.

In addition to lipotoxicity, ROS and IR become elevated through increased chronic levels of blood-glucose, leading to glucotoxicity [96]. This increase leads to glucose auto-oxidation, glucotoxicity, damaged pancreatic islet cells, and ultimately the decrease of insulin production. However, it is obvious that since these elevations affect insulin production, they also affect lipolysis, IMCT formation and breakdown, and ROS production not only as a direct result of the glucose elevation, but also from some of the lipid-induced processes discussed above. Clearly, manipulation of one or more aspects of diabetes metabolism has effects on outcome measures such as IR and glucose tolerance. These systems are part of an ongoing cycle that play upon each other to exacerbate the breakdown of T2D metabolism. These kinds of interactions of the different systems affecting diabetes metabolism and IR have not been studied as systems working together and against each other simultaneously, and as a result are not fully understood.

There is very little evidence indicating how and why ROS is elevated in diabetes, or how it acts to increase IR. It has been suggested that changes in metabolic function (via hyperglycemia or hyperlipidemia) can adversely affect the body's ability to handle increased ROS production leading to oxidative stress. However because of a lack of agreement in the literature, it is unclear whether this is due to increased ROS production or decreased level, changes in chronic inflammation, and/or activity of endogenous antioxidants. ROS overproduction is blamed for the production of oxidative stress, however it is not possible to draw these kinds of conclusions unless other aspects are also measured simultaneously, including antioxidant bioavailability/activity and efficient repair of oxidative damage. So far, there have been no studies that have conducted this kind of comprehensive examination of the oxidative model in T2D.

It has been shown that increases in ROS and oxidative damage are closely related not only to gluco- and lipotoxicity, but also increased circulating cytokines. However, since no initial cause has been identified, there is no known starting point for this cycle. One novel idea used by several researchers is that an increase in ROS production leads to mitochondrial damage. This damage then triggers the body to protect itself against further oxidative damage by down-regulating mitochondrial DNA expression to reduce metabolism, thereby reducing the production of ROS. However, this theoretical protective measure is not without consequence. The decreased mitochondrial activity (or mitochondrial dysfunction) inhibits the muscle from utilizing the IMCT completely causing increasing levels of IMCT, and a back-up of both circulating long-chain fatty acid and glucose in the blood and in the electron transport chain, leading to proton leaking and further oxidative damage [126]. These in turn further inhibit the cell's ability to respond to insulin, leading to yet more increases of insulin production, followed

by further decreases in lipolysis and ultimately the progression of T2D. Interestingly, this concept is directly and indirectly connected with other prevailing theories IR development and oxidative stress in that it leads to a cycle whereby increased ROS production leads to oxidative stress, IR, elevated FFA and IMCT, glucotoxicity, and likely to inflammation. However since this is cycle is a prevailing theme in T2D research, there is still not clear starting point to this process, nor is there a good understanding of how these connected factors influence each other.

- Exercise and T2D

Acute and chronic exercise has been shown to decrease oxidative stress by increasing endogenous antioxidant production and activity [148] leading to an overall decrease in oxidative damage. These adaptations may precede the reduced IR that also accompanies exercise. The decreased IR may be due to lower blood-glucose from insulin-independent glucose transport, or from possible enhanced, or complete metabolism of lipid, which in turn can also decrease fasting oxidative stress. There seems to be no starting point in the deleterious chain that leads to IR and diabetes, however both strength and aerobic exercise seem to intervene on several levels to help better regulate glucose and fat metabolism, and lower IR and oxidative stress. It is difficult to say with any authority exactly HOW exercise affects the diabetic model since there is no universal testing model in place. So far, much of what has been done has a small focus and ignores much of the confounding factors that ALSO affect the outcome measures being examined. For example, looking at decreases in oxidative stress after exercise training is interesting, but is that a result of the exercise, of lower triglycerides, of non-insulin stimulated glucose uptake, metabolic increases both during and after exercise (EPOC), etc.? The scales are tipped towards reduced IR and improved glucose uptake, but by ignoring other aspects of the T2D model, questions like these cannot be answered.

- Future Research

Given the current literature and its lack of *in vivo* human studies, future studies should include aspects of diabetes like chronic blood-glucose and FFA regulation and how they may be related to ROS and antioxidant concentrations, and how that balance affects oxidative damage markers and IR. For example, higher HbA1c is due to poor glucose regulation, which may lead to glucose toxicity and glucose auto-oxidation causing increased ROS, higher islet apoptotic rates, and reduced insulin secretion. These can easily be estimated using an at-home HbA1c test, measuring pro- and antioxidants in plasma as well as damage markers, and then determining if a shift in the pro and antioxidant balance that leads to oxidative stress is related to a decline in OGTT. These kinds of studies are lacking in the current literature. Much of the research done is extremely narrow in focus when in fact the problem of IR in T2D is broad and complex.

Little is known of the effects of exercise interventions on fasting oxidative stress as it relates to lipid and glucose metabolism and insulin sensitivity. Even less is known of the effects of resistance training on oxidative stress or glucose tolerance and IR. Future research is needed to determine if continuous resistance training can aid in restoring redox balance in diabetes and pre diabetes. Several studies have shown increases in glucose tolerance and reductions in insulin resistance with aerobic training, however these effects are quickly lost after as little as three days without exercise. One possible explanation is that aerobic training does not increase muscle mass, making any possible changes seen based on long-term recovery kinetics rather than longer-lasting metabolic adaptations. It is possible that the increased muscle mass that accompanies resistance training may allow such beneficial adaptations to remain longer after cessation of training.

Furthermore, offspring of diabetics have been targeted as a highly likely group to develop T2D if glucose tolerance is low. It is critical to be able to identify and target specific mechanisms in which these chances are decreased. With the effects of aerobic training on glucose tolerance being short-lived, perhaps incorporating resistance into an intervention will yield longer-lasting results. Since both glucotoxicity and lipotoxicity lead to diabetes, any exercise interventions should include measures of glycated hemoglobin and circulating NEFA, and determine if resistance training has an effect on these measures as well as resting glucose and fat oxidation and oxidative stress.

All things considered, there is much research still needed to determine how ROS affects the development and progression of T2D, and how different modes of exercise can prevent and even alleviate some of the problems leading to its continued progression. However, to date, most of the research on ROS, lipotoxicity, glucotoxicity, antioxidants, and inflammation on T2D outcomes have been extremely narrow in focus. There is no uniform method for testing the effects of manipulating ANY of these variables in IR or glucose tolerance. Since each of these variables interacts with one another, ignoring these interactions prohibits a true understanding of the complexity of this disease and negates much of the conclusions made by recent narrow-focused studies. Before any real understanding can be reached, a model for testing should be developed that incorporates a balance of positive and negative interactions affecting the development and progression of T2D. For example, this model should include both lipo- and glucotoxicity models, and examine the balance of ROS/NOS production and how endogenous antioxidants are able/unable to defend the body against a shift towards oxidative stress as measured by oxidative damage. With this, we can determine what magnitude of redox regulation shift is required to cause damage. Furthermore, oxidative damage markers alone are insufficient

to elucidate why oxidative damage may be developing in T2D. Therefore, a balance of pro- and antioxidants should be assessed to better understand whether those with T2D display over-abundant ROS production, or a lack of endogenous antioxidants to help protect the system from such damage. Lastly, this balance model of the oxidative damage system (pro- and antioxidants in addition to damage markers) are only an outcome of a physiologic mal-adaptation that seems to develop long before increased oxidative damage occurs, therefore factors such as glucotoxicity, lipotoxicity, and chronic inflammation should be examined those displaying the earliest signs of metabolic dysfunction, such as FH+.

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APPENDIX II: EXERCISE QUESTIONNAIRE

Name: _____ Date: _____

- 1) In the last 3 months, how many times each week have you:

Lifted weights: _____ Done cardio/aerobic workouts: _____ been highly
active: _____

- 2) In a given week, how many hours do you spend exercising? _____

- 3) Of those exercises, how much time do you spent performing resistance?
_____ What type? _____

- 4) How much time do you spend performing aerobic/cardiovascular training?

- 5) _____ What type? _____

- 6) Do you take medications on a regular basis? Y / N If so, what are they?
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- 7) Do you have diabetes or pre-diabetes?

- 8) Does anyone in your family have diabetes or pre-diabetes?

VITA

Ryan Russell has been independently studying diabetes metabolism for 2 years under the guidance of Dr. Arnold Nelson for a doctorate in philosophy in kinesiology. Recent projects and publications include: *Metabolic Inflexibility: The link Connecting Diabetics with First and Second Degree Offspring of Diabetes?*, *Reduced Lipotoxicity Markers in Diabetic Offspring and Controls with Resistance Training*, *Effects of Acute Cyclooxygenase Inactivation on Glucose Tolerance in Diabetic Offspring*, and *Fasting Blood-glucose Changes in Diabetic Offspring and Controls with Seven Weeks of Resistance Training*. Future work includes stratifying metabolic and cardiovascular risk factors in diabetes offspring. In May of 2010, Ryan was invited by Luc van Loon to spend the Fall semester studying continuous 24-hour glucose monitoring systems (CGMS) in his lab at Maastricht University in the Netherlands where he collaborated on multiple independent projects with students in Dr. van Loon's lab. Ryan has presented at FASEB on Metabolic Inflexibility and has an upcoming presentation at the American College of Sports Medicine National conference in Denver, CO. Following his return from Maastricht, Ryan was invited to study as a visiting scholar in the lab of Matthew Hulver in Virginia Polytechnic Institute and State University, and the Boshell Diabetes research group at Auburn University. Ryan was recently invited to give a seminar to the University of Maryland School of Medicine, and accepted an offer for an NIH-funded T-32 postdoctoral fellowship as a result. Ryan is expected to finish his doctorate, graduate with honors, and start on his new career in the Summer of 2011.