CUT-OFF VALUES OF BODY FAT PERCENTAGE AND INSULIN FOR PREDICTING LOW ADIPONECTIN / LEPTIN RATIO

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CUT-OFF VALUES OF BODY FAT PERCENTAGE AND INSULIN FOR PREDICTING LOW ADIPONECTIN / LEPTIN RATIO (Abstract): This study **aims** to establish a reliable parameter and its optimal cut-off point for accurately estimating adipose tissue dysfunctionality in apparently healthy, but abdominally obese subjects. **Materials and methods:** The 104 subjects included in the study, all with no prior chronic diseases, underwent biochemical (TG, HDL-c, glucose, insulin, adiponectin, leptin) and anthropometric measurements (waist circumference, height, adipose tissue percentage). A ROC curve was performed to assess the overall performance of these parameters to determine the dysfunctionality of the adipocytes, as assessed by adiponectin: leptin ratio (ALR) lower than 0.5. Logistic regression identified models that included 2 or 3 parameters to further increase the accuracy. The software Excel and SPSS were used for the statistical analysis. **Results:** From all individual parameters, trunk adipose tissue percentage had an AUC value of 0.812, suggesting a high accuracy for predicting a value of ALR<0.5. The optimal cut-off point for trunk adipose tissue percentage was established at 42.45% with a sensitivity of 0.725 and a specificity of 0.766. A higher AUC (0.857) was observed for the model that included whole-body adipose tissue (cut-off 41.7%) and insulin (cut-off 14.6 μU/mL), while the highest accuracy (AUC=0.889) was reported in the model for metabolically unhealthy non-obese subjects. **Conclusions:** Apparently healthy individuals, but abdominally obese, report adipocyte dysfunctionality together with high cardiometabolic risk at values for trunk adipose tissue percentage surpassing 42.45%, or combined values of 41.7% for whole-body adipose tissue and 14.6 μU/mL for insulin. **Keywords:** CUT-OFF VALUES, BODY FAT, INSULIN, ADIPONECTIN / LEPTIN RATIO.

INTRODUCTION

It is well-established that quantity and quality of adipose tissue play a critical role in metabolic health, as both excessive or insufficient adipose tissue are associated with significant health risks. In conditions such as congenital or acquired lipoatrophy insufficient adipose tissue leads to severe metabolic disturbances and conversely, obesity is also linked to similar metabolic complications (1, 2). Adipocytes have a unique secretory profile that includes adipokines like leptin and adiponectin, cytokines or various metabolites, which collectively interact to establish communication pathways between organs (3). Their bal-

ance is vital for organism physiology, therefore accurately identifying adiposopathy is pivotal in a clinical setting for prevention and diagnostics strategies, while particularly challenging to attain. Previous studies have confirmed adiponectin/leptin ratio, as opposed to single biomarker approaches, as a diagnostics tool for adipocyte metabolism dysfunctionality, with lower values than 1 being associated with cardiometabolic risk (4, 5).

This study aims to establish a reliable parameter and its optimal cut-off point for accurately estimating adipose tissue dysfunctionality defined as adiponectin/leptin ratio < 0.5. There are some key points we consider as arguments for this analysis. While adiponectin and leptin together profile the status of adiposopathy and that of cardiometabolic risk, these assays are not performed usually as screening tests in individuals and their cost is handled by the patient, therefore a balanced solution would be highly valued. Judging from an economical and practical point of view, the detailed profile of an individual that represents the core of personalized medicine is still hard to portray. However, modern technologies including artificial intelligence or advanced statistics aid in generating a more complete profile as possible. To generate algorithms solely for adiponectin and leptin with the help of hands-on and easy to obtain parameters is a challenging task. Therefore, our study focused on predicting the reduced ratio levels between the two which has a broader significance and could tell us more about the functionality of the adipose tissue, despite its limited focus on specific outcomes. We expect to have a more detailed picture of the apparently healthy individual that also has abdominal obesity, with respect to adipocyte metabolism and cardiometabolic implications.

MATERIALS AND METHODS

This observational cross-sectional study included 104 subjects, aged 35-75 years old (25.96% men and 74.04% women), with abdominal obesity and no prior known chronic disorders or acute atherosclerotic events in antecedents. All patients underwent biochemical and anthropometric measurements in the morning, with blood samples being collected after a 12 hrs. overnight fast. Serum samples for marker analysis were stored at -20ºC. Glucose, TG, HDL-c levels were determined using the spectrophotometric method, insulin using chemiluminescence, adiponectin and leptin with ELISA technique.

Visceral adiposity index was calculated based on previous validated equations (6). For adipose tissue mass and percentage, dual-energy X-ray absorptiometry (DEXA) was used, as a gold standard for this measurement.

The population included in the cohort was apparently healthy, but after investigations some subjects presented metabolic syndrome (MetS). The presence of MetS included waist circumference > 88 cm (women)/104 cm (men) and at least 2 of the following criteria: glucose ≥ 100 mg/dL, HDL < 40 mg/dL (men) / < 50 mg/dL (women), TG ≥ 150 mg/dL, SBP/DBP ≥ 130/85 mmHg (7).

Data was processed with Excel and SPSS. Distribution was assessed for all variables with the Kolmogorov-Smirnov test and confirmed visually, analyzing the histograms. Correlations between variables were performed with Spearman and Kendall's tau-b test. Logistic regression delivered models with high performance and the predicted probability calculated for these models was further used as input for the

receiver operating characteristic (ROC) curve for determining specific biomarker values to better predict a low ALR.

RESULTS

All analyzed variables are non-normally distributed, except trunk adipose tissue percentage which has a normal distribution. In this study 74.04% of the subjects were women and 25.96% were men, with a mean age of 59.51±8.18. Baseline characteristics of the study are presented in first table.

TABLE I.
Baseline characteristics of the study group

Parameter	Median (Q3-Q1)	Min	Max
Age (years)	62 (53-65)	39	75
BMI (kg/m^2)	30.99 (28.57-34.39)	21.91	53.31
BMI (kg/m^2) - women	30.23 (28.01-34.23)	21.91	49.50
BMI (kg/m^2) - men	32.43 (30.17-35.61)	22.75	53.31
Waist circumference (cm)	106 (99-115)	89	160
Insulin (µU/mL)	15 (9.38-21.45)	2.13	52
Glucose (mg/dL)	103 (94-116)	76	336
HOMA-IR	3.78 (2.38-5.99)	0.49	27.25
Adiponectin (µU/mL)	13.02 (10.33-16.77)	4.53	28.53
Leptin (ng/mL)	19.63 (11.22-39.67)	2.60	58.20
ALR	0.59 (0.33-1.36)	0.13	4.98
eVAI	4.50 (3.15-7.64)	1.38	33.66
Whole-body adipose tissue (%)	41.10 (36.32-44.27)	18.90	53.20
Parameter	**Mean±S.D.**	**C.I. (95%)**	
		Lower limit	**Upper limit**
Trunk adipose tissue (%)	40.30 ± 6.42	39.05	41.55

* S.D.= standard deviation, Q3-Q1=difference between superior quartile and inferior quartile, ALR=adiponectin/leptin ratio, eVAI=estimated visceral adipose index.

The correlation matrix between biomarkers and parametric variables describing adipose tissue health report ALR as having stronger correlations with obesity parameters than adipokines alone. The eVAI parameter reports little to no correlation with all the other variables, adipose tissue percentage presents a stronger correlation with ALR than BMI and insulin is stronger correlated with ALR than HOMA-IR. All correlation coefficients over 0.200 are statistically significant (tab. II).

Logistic regression rendered two best models presented in third table for estimating the status of ALR < 0.5. Variables with the strongest correlation were the ones that performed best, with whole-body adipose tissue percentage and insulin being the most important influencers for low ALR. In order to calculate the accuracy of each parameter and model for assessing low ALR, ROC curve analysis was performed (fig. 1).

The AUC values of factors predicting adipose tissue dysfunctionality confirms high accuracy for adipose tissue percentage, insulin and the 2 models (tab. IV). Optimal cut-off points were chosen for these parameters, preserving in the same time the highest sensitivity and specificity possible (tab. V).

TABLE II.

Correlations between biochemical and anthropometric parameters of obesity

	Insulin	Glucose	HOMA-IR	Adiponectin	Leptin	ALR	BMI	Waist circumference	eVAI	Trunk adipose tissue %	Total adipose tissue %
Insulin	1	0.336	0.946	-0.404	0.392	-0.575	0.386	0.405	0.291	0.206	0.069
Glucose	0.336	1	0.552	-0.236	-0.07	-0.047	0.13	0.38	0.248	-0.095	-0.127
HOMA-IR	0.946	0.552	1	-0.414	0.304	-0.496	0.368	0.464	0.314	0.133	0.007
Adiponectin	-0.404	-0.236	-0.414	1	0.119	0.362	-0.286	-0.308	-0.216	0.052	0.234
Leptin	0.392	-0.068	0.304	0.119	1	-0.86	0.416	0.225	0.038	0.744	0.778
ALR	-0.575	-0.047	-0.496	0.362	-0.86	1	-0.522	-0.367	-0.157	-0.674	-0.615
BMI	0.386	0.13	0.368	-0.286	0.416	-0.522	1	0.788	0.172	0.381	0.295
Waist circum-ference	0.405	0.38	0.464	-0.308	0.225	-0.367	0.788	1	0.277	0.222	0.056
eVAI	0.291	0.248	0.314	-0.216	0.038	-0.157	0.172	0.277	1	0.157	0.014
Trunk adipose tissue %	0.141	-0.061	0.086	0.034	0.557	-0.479	0.381	0.222	0.157	1	0.754
Total adipose tissue %	0.069	-0.127	0.007	0.234	0.778	-0.615	0.295	0.056	0.014	0.908	1

*Spearman test correlation was performed for all variables, except for “Trunk adipose tissue percentage” which required Kendall’s tau -b correlation test

TABLE III.

Models for estimating the status of ALR < 0.5.

Model 1				
Parameters	**B**	**Std. Error**	**Exp(B)**	**p**
Whole-body adipose tissue percentage	0.332	0.076	1.394	<0.001
Insulin	0.105	0.028	1.110	<0.001
Constant	-16.158	3.543	0.000	<0.001
Model 2				
Parameters	**B**	**Std. Error**	**Exp(B)**	**p**
Whole-body adipose tissue percentage	0.333	0.078	1.395	<0.001
Insulin	0.097	0.030	1.102	0.001
Clinical obesity phenotype				
• MHO				0.07
• MUO	1.967	0.875	7.146	0.025
• MHNO	1.988	0.804	7.302	0.013
• MUNO	1.356	1.071	3.880	0.205
Constant	-17.522	3.723	0.000	<0.001

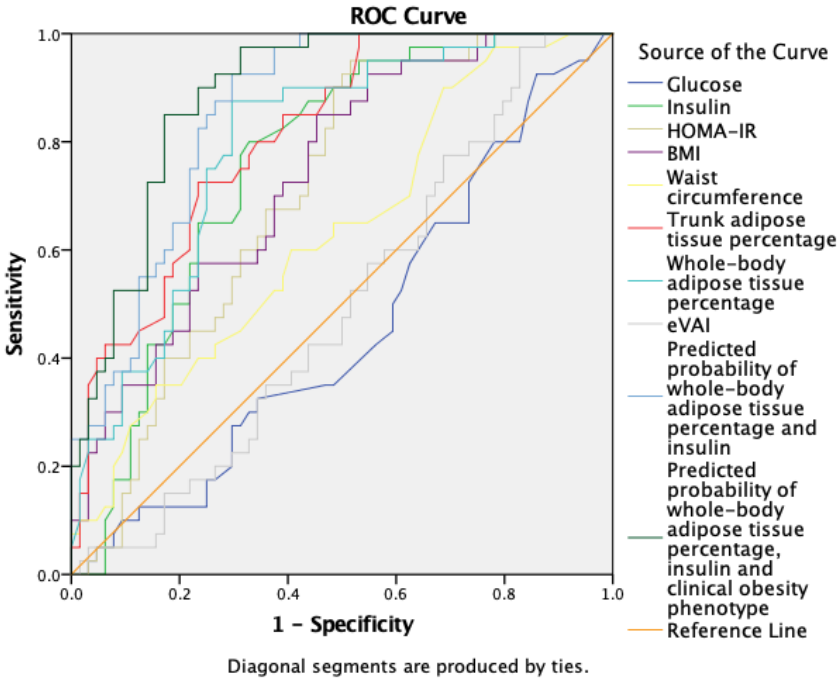

Fig. 1. Receiver operating characteristics (ROC) curve analysis on the prediction of dysfunctional adipose tissue

TABLE IV.

Accuracy estimates for factors predicting adipose tissue dysfunctionality

Parameter	AUC	Std. Error	p	Confidence Interval (95%)	
				Lower Bound	Upper Bound
Glucose	0.456	0.058	0.450	0.343	0.569
Insulin	0.758	0.047	<0.001	0.666	0.850
HOMA-IR	0.705	0.050	<0.001	0.607	0.803
BMI	0.738	0.048	<0.001	0.644	0.833
Waist circumference	0.632	0.055	0.024	0.524	0.740
Trunk adipose tissue percentage	0.812	0.041	<0.001	0.731	0.892
Whole-body adipose tissue percentage	0.795	0.044	<0.001	0.709	0.880
eVAI	0.497	0.057	0.957	0.386	0.608
Model 1*	0.857	0.035	<0.001	0.788	0.926
Model 2**	0.889	0.031	<0.001	0.828	0.950

Note: AUC = area under the curve.

*Model 1 = Predicted probability for whole-body adipose tissue percentage and insulin.

**Model 2 = Predicted probability for whole-body adipose tissue percentage, insulin and clinical obesity phenotypes.

TABLE V.

Optimal cut-off points for predicting adipose tissue dysfunctionality

Parameter	Optimal cut-off	Sensitivity	Specificity	Youden Index
Insulin (μU/mL)	14.90	0.80	0.672	0.472
Whole-body adipose tissue percentage (%)	40.85	0.875	0.703	0.578
Trunk adipose tissue percentage (%)	42.45	0.725	0.766	0.491
Model 1* - Associated variables	0.32 - Whole-body adipose tissue (%): 41.7 - Insulin (μU/mL): 14.6	0.925	0.703	0.628
Model 2** - Associated variables	0.45 - Whole-body adipose tissue (%): 39.1 - Insulin (μU/mL): 15 - Metabolically unhealthy non-obese	0.85	0.828	0.678

*Model 1 = Predicted probability for whole-body adipose tissue percentage and insulin.

**Model 2 = Predicted probability for whole-body adipose tissue percentage, insulin and clinical obesity phenotypes.

DISCUSSION

In the present study we identified predictive values of biomarkers and representative parameters of body composition for adipose tissue dysfunctionality. The main finding is that adipose tissue percent-

age together with insulin identify the presence of ALR lower than 0.5 with a high accuracy rate. The optimal cut-off point for trunk adipose tissue percentage is 42.45% and for insulin is 14.9 μU/mL. These values are referenced against other established thresholds for cardiometabolic risk in several studies. Bawasi H. *et al*. proposed sex-specific cut-off points for adipose tissue percentage in people over 40 years old, with values of 34.8% (men) and 46.3% (women) for total adipose tissue or 21.6% (men) and 23.4% (women) for trunk adipose tissue (8). The collaborative group of Macek P. reports optimal cut-off points for body fat percentage of 25.8% for men and 37.1% for women (9). These values are not standardized as they reflect a threshold for cardiovascular risk that is measured distinctly for every study, therefore it remains difficult to compare them between each other. Furthermore, to the best of our knowledge, this is the first study that calculates a cut-off point of body fat percentage for determining adiposopathy defined as low ALR and moreover, it predicts severe cardiometabolic risks since the value presents an important decrease (inferior to 0.5).

A recent comprehensive review emphasizes that besides BMI, more essential to measuring the extent of adiposity are waist circumference and body fat percentage. Together with the clinical component these are critical for a complications-centric approach to obesity care, which aligns with the Adiposity-Based Chronic Disease framework (10). A position statement on visceral and ectopic fat highlights the necessity of creating and implementing methods to detect individuals with excessive adiposity, both in clinical settings and within public health initiatives (11). Moreover, trunk adipose tissue percentage, particularly visceral fat is strongly associated with metabolic risk factors, including insulin resistance, reinforcing the utility of adipose tissue percentage as a reliable predictive marker (12), together with adiponectin and leptin as primarily derived molecules from adipose tissue (13) as promising candidates for distinguishing between insulin-sensitive and insulin-resistance obesity phenotypes (14).

The variables included in the models were depicted based on their influence on adiponectin-leptin ratio, which was assessed with logistic regression. Even though the clinical obesity phenotype variable does not have a significant p value as a whole group, model 2 outperformed model 1. Insulin is present in both models in our study, confirming its key influence on ALR, since insulin resistance likely results from the dynamic interrelationship between multiple adipokines rather than the action of a single molecule. Important pathophysiological processes take place when leptin is hyper secreted and adiponectin underproduced, like: pancreatic β-cells damage reducing insulin production, exacerbation of glucose intolerance (15), suppression of glucose transporter-4 and insulin receptor substrate-1 expression (16, 17). In our study HOMA-IR reported a lower accuracy rate for predicting adipose dysfunctionality than insulin alone, however the values demonstrated proximity. To confirm whether indeed insulin or HOMA-IR is more relevant in a clinical setting to predict adipose dysfunctionality, we would need to extend the study to a larger cohort.

The ROC analysis used in our study depicts the trade-off between sensitivity and specificity over a series of established thresholds for different obesity linked pa-

rameters. The optimal cut-off points were chosen based on the Younden index, the global measure of biomarker effectiveness (18). However, the choice could be subjective depending on the severity of the disease. In our case, identifying adiposopathy is mostly used in preventive or cardiovascular stratifying programs, therefore we can accept a balance between correctly and incorrectly classifying individuals with the disease. In severe pathologies, we would need to choose a cut-off point that provides 100% sensitivity, even if the specificity in this case would be low.

Since it is difficult in clinical settings to find the specific values for all variables in one patient, these models are difficult to implement in a day by day practice. Therefore, individual variables could be of better use to clinicians, since they provide a clear percentage of false positives or false negatives regarding adiposopathy. Since body composition analysis implies a high cost, artificial intelligence networks can be used to calculate adipose tissue percentages with high accuracy, therefore making it almost cost-free to estimate ALR. The limitation is, of course, the false positive and false negative rates that have to be considered. Our group worked on an algorithm that predicts with high accuracy trunk adipose tissue mass from easy to measure anthropometric parameters (19), while there are also other models performed on larger cohorts sustaining the need for this cost-effective prediction tool (20-22).

CONCLUSIONS

Apparently healthy individuals, but abdominally obese, report adipocyte dysfunctionality together with high cardiometabolic risk at values for trunk adipose tissue percentage surpassing 42.45%, or combined values of 41.7% for whole-body adipose tissue and 14.6 μU/mL for insulin. Adipose tissue percentages can be easily and accurately estimated based on already developed algorithms, therefore the severe cardiometabolic risk linked to ALR values lower than 0.5 can be estimated almost cost-free.

These findings have clinical relevance for patient care and contribute significantly to the advancement of personalized medicine, particularly within the context of preventive strategies and cardiovascular risk stratification.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest or funding.

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