Extant and Composition of Coronary Lesions in Relation to Fat Distribution in Women Younger Than 50 Years of Age

Marja-Leena Kortelainen, Terttu Särkioja

Abstract—To ascertain the relationship between the extent and composition of coronary arterial lesions and the regional distribution of fat in healthy women younger than 50 years of age, a series of 30 forensic autopsy cases were investigated. Body height and weight, waist and hip circumferences, and the thickness of the subscapular and abdominal subcutaneous fat were measured; the body mass index (BMI) and waist-to-hip ratio (WHR) were calculated, and omental and mesenteric fat deposits were weighed. The extent of coronary lesions was measured by planimetry, and the thickness of the intima-media was measured by computerized image analysis. Intimal macrophage foam cells and smooth muscle cells were detected by immunohistochemistry, and macrophages were quantified. The intima-media thickness in the left anterior descending artery, circumflex artery, and right coronary artery varied significantly across the tertiles of WHR when age and BMI were adjusted, being highest when WHR exceeded 0.87. The thickest lesions also contained the largest numbers of macrophage foam cells. The intima-media thicknesses were highest with increased amounts of intraperitoneal fat. These results indicate that the severity of clinically silent coronary lesions in younger female individuals is associated with increased WHR and increased amounts of intraperitoneal fat. These results emphasize the importance of WHR as a coronary risk indicator in younger women. (Arterioscler Thromb Vasc Biol. 1999;19:695-699.)

Key Words: coronary artery disease ■ obesity ■ body fat distribution ■ heart

The accumulation of fat in the abdominal region is a well-known independent risk factor for coronary heart disease in both men and women. Angiographic studies have revealed a positive association between clinically significant coronary narrowings and abdominal obesity in both sexes,1–4 and our previous study of ante mortem healthy female forensic autopsy cases indicates that both coronary lesions are more advanced as the numerical value for waist-to-hip ratio (WHR) increases.5 The subjects in that study were both young and postmenopausal women, and the severity of coronary involvement was evaluated only by visual inspection. Because the degree of less severe lesions can often be underestimated when only a visual method is used,6 a more detailed investigation of the lesions is needed when obesity-associated cardiovascular pathology is studied in younger individuals. The type of plaque, especially its tendency to rupture, is considered more important than the mere narrowing caused by the lesion.7,8

Acute cardiovascular events are very rare in young or premenopausal women, and even in Finland, where cardiovascular morbidity and mortality are high, the incidence of fatal acute myocardial infarction in 1995 was no more than 8/100 000 among women from 45 to 49 years of age and only 1.5/100 000 in the age group 40 to 44 years,9 compared with the incidences of 116/100 000 and 42/100 000, respectively, in the corresponding male age groups.9 The protective effect of estrogens on the cardiovascular system has been concluded to be a major factor associated with the sex difference in cardiovascular disease.10 It would be of great importance to focus research on young women who develop premature coronary artery disease despite the protective effect of estrogen. To test the hypothesis that younger women with an android type of body fat distribution would probably have a greater risk of a future cardiovascular event, we designed a prospective autopsy study in which a detailed examination of coronary lesions was carried out. The purpose was to find out whether the actual degree of coronary pathology, evaluated in terms of the extent of intima involved, narrowing caused by the lesions, and the microscopic composition of the lesions, is associated with increased abdominal accumulation of fat.

Methods

The material was collected from medicolegal autopsies performed at the Department of Forensic Medicine, University of Oulu, Finland, from 1995 to 1997. Thirty women from 19 to 49 years of age (median 37) were examined. The subjects were not known to have had any ante mortem clinical signs of coronary artery disease, hypertension, lipid disorders, or any other cardiovascular abnormalities. Chronic alcoholics, diabetics, and individuals with other chronic diseases or marked changes in body structure (eg, severe crush injuries or marked ante mortem changes in body weight) were also excluded. The cases consisted of violent deaths from accidental...
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causes (33%), homicide (7%), suicide (33%), or sudden unexpected natural causes (27%) including subarachnoid hemorrhage, acute pulmonary embolism, and sligt obesity-associated cardiomegaly without any specific etiology.

The data were collected from the medical and police records. The police records included a standard form containing the results of scene investigations, interviews with family members, as well as information on diseases, alcohol consumption, and medication. The medical records were obtained from the hospitals and/or medical reception centers; the medical records contained the essential information available on each individual. No heavy alcohol consumption was reported in the records of any individual included in the series. Because no quantitative estimates of smoking habits were available, it was considered reasonable to try to reduce its confounding effect by excluding those who were reported to be regular smokers either in their medical records or according to the information given by the police. None of the individuals had received any long-term medication. Occasional use of minor tranquilizers or analgesics was mentioned in the medical records of some individuals.

Anthropometric Measurements

After the cadavers were weighed, they were placed naked on the autopsy table in the supine position and the following measurements were made by 2 trained autopsy technicians: height (cm), weight (kg), waist circumference (cm), hip circumference (cm), and thickness of the abdominal and subscapular subcutaneous fat (mm). The levels for measuring the circumferences and fat thicknesses were based on the same skeletal reference points as used for clinical purposes. Body mass index (BMI; weight divided by height squared) and WHR were calculated from the measurements.

Intraperitoneal Fat

The greater omentum was excised free of intra-abdominal tissues, and the mesenteric fat was excised from the gut. Both fat deposits were weighed while fresh. The sum of the weights of the 2 fat deposits was calculated and is referred to in this study as intraperitoneal fat. In our previous autopsy studies, we had also measured perirenal retroperitoneal fat, but we now decided to measure only the intraperitoneal fat, which is drained by the portal circulation and thus produces unfavorable metabolic effects because of increased FFA flux.

Coronary Arteries

The coronary arteries were opened longitudinally and the left and right coronary arteries were excised along a line around the orifices of the left main trunk and the RCA. The left and right coronary arteries were then removed en bloc so that the smaller branches were also excised free of surrounding tissues as far as could be judged with naked eye. Fat degree of coronary narrowing was visually estimated in the LM, LAD, CX, and RCA, separately. A numerical value was given for each artery according to the following criteria: 0, no detectable lesions; 1, stenosis 20% or less; 2, stenosis 20% to 40%; 3, stenosis 40% to 60%; 4, stenosis 60% to 80%; and 5, stenosis >80%. The total score for each individual was obtained from the sum of the scores in the 4 arteries (maximum score, 20). Each artery was then trimmed free of the surrounding muscle and fat tissue, placed intimal surface upward on a piece of cardboard, and fixed in 10% neutral formalin for 24 hours. Transversely cut samples, 2 mm thick, were taken from the lesion-occupied region of the LM, LAD, CX, and RCA. All of the samples from vessels with no visible lesions were taken from the proximal parts of the LAD, CX, and RCA. The length of the LM was variable, being very short in some cases, and therefore the samples were taken either from the lesion site or from the middle of the vessel in a macroscopically normal left main trunk.

The arteries were stained overnight at room temperature with Sudan IV. The total area of the vessels and the areas of the lipid-stained lesions and the more advanced lesions with or without positive staining were measured planimetrically (Ushikata Area-Curvimeter X-plan 360 days, Ushikata Mfg Co, Ltd). The total area covered by the lesions was calculated, and the percentage of the intima covered by the lesions was determined according to the following formula: (Intimal Area Covered by Lesions/Total Intimal Area)×100.

Histology and Immunohistochemistry

The paraffin-embedded coronary artery samples, sectioned transversely at 5 μm, were stained with the Verhoeff-Masson trichrome method, in which collagen appears green, smooth muscle red, and elastic fibers black. Intimal macrophage foam cells were detected immunohistochemically with the monoclonal mouse anti-human antibody HAM56 (Dako), and smooth muscle cells were detected with a monoclonal mouse anti-human smooth muscle actin antibody (Dako).

Morphometric Measurements and Quantification of Macrophages in Coronary Artery Samples

Only those sections in which all the layers were completely visible without any distortion or other damage produced by tissue processing were chosen for morphometric analysis. Multiple sections were made from each paraffin-embedded tissue block to obtain acceptable material. The thickness of the intimal layer was first measured in the samples in which the intima was of a normal appearance or showed only diffuse thickening. In the samples with more advanced lesions, the internal elastic membrane often showed variable degrees of destruction, so that the exact limit of the intimal and medial layer was no longer visible. We therefore measured the combined thickness of the intima and media in each sample to compare the results between the cases as described in our previous study. All measurements were made at 40× magnification on a microscope connected to a computerized image analysis system (Imaging Research Inc). The results represent means of at least 5 measurements made on each vessel.

Macrophages were counted at a magnification of 200× in the immunostained sections using the computerized image analysis system. Three intimal areas were measured from the cross-sections in which the lesions were found. The fields were selected either from the shoulder regions of the lesions, where maximum density of macrophages is usually seen, or from other parts of a plaque with maximal amount of macrophages. Three fields were examined also from cross-sections with only a few macrophages and no apparent lesion formation. The results represent means of the 3 measurements.

Statistical Analysis

All the analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. All variables except height showed some skewness in their distribution, and therefore logarithmic transformation was performed on them. A small constant (0.01) was added to the percentage of coronary plaques, coronary narrowing score and macrophages/mm² to avoid the logarithm of zero. Normal distributions were achieved with BMI, abdominal and subscapular fat, and the intima-media thickness of LM, LAD, and CX. The distributions of the other variables still remained somewhat skewed. Pearson correlation coefficients were calculated between age and the other variables, and between the cardiac parameters. Tertiles of BMI, WHR, abdominal and subscapular fat, and intraperitoneal fat were calculated. The cutoff points were 20.3 kg/m² and 25.4 kg/m² for BMI, 0.78 and 0.87 for WHR, 18 mm and 29 mm for abdominal fat, 4 mm and 11 mm for subscapular fat, and 243 g and 475 g for intraperitoneal fat. An analysis of covariance with age as a covariate was used to compare the means of coronary parameters across the tertiles of BMI, WHR, subcutaneous fat thicknesses, and intraperitoneal fat. An analysis of covariance was also carried out with age and BMI together as covariates, comparing the means of the various cardiac parameters across tertiles of WHR and the fat tissue measurements.

Results

The descriptive statistics, including means, standard deviations and ranges, are presented in Table. The variation in the various indicators of body size and obesity was wide. The extent of coronary lesions ranged from 0% to 30%, but no
significant variation across the tertiles of BMI was seen. In 15 cases, no fatty streaks were seen at all. The visually determined degree of coronary narrowing was generally mild. In only 2 cases was 50% of the coronary arterial lumen estimated to be narrowed.

The proximal part of the LAD was most often seen to be affected on visual inspection (15 cases), followed by the RCA (10 cases). The intima-media was thickest in the LAD, followed by LM, RCA, and finally CX (Table). Many of the macroscopically normal arteries showed diffuse intimal thickening with smooth muscle proliferation. The maximal

thicknesses of the raised lesions with a visible lipid core all exceeded 900 μm, and all these lesions were rich in macrophages, which were occasionally seen also in the lesions without distinct lipid cores. A raised lesion with a lipid core, together with the dimensions of the plaque, is shown in Figure 1.

Age was positively correlated with the various coronary parameters, but statistical significance was reached only with the thickness of the intima-media in the CX (r=0.441, P<0.05) and RCA (r=0.444, P>0.05). The various indicators of obesity also showed positive correlations with age, but these were not statistically significant.

The intima-media thicknesses of the LM, LAD, CX, and RCA were highest in the third tertile of WHR (>0.87). The intima-media thicknesses of the LAD and CX varied significantly across the tertiles of WHR when adjusted for age and BMI together (Figure 2). The intima-media thickness of the RCA varied significantly across the tertiles of WHR both when adjusted for age alone and when adjusted for age and BMI together (Figure 2). The number of macrophages/mm² in

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<th>Descriptive Statistics for Series of 30 Autopsy Cases</th>
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<tr>
<td>Age, y</td>
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Subcutaneous fat, mm
Abdominal | 28±17 | 5–75 |
Subcapular | 9±8 | 1–32 |
Intraperitoneal fat, g | 517±448 | 86–2322 |
Mesenteric | 245±213 | 61–1037 |
Omental | 272±261 | 25–1285 |

Coronary plaques, % of intimal surface area | 5±8 | 0–30 |
Coronary narrowing, score 0–20 | 2±2 | 0–10 |

Thickness of intima-media, μm
LM | 631±192 | 257–1056 |
LAD | 663±363 | 224–1713 |
CX | 414±246 | 85–1024 |
RCA | 457±303 | 200–1733 |

Macrophage foam cells/mm²
LM | 2±9 | 0–50 |
LAD | 120±241 | 0–837 |
CX | 36±112 | 0–481 |
RCA | 36±168 | 0–920 |

Figure 1. Transverse section of a raised lesion in the LAD of a 34-year-old woman with WHR 0.87 stained by the Verhoeff-Masson trichrome method. (i) indicates intima; (m), media; and (l), lipid. Magnification ×100; bar=500 μm.

Figure 2. Means of LAD, CX, and RCA intima-media thickness across tertiles of WHR. Values were compared by analysis of covariance. LAD: Age adjusted, F=2.404, P=0.090; Age and BMI adjusted, F=4.717, P=0.006. CX: Age adjusted, F=2.800, P=0.060; Age and BMI adjusted, F=4.341, P=0.008. RCA: Age adjusted, F=3.849, P=0.022; Age and BMI adjusted, F=7.050, P=0.001.
the RCA and CX varied significantly across the tertiles of WHR when adjusted for age and BMI together (F = 3.413, P = 0.023 for RCA; F = 2.806, P = 0.047 for CX), being highest when WHR exceeded 0.87. The visually estimated coronary narrowing and the percentage of intima covered by the lesions did not show any significant variation over the tertiles of WHR. The percentage of coronary lesions and the intima-media thickness of the LAD and CX were highest in the second tertile of BMI (20.3 to 25.4 kg/m²), but the intima-media thickness of the RCA was highest in the leanest group, with BMI < 20.3 kg/m². The degree of coronary narrowing was highest in the second tertile of BMI, but this was not statistically significant. Most of the coronary parameters attained their highest values in the second tertile of abdominal subcutaneous fat (18 to 29 mm) but in the highest tertile of subscapular subcutaneous fat (4 to 11 mm). The intima-media thicknesses of the LM, LAD, CX, and RCA were highest in the third tertile of intraperitoneal fat, the variation across the tertiles being significant in the RCA and CX (Figure 3).

The correlations between the various coronary parameters were generally positive and significant, the degree of coronary narrowing being associated with the percentage of intimal plaques (r = 0.577, P < 0.01) and with the intima-media thickness of the LAD (r = 0.471, P < 0.05), CX (r = 0.527, P < 0.01), and RCA (r = 0.635, P < 0.001). There were also significant positive correlations between intima-media thickness and the numbers of macrophages/mm² in the LAD (r = 0.645, P < 0.001), CX (r = 0.412, P < 0.05), and RCA (r = 0.843, P < 0.001).

**Discussion**

The present autopsy study revealed a positive association between the abdominal type of body fat accumulation and the severity of coronary artery pathology in ante mortem healthy women from 19 to 49 years of age. Advanced coronary lesions with lipid cores and large amounts of macrophage foam cells were seen in women with high WHR, even women in their twenties and thirties.

Previous autopsy-based studies have brought out some positive associations between obesity and coronary atherosclerosis in younger individuals, but mainly in men. Significant associations between the extent of intimal fatty streaks and raised lesions in the RCA and both BMI and the thickness of the panniculus adiposus in men younger than 34 years of age were reported by McGill et al., but they used only visual estimation of the RCA. We have previously found a positive association between increased WHR and clinically silent coronary lesions in men younger than 40 years of age. Our other previous autopsy study on females also included postmenopausal women, and the results of both that and the present survey suggest that the severity of coronary atherosclerosis does not increase with increasing BMI values in women although it does so with increasing WHR and with the size of the intraperitoneal fat deposits.

The general limitations of autopsy studies must be considered when evaluating the present results: the effects of early autolytic changes in a cadaver, with consequent effects on tissue processing; the reliability of the ante mortem data; and the difficulties in adjusting for the various confounding lifestyle factors such as smoking habits and habitual alcohol consumption, physical activity, dietary habits, and emotional stress. As in our previous autopsy studies, we did not find any evidence of heavy alcohol consumption at autopsy, but post mortem lipid analyses were not performed because they can be regarded as somewhat unreliable even during the first 24 hours after death. Thiocyanate measurements would have revealed recent smoking, but lifetime smoking habits would still have remained unknown. It is significant that women with high WHR have been reported to smoke more frequently. Individuals with impaired glucose tolerance could also not be excluded from the present study.

The android type of body fat accumulation is associated with severity of coronary atherosclerosis as measured by various methods in ante mortem healthy women younger than 50 years of age. The estimation of the degree of coronary narrowing in this study was entirely subjective, but it was significantly positively correlated with the intima-media thicknesses measured. Even normal-weight women with high WHR have plaques with lipid cores and an abundance of macrophage foam cells. Young women with an android type of body structure would probably need very careful elimination of other major coronary risk factors to stabilize the lesions and alleviate their progression.

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**References**


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