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CREATINE PHOSPHOKINASE ELEVATIONS FOLLOWING
EXERCISE IN INDIVIDUALS INFECTED WITH THE HUMAN
IMMUNODEFICIENCY VIRUS

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By

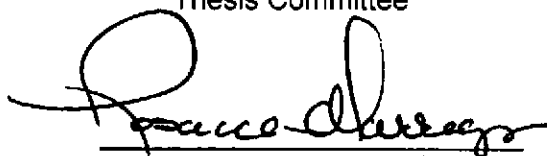
Larry John Day

Thesis Committee:

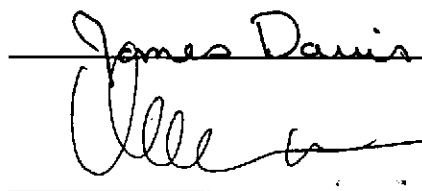
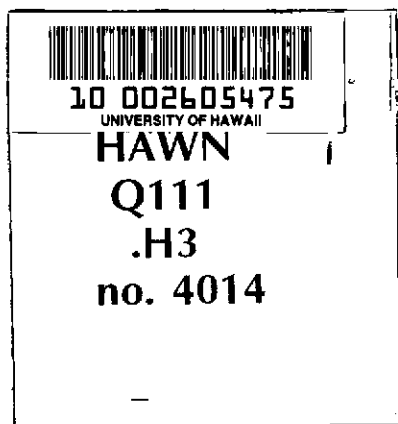
Rosanne Harrigan-Chairperson
Jim Davis
David Easa

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Biomedical Sciences.

Thesis Committee



Chairperson.



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ABSTRACT

Problem: Muscle disease is a well described consequence of HIV infection and its therapy. While symptomatic myopathy is rare, elevations in serum markers of muscle injury are seen more commonly. These elevations may represent subclinical muscle damage due to human immunodeficiency virus (HIV) infection and/or antiretroviral therapy. Provocative testing of muscle by resistance training may elicit abnormal patterns of the enzyme creatine phosphokinase (CK) in HIV-infected individuals with muscle abnormalities.

Methods: Thirteen untrained HIV-infected individuals on antiretroviral therapy and six age- and gender-matched HIV-seronegative controls took part in a single session of isokinetic leg exercise. The degree of CK elevation was assessed at several intervals post-exercise. The magnitude and duration of CK elevation was related to demographic, anthropomorphic, functional and HIV disease-related variables.

Results: The HIV-positive participants had lower body fat percentage and body mass index compared to controls, but comparable muscle mass and function. There were no significant differences between the HIV-positive participants and the control group with regard to baseline or peak CK values, nor were there differences in the time to peak CK values or to normalization post-exercise.

Conclusion: This feasibility study did not reveal significant variables correlating to CK elevations in those with HIV-disease. Further investigation utilizing a larger sample population may be warranted.

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LIST OF ABBREVIATIONS

AIDS	acquired immune deficiency syndrome
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AZT	zidovudine
CD4	cluster of differentiation four
CK	creatine phosphokinase
DLS	Diagnostic Laboratory Services
DNA	deoxyribonucleic acid
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HMG CoA	3-hydroxy-3-methylglutaryl coenzyme A
IU/L	international unit per liter
mEq/L	milliequivalent per liter
mg/dL	milligram per deciliter
mtDNA	mitochondrial deoxyribonucleic acid
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
PI	protease inhibitor
RNA	ribonucleic acid
SST	serum separator tube
ULN	upper limit of normal

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Muscle injury is an increasingly recognized phenomenon described in individuals infected with the human immunodeficiency virus (HIV). A spectrum of findings attributable to muscle abnormalities are seen, from asymptomatic elevations of muscle enzymes to clinical myopathy characterized by weakness, exertional intolerance and decreased muscle bulk. Whereas HIV-associated muscle wasting and opportunistic infections accounted for the majority of muscle disease prior to the advent of antiretroviral therapy, nucleoside reverse transcriptase inhibitors (NRTIs) used to treat HIV infection are probably responsible for most contemporary myopathy cases^{3,6}.

Antiretroviral-associated muscle damage:

Zidovudine (AZT), the first NRTI used in HIV treatment, is the most well described cause of myopathy. Both skeletal and cardiomyopathy have been attributed to its use. Abnormal muscle findings were more commonly seen with high-dose AZT monotherapy utilized prior to the availability of multi-drug highly active antiretroviral therapy (HAART). However, myopathy has also been described with lower doses of the drug included as a component HAART. Muscle disease has been attributed to other NRTIs as well, suggesting a class-specific mechanism causing muscle injury.

NRTIs function through limiting HIV ribonucleic acid (RNA) replication by inhibition of the viral enzyme reverse transcriptase. Unfortunately, NRTIs are not specific in their action, as they can also inhibit cellular deoxyribonucleic acid (DNA) polymerases. Many chronic NRTI toxicities, including myotoxicity and lipodystrophy, are thought to occur via mitochondrial dysfunction. Such dysfunction may occur via NRTI-mediated inhibition of mitochondrial DNA polymerase γ . This enzyme is responsible for mitochondrial DNA (mtDNA) replication. MtDNA encodes many of the proteins necessary for aerobic metabolism via oxidative phosphorylation; if this process is impaired, metabolically active tissue such as muscle may be damaged. Findings of mitochondrial myopathy, including “ragged red” muscle fibers with altered mitochondrial ultrastructure, and diminished mtDNA content in muscle biopsies of individuals with AZT-associated myopathy support this model of disease pathogenesis¹⁻⁴.

Creatine phosphokinase as a marker of muscle damage:

Damaged muscle may release intracellular enzymes into the circulation, providing a readily measurable surrogate of muscle injury. The most commonly utilized serum indicator of muscle damage is creatine phosphokinase (CK). CK is a dimeric enzyme which facilitates the transfer of high-energy phosphate groups from creatine phosphate to adenosine diphosphate, yielding creatine and

adenosine triphosphate (ATP). ATP is then utilized for the immediate energy needs of metabolically active cells.

Normally the enzyme is contained within the plasma membrane of cells, principally in skeletal muscle but also in cardiac muscle and the brain⁵. Damage to any of these organs may cause the enzyme to be released into the circulation, mediated either through cell death or increased membrane permeability. This release may be acute and severe, as in rhabdomyolysis following muscle ischemia, crush injury or heat stroke, and to a lesser extent, cardiac damage following myocardial infarction. Additionally chronic low-grade to moderate elevations in CK are seen in myopathies such as muscular dystrophy⁵.

Alternatively, aberrant rises in CK may only follow exertional activity in individuals with other forms of muscle disease, including some heritable mitochondrial disorders. The latter individuals may serve as a model for antiretroviral-associated CK abnormalities, given the known mitochondrial toxicity associated with chronic NRTI use.

Effect of exertion on CK levels:

However, the magnitude and duration of CK elevation in response to exertion varies considerably, even in healthy individuals. Patterns of CK release may differ according to age, gender, race, and baseline activity level^{9,10}. Among exercising individuals, the type of activity undertaken is also important. For a

given amount of work output, higher intensity exercise has a more dramatic effect on CK release than lower intensity exercise over a longer duration.

Accordingly, resistance exercise generally creates a greater release of CK than aerobic endurance exercise⁵.

Additionally, the pattern of CK release varies with the mode of muscle contraction. In particular, eccentric muscle contraction in response to forced muscle lengthening, as occurs in downhill walking or lowering of weight against gravity, creates a delayed CK response. Such activity promotes sustained CK elevations up to 7-10 days following exercise¹¹⁻¹². This contrasts to the pattern of CK release following intense aerobic activity, with peaks seen within 24-48 hours and normalization after three to four days. Weight training primarily employs concentric muscle contraction, along with a minor eccentric component, which results in an intermediate time course of CK elevation. Finally, there appears to be a training effect whereby the magnitude of CK released following a given activity lessens over time as an individual repeatedly performs an exercise¹³⁻¹⁵. This latter observation is thought to result from physiologic adaptation in an exercising muscle, with enhanced blood flow and improved metabolic capacity, in part due to mitochondrial biogenesis.

HIV and CK elevation:

HIV-infected individuals appear to have CK elevations more commonly than the general population. These elevations may be intermittent or sustained, but not

consistently associated with myopathic symptoms. Among 875 HIV-seropositive subjects receiving HAART, prospectively followed over nine months, a 15% incidence of CK abnormalities was found. While most subjects had only an isolated CK elevation, thirty-three subjects had repeatedly abnormal values. Male gender and increased duration of the NRTI stavudine was seen more commonly in those with CK elevations, though other variables including age, overall length of antiretroviral therapy, CD4+ lymphocyte count, and HIV viral load were not significant. Additionally, symptoms such as muscle weakness and fatigue were not more prevalent among those with elevations in CK compared to those without CK abnormalities³. Another prospective study of nearly 700 patients revealed CK elevations of at least grade 3 (4-6 times the upper limit of normal) in 5 percent of subjects. A trend towards increased AZT use was seen in subjects with CK elevations. These elevations were most pronounced in regimens containing the combination of AZT and zalcitabine for twenty weeks or greater. However, symptoms of muscle damage again did not reliably correlate to CK abnormalities⁶. Furthermore, neither of these studies assessed the relationship between CK measures and differences in activity level among subjects.

HIV and exercise:

The effect of various forms of exercise in HIV-infected individuals has been studied in other contexts. Most evaluations have involved aerobic exercise

interventions, with a lesser emphasis on resistance training. A meta analysis of randomized controlled trials investigating the effects of aerobic training in HIV-seropositive subjects found moderate intensity exercise to be well tolerated, with beneficial effects on cardiopulmonary and psychologic status. No adverse effects on CD4+ lymphocyte count or virologic control was observed. Similarly, combined aerobic exercise and resistance training has been shown to improve cardiopulmonary status without compromising HIV controls¹⁶. Individual studies employing less rigorous methodology have shown exercise-related improvements in serum lipids, blood pressure and body composition (primarily decreased abdominal fat), as well as slowing disease progression to acquired immune deficiency syndrome (AIDS)^{17,18}. Furthermore, the combination of resistance training and androgen therapy has been shown to improve muscle strength and lean body mass in hypogonadal and eugonadal HIV-infected individuals^{19,20}.

Thesis Statement:

NRTIs likely contribute to low-level myopathy in a portion of HIV-infected individuals chronically on HAART. This may first become apparent through asymptomatic CK elevations at rest or aberrant patterns of CK release in response to exertion. While symptomatic muscle disease can result in significant debility and limit therapeutic options for the suppression of HIV, the

consequences of intermittent or sustained muscle enzyme elevations in asymptomatic HIV-infected individuals are unknown. Conceivably these markers may be the first indication of a subclinical myopathy, which could potentially be addressed before symptoms develop.

Muscle damage following resistance exercise (weight training) is commonly seen and may be necessary for strength gains and muscle growth. This study was performed to clarify the patterns of CK release following a single session of moderate intensity resistance training in HIV-infected individuals and healthy volunteers, and to relate these patterns to strength, muscle bulk, and exercise capacity. The information gained from this study will serve as the basis for the development of individualized progressive resistance training regimens, which may improve the muscle abnormalities associated with HIV and its therapy.

CHAPTER 2: METHODOLOGY

Design:

This pilot study explored the patterns of serum CK elevation following a single session of resistance exercise in untrained individuals. Two groups of untrained subjects were studied; an HIV-seropositive group and a HIV-negative control group. Participants were considered untrained if they exercised less than two days a week and had not taken part in weight training in the preceding six months.

There were three study visits and five blood draws per subject. Blood draws either took place at Le'ahi Hospital or a satellite of Diagnostic Laboratory Services, Incorporated. The initial visit at Le'ahi Hospital reviewed the study details with the subject and provided informed consent for study participation (Appendix A). Past medical history and current medication use, including herbals and over-the-counter drug use, were reviewed. The use of alcohol and illicit substances was also determined by subject report. For the HIV-seropositive subjects, additional history pertaining to antiretroviral use was elicited, as was the subject's most recent CD4+ lymphocyte count and viral load. Finally, activity level (type and frequency of exercise) was determined for all subjects.

A brief physical exam was performed to determine suitability for the exercise requirements of the study. Review of systems emphasizing weakness, exertional

fatigue, and muscle soreness was determined for each subject. A blood draw for baseline CK, chemistries including renal function, and venous lactate was also performed at the initial visit. Finally, anthropomorphic measures (height, weight, waist and hip circumference) and body composition by Dual Energy X-ray Absorptiometry (DEXA) was obtained in participants suitable for study inclusion.

Participants were excluded from the protocol if they had a history of medical illnesses (cardiac, thyroid, rheumatologic, renal, neurologic, muscle, or other severe disease) which would limit their exercise tolerance or took medications or substances associated with CK abnormalities (Figure 1).

Table 1. Exclusionary Substances/Medications

Current illicit substance abuse	
Alcohol in excess of 20g/day	
Anabolic steroid or ergogenic compounds (i.e. creatine)	
<u>Myopathic Medications</u>	
Amiodarone	Lipid-lowering agents
Antibiotics	HMG CoA reductase inhibitors
Daptomycin	Fenofibrates
Corticosteroids (systemic)	Niacin
Colchicine	Isoniazid
Chloroquine/hydroxychloroquine	Pentamidine
D-penicillamine	Lamotrigine
Beta Blockers	Valproic Acid
Cimetidine	Neuroleptics/Phenothiazines

HIV-seropositive subjects also were required to be taking stable NRTI-containing antiretroviral therapy. For the purposes of the study, stable was defined as maintaining HAART for at least three months prior to study's entry. CD4+ lymphocyte count was required to be greater than or equal to 200×10^6 cells/L and viral load less than 1000 copies/mL within 3 months of screening to qualify for study inclusion.

The second visit took place at the University of Hawaii at Manoa in collaboration with the Department of Leisure Science and Kinesiology. The subjects' strength was quantified through determination of the maximal torque generated during dominant leg knee extension and flexion utilizing a Biodex System 3 exercise machine (Biodex Medical Systems, Inc., Shirley, NY).

A moderate intensity exercise regimen employing isokinetic resistance was utilized. This type of training provides variable resistance at a constant velocity to accommodate changes in muscle strength due to mechanical variability throughout the range of motion for a given exercise. It involves both concentric and eccentric components of muscle contraction. A moderate intensity resistance protocol was utilized for knee flexion and extension consisting of 3 sets at a rotational velocity of 180 degrees/sec. Each set was limited to the number of repetitions that could be completed at $\geq 50\%$ of the participant's baseline power; the participant continued the exercise until they could not

generate 50% of their starting maximal power. Subjects rested 2 minutes between sets, according to the progressive resistance exercise concept originally proposed by DeLorme *et al*²¹. CK values were drawn 24, 48, 96, and 148 hours following the resistance exercise. A single repeat venous lactate value was obtained 24 hours after exercise as well.

The final study visit occurred after the last CK blood draw, approximately one-week post-exercise. Subjects were assessed for residual muscle soreness, weakness, or other complications of the protocol by patient report. The subjects were compensated \$60 for their time and inconvenience related to participation in the study. This amount was paid to the participants in three \$20 increments following the three study visits.

The study was reviewed and approved by the University of Hawaii's institutional review board.

Sample:

The subjects consisted of untrained HIV-infected subjects on NRTI-containing HAART and HIV-seronegative controls. HIV-infected adults (age >18) were accrued through the patients and subjects attending the Clint Spencer Clinic and AIDS Clinical Research Program at Le'ahi Hospital in Honolulu, Hawaii. Age- and gender-matched controls were recruited from students at the University of

Hawaii at Manoa and Kapiolani Community College. Additionally, seronegative control subjects were recruited at Le'ahi Hospital.

Measures:

Demographic variables including age, gender, and race were recorded for all participants. HIV-related variables including CD4 count, current antiretroviral medications and duration of antiretroviral therapy were also determined. Lipodystrophy, defined as body fat maldistribution among HIV-infected participants, was also determined by participant and examining physician report (See Appendix B for complete data collection sheet).

Subjects meeting eligibility criteria had whole body composition performed by DEXA scan at Le'ahi Hospital using a GE Lunar Prodigy Advance densitometer (GE Healthcare, Waukesha, WI). The protocol for whole body composition has been described elsewhere²². Briefly, DEXA machines are composed of a low-radiation source which emits x-rays at two different photon-energies from the generator to a scanning table, where subjects lie. The differential penetration of these x-rays through the body allows for accurate calculation of body composition, subdivided into soft tissue (adipose and muscle) and bone density analysis. Whereas precision for bone mineral density has been calculated at less than 1%, variation in lean body mass (bone + muscle) may be somewhat greater, between 4.9 to 5.3%. Interobserver variation was minimized by the scans being performed by a single operator, the principal investigator. Body

composition analysis was accomplished using the standard software accompanying the DEXA, encore 2004 for Windows, version 8.1 (GE Healthcare, Waukesha, WI).

A Biodex System 3 machine was used for all portions of the exercise protocol. This machine provides individualized adjustments and impact free acceleration to minimize joint trauma from isokinetic resistance exercise. The machine has been clinically validated in a variety of settings including the management of movement disorders and other neurologic diseases, rehabilitation following orthopedic or sports injuries, and for the treatment of general deconditioning. Data for muscle torque were generated by the software included with the exercise apparatus (System 3 Advantage Software, Biodex Medical Systems, Inc., Shirley, NY). Normative values for this variable and a technical description of the machine are provided at the company's website (www.biodex.com).

CK assays were drawn on-site at Le'ahi Hospital or at the processing lab, Diagnostic Laboratory Services (DLS), Inc (Honolulu, HI). The assay employed was an automated paramagnetic chemiluminescent sandwich immunoassay utilizing the UniCel Dxl 800 Access® Immunoassay System (Beckman-Coulter, Fullerton, CA). Each specimen was collected in a serum-separating tube (SST). Baseline chemistries and lactate were also obtained through DLS. They were collected in SST and sodium fluoride-containing specimen tubes, respectively.

Statistics:

Mean CK values pre- and post-exercise among HIV-infected and healthy individuals were compared. The peak CK values and the duration of CK elevation were also evaluated. These values were also correlated to lower extremity muscle mass by DEXA, as well as quadriceps femoris muscle strength (peak torque). Other variables including demographic parameters, specific antiretroviral history and duration of HAART, and CD4+ lymphocyte count were assessed for correlation to CK elevations. Means of continuous variables were compared utilizing paired t tests, while dichotomous variables were compared using chi-square methodology. The threshold for statistical significance was set at $p = 0.05$. All database preparation and analysis was carried out using SAS statistical software version 9 (SAS Institute Inc., Carl, NC).

Chapter 3: RESULTS

Demographics:

There were a total of nineteen participants who met inclusion criteria for the study. Thirteen subjects were HIV-positive with six HIV-negative controls. The mean age was 43.3 years (range 32 - 62 years) with a standard deviation of 7.8 years. Sixteen (84%) of the subjects were male. Sixty-three percent of the participants were Caucasian, 21% were Asian, while the remainder were divided equally among African American, Pacific Islander, and mixed ethnicity. There were no significant differences between the HIV-positive and control group with regard to any of these demographic variables.

HIV disease variables:

Thirteen HIV-positive individuals were studied. The mean CD4 lymphocyte count was 528 cells $\times 10^6/\text{ml}$ (range 245-1061 cells $\times 10^6/\text{ml}$, standard deviation 256 cells $\times 10^6/\text{ml}$). The mean duration of antiretroviral therapy among these individuals was 36.9 months (range 12-75 months). The components of the participants' current HAART are listed in Table 1. The presence of lipodystrophy in five participants was not correlated to CD4 lymphocyte count, antiretroviral duration, or any specific medication used in the participants' current HAART. While not statistically significant, all three of the participants taking stavudine were noted to be lipodystrophic, however. By design, all of the HIV-infected participants had undetectable (less than 50 copies/mL) HIV RNA.

Table 2. Current HAART Components

Medication Class/Medication	Number Prescribed (Group n=13)
NRTI	13
Lamivudine	9
Zidovudine	8
Tenofovir	4
Stavudine	3
Abacavir	1
Didanosine	1
Emtricitabine	1
PI	8
NNRTI	5

Laboratory and Body Composition/Anthropometric Measures:

Baseline laboratory evaluation of blood was performed prior to participation in the exercise portion of the protocol. The mean lab values, anthropometric measures and body composition findings are listed in Table 2. Compared to controls, HIV-infected participants had slight elevation of their liver function tests, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). These values were only slightly higher than the upper limit of normal (ULN) for the assays, however (38 and 41 IU/L, respectively). Renal function and baseline lactate values were

not different between the study groups. Three participants (all HIV-positive) had mild elevation in baseline lactate values, less than 2 times the ULN (2.2 mEq/L). These values remained mildly elevated twenty-four hours post-exercise in all three participants. Additionally three other subjects (2 HIV-positive) with normal baseline values had lactate elevations of similar magnitude twenty-four hours post-exercise.

Table 3. Mean Lab Values and Body Composition/Anthropometrics

Measure	HIV+ (n=13)	HIV- (n=6)	p value
<u>Laboratory</u>			
AST (IU/L)	42.8	24	0.02
ALT (IU/L)	55.8	31.7	0.06
Creatinine (mg/dL)	0.9	1.0	0.45
Lactate (mEq/L)	1.5	1.4	0.54
<u>Body Composition/Anthropometrics</u>			
Body Fat (%)	25.4	34.4	0.02
Leg Muscle Mass (grams)	8029.5	9508.5	0.08
BMI	25.2	30.7	<0.01
Waist:Hip Ratio	1.0	1.0	0.32

Body composition was different between HIV-infected participants and controls. Compared to HIV-negative participants, subjects with HIV had statistically lower BMIs and body fat percentage. However, only one of the HIV-positive participants was underweight (BMI < 18.5) with a BMI of 18. Nine subjects (47%) were overweight (BMI > 25) and four (21%) were obese (BMI >

30, three HIV-negative). Additionally, there was a trend towards less muscle mass in the legs of HIV-infected subjects.

The findings of the resistance training portion of the protocol are summarized in Table 3. Although HIV-positive participants generated less torque for both leg flexion and extension, HIV-negative subjects fatigued earlier (fewer set three repetitions). However, none of these differences were statistically significant.

Table 4. Mean Resistance Training Values

Parameter	HIV + (n=13)	HIV- (n=6)	p Value
<u>Peak Torque (foot-pounds)</u>			
Extension	95.4	124.7	0.15
Flexion	54.2	70.2	0.15
<u>Repetitions</u>			
Set 1	29.8	25.0	0.17
Set 2	23.1	21.2	0.52
Set 3	19.2	17.0	0.37

CK values were assessed at five points during the experimental protocol. At baseline, there were no differences between the study groups. Four participants had elevated CK values at baseline, all of which were HIV-positive. Of note, two of these were lipodystrophic. Additionally the magnitude and timing

of peak CK values did not differ between groups. Finally the time to CK normalization (less than 232 IU/L) was not significantly different between HIV-positive and control participants. Six subjects (four HIV-positive, one lipodystrophic) did not normalize their CK values one week after resistance training, although all were down trending at the last blood draw. These data are summarized in Table 4.

Table 5. Characteristics of CK Values

Parameter	HIV + (n=13)	HIV - (n=6)	p Value
CK (IU/L)			
Baseline (Mean)	210.9	146.3	0.26
Range	47-567	96-213	—
Maximum (Mean)	874.5	505.2	0.31
Range	60-4257	176-908	—
Time to Maximum (Hours)	38.7	32	0.65
Time to Normalization (Hours)	97.8	100	0.95

A frequency distribution of maximal CK measurements, stratified by HIV status is shown in Figure 1.

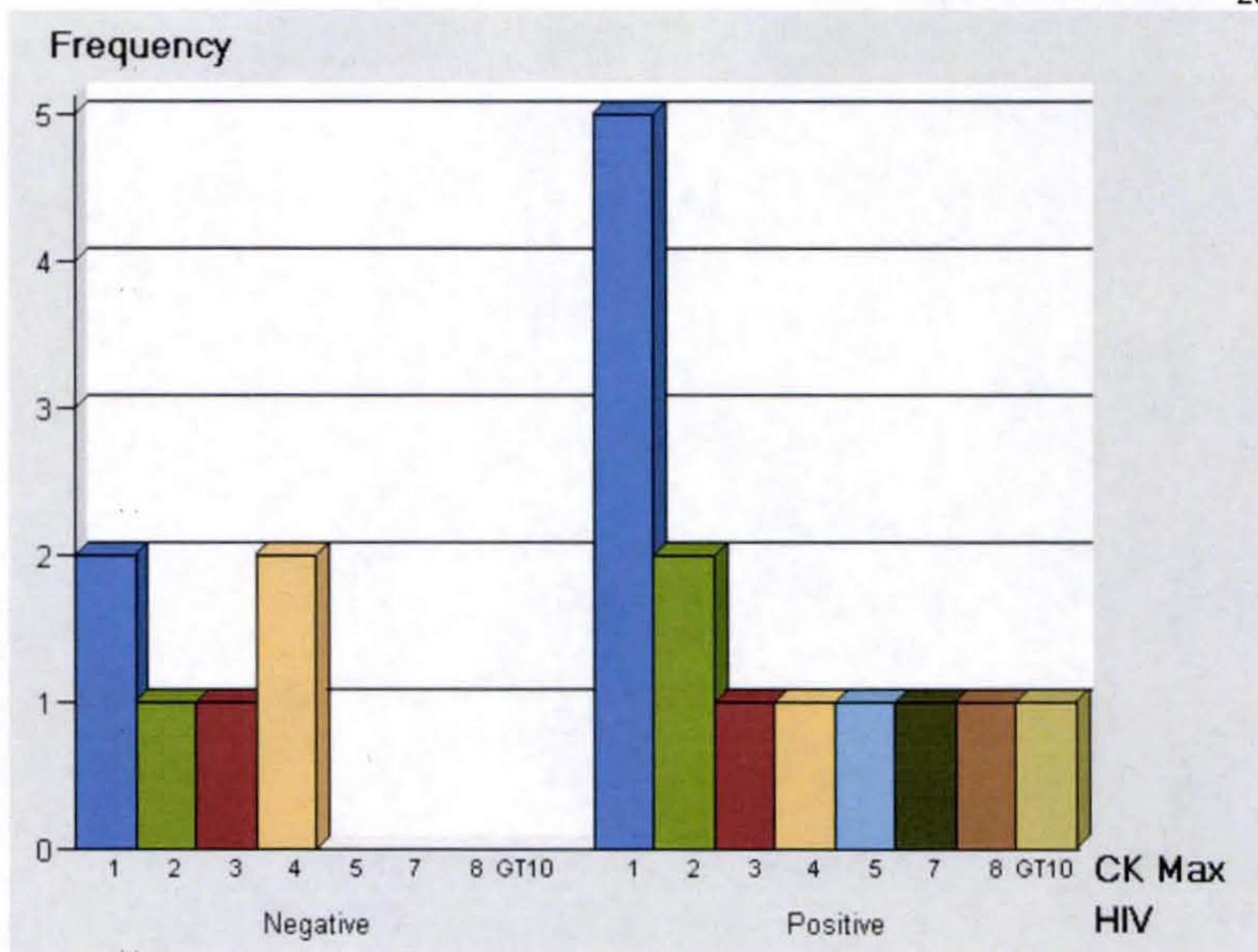


Figure 1. Frequency of Maximal CK Measurement

Maximal CK values are listed as a factor of the ULN (232 IU/L). HIV-negative participants had a smaller range of measured CK values, all of which were less than four times the ULN. In contrast, four HIV-positive participants had maximal CKs greater than four times the ULN. Subset analysis of this outlying group did not find statistically significant differences between it and the remaining study population, however. Timing of maximal CK measurement, plotted according to frequency and HIV status, was similar for both groups as well, as seen in Figure 2.

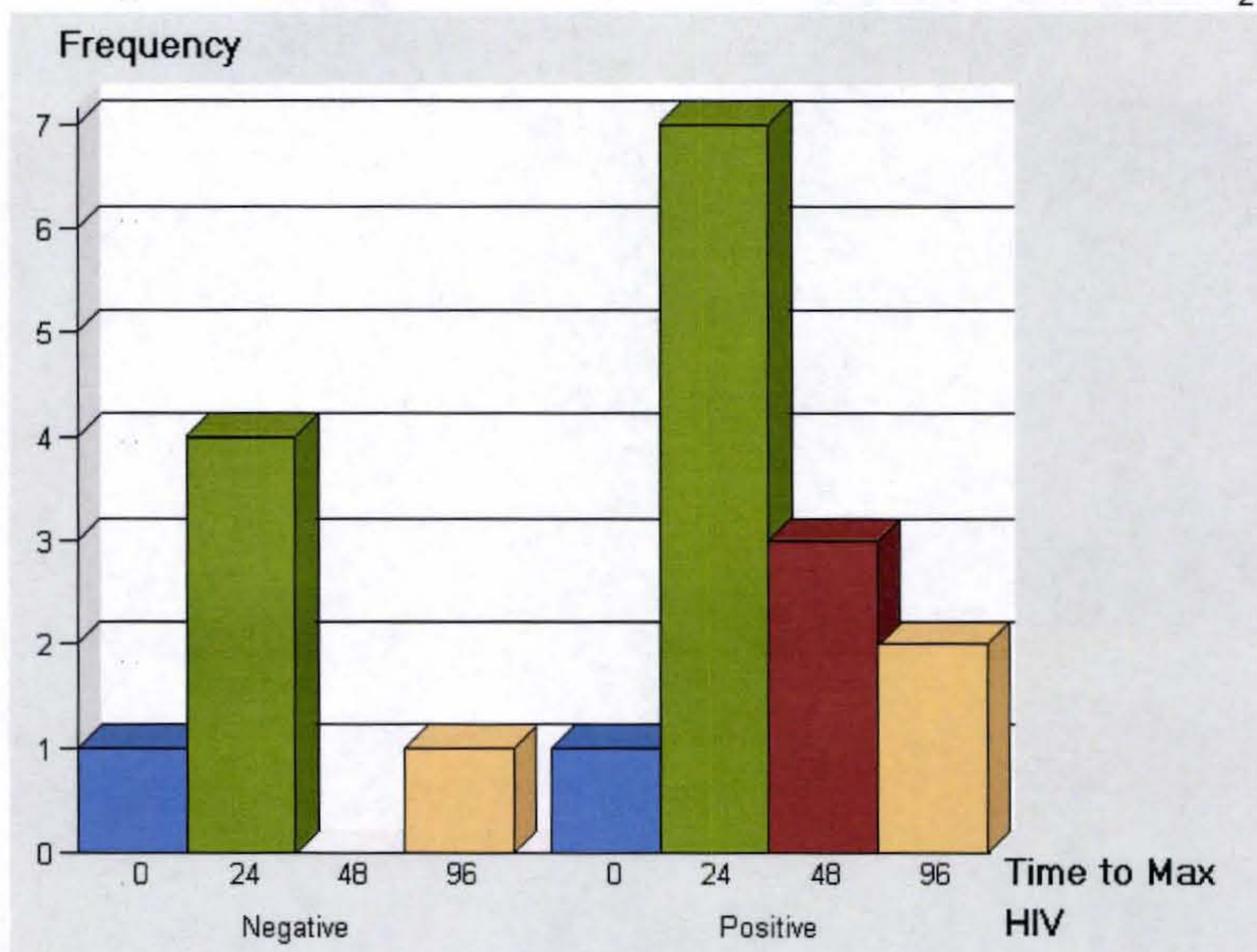


Figure 2. Timing of Maximal CK Measurement

Finally, the frequency distribution for the time to CK normalization is shown in Figure 3. A bimodal pattern of CK normalization is seen in both HIV-positive and the control groups. The first peak occurred within 24 hours post-exertion. One-third of the HIV-negative participants had normal CK values in this time period, while 38% of the HIV-infected subjects did the same. Of the remaining participants, approximately one-half in each group took one week or longer for CK values to normalize post-exertion.

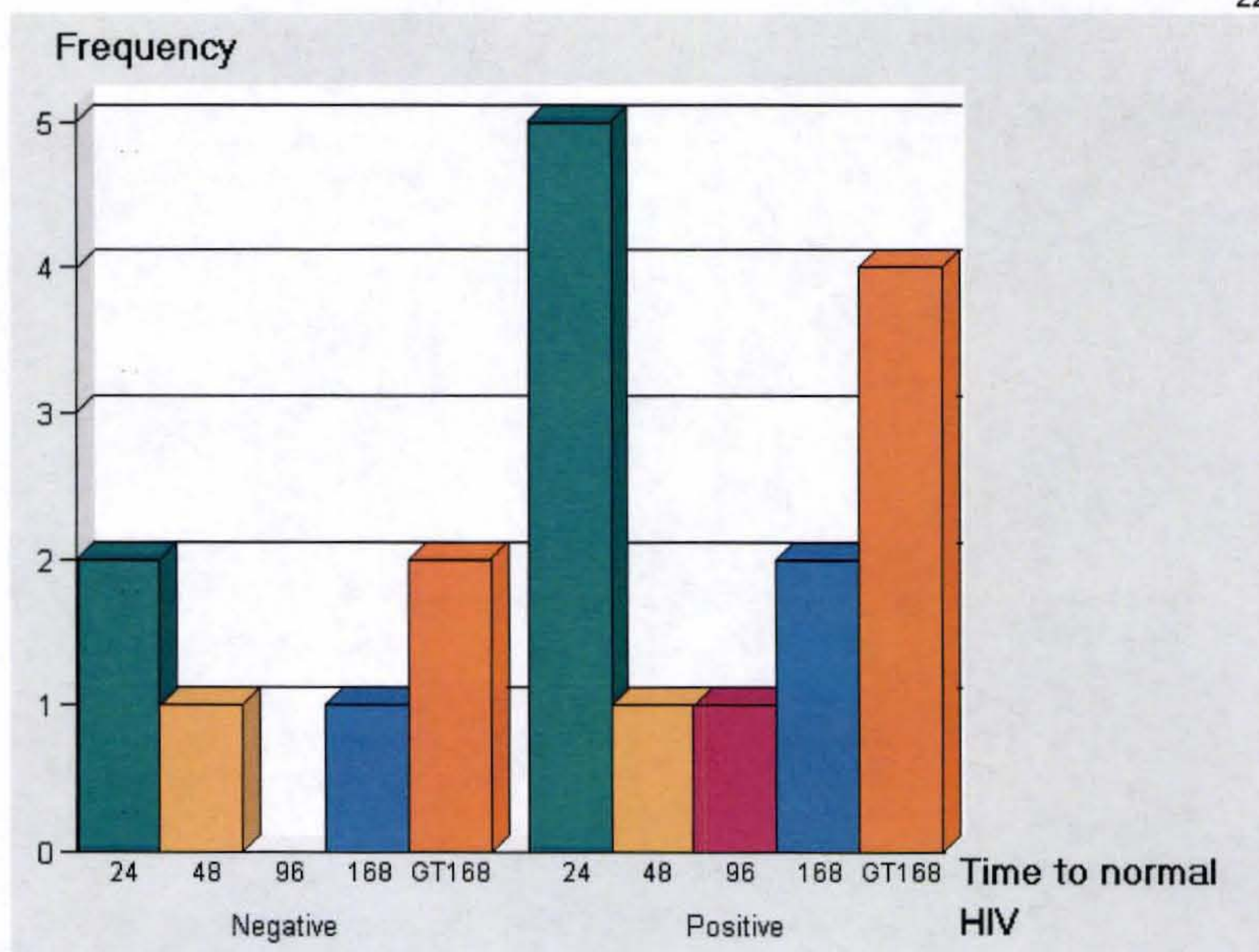


Figure 3. Time to CK Normalization

Chapter V: Discussion

The introduction of HAART has dramatically changed the course of HIV infection. Suppression of viral replication and immune preservation has limited the impact of opportunistic infections and progression to AIDS. Meanwhile, antiretroviral medication toxicity has become a more important contributor to disease-related morbidity and mortality. Myopathy characterized by muscle pain, weakness, and fatigue is one condition which is thought to result from chronic HAART toxicity. NRTI-mediated mitochondrial dysfunction may underlie its development.

Independent of HAART, HIV infection *per se* can also affect muscle. HIV wasting syndrome, defined as involuntary loss of greater than 10% of one's body weight, is an AIDS-defining illness. It can occur in antiretroviral-naïve or antiretroviral-treated individuals. The weight loss is principally lean muscle tissue, as opposed to the fat loss seen in antiretroviral-associated lipoatrophy. Wasting syndrome remains common in the HAART era, seen in up to 18% of HIV-infected individuals. Moreover, the syndrome can persist despite adequate virologic suppression by HAART.

In the absence of symptomatic myopathy or wasting syndrome, the prevalence of subclinical muscle disease in HIV-positive individuals is unknown. Unexplained elevations of enzymes indicative of muscle injury have been described in HIV-infected patients with few or no symptoms, suggesting the possibility of

undiagnosed muscle abnormalities. The goal of this study was to define differences between HIV-infected subjects and HIV-seronegative controls with regards to blood levels of the muscle enzyme CK. A moderate intensity session of resistance exercise was utilized to stress the subjects' muscles beyond their baseline activity level, with the assumption that those with incipient muscle dysfunction may manifest differences in post-exertional CK release.

In the studied population of untrained individuals with well-controlled HIV disease on stable antiretroviral therapy, no clear differences in the pattern of CK release following resistance training was seen. Though the HIV-infected participants were leaner with less leg muscle bulk than their seronegative counterparts, muscle strength and endurance were similar. At the extremes, the HIV-infected participants had the highest absolute CK measures, though these differences were not attributable to body composition differences, peak torque generation, or HIV disease-specific variables such as CD4+ lymphocyte count or HAART components. Moreover, there were no significant symptoms attributable to these CK elevations, with all participants denying fatigue or muscle soreness within 24 hours post-exercise.

The time to peak CK measurement is consistent with other studies assessing the enzyme's value following resistance training; most peak values were obtained within 24-48 hours after exercise with no difference between groups. The bimodal distribution of time to CK normalization likely reflects increased time for

CK clearance, as all ten participants with peak CK values greater than two times the ULN required one week or greater to normalize. Conversely, none of the patients with lower CK peaks required greater than ninety-six hours to return to normal, with most normalizing after the 24-hour blood draw. Alternatively, it is possible that those with prolonged time to CK normalization continued to release the enzyme from damaged muscle, though the lack of muscle soreness one day post-exercise suggests this is not the case.

If mitochondrial damage underlies antiretroviral-associated myopathy, one might expect those with other evidence of mitochondrial dysfunction to exhibit abnormalities in CK release post-exercise. Lipodystrophy and lactic acidemia are two well-described consequences of NRTI-mediated mitochondrial dysfunction, though additional factors also contribute to their pathogenesis. Among the studied individuals, the five lipodystrophic subjects had no statistically significant differences in their CK release characteristics or muscle function compared to the non-lipodystrophic HIV-infected individuals or controls. As noted previously, two of four participants with abnormal baseline CK measurements were lipodystrophic, though this finding did not correlate with other variables suggestive of muscle abnormality. Interestingly, both the highest peak CK value (4257 IU/L) and two of the three lowest peak values occurred among lipodystrophic participants. Also of note, all three of the participants taking stavudine reported lipodystrophy, consistent with this drug's well-described association with mitochondrial dysfunction and fat wasting.

Lactate is a product of anaerobic metabolism increased in those with severe mitochondrial dysfunction. Among those with elevated lactate values, no correlation to CK magnitude or release characteristics was found. Additionally, hyperlactatemia was not associated with lipodystrophy. Liver function test abnormalities, likely due to direct HAART toxicity and/or hepatic steatosis, may also be associated with mitochondrial dysfunction. Although the underlying etiology for the mild liver function abnormalities in the HIV-infected participants was not clarified, there was no association between these tests and CK values.

There are several limitations which may help to explain the study findings. Perhaps most importantly, the sample size for this feasibility study may not have been large enough to detect meaningful differences between the study groups. As standardized values for the mean and standard deviation of CK assays following exercise are not available, power analysis could not be calculated. Inclusion of subjects with lipodystrophy, in whom a suspicion for mitochondrial dysfunction existed *a priori*, was pursued under the assumption that these individuals would have a higher likelihood of muscle mitochondrial dysfunction. Although different mitochondrial toxicities may coexist within the same individual, they are not always found together. This may be due to differential tissue penetration and metabolism of individuals NRTIs, as well as differences in genetic susceptibility to specific antiretroviral toxicities. It is also possible that CK assays may not be sensitive or specific enough to discern subtle muscle

abnormalities. Although this assay was selected for its clinical familiarity as a marker of muscle damage, others have argued the utility of other markers including aldolase. Finally, the role of uncontrolled HIV infection and muscle disease could not be assessed from the study participants, as all subjects had undetectable viral loads by virtue of the study design. As previously discussed, muscle disease including wasting syndrome has been described in those with good virologic control, such that HIV RNA level was thought to be less important in disease pathogenesis.

Conclusion and future direction

Muscle disease remains an important complication of HIV infection and its therapy. Little is known concerning the early events leading up to symptomatic muscle disease, when intervention may prevent its progression. Creatine phosphokinase abnormalities seen in some HIV-infected individuals may serve as a harbinger of clinical myopathy. Although the present study did not find significant morphologic, functional, or HIV-associated disease correlates of elevated CK values, future investigation of this common lab abnormality is probably warranted. A larger study population including those with symptomatic myopathy may clarify the utility of CK as a surrogate of muscle injury. Once individuals with preclinical myopathy are identified, targeted interventions including adjustment of myotoxic medications and preemptive strength training may limit the impact of muscle damage on functionality.

Appendix A: Data Collection Sheet

Patient ID Number – _____ DOB – _____ Date – _____

Gender - ☐ M ☐ F Ethnicity - ☐ Hispanic ☐ Non-Hispanic

Race - ☐ Caucasian ☐ African-American ☐ Pacific Islander

☐ Asian (Specify) _____ ☐ Mixed (Specify) _____

HIV Status ☐ Positive ☐ Negative Date of Diagnosis _____

CD4 + (Date) _____ Viral Load (Date) _____

Past Medical History/Review of Systems _____

Current Medications (Include duration) _____

Stable HAART x 3 months? ☐ Yes ☐ No

Exclusionary Medications? ☐ Yes ☐ No

Alcohol Consumption? ☐ Yes, Quantify _____ ☐ No

Exercise? ☐ Yes, Frequency and Type _____ ☐ No

PE – Vitals: BP _____ P _____ R _____ T _____ Waist, Hip Circ. _____

Lipodystrophy? ☐ Yes ☐ No

CV ☐ NI ☐ Abnl Pulm ☐ NI ☐ Abnl

MSK ☐ NI ☐ Abnl Neuro ☐ NI ☐ Abnl

Other ☐ NI ☐ Abnl _____

Abnormal Findings: _____


Appendix B: Informed Consent

SEP-21 1990 17:40 10/01/01 02:10
UNIVERSITY OF HAWAII
 Committee on Human Studies

MEMORANDUM

January 12, 2005

TO: Larry Day, M.D.
 Principal Investigator
 Hawaii AIDS Clinical Research Program

FROM: William H. Dendle 
 Executive Secretary

SUBJECT: CHS # 13293, "Creatine Phosphokinase Elevations Following Exercise in Individuals Infected with the Human Immunodeficiency Virus"

This acknowledges receipt of your email response dated January 9, 2005, to the recommendations made by the Committee on Human Studies during its review of this project at its meeting of November 16, 2004. This information satisfactorily addresses the CHS concerns.

On behalf of the Committee, your project as revised, is granted approval for one year effective November 16, 2004.

If, during the course of your project, you intend to make changes which may significantly affect the human subjects involved, you should obtain CHS approval prior to implementing these changes. Any unanticipated problems related to your use of human subjects must be promptly reported to the CHS. The CHS may be contacted through its office. This is required so that the CHS can update or revise protective measures for human subjects as may be necessary. In addition, under the University's Assurance with the U.S. Department of Health and Human Services, the University must report certain situations to the federal government. Examples of these reportable situations included deaths, injuries, adverse reactions or unforeseen risks to human subjects. These reports must be made regardless of the source of funding for your project.

In accordance with the University policy, you are expected to maintain as an essential part of your project records, any records pertaining to the use of humans as subjects in your research. This includes any information or materials conveyed to, and received from, the subjects, as well as any executed forms, data and analysis results. These records must be maintained for at least three years after project completion or termination. If this is a funded project, you should be aware that these records are subject to inspection and review by authorized representatives of the University, State of Hawai'i, and the federal government.

Please note that the CHS approval cannot exceed one year. If you expect your project to continue beyond this approval period, you must submit continuation applications to the CHS for renewal of CHS approval. CHS approval must be obtained and maintained for the entire term of your project or award.

SEP-11-2005 11:45

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Larry Day, M.D.

Page 2

January 12, 2005

Please notify this office when your project is completed. We may ask that you provide information regarding your experiences with human subjects and with the CFIS review process. Upon notification, we will close our files pertaining to your project. Any subsequent reactivation of the project will require a new CHS application.

Please do not hesitate to contact this office if you have any questions or require assistance. We will be happy to assist you in any way we can.

Thank you for your cooperation and efforts throughout this review process. We wish you success in this endeavor.

Enclosure

SEP-21-2005 17:40

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SEP 23 2005 P.04:05


UNIVERSITY OF HAWAII

Committee on Human Studies

MEMORANDUM

January 12, 2005

TO: Larry Day, M.D.
Principal Investigator
Hawaii AIDS Clinical Research Program

FROM: William H. Dendle 
Executive Secretary

SUBJECT: CHS #13293 - "Creatine Phosphokinase Elevations Following Exercise in Individuals Infected with the Human Immunodeficiency Virus"

The proposed revisions to the protocol, for the project identified above, as explained in your email received January 10, 2005, were reviewed by the Chair of the Committee on Human Studies through Expedited Review procedures. The proposed changes qualify for expedited review by CFR 46.110 AND 21 CFR 56.110, Category (b) of the DHHS list of expedited review categories.

These revisions were approved on January 11, 2005, for the current approval period. Please ensure that these revisions replace the previous version approved by the Committee. Should future revisions be considered, please contact this office for guidance as to whether Committee approval is required.

Thank you for your cooperation, and please do not hesitate to contact this office at 539-3955 if you have any questions or require assistance.

SEP-21-2005 10:44

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UNIVERSITY OF HAWAII

Committee on Human Studies

MEMORANDUM

January 20, 2005

TO: Larry Day, M.D.
Principal Investigator
Hawaii AIDS Clinical Research Program

FROM: William H. Dendle 
Executive Secretary

SUBJECT: CHS #13293 - "Creatine Phosphokinase Elevations Following Exercise in Individuals Infected with the Human Immunodeficiency Virus"

The proposed revisions to the consent form, for the project identified above, as explained in your email received January 18, 2005, were reviewed by the Chair of the Committee on Human Studies through Expedited Review procedures. The proposed changes qualify for expedited review by CFR 46.110 AND 21 CFR 56.110, Category (b) of the DHHS list of expedited review categories.

These revisions were approved on January 19, 2005, for the current approval period. Please ensure that these revisions replace the previous version approved by the Committee. Should future revisions be considered, please contact this office for guidance as to whether Committee approval is required.

Thank you for your cooperation, and please do not hesitate to contact this office at 539-3955 if you have any questions or require assistance.

SEP-21-2005 17:46

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UNIVERSITY OF HAWAII

Committee on Human Studies

MEMORANDUM

January 28, 2005

TO: Larry Day, M.D.
Principal Investigator
Hawaii AIDS Clinical Research Program

FROM: William H. Dendle 
Executive Secretary

SUBJECT: CHS #13293 - "Creatine Phosphokinase Elevations Following Exercise in Individuals Infected with the Human Immunodeficiency Virus"

The proposed revisions to the protocol, for the project identified above, as explained in your email received January 25, 2005, were reviewed by the Chair of the Committee on Human Studies through Expedited Review procedures. The proposed changes qualify for expedited review by CFR 46.110 AND 21 CFR 56.110, Category (b) of the DHHS list of expedited review categories.

These revisions were approved on January 27, 2005, for the current approval period. Please ensure that these revisions replace the previous version approved by the Committee. Should future revisions be considered, please contact this office for guidance as to whether Committee approval is required.

Thank you for your cooperation, and please do not hesitate to contact this office at 539-3955 if you have any questions or require assistance.

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