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Measures of fat distribution as determinants of serum lipids in healthy volunteers consuming a uniform standardized diet

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Abstract. The relation was investigated between body fat topology and serum lipoproteins in healthy, non-obese men and women. Twenty-four men and 24 women consumed a standardized affluent diet for 17 days. Serum was obtained after 14 and 17 days. Regression coefficients of lipids and lipoproteins with age, body mass index, measures of fat distribution, and estimates of fat areas at a cross-section of the body at the level of the umbilicus, were calculated. Waist-to-thigh circumference ratio proved to be the strongest correlate of serum lipids compared with other measures of fat distribution. Upon multiple regression analysis, the waist-to-thigh ratio proved to be a stronger predictor of total cholesterol, LDL-cholesterol and triglyceride levels in men than either age or body mass index. In women this was only true for HDL_3-cholesterol. In addition, no significant relations were observed any more of age and body mass index with these serum lipids, with the exception of age for IDL-cholesterol in men. Estimates of intra-abdominal fat area showed in general a stronger correlation with serum lipids than estimates of subcutaneous fat area. It is concluded that, in non-obese subjects, fat distribution is a stronger determinant of serum lipids than either body mass index or age.

Keywords. Obesity, fat distribution, serum lipids, cholesterol, lipoproteins.

Introduction

Many reports from various parts of the world have shown that, in addition to indices of general adiposity, measures of fat distribution are predictive of cardiovascular diseases [1]. These associations may, at least partly, be explained by associations of fat distribution with risk factors for cardiovascular disease like total serum cholesterol concentrations, blood pressure and glucose intolerance. Results of studies in which relations between fat distribution and serum lipids were investigated are, however, inconsistent [2–8]. This might be due to differences in study populations, most studies having been carried out in obese subjects, and to different indices used for measuring fat distribution. In addition, the diet of the subjects was not controlled in any of these studies; this might have confounded the results. We have now examined relationships between serum lipoproteins and several measures of fat distribution, including an estimation of intra-abdominal fat mass, in apparently healthy non-obese volunteers while they were consuming a standardized uniform diet.

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Patients and methods

Subjects

Fifty-seven subjects participated in a strictly controlled dietary experiment to compare the effects of an olive-oil-rich and a carbohydrate-rich diet on serum lipoproteins. Their total serum cholesterol ranged from 3·43 to 7·58 mmol l^{-1} (mean 5·07 mmol l^{-1}), HDL-cholesterol from 0·72 to 2·58 mmol l^{-1} (mean 1·38 mmol l^{-1}), and serum triglycerides from 0·33 to 2·77 mmol l^{-1} (mean 0·98 mmol l^{-1}). Criteria for participation in the study were the absence of anaemia, glycosuria, proteinuria, and hypertension. In addition, none of the volunteers received medication known to affect serum lipids at least 2 months before or during the study. The protocol of the study, approved by the Ethical Committee of the Department, was fully explained to the volunteers, and written informed consent was obtained.

Before the experiment subjects were asked to weigh and record their food intake on 3 separate days, including 1 weekend day. Foods were coded and energy intake calculated using the 1985 edition of the computerized Dutch food composition table (*UCV Table*). During the experiment all subjects consumed a standardized affluent diet for 17 days, which provided 14% of energy (en%) as protein, 38 en% as total fat, 20 en% as saturated fatty acids, 4 en% as polyunsaturated acids, 48 en% as carbohydrates, 1 en% as alcohol and, on average, 390 mg day^{-1} cholesterol. These 17

days constituted the baseline period of the experiment, which has been reported in full detail elsewhere [9]. All foodstuffs were supplied individually tailored to each person's energy requirement, except for some free-choice items, free from fat and cholesterol, which provided a fixed amount of energy ranging from 4·6 to 10·0 en% of the total daily energy intake. Body weights were recorded twice weekly and energy intake was adjusted to counteract weight changes. Individual energy intakes ranged from 6·1 to 19·9 MJ (mean 11·1 MJ or 2660 kcal) per day.

Data from nine subjects were excluded from analysis because of intercurrent illness, departure from the protocol, or weight loss of more than 2·5 kg during the study. Thus, 48 subjects (24 men, 24 women) completed the experiment. Six women used oral contraceptives and two women were post-menopausal.

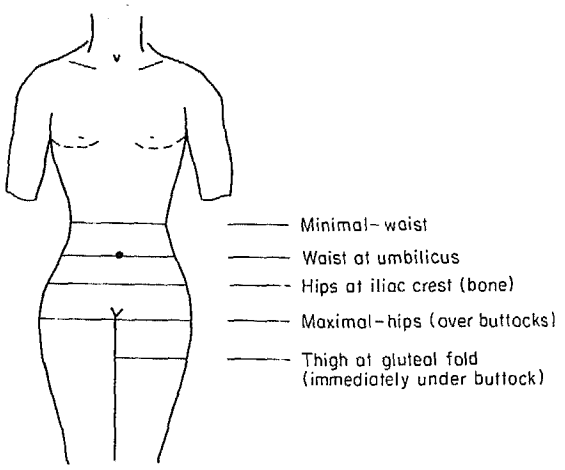

Figure 1. Schematic representation of levels at which body circumferences were measured. The amount of surface taken up by fat tissue in a planar cross-section of the trunk at the level of the fourth lumbar vertebra was estimated using published equations [16].

Blood sampling and analysis

Blood samples were obtained on days 14 and 17 after an overnight fast. Serum was obtained by low speed centrifugation within 1 h after venepuncture and checked for the presence of chylomicrons [10]. All samples were negative, indicating a true fasting state of the subjects. Aliquots of serum were stored at −80°C and analysed for total cholesterol, HDL-cholesterol and triglycerides at the end of the study [11–14]; other aliquots were stored at 4°C until ultracentifugation. On day 17, equal amounts of sera of days 14 and 17 from each subject were pooled, and lipoproteins were fractionated by density ultracentifugation [15]. The following density classes were isolated (d in g ml^{-1}) and stored at −80°C until analysis: VLDL ($d < 1{\cdot}010$), IDL ($1{\cdot}010 < d < 1{\cdot}019$), LDL ($1{\cdot}019 < d < 1{\cdot}055$), HDL_1 plus lipoprotein (a) ($1{\cdot}055 < d < 1{\cdot}075$), HDL_2 ($1{\cdot}075 < d < 1{\cdot}100$), HDL_3 ($1{\cdot}100 < d < 1{\cdot}180$) and a bottom fraction ($d > 1{\cdot}180$). Cholesterol concentrations were estimated in the lipoprotein fractions and in aliquots of the original sera. The mean recovery of cholesterol was 93±5·3%. The within-run CV for control sera was 0·9% for total cholesterol, 1·8% for HDL-cholesterol, and 1·0% for total triglycerides. Accuracy was checked by analysis of serum pools of known value provided by the Centers of Diseases Control (Atlanta, GA, U.S.A.). Mean bias with regard to CDC target values was +0·1% for total cholesterol, −3·2% for HDL-cholesterol and −1·5% for total triglycerides. All samples of one subject were analysed within one run.

Anthropometric measurements

Weight and height were measured in the fasting state at the start of the study. Subjects were weighed without shoes or heavy clothing on a calibrated balance weighing scale to the nearest 0·1 kg. Height was measured to the nearest 0·1 cm. Body mass index, expressed in kg m^{-2}, was calculated. Halfway through the study, anthropometric measurements were performed by two trained investigators, one for the female subjects and one for the male subjects. Skinfolds were measured with a Holtane caliper. The supra-iliac and para-umbilical skinfolds were used for the estimation of cross-sectional fat areas, as described below. Circumference measurements were made with a flexible plastic tape measure with the subject standing upright, the weight equally balanced on both feet, and breathing lightly. Waist circumferences were measured in each subject at the level of minimal girth and at the level of the umbilicus, and hip circumferences at the level of the iliac crest and at the level of the maximal circumference over the buttocks. The thigh circumference was measured at the level of the gluteal fold (Fig. 1). The following ratios were calculated: minimal-waist:thigh; umbilical-waist:thigh; minimal-waist:maximal-hip; umbilical-waist:hips at iliac crest; umbilical-waist:maximal-hip.

Cross-sectional intra-abdominal and subcutaneous abdominal fat areas

We have shown earlier [16] that the area of intra-abdominal and subcutaneous fat, as measured by computed tomography scans of a cross-section of the body at the level of the fourth lumbar vertebra (L4), which usually corresponds with the level of the umbilicus, can be estimated from regression equations employing the anthropometric variables measured in the present study. The equations used explained 79–84% of the variance in the intra-abdominal and subcutaneous abdominal fat areas in men and women, and 32% (in women) or 49% (in men) of the ratio of intra-abdominal to subcutaneous abdominal fat areas [16].

Statistical methods

None of the variables deviated from normality, as indicated by the Kolmogorov-Smirnov test. Differences between men and women were tested with Student's unpaired *t*-test. Univariate and multiple linear regression was performed with blood lipids as dependent variables and the various circumference ratios, age, and body mass index as independent variables [17]. Analysis of residuals was performed to check the linearity of the relationships.

Results

Table 1 shows some details of the study population. All circumference ratios were significantly ($P<0{\cdot}01$) higher in men than in women, confirming the popular metaphor describing men as 'apple-shaped' and women as 'pear-shaped'. Total HDL-, HDL_2-, and HDL_3-cholesterol were all higher in women.

Regression coefficients of blood lipids with age and anthropometric variables are shown in Table 2 (men) and Table 3 (women). In men, total cholesterol was positively associated with virtually all variables displayed. For triglyceride concentrations the most significant regression coefficients were found with age and the minimal-waist to thigh circumference ratio. Statistically significant relations of VLDL-, IDL-, and LDL-cholesterol with the various independent variables were also found. For HDL-cholesterol, no significant regression coefficient was found. In women, a significant correlation with total cholesterol was found only for the estimate of the ratio of intra-abdominal fat to subcutaneous abdominal fat. The minimal-waist to maximal-hip circumference ratio displayed a negative association with HDL-cholesterol. In females, none of the variables presented showed a significant relation with triglycerides. For both men and women, the associations with serum lipids of the estimated fat area within the abdominal cavity were, in general, more significant than those of the estimated subcutaneous fat area.

The associations of blood lipids with the umbilical-waist to thigh circumference ratio were less pronounced than with the minimal-waist to thigh circumference ratio, especially for men. Also, the circumference ratio of umbilical-waist to hips at the iliac crest was less strongly associated with each of the serum lipids than the other two waist to hip circumference ratios.

Tables 4 and 5 show the results of multiple linear regression analysis, using serum lipids as the dependent variables, and age, body mass index, and the minimal-waist to thigh circumference ratio as independent variables. In men, the minimal-waist to thigh circumference ratio was independently related ($P<0{\cdot}05$) with total cholesterol, triglycerides, and LDL-cholesterol. In women this was true for HDL_3-cholesterol. No significant regression coefficients of

Table 1. Anthropometric variables and serum lipids and lipoproteins in 48 healthy non-obese volunteers consuming a standardized high-fat diet

	Men ($n=24$)			Women ($n=24$)		
Variable	Mean	SD	Range	Mean	SD	Range
Age (years)	25·6	8·0	19–53	27·9	10·9	19–59
Height (cm)***	184·0	7·2	171–202	171·0	6·6	160–186
Weight (kg)***	75·0	6·7	63·2–87·5	66·7	6·8	52·8–77·9
Body mass index (kg m^{-2})	22·3	2·2	18·4–26·7	23·0	2·3	19·3–28·4
Waist to hip ratio 1†***	0·84	0·06	0·76–0·96	0·72	0·03	0·66–0·78
Waist to thigh ratio 1‡***	1·44	0·09	1·31–1·68	1·23	0·06	1·08–1·35
Waist to hip ratio 2§**	0·94	0·05	0·86–1·03	0·87	0·05	0·78–0·96
Waist to thigh ratio 2¶***	1·49	0·09	1·34–1·71	1·37	0·11	1·16–1·60
Waist to hip ratio 3††**	0·86	0·05	0·78–0·98	0·81	0·06	0·70–0·95
Total cholesterol (mmol l^{-1})	4·91	0·96	3·68–7·66	5·25	0·67	4·31–6·71
HDL-cholesterol (mmol l^{-1})	1·30	0·32	0·76–2·16	1·60	0·36	0·98–2·51
Triglycerides (mmol l^{-1})	0·98	0·67	0·27–3·33	0·85	0·28	0·42–1·45
VLDL-cholesterol (mmol l^{-1})	0·41	0·34	0·08–1·72	0·29	0·12	0·00–0·52
IDL-cholesterol (mmol l^{-1})	0·25	0·17	0·09–0·84	0·21	0·08	0·08–0·37
LDL-cholesterol (mmol l^{-1})	2·50	0·62	1·30–4·12	2·60	0·60	1·48–3·91
HDL_2-cholesterol (mmol l^{-1})**	0·40	0·20	0·11–0·93	0·57	0·23	0·26–1·04
HDL_3-cholesterol (mmol l^{-1})*	0·82	0·12	0·58–1·07	0·92	0·18	0·63–1·35

*$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.
†Minimal-waist to maximal-hip circumference ratio.
‡Minimal-waist to thigh circumference ratio.
§Umbilical-waist to hips at iliac crest circumference ratio.
¶Umbilical-waist to thigh circumference ratio.
††Umbilical-waist to maximal-hip circumference ratio.

Table 2. Regression coefficients of age and anthropometric variables with serum lipids (mmol l^{-1}) in 24 healthy non-obese men consuming a standardized high-fat diet

	Age (years)	BMI (kg m^{-2})	WTR†	WHR‡	Intra-abdominal fat area (mm^2)	Sub-cutaneous fat area (mm^2)	Intra to subcutaneous fat area
Total cholesterol							
Constant	2·498	0·142	−7·022	−3·813	3·858	4·043	3·758
Coefficient	0·094***	0·214	8·260***	10·379**	0·023***	0·009*	2·353
HDL-cholesterol							
Constant	1·440	2·186	2·601	2·028	1·355	1·239	1·714
Coefficient	−0·006	−0·040	−0·904	−0·872	−0·001	0·001	−0·856
Triglycerides							
Constant	−0·515	−1·753	−6·750	−4·000	0·387	0·584	0·040
Coefficient	0·058***	0·122	5·346***	5·913	0·013**	0·004	1·906
VLDL-cholesterol							
Constant	−0·272	−1·095	−3·245	−2·095	0·128	0·240	−0·144
Coefficient	0·027**	0·068*	2·533***	2·986*	0·006**	0·002	1·142*
IDL-cholesterol							
Constant	−0·182	−0·751	−1·663	−1·320	0·069	0·122	−0·016
Coefficient	0·017***	0·045**	1·323***	1·866**	0·004***	0·001*	0·539*
LDL-cholesterol							
Constant	1·294	−0·249	−4·267	−2·684	1·935	2·033	1·895
Coefficient	0·047**	0·124*	4·686***	6·172**	0·013**	0·005*	1·243
HDL_2-cholesterol							
Constant	0·405	0·581	1·046	0·615	0·392	0·328	0·618
Coefficient	0·000	−0·008	−0·449	−0·259	0·000	0·001	−0·451
HDL_3-cholesterol							
Constant	0·832	0·873	1·007	0·815	0·828	0·823	0·853
Coefficient	0·000	−0·002	−0·128	0·008	0·000	0·000	−0·063

* $P<0·05$, ** $P<0·01$, *** $P<0·001$.
†WTR = Minimal-waist to thigh circumference ratio.
‡WHR = Minimal-waist to maximal-hips circumference ratio.

Table 3. Regression coefficients of age and anthropometric variables with serum lipids (mmol l^{-1}) in 24 healthy non-obese women consuming a standardized high-fat diet

	Age (years)	BMI (kg m^{-2})	WTR†	WHR‡	Intra-abdominal fat area (mm^2)	Sub-cutaneous fat area (mm^2)	Intra to subcutaneous fat area
Total cholesterol							
Constant	4·676	4·361	2·739	3·548	4·855	5·255	3·440
Coefficient	0·020	0·038	2·037	2·341	0·010	0·000	8·032**
HDL-cholesterol							
Constant	1·904	2·327	4·059	5·265	1·791	1·924	1·700
Coefficient	−0·011	−0·032	−2·005	−5·068*	−0·005	−0·002	−0·471
Triglycerides							
Constant	0·600	0·584	−0·552	−0·452	0·656	0·589	0·618
Coefficient	0·009	0·011	1·135	1·788	0·005	0·001	1·005
VLDL-cholesterol							
Constant	0·143	0·018	−0·701	−0·754	0·166	0·165	0·083
Coefficient	0·005*	0·012	0·803*	1·436	0·003*	0·001	0·905
IDL-cholesterol							
Constant	0·107	0·083	−0·549	−0·597	0·127	0·147	0·029
Coefficient	0·004*	0·005	0·613*	1·108*	0·002	0·000	0·783*
LDL-cholesterol							
Constant	1·982	0·776	1·365	0·711	2·197	2·402	1·337
Coefficient	0·022*	0·080	1·007	2·612	0·010	0·001	5·635*
HDL_2-cholesterol							
Constant	0·788	1·114	1·865	2·502	0·704	0·833	0·533
Coefficient	−0·008	−0·023	−1·056	−2·672	−0·003	−0·002*	0·150
HDL_3-cholesterol							
Constant	0·906	0·796	2·356	2·632	0·908	0·910	1·029
Coefficient	0·000	0·005	−1·168*	−2·364*	0·000	0·000	−0·489

* $P<0·05$, ** $P<0·01$.
†WTR = Minimal-waist to thigh circumference ratio.
‡WHR = Minimal-waist to maximal-hips circumference ratio.

Table 4. Multiple regression coefficients of age, body mass index (BMI), and minimal-waist to thigh circumference ratio (WTR) in relation to serum lipids (mmol l^{-1}) in 24 healthy non-obese men consuming a standardized high-fat diet

Independent variables	Total cholesterol		HDL-cholesterol		Triglycerides		VLDL-cholesterol	
	b	SE	b	SE	b	SE	b	SE
Constant	−4·092		3·314		−5·159		−2·748	
Age (years)	0·046	0·022	0·008	0·013	0·024	0·019	0·009	0·011
BMI (kg m^{-2})	0·041	0·060	−0·030	0·036	0·008	0·050	0·017	0·029
WTR (cm cm^{-1})	4·785*	1·957	−1·066	1·174	3·710*	1·647	1·772	0·943
Multiple *r*	0·72		0·11		0·60		0·50	
	IDL-cholesterol		LDL-cholesterol		HDL_2-cholesterol		HDL_3-cholesterol	
	b	SE	b	SE	b	SE	b	SE
Intercept	−1·017		−3·742		1·707		1·139	
Age (years)	0·011*	0·004	0·010	0·019	0·009	0·008	0·002	0·005
BMI (kg m^{-2})	0·019	0·011	0·031	0·051	−0·002	0·021	0·000	0·014
WTR (cm cm^{-1})	0·386	0·355	3·659*	1·672	−1·046	0·722	−0·250	0·451
Multiple *r*	0·71		0·52		0·10		0·02	

*$P<0·05$.

Table 5. Multiple regression coefficients of age, body mass index (BMI), and minimal-waist to thigh circumference ratio (WTR) in relation to serum lipids (mmol l^{-1}) in 24 healthy non-obese women consuming a standardized high-fat diet

Independent variables	Total cholesterol		HDL-cholesterol		Triglycerides		VLDL-cholesterol	
	b	SE	b	SE	b	SE	b	SE
Constant	5·057		4·337		0·628		−0·499	
Age (years)	0·026	0·021	−0·005	0·011	0·012	0·008	0·004	0·003
BMI (kg m^{-2})	−0·044	0·090	−0·020	0·047	−0·025	0·037	0·000	0·015
WTR (cm cm^{-1})	0·383	2·573	−1·760	1·327	0·384	1·056	0·556	0·431
Multiple *r*	0·13		0·19		0·16		0·28	
	IDL-cholesterol		LDL-cholesterol		HDL_2-cholesterol		HDL_3-cholesterol	
	b	SE	b	SE	b	SE	b	SE
Constant	−0·261		2·054		1·845		3·058	
Age (years)	0·003	0·002	0·021	0·018	−0·005	0·007	0·006	0·005
BMI (kg m^{-2})	−0·005	0·009	0·012	0·080	−0·001	0·030	−0·164	0·023
WTR (cm cm^{-1})	0·396	0·259	−0·256	2·265	−0·763	0·842	−1·574*	0·648
Multiple *r*	0·37		0·17		0·18		0·23	

*$P<0·05$.

body mass index or age with serum lipids were found any more after adjustment for fat distribution, with the exception of age with IDL-cholesterol in men.

When substituting any other circumference ratio for the minimal-waist to thigh circumference ratio into the regression model equation, the relations of the dependent variable and the particular circumference ratio were less pronounced in men. For example, in none of the equations employed was the regression coefficient for the minimal-waist to maximal-hip and the umbilical-waist to hips at iliac crest circumference statistically significant, in contrast to the coefficients for age. In women, none of the circumference ratios was superior to any other. However, the relationships observed between blood lipoproteins and age observed in univariate analysis (Table 3) disappeared after correction for the minimal-waist to thigh circumference ratio.

Discussion

This study shows that, in multiple regression analysis, the relationship of serum lipids with measures of fat distribution was stronger than that with well-known determinants such as age and body mass index. Thus, the rise of serum cholesterol with age, which is so pronounced in men aged 20–40 years, might conceivably be due to an increase in the mass of fat, especially intra-abdominal fat, rather than to a physiological and inevitable ageing process. The strength and specificity of this relationship may have escaped attention because indicators of fatness are usually based on total body mass or composition, and do not reflect specific accumulation of fat inside the abdominal cavity. The relations observed by us were more evident for men than for women. The reason for this finding is not clear.

Associations between fat distribution and serum lipids have been shown before [2–8]. However, to our knowledge, studies have never been performed on subjects who were consuming a standardized diet. In addition, most of the available evidence for a relation between fat distribution and serum lipids is based on studies with selected populations (in most cases overweight or obese women). We have compared our present results with those obtained when the same subjects were eating their habitual diets, prior to the controlled diet period. Those results showed even stronger relationships between fat distribution and serum lipids, especially for HDL-cholesterol in women. This suggests that part of the observed associations in previous studies may have been due to dietary habits rather than to fat distribution. In addition to the five circumference ratios we also investigated the ratio of the triceps to the subscapular skinfold thickness, which is often used as a measure of subcutaneous fat patterning. Without exception, the correlations of this skinfold ratio with serum lipids were considerably weaker than those of the body circumference ratios with serum lipids.

The first evidence for a relation between fat distribution and serum lipids originated more than two decades ago when Albrink and Meigs showed, in their study on male factory workers in 1964, that a trunk skinfold thickness was more highly correlated with serum triglyceride levels than the triceps skinfold [2]. This association of blood lipids with trunk rather than limb skinfolds has since then been confirmed by others [3–5]. The waist to hip circumference ratio has proven to be a more satisfactory measure of fat distribution than indices based on skinfolds and has in some, but not all, studies been shown to be related positively to triglyceride levels and negatively to HDL-cholesterol levels. The associations of fat distribution with total cholesterol in other studies [6–8] were usually less evident than those in our study. This may be due to the fact that other investigators used the waist to hip circumference ratio. In our experience this ratio also yields lower correlations with serum lipids than the waist to thigh circumference ratios.

We have used regression equations obtained from another study population for the estimation of cross-sectional body fat areas. This could introduce random errors, even though most of the R^2 values of these equations were rather high (see Patients and methods section). Therefore it can be expected that the real associations between abdominal fat areas and blood lipids are even stronger.

It has been suggested that the relationships between circumference ratios and metabolic parameters or disease endpoints, such as cardiovascular disease and diabetes mellitus, reflect associations of intra-abdominal fat mass with these diseases [18]. We found that the estimated area of intra-abdominal fat at the level of the L4 vertebra had a stronger relationship with each of the serum lipids in men, and with total cholesterol in women, than the estimated area of subcutaneous fat. It has been proposed [8, 19] that fat stores within the abdomen are of particular importance for the development of metabolic abberrations, because the free fatty acids that are very easily released from this depot drain directly into the portal vein, in which they are transported to the liver. Exposure of the liver to high free fatty acid concentrations may cause enhanced hepatic synthesis of VLDL-triglycerides and apolipoprotein B, leading to elevated triglyceride concentrations and possibly also decreased HDL-cholesterol concentrations in the blood. According to Kissebah and his colleagues [8], the association between abdominal fat mass and serum lipids may be attributed to indirect or direct effects of increased levels of unbound androgens. The level of unbound androgens has been shown to be related to higher waist to hips circumference ratios in women [20] and thus to increased intra-abdominal fat mass [16] but, in addition, Kissebah cited evidence for a direct effect of male sex steroids on serum lipids [8]. The reason why the ratio of intra-abdominal fat to subcutaneous fat showed stronger associations with total cholesterol than any other variable in women is not clear. This ratio has also been shown to have, among other measures of fat distribution, the highest correlation with fasting blood glucose [8, 21]. It may be that this ratio reflects the degree of androgeneity, but this requires further study.

In summary, our results suggest that fat distribution, as expressed by the minimal-waist to thigh circumference ratio in non-obese subjects, is a better predictor of serum lipids than age or body mass indexes. In addition, the amount of intra-abdominal fat was more strongly associated with lipoproteins than the amount of subcutaneous fat. However, it should be stressed that most of our subjects were younger than 30 years of age and were non-obese. Therefore, further studies are needed to establish whether our findings can be extended to other population groups.

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