

Ideal Cardiovascular Health and Job Strain: A Cross-Sectional Study from the Amazon Basin

Davi Dantas Muniz,¹ Kamile Santos Siqueira,² Cristina Toledo Cornell,³ Miguel Morita Fernandes-Silva,⁴ Pascoal Torres Muniz,¹ Odilson Marcos Silvestre¹⁰

Universidade Federal do Acre (UFAC),¹ Rio Branco, AC – Brazil Universidade Federal Fluminense,² Niterói, RJ – Brazil Brigham and Womens Hospital - Harvard University,³ Boston, Massachussets – USA Pontifícia Universidade Católica do Paraná,⁴ Curitiba, PR – Brazil

Abstract

Background: Ideal Cardiovascular (CV) Health is characterized by four ideal lifestyle parameters and absence of cardiovascular risk factors. The prevalence of ideal CV health in the Amazon Basin and the influence of job strain on CV health in this setting are uncertain.

Objective: To evaluate the prevalence of ideal CV health and its relationship with job strain in a secluded area from a developing country.

Methods: Job strain was evaluated in 478 employees from an university in the Amazon Basin by a questionnaire that classified participants as passive, active, low or high strain, according to the demand-control model. CV health was evaluated using the American Heart Association 7 health factors (diet, physical activity, body mass index (BMI), smoking, hypertension, diabetes and hypercholesterolemia). Participants were classified as having ideal, intermediate or poor CV health. The level of significance was set at p < 0.05.

Results: The mean age was 44.3 ± 12 years, 65% were men, and 35% were faculty. No participant fulfilled the criteria for ideal CV health. Intermediate CV health was found in 44 (9%) and poor in 434 (91%) individuals. Considering low strain as a reference group, individuals classified as high strain, active and passive had a non-significant (p > 0.05) increase in the chances of having poor CV health. When adjusting for possible confounders, high job strain was associated with poor BMI (> 30 kg/m²), (OR 2.11, 95%CI 1.06-4.22; p = 0.034) and poor diet (OR 2.31, 95% CI 1.29-4.13; p = 0.005).

Conclusion: Job strain was not associated with cardiovascular health, but high job strain was related to obesity and poor diet. Given the high prevalence of poor CV health and lack of participants with ideal CV health, policies focusing on health education and lifestyle interventions are paramount to this population. (Arq Bras Cardiol. 2019; 112(3):260-268)

Keywords: Cardiovascular Diseases/epidemiology; Risk Factors; Prevention and Control; Amazonian Ecosystem; Stress, Psychological; Obesity; Eating Disorders.

Introduction

Cardiovascular diseases (CVD) remain the leading cause of death in both the developed and developing world.¹⁻³ In order to combat CVD, the American Heart Association (AHA) launched the 2020 impact goal and the Ideal Cardiovascular Health initiative.⁴ Ideal Cardiovascular (CV) Health is defined as the presence of both ideal health behaviors (nonsmoking, body mass index (BMI) < 25 kg/m², physical activity at goal levels, and pursuit of a diet consistent with current guideline recommendations) and ideal health factors (untreated total cholesterol < 200 mg/dL, untreated blood pressure < 120/80 mmHg, and fasting blood

Mailing Address: Odilson Marcos Silvestre •

Clínica Silvestre Santé - Rua Quintino Bocaiúva, 1108, Postal Code 69900-785; Rio Branco, AC – Brazil

E-mail: odilsonsilvestre@yahoo.com.br

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glucose < 100 mg/dL). Subjects in ideal status have a lower incidence of CVD and an increased life expectancy when compared with those in poor status.⁴⁻⁶

CV health seems to be influenced by workplace conditions.^{7,8} In a study by Karasek et al.,⁸ high job strain was associated with higher chances of having CV disease.⁸ The authors proposed that demand and control at the workplace influence health status. Control is defined as how much leeway in decision making the employee has and how much intellectual skills are required when working. Demand is the intellectual pressure that can be either quantitative, for example, time and velocity to work, or qualitative, defined as conflicts between contradictory demands.⁷

Although few studies in this regard have been performed in developed countries, the relationship between job strain and CV health is not completely known in the setting of a developing country. Using the above framework, we hypothesized that ideal CV health has low prevalence in the Amazon Basin. Furthermore, we believe that job strain is associated with a higher prevalence of poor CV health.

Objective

The aim of this study is to evaluate the prevalence of ideal CV health and its relationship with job strain in a secluded area from a developing country.

Methods

The study was performed between 2012 and 2013 in Rio Branco, Brazil. All 759 employees at the Federal University of Acre were invited to participate. Employees received a call or email to setup an appointment with two trained researchers. A total of 478 employees accepted to participate, including 166(35%) faculty professionals and 312(65%) non-faculty staff. A questionnaire was used to collect information regarding demographics, CV behavior and risk factors. Weight, height, waist circumference and blood pressure were measured twice at different appointments. The average of the two measurements was considered the final value. We excluded those subjects who declined to sign an informed consent. Twenty-seven participants with missing data regarding job strain were not included in the comparisons among CV health factors and job strain. This study was approved by the Institutional Review Board at the Federal University of Acre.

We used the validated Portuguese-language adapted Job Content Questionnaire based in Robert Karesek theoric model to assess job strain.⁹ It is composed of 6 questions to evaluate control, 5 questions to assess demand, and 6 questions to assess social support. Each question was composed of 4 possible answers ranging from 1 (strongly disagree) to 4 (strongly agree). We defined as having high demand those participants with scores above the 50th percentile on the Portuguese-language adapted Job Content Questionnaire; low control when below the 50th percentile; High job strain was defined as the combination of high demand and low control; low job strain as low demand and high control; active as high demand and high control; passive as low demand and low control.

This study selected seven health indicators proposed by the AHA as markers of CV health. These seven parameters include four lifestyle variables (smoking, physical activity, diet, and BMI) and three CV risk factors (diabetes, hypercholesterolemia, and high blood pressure). Smoking status was classified as never smoker, former smoker and current smoker. Ideal physical activity was defined by more than 150 minutes/week of moderate intensity exercise, any physical activity lower than 150 minutes/week as intermediate and no physical exercise as poor. BMI was calculated as body weight in kilograms divided by the square of body height in meters (kg/m²). Values lower than 25 were considered ideal, between 25 and 29.9 intermediate, and greater than 30 were considered poor. Diabetes and hypercholesterolemia were self-reported in a questionnaire as yes or no responses. Blood pressure was obtained by trained researchers. High blood pressure was defined as a measured value greater than 140/90 mmHg or self-reported high blood pressure in use of anti-hypertensive medication or not. Systolic blood pressure between 120 and 139 or diastolic blood pressure between 80 and 89 were classified as intermediate. Blood pressure < 120/80 was considered ideal. According to the AHA, an ideal diet pattern was defined as consumption of 4 portions or more of fruits and/or vegetables per day, less than 1 liter of sugar-sweetened beverages per week and more than two 3.5-oz servings per week of fresh fish. We used a food frequency questionnaire previously validated in a Brazilian cohort.¹⁰ Sodium and fiber-rich whole grains consumption couldn't be accessed by the questionnaire. Individuals were classified as having ideal CV health (ideal lifestyle and absence of CV risk factors), poor (when any of the seven factors was evaluated as poor) or intermediate (participants who did not belong to ideal or poor CV health group).

The covariates used for data analysis were age, gender, income, education level and occupation, which were self-reported in a questionnaire. Income was measured in US dollars and subjects were split in two categories, more or less than \$20,000/year. Occupation was classified as teaching and non-teaching staff. We modeled education as a dichotomous variable with subjects classified as college or more or high school or less. Low social support was considered for those participants bellow 50th percentile on the adapted Job Content Questionnaire.

Statistical analysis

The study was designed to detect a difference in the proportion of poor CV health of 15% between high strain (85%) and low strain (70%). Considering a power set at 0.8, an alpha level of 0.05, we estimated a sample size of at least 121 patients per group and a total of 424 participants. We planned including 478 individuals, allowing for non-respondents or incomplete responses up to 12% of participants.

Categorical data was reported as percent frequencies and compared by chi-squared test. Continuous normally distributed variables were displayed as mean and standard deviation, and continuous non-normally distributed variables were displayed as median and 25th and 75th percentiles. Significant pairwise comparisons were adjusted for multiple testing using Bonferroni correction and are shown only for variables in which a significant global difference was detected using one-way ANOVA or Kruskal-Wallis tests. The association between job strain and CV health was assessed using multivariable logistic regression using the low strain group as reference. We also used multivariable logistic regression to assess the association between job strain and CV risk factors. We built two models for analyses; the crude analyses without any covariate and the adjusted analyses, which controlled for age, sex, education, income, and occupation. Exposures included high job strain and also high demand and low control, separately. All statistical analyses were performed with STATA version 13.1 (Stata Corp., College Station, TX, USA). P-values < 0.05 were considered statistically significant.

Results

Participants had a mean age of 44.3 ± 12 years, and 65% were men; In terms of job strain, the most prevalent group was low job strain with 144 (32%) individuals. A total of 86 (19%) participants were found to have high job strain, 93 (21%) were considered active, and 128 (28%) were considered to have passive work. Teaching professionals were less likely to

be among the high strain job group. No significant differences were found between high strain and low strain group in terms of age, sex, and income. Passive participants were more likely to be older and low educated than low strain subjects. Active participants had higher income and were more likely to be professors than low strain participants (Table 1).

Ideal CV health was not found in this sample. Intermediate CV health was found in 44 (9%) and poor in 434 (91%) individuals. Poor physical activity (53%) and poor diet (55%) were the factors with the highest prevalence among the participants. Poor BMI was found in 22% of participants, smoking in 7%, hypertension in 36%, hypercholesterolemia in 26%, and diabetes in 7% (Figure 1). Most participants had one to three CV health factors classified as poor (Table 2).

Considering the outcome CV health and low strain as reference group for comparisons, we found that high strain, active and passive individuals have a non-significant increase in the chances of having poor CV health (Table 3 and Figure 2). Either high demand or low control category when analyzed separately or combined was not associated with poor CV health (Table 3). Individually, the active group had a trend to increase the odds of poor physical activity (OR 1.67, 95% CI 0.96-2.92; p = 0.07). High job strain increased the chance of poor diet by 2.3-fold in comparison with the low strain group (p = 0.005). Similarly, high job strain was associated with poor BMI (> 30 kg/m²), OR 2.11, 95%CI 1.06-4.22; p = 0.034. We did not find an association between job strain and poor smoking, high blood pressure, hypercholesterolemia, or diabetes (Table 4). When demand and control categories were analyzed separately, low control was associated with poor diet. (Table 5)

Discussion

This study included 451 from an academic center and, to our knowledge, it is the first to describe the prevalence of CV health and explore a possible relationship with job strain in the Amazon Basin. Our main finding was a high prevalence of poor CV health with no individuals in ideal

Table 1 - Characteristics of subjects according to job strain (active, passive, low strain, high strain)

Demonstern	Low strain	Active	Passive	High Strain	
Parameters	n = 144 (32%)	n = 93 (21%)	n = 128 (28%)	n = 86 (19%)	
Age (years)	42.9 ± 11.9	43.6 ± 10.7	46.7 ± 13.2 [†]	42.8 ± 13.3	
Gender - male n (%)	94 (65)	56 (60)	82 (64)	55 (64)	
Income - > \$20.000/yr n (%)	57 (42)	62 (67) [*]	42 (35)	31 (37)	
Highly educated n (%)	112 (78)	82 (88)	63 (49) [†]	64 (76)	
Occupation- professor n (%)	63 (44)	65 (70) [*]	10 (8) †	18 (21) [‡]	
Low social support N (%)	66 (40)	59 (66)*	39 (31)	56 (66) [‡]	
Smoking n (%)					
Poor	8 (6)	7 (8)	9 (7)	8 (9)	
Intermediate	31 (22)	14 (15)	42 (33)	11 (13)	
Ideal	105 (73)	72 (77)	77 (60)	67 (78)	
Hypercholesterolemia n (%)	34 (24)	24 (26)	39 (30)	17 (20)	
Physical activity n (%)					
Poor	66 (46)	53 (57)	73 (57)	46 (54)	
Intermediate	38 (26)	21 (23)	31 (24)	21 (24)	
Ideal	40 (28)	19 (20)	24 (19)	19 (22)	
Diet n (%)					
Poor	71 (49)	45 (48)	72 (56)	58 (67)‡	
Intermediate	38 (26)	32 (34)	36 (28)	17 (20)	
Ideal	35 (24)	16 (17)	20 (16)	11 (13)	
Diabetes n (%)	8 (6)	6 (6)	11 (9)	5 (6)	
High blood pressure n (%)					
Poor	51 (35)	28 (30)	48 (38)	32 (37)	
Intermediate	72 (50)	50 (54)	70 (55)	49 (57)	
Ideal	21 (15)	15 (16)	10 (8)	5 (6)	
Body mass index (kg/m ²)	26.5 ± 3.8	26.4 ± 4.2	27.3 ± 4.6	27.5 ± 5.0	

*p < 0.05 active vs. low strain; †p < 0.05 passive vs. low strain; ‡p < 0.05 high strain vs low strain.



Figure 1 – Stacked bars showing the prevalence of cardiovascular risk factors. BMI: Body mass index (kg/m²).

Table 2 – Distribution	(count) of	poor cardiovascular	health factors	according to	iob strain*
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Number of poor CV factors	Low strain n = 144(32%)	Active n = 93(21%)	Passive n = 128(28%)	High Strain n = 86(19%)
0	16 (11)	9 (10)	12 (9)	6 (7)
1	52 (36)	31 (33)	27 (21)	18 (21)
2	37 (26)	23 (25)	35 27)	27 (31)
3	29 (20)	17 (18)	35 (27)	25 (29)
4	5 (4)	9 (10)	13 (10)	8 (9)
5	3 (2)	4 (4)	4 (3)	2 (2)
6	2 (1)	0	2 (2)	0
7	0	0	0	0
Mean of poor CV factors (n)	1.8	2	2.2	2.2

p >0.05 for all group comparisons. CV: cardiovascular.

CV health. High job strain was associated with a poor diet and obesity. These findings highlight the influence of workplace condition in CV health of employees and the need to implement policies to improve their health.

We found no individuals in our sample that would be classified as having ideal CV health as defined by the AHA guideline. More than 90% were classified as having poor CV health. Surprisingly, these findings are worse than those from studies in developed countries. In a United States community-based study with 1933 individuals, only one individual fulfilled criteria for ideal CV health. In the same study, 17% had intermediate CV health, and 83% had poor CV health.¹¹ We would expect a better CV health profile in our population since we recruited employees from an academic center with wide access to health information, particularly given the presence of nutrition, medicine and other health-related disciplines on campus. Furthermore, our sampled population was approximately 10 years younger than

the population of other similar studies.^{11,12} In an Asian study, the prevalence of ideal CV health was 0.2%, intermediate 21% and poor 79% among hospital workers.¹³ Comparing this sample with the current study participants, the main difference of CV parameters can be explained by better blood pressure control and low index of overweight and obesity in the Asian population. Importantly, this worse scenario found in our sample could not be explained by differences in methodology among studies because we used similar definitions for CV health factors as defined by the AHA.⁴

In regards to the difference in job strain and CV health, no relationship between job strain and poor CV health was demonstrated. Previous studios with larger sample size than our study demonstrated that job strain was associated with CV health scores or established CV disease.¹⁴ The current study included 451 participants which limits the power to find small differences. Moreover, socio-cultural differences between this study sample and other studies mainly from developing

		Poor Cardiovascular Health	(Crude)	Poor Cardiovascular Health (Adjusted*)		
	n (%)	Odds Ratio (95% CI)	р	Odds Ratio (95% CI)	p	
Isolated parameters						
High demand	180 (40)	1.24 (0.64-2.39)	0.52	1.49 (0.76-2.94)	0.24	
Low Control	229 (49)	1.29 (0.69-2.40)	0.43	1.09 (0.54-2.20)	0.81	
Combined parameters						
Low strain	144 (32)	Ref	-	Ref	-	
Active	93 (21)	1.17 (0.49-2.76)	0.73	1.21 (0.49-2.97)	0.68	
Passive	128 (28)	1.21 (0.55-2.66)	0.64	0.91 (0.38-2.18)	0.84	
High strain	86 (19)	1.67 (0.63-4.44)	0.31	1.79 (0.65-4.92)	0.26	

Table 3 - Association between job demand, control, or strain with poor cardiovascular health

CI: confidence interval; *Adjusted for age, sex, income, education and profession.



Figure 2 – Bar graph with percentages of intermediate and poor cardiovascular health according to tob strain categories are low strain (low demand and high control), active (high demand and high control), passive (low demand and low control), and high strain (high demand and low control). Ideal cardiovascular health is not represented because none individual was in this group.

countries might influence to change the impact of job strain in CV health. Other authors explored individual CV health factors and found association between high job strain and physical inactivity,^{15,16} diabetes, smoking and obesity.¹⁶

In the current study, diet pattern was associated with job strain, perhaps due to the fact that people with higher demand and lower control usually have less time to prepare or buy healthy food. We also found an association between job strain and obesity, which may be a consequence of a poor diet; it is reasonable to hypothesize that a poor diet is a mediator factor for the association between job strain and obesity. However, we did not found mediator effect for this association (data not shown). In addition, the control-demand model was built to evaluate psychosocial factors that affect mental health.⁷ Participants with job strain are more prone to have eating disorders resulting in weight gain, which may explain our findings. In our questionnaires, we did not explore mental health disorders to add on this discussion. In the current study, physical inactivity was not associated with job strain, which is different from a previous publication that found it as the only risk factor associated with job strain.¹⁵ Our data showed a trend towards worse CV health factors and job strain, albeit without statistical significance. This is likely due to the lack of sample power given the relatively small sample size. In addition, it is also possible that the busiest and healthiest employees may have declined to participate in the study, introducing a bias towards the null. On the other hand, job stress might determine less influence in CV health in an academic environment in the Amazon region because employees would work in a less competitive behavior.

This study has some limitations. Data on diabetes and hypercholesterolemia were self-reported, which could introduce misclassification bias. On the other hand, self-reported questionnaires have low sensitivity for detecting these

Table 4 - Odds ratio for cardiovascular health factors according job demand, job control and job strain

	n (%)	Crude Odds Ratio (95% CI)	р	Adjusted Odds Ratio (95% CI)*	р
Poor physical activity					
Low strain	66 (46)	Ref	-	Ref	-
Active	53 (57)	1.57 (0.93-2.65)	0.09	1.67 (0.96-2.92)	0.07
Passive	73 (57)	1.57 (0.97-2.53)	0.066	1.19 (0.70-2.04)	0.52
High strain	46 (53)	1.36 (0.80-2.32)	0.26	1.40 (0.80-2.46)	0.24
Poor diet					
Low strain	71 (49)	Ref	-	Ref	-
Active	45 (48)	0.96 (0.57-1.62)	0.89	0.95 (0.55-1.65)	0.86
Passive	72 (56)	1.32 (0.82-2.13)	0.25	1.38 (0.81-2.35)	0.24
High strain	58 (67)	2.13 (1.22-3.18)	0.008	2.31 (1.29-4.13)	0.005
BMI > 30 kg/m²					
Low strain	22 (15)	Ref	-	Ref	-
Active	21 (23)	1.62 (0.83-3.15)	0.16	1.75 (0.86-3.53)	0.12
Passive	34 (26)	2.01 (1.10-3.65)	0.023	1.83 (0.94-3.57)	0.07
High strain	23 (27)	2.02 (1.05-3.91)	0.036	2.11 (1.06-4.22)	0.034
Smoking					
Low strain	8 (6)	Ref	-	Ref	-
Active	7 (8)	1.38 (0.48-3.95)	0.55	1.65 (0.53-5.11)	0.39
Passive	9 (7)	1.29 (0.48-3.44)	0.62	1.01 (0.34-3.04)	0.98
High strain	8 (9)	1.74 (0.63 (4.83)	0.29	2.06 (0.69-6.12)	0.19
High blood pressure					
Low strain	51 (35)	Ref	-	Ref	-
Active	28 (30)	0.79 (0.45-1.37)	0.4	0.82 (0.44-1.51)	0.53
Passive	48 (38)	1.09 (0.67-1.79)	0.72	0.72 (0.41-1.28)	0.27
High strain	32 (37)	1.08 (0.62-1.88)	0.78	0.93 (0.51-1.71)	0.82
Dyslipidemia					
Low strain	34 (24)	Ref	-	Ref	-
Active	24 (26)	1.13 (0.62-2.06)	0.7	1.23 (0.64-2.36)	0.53
Passive	38 (31)	1.42 (0.83-2.43)	0.2	0.92 (0.50-1.69)	0.78
High strain	17 (19)	0.80 (0.41-1.53)	0.5	0.72 (0.36-1.45)	0.35
Diabetes					
Low strain	8 (6)	Ref	-	Ref	-
Active	6 (6)	1.17 (0.39-3.49)	0.78	1.05 (0.33-3.30)	0.94
Passive	11 (9)	1.60 (0.62-4.11)	0.33	1.20 (0.42-3.49)	0.73
High strain	5 (6)	1.05 (0.33-3.32)	0.93	1.07 (0.32-3.56)	0.91

CI: confidence interval; BMI: body mass index; *Adjusted for age, sex, income, education and profession.

diseases¹⁷ and the prevalence of CV risk factors may have been underestimated, which increases the concern regarding the burden of CVD among employees at the University. Importantly, this need to be addressed at a larger scale, as it may reflect a high prevalence of cardiovascular risk factors in Rio Branco, the Brazilian capital with the highest prevalence of obesity.¹⁸ However, the prevalence of these cardiovascular factors in our study was similar to data on cardiovascular risk factors reported in the literature.¹⁸⁻²⁰ The food questionnaire used couldn't capture sodium and fiber-rich whole grains consumption, which limited the food related factors to only three items of the diet guideline proposed by the AHA.⁴ Furthermore, some employees declined to respond the questionnaire, which could introduce bias, as employees who declined to fill out the survey could have different characteristics such as higher strain jobs and lower education compared to employees who filled out the survey. We considered that the current study sample is significant, since most part of

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Demand			Control				
	n (%)	Adjusted Odds Ratio (95% CI)	р		n (%)	Adjusted Odds Ratio (95% CI)*	р
Poor physical activity							
Low demand	99 (39)	Ref	-	High control	121 (49)	Ref	-
High demand	141 (59)	1.34 (0.90-1.98)	0.15	Low control	126 (51)	1.03 (0.68-1.56)	0.88
Poor diet							
Low demand	160 (61)	Ref	-	High control	123 (47)	Ref	-
High demand	103 (39)	1.21 (0.82-1.80)	0.34	Low control	140 (53)	1.70 (1.12-2.58)	0.012
BMI > 30 kg/m²							
Low demand	45 (44)	Ref	-	High control	59 (58)	Ref	-
High demand	58 (56)	1.49 (0.93-2.41)	0.1	Low control	43 (42)	1.50 (0.91-2.49)	0.12
Smoking							
Low demand	18 (55)	Ref	-	High control	18 (54)	Ref	-
High demand	15 (45)	1.98 (0.91-4.29)	0.085	Low control	15 (45)	1.17 (0.52-2.65)	0.71
Hypertension							
Low demand	31 (61)	Ref	-	High control	17 (32)	Ref	-
High demand	20 (39)	0.70 (0.37-1.35)	0.29	Low control	36 (68)	0.78 (0.38-1.60)	0.5
Dyslipidemia							
Low demand	74 (64)	Ref	-	High control	62 (52)	Ref	-
High demand	42 (36)	1.00 (0.62-1.59)	0.99	Low control	58 (48)	0.83 (0.51-1.34)	0.44
Diabetes							
Low demand	19 (63)	Ref	-	High control	14 (44)	Ref	-
High demand	11 (37)	1.01 (0.19-1.16)	0.98	Low control	18 (56)	1.21 (0.52-2.79)	0.66

Table 5 – Association between job demand or job control with cardiovascular health factors

CI: confidence interval; BMI: body mass index; *Adjusted for age, sex, income, education and profession.

employees responded the questionnaire (still representative of our community as most people invited to participate responded to the questionnaire). Moreover, unmeasured and residual confounding of the relationship between change job stress and CV health could not be completely addressed by multivariate modeling. Finally, as the design of the study is cross-sectional, only associations can be established. A further study with a longitudinal design would explore causality between job strain and CV health factors.

Conclusion

The findings of high prevalence of poor CV health provide important information to public health officials in middle-income countries. Furthermore, a trend to worse CV health in high strain jobs is of interest as these factors could potentially worsen overtime with poor working conditions in developing countries. Strategies for promoting healthy behaviors such as healthier foods offered on campus restaurants, fitness projects, education campaigns, in conjunction with good primary care on campus could potentially have a great impact on life expectancy and improved CV health in this population.

Author contributions

Conception and design of the research: Muniz DD, Siqueira KS, Muniz PT, Silvestre OM; acquisition of data: Muniz DD, Siqueira KS, Muniz PT; analysis and interpretation of the data: Muniz DD, Cornell CT, Silva MMF, Silvestre OM; statistical analysis: Silva MMF, Silvestre OM; writing of the manuscript: Muniz DD, Cornell CT, Silvestre OM; critical revision of the manuscript for intellectual content: Muniz DD, Siqueira KS, Cornell CT, Silva MMF, Muniz PT, Silvestre OM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Universidade Federal do Acre under the protocol number 23107.017363/2011-52. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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