EFFECT OF RESISTANCE TRAINING ON CYTOKINES IN HIV+ MEN WITH CHEMICAL DEPENDENCE

John Harper Curtis

Thesis Prepared for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF NORTH TEXAS

May 2012

APPROVED:

Jakob L. Vingren, Major Professor
David W. Hill, Committee Member
Alan Jackson, Committee Member and Chair of the Department of Kinesiology, Health Promotion, and Recreation
James D. Meernik, Acting Dean of the Toulouse Graduate School

Human immunodeficiency virus (HIV) and substance abuse (drug and/or alcohol) independently impair the immune system; importantly, the combination of HIV infection and substance abuse might produce more than an additive effect on this system. Tumor necrosis factor-alpha (TNF-α) and Interferon gamma (IFNγ) are pro-inflammatory cytokines involved in differentiation of T_h0 cells into T_h1 cells. Interleukin 4 (IL-4) and Interleukin 10 (IL-10) are anti-inflammatory cytokine involved in differentiation of T_h0 cells to T_h2 cells. Unbalanced T_h1 and T_h2 cells can lead to immune suppression. Thus, changes in these cytokines could have important implications for people infected with HIV (HIV+). Resistance training can counteract muscle wasting, improve strength, and improve muscle mass.

The purpose of this study was to examine the effect of resistance training on resting concentrations of circulating TNF-α, IFN-γ, IL-4, and IL-10. Sixteen men (42 ± 11 years, 180.4 ± 9.1 cm, 89.2 ± 20.7 kg) infected with HIV and enrolled in an intensive 60-day in-patient substance addiction/abuse treatment program were recruited shortly after admission to the treatment facility. Participants were assigned to one of two groups using randomization: supervised resistance exercise 3 times per week using a progressive and non-linear periodized program (Exercise) or no exercise training (Non-Exercise) for six weeks. Before (Pre) and after (Post) the 6-week period, resting and fasted blood samples were obtained and analyzed for serum TNF-α, IFN-γ, IL-4, and IL-10 concentrations using a high-sensitivity ELISA.

TNF-α did not change following the 6-week period for Exercise (Pre: 4.8 ± 2.7 pg·ml⁻¹; Post: 4.6 ± 2.4 pg·ml⁻¹) or Non-Exercise (Pre: 3.0 ± 1.3 pg·ml⁻¹; Post: 2.7 ± 0.8 pg·ml⁻¹). IFN-γ,
IL-4, and IL-10 concentrations were below detectable limits. No adverse effects of the intervention were reported. A six-week resistance training program does not elicit changes in circulating TNF-α concentrations in men infected with HIV and undergoing an intensive in-patient substance addiction/abuse treatment program. Concentrations of IFN-γ, IL-4, IL-10 were below detectable levels. Six weeks of resistance training was not sufficient to affect circulating TNF-α, nor enough to increase concentrations of IFN-γ, IL-4, IL-10 to detectable levels. The lack of adverse effects shows that adding resistance training to the current in-patient treatment regimen for substance abuse among men infected with HIV is safe.
Copyright 2012

by

John Harper Curtis
ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Jakob Vingren for his research and initial work in preparation of this study, and in locating a place in which to conduct it. Your help and guidance during this study has been invaluable. You took a person who wanted to do a thesis, but with no idea on how to start, and helped me move to this finished product.

I would like to also thank Homeward Bound, Inc. They allowed a small North Texas team to go down and be able to spend time with them. Over the course of the study, the participants moved from subjects to “family”. I think that even though they might have learned how to lift weights, I feel I learned more about life from them than they learned from me.

I would like to thank my committee members, Drs. Elaine Lee and David Hill, who have reviewed, re-reviewed, and reviewed some more to get this product to its final conclusion. I have always said it is one thing to get published, another to have it stand out. I feel we accomplished our goal.

Last, and certainly not least, I would like to thank my wife, Christi. You may have helped out with the writing, but your most important contribution was to convince me to believe in myself; to convince me to go back to school and chase my dream of a Masters degree. Your belief and confidence never wavered. This is the result of your faith in me.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>5</td>
</tr>
<tr>
<td>RESULTS</td>
<td>8</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>11</td>
</tr>
<tr>
<td>APPENDIX A: REVIEW OF LITERATURE</td>
<td>17</td>
</tr>
<tr>
<td>APPENDIX B IRB CONSENT FORM</td>
<td>24</td>
</tr>
<tr>
<td>APPENDIX C AMENDED IRB CONSENT FORM</td>
<td>32</td>
</tr>
<tr>
<td>APPENDIX D BLOOD TESTING SHEET</td>
<td>37</td>
</tr>
<tr>
<td>APPENDIX E EXERCISE TESTING SHEET</td>
<td>39</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>41</td>
</tr>
</tbody>
</table>
INTRODUCTION

The Centers for Disease Control (CDC) estimate that over one million people in the United States are currently infected with the human immunodeficiency virus (HIV); this estimate represents a 7% increase from 2006 data (CDC, 2010a). HIV-infected patients whose CD4+ T cells counts fall below 200 cells per 1 µl of blood are diagnosed with acquired immunodeficiency syndrome (AIDS). In 2008, of the 490,696 people diagnosed with AIDS in the United States who responded to the CDC survey, 32% reported that they received the AIDS diagnosis within 12 months of their initial diagnosis of HIV infection (CDC, 2010b).

Currently, individuals who are diagnosed with HIV in their 20s are able to live into their 60s, due to the introduction of highly active antiretroviral therapies (Antiretroviral Therapy Cohort Collaboration, 2008) which substantially delay the progression to AIDS (Hirschel et al., 1999; Montaner et al., 1998). Despite the increase in the total number of people living with HIV in the United States, the number of new, HIV diagnoses (~56,000 in 2008) (CDC, 2010a) has stabilized over the past 5 years. The annual number of new AIDS diagnoses has also stabilized (~34,000 in 2008) (2010b).

Despite promising therapies that reduce AIDS development in HIV-infected individuals, external factors, such as substance abuse, decrease this benefit in some patient populations (Hicks et al., 2010). Recently, 64% of HIV/AIDS patients reported to the National Institute on Drug Abuse as having used an illicit drug (NIDA, 2011). Of those, one in four patients reported use of drugs or alcohol at a level that warranted treatment (NIDA, 2011). Such drug or alcohol use exacerbates HIV virulence and disease severity (NIDA, 2005). Substances such as alcohol, marijuana, cocaine, methamphetamines, and heroin are immunosuppressive (Friedman et al., 1995; Nelson & Kolls, 2002), causing decreased activity of natural killer cells and
decreased lymphocyte numbers (Nelson & Kolls, 2002). Furthermore, the use of opioids (e.g., morphine and heroin) and cocaine increases the rate of HIV replication, which can lead to larger viral load (Sobel, 1991; Peterson et al., 1990; Novick et al., 1991).

The pathological mechanism of HIV is to directly infect human immune cells, resulting in a reduced defense against opportunistic infectious diseases; the inability to ward off opportunistic infectious diseases and is the most common course of premature death in people living with HIV (CDC, 2009). Specifically, HIV targets macrophages, dendritic cells, and CD4+ T helper (Th) cells. The Th0 cells are a group of naïve lymphocytes produced in the thymus that develop into Th1 or Th2 or other cell subtypes. Th1 cells primarily function in phagocytic, innate immune responses (Szabo et al., 2003), while Th2 cells function in B-cell (lymphocytes formed in bone marrow) mediated adaptive immune responses. Both Th1 cells, Th2 cells have antigenic memory potential (Szabo et al., 2003). The Th cell differentiation into subtypes (Th1 or Th2) is regulated by separate groups of cytokines, which are the primary signaling molecules for the immune system (Gilman et al., 2001). Modulations in circulating cytokine concentrations, therefore, can affect the immune system and its responsiveness to opportunistic infections.

Differentiation of a naïve Th0 cell into either a Th1 or a Th2 T cell is regulated by cytokines, in particular, Interferon gamma (INF-γ), Tumor necrosis factor alpha (TNF-α), Interleukin-4 (IL-4), and Interleukin-10 (IL-10). All four cytokines serve in the differentiation of a naïve Th0 cell towards either a Th1 cell, or a Th2 cell. IFN-γ, and TNF-α are associated with the differentiation of a naïve Th0 cell towards a Th1 cell, while IL-4, and IL-10 promote differentiation towards a Th2 cell (Bradley et al., 1996). INF-γ inhibits HIV replication in latently infected cells (Sarol et al., 2002) and low production of IFN-γ is correlated with disease progression to AIDS in people infected with HIV (Ullum et al., 1997). TNF-α attracts and
activates neutrophils (the most abundant type of white blood cell), and enhances the phagocytic function of both neutrophils and macrophages (Elgert, 2009). However, HIV infection of monocytic cells increases TNF-α production and the resultant increase in TNF-α concentration increases HIV replication (Osborn et al., 1989; Folks et al., 1989). IL-10 down regulates the expression of the Th1 cytokines and major histocompatibility complex II (MHC II) antigens. MHC II antigens are involved in activation of CD4+ T_h cells (Zhou et al., 2011). IL-10 increases with disease progression in HIV infected individuals (Stylianou et al., 1999), and contributes to HIV persistence by suppressing the T_h cell response to infections (Brooks et al., 2006). IL-4 stimulates and induces the differentiation of a naïve T_h0 cell into a T_h2 cell (Sokol et al., 2008) and suppresses the production of T_h1 cells (Tanaka et al., 1993).

Both aerobic fitness and resistance training can improve the health of people living with many diseases, including HIV (O’Brien et al., 2010), and the prevention of muscle wasting (Greiwe et al., 2001). The mechanism of such exercise benefits may be via cytokine regulation, as is suggested in studies of other autoimmune diseases such as multiple sclerosis (Golzari et al., 2010). In patients with multiple sclerosis, combined aerobic and resistance training results in a decrease in circulating IFN-γ concentrations, but no change in circulating plasma IL-4 (Golzari et al. 2010). Resistance exercise training alone acutely reduces IFN-γ for 48 hours post exercise in patients with multiple sclerosis (White et al., 2006), with significant decrease also in resting concentrations of IL-4. These results suggest possible differences in resistance vs. aerobic or combined training program effects on circulating cytokines in autoimmune diseases. No study has yet defined how exercise training may affect cytokine concentrations in HIV patients, or HIV patients with chemical dependence. Given that resistance training seems to cause an overall reduction in TNF-α concentrations in elderly populations (Greiwe et al., 2001), which is relevant
to aging HIV-infected patients, and that exercise clearly affects HIV-relevant cytokines TNF-α (Ostrowski et al., 1999) and IL-10 (Smith et al., 2000) in healthy subjects, both acutely and chronically, it is likely that exercise training may affect cytokine signaling in HIV patients and thereby to affect disease prognosis.

The aim of this study was to determine the effect of resistance training on circulating cytokine (Th1 vs. Th2: IFN-γ, TNF-α, IL-4, IL-10) concentrations in men living with HIV and who had a recent history of substance abuse. The interaction between exercise and cytokines has never been studied and we hypothesize that an exercise treatment program could be associated with Th1 or Th2 cytokine regulation to indicate possible immune-related benefits of exercise in HIV infection and chemical dependence.
METHODS

Sixteen HIV\(^+\) men (42 ± 11 years, 180 ± 9 cm, and 90 ± 21 kg), who had a recent history of substance abuse, were assigned to one of two interventions: (1) a 6-week resistance training program (Exercise, \(n = 8\)), which comprised of three weekly resistance training sessions for 6 weeks, or (2) a 6-week control condition with no exercise (Non-Exercise, \(n = 8\)). Strength, body composition, and circulating cytokines were measured before (pre) and after (post) the 6-week intervention.

Participants were recruited by word-of-mouth and flyers at Homeward Bound Inc. (Dallas, TX), a substance abuse/addiction treatment facility. HIV\(^+\) Homeward Bound residents (≥21 years), recovering from chemical addiction/abuse and not resistance trained, provided informed consent and after physician-approved clearance to resistance train. After consent, participants were randomly assigned to either Non-Exercise or Exercise groups. Due to the controlled nature of the treatment facility in which the participants live, sleep pattern, diet, and involvement in non-study related substance abuse treatment activities (e.g., counseling, and community service) were similar for all participants. Similarly, adherence to individual medication plans was tightly monitored and enforced by the facility staff.

Fasting (12h overnight) and resting blood samples were collected via venipuncture of an antecubital vein at 0700-0800h. Whole blood was centrifuged (1500g, 15 minutes, 4\(^\circ\)C) and serum was stored (−80\(^\circ\)C) in multiple aliquots until analysis. Participants did not exercise ~72 hours before blood sample was taken. Anthropometric measurements were taken after blood sample. Measurements included skinfold and circumference measurements. These measurements were used to calculate muscle mass (Martin et al. (1990)).
Participants were familiarized with testing exercises 48 to 72 hours before test day. Pre-testing of the participants was performed at least 48 to 72 hours after familiarization. The post training test day was separated from the last training day by at least 72 hours to avoid short-term, carry-over effects from the last exercise bout. Each testing session began with a standardized dynamic warm-up (lunges, heel kicks, no load squats, high-knee raises, and high kicks). Strength was measured after the warm-up with isometric squat, indirect bench press 1 repetition maximum, and vertical jump test. The isometric squat test required maximal effort on a force plate (AMTI, Watertown, MA) for 10 seconds. The indirect bench press test consisted of a light warm-up (50% estimated weight predicted for maximal exercise) and a maximal test phase. During the test phase, each participant completed as many repetitions as possible at a weight estimated to allow for 2-20 repetitions. (One-repetition maximum = load × (1 + (0.033 × number of repetitions)) (Epley, 1985)). The vertical jump test consisted of the participant standing on a force plate (AMTI, Watertown, MA), with hands placed on their hips, performing three consecutive jumps.

Exercise training was performed 3 times per week (Monday-Wednesday-Friday) for 6 weeks, under supervision of an experienced trainer. Each resistance training session involved 3 to 5 sets of 5 to 12 repetitions of standard resistance exercises designed to target all major muscle groups including: (1) free weight exercises (bench press, incline bench press, back squat, calf raise, military press, sit-up, upright row, and weighted sit-ups) and (2) cable machine exercises (lateral pull down and seated row). Different weights and exercises were used on different days to provide variation in the exercise stress and follow progressive overload format modeling (Kraemer et al., 2006). The resistance used by each participant was determined by the trainer and was increased when participants were able to perform the prescribed repetitions using proper
technique. Table 1 illustrates the overload training and resistance exercise prescription (Kraemer et al., 2006) for this study.

Table 1

<table>
<thead>
<tr>
<th>Exercise Prescription Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

Note. number of sets × number of repetitions per exercise following non-linear exercise programming.

Serum cytokine concentrations were determined (in duplicate) using enzyme-linked immunosorbent assays (ELISA) according to manufacturer guidelines. The sensitivity of the TNF-α assay (Life Technologies, Grand Island, NY) was 0.09 pg·µl⁻¹. The sensitivity of the IL-4 assay (Life Technologies, Grand Island, NY) was 2.00 pg·µl⁻¹. The sensitivity of the IFN-γ assays (Abnova, Taipei City, Taiwan) was 0.99 pg·µl⁻¹. The sensitivity of the IL-10 assay (Abcam, Cambridge, MA) was 1.3 pg·µl⁻¹.

Data was analyzed using repeated measures (time), two-way analysis of variance (ANOVA) (Time × Treatment) (PASW Statistics version 18, Chicago, IL). Alpha level was set at \( p \leq 0.05 \). Data is presented as mean ± standard deviation (SD).
RESULTS

Strength measures improved within the exercise group, but not for the non-exercise group. The estimated 1-repetition maximum (RM) bench press (Figure 1) increased from pre-test to post test in the exercise group (pre-test: 73.4 ± 23.4 kg, post test: 85.0 ± 30.4, \( p < 0.05 \)). The non-exercise group did not change pre to post (pre-test: 54.9 ± 24.6 kg, post test: 57.3 ± 27.0 kg). Isometric squat peak force (Figure 2) improved from pre-test to post test in the exercise group (pre-test: 2627 ± 1071 N, post test: 2892 ± 1171 N, \( p < 0.05 \)). The non-exercise group did not change pre to post (pre-test: 2411 ± 675 N, post test: 394 ± 592 N). Furthermore, muscle mass (Figure 3) also improved within the exercise group: (pre-test: 46.5 ± 8.2 kg, post test: 50.5 ± 8.1 kg, \( p < 0.05 \)). The non-exercise group showed no improvement pre to post: (non-exercise group: pre-test: 46.9 ± 13.0 kg, post test: 47.8 ± 12.4 kg).

![1-RM Bench Press](image)

*Figure 1. 1 RM Bench Press (kg) before (Pre) and after (Post) the 6-week intervention for the non-exercise group and the exercise group. (*significantly different from corresponding pre-test.*)
Figure 2. Isometric squat peak force (N) before (pre) and after (post) the 6-week intervention for the non-exercise group and the exercise group. (*significantly different from corresponding pre-test.)

Figure 3. Muscle mass (kg) before (pre) and after (post) the 6-week intervention for the non-exercise group and the exercise group. (*significantly different from corresponding pre-test.)

Despite randomization, there was a pre-existing difference between TNF-α resting
concentrations in the exercise group and non-exercise group ($p = 0.012$, non-exercise: pre-test: $3.00 \pm 1.28 \text{ pg} \cdot \text{µl}^{-1}$ (post test: $2.73 \pm 0.79 \text{ pg} \cdot \text{µl}^{-1}$) exercise: pre-test: $4.85 \pm 2.68 \text{ pg} \cdot \text{µl}^{-1}$ (post test: $4.61 \pm 2.43 \text{ pg} \cdot \text{µl}^{-1}$)). Figure 4 shows normalized TNF-α values (normalized to each group’s respective pre-test value, Pre-test = 1.00).

Figure 4. Normalized TNF-α values before (pre) and after (post) the 6-week intervention for the control group (non-exercise) and the exercise training group (exercise).

The serum concentrations of IL-4, IL-10, and IFN-γ were below detectable limits pre intervention and post intervention for both Exercise and the Non-Exercise groups; therefore, possible changes in resting concentration levels could not be determined.
DISCUSSION

The aim of this study was to determine the effect of resistance training on circulating concentrations of cytokines (T_h1 vs. T_h2: IFN-γ, TNF-α, IL-4, IL-10) in HIV+ men who had a recent history of chemical dependency/abuse. No study has investigated or characterized the cytokine response to resistance training in this clinical population. Exercise training is beneficial for body composition, reduction in resting heart rate, and increases in strength for people diagnosed with HIV+ (Rigsby et al., 1992; O’Brien et al., 2010). Exercise also clearly affects cytokine signaling (Smart et al., 2011; Golzari et al., 2010; Steensberg et al., 2003; Calle & Fernandez, 2010; White et al., 2006). It is important to determine how exercise may affect the major cytokine signaling in HIV+ infection.

This study found that these participants exhibited increased strength, muscle mass, and power with training, but showed no consistent circulating TNF-α changes. In addition, IL-4, IL-10, and IFN-γ concentrations were below detectable concentration levels before and after the 6-week period for both groups.

TNF-α recruits additional macrophages without triggering necrosis (Hernandez-Pando & Rook, 1994). HIV-1 infection of monocytic cells increase TNF-α production. This increase in TNF-α has been associated with increases HIV-1 replication (Osborn et al., 1989; Folks et al., 1989). TNF-α concentrations are down-regulated by increased levels in IL-6 (Keller et al., 2005). Resting levels of TNF-α for healthy men has been reported to be between 4 pg·µl⁻¹ to 12 pg·µl⁻¹ (Çalıkoglu et al., 2004; Erken et al., 1993). The TNF-α resting concentration levels observed in this study were unaffected by the 6-week intervention (exercise and non-exercise).

TNF-α has, however, been shown to change with exercise training (Smart, et al., 2011). Even though IL-6 was not measured in this investigation, IL-6 acute concentrations are generally
elevated following resistance training (Bruunsgaard et al., 1997). TNF-α has also been observed to be expressed in type II muscle fibers (Plomgaard, et al., 2005), suggesting that the secretion of TNF-α could be manipulated by resistance training. TNF-α levels for elderly participants in a resistance training program showed lower concentrations of resting TNF-α than in younger participants (Greiwe et al., 2001). Greiwe et al. (2001) suggesting that resistance exercise might attenuate muscle wasting in elderly populations by suppressing muscle TNF-α expression.

Studies that involve distance running have noted small, but significant increased resting concentrations up to 4 hours post-exercise (Ostrowski et al., 1999; Ostrowski et al., 1998). As increased concentrations of TNF-α increase HIV replication (Osborn et al., 1989; Folks et al., 1989), no change in resting concentration levels during this 6 weeks intervention would indicate that resistance training does not enhance an HIV viral replication signal.

IFN-γ concentrations were below detectable limits in this current study. IFN-γ concentrations in healthy populations range from 3 pg·µL⁻¹ to 10 pg·µL⁻¹ (Castellano et al., 2008). IFN-γ up-regulates the quantity and diversity of peptides presented on the cell surface of Major Histocompatibility Complex I (MHC I). This is an important function in the immune system as it promotes the induction of cell mediated immunity by increasing cytotoxic T cell recognition of foreign peptide or amino acid (Schroder et al, 2004). In addition, IFN-γ acts as a positive feedback mechanism on the phagocytic cells by activating them and enhancing their ability to produce additional pro-inflammatory cytokines such as IL-12, IL-23, and IL-27 (Watford et al., 2003). IFN-γ might also inhibit HIV replication in latently infected cells (Sarol et al., 2002) and low IFN-γ production has been correlated to disease progression in HIV infection (Ullum et al., 1997). IFN-γ responses to exercise appear to be mixed. Goldhammer et al. (2005) found that resting IFN-γ concentrations were lower immediately following a bout of aerobic exercise.
Castellano et al. (2008) demonstrated elevated levels of IFN-γ in participants diagnosed with multiple sclerosis after participating in an eight-week training study involving a cycle ergometer. White et al. (2006) showed that resistance training reduced IFN-γ concentrations following an acute bout of exercise. Increased concentrations of IFN-γ would directly benefit people diagnosed with HIV as it could help delay the progression to AIDS.

This study found IL-4 resting concentrations were below detectable limits in both groups pre and post in this intervention. IL-4 is an important cytokine in the progression of HIV (Clerici & Shearer, 1993), and has previously been found to respond with increased circulations to aerobic exercise (Peake et al., 2005), and with resistance training (White et al., 2006). IL-4 stimulates the differentiation of naïve T<sub>h</sub>0 cells into T<sub>h</sub>2 cells (Sokol, et al., 2008) and stimulates the activated B-cell (Hinton & Welham, 1999). In addition, IL-4 decreases the production of T<sub>h</sub>1 cells and IFN-γ (Tanaka, et al., 1993). Valentin et al. (1998) identified IL-4 as an important regulator of HIV, and suggest that a critical role of IL-4 is in the control of virus replication leading to accelerated disease progression. IL-4 induces HIV replication with the selected loss of the CD4<sup>+</sup> subset in certain cultures, and stimulates HIV replication (Foli et al., 1995). Previous studies have found resting IL-4 levels from below detectable range (via ELISA) to as high as 38 pg·µl<sup>-1</sup> (Kilciler et al., 2008; Koh et al., 2001). Rincon et al. (1997) showed that IL-6 stimulates the production of IL-4. IL-6 can be stimulated through resistance exercise (Keller et al., 2005; Ostrowski et al., 1999). Prokopchuk et al. (2007) proposed that IL-4 is involved in muscle hypertrophy, and that IL-4 is involved in the promotion of muscle regeneration. White et al. (2006) found that 48 hours after an 8 week resistance training program, plasma concentrations of both IL-4 and IL-10 showed lower levels than pre-test samples in participants diagnosed with
multiple sclerosis. There are no studies on the effect of resistance training on IL-4, and IL-10 in people living with HIV.

This study found IL-10 resting concentrations were below detectable limits in all participants. The role of IL-10 is to down regulate the expression of \( T_{h1} \) cytokines and MHC II antigens (Tze et al., 2011) that are involved in the activation of CD4\(^+\) T\(_h\) cells (Zhou et al., 2011). IL-10 levels increase with disease progression in HIV infected individuals (Stylianou et al., 1999). This progression is likely through the acceleration of viral replication. IL-10 may promote viral persistence through inactivation of effector immune mechanisms (Dshanta et al., 2009). A decrease in IL-10 or a blockade of the IL-10 pathway may enhance T cell immune responses, which then could result in the rapid elimination of virus and the development of antiviral memory T cell responses (Brockman et al., 2009). A normal concentration range for IL-10 vary from undetectable (<3 pg·µl\(^{-1}\)) to 14 pg·µl\(^{-1}\) for healthy individuals (Nemunaitis et al., 2001; Fayad, et al., 2001).

Following strenuous aerobic exercise, high circulating levels of IL-6 are followed by an increase in IL-10 (Steensberg et al., 2003). IL-10 plasma levels were recorded as high as a 27 fold increase in athletes that had just completed running a marathon distance compared to resting levels (Ostrowski et al., 1999). IL-10 does respond to acute resistance exercise (Steensberg et al., 2003; Calle & Fernandez, 2010), with IL-10 concentration increases noted as far as 45 minutes post exercise (Izquierdo et al., 2009). Elevated levels of IL-10 were recorded 72 and 144 hours and were elevated as long as 6 days post exercise after completion of a program that included resistance training and aerobic exercises (Smith et al., 2000). There are no studies on the effect of resistance training on IL-10 in people living with HIV.
Exercise has been able to affect resting concentrations of TNF-α, IFN-γ, IL-4, and IL-10 through exercise, the lack of such a finding in this study does not necessarily exclude possible hypotheses about exercise effects on cytokine signaling in HIV+ patients. As TNF-α drives Th1 cell production and IL-10 along with IL-4 inhibits Th1 cell production, a finding of no change of TNF-α resting concentrations can support Rigsby et al. (1992) in which CD4+ cell concentrations were unaffected by exercise. As the cytokines IFN-γ, IL-4, and IL-10 specifically affect the CD4+ T cell, and CD4 T cell numbers do not change by exercise, these cytokines might remain unchanged through resistance training. Another potential explanation of our results is that the concentrations might respond acutely to the resistance exercise, but do not persist 48 hours after exercise. Levels that were undetectable could be due to abnormally low resting levels in each of the four cytokines (TNF-α, IFN-γ, IL-4, IL-10) within participants within this study.

Considerations for future research would include using more exact measurement methods such as flow cytometry that could measure more precise cytokine concentrations than standard ELISA assays, or the acquisition of more sensitive ELISA kits. Additionally, an extension of the program length might provide a more potent stimulus than resistance exercise alone for impacting the activity of the cytokines. Other studies have used eight to twelve week resistance training interventions (Golzari, et al., 2010; Ostrowski, et al., 1999). Additionally, the addition of aerobic training might extend the time in the training program each day, and create a more pronounced inflammatory environment for the recruitment of elevated levels of the cytokines investigated in this study.

In conclusion, the present study found no changes in concentrations of TNF-α after a 6-week progressive resistance training program; the concentrations of IFN-γ, IL-4, or IL-10 were below detectable limits pre and post intervention. Although previous studies have demonstrated
increased concentrations of these cytokines acutely after resistance exercise, with at least one study showing these elevated levels of IL-10 up to 6 days post intervention, we were unable to produce altered levels of these cytokines with a six week intervention given this population. Increased concentrations of TNF-α increase HIV replication (Osborn et al., 1989; Folks et al., 1989), no change in resting concentration levels would suggest that resistance training does not enhance HIV viral replication signal. Thus, the 6 week training program induced positive fitness benefits without compromising immune status.
APPENDIX A

REVIEW OF LITERATURE
Human immunodeficiency virus (HIV) infects cells in the human immune system including helper T cells (specifically CD4+ T cells), macrophages, and dendrite cells. Once diagnosed, patients that live with this virus are tested regularly for viral load and CD4+ T cell counts. The standard CD4+ count test measures the number of T cells expressing CD4. Normal blood values are 500-1200x10^6/L (Bofil, 1992). When CD4+ T cell numbers decline below 200-350 cells per microliter, cell-mediated immunity is lost, and the body becomes more susceptible to opportunistic infections (Cunningham, 2010). When CD4+ T cell numbers are less than 200 cells per microliter, an HIV positive individual is then diagnosed as developing AIDS (Piot, 2007).

The standard therapy for the management of HIV is done through a combination of anti-retroviral drugs. These drugs are known as the highly active anti-retroviral therapy (HAART). This combination is composed of at least three drugs: a nucleotide reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), and a protease inhibitor (PI) (Hirschel et al., 1999, Montaner et al., 1998).

It is important to understand the relationship of CD4 and T cells. CD4 is a glycoprotein which is expressed on the surface of a T helper cell (Ryu, 1994). CD4 is a co-receptor that assists the T cell receptor with an antigen-presenting cell. Using its portion that resides inside the T cell, CD4 amplifies a T cell receptor signal which is essential for activating several molecules involved in the signaling cascade of an activated T cell. (Brady, 1993) T cells play various roles in orchestrating the immune responses. They are important in cell-mediated immunity, which is the defense against tumor cells and pathogenic organisms inside tissue cells (Waldmann, 2006). Mature T helper cells which express the surface protein CD4 are referred to as CD4+ T cells.
Since the CD4$^{+}$ T cell is a signaler to an immune response, it is important to know the CD4$^{+}$ T cell level for someone diagnosed with an immune disease such as HIV.

Cytokines contribute to the growth and function of the cellular immune system. T cells play a dominant role in the network since they are the main source of many cytokines (Mosmann et al., 1986). A naïve T helper cell (T$_{n}$0) is divided into one of two major subtypes. The T$_{n}$0 cells will differentiate into T$_{h}$1 or T$_{h}$2 cells depending on cytokine environment. T$_{h}$1 cells produce cytokines such as Interferon-γ, and Tumor Necrosis Factor – α which promote cell mediated immunity. T$_{h}$2 cells produce cytokines such as Interleukin-4 (IL-4), Interleukin-6 (IL-6), and Interleukin-10 (IL-10) that promote antibody-mediated immunity (Mosmann et. al., 1986). Interferon Gamma (IFN-γ) drives T$_{h}$1 cell production while Interleukin 10 (IL-10) and Interleukin 4 (IL-4) inhibit T$_{h}$1 cell production. Conversely, IL-4 drives T$_{h}$2 cell production and IFN-γ inhibits T$_{h}$2 cells (Mosmann & Sad, 1996). This study will research the following cytokines and the relationship to exercise in this population:

Interferon – γ (INF-γ)

Interferon gamma (IFN-γ) is the only type II class of interferon (Gray, 1982). IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the immune response, as well as by Th1 CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen specific immunity develops (Schoenborn, 2007). IFN-γ is crucial for immunity against intracellular pathogens, and also has antiviral and antitumor properties (Schroder et al., 2004) This cytokine, IFN-γ, is the primary cytokine that defines T$_{h}$1 cells: T$_{h}$1 cells secrete IFN-γ which in turn causes more naïve CD4 cells (T$_{n}$0 cells) to differentiate into T$_{h}$1 cells, while suppressing T$_{h}$2 cell differentiation (Bradley et al., 1996). IFN-γ has the ability to up-regulate the antigen presenting peptides on the cell surface of a Major Histocompatibility
Complex (MHC) I. This is important as it promotes the induction of cell mediated immunity by increasing cytotoxic T cell recognition of foreign peptide (or amino acid) (Schroder et al, 2004). In addition, IFN-γ acts as a positive feedback mechanism on the phagocytic cells by activating them and enhancing their ability to produce pro-inflammatory cytokines (Watford et al., 2003). A study done in 2002 (Rueben, 2002) reported that immune restoration of HIV infected children receiving HAART medication might be related to an increase in IFN-γ concentrations and a decrease in the IL-10 concentrations. IFN-γ might also inhibit HIV replication in latently infected cells (Sarol et al., 2002). Low production of IFN-γ has been correlated to disease progression in HIV+ infection (Ullum et al., 1997).

IFN-γ responses to exercise appear to be mixed. A study by Baum et al. (1997) showed that moderate exercise was followed by an improved INF-γ production. Studies that looked at aerobic exercise responses show negative responses. Goldhammer et al. (2005) found that aerobic exercise actually lowers IFN-γ levels. Golzari et al. (2010) found that there was a significant decrease in IFN-γ levels in participants that were diagnosed with Multiple Sclerosis (MS) following an intervention of aerobic exercise and resistance training. White et al. (2006) showed that resistance training statistically reduced IFN-γ after the last workout.

**Tumor Necrosis Factor – α (TNF-α)**

Tumor necrosis factor alpha (TNF-α) is a cytokine secreted by the macrophages, and is best known for mediating immune responses in acute infection. This cytokine has also been linked to wasting disease and increased HIV replication in people diagnosed with HIV (Drexler, 1995). TNF-α does provide beneficial activities. TNF-α attracts and activates neutrophils (white blood cells), as well as enhances the phagocytic function of both neutrophils and macrophages. It also stimulates the endothelium to become more permeable, in which allows plasma and blood
cells to enter the site of an injury. TNF-α also stimulates wound healing by stimulating the growth of tissue and blood cells (Drexler, 1995). HIV forms a symbiotic relationship with TNF-α. HIV-1 infection of monocyctic cells will increase TNF-α production. This increase in TNF-α then further increases HIV-1 replication (Osborn et al., 1989; Folks et al., 1989). TNF-α elevated levels are also used to identify wasting disease in patients diagnosed with AIDS (Abad et al., 2002).

TNF-α’s relationship to exercise has been studied. The studies appear to have mixed findings, depending on the participant’s age. Resistance training seems to have an overall reduction in skeletal muscle concentrations in elderly populations (Greiwe, et al., 2001). Studies in younger populations show elevated levels of TNF-α in participants that have either participated in a marathon race (Ostrowski et al., 1999). TNF-α was also observed to be expressed in type II muscle fibers (Plomgaard et al., 2005), leading to suggest that levels could be manipulated through resistance training.

Interleukin 10 (IL-10)

Interleukin – 10 (IL-10) is a secreted protein cytokine that is considered anti-inflammatory (Eksdale et al., 1997). Once a naïve T_h,0 cell is stimulated through high concentrations of the presenting antigen (typically interleukin – 4), the naïve cell converts to a T_h,2 cell. This T_h,2 cell is then responsible for the secretion of the IL-10 cytokine. IL-10’s main responsibility is to down regulate the expression of T_h,1 cytokines, and MHC II antigens (Tze et al., 2011) that are used in the activation of CD4⁺ T helper cells (Zhou et al., 2011). IL-10 also reduces HIV viral replication in macrophages (Akridge et al., 1994), but contributes to HIV persistence by suppressing T cell responses (Brooks et al., 2006). IL-10 levels increase with disease progression in HIV infected individuals (Stylianou et al., 1999). This progression is
likely through the acceleration of viral replication. IL-10 may promote viral persistence through inactivation of effector immune mechanisms (Dshanta et al., 2009). A decrease in IL-10 or a blockade of the IL-10 pathway may enhance T cell immune responses, which then could result in the rapid elimination of virus and the development of antiviral memory T cell responses (Brockman et al., 2009).

IL-10 plays a part in response to exercise. Following strenuous aerobic exercise, high circulating levels of Interleukin – 6 (IL-6) are followed by an increase in two anti-inflammatory molecules, IL-1ra and IL-10 (Steensberg et al., 2003). Therefore, IL-6 induces an anti-inflammatory environment. IL-10 plasma levels were recorded as high as a 27 fold increase in athletes that had just completed running a marathon distance compared to resting levels (Ostrowski et al., 1999). IL-10, being considered an anti-inflammatory, has also been found to respond to an acute bout of resistance training, (Calle & Fernandez, 2010). Elevated levels of IL-10 were recorded 72 and 144 hours post exercise. High levels were recorded as long as 6 days post exercise (Smith et al., 2000).

Interleukin 4 (IL-4)

Interleukin – 4 (IL-4) is a cytokine that stimulates and induces the differentiation of a naïve T₈0 cell into a T₈2 cell (Sokol et al., 2008), as well as the stimulation of the activated B-cell (Hinton & Welham, 1999). IL-4 decreases the production of Th1 cells, as well as IFN-gamma (Tanaka et al., 1993). This cytokine has also been shown to cause B lymphocytes to increase and to make antibodies. IL – 4 has been studied in relationship to exercise. Peake et al (2005) showed that plasma levels of IL – 4 were unchanged in groups that were subjected to moderate intensity running, high intensity running, and downhill running. In regards to Resistance training, opinion appears to be mixed. White et al (2006) found reduced resting
concentrations in the blood after a series of twice weekly progressive resistance training in people diagnosed with Multiple Sclerosis (MS). However, Golzari et al (2010) did not find any statistical change in plasma concentrations in an 8 week program which included resistance training, and aerobic exercise in a population of people diagnosed with MS.
APPENDIX B

IRB CONSENT FORM
Informed Consent Form

University of North Texas Institutional Review Board

Before agreeing to participate in this research study, it is important that you read and understand the following explanation of the purpose, benefits and risks of the study and how it will be conducted. Signing this form also gives permission for use and disclosure of your health information as part of this research study.

**Title of Study:** Weight training: A treatment for muscle wasting from the interaction of long-term alcohol addiction and infection with HIV

**Principal Investigator:** Jakob Vingren, Ph.D., University of North Texas (UNT) Department of Kinesiology, Health Promotion and Recreation.

**Purpose of the Study:** You are being asked to participate in a research study which involves investigating the effect of resistance training (weight training) on muscle in individuals with HIV and a recent history of long-term alcohol addiction.

**Study Procedures:** You will be asked to complete tests before and after a 6-week period. The tests will include measurement of body size, muscle strength and psychological aspects such as anxiety, loneliness, depression, alcohol relapse, medication adherence, stress, and shame. During the 6 week between the test sessions you will participate in either a resistance training (weight training) program 3 times per week (about 1 hour per session) or no resistance exercise. You will be assigned to one of the two groups using random assignment (coin-flip). You must be willing to participate in either of the 2 groups to be eligible for this study.

**Resistance training:** The training protocol will vary across sessions and weeks and include heavy, medium and light intensity sessions; each session will train all major muscle groups and will begin with a standardized warm-up.

**Testing procedures:**

*Medical Screening:* After you provide informed consent to participate, you will complete a medical history questionnaire to ensure you do not have medical conditions that might put you at risk during the testing procedures.

*Familiarization:* Prior to each test visit you will be familiarized with the exercise procedures described below (familiarization will occur at Homeward Bound, Inc Dallas).
Testing:
You will be transported to the laboratory located on the UNT campus in Denton in the morning after a 12 hour overnight fast to complete the battery of tests. You will be transported back to Homeward Bound Inc., Dallas) at the end of the testing.

Battery of tests:
Body size:
Upon arrival to the lab your height, weight, and body composition will be measured. Body composition will be measured using Dual-energy x-ray absorptiometry (whole body scan); which is the gold standard for this measurement. You will lie on a table for approximately 8 minutes while a small scan arm moves over your body.

Blood tests:
A 20 ml (1.3 table spoons) blood sample will be obtained from a forearm vein for analysis of hemoglobin, hematocrit, blood lipids (cholesterol) and glucose.

You will begin by warming up using a standardized protocol involving light stationary cycling followed by light dynamic stretching. After the warm-up you will complete the strength and power measurements

Vertical jump:
You will complete 3-5 maximal vertical jumps each separated by 2 minutes. The jumps will be performed on a force plate which will measure jump height, power and force.

Maximal strength:
Maximal isometric (static) grip strength will be measured using a hand grip dynamometer. You will hold the dynamometer in your dominant hand at the side of the body. The handle of the dynamometer is adjusted if required - the base should rest on first metacarpal (heel of palm), while the handle should rest on middle of four fingers. When ready you will squeeze the dynamometer with maximum isometric effort, which is maintained for about 5 seconds. No other body movement will be allowed.

Indirect bench press and squat maximal strength will be measured using a smith machine. This device allows for only vertical movement of the bar. After a light warm-up set, you will complete as many repetitions as possible at a load estimated to allow for 2-20 repetitions (load determined during familiarization). The following formula will be used to estimate maximal strength for each exercise: One-repetition maximum = load × (1 + (0.033 × Number of repetitions)).

Balance:
The balance test will be performed on the Biodex Balance System (BBS). You will stand (balance) in the center of the platform for three, 30-second trials to measure dynamic balance. The BBS is a device that measures and records an individual’s ability to stabilize.
Psychological questionnaires:
You will complete a series of questionnaires on a laptop computer. Each questionnaire is described below.

Forgiveness: The Heartland Forgiveness Scale (Thompson et al., 2005) is a measure of dispositional forgiveness.

Risky Sexual Behavior: A standard risky sexual behavior inventory will assess frequency of participation in risky behaviors.

Self-esteem: The Rosenberg Self-Esteem Scale is a self-report measure used to assess global self-esteem.

Coping: Coping strategies utilized by participants in the past three months will be measured with the Brief Cope.

Social support: The UCLA Social Support Scale is a measure of interpersonal relationships and their dynamics.

Quality of life: The Short-Form 36 is a 36 question measure of quality of life.

Self-efficacy for managing a chronic disease: Self-efficacy for Managing Chronic Disease scale measures self-efficacy when trying to manage disease.

Loneliness: The UCLA Loneliness Scale is a 10-item self-report measure that assesses the extent to which an individual feels lonely.

Adherence (Medication Usage): Adherence to HIV medications will be assessed with the Adult Clinical Trials Group Medical Adherence Questionnaire.

Stress: The Perceived Stress Scale is designed to measure the degree to which situations in one's life are appraised as stressful.

Stigma: The HIV Stigma Scale measures HIV-related stigma experiences.

Anxiety:
State/Trait Anxiety Inventory: measures the level of anxiety that the participant is currently in as well as anxiety as more of a characteristic of the individual.

Depression:
Center for Epidemiological Scale for Depression is designed to measure current levels of depressive symptoms.

Exercise Motivation:
Exercise Motivation Scale Revised: This measures motivation to exercise on a continuum that includes no motivation, external motivation, and internal motivation.
Future Time Orientation:
Consideration of Future Consequences measures an individual’s propensity to consider future consequences in decision making.

Mindfulness:
Kentucky Inventory of Mindfulness measures mindfulness.

Resilience:
Conner-Davidson Resilience Scale is used to measure resilience.

Potential for alcohol abstinence/relapse:
Alcohol Abstinence Self-Efficacy Scale, AASE tool
AWARE, Advanced Warning of Relapse tool

Study controls:
You will be asked to write down everything that you eat and drink during the 3 days leading up to the first testing session, you will be asked to repeat that diet for the 3 days leading up to the final testing session. Additionally, you must refrain from resistance exercise and intense endurance exercise for 3 days prior to each testing session.

All experimental trials will be performed at the same time of day (± 1 hour) to avoid confounding influences of diurnal hormonal variations. Additionally, you will be instructed to drink at least 0.5 liter of water the night before and the morning of the experimental trials to ensure adequate hydration. Hydration state will be determined before the SEP via urine analysis.

Diet and physical activity logs:
You will be asked to write down your daily physical activity during weeks 1 and 6 of the study and your diet for 3 days during week 3 and 6.

Viral load and CD4/CD8 counts immediately prior to and after the training period will be assessed from your medical charts.

Time commitment:
Your total time involvement in this study will be approximately 23 hours if you are placed in the training group and 5 hours if you are placed in the control group.

Foreseeable Risks: The potential risks involved in this study are as follows:

Exercise: Even though the exercise protocol is designed to be very safe there is the risk that you may become injured. The researchers have extensive experience in conducting exercise-testing studies, and they will do everything possible to reduce the chance of injury. Every effort will be made to make this study safe for you through supervision, screening, monitoring while training and testing, familiarization and technique instruction, and proper
warm-up and cool-down practices. However, if at any time during exercise testing you experience pain, unexpected discomfort, soreness, headache, loss of concentration, dizziness, unusual fatigue or difficulty breathing you should immediately inform one of the supervising researcher team members, who will bring this to the attention of the principal investigators and the medical monitor.

The performance of resistance exercise can entail a certain degree of risk from overexertion and/or accident. With strength and power testing, minimal risks exist for muscle strain or pulls of the exercised muscles, muscle spasm, and in extremely rare cases, muscle tears. Some muscle soreness may be experienced 24 to 48 hours after exercise, and this should completely subside within a few days and have no long-lasting effect. A CPR certified individual will be present at all testing and the Principal Investigator (Dr. Jakob Vingren) is a National Strength and Conditioning Association (NSCA) Certified Strength and Conditioning Specialist recertified with Distinction (CSCS*D).

**Blood Draws.** While blood draws using single use sterile supplies are normally a safe procedure, it is possible that short-term side effects can occur, such as dizziness, bruising, or fainting. Although the chances are remote, it is also possible that bruising around the vein, an infection, or nerve damage can develop. All possible precautions to avoid infection will be taken including use of sterile disposable needles, drapes and gauze and the practice of sterile techniques during blood sampling. All blood samples will be obtained by a trained phlebotomist adhering to the standard operating procedures of the laboratory. In case of emergency, we will call 911. It is possible that the fasting prior to the blood draw might increase the chance of relapse. To minimize this risk we will provide a breakfast after the blood sample has been collected.

**Body Composition.** You will be exposed to a very small amount of radiation by the scanner used to measure your body composition. Exposure to any amount of radiation, no matter how low, may cause abnormal changes in cells. However, the body continuously repairs these changes and the amount of radiation is very low in this study. The total exposure for a whole body scan is approximately 125 times less than the average radiation from a standard chest x-ray or 150 times less than a round trip transcontinental flight. Thus, the radiation levels are extremely low and the health risk minimal. The scan will be performed by a technician who has received training required by the State of Texas for operating the scanner. You will complete a separate “Acknowledgement of risk” form for the scan as well as a safety questionnaire prior to any scan to ensure you are not at risk during the scan.

**Questionnaires.** The topics addressed throughout the questionnaires may prove a source of emotional discomfort. Although this is unlikely to occur, the researchers will be vigilant to identify signs of emotional and mental distress. In the event you experience a significant amount of distress as result of the questionnaires, the researchers will provide you with a list of local referral sources and phone numbers.

**Benefits to the Subjects or Others:** We expect the project to benefit you by teaching you how to properly complete the exercises involved in the study. You will also learn about
your body composition, strength, and balance, as well as how these change over the 6-week intervention period. All of your data will be explained to you and interpreted for you so that a maximum amount of educational understanding and use of the data will be achieved. The benefit to the HIV and alcohol addiction community might be large if a beneficial effect of the training is found.

**Compensation for Participants:** If you are placed in the training group and complete all aspects of the study you will receive gift cards to stores such as Wal-Mart in the amount of $200. If you are placed in the non-training control group and complete all aspects of this study you will receive gift cards in the amount of $60. If you cannot or choose not to complete the study, you will receive partial compensation.

**Procedures for Maintaining Confidentiality of Research Records:** All data will be kept in coded participant files in the primary investigator’s locked files. Participant codes will be used when statistical analyses are performed or when experimental feedback sheets are provided to you. All investigators, professional staff, and technicians are aware of the confidentiality involved with this study and have completed the confidentiality training required by the University. Your data will not be available or divulged to anyone outside of the experimental research team. The data files will be kept for 3 years after the study is terminated. The confidentiality of your individual information will be maintained in any publications or presentations regarding this study.

**Questions about the Study:** If you have any questions about the study, you may contact *Dr. Jakob Vingren* at telephone number (940) 565 3899.

**Review for the Protection of Participants:** This research study has been reviewed and approved by the UNT Institutional Review Board (IRB). The UNT IRB can be contacted at (940) 565-3940 with any questions regarding the rights of research subjects.

**Research Participants’ Rights:**
Your signature below indicates that you have read or have had read to you all of the above and that you confirm all of the following:

- *Jakob Vingren, Ph.D or another researcher* has explained the study to you and answered all of your questions. You have been told the possible benefits and the potential risks and/or discomforts of the study. You have been told how my health information will be used and disclosed for the study.
- You understand that you do not have to take part in this study or authorize use and disclosure of your health information, and your refusal to participate or your decision to withdraw will involve no penalty or loss of rights or benefits. If you decide to withdraw from the study, the study personnel may only use and disclose your health information already collected. If you decide to revoke your authorization to use and disclose your health information, you may not be allowed to continue in the study. The study personnel may choose to stop your participation at any time.
- You understand why the study is being conducted and how it will be performed.
- You understand your rights as a research participant and you voluntarily consent to
participate in this study. You also consent to use of your health information in this study.

- You have been told you will receive a copy of this form.

________________________________________
Printed Name of Participant

________________________________________  ____________
Signature of Participant                                      Date

**For the Principal Investigator or Designee:**

I certify that I have reviewed the contents of this form with the subject signing above. I have explained the possible benefits and the potential risks and/or discomforts of the study and the use and disclosure of health information. It is my opinion that the participant understood the explanation.

________________________________________  ____________
Signature of Principal Investigator or Designee                                      Date
APPENDIX C

AMENDED IRB CONSENT FORM
Informed Consent Form

University of North Texas Institutional Review Board

Before agreeing to participate in this research study, it is important that you read and understand the following explanation of the purpose, benefits and risks of the study and how it will be conducted. Signing this form also gives permission for use and disclosure of your health information as part of this research study.

Title of Study: Weight training: A treatment for muscle weakness from long-term alcohol addiction and infection with HIV

Principal Investigator: Jakob Vingren, Ph.D., University of North Texas (UNT) Department of Kinesiology, Health Promotion and Recreation.

Purpose of the Study: You are being asked to participate in a research study which involves investigating the effect of resistance training (weight training) on muscles in individuals with HIV and a recent history of long-term alcohol addiction.

Study Procedures: You will be asked to complete questionnaires and physical tests before and after a 7-week period. You will be asked to write down everything that you eat and drink during the 3 days leading up to the first testing session, you will be asked to repeat that diet for the 3 days leading up to the final testing session. Additionally, you must refrain from resistance exercise and intense endurance exercise for 3 days prior to each testing session. You also will be asked to write down your daily physical activity during weeks 1 and 6 of the study and your diet for 3 days during week 1 and 6. The questionnaires will be about social and psychological issues. While you are answering the surveys, you can take a break at any time to use the restroom or get some water, etc. The physical tests will include measurement of body size, muscle strength and we will take a blood sample from you. For the blood test you will need to fast overnight (no food or drink except water) for 12 hours. During the 7 weeks between the test sessions you will participate in either a resistance training (weight training) program 3 times per week (about 1 hour per session) or no resistance exercise. You will randomly be assigned to one of two groups (an exercise group and a non-exercise group). You must be willing to participate in either of the 2 groups to be eligible for this study.

If you are NOT diagnosed as HIV+, you will only participate in one rested and fasted blood draw and the body measurements but will not participate in the other testing or exercise described in this form.

Resistance training: The training will vary across sessions and weeks and include heavy, medium and light sessions.

Medical Screening: Before you start the study, you will fill out a medical history questionnaire to make sure you do not have medical conditions that might put you at risk during the testing procedures.

Follow-up questionnaires: 2-months after the completion of the study you will be asked to complete the questionnaires again.
**Time commitment:** The total time you will spend in this study will be approximately 27 hours total (21 hours of strength training, 3 hours of exercise and medical testing, and 3 hours of filling out questionnaires) if you are placed in the exercise group and 6 hours total (3 hours of exercise and medical testing, and 3 hours of filling out questionnaires) if you are placed in the non-exercise group. In addition, you will need a little time to write down your diet and physical activity. If you are NOT diagnosed as HIV+, your time commitment will be about 1 hour total.

**Foreseeable Risks:** The potential risks involved in this study are as follows:

*Exercise:* Even though the exercises you will do are designed to be very safe there is a slight risk that you may become injured. The researchers have extensive experience in conducting exercise-testing studies, and they will do everything possible to reduce the chance of injury. However, if at any time during exercise testing you experience pain, unexpected discomfort, soreness, headache, loss of concentration, dizziness, unusual fatigue or difficulty breathing you should immediately inform one of the supervising researcher team members, who will bring this to the attention of the principal investigators and the medical monitor.

Additionally exercise can entail a certain degree of risk from overexertion and/or accident. Minimal risks exist for muscle strain or pulls of the exercised muscles, muscle spasm, and in extremely rare cases, muscle tears. Some muscle soreness may be experienced 24 to 48 hours after exercise, and this should completely subside within a few days and have no long-lasting effect.

*Blood Draws.* While blood draws are normally a safe procedure, it is possible that short-term side effects can occur, such as dizziness, bruising, or fainting. Although the chances are remote, it is also possible that bruising around the vein, an infection, or nerve damage can develop. All possible precautions to avoid infection will be taken including use of sterile disposable needles, drapes and gauze and the practice of sterile techniques during blood sampling. All blood samples will be obtained by a person trained in drawing blood who will use standard laboratory operating procedures. In case of emergency, we will call 911. It is possible that the hunger from fasting prior to the blood draw might increase the chance of alcohol relapse. To minimize this risk we will collect the sample in the early morning so most of the fasting will be while you are sleeping.

*Questionnaires.* The topics addressed throughout the questionnaires may prove a source of emotional discomfort. Although this is unlikely to occur, the researchers will be vigilant to identify signs of emotional and mental distress. If you experience a significant amount of distress as result of the questionnaires, the testing will be postponed and the researchers will provide you with a list of local referral sources and we will inform the staff of Homeward Bound of your distress.

**Benefits to you or Others:** Although we cannot guarantee any benefit to you from participating in this study, we expect the project to teach you how to properly complete the exercises involved in the study. You will also learn about your body composition and strength, as well as how these change over the 7-week period. All of the data we collect on you will be explained and interpreted for you so that you can learn more about yourself. Should our findings be positive, what we learn may benefit the development of treatments in the HIV and alcohol addiction communities.
Compensation for participation: If you are placed in the exercise group and complete all aspects of the study you will receive gift cards to stores such as Wal-Mart in the amount of $200. If you are placed in the non-exercise group and complete all aspects of this study you will receive gift cards in the amount of $60. If you cannot or choose not to complete the study, you will receive partial compensation based on the time you have spent in the study. When you complete the 2 month follow up you will be entered into a drawing for one of four $50 gift cards. If you are in the no-HIV group you will not receive any compensation.

In addition, if you are placed in the non-exercise group you will be offered supervised training (identical to that of the exercise group) after you have completed the study. This training will be offered at the UNT campus in Denton, TX.

Procedures for Maintaining Confidentiality of Research Records: All data will be kept in coded participant files in the primary investigator's locked office. Participant codes will be used instead of your name to keep you information confidential. Only the research team will have access to the code book. The data files will be kept for 3 years after the study is terminated. The confidentiality of your individual information will be maintained in any publications or presentations regarding this study.

Questions about the Study: If you have any questions about the study, you may contact Dr. Jakob Vingren at telephone number (940) 565 3899.

Review for the Protection of Participants: This research study has been reviewed and approved by the UNT Institutional Review Board (IRB). The UNT IRB can be contacted at (940) 565-3940 with any questions regarding the rights of research subjects.

Research Participants’ Rights: Your signature below indicates that you have read or have had read to you all of the above and that you confirm all of the following:

- *Jakob Vingren, Ph.D or another researcher* has explained the study to you and answered all of your questions. You have been told the possible benefits and the potential risks and/or discomforts of the study. You have been told how my health information will be used and disclosed for the study.
- You understand that you do not have to take part in this study or authorize use and disclosure of your health information, and your refusal to participate or your decision to withdraw will involve no penalty or loss of rights or benefits. If you decide to withdraw from the study, the study personnel may only use and disclose your health information already collected. If you decide to revoke your authorization to use and disclose your health information, you may not be allowed to continue in the study. The study personnel may choose to stop your participation at any time.
- You understand why the study is being conducted and how it will be performed.
- You understand your rights as a research participant and you voluntarily consent to participate in this study. You also consent to use of your health information in this study.
- You have been told you will receive a copy of this form.

________________________________________
Printed Name of Participant

________________________________________

For the Principal Investigator or Designee: I certify that I have reviewed the contents of this form with the subject signing above. I have explained the possible benefits and the potential risks and/or discomforts of the study and the use and disclosure of health information. It is my opinion that the participant understood the explanation.

____________________________________                    ____________
Signature of Principal Investigator or Designee                    Date
APPENDIX D

BLOOD TESTING SHEET
ROP Study 2010

Blood Testing

Dr. Jakob Vingren – University of North Texas

ID_____________  ___Pre or ___Post testing  Date______ Time_______

Phlebotomist:________________________________________

Tester______________________________________________

Fasted: ___yes___no

Total Chol:____________

HDL: ____________

Trig: ____________

Calc. LDL: ____________

Glucose: ____________

Comments:
APPENDIX E

EXERCISE TESTING SHEET
ROP Study 2010

Exercise Testing

Dr. Jakob Vingren – University of North Texas

ID___________  __Pre or ___Post testing  Date_____  Time_______

Tester____________________________

Warm up:

___Dynamic Warm up

Knee extension: Back location:_____

Warm up: Load_____ lbs  Reps:____  Test: Load_____ lbs  Reps:____

Bench Press: Handle: ___Short or ___long

Warm up: Load_____ lbs  Reps:____  Test: Load_____ lbs  Reps:____

Isometric Squat: Bar position #: _____  Max force:_________

Isometric Grip: handle position: ____mm, 1st____kg  2nd____kg  3rd_______kg

Vertical jump: Jump height____  Max power_______  Max force_______

Comments:
REFERENCES


