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Importance of anthropometry in assessing insulin resistance as a pre-alarming sign before the onset of metabolic syndrome: a study among apparently healthy subjects

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Abstract Insulin resistance (IR) and obesity are inter-related causes of metabolic syndrome. Early identification before the onset of metabolic syndrome will be useful to lead a healthy life. The purpose of the present study was to identify the importance of IR before the onset of metabolic syndrome in apparently healthy, non-diabetics subjects. Data of 227 apparently healthy non-diabetics (20-70 years) who reside in a suburban area in Colombo district, Sri Lanka, were recruited for this study. Fasting blood glucose (FBG), fasting serum insulin (FSI), weight, height, waist circumference (WC), hip circumference (HC), and mid-upper arm circumference (MUAC) were measured and homeostatic model assessment for insulin resistance (HOMA-IR) was calculated. Body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated. Data were analyzed using Statistical Package for Social Science (ver.17). Majority were females (61.8 %). Prevalence of IR was 59.9 %. Mean BMI of IR subjects was 28.3 ± 2.7 kg $m^{-2}$where 75.3 % of obese had IR. All anthropometric variables except height had significant positive correlations ($P<0.01$) with IR. Linear regression analysis indicated that BMI is useful in predicting IR while logistic regression analysis showed that BMI and WC are the best predictors of IR in males whereas it was WHtR and WC in females. Even though study subjects were apparently healthy and not diagnosed as diabetes, those with elevated anthropometric parameters had higher prevalence of IR. Best anthropometric predictors of IR for a specific sex should be used as an easy self-monitoring alarming sign before the onset of metabolic syndrome.

Keywords Insulin resistance · Body mass index · Waist circumference · Apparently healthy · Waist-to-height ratio · Waist-to-hip ratio

Introduction

Insulin resistance (IR) is a state in which normal amounts of serum insulin are not adequate to produce the expected biologic response in target tissues like adipocytes, muscle, and liver [1]. IR is a characteristic feature of type 2 diabetes (T2D) which is mainly linked with metabolic syndrome (MS). IR is considered as one of the major causative factors for MS, a cluster of metabolic abnormalities including diabetes, high blood pressure, and high cholesterol levels along with obesity. Over the past two decades, worldwide prevalence of MS has increased significantly. Approximately 20–25 % of the worlds' adult population accounts for MS, and they are prone to fivefold greater risk of developing T2D [2]. It is estimated that around 90–95 % of diabetes worldwide are diagnosed as T2D with IR. Furthermore, prevalence of MS among South Asians is estimated to be 20–25 %, and early onset of T2D and cardiovascular diseases (CVD) is common among Asians [3]. In 2005, the prevalence of T2D among Sri Lankans was approximately 11 % and one fifth of adults were found to be dysglycemic [4]. Obesity, as one of the major components of MS, has reached epidemic proportions during the last three

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decades [5]. A global pandemic of obesity is driving the increased incidence and prevalence of T2D and, on the other hand, increased IR causes some degree of obesity. Overweight and obesity prevalence was at peak in the USA out of all the WHO regions and lowest was reported in South East Asia. Women have higher tendency to be obese than men in the world. Incidence of overweight and obesity among Sri Lankans were at elevated levels. In year 2005/2006, the prevalence of overweight of both genders was 25.2 %, and in 2006/2007, 31.2 % of women were found to be overweight [6].

Studies have shown that increased IR results in failure of suppression of hepatic glucose production and peripheral IR impairs peripheral glucose uptake. Thus, it leads to fasting and postprandial hyperglycemia with high fasting serum insulin (FSI) levels with the progression to T2D. Later in the course of T2D, IR begets IR by reduced non-oxidative glucose metabolism in muscles, further exacerbating hyperglycemia [7]. Yet, many studies consider IR as a diagnostic criterion in overt T2D individuals despite the fact that increase in IR makes a strong predictor for early development of MS and future diabetes [8]. Therefore, assessing IR is a useful indicator to know the state of hyperinsulinemia before the onset of T2D or to alarm the individuals who have the high potential to develop MS [9]. A variety of methods are being used to measure IR, where hyperinsulinemic euglycemic clamp is considered as the gold standard. The homeostasis model assessment of IR (HOMA-IR) was developed based on the above to provide a simple, consistent, and inexpensive method to detect IR in individuals. It involves only measuring FSI value as well as fasting blood glucose (FBG) value of a subject. Thus, it indicates fasting steady state levels of blood glucose and serum insulin for any dynamic function of pancreatic beta cells and insulin sensitivity. HOMA index ≥ 1 is considered as insulin resistant [10]. Therefore, moderate or large increases in HOMA-IR can have an effect on apparently healthy, non-diabetic, but early MS subjects.

Conventionally, multiple methods of cheap costless anthropometry are being used to assess obesity and body fat distribution. As obesity itself could be used as a marker to predict IR, anthropometric measures and indices such as waist circumference (WC), body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) are used to classify general obesity and regional adiposity, and also to define the risk groups who have the tendency to develop metabolic abnormalities. Some studies have correlated different anthropometric parameters with IR [11] in MS subjects.

In genetically predisposed subjects, defects in insulin secretion can lead to impaired fasting glucose (IFG) which can be assessed by the anthropometric measures. Some individuals with obesity might be metabolically healthy and some lean individuals with an increased amount of visceral body fat distribution might have significant IR. Therefore, it is vital to identify which anthropometric parameter has the significant association with IR and which parameter enables optimizing the clinical prediction of IR. Further, detecting insulin-resistant subjects in a general population provides an evaluation of early diagnosis of apparently healthy but early MS individuals. To our knowledge, no study has been carried out to identify the significance anthropometric parameters in measuring IR as an early detection method before the development of MS. Hence, the aim of the present study was to identify the significance of costless, self monitoring anthropometric parameters that would optimize clinical prediction of IR in apparently healthy, non-diabetic, male and female subjects.

Methods

Study design and participants

A descriptive cross-sectional study was carried out at the Family Practice Centre, University of Sri Jayewardenepura, located at Nugegoda, a city exceeding 250,000 of multi-ethnic population. Non-probability, convenience sampling was used and sample size was determined by the equation for estimating mean.

The study was approved by the Ethics Review Committee of University of Sri Jayewardenepura and informed written consent was obtained from all individual participants prior to the study. This study involved 227 study participants between 20 and 70 years of age. The subjects enrolled in this study were fully informed about the study protocol, and informed written consent was obtained from all the subjects, prior to the study. Apparently healthy subjects who were not diagnosed as having diabetes (FBG <6.9 mmol/L) were included in the study, whereas subjects who were pregnant, on steroidal drug, and having severe diseases; who have physical and cognitive impairments; and who dislike to participate in the study were excluded.

Anthropometric measurements and indices

All the anthropometric measurements were taken of the standard protocol according to NHANES [12]. Individuals' height was measured using a stadiometer to the nearest 0.1 cm. Body weight was measured using an electronic digital weighing scale (Chyo, Mu-150 K, Japan) to the nearest 0.1 kg. WC was measured at the approximate midpoint between the lower margin of the last palpable rib and the crest of the ileum (top of the hip bone), placing the non-stretchable tape around the trunk in a horizontal plane. The tape was parallel to the floor and it was without compressing the skin. The measurement was made at the end of a normal expiration to the nearest 0.1 cm. Similarly, HC was measured by placing the non-stretchable tape around the widest portion of the buttocks, and the sides of it were adjusted to ensure that it is in a horizontal plane. The measurement was taken from the right side of the subject to the nearest 0.1 cm. Mid-upper arm circumference (MUAC) was measured when the subjects were standing upright, shoulders relaxed, and the right arm bent at 90°. The tape was wrapped around the midpoint of the arm between the shoulder (Acromian process) and the tip of the elbow (Olecranon process), and the measurement was taken to the nearest 0.1 cm.

Anthropometric index BMI was calculated dividing body weight in kilograms by the square of the height of the body in meters. Anthropometric ratios such as WHR and WHtR were calculated as the ratio of the circumference of the waist to the hip and the circumference of the waist-to-height, respectively [13, 14].

Blood samples and biochemical analysis

The subjects were instructed to come with an overnight fasting period. Prior to phlebotomy, all the required materials for blood drawing were assembled and fasting blood samples were collected from each individual by a trained phlebotomist in an aseptic environment while the subject was in a seated position preceded by a 10–15-min rest. Blood samples were collected into empty sterile centrifuge tubes without an anticoagulant and allowed to clot for 30–40 min at room temperature, for serum separation. About 150 μL blood was pipetted simultaneously, into an Eppendorf tube with NaF for FBG analysis. Collected samples were taken and analyzed at the Faculty of Medical Sciences, University of Sri Jayewardenepura.

Blood in Eppendorf tubes was centrifuged at 3600 rpm for 10 min and serum was separated for FBG analysis. The rest of the blood in centrifuge tubes was centrifuged at 3600 rpm for 10 min at room temperature to separate the serum. Serum was stored in a deep freezer at −20 °C for batch assay of serum insulin while analysis of FBG was done on the same day.

FBG and FSI levels were analyzed using glucose oxidase (GOD-PAP) kit method and ELISA method, respectively. All the required steps of the procedure were carried out as indicated in the kits (Biolabo reagent, Maizy; DRG International, Inc, USA) according to the manufacturers' guidelines. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) defined as

$$HOMA\text{-}IR = [FSI\ (\mu U/mL)\ x\ FBG\ (mmol/L)]/22.5$$

Definitions of outcome and covariates

Subjects were classified as normal and impaired fasting glucose according to IDF/WHO criteria [15]. WC and WHR were divided into two categories, as metabolic risk and normal based on WHO classification for males and females separately [13]. Obese and non-obese categories were derived from WHO South Asian classification for BMI [16]. MUAC was also classified as metabolically normal and risk, based on cutoff values given in literature [17]. WHtR was classified into metabolic risk and normal groups based on the findings of Ashwell and Hseih [14]. As a measure of IR, subjects having HOMA-IR ≥1 was taken as insulin resistant [10].

Statistical analysis

All data were double entered and cross-checked for consistency. Data were analyzed using the Statistical Analysis Package for Social Sciences version 17 (SPSS Inc, Chicago, IL, USA). Means and standard deviations were computed for anthropometric and biochemical variables. Independent sample *t* - test was done to find out the significant differences among IR and normal groups for anthropometric parameters.

Stepwise linear regression analyses, utilizing continuous anthropometric measures as potential independent predictors, were performed to predict HOMA-IR. The parameters used were weight, height, WC, HC, MUAC, BMI, WHR, and WHtR. A stepwise regression was performed on all participants, using all anthropometric parameters as predictive variables and HOMA-IR as the dependent variable. A second stepwise regression was done, with the same conditions, after dividing the participants into two groups as females and males.

Binary logistic regression analysis was carried out in dichotomous variables, to identify the relative risk factor and the most suitable anthropometric parameter to predict the risk of IR. All the analysis was carried out for the total population as well as for males and females separately. Logarithmic values of HOMA-IR were used in the analysis to normalize its distribution.

Results

The study population had only 40.1 % males and females were the majority. The comparison of the biochemical and anthropometric data with HOMA-IR ≥1 and <1 is given in Table 1. The study indicates that, except age and height, all the other clinical parameters are significantly higher in insulin resistant group. The percentage of subjects with increased IR in this study was 59.9 % (n=136). Among the subjects with increased IR, female population was high with 61.8 %, and 38.2 % were males.

Among the insulin-resistant subjects, 49.3 % of both sexes were obese (BMI ≥25 kg m^{-2}) having a mean BMI of 28.3± 2.7 kg m^{-2} even though the mean BMI of subjects having HOMA-IR ≥1 was 25.1±9.7 kg m^{-2}. This was higher than

Table 1 Baseline parameters of apparently healthy non-diabetic subjects with HOMA-IR values

Characteristics	Non-diabetic, apparently healthy subjects (n=227)		P value
	IR (HOMA-IR ≥1) (n=136)	Normal (HOMA-IR <1) (n=91)	
Age (years)	41±14	41±13	$P>0.05$
Anthropometric data			
Weight (kg)	64.2±14.2	58.4±11.1	$P<0.05$
Height (cm)	159.8±9.8	159.5±14.6	$P>0.05$
WC (cm)	86.1±11.1	79.9±9.9	$P<0.05$
HC (cm)	98.6±8.8	95.4±7.6	$P<0.05$
MUAC (cm)	30.3±3.7	28.1±3.4	$P<0.05$
BMI (kg m^{-2})	25.1±9.7	22.6±3.7	$P<0.05$
WHR	0.9±0.1	0.8±0.1	$P<0.05$
WHtR	0.5±0.1	0.5±0.1	$P<0.05$
Biochemical data			
FBG (mmol/L)	4.8±0.7	4.5±0.5	$P<0.05$
FSI (pmol/L)	63.1±1.7	20.9±1.5	$P<0.05$
HOMA-IR	2.5±1.9	0.6±0.2	$P<0.05$

All the characteristics are described as mean±standard deviation. Means were significant (two-tailed) at $P<0.05$

WC waist circumference, *HC* hip circumference, *MUAC* mid-upper arm circumference, *BMI* body mass index, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio, *FBG* fasting blood glucose, *FSI* fasting serum insulin, *HOMA-IR* homeostatic model assessment of insulin resistant

the mean of whole study population which was 24.1± 4.1 kg $m^{-2}$and much higher than the BMI of subjects who had HOMA-IR less than 1 which was only 22.6± 3.7 kg m^{-2}. But, among the insulin-resistant subjects, 60.8 % were overweight or above (BMI ≥23 kg m^{-2}). The percentage of participants classified as insulin-resistant was highest among obese subjects, which was 75.3 %.

All the continuous, anthropometric parameters were used in a standard regression analysis to predict IR as independent variables. Stepwise multiple linear regression analysis was performed for the continuous variables in the total population to find out significant determinants for HOMA-IR. The raw and standardized regression coefficients of the predictors together with their correlations with IR, their squared semi-partial correlations, and their structure coefficients are shown in Table 2. Even though BMI, WHR, and WC were selected, and both WHtR and MAC were excluded in both males and females, the correlation of the variables, except height, had statistically significant ($P=0.000$) difference. According to the stepwise regression analysis carried out, the prediction model had two of the eight predictors for the total population. The model was statistically significant, F (227) = 29.269, $P<0.01$. It suggests that the effect of BMI on HOMA-IR was dominant compared with other parameters ($P=0.000$) and to a lesser extent by higher WHR levels. In male population, HOMA-IR was primarily predicted by BMI (F=46.128, $P<0.01$), whereas in females, it was WC (F=23.450, $P<0.01$).

In binary logistic regression analysis, odds ratios of the anthropometric parameters are given in Table 3. Among the total population, relative risk of BMI and WHtR had a higher association with IR. In males, BMI as well as WC had high relative risk for IR, and in females, WHtR and WC had high relative risk.

Discussion

Variety of anthropometric techniques is being used to identify obesity and body fat distribution, and obesity itself is used as a marker to predict IR. Central obesity identified by WC and IR are considered as underlying causative factors of metabolic syndrome and T2D. Several studies have been carried out to identify the association of one or more anthropometric parameters with the high risk for developing above metabolic abnormalities in different populations with various conditions. Measures of central obesity as well as generalized obesity have been studied for positive predictive value in different populations with varying results. The goal of this prospective study was to identify the link between anthropometric parameters and IR using HOMA-IR index and to identify the most suitable anthropometric parameter which would optimize clinical prediction of IR in apparently healthy, non-diabetic, male and female subjects specifically. To our knowledge, this is the study first to predict IR using anthropometric parameters among apparently healthy subjects, carried out in a Sri Lankan population. It showed that apparently healthy, non-

Table 2 Stepwise regression analysis for predicting IR

Model	b	SE-b	Beta	Pearson correlation	sr^2	Structure co-efficient
Total population (*n*=227)						
Constant	−0.794	0.125				
BMI*	0.037	0.005	0.431	0.431	0.185	1.010
WHR*	0.674	0.276	0.158	0.303	0.025	0.665
Males (n=91)						
Constant	−1.371	0.219				
BMI*	0.061	0.009	0.584	0.584	0.341	1.000
Females (*n*=136)						
Constant	−0.882	0.202				
WC*	0.012	0.002	0.386	0.386	0.144	0.999

The dependent variable is insulin resistance. sr^2 is the squared semi-partial correlation

WC waist circumference, *BMI* body mass index, *WHR* waist-to-hip ratio

*Significance at $P<0.05$

diabetic individuals had higher prevalence of IR with elevated anthropometric parameters. Linear and logistic regression analyses revealed that, among all the anthropometric parameters, BMI and WC are considered as the best predictors of IR in males and WHtR and WC in females.

Hyperinsulinemia and IR are two markers that are being used to identify subjects who have high tendency to develop IR-related metabolic abnormalities such as T2D and cardiovascular diseases [7]. Most of the studies related to IR have been carried out among subjects with either T2D or some other disease conditions [18–20]. Only a few studies have used random samples from the general populations [21]. The incidence rate of IR in our apparently healthy study population was 59.9 % which was comparatively higher than the prevalence among Chennai (11.2 %) urban subjects, [22] and the recorded IR prevalence in a Chinese population (7.2 %) [23]. This greater incidence rate of IR among non-diabetic subjects may account for racial and ethnic differences among the populations, and furthermore, studies have also showed that South Asians are more insulin resistant than Caucasian individuals independent of their adiposity and body fat distribution [22]. According to literature, South Asian Indians have a higher tendency to develop MS and T2D, perhaps due to genetic predisposition at smaller body sizes with increased central adiposity in the presence of a lower BMI [16]. Thus, screening of both glucose and serum insulin levels and calculating IR even at a lower level of BMI would be advantageous to minimize the risk of developing metabolic abnormalities.

Overweight and obesity had been positively associated with IR and hyperglycemia especially in subjects with T2D and CVD risk factors [24]. Even in non-diabetic individuals, if they are overweight or obese, there is a high tendency to become IR or hyperinsulinemic [25]. In addition, excess regional adiposity is considered as a major causative factor of IR [26]. The incidence of obesity among IR subjects was 49.3 % with a mean BMI of 28.3±2.7 kg m^{-2}, which was greater than the average BMI of whole study population (24.1± 4.1 kg m^{-2}), and 60.8 % of insulin-resistant subjects were overweight or above. This shows that subjects with overweight have a tendency to have high IR. South Asians with high IR were found to be obese. The occurrence of high percentage of obesity (75.3 %) among the more IR population indicates that Sri Lankans, too, have a high tendency to develop MS. These findings are in line with the several other

Table 3 Odds ratio for the anthropometric risk factors associated with insulin resistance.

Anthropometric parameters	Total (*n*=227) OR (95 % CI)	Males (*n*=93) OR (95 % CI)	Females (*n*=134) OR (95 % CI)
BMI (kg m^{-2})	2.91 (1.668–5.101)*	4.35 (1.740–10.869)*	2.33 (1.140–4.776)*
WHtR	2.87 (1.638–5.045)*	2.86 (1.188–6.870)*	2.88 (1.384–6.009)*
WHR		2.25 (0.942–5.374)	2.52 (1.234–5.160)*
WC (cm)		3.92 (1.309–11.708)*	2.72 (1.307–5.660)*

WC waist circumference, *BMI* body mass index, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio, *OR* odds ratio

*Odds ratio significant at $P<0.05$

earlier study findings [27, 28]. Further, the elevated IR level was positively associated with increased anthropometric parameters. Increasing IR has been found to be linked with increased subcutaneous as well as abdominal adipose tissue in Asian Indians [29]. Thus, increasing IR is inclined to give increased anthropometric parameters in non-diabetics. These findings indicate that IR directly effects by causing obesity and it influences individuals to gain weight physiologically [28]. Therefore, the above outcomes ensure that identifying generalized or regional adiposity has a significant relationship with identifying IR related metabolic abnormalities. Since the prevalence of IR was higher in our study, it would be better to carry out further investigations on a larger sample to find out the prevalence among healthy Sri Lankans.

When we assess the IR prevalence as well as the metabolic risk among the two genders, based on WC and WHR, the percentage of female insulin-resistant subjects, who were at the metabolically risk category, was greater than the percentage of males. Deposition of body fat varied among two genders. Females tend to deposit more fat peripherally when compared to central deposition in males. And, these findings were supported by the earlier reports of Alemzadeh and Kichler [30]. Therefore, female subjects have a high tendency to develop IR-related metabolic abnormalities which suggests that lower total lean body mass and greater fat mass may play a strong role in pathogenesis of IR and related obesity among females.

Regression analysis indicates that measures of central adiposity or measures of general obesity alone cannot predict the overall adiposity vis-à-vis IR among the apparently healthy subjects. In contrast, the results indicate that both anthropometric measurements and indices in determining central or generalized adiposity are collectively corporate in predicting IR. Present study found that both WHtR and BMI are better measures to assess the metabolic abnormalities in a general population with both genders. Further, we found BMI and WC as better predictors of IR among males and WHtR and WC for females.

BMI is an index which reflects the measures of relative weight. Further, categorizing of body weight which includes degrees of underweight and overweight is based on BMI. Hence, it is an acceptable alternative for thinness and fatness that have been directly related to health risks and death rates in many populations [31]. It has been used traditionally as an indicator. However, classical cutoffs of BMI among South Asians showed that even at relatively low levels of obesity, subjects are more prone to develop T2D as South Asians have higher amounts of body fat deposition in abdominal areas [16]. In comparison with general obesity, defined by BMI alone, site of excess body fat deposition is a predominant factor of determining the tendency to develop MS and related diseases like T2D and coronary heart diseases as abdominal obesity is significantly linked with MS [19]. Therefore, involvement of a central adiposity measurement adds to a great advantage.

Out of the measures of central obesity, waist circumference (WC) is the most widely used central adiposity measure as it has a good prognostic value in identifying metabolic abnormalities [13]. According to our study findings, even though WC is a primary predictor of IR in females, males with high WC also have a high risk of developing IR. WC has gender specific cutoff points to assess risk of getting co-morbidities of obesity, as males have a greater total lean mass, bone mineral mass, and a comparatively lower fat mass than females. Female subjects have considerably visceral adiposity than males. Hence, WC is attributed to predict IR in males and females separately, using different cutoff values.

However, WC is dependent upon the height measurement of a subject; hence, WHtR provides a better tool for assessing the metabolic risk in a population with short stature such as South Asians [19]. Ashwell and Hseih [14] have shown that WHtR is more sensitive than BMI as an early predictor of health risk. In our study, RR of WHtR in predicting IR among both males and females was higher (more than two times). Thus, our study findings stated that WHtR is a dependable and an effective anthropometric index to identify metabolic risk among Sri Lankan general population, and it was in corroboration with the findings of Jayawardana et al. [19]. Thus, the very high odds ratios of WHtR ≥0.5 found for IR in general Sri Lankan adults can be used to detect the risk of developing metabolic abnormalities.

WHtR was found to be a better predictor of IR in females, and such findings were earlier reported in Asian populations, such as Taiwanese and Japanese. This indicates that subjects with short stature and a large waist would have a high risk in developing obesity related co-morbidities and thus proving the concept of "keeping your waist to less than half your height". In contrast to our findings, Jayawardana et al. [19] have shown a higher correlation between WHtR and disease risk in males. Therefore, further studies based on gender-specific WHtR cutoffs to identify the risk of metabolic abnormalities are needed to validate the above findings.

The limitations of the present study included a convenience sample with cross-sectional study design. Furthermore, HOMA-IR assessment has a limitation of being a static measurement of insulin function and requirement of a defined HOMA-IR cutoff for apparently healthy subjects. Comparatively large sample size, representing the majority of the society, and its applicability to the real world are the strengths of this study as the expenses were at low, and evaluation in a community setting. Our emphasis on an apparently healthy, non-diabetic population is the strength of this study, and it would be of advantage if apparently healthy subjects with elevated, abnormal BMI, WC, and WHtR calculate their IR in order to identify the risk of developing IR and metabolic abnormalities prior to onset. These costless, self-monitoring anthropometric measures are reliable tools to encourage the increased practice of screening for IR, in a community setting with apparently healthy subjects.

Conclusions

This study demonstrates that with elevated anthropometric parameters, higher prevalence of IR is present in apparently healthy individuals. Among the anthropometric parameters, BMI and WC have been found to be the best predictors of IR in males and WHtR and WC in females. IR levels could be used in healthy individuals as a "warning" sign which could be made easier by measuring simple anthropometric measures suitable for different sexes.

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Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

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