Title: The relationships among immune, endothelial function, and cardiometabolic markers in response to treatment with a polysaccharide in adults with HIV

Running Title: Immune, endothelial function, and cardiometabolic relationships and polysaccharide treatment

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Abstract

Introduction. People living with HIV (PLWH) are subject to many immune, endothelial function, and cardiometabolic insults. Rice Bran Arabinoxylan Compound (RBAC) is a nutritional supplement that is a potent immunomodulator. The purpose of this study was to assess the relationships among immune, endothelial function, and cardiometabolic markers in response to RBAC treatment in PLWH.

Methods. Forty-seven HIV positive adults on a stable antiretroviral therapy regimen were enrolled and randomly assigned to one of the two study conditions (n=22 RBAC and n=25 placebo) and consumed 3 gram/day of either for six months. Subjects were assessed at baseline and three and six months for immune, endothelial function, and cardiometabolic markers.

Results. No adverse effects were reported. Various significantly beneficial relationships were noted among immune, endothelial function, and cardiometabolic markers at 3 and 6 months in the RBAC group compared to placebo. For example, the CD4+/CD8+ ratio was positively correlated at 3 months with sudomotor function (NO sweat peak; r=0.50, p=0.043) and negatively correlated with cardiac autonomic neuropathy (CAN; r=-0.54, p=0.05) at 6 months in the RBAC group. At 3 months in the RBAC group, CD4+ was positively correlated with PTGi, the primary assessment of endothelial function (r=0.66, p=0.004), and total power, the main indicator of autonomic nervous system activity (r=0.54, p=0.03).

Conclusions. Our results showed important relationships among immune, endothelial function, and cardiometabolic markers in the RBAC group compared to placebo. Thus, the results of this study suggest that the immunomodulatory and anti-senescent activities of RBAC are promising for PLWH, who are subjected to accelerated aging and a host of medical complications.

Introduction

Over one million people are living with HIV (PLWH) in the United States. Once a lethal disease that was the leading cause of death among Americans aged 25 to 44 in the 1990s, HIV has now been transformed into a manageable chronic condition with advances in pharmacology, but still remains in the top 10 leading causes of death for this age group [1, 2]. Studies conducted before the widespread use of antiretroviral therapy (ART) suggest that HIV infection is associated with a pro-atherogenic lipid profile characterized by an increase in insulin resistance and triglyceride levels, a decrease in HDL cholesterol levels, and the presence of small, dense LDL particles [3-5]. The addition of ART, particularly the use of protease inhibitors (PI), has had further deleterious effects on cardiometabolic risk factors in this already vulnerable population. Specifically among PLWH, the initiation of PI-based ART is associated with the development of insulin resistance in 25-62% and the development of overt new-onset diabetes mellitus in 6-7% [6-8]. Increases in LDL cholesterol and triglyceride levels following ART have also been observed [9, 10]. Thus, PLWH on ART suffer from chronic microvascular disease and endothelial dysfunction, which lead to increased inflammation. As a result, an increased prevalence of many chronic conditions is seen in this population, including: metabolic syndrome [11, 12], insulin resistance and type 2 diabetes [6-8], dyslipidemia and hypertriglyceridemia [9, 10], and cardiovascular disease [13-15].

Endothelial dysfunction is recognized as a starting point for HIV-related atherosclerosis ultimately leading to cardiovascular events [16]. In the general population, the endothelium actively participates in both the innate and adaptive immune responses, including triggering pro- and anti-inflammatory responses and serving as antigen-presenting cells [17, 18]. The clinical detection of endothelial dysfunction is challenging, but given the elevated cardiometabolic risk

among PLWH on ART, development of such a screening tool is important. A validated, non-invasive device to detect early endothelial dysfunction would be invaluable in PLWH and allow for early intervention to prevent catastrophic cardiovascular complications in this population.

Therapeutic nutritional supplementation holds promise as an adjunct to ART in the treatment of HIV disease. A wide range of physiologic targets are described in the literature, outlining clear biologic plausibility of nutritional therapies in PLWH. For example, antioxidants enhance mitochondrial energy production, decrease the release of lactic acid into the bloodstream, and enhance T and B lymphocyte proliferation [19], which would counteract elevated reactive oxygen species, commonly known as oxidative stress [20]. Additionally, several *in vitro* and *ex vivo* studies have shown Rice Bran Arabinoxylan Compound (RBAC) to possess a biologic response modifier effect on immune system function, particularly in natural killer (NK) cell activity. One *in vitro* study showed RBAC blocked HIV-1 replication by inhibiting p24 antigen production in a dose-dependent manner [21]. Another study found significant increases in NK cell cytotoxicity compared to baseline, when a similar RBAC-based agent was administered orally to human subjects [22]. RBAC has also been shown to enhance macrophage phagocytic activity and nitric oxide release and scavenge free radicals in a dose-dependent manner. Thus, it may also function in an antioxidant capacity [23, 24]. In our lab, we showed that RBAC demonstrated true immunomodulation by enhanced NK cell cytotoxicity, significant changes in 9 out of 12 cytokines and growth factors, and safety and tolerability of the product among a sample of healthy adults [25].

Although nutritional supplementation offers promise as an adjunct to HIV therapy, we are aware of no study that has investigated the relationships among immune, endothelial function, and cardiometabolic biomarkers in response to treatment with a polysaccharide

nutritional supplement such as RBAC in PLWH. Thus, the purpose of this study is to determine the change in relationships among immune, endothelial function, and cardiometabolic markers in response to six months of RBAC treatment in PLWH.

Methods

Subjects. The study was conducted with the approval of the University of Miami Institutional Review Board for human subjects research (registry name: www.clinicaltrials.gov; registry number: NCT02214173; available at: https://www.clinicaltrials.gov/ct2/show/NCT02214173). Potential subjects were initially identified from physician referrals, the Medical Wellness Center, and the Departments of Psychiatry and Behavioral Sciences and Medicine at the University of Miami Miller School of Medicine, where the data were collected. Recruitment began in January 2015 and ended in October 2015, after sufficient subjects were enrolled. Inclusion criteria were: (a) age 18 or older; (b) confirmed HIV infection; (c) CD4+ T cell counts >50/μL and <250/μL; (d) on a stable ART regimen before (≤6 months) and during the intervention; (e) planning to maintain current medication during the course of the intervention; (f) not on any lipid-lowering agents for a minimum of 3 months before the enrollment; (g) previous nutritional supplement usage of similar polysaccharide formulas permitted, but must be discontinued 2 weeks before and for the duration of the trial; (h) willing to follow recommendations for assessment and intervention study protocol; and (i) able to provide informed consent. Exclusion criteria were: (a) currently enrolled in another research trial for similar investigative nutritional therapies; (b) known allergy to rice, rice bran, mushrooms, or related food products; (c) any gastrointestinal disorders that could lead to uncertain absorption of the study supplement; (d) other medical complications that

might preclude one from participating in the study, e.g., recent heart attack or stroke or chronic kidney disease; (e) currently taking immunomodulatory medication, e.g., interferon; (f) currently taking chemotherapeutic agents; (g) multiple drug resistance to ART; (h) current smoker; (i) severe anemia or other medical condition that would not permit a safe blood draw; (j) bleeding disorder; or (k) active pregnancy or attempting conception.

Seventy-three subjects were screened for inclusion and exclusion criteria. Forty-seven subjects met the criteria and were enrolled in the study after signing the informed consent and HIPAA privacy forms prior to study entry. The participants were assigned using a simple randomization procedure to one of two conditions: (a) RBAC or (b) placebo, using a random permutations table. All subjects and investigators were blinded to the treatment condition and remained blinded until after data analysis. Placebo and supplements were provided by Daiwa Health Development (Gardena, CA, USA) labeled as Protocol A and Protocol B. Only a staff member at Daiwa Health Development knew the assignment of treatment to Protocol A or B. After randomization, participants were scheduled for assessments at baseline and three and six months follow-up, and blood was drawn at each time point to assess the biological markers. Subjects were compensated $40 for completing the assessment at each time point. Ten participants dropped out of the study at 3 months, three more dropped out at 6 months, and thus 34 subjects completed the study.

Intervention. Participants enrolled in the study were randomly assigned to either (a) RBAC (n=22) or (b) placebo (n=25). Regardless of study arm assignment, subjects were instructed to take 2 capsules 3 times per day (3 g/day total) for the 6-month intervention period. Subjects were advised to not modify dietary or physical activity habits or prescription medication use. Subjects were also instructed not to consume any known immune-active pharmaceutical

agents or any nutritional supplements containing mushroom products for two weeks prior to having the baseline assessment and until the conclusion of the 6-month intervention period. Because of the way RBAC is produced by Daiwa Health Development, consuming this product is virtually no different than consuming rice bran and should be tolerated no differently than common foods. We are not aware of any documented side effects of RBAC, and our first study with this product showed no adverse events [25]. According to the company's literature, RBAC is a water soluble extract of rice bran that has been hydrolyzed by an enzyme complex extracted from shiitake mushroom. In addition, RBAC contains: microcrystalline cellulose, hypromellose, sucrose fatty acid ester, gellan gum, and potassium acetate. Each capsule contained 500 mg of RBAC. The placebo capsules were indistinguishable from the RBAC, but contained cellulose.

Outcomes and Assessments. Each participant completed a basic demographics and medical history questionnaire at baseline. Subjects were also asked to list their current medications and note any changes in type or amount during the course of the study. All outcome variables were assessed at baseline and at the end of 3 and 6 months (±1 week). Criteria used to select the assessment instruments included: (a) appropriateness for the population; (b) ease of administration and scoring; (c) experience administering these measures; and (d) employment of measures involving a multi-method (i.e., self-report and biological values) approach to enhance the validity of the overall assessment.

Immune Function. CD4+ and CD8+ T cell counts were obtained at each assessment.

Glucose, Lipids, and Triglycerides. Participants abstained from caffeine and alcohol consumption for 24 hours before testing, which was conducted in the post-absorptive state following an overnight (12 hour) fast between 8:00-10:00 AM. Ten mL of whole blood were

drawn into citrate and gel and lysis activator (serum) tubes. Blood clotted at room temperature for 30 minutes before centrifugation at 1,500 x *g*, followed by serum recovery. Platelet-poor plasma was prepared from citrate tubes by centrifuging at 3,000 x *g* for 30 minutes and the top 2/3 of plasma decanted. Total cholesterol and triglycerides were assayed using automated methods (Roche Cobas-Mira) and commercially available kits according to the manufacturer's instructions and run procedures. HDL cholesterol was assayed after removal of apoB-containing lipoproteins by polyanion precipitation [26]. LDL cholesterol was computed by a standardized method [27].

Endothelial Function. The ANS-1 (LD Technology, Inc., Miami, FL, USA) is an FDA cleared and patented system that contains three modules: (a) a sympathetic skin response (SSR) device, (b) a plethysmography sensor, and (c) a blood pressure device. The ANS-1 SSR module assesses sudomotor function by generating a low-voltage signal with a constant weak direct current that is fed to the active electrode. For a complete description of the technical specifications and algorithmic operations of the ANS-1 system, please refer to the details in our most recent paper [28]. In two separate studies in persons with type 2 diabetes and retinopathy, we showed that ANS-1 markers were highly correlated with glucose, insulin, and C-reactive protein, among others, and the system could discriminate between type 2 diabetes and retinopathy patients and healthy participants [28, 29]. We also demonstrated the system's accuracy for the assessment of body composition and cardiac output in additional studies [30]. Other investigators have found the ANS-1 to be accurate in detecting peripheral distal neuropathy symptoms [31] and insulin resistance [32]. Given all of the prior positive findings, the current study is the first attempt to use the ANS-1 system in PLWH to evaluate endothelial

function along with immune and cardiometabolic markers and to determine if these variables are interrelated.

As per standard protocol, every team member was trained by the manufacturer. Participants were instructed to sit in a chair in front of the ANS-1 unit, bare feet on the metal plates, right index finger in the pulse oximeter, and blood pressure cuff on the left arm. After entering demographic, anthropometric, and physical activity information, the assessment was initiated for 2 minutes while the participant was sitting down. Then, the participant was asked to perform the Valsalva maneuver by squeezing the nose with the left hand while trying to breathe out for 15 seconds with the mouth closed to build pressure. After releasing the nose, the participant was told to breathe deeply for 30 seconds, inhaling and exhaling for 5 seconds each. Finally, the participant was required to stand up until the assessment was completed, while straightening the left arm to the side and keeping the index finger in the pulse oximeter. The entire ANS-1 assessment lasted about 12 minutes.

Descriptive and Control Variables. Demographics such as age, race/ethnicity, socioeconomic status, education, employment status, and current living situation were assessed at baseline. Medical History. The basic health assessment questionnaire included past medical history with an emphasis on development of opportunistic infections, family history, and a review of systems. Information regarding history of infectious illness, respiratory diseases, diabetes, coronary artery vascular disease, oral diseases, history of cancer, and drug, alcohol, and tobacco use was obtained. At the follow-up visits, subjects were asked about the occurrence of opportunistic infections and hospitalizations during that time. ART-related effects were assessed at each visit, and current non-HIV related medications were documented. Past history of ART use was recorded and confirmed with medical records.

Adverse Events. Participants were monitored until the end of the study. Potential adverse effects were explained to each participant during informed consent.

Compliance was measured using a modified version of the 8-item Morisky Medication Adherence Scale (MMAS-8). MMAS-8 is a generic, validated, self-report measure of medication-taking behavior that does not target a specific age, disease, or treatment group.

Statistical Analyses. Frequency and descriptive statistics were calculated on all variables. Independent samples t-tests and chi-squares were utilized to evaluate differences in sociodemographic and clinical history characteristics between groups at baseline. Pearson product-moment correlations were used to assess the relationships between the immune, endothelial function, and cardiometabolic markers at each time point. SPSS 24 for Windows (IBM, Inc., Chicago, IL, USA) was used for statistical analyses, and $\alpha<0.05$ was considered statistically significant.

Results

Sociodemographics, Comorbid Disorders, and Medication Use. See Table 1 for the descriptive information of the sample for age, gender, race/ethnicity, education, and marital status, which were all non-significantly different between the RBAC and placebo groups. The most prevalent co-morbid conditions were depression (n=4 [8.5%] RBAC and n=12 [25.5%] placebo), hypertension (n=15 [32%]), anxiety (n=11 [23%]), and dyslipidemia (n=11 [23%]). The difference between groups for depression was statistically significant (χ^2=4.6 [1], p=0.03), whereas the differences for all other disorders were insignificant. Subjects were on the following HIV medication regimens (by drug categories): (a) two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI; n=17), (b) two

NRTIs and boosted PI (n=16), (c) two NRTIs and one integrase inhibitor (n=10), (d) two NRTIs, boosted PI, and one integrase inhibitor (n=3), and (e) boosted PI (n=1). The frequency of these regimens was not significantly different between the two groups ($\chi^2(4)=1.5$, p=0.83). Other commonly taken medications were: Crestor (n=4), Metformin (n=4), Lisinopril (n=4), Acyclovir (n=3), and Bactrim (n=3).

Compliance and Safety. According to the MMAS-8 total scores, 51.4% of the sample had medium to high compliance at 3 months, and 64.7% of the sample had medium to high compliance at 6 months. During the entire study period, no adverse event was reported by any subject.

Correlations among Immune, Endothelial Function, and Cardiometabolic Markers for Placebo Group. At baseline for the placebo group (See Table 2A.), HDL cholesterol was positively correlated with systematic arterial stiffness photoplethysmography (AIPTG; r=0.60, p=0.004) and inversely associated with plethysmography total power index (PTGi; r=-0.55, p=0.01). At 3 months (See Table 3A.), HDL cholesterol was positively correlated with PTGi (r=0.65, p=0.01) and inversely associated with stress index (r=-0.49, p=0.04). At 6 months (See Table 4A.), CD8+ was positively correlated with stress index (r=0.55, p=0.03) and cardiac autonomic neuropathy (CAN; r=0.68, p=0.04). CD4+/CD8+ was inversely correlated with sweat peak (r=-0.55, p=0.03).

Correlations among Immune, Endothelial Function, and Cardiometabolic Markers for RBAC Group. For the RBAC group at baseline (See Table 2B.), CD8+ was positively correlated with AIPTG (r=0.63, p=0.002) and cardiometabolic risk score (CMRS; r=0.45, p=0.04) and negatively correlated with PTGi (r=-0.57, p=0.01). CD4+/CD8+ was negatively correlated with AIPTG (r=-0.57, p=0.006) and positively correlated with PTGi (r=0.54, p=0.01). At 3 months

(See Table 3B.), CD8+ was positively correlated with stress index (r=0.50, p=0.04), CAN (r=0.56, p=0.02), and CMRS (r=0.49, p=0.05). CD4+ was positively correlated with total power (r=0.54, p=0.03), and PTGi was positively correlated with CD4+ (r=0.66, p=0.004) and HDL (r=0.53, p=0.03). CD4+/CD8+ was positively correlated with (nitric oxide) NO sweat peak (r=0.50, p=0.043). Stress index was positively correlated with total (r=0.52, p=0.03) and VLDL cholesterol (r=0.63, p=0.01) and triglycerides (r=0.63, p=0.01). At 6 months (See Table 4B.), CD8+ was positively correlated with CMRS (r=0.67, p=0.01). CD4+/CD8+ was negatively correlated with CAN (r=-0.54, p=0.05). AIPTG was positively correlated with total (r=0.75, p=0.001) and LDL cholesterol (r=0.66, p=0.01). Total cholesterol was negatively correlated with PTGi (r=-0.52, p=0.05). Stress index was positively correlated with VLDL cholesterol (r=0.63, p=0.01) and triglycerides (r=0.63, p=0.01).

Discussion

Studies indicate that PLWH taking ART are at increased risk of endothelial dysfunction and chronic inflammation, placing them at increased risk of metabolic syndrome, type 2 diabetes, and cardiovascular disease [6, 10, 14, 15]. Additionally, PLWH are a model of accelerated aging due to inmmunosenescence and a host of other physical factors.

In the current study, the CD4+/CD8+ ratio was positively correlated at 3 months with sudomotor function (NO sweat peak) and negatively correlated with cardiac autonomic neuropathy (CAN) at 6 months in the RBAC group. Neurological complications such as peripheral neuropathy have commonly evolved from the use of ART [33-35]. Although not widely researched, CAN has been shown in two studies to be at least partially attributed to HIV [36, 37]. To our knowledge, the current study is the first to show relationships between

CD4+/CD8+ ratio with sudomotor function (positive) and cardiac autonomic neuropathy (inverse) in response to nutritional supplementation with a polysaccharide. Thus, RBAC shows some ability to positively impact the relationship of CD4+/CD8+ ratio with both sudomotor function and cardiac autonomic neuropathy, which are important issues for PLWH regarding risks of type 2 diabetes and cardiovascular disease.

CD8+ count was consistently and positively correlated with CMRS, which is an overall indicator of cardiometabolic risk according to the ANS-1 assessment. RBAC may be initiating a process of immune surveillance linked to endothelial function that was not evident in the placebo group. This would be a novel effect of RBAC that has not been identified prior to this study. Previously, we showed another polysaccharide complex's effect on CD14+ count, while simultaneously lowering VEGF in subjects with Alzheimer's disease [38]. Thus, the current result may be similar in metabolic consequence, although we did not measure VEGF in this study, nor did we utilize the ANS-1 in the Alzheimer's study, so this similarity between studies is only theoretical at this point.

At 3 months in the RBAC group, we noted that CD4+ was positively correlated with PTGi, the primary assessment of endothelial function, and total power, the main indicator of autonomic nervous system activity, although these relationships did not hold at 6 months. Nonetheless, these findings at 3 months are important and consistent with the evolving interactions noted between the immune and endothelial systems [17]. Once again, the ability of RBAC to improve the relationship between these two organ systems in PLWH could be very significant for counteracting disease progress in this population. Another recent study showed little relationship between baseline CD4+ count or change in CD4+ count from baseline to four months and autonomic function after initiation of ART, with the exception of changes in high

and low frequency responses during the head-up tilt test [36]. To our knowledge, our study is the only one documenting a relationship between immune, endothelial, and autonomic function in response to a nutritional supplement of any kind, particularly this type of polysaccharide as RBAC.

Overall, we found significant relationships among immune, endothelial, and cardiometabolic markers in the RBAC group compared to placebo. These findings are significant, given that the relationships between chronic inflammation, i.e., immune dysregulation, resulting in endothelial dysfunction and atherosclerosis and the risk of type 2 diabetes and cardiovascular disease in PLWH have now been shown consistently [15, 39-41].

Limitations. PLWH taking ART is a challenging population for nutritional supplement intervention due to the multiple medical co-morbidities in this population. PLWH are dealing with constant medical surveillance and appointments with various clinicians, so they have limited time and interest in participating in research for what would be considered secondary to their basic needs. Thus, confounding from so many different comorbid complications cannot be entirely ruled out in a study such as this. Clearly, this type of intervention is significantly different from what is typically provided to PLWH. Thus, recruiting interested, willing, and reliable candidates for this study proved challenging. Nonetheless, even with such challenges we still showed favorable and promising effects of RBAC in this study. Additionally, the small sample size and minor rate of attrition likely had a negative impact on the power of the study, which may have further limited the findings of our study. What may happen in a larger sample of participants is unknown at this time.

Conclusions. A high-quality nutritional supplement that could attenuate endothelial dysfunction and cardiometabolic risk in PLWH on ART would be beneficial, but studies are

limited. HIV is now considered a chronic disease, hence efficacious nutritional supplements can provide PLWH alternatives to counteracting endothelial and cardiometabolic consequences. Based on the positive statistically significant findings, RBAC may offer a tool to counteract the negative effects of complications of HIV. The use of the ANS-1 system demonstrated an invaluable component of this study by uncovering links between immune, endothelial, and cardiometabolic function that otherwise would not have been shown. Additionally, reliable and noninvasive methods are needed to detect endothelial and autonomic nervous system function in PLWH taking ART, and the current results support our previous work with the ANS-1 and its usefulness in the primary care setting [30, 42]. Given that RBAC is an all-natural product causing no adverse effects and has no known negative interactions with pharmaceuticals, it may help delay disease progression in PLWH. The next step in the evaluation of RBAC would be to replicate the current findings for a longer period of time in a larger sample size.

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Authors' Contributions. JEL, SEA, ET, JMW, SG, AM, AR, and JK designed the research. JEL, SEA, AR, AF, HA, LAL, LCL, FM, LG, OLH, AF, ET, JMW, SC, RS, SG, AM, AR and JK conducted the research. JMW and AM provided essential reagents and essential materials. JEL and SC performed the statistical analysis. JEL wrote the paper. All authors had primary responsibility for the final content.

Table 1. Sociodemographic Characteristics of the Sample

Variable	Category	RBAC (n=22)	Placebo (n=25)	Statistic
Age	-	M=50.3, SD=10.5, R=18, 64	M=48.1, SD=9.8, R=24, 66	t=0.8 (45), p=0.45
Gender	Male	9 (41%)	13 (52%)	χ^2=0.6 (1), p=0.45
	Female	13 (59%)	12 (48%)	
Race/Ethnicity	White, non-Hispanic	-	2 (8%)	χ^2=0.6 (1), p=0.45
	Black, non-Hispanic	16 (73%)	16 (64%)	
	Hispanic	6 (27%)	6 (24%)	
	Other	-	1 (4%)	
Education	Up to high school	7 (32%)	5 (21%)	χ^2=4.4 (3), p=0.22
	High school graduate	7 (32%)	6 (25%)	
	Post high school training/some college	8 (36%)	9 (38%)	
	College graduate	-	4 (17%)	
Marital Status	Never Married	12 (55%)	18 (72%)	χ^2=4.0 (2), p=0.14
	Married	1 (5%)	3 (12%)	
	Widowed, divorced, or separated	9 (41%)	4 (16%)	

Note: M=mean, SD=Standard Deviation, and R=Range.

Table 2A. Correlations among Endothelial Function and Cardiometabolic Markers for the Placebo Group at Baseline

		AIPTG	PTGi	PTGr	ESRCl	SweatP	StressI
HDL Cholesterol (mg/dL)	r	.60	-.55	.52	-.43	-.44	.54
	p value	.004	.01	.02	.05	.05	.01
VLDL Cholesterol (mg/dL)	r				-.57	-.43	
	p value				.01	.05	
Triglycerides (mg/dL)	r				-.57	-.43	
	p value				.01	.05	

Note: AIPTG - systematic arterial stiffness photoplethysmography; PTGi - plethysmography total power index; PTGr - plethysmography ratio; ESRCl - electric skin response to the chloride ion; SweatP - sweat peak; StressI - stress index; HDL - high-density lipoprotein; VLDL - very low-density lipoprotein

Table 2B. Correlations among Immune, Cardiometabolic, and Endothelial Function Markers for the RBAC Group at Baseline

		Total Power	AIPTG	PTGTP VLF	PTGTP VLFi	PTGi	PTGr	CMRS
Total Cholesterol (mg/dL)	r			.62			.52	
	p value			.002			.01	
LDL Cholesterol (mg/dL)	r			.53			.45	
	p value			.01			.03	
VLDL Cholesterol (mg/dL)	r	.44		.51			.44	
	p value	.04		.02			.04	
Triglycerides (mg/dL)	r	.43		.52			.44	
	p value	.04		.02			.04	
CD8+ (cells/uL)	r		.63		.57	-.57		.45
	p value		.002		.01	.01		.04
CD4+/CD8+ Ratio	r	.51	-.57		-.49	.54		
	p value	.02	.006		.02	.01		

Note: Total Power - main indicator of autonomic nervous system activity; AIPTG - systematic arterial stiffness photoplethysmography; PTGTP VLF - Plethysmography very low frequency; PTGTP VLFi - plethysmography very low frequency index; PTGi - Plethysmography total power index; PTGr - plethysmography ratio; CMRS - cardiometabolic risk score; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein.

Table 3A. Correlations among Immune, Cardiometabolic, and Endothelial Function Markers for the Placebo Group at 3 Months Follow-Up

		PTGTP VLFi	PTGi	NO Sweat Peak	StressI
HDL Cholesterol (mg/dL)	r		.65		-.49
	p value		.01		.04
Glucose (mg/dL)	r			.51	
	p value			.04	
CD8+ (cells/uL)	r	.526			
	p value	.03			

Note: PTGTP VLFi - plethysmography very low frequency index; PTGi - Plethysmography total power index; NO Sweat Peak - nitric oxide sweat peak; StressI - stress index; HDL - high-density lipoprotein

Table 3B. Correlations among Immune, Cardiometabolic, and Endothelial Function Markers for the RBAC Group at 3 Months Follow-Up

		Total P.	PTG VLF	PTG VLFi	PTGi	PTGr	NO Sweat Peak	StressI	CAN	CMRS
Total Cholesterol (mg/dL)	R							.52		
	p value							.03		
HDL Cholesterol (mg/dL)	R				.53					
	p value				.03					
VLDL Cholesterol (mg/dL)	R		.51			.59		.63		
	p value		.04			.01		.01		
Triglycerides (mg/dL)	R		.51			.59		.63		
	p value		.04			.01		.01		
Glucose (mg/dL)	r			.54						
	p value			.03						
CD4+ (cells/uL)	r	.54			.66					
	p value	.03			.004					
CD8+ (cells/uL)	r							.50	.56	.49
	p value							.04	.02	.05
CD4+/CD8+ Ratio	r						.50			
	p value						.043			

Note: Total P. - main indicator of autonomic nervous system activity; PTGTP VLF - Plethysmography very low frequency; PTGTP VLFi - plethysmography very low frequency index; PTGi - Plethysmography total power index; PTGr - plethysmography ratio; NO Sweat Peak - nitric oxide sweat peak; StressI - stress index; CAN - cardiac autonomic neuropathy;

CMRS - cardiometabolic risk score; HDL - high-density lipoprotein; VLDL - very low-density lipoprotein

Table 4A. Correlations among Immune, Cardiometabolic, and Endothelial Function Markers for the Placebo Group at 6 Months Follow-Up

		PTGTPi	PTGTP VLFi	PTGr	ESRCl	SweatP	StressI	CAN
Total Cholesterol (mg/dL)	r			-.49				
	p value			.05				
CD8+ (cells/uL)	r						.55	.68
	p value						.03	.004
CD4+/CD8+ Ratio	r	.52	.55		-.51	-.55		
	p value	.04	.03		.05	.03		

Note: PTGTPi - plethysmography total power index; PTGTP VLFi - plethysmography very low frequency index; PTGr - plethysmography ratio; ESRCl - electric skin response to the chloride ion; SweatP - sweat peak; StressI - stress index; CAN - cardiac autonomic neuropathy

Table 4B. Correlations among Immune, Cardiometabolic, and Endothelial Function Markers for the RBAC Group at 6 Months Follow-Up

		AIPTG	PTGTP	PTG VLFi	PTGi	StressI	CAN	CMRS
Total Cholesterol (mg/dL)	r	.75			-.52			
	p value	.001			.05			
LDL Cholesterol (mg/dL)	r	.66						
	p value	.01						
VLDL Cholesterol (mg/dL)	r					.63		
	p value					.01		
Triglycerides (mg/dL)	r					.63		
	p value					.01		
CD8+ (cells/uL)	r		.64	.78				.67
	p value		.01	.001				.01
CD4+/CD8+ Ratio	r						-.54	
	p value						.05	

Note: AIPTG - systematic arterial stiffness photoplethysmography; PTGTPi - plethysmography total power index; PTGTP VLFi - plethysmography very low frequency index; PTGi - plethysmography total power index; StressI - stress index; CAN - cardiac autonomic neuropathy; CMRS - cardiometabolic risk score; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein

References

1. Centers for Disease Control and Prevention. HIV prevalence estimates--United States, 2006. MMWR Morb Mortal Wkly Rep. 2008;57(39):1073-6.

2. Centers for Disease Control and Prevention. HIV Surveillance Report, 20152016 May 12, 2017; 27. Available from: http://www.cdc.gov/hiv/library/reports/hiv-surveillance.html.

3. Feingold KR, Krauss RM, Pang M, Doerrler W, Jensen P, Grunfeld C. The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low density lipoprotein subclass pattern B. J Clin Endocrinol Metab. 1993;76(6):1423-7.

4. Grunfeld C, Kotler DP, Hamadeh R, Tierney A, Wang J, Pierson RN. Hypertriglyceridemia in the acquired immunodeficiency syndrome. Am J Med. 1989;86(1):27-31.

5. Shor-Posner G, Basit A, Lu Y, Cabrejos C, Chang J, Fletcher M, et al. Hypocholesterolemia is associated with immune dysfunction in early human immunodeficiency virus-1 infection. Am J Med. 1993;94(5):515-9.

6. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet. 1999;353(9170):2093-9.

7. Dever LL, Oruwari PA, Figueroa WE, O'Donovan CA, Eng RH. Hyperglycemia associated with protease inhibitors in an urban HIV-infected minority patient population. Ann Pharmacother. 2000;34(5):580-4.

8. Walli R, Goebel FD, Demant T. Impaired glucose tolerance and protease inhibitors. Ann Intern Med. 1998;129(10):837-8.

9. Calza L, Manfredi R, Farneti B, Chiodo F. Incidence of hyperlipidaemia in a cohort of 212 HIV-infected patients receiving a protease inhibitor-based antiretroviral therapy. Int J Antimicrob Agents. 2003;22(1):54-9.

10. Nolan D. Metabolic complications associated with HIV protease inhibitor therapy. Drugs. 2003;63(23):2555-74.

11. Samaras K, Wand H, Law M, Emery S, Cooper D, Carr A. Prevalence of metabolic syndrome in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes Foundation and Adult Treatment Panel III criteria: associations with insulin resistance, disturbed body fat compartmentalization, elevated C-reactive protein, and [corrected] hypoadiponectinemia. Diabetes Care. 2007;30(1):113-9.

12. Tiozzo E, Konefal J, Adwan S, Martinez LA, Villabona J, Lopez J, et al. A cross-sectional assessment of metabolic syndrome in HIV-infected people of low socio-economic status receiving antiretroviral therapy. Diabetol Metab Syndr. 2015;7:15.

13. Sinha A, Ma Y, Scherzer R, Hur S, Li D, Ganz P, et al. Role of T-Cell Dysfunction, Inflammation, and Coagulation in Microvascular Disease in HIV. J Am Heart Assoc. 2016;5(12).

14. Iantorno M, Schar M, Soleimanifard S, Brown TT, Moore R, Barditch-Crovo P, et al. Coronary artery endothelial dysfunction is present in HIV-positive individuals without significant coronary artery disease. AIDS. 2017;31(9):1281-9.

15. Hove-Skovsgaard M, Gaardbo JC, Kolte L, Winding K, Seljeflot I, Svardal A, et al. HIV-infected persons with type 2 diabetes show evidence of endothelial dysfunction and increased inflammation. BMC Infect Dis. 2017;17(1):234.

16. Auclair M, Afonso P, Capel E, Caron-Debarle M, Capeau J. Impact of darunavir, atazanavir and lopinavir boosted with ritonavir on cultured human endothelial cells: beneficial effect of pravastatin. Antivir Ther. 2014;19(8):773-82.

17. Mai J, Virtue A, Shen J, Wang H, Yang XF. An evolving new paradigm: endothelial cells--conditional innate immune cells. J Hematol Oncol. 2013;6:61.

18. Choi J, Enis DR, Koh KP, Shiao SL, Pober JS. T lymphocyte-endothelial cell interactions. Annu Rev Immunol. 2004;22:683-709.

19. Kalebic T, Kinter A, Poli G, Anderson ME, Meister A, Fauci AS. Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and N-acetylcysteine. Proc Natl Acad Sci U S A. 1991;88(3):986-90.

20. Ivanov AV, Valuev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES, Bartosch B, et al. Oxidative Stress during HIV Infection: Mechanisms and Consequences. Oxid Med Cell Longev. 2016;2016:8910396.

21. Ghoneum M. Anti-HIV activity in vitro of MGN-3, an activated arabinoxylane from rice bran. Biochem Biophys Res Commun. 1998;243(1):25-9.

22. Ghoneum M. Enhancement of human natural killer cell activity by modified Arabinoxylan from rice bran (MGN-3). Int J Immunotherapy. 1998;14(2):89-99.

23. Ghoneum M, Matsuura M. Augmentation of macrophage phagocytosis by modified arabinoxylan rice bran (MGN-3/Biobran). Int J Immunopathol Pharmacol. 2004;17(3):283-92.

24. Tazawa K, Namikawa H, Oida N, Masada M, Maeda H. Scavenging activity of modified arabinoxylane from rice bran (biobran/mgn-3) with natural killer cell activity on free radicals. Biotherapy. 2000;14:493-5.

25. Ali K, Melillo A, Leonard S, Asthana D, Woolger J, Wolfson A, et al. An open-label, randomized clinical trial to assess the immunomodulatory activity of a novel oligosaccharide compound in healthy adults. Functional Foods in Health and Disease. 2012;2(7):265-79.

26. Bachorik PS, Albers JJ. Precipitation methods for quantification of lipoproteins. Methods Enzymol. 1986;129:78-100.

27. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

28. Lewis JE, Atlas SE, Rasul A, Farooqi A, Lantigua L, Higuera OL, et al. New method of sudomotor function measurement to detect microvascular disease and sweat gland nerve or unmyelinated C fiber dysfunction in adults with retinopathy. J Diabetes Metab Disord. 2017;16:26.

29. Lewis JE, Lantigua L, Atlas SE, Lopez J, Mendez A, Goldberg S, et al. A cross-sectional assessment to detect type 2 diabetes with endothelial and autonomic nervous system markers using a novel system. J Diabetes Metab Disord. 2014;13(1):118.

30. Lewis JE, Tannenbaum SL, Gao J, Melillo AB, Long EG, Alonso Y, et al. Comparing the accuracy of ES-BC, EIS-GS, and ES Oxi on body composition, autonomic nervous system activity, and cardiac output to standardized assessments. Med Devices (Auckl). 2011;4:169-77.

31. Gandhi P, Rao, G. Detection of neuropathy using a sudomotor test in type 2 diabetes. Degenerative Neurological and Neuromuscular Disease. 2015;5:1-7.

32. De Souza AL, Batista GA, Alegre SM. Assessment of insulin sensitivity by the hyperinsulinemic euglycemic clamp: Comparison with the spectral analysis of photoplethysmography. Journal of diabetes and its complications. 2017;31(1):128-33.

33. Cherry CL, Skolasky RL, Lal L, Creighton J, Hauer P, Raman SP, et al. Antiretroviral use and other risks for HIV-associated neuropathies in an international cohort. Neurology. 2006;66(6):867-73.

34. Evans SR, Ellis RJ, Chen H, Yeh TM, Lee AJ, Schifitto G, et al. Peripheral neuropathy in HIV: prevalence and risk factors. AIDS. 2011;25(7):919-28.

35. Maritz J, Benatar M, Dave JA, Harrison TB, Badri M, Levitt NS, et al. HIV neuropathy in South Africans: frequency, characteristics, and risk factors. Muscle Nerve. 2010;41(5):599-606.

36. Chow D, Kocher M, Shikuma C, Parikh N, Grandinetti A, Nakamoto B, et al. Effects of antiretroviral therapy on autonomic function in early HIV infection: a preliminary report. Int J Med Sci. 2012;9(5):397-405.

37. Rogstad KE, Shah R, Tesfaladet G, Abdullah M, Ahmed-Jushuf I. Cardiovascular autonomic neuropathy in HIV infected patients. Sex Transm Infect. 1999;75(4):264-7.

38. Lewis JE, McDaniel HR, Agronin ME, Loewenstein DA, Riveros J, Mestre R, et al. The effect of an aloe polymannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease. J Alzheimers Dis. 2013;33(2):393-406.

39. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013;173(8):614-22.

40. Hsue PY, Deeks SG, Hunt PW. Immunologic basis of cardiovascular disease in HIV-infected adults. J Infect Dis. 2012;205 Suppl 3:S375-82.

41. Worm SW, De Wit S, Weber R, Sabin CA, Reiss P, El-Sadr W, et al. Diabetes mellitus, preexisting coronary heart disease, and the risk of subsequent coronary heart disease events in

patients infected with human immunodeficiency virus: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D Study). Circulation. 2009;119(6):805-11.

42. Lewis JE, Lantigua L, Atlas SE, Lopez J, Mendez A, Goldberg S, et al. A cross-sectional assessment to detect type 2 diabetes with endothelial and autonomic nervous system markers using a novel system. J Diabetes Metab Disord. 2014;13(1):118.