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RESEARCH

Evaluation of the psychometric properties and convergent validity of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) questionnaire in Brazilian young adults

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Abstract

Background This cross-sectional study aims to evaluate the psychometric properties and the convergent validity of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST). Confirmatory factor analysis (CFA) and linear regression adjusted for confounding factors were performed to obtain the adjusted beta value (*ß*adj) for sleep quality (Pittsburgh Sleep Quality Index), burnout (Copenhagen Burnout Inventory), and depressive symptoms (Patient Health Questionnaire-9).

Results The study population consisted of 3187 young adult university students. The CFA indicated adequate fits for the one- and two-factor structure of the ASSIST, with better indices for the two-factor model. Convergent validity results confirmed the best fit of the one-factor model (version 3.1), in which individuals classified as "suggestive of dependence" showed a greater effect on worse sleep quality (*ß*adj 2.16, 95% confidence interval (CI) 1.48–2.84), burnout (*ß*adj 12.25, 95% CI 8.43–16.06), and depressive symptoms (*ß*adj 6.69, 95% CI 5.37–8.00).

Conclusions The results and the literature review indicated that the one-factor presents an adequate factor structure and better convergent validity.

Keywords Consumption of alcoholic beverages, Psychometrics, Young adult

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Background

Alcohol is one of the oldest psychoactive substances used by mankind. Its use can lead to several negative health effects, including dependence [1]. It is estimated that in 2019, alcohol abuse was responsible for over 168,000 deaths worldwide, especially among men (approximately 143,000 deaths) [2]. Although mortality occurs predominantly in individuals over 30 years of age [2, 3], the habit of consumption and problematic alcohol use [4, 5] can start in adolescence and at certain transitional times, such as during higher education [1]. In addition, alcohol use can have a harmful impact on young adults' mental health, reinforcing the importance of further investigating this topic in this population [6, 7].

There is no consensus on the best subjective method to assess alcohol consumption [8]. In general terms, the analyses on this topic can be divided into those that focus on the consumption, i.e., yes or no consumption, frequency of consumption [9, 10] and binge drinking [4, 5], and analyses focusing on general use, as ranging from consumption to the impact on users' lives and routines [11, 12]. Given the complexity of the subject and aiming to propose a quick application tool to assess the consumption and impact of several psychoactive substances, the World Health Organization coordinated a working group that developed the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) [13].

The first version (1.0) contained 12 questions, which was reduced to an 8-question version, called the ASSIST 2.0 [13, 14]. Subsequently, multicenter studies were conducted with version 2.0, leading to its refinement (version 3.0), which, after revision, resulted in version 3.1 [11]. The differences between versions 2.0 and 3.1 are subtle, with important changes in the weight assigned to each answer, which affect the sum and the cutoff scores.

Although version 3.1 resulted from an improved study process [11], the analysis of psychometric properties, an important technique for analyzing measurement instruments [15, 16], has shown inadequate fits for the ASSIST scale in one-factor, with possible two-factor behavior [17]. This empirical evidence indicates that the seven ASSIST questions that receive scores may be organized into one factor (alcohol abuse) and into two correlated factors. However, the analysis of the psychometric properties of the ASSIST lacks consensus on the organization of the factors, and no progress has been made regarding its application in epidemiological studies, i.e., on the benefits of interpreting the ASSIST in models that are no one-factor models [17–19].

The advantages of the ASSIST (easy to use, quick application, and indication of clinical interventions) make it one of the most widely used tools for the subjective assessment of alcohol consumption [10, 20]. However, the use of different versions of the questionnaire [18, 21] is still observed in the literature, as well as gaps regarding the one-factor behavior [17–19, 22], which may compromise the extrapolation of results and comparisons between studies. Thus, considering the importance of analyzing subjective measures of alcohol consumption, especially in young adults [3, 7, 10], the present study aims to (1) analyze the psychometric properties of the ASSIST to identify the number of factors that comprise it and (2) examine the convergent validity of different versions and factor behaviors of the ASSIST.

Methods

Delineation

This is a cross-sectional epidemiological study, part of the GraduaUEL: Analysis of the Health and Lifestyle Habits of Undergraduate Students at UEL project, which aimed to investigate the health and lifestyle of undergraduate students at the State University of Londrina (UEL), in Londrina, Paraná, Brazil. The young adult population studied was composed of students regularly enrolled in one of the 50 undergraduate courses of UEL in 2019.

The students were selected based on the following inclusion criteria: active enrollment in an undergraduate course and being 18 years of age or older at the time of the interview. Students who did not answer the questions related to sleep, alcohol consumption, or adjustment variables were considered losses. The data were collected in the first semester of 2019 using a questionnaire administered on a digital platform. The research objectives and participation methods were widely publicized to encourage participation among the student population.

Characterization variables

The characterization variables considered were sex (male or female), age (continuous), ethnic identity (non-White or White), full-time course enrollment (yes or no), workload (does not work, works up to 30 h per week, or works 31 h or more per week), and daily social media usage time (≤ 179 min, 180–299 min, or ≥ 300 min).

The variables related to alcohol consumption (independent) included frequency of consumption, binge drinking, and assessment of alcohol consumption based on the ASSIST to identify alcohol abuse (high consumption with consequences for the individuals' routine) [11]. The frequency of consumption was assessed considering the drinking history in the last 3 months and categorized as frequent consumption (daily and weekly), occasional consumption (monthly and once in the last 3 months), and does not consume (never). Binge drinking was determined based on the consumption of four doses for women and five doses for men on a single occasion in the 30 days prior to the interview, categorized as yes or no. The ASSIST scale was applied according to version 3.1 [11]. The calculations based on the weights and cutoff scores of versions 2.0 [13, 14] and 3.1 [11] were performed separately. Details about the scoring system and differences between the versions can be found in Additional file 1: Supplement 1. For a better understanding of the tool, when identified with two factors, the ASSIST factors obtained through exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) were identified and combined in a quadrant to be analyzed (Fig. 1).
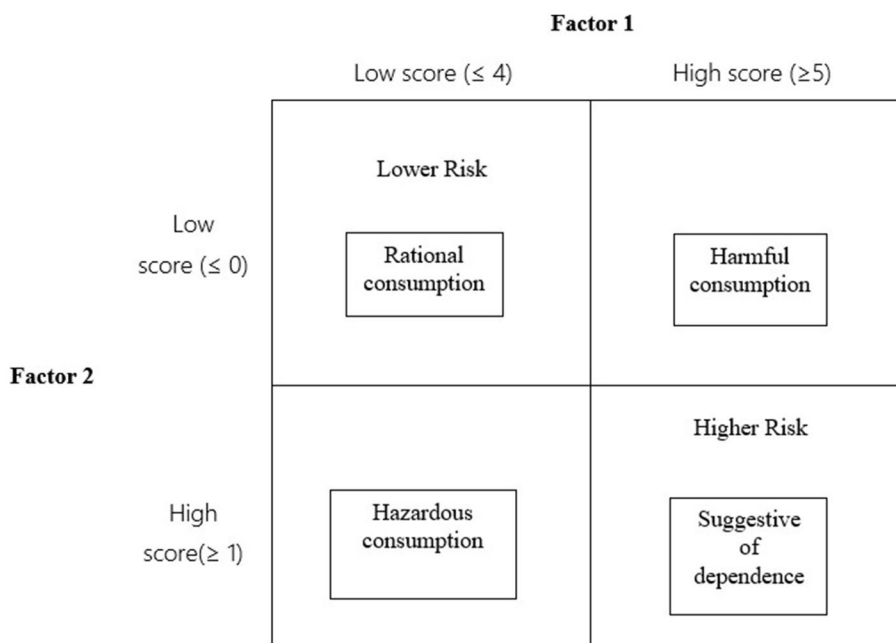
Fig. 1 Quadrant of classification according to the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) questionnaire administered to students from a public university in Londrina, Paraná, Brazil, 2019

Variables used for convergent validity

Convergent validity was verified for worse sleep quality [10, 23], burnout [24], and depressive symptoms [25], which are mental health constructs associated with higher alcohol consumption. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) [26, 27]. After applying the PSQI, individuals who had sleep duration < 3 h, sleep latency > 300 min, or sleep efficiency > 100% (with a difference > 30 min) in the 30 days prior to the interview were excluded from the analyses as quality criteria [28]. Data on academic burnout were obtained using the Copenhagen Burnout Inventory (CBI), based on the sum of the four dimensions of burnout (personal, study-related, peer-related, and teacher-related) [29, 30]. The presence of depressive symptoms was assessed according to the Patient Health Questionnaire-9 (PHQ-9) scale [31, 32]. All three scales were analyzed continuously, with higher scores indicating the worst condition, i.e., worse sleep quality [26, 27], higher burnout [29, 30], and more depressive symptoms [31, 32].

Factor analysis and internal consistency

Since ASSIST versions 2.0 [13, 14] and 3.1 [11] differ in the scoring system and cutoff scores but not in individual responses, the factor analysis of the different versions yields the same results. Thus, the EFA of the ASSIST 3.1 was performed with the seven items that receive scores (questions 2 to 7), using the fit indices and factor loadings to identify the number of factors in the scale.

CFA was performed on the models proposed in the previous step using the weighted least squares mean and variance adjusted (WLSMV) estimator with a polychoric correlation matrix. The parameters estimated by the proposed models in the CFA included standardized loadings (λ) with the respective 95% confidence interval (95% CI), standard error (SE), and correlations between the factors (ϕ). Factor loadings (λ) > 0.40 were considered satisfactory. To analyze the fit of the models tested in the CFA, four indices were used: the root mean square error of approximation (RMSEA; < 0.08 ideal and < 0.10 acceptable), the comparative fit index (CFI; > 0.95 adequate), and the Tucker–Lewis Index (TLI; > 0.90 adequate) [15, 16].

Residual analysis was performed, and internal consistency was assessed using composite reliability and convergent factor validity, considering the average variance extracted and the correlation between factors [33]. Additionally, the internal consistency of the complete tool and the extracted factors was evaluated using Cronbach's alpha [34]. EFA and CFA were performed in the Mplus software, and Cronbach's alpha was performed in the SPSS software.

Convergent validity analysis

Convergent validity was conducted using linear regression models to obtain the beta value and 95% confidence

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intervals (95% CI) between the alcohol-related variables and mental health-related variables (dependent). In addition to the crude analyses, regressions were adjusted by age (continuous), sex, ethnic identity, full-time course enrollment, workload, and daily social media usage time.

Furthermore, a variable related to alcohol consumption was created, resulting from the combination of the two factors extracted in the CFA. Therefore, the individuals were classified into higher or lower scores for the first (F1) and second (F2) factors, based on medians of 4 and 0, respectively. The factors were combined into a possibility quadrant using the ASSIST quadrant (QA) model (Fig. 1), considering individuals with lower scores in both factors as the lowest risk category, individuals with higher scores in F1 and lower scores in F2 or lower scores in F1 and higher scores in F2 as intermediate categories, and individuals with higher scores in both F1 and F2 as the highest potential risk category. Convergent validity analyses were processed in the SPSS software.

Ethical issues
This study was approved by the Research Ethics Committee of the State University of Londrina (CAAE 04456818.0.0000.5231). All participants were informed about the research objectives and signed the informed consent form before the questionnaire was carried out.

Results
Of the 12,536 university students who were eligible for the study, 3252 completed the questionnaire. Of these, 547 were considered losses for lack of information or for exceeding the threshold values for sleep quality information, resulting in a final sample of 3187 (21.6%) university students (Additional file 1: Supplement 2).

The mean age was 21.9 years (± 4.5), and the majority were female (68.1%), not working (64.5%), and using social media for up to 3 h a day (62.0%). Regarding sleep quality, the mean PSQI score was 7.6 points (± 3.3), with a mean sleep duration of 385.6 min (± 69.5) (Table 1).

Factor analysis and internal consistency results
The EFA identified a two-factor behavior in the ASSIST questionnaire. In the CFA, after analyzing residuals and inserting interactions, the one- and two-factor models showed satisfactory indices, with a better fit for the two-factor model (Table 2) when compared to the one-factor model (Additional file 1: Supplement 3). Cronbach's alpha was 0.714 for the one-factor model and 0.718 and 0.538 for F1 and F2, respectively.

Table 1 Sociodemographic characterization of students from a public university in Londrina, Paraná, Brazil, who composed the final sample ($n = 3187$) in 2019

Variables	Number	Percent
Sex		
Female	2187	68.6
Male	1000	31.4
Ethnic identity[a]		
White	2227	69.9
Non-White	958	30.1
Full-time course enrollment[a]		
Yes	1412	44.3
No	1773	55.7
Workload*		
Does not work	2081	65.4
Works up to 30 h per week	730	22.9
Works 31 h or more per week	374	11.7
Average daily social media usage time[a]		
≤179 min	1930	61.0
180–299 min	745	23.6
≥300 min	487	15.4
	Average	**Standard deviation**
Age (in years)	21.8	4.1
PSQI	7.8	3.1
CBI[a]	75.2	18.4
PHQ9[a]	13.5	6.7

PSQI Pittsburgh Sleep Quality Index, *CBI* Copenhagen Burnout Inventory, *PHQ9* Patient Health Questionnaire-9

[a] Smaller sample number due to lack of response to one or more questions of the variable

Results of the convergent validity analysis
Alcohol consumption in the 3 months prior to the interview was reported by 79.1% of university students, with 40.3% reporting a history of binge drinking (Additional file 1: Supplement 4). In the crude analyses (data not shown) and in the regression models adjusted for confounding factors, frequent alcohol consumption, history of binge drinking, alcohol abuse, and higher ASSIST scores were associated with worse sleep quality, greater burnout symptoms, and depressive symptoms (Table 3).

The results of the present study were consistent with the fact that alcohol consumption, regardless of the method of analysis (frequency, binge drinking, and alcohol abuse), has an effect on the mental health-related variables investigated. CFA showed adequate results for both the one- and two-factor models of the ASSIST, with better-fit indices for the two-factor model. Analyses using the one-factor model, regardless of the scoring system used (version 2.0 or 3.1), demonstrated a greater magnitude of effect for the three health outcomes investigated, as well as greater ease of interpretation and use.

Table 2 Confirmatory factor analysis of the ASSIST scale, two-factor model, in students from a public university in Londrina, Paraná, Brazil: standardized factor loadings (λ), standard error (SE), compatibility intervals (CI), and model fit indices

Scale items	Load (λ)	95% CI	SE
Moderate problems associated with alcohol consumption (F1)			
1. In the last 3 months, how often have you used alcohol?	0.666	0.632; 0.700	0.020
2. During the last 3 months, how often have you had a strong desire or urge to use alcohol?	0.733	0.705; 0.761	0.017
3. How often has your alcohol use led to health, legal, social, or financial problems?	0.869	0.842; 0.897	0.017
4. How often have you failed to do what was normally expected of you because of your alcohol use?	0.818	0.789; 0.847	0.018
Serious problems associated with alcohol consumption (F2)			
5. Has a friend or relative or anyone else ever expressed concern about your alcohol use?	0.800	0.757; 0.844	0.026
6. Have you ever tried to cut down on using alcohol but failed?	0.718	0.676; 0.793	0.026
Correlations between the factors	**Φ**	**95% CI**	**SE**
F1 $\leftrightarrow$ F2	0.753	0.712; 0.760	0.025
Fit indices (adequate reference value)		**95% CI**	
RMSEA (< 0.08)	0.033	0.020; 0.047	
CFI (> 0.95)	0.996		
TLI (> 0.90)	0.993		
F1 factor composite reliability (≥ 0.7)	0.852		
Average variance extracted from the F1 (≥ 0.5)	0.592		
F2 factor composite reliability (≥ 0.7)	0.731		
Average variance extracted from the F2 (≥ 0.5)	0.577		

Questions 1 to 4 are based on the last 3 months

RMSEA root mean square error of approximation, *CFI* comparative fit index, *TLI* Tucker–Lewis Index

Discussion

The CFA of the ASSIST showed indices compatible with one- and two-factor behavior. However, in the convergent validity analysis, the two-factor model, assessed using the QA, showed a lower magnitude of effect with the evaluated constructs than with those obtained using the one-factor structure of ASSIST.

Factor analysis and internal consistency

The CFA identified the presence of two distinct factors. Other studies that also evaluated the psychometric properties of the ASSIST instrument regarding alcohol consumption showed one-factor [20] or two-factor [19] behavior for university students. Such observations could indicate a variation in ASSIST behavior and the need to perform CFA on this scale before its analysis in new studies, especially considering the differences between the factors found in this study and those present in the literature [17–19]. However, this assumption is fragile, as the analyses conducted by other studies [17–19] did not include adjustment terms, or they do not use the short version most used in the literature [18]. Thus, a more comprehensive analysis of the use of the two-factor structure of the ASSIST is necessary.

The internal consistency of the extracted factors showed an adequate result for F1 but an unsatisfactory result for F2, a result similar to that reported by Mostardinha et al. [19] when investigating the internal consistency for a factor composed of only two questions. Despite the unsatisfactory Cronbach's alpha for F2, this result can be attributed to the small number of questions in this factor, since the formula is sensitive to the number of questions, which artificially generates lower reliability for factors/tools with two questions [34, 35]. Due to the fragility of this analysis, a theoretical evaluation of the extracted components is essential, as well as the evaluation of other psychometric properties [35] and convergent validity, which does not support the use of ASSIST in two-factor in the present study.

The questions that comprise F2 in the CFA (relatives' or friends' concern about alcohol consumption and failing to stop/reduce drinking) have a lower maximum score than some of the other questions and only three possible responses, which may have led to their underestimation in the CFA using only one factor and benefited the two-factor model. From a theoretical perspective, the two identified factors differed mainly in the possible severity of the behavioral change in relation to alcohol consumption. While the first factor (F1) was composed of questions related to the frequency of consumption and possible moderate severity consequences, the second factor (F2) included questions that may indicate greater severity in the individuals' alcohol consumption. Attempting to stop or to reduce alcohol consumption

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Table 3 Crude and adjusted multivariate analysis between variables related to alcohol consumption and variables related to mental health in students from a public university in Londrina, Paraná, Brazil, 2019

Independent variables	βadj (95% CI, *p* value)		
	PSQI	CBI	PHQ9
Frequency of alcohol consumption in the last 3 months			
Frequent	**0.540 (0.208; 0.871, 0.001)**	**2.949 (1.177; 4.721, 0.001)**	**1.667 (1.028; 2.306, < 0.001)**
Occasional	0.228 (− 0.086; 0.543, 0.155)	**2.088 (0.409; 3.767, 0.015)**	**0.683 (0.076; 1.290, 0.027)**
Does not consume	0	0	0
History of binge drinking in the last 3 months			
Yes	**0.749 (0.507; 0.991, < 0.001)**	**3.312 (1.806; 4.417, < 0.001)**	**1.537 (1.067; 2.008, < 0.001)**
No	0	0	0
Classification of alcohol consumption (ASSIST 2.0)			
Suggestive of dependence	**2.812 (0.812; 4.813, 0.006)**	**18.104 (7.202; 29.006, 0.001)**	**7.837 (4.242; 11.432, < 0.001)**
Suggestive of abuse	**1.150 (0.888; 1.412, < 0.001)**	**4.994 (3.571; 47.309, < 0.001)**	**2.741 (2.235; 3.246, < 0.001)**
Occasional use	0	0	0
Classification of alcohol consumption (ASSIST 3.1)			
Suggestive of dependence	**2.157 (1.478; 2.835, < 0.001)**	**12.246 (8.429; 16.063, < 0.001)**	**6.685 (5.368; 8.002, < 0.001)**
Suggestive of abuse	**1.351 (1.067; 1.634, < 0.001)**	**5.128 (3.589; 6.668, < 0.001)**	**2.806 (2.263; 3.349, < 0.001)**
Occasional use	0	0	0
Consumption-consequences quadrant (ASSIST factors)			
Suggestive of dependence	**1.841 (1.500; 2.183, < 0.001)**	**8.769 (6.914; 10.624, < 0.001)**	**4.524 (3.868; 5.180, < 0.001)**
Harmful consumption	**1.284 (0.840; 1.728, < 0.001)**	**7.161 (4.766; 9.555, < 0.001)**	**3.591 (2.734; 4.448, < 0.001)**
Hazardous consumption	**0.623 (0.294; 0.953, < 0.001)**	0.682 (− 1.086; 2.450, 0.450)	**0.648 (0.019; 1.277, 0.043)**
Rational consumption	0	0	0

βadj by age (continuous), sex, ethnic identity, full-time course enrollment, workload, and social media use

95% CI 95% confidence interval, *PSQI* Pittsburgh Sleep Quality Index, *ASSIST* Alcohol, Smoking and Substance Involvement Screening Test, *CBI* Copenhagen Burnout Inventory, *PHQ9* Patient Health Questionnaire-9

and failing has been associated with a pattern of heavy drinking [36], which may indicate that the questions for this factor are appropriate for identifying heavy drinking. However, no studies that could help in the theoretical understanding of the selection of these questions in a single factor were found.

Despite the possibility that dividing the ASSIST into two factors occurred due to issues inherent to the tool's construction (score and/or number of response categories), it is important to consider that other quality criteria were explored in this study, such as residual analysis, composite reliability, and average variance extracted. These criteria allow us to evaluate the interaction between questions and the tool's accuracy [15, 33], reinforcing the relevance of a special analysis for the questions in F2. Despite the advances made with CFA findings, the absence of these calculations to analyze the results is a recurrent limitation in the literature [17–19], especially for tools with established one-factor structure [35], such as the ASSIST. Thus, the QA-based analysis provided a verifiable analysis approach for the two-factor structure of the ASSIST, which can be replicated in other studies.

Convergent validity

The use of a more comprehensive method to assess alcohol consumption, such as the ASSIST, has demonstrated greater convergent validity than consumption measurement methods, as pointed out in the literature [10, 23–25]. Among the ASSIST versions, 3.1 showed a greater number of individuals in the "suggestive of abuse" and "occasional use" categories, influenced by the greater weight attributed to certain behaviors when compared to version 2.0. The modifications made between versions 2.0 and 3.1 seem to be justified by the fact that alcohol consumption is a very frequent behavior [3, 10].

Considering the strong association between greater alcohol consumption, especially alcohol abuse, and poorer sleep quality [10, 23], burnout [24], and depressive symptoms [25], it can be concluded that although the two-factor model showed better fits in the CFA, it did not impact the convergent validity. In contrast, dividing the ASSIST into two factors increased the complexity of the analysis and decreased the magnitude of the effect.

Given the lack of literature evaluating the different methods to analyze the two-factor structure of the ASSIST, it was not possible to compare the present

results with those of other studies. Regardless, EFA and CFA should aid in the understanding of the tools and not be insurmountable barriers [35]. Therefore, the interpretation of CFA in conjunction with convergent validity seems to safely indicate the use of the one-factor versions (2.0 and 3.1) of the ASSIST.

Regarding their comparisons, although the category "suggestive of dependence" in version 2.0 presented a greater magnitude of effect, it is important to note that, due to the scoring system and cutoff score adopted in this version, this result was found for only a very small stratum of the sample (0.5%). Thus, although the present study did not employ a gold standard for alcohol dependence, it is possible to infer that version 2.0 has greater specificity but low sensitivity for identifying individuals who fit in the "suggestive of dependence" category. In contrast, version 3.1, corroborating the improvements made [11], showed adequate convergent validity and higher sensitivity, compatible with the objective of the ASSIST, i.e., to be a screening instrument and therefore to have higher sensitivity [11, 13, 14]. Thus, the results of the present study reinforce the use of version 3.1 with a one-factor structure.

This study evaluated different ways of investigating alcohol consumption in a population of university students to understand the factor behavior and convergent validity of the ASSIST. However, all the variables used were self-reported, and the tests did not employ a gold standard for alcohol dependence that could be used in estimating the sensitivity and specificity of the studied versions. Despite these limitations, it is noteworthy that this study used a large sample, investigating in depth the individuals' psychometric properties and employing several quality indices to evaluate the models, as well as an analysis of the results based on constructs that have a well-established association.

The ASSIST tool has a high clinical and epidemiological relevance. In this sense, the present study contributes through the analysis of the psychometric properties in a considerable population of young adults, identifying that the results are adequate to the one-factor model. Nevertheless, an analysis proposal was made for the possible two-factor behavior, which may contribute to future investigations.

Conclusion

Based on the evidence presented, it is possible to conclude that the assessment of alcohol consumption should preferably be performed using comprehensive tools, such as the ASSIST. Although the assessment of psychometric properties is essential for the understanding of epidemiological scales, the two-factor behavior of the ASSIST was not adequate in terms of convergent validity. Thus, considering the adequate results of the one-factor model

with residual analysis and insertion of interactions, the results of this study indicate that the use of version 3.1 with a one-factor structure is more suitable for assessing alcohol consumption in young adults.

Abbreviations

ASSIST	Alcohol, Smoking and Substance Involvement Screening Test
CFA	Confirmatory factor analysis
βadj	Adjusted beta value
95% CI	95% Confidence interval
UEL	State University of Londrina
EFA	Exploratory factor analysis
PSQI	Pittsburgh Sleep Quality Index
CBI	Copenhagen Burnout Inventory
PHQ-9	Patient Health Questionnaire-9
WLSMV	Weighted least squares mean and variance adjusted
λ	Standardized loadings
SE	Standard error
φ	Correlations between the factors
CFI	Comparative fit index
TLI	Tucker–Lewis Index
QA	ASSIST quadrant
F1	Factor 1
F2	Factor 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43045-024-00392-y.

> **Additional file 1: Supplement 1.** Comparison of versions 2.0 and 3.1 of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) questionnaire. **Supplement 2.** Flowchart of losses and exclusions. **Supplement 3.** Confirmatory factor analysis of the ASSIST scale, single-factor model, in undergraduate students from a public university: standardized factor loadings (λ), standard error (SE), confidence intervals (CI) and model fit indices. **Supplement 4.** Variables related to alcohol consumption in undergraduate students of a public university, Brazil, 2019.

Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All procedures followed were in accordance with the ethical standards of the ethical committees of Universidade Estadual de Londrina and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent for participation was obtained from all participants for being included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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