

Original Article

Relation of anthropometric parameters to the functional assessment of HDL particle size in three ethnic cohorts

Hamidreza Zakeri MD,¹ Jiri J Frohlich MD,^{2,3} and Scott A Lear PhD^{3,4}

¹ St. Paul's Hospital and University of British Columbia, Vancouver, B.C., Canada and Mazandaran Medical Science University, Iran

² Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada

³ Healthy Heart Program, St. Paul's Hospital, Canada

⁴ School of Kinesiology, Simon Fraser University, Canada

The purpose of this study was to characterize the association between HDL particle size (assessed by fractional esterification rate in apo B depleted plasma (FER_{HDL})), and anthropometric measures in men and women of Chinese, European and South Asian origin and to determine if ethnic background is a modifier of this relationship. A convenience sample of apparently healthy men and women of Chinese (n=56), European (n=66) or South Asian (n=76) descent were recruited and assessed for body mass index, waist circumference, blood pressure, lipids, insulin, glucose and FER_{HDL}. Univariate correlations with FER_{HDL} were determined within each ethnic-gender group. Regression analysis was used to determine if ethnicity was a significant modifier of the relationship between FER_{HDL} and waist circumference. FER_{HDL} was significantly correlated with various anthropometric measures within the ethnic and gender groups. The relationship between waist circumference and FER_{HDL} was significantly different between the European, and Chinese and South Asian groups combined in women, but not in men. After adjustment for age, body mass index and insulin, ethnicity was no longer a significant predictor. However, ethnic background (European vs. Chinese/South Asian) was a significant predictor ($P=0.034$) of the relationship between FER_{HDL} and waist circumference adjusted for age, gender, body mass index and insulin in the entire cohort. Increasing adiposity in Chinese and South Asian men and women is associated with a less protective HDL particle profile. Ethnic background (Asian compared to European) is a significant modifier of the relationship between FER_{HDL} and waist circumference.

Key Words: anthropometry; lipoproteins, HDL; Asians

Introduction

Obesity, particularly an increase in abdominal fat, is associated with dyslipidemia, insulin resistance, the metabolic syndrome, diabetes mellitus and an increase in cardiovascular disease (CVD) risk.¹⁻³ Previous reports have suggested a relationship between the degree of adiposity, insulin resistance and qualitative changes in plasma lipoproteins, specifically decreased lipoprotein particle size as assessed by gel electrophoresis or nuclear magnetic resonance methods.⁴⁻⁸ Of the lipoprotein particles, high density lipoproteins (HDL) are inversely associated with abdominal fat and CVD risk.⁴ However, subclasses of the HDL particles have differing effects with HDL2 having a greater protective effect than does HDL3.⁹

Our group and others have previously identified that the association between abdominal fat and CVD risk factors differs along ethnic lines, such that individuals of Chinese and South Asian origin tend to present with more CVD risk factors at a similar waist circumference (WC) and body mass index (BMI) than those of European origin.¹⁰⁻¹³ Whether this finding extends to HDL particle size is unknown. The purpose of this study was to characterize the association between HDL particle size (using a functional assay of the fractional esterification rate in apo

B depleted plasma (FER_{HDL})), and BMI and WC in men and women of Chinese, European and South Asian origin and to determine if ethnic background is a modifier of this relationship.

Materials and methods

The participants were recruited from staff, students, volunteers and their friends of three local hospitals and the surrounding communities. Apparently healthy subjects were eligible if they were over 18 years of age and either of Chinese, European or South Asian descent. Ethnicity was determined by interview; only those individuals who reported having all known ancestors exclusively of either European, Chinese or South Asian descent were invited to participate. Participants were excluded if they had a history of either diabetes, CVD, taking medications that would

Correspondence address: Dr Scott A. Lear, Healthy Heart Program, St. Paul's Hospital 180 – 1081 Burrard St, Vancouver, BC, Canada V6Z 1Y6
Tel: (604) 682-2344 ext. 62778; Fax: (604) 806-8590
slear@providencehealth.bc.ca
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Table 1. Demographic and Laboratory Data within Women of each ethnic group

	Chinese (N=31)	European (N=38)	South Asian (N=44)
Age	45 ± 14	45 ± 10	39 ± 14
Total cholesterol (mmol/L)	4.85 ± 0.75	4.84 ± 0.82	4.50 ± 0.96
LDL-C (mmol/L)	2.75 ± 0.60	2.69 ± 0.72	2.52 ± 0.87
HDL-C (mmol/L)	1.50 ± 0.36	1.66 ± 0.35	1.41 ± 0.36*
Triglycerides (mmol/L)	1.33 ± 0.87	1.10 ± 0.59	1.25 ± 0.79
TC/HDL-C	3.39 ± 0.94	3.03 ± 0.73	3.42 ± 1.25
FER _{HDL}	16.1 ± 6.9	14.7 ± 7.4	17.7 ± 6.2
Insulin	52.4 ± 36.6	43.7 ± 23.7	59.9 ± 32.1
Glucose (mmol/L)	5.3 ± 0.4	5.1 ± 0.4	5.2 ± 1.7
HOMA-IR	1.76 ± 1.34	1.46 ± 0.80	1.92 ± 1.09*
Systolic blood Pressure (mmHg)	110 ± 19	113 ± 12	106 ± 14
Diastolic blood Pressure (mmHg)	70 ± 11	73 ± 9	67 ± 8*
Smokers (%)	0	0	1
BMI (kg/m ²)	23.6 ± 3.6	27.0 ± 6.0	25.4 ± 4.5*
Waist circumference (cm)	78.2 ± 11.4	82.2 ± 12.3	80.5 ± 11.7
WHR	0.83 ± 0.10	0.78 ± 0.05	0.81 ± 0.07*

* $P < 0.05$

affect the risk factors under assessment or had increased abdominal girth not related to increased adiposity (such as pregnancy, peritoneal dialysis, or ascites). Those with untreated hyperlipidemia or elevated blood glucose were not excluded. All participants read and provided informed consent before commencing the study (approved by the Providence Health Care Research Ethics Committee). A sample size of convenience was limited to 56 Chinese, 66 European and 76 South Asian participants.

Participants' assessment included medical history, BMI, WC, hip circumference (HC), blood pressure (BP), and fasting blood samples for plasma total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG), insulin and glucose. Plasma and serum samples were kept frozen at -70°C until analyzed. Weight was measured to the nearest 0.1kg on a balance beam scale, and participants were asked to remove their shoes and any heavy items from their pockets. Height was measured at the same time to the nearest 0.5cm. Body mass index was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured (to the nearest 0.1 cm) directly over the skin at the point of maximal narrowing of the trunk as viewed from the anterior position with the participant standing upright after a normal expiration. Hip circumference was measured (to the nearest 0.1 cm) over undergarments at the point of the greatest gluteal protuberance as observed from the lateral view. Waist-to-hip ratio (WHR) was calculated by dividing WC by HC. Blood pressure was the average of two measures taken in the left arm following seated rest and five minutes apart. Serum TC, HDL-C, TG, glucose and insulin were measured using standard procedures in a quality controlled, certified clinical laboratory. LDL-C was calculated using the Friedewald equation.¹⁴ Insulin resistance was assessed by the homeostasis model (HOMA).¹⁵

Measurement of FER_{HDL} in plasma depleted of apo B containing lipoproteins has been previously described in detail.¹⁶⁻¹⁸ In this method, apoB-containing lipoproteins are precipitated from EDTA plasma (that can be stored at -20°C up to 3 months or at -70°C up to several years) by

phosphotungstic acid and MgCl₂. A filter paper disk containing a trace of 3H-cholesterol is added to the supernatant. After an overnight incubation at 4°C the disk is removed, and the plasma containing labelled HDL is incubated at 37°C for 30 min (the esterification reaction is always linear during this time period). After the incubation lipids are extracted and separated by thin layer chromatography. The fractional esterification rate of cholesterol is calculated from the ratio of radioactivity of free and esterified cholesterol. Values are percentages of HDL cholesterol esterified per hour (%/h).

Statistical analysis

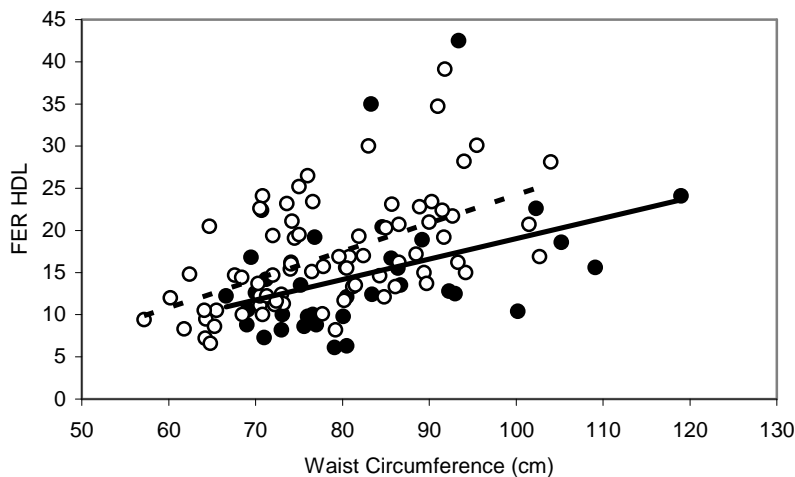
Data are reported as means and standard deviations. Comparisons between groups were analysed using a three-way ANOVA stratified by gender. Pearson correlation coefficients were determined for FER_{HDL} with age, anthropometric variables and CVD risk factors within each gender and ethnicity. Three regression analyses (Chinese vs. European, Chinese vs. South Asian and European vs. South Asian) were performed within each gender with FER_{HDL} as the dependent variable and ethnicity or WC as the independent variables. As ethnicity was not a significant predictor of FER_{HDL} in the model of Chinese vs. South Asian, these data were combined in a second model grouping Chinese and South Asian participants together vs. European participants for the ethnicity variable and WC for the other independent variable. This model was adjusted for age, BMI and insulin and conducted separately by gender. A third model was performed combining men and women, with ethnicity (as stratified in the second model) and WC as the independent variables adjusted for age, gender, BMI and insulin. All tests were conducted with p set at 0.05 for significance using SPSS version 10.0 software.

Results

A total of 113 women and 85 men were recruited for this study. Tables 1 and 2 outline the metabolic and anthropometric data for women and men, respectively, stratified by ethnic background. There was a significant difference

Table 2. Demographic and Laboratory Data within Men of each ethnic group

	Chinese (N=25)	European (N=28)	South Asian (N=32)
Age	39 ± 11	42 ± 9	42 ± 14
Total cholesterol (mmol/L)	5.16 ± 0.92	4.77 ± 1.07	5.08 ± 1.21
LDL-C (mmol/L)	3.13 ± 0.84	2.95 ± 0.96	3.10 ± 0.97
HDL-C (mmol/L)	1.26 ± 0.22	1.24 ± 0.31	1.13 ± 0.26
Triglycerides (mmol/L)	1.72 ± 0.94	1.28 ± 0.61	2.13 ± 2.17
TC/HDL-C	4.24 ± 1.09	4.05 ± 1.24	4.73 ± 1.74*
FER _{HDL}	23.9 ± 8.8	22.0 ± 7.9	24.9 ± 11.0
Insulin	68.8 ± 104.8	54.6 ± 30.3	75.4 ± 52.7
Glucose (mmol/L)	5.4 ± 0.5	5.3 ± 0.6	5.2 ± 1.1
HOMA-IR	2.53 ± 4.69	1.77 ± 0.99	2.52 ± 2.07
Systolic blood Pressure (mmHg)	111 ± 15	116 ± 10	118 ± 14
Systolic blood Pressure (mmHg)	75 ± 12	77 ± 8	72 ± 9
Smokers (%)	1	3	2
BMI (kg/m ²)	23.2 ± 2.5	26.3 ± 3.5	26.1 ± 3.5*
Waist circumference (cm)	78.4 ± 8.4	89.4 ± 11.0	89.5 ± 11.2*
WHR	0.85 ± 0.09	0.89 ± 0.08	0.90 ± 0.09

* $P < 0.001$ **Figure 1.** Relationship between waist circumference and FER_{HDL} in women stratified by European origin (solid circles and line) and Chinese/South Asian origin (open circles and dashed line).

between the three groups of women for HDL-C, HOMA-IR score, diastolic BP, BMI and WHR. For the men, only TC/HDL-C ratio, BMI and WC were significantly different between the three groups.

Tables 3 and 4 outline the correlation coefficients for the metabolic and anthropometric variables with FER_{HDL} in women and men, respectively, stratified by ethnic group. HDL-C was negatively correlated with FER_{HDL} in all three ethnic groups for both genders. Triglycerides and the TC/HDL-C ratio were positively correlated with FER_{HDL} in all three groups and both genders. Insulin, HOMA-IR, BMI and WC were positively associated with FER_{HDL} in all three groups of women.

Based on linear regression analysis, ethnicity was a significant predictor ($P = 0.029$) of FER_{HDL} adjusted for WC and age when the European and South Asian groups in women were compared, while ethnicity was not a significant predictor ($P = 0.119$) when the Chinese and European groups were compared. In men, ethnicity was a significant predictor ($P = 0.017$) adjusted for WC and age when the Chinese and European groups were compared,

while ethnicity was not a significant predictor ($P = 0.244$) when the European and South Asian groups were compared. As the relationship between WC and FER_{HDL} was similar between Chinese and South Asian women and men (data not shown), these groups were combined and compared to the European group. In women the relationship between WC and FER_{HDL} was significantly different between the European and Chinese/South Asian groups (Fig. 1). However, after adjustment for age, BMI and insulin, ethnicity was no longer a significant predictor. Ethnicity was not a significant predictor in this relationship among the men. When the entire cohort was combined ethnic background (discriminating between European and Chinese/South Asian) was a significant predictor ($P = 0.034$) of the relationship between FER_{HDL} and WC after being adjusted for age, gender, BMI and insulin (Fig. 2).

Discussion

Our results provide novel information indicating that FER_{HDL} (a marker of HDL particle size) is positively

Table 3. Women, univariate correlations with FER_{HDL}

	Chinese (N=31)	European (N=38)	South Asian (N=44)
Age	0.462**	-0.127	0.211
Total cholesterol (mmol/L)	0.346	0.213	0.150
LDL-C (mmol/L)	0.252	0.264	0.230
HDL-C (mmol/L)	-0.515**	-0.605***	-0.639***
Triglycerides (mmol/L)	0.754***	0.743***	0.516***
TC/HDL-C	0.693***	0.763***	0.519***
Insulin	0.685***	0.436**	0.315*
Glucose (mmol/L)	0.506**	0.235	0.320*
HOMA-IR	0.714***	0.433**	0.399**
Systolic blood pressure (mmHg)	0.577**	0.133	0.251
Diastolic blood pressure (mmHg)	0.548**	0.028	0.098
BMI (kg/m ²)	0.566**	0.437**	0.355*
Waist circumference (cm)	0.549**	0.407**	0.487**
WHR	0.254	0.328*	0.419**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

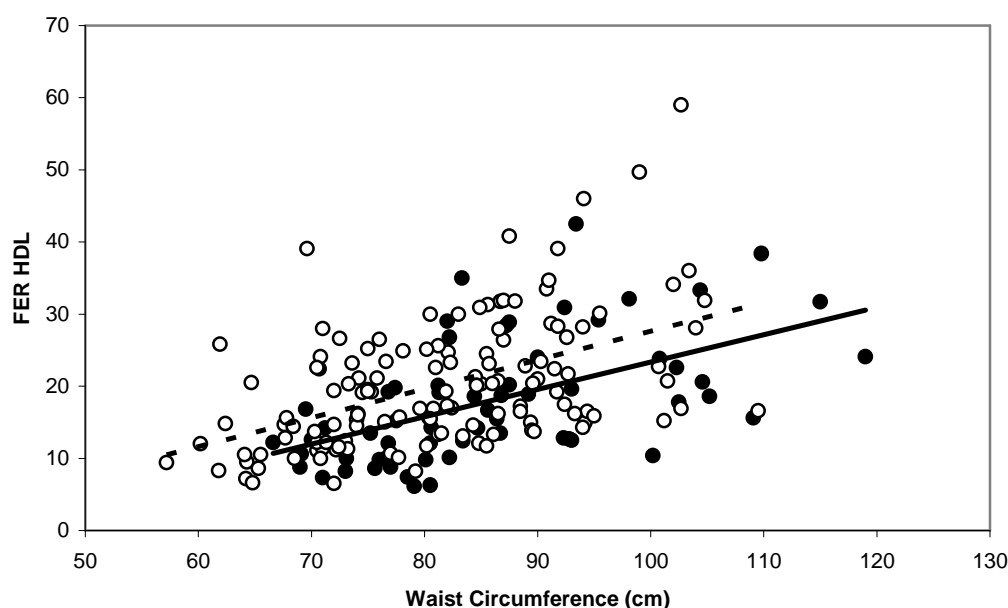


Figure 2. Relationship between waist circumference and FER_{HDL} in men and women combined stratified by European origin (solid circles and line) and Chinese/South Asian origin (open circles and dashed line)

associated with simple anthropometric measures in Chinese and South Asian men and women. Therefore, as in Europeans, increasing adiposity in these populations results in a greater proportion of HDL3 compared to HDL2 particles which may contribute to an increase in CVD risk. In addition, we found that ethnic background modifies the relationship between FER_{HDL} and WC after adjustment for age, gender, BMI and insulin, such that those of Chinese and South Asian origin have higher FER_{HDL} values at a similar WC than the Europeans.

While no published reports exist measuring FER_{HDL} in either Chinese or South Asian populations, a few studies have reported measuring HDL particle size in these groups. Studies in Chinese populations have found the concentration of HDL particles or the ratio of particles to be similar^{19,20} or less^{21,22} than those of European origin. Reports of South Asian populations have found that HDL concentrations were lower compared to Europeans.²¹⁻²⁴ None of these studies however, adjusted for apparent differences in BMI.

Previous studies investigating the relationship between HDL particle size and simple anthropometric measures

(such as WC and BMI) have been conducted in populations of predominantly European origin.²⁵ To our knowledge, only one study exists investigating this relationship in Chinese men,²⁶ which found BMI and the waist to hip ratio to be inversely associated with HDL2 concentrations. No studies have been conducted in Chinese women or in South Asians. In our study we found that FER_{HDL} was positively associated with BMI, WHR and WC in each of the Chinese, European and South Asian groups, while this relationship was not as clear in men, most likely due to the smaller sample sizes. These data indicate that increasing adiposity is associated with a pro-atherogenic distribution of HDL particles regardless of ethnicity and add to the cluster of atherogenic risk factors associated with adiposity in these groups. Previous work by our group has indicated that ethnic background significantly modifies the relationship between WC with other CVD risk factors: LDL-C, HDL-C, triglycerides, insulin, glucose and C-reactive protein.¹⁰⁻¹³ Others have reported similar findings and have also indicated that at a similar BMI, those of Chinese and South Asian have a higher percent of body fat than those of European origin.²⁷

Table 4. Men, univariate correlations with FER_{HDL}

	Chinese (N=25)	European (N=27)	South Asian (N=32)
Age	0.143	0.057	-0.054
Total cholesterol (mmol/L)	0.982***	0.303	0.473**
LDL-C (mmol/L)	0.595**	0.360	0.342
HDL-C (mmol/L)	-0.627**	-0.759***	-0.393*
Triglycerides (mmol/L)	0.627**	0.769***	0.729***
TC/HDL-C	0.825***	0.789***	0.691***
Insulin	0.212	0.334	0.634***
Glucose (mmol/L)	0.170	-0.068	0.008
HOMA-IR	0.204	0.356	0.540**
Systolic blood pressure (mmHg)	-0.046	0.228	0.211
Diastolic blood pressure (mmHg)	0.239	0.388*	0.362*
BMI (kg/m ²)	0.490*	0.600**	0.189
Waist circumference (cm)	0.255	0.590**	0.288
WHR	0.214	0.520**	0.362*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

origin. While we did not directly measure body fat, increasing WC levels are likely the result of increased abdominal fat. Compared to BMI, WC is a better indicator of CVD risk as it is more strongly associated with various CVD risk factors.² This is most likely due to WC being associated with visceral adipose tissue (VAT),² which is strongly associated with TC, LDL-C, low HDL-C, TG, apolipoprotein B, blood pressure, insulin resistance and C-reactive protein.^{2,28,29} Few studies have investigated the relationship between VAT and HDL particle size, but they have indicated that increased levels of VAT are negatively associated with HDL2 and HDL3 levels.³⁰⁻³² When women with and without glucose intolerance were matched by VAT, fat mass and menopausal status, the difference in HDL2 concentrations was no longer apparent.³¹

The obvious limitation of this study is its small sample and it is possible some of the relationships that failed to reach significance did not have sufficient power (Type II error). However, we are confident in the findings that did reach significance and believe these are unlikely the result of a Type I error. These findings should be confirmed in larger populations. While not a direct measure of HDL particle size, FER_{HDL} is a functional assessment of lipoprotein quality that gives information on both LDL and HDL particle size.^{17,33} This method is more convenient than measurement of individual lipoprotein particle sizes as it is faster and less expensive. Higher values of FER_{HDL} correlate well with decreased lipoprotein particle sizes and atherogenic lipid profile such as decreased HDL-C, increased triglycerides.¹⁸ In addition, this parameter has been shown to correlate well with findings on selective coronary angiography.^{17,34} The results presented in this paper fall within expected normal ranges for European men and women.³⁵

In conclusion, we have found that the relationship between HDL particles, as assessed by FER_{HDL}, and anthropometric measures in Chinese and South Asian men and women is similar in terms of direction to that reported in previous populations of European origin and add to the cluster of metabolic risk factors associated with increased adiposity. In addition, ethnic background appears to be a significant modifier of the relationship between FER_{HDL} and WC when compared between the Asian groups combined and the Europeans. This indicates that at a similar

WC, those of Chinese and South Asian origin have higher FER_{HDL}, and therefore a lower HDL2:HDL3 than those of European origin. This adds to the mounting evidence to suggest that those of Asian origin require lower WC targets than those currently used for European populations. While the mechanism for this is at present unknown, it is possible that those of Asian origin have higher amounts of VAT at the same BMI or WC compared to those of European origin. This hypothesis will need to be tested in a larger sample size.

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Original Article

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Hamidreza Zakeri MD,¹ Jiri J Frohlich MD,^{2,3} and Scott A Lear PhD^{3,4}

¹ *St. Paul's Hospital and University of British Columbia, Vancouver, B.C., Canada and Mazandaran Medical Science University, Iran*

² *Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada*

³ *Healthy Heart Program, St. Paul's Hospital, Canada*

⁴ *School of Kinesiology, Simon Fraser University, Canada*

三个种族队列人体测量学参数与 HDL 脂蛋白颗粒大小功能评价的关系

本科题的目的是阐明华人、欧洲人、南亚后裔高密度脂蛋白 (HDL) 颗粒大小 (以去除 apo B 后的高密度脂蛋白酯化速率来评价(FER_{HDL}))和人体测量学参数之间的相关性, 种族背景是否是这种相关性的预测指标。招募明显健康华人($n=56$)、欧洲人($n=66$)和南亚后裔 ($n=76$) (包括男性和女性) 作为受试者, 测定受试者的体重指数、腰围、血压、脂类、胰岛素、葡萄糖和 FER_{HDL} 。以 FER_{HDL} 在每一种族-性别组内进行单变量相关性分析。采用回归分析来决定种族背景是否是 FER_{HDL} 和腰围相关性的重要预测指标。每一种族组内和性别组内, FER_{HDL} 显著地与各种人体测量学参数相关。腰围和 FER_{HDL} 的相关性在欧洲人、华人、南亚后裔女性组间显著的不同, 校正年龄、体重指数和胰岛素等因素后, 种族性不再是腰围和 FER_{HDL} 相关性的重要预测指标。然而在整个队列中, 校正年龄、性别、体重指数和胰岛素等因素后, 种族背景(欧洲比较华人/南亚后裔)是 FER_{HDL} 和腰围相关性的重要预测指标 ($p=0.034$)。中国和南亚人日益增加的肥胖与低的保护性 HDL 脂蛋白颗粒组成相关。种族背景 (亚洲人与欧洲人比较) 是 FER_{HDL} 和腰围相关性的显著指标。

关键字: 人体测量学、脂蛋白、HDL、亚洲人。