Virchows Arch [Pathol Anat] (1985) 407:431–439

Virchows Archiv A

Arterial calcification in diabetics*

U. Fuchs[1], P. Caffier[1], H.-G. Schulz[2], and P. Wieniecki[3]

[1] Dept. Pathology, Sector of Medicine
[2] Radiological Clinic, Sector of Medicine
[3] Institute for Medical Statistics and Documentation, Sector of Medicine;
Karl-Marx-University, Leipzig, German Democratic Republic

Summary. Calcification of the media of the arteries of the lower limbs, giving a linear type of calcification roentgenologically is typical for diabetic arteriosclerosis. Spotty calcification of the arteries of the lower extremity, which histologically is found in the intima, is also seen a little more often in diabetics with gangrene. There are no major differences between the other histological vascular structures of diabetics and arteriosclerotics. Maximal stenosis of the lumen is found more peripherally in diabetics than in persons with normal carbohydrate metabolism. The results favour the idea of predominant femoropopliteal occlusion in arteriosclerosis and popliteal-tibial occlusion in diabetics.

Key words: Diabetes mellitus – NIDDM (non insulin dependent diabetes mellitus) – Arteriosclerosis – Gangrene – Calcification – Lumen stenosis

1. Introduction

Arteriosclerosis has a higher morbidity and mortality in diabetics than in non-diabetics (Bibergeil and Michaelis 1983; Bloodworth jr. 1968). Diabetics have a higher incidence of severe arteriosclerosis and its complications (Bloodworth jr. 1968). For example, in patients with chronic arteriosclerotic occlusion of the femoral artery, gangrene developed in 40% of the diabetic against 9% of the non-diabetic patients (Schadt et al. 1961). In the attempts to discover some structural and chemical differences between the arteriosclerosis of diabetics and of persons with normal carbohydrate metabolism four reports are of special interest. Randerath and Diezel (1959) suggest a mucopolysaccharide plaque in the muscular arteries in diabetics (see criti-

* The findings reported in this paper are based on the First Degree Thesis of Dietlinde Barth and Martina Uhlmann (1979), Isolde Fleischhauer, T. Frank, Petra Hollmann, A. Weber (1979), Angela Lieberwirth (1981) and C. Reinknecht (1981)

Offprint requests to: U. Fuchs at the above address

cal discussion in Bloodworth jr. 1968 and Kunz 1975). The serum of diabetics harbors substances which stimulate arterial smooth muscle cells to proliferate. This factor is not glucose, insulin, or lipid (Ledet et al. 1974 and 1976, Ledet and Vuust 1980). Hevelke (1959) found more galactosides, cholesterol and magnesium in the aorta of diabetics. Lundbaek (1977) stressed the importance of media sclerosis. Comparable differences are not described in X ray findings in the Encyclopedia of Medical Radiology (Seldinger 1964) and are not mentioned by Wissler (1976). Lundbaek and Petersen (1953) found a lower calcium content of arteries in diabetics than in non-diabetics.

In looking for variables to distinguish arteriosclerosis in subjects having a normal metabolism from that in diabetics the pattern of calcification was studied using histological, morphometrical, and roentgenological methods.

2. Material and methods

206 patients were investigated. Two series of examinations were made independently in vascular disease; one group of patients had a gangrene of the lower extremity and the other did not (Table 1).

The first series answers the question of whether radiological differences between diabetic and banal arteriosclerotic vascular damage are found in patients with a comparatively early stage of the disease, where the disease has not yet progressed to the critical stage of gangrene (group a). The arteries of the lower extremity, the aorta and coronary arteries of autopsy cases were isolated and processed by the same roentgenological technique (6 pulse generator, accelerating voltage 40 kV, focus 1.2×1.2 mm, film distance 130 cm, Orwo Roentgen Film HS 11, developing automate Pentacon EAR 301). This technique avoids difficulties of projection as with arteries in situ, but causes difficulties of the definition of the consecutive arterial parts of the lower extremity. Therefore these results are pooled. Certain definitions have been introduced for morphometric analysis. More or less continuous striae of longer than two centimeters along one or both sides of the artery are arbitrarily referred to as lines, whereas smaller dense stripes along the longitudinal axis of the artery or irregular deposits are called spots (see Lindbom 1950). Parts of the vascular wall defined by their radiological pattern of calcification were excised and histologically studied after staining with von Kóssa (group b). In the same cases the thickness of intima and media, the amount of collagen, fibrin, cholesterol crystals, necroses, foam cells and vascularization was quantified by morphometric methods (group b). A square lattice incorporated into an eye piece was directly superimposed on histological sections. In this point-counting procedure the number of cross-points lying on the examined structures are counted as "hits" (Glagolev 1933, 1934, Weibel, Kistler, Scherle 1966, Weibel, Elias 1967). The thickness of the intima and media was measured with a calibrated ocular measuring plate.

In the fully developed disease, where diabetic or arteriosclerotic *gangrene* made amputation of the lower extremity necessary, the histological pattern of calcification (group c) and the localization of vascular stenosis (group d) were examined. Histologically, regarding the calcification, an arbitrary distinction has been made. Particles bigger or smaller than 27 μm are called granules or clods, resp. The sum of the values for granules and clods gives the total calcification. It is well known that excised arteries change their length and diameter, but not their relative areas. Therefore morphometric analysis was also done. The lumen is given as a percentage of the total cross sectional area of the vascular wall (lumen plus intima plus media). Statistical tests were done with the *U*-test of Mann and Whitney using the electronic data processing machine ES 1040. To be sure that arteries of persons of the same height were compared, the length of the lower legs and feet were measured, comparing the values of the diabetics and non diabetics with one another (37.5/41.8 vs 22.3/22.0 cm). The diabetes

Table 1. Groups investigated

	Patients			
	Without gangrene (autopsy cases)		With gangrene (amputated legs)	
Group Examination	**a** Radiological	**b** Histological	**c** Histological (calcification)	**d** Histological (lumen stenosis)
Aorta thoracica	+	+	−	−
Aorta abdominalis	+	+	−	−
Art. coronaria	+	+	−	−
Art femoralis	+	+	+	+
Art. poplitea	+	−	+	+
Art. tibialis ant.	+	−	+	+
Art. tibialis post.	+	−	+	+
Art. dorsalis ped.	+	−	+	+
n (diabetes/control)	46/34	20/12	47/47	40/45
age (years) (diabetics/controls)	76/76	73/73	69/76	67/71

mellitus had lasted up to 25 years and was of type II. As expected, patients afflicted with diabetic gangrene are several years younger than the patients with arteriosclerotic gangrene (Table 1, group c, d).

In this paper the term "arteriosclerotic" concerns banal arteriosclerosis in patients with normal carbohydrate metabolism and "diabetic" in the corresponding context, arteriosclerosis in diabetic patients.

3. Results

3.1. Radiological findings

Linear calcification is more often seen in the arteries of the lower extremity (arteria femoralis-arteria dorsalis pedis) and in the coronary arteries of diabetics than in those of persons with normal metabolism (Table 2). This difference is especially pronounced in men and in comparing hypertensive diabetics with hypertensive controls. Patients older than 80 or who have been diabetic for more than 10 years have more linear calcification.

Spotty calcification is also more frequent in the arteries of the lower limbs of diabetics than of the controls (Table 2). This difference is also particularly clear in hypertensives. With the technique used spots were only registered in the thoracic and abdominal aorta ($8.60 \pm 1.90\%$ and $13.24 \pm 2.65\%$ for the diabetics; $8.64 \pm 1.48\%$ and $8.14 \pm 1.22\%$ for the controls). Histologically, the linear type of calcification corresponds to a medial calcification. In the spotty form the intima shows a positive von Kóssa reaction.

Table 2. Radiological findings. Morphometric data. The numbers give intersection points ($\bar{x} \pm s_{\bar{x}}$ (%)). The results of the statistical analysis are marked ($p < 0.05$)

	Diabetes mellitus		Normal metabolism
Arteries of the lower extremity			
Linear calcification	45.70 ± 9.99	>	30.77 ± 12.05
	v		v
Spotty calcification	22.98 ± 5.03	>	14.80 ± 4.96
Coronary Arteries			
Linear calcification	66.60 ± 3.00	>	34.44 ± 2.89
	v		v
Spotty calcification	20.22 ± 1.65	=	20.41 ± 2.74

Table 3. Histological findings. Calcification of the *media*. Morphometric data (number of calcified fields/cross section ($\bar{x} \pm s_{\bar{x}}$))

	Diabetes mellitus	Normal metabolism
Arteria femoralis	22.44 ± 4.59^{a}	9.44 ± 2.79^{b}
Arteria poplitea	26.68 ± 4.81^{c}	7.96 ± 1.52^{d}
Arteria tibialis anterior	26.22 ± 2.82^{e}	6.00 ± 1.31^{f}
Arteria tibialis posterior	21.25 ± 2.79^{g}	4.05 ± 1.24^{h}
Arteria dorsalis pedis	11.40 ± 1.99^{i}	1.32 ± 0.52^{k}

Statistically significant findings (* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$)
a–b**; c–d***; e–f***; g–h***; i–k***; a–i*; c–i*; e–i*; g–i*; b–k*; d–k*; f–k*

3.2. Histological findings

The diabetic patients more often have *calcification* in the *media* of the arteries of the lower limbs than patients with banal arteriosclerosis (Table 3). This is true for total calcification (Table 3) and also for the granules and the clods independently. In these two last mentioned cases the statistical error of the differences amounts to $p < 0.05$ and 0.01 for the granules (exception: Art. femoralis) and for $p < 0.001$ for the clods. The diabetics have approximately 2 to 10 times more calcification. In the diabetics, but not in the arteriosclerotic patients, a negative correlation (coefficient – 0.47) is found between the area of the media without and with calcification ($y = 35.08 - 0.62x$). The art. femoralis has more calcification than the art. dorsalis pedis (Table 3). Beneath fibrolipidic plaques the media has a tendency to more calcification. In diabetic and non diabetic patients with calcification of the media, calcification may occupy the same proportion of the media.

Statistically shown differences in the calcification of the *intima* of the diabetics and banal arteriosclerotics are only found for the total calcification of the arteria femoralis and poplitea in banal arteriosclerosis (Table 4). The

Table 4. Histological findings. Total calcification of the *intima*. Morphometric data (number of calcified fields/cross section $\bar{x} \pm s_{\bar{x}}$)

	Diabetes mellitus	Normal metabolism
Arteria femoralis	5.43 ± 1.41^{a}	11.27 ± 3.29^{b}
Arteria poplitea	6.35 ± 1.68^{c}	14.13 ± 2.45^{d}
Arteria tibialis posterior	6.13 ± 1.19^{e}	9.62 ± 1.91^{f}
Arteria tibialis anterior	4.94 ± 1.09^{g}	9.62 ± 2.32^{h}
Arteria dorsalis pedis	3.00 ± 1.33^{i}	2.70 ± 0.68^{k}

Statistically significant findings * $P<0.1$; ** $P<0.05$)
a–b**; c–d**; a–i*; c–i*; e–i*; g–i*; b–k*; d–f*; d–k*; f–k*; h–k*; d–h*

Table 5. The lumen ($\bar{x} \pm s_{\bar{x}}$% of total area of artery) of the arteries of the limb

	Arteriosclerotic gangrene	Diabetic gangrene	$P<$
Arteria femoralis	22.42 ± 3.24	30.10 ± 3.43	0.05
Arteria poplitea	30.43 ± 3.25	33.02 ± 3.28	–
Arteria tibialis anterior	25.46 ± 3.50	31.37 ± 3.92	0.01
Arteria tibialis posterior	30.75 ± 3.15	24.32 ± 3.48	0.01
Arteria dorsalis pedis	24.67 ± 3.77	28.94 ± 3.71	0.01

patients with arteriosclerotic gangrene have 2 times more calcification in these arteries than the diabetics. The art. femoralis has more calcification than the art. dorsalis pedis (Table 4). Histologically, fibrolipidic plaques of diabetics and controls have similar amounts of calcareous material. The same is true for calcareous plaques.

There are no differences between the other *histological structures* of diabetics and arteriosclerotics (group b). The intima-media index, as a parameter of the severity of the arteriosclerosis, is higher in the coronary arteries than the aorta thoracica and abdominalis with regard to the diffuse intima thickening and the plaques. Fibrolipidic plaques are 7.6 to 10.9 times thicker in diabetics and in arteriosclerotics than the macroscopically unchanged intima of the same cases. The underlying media is equally thinned in both groups, to 1/2 to 1/3 of the normal thickness. Under plaques the media of diabetics and arteriosclerotics contains more collagen.

Regarding the mean values, the *lumen* (Table 5) is wider proximally in the arteries in diabetic compared with arteriosclerotic gangrene. The frequency polygon (Table 6) shows the severest stenosis (lumen $<10\%$) to be twice as frequent in the proximal arteris of arteriosclerotics, whereas wider lumina (lumen 41 to 50%) in the arteria femoralis and poplitea are 2 to 4 times more frequent in diabetics. The column of the lumen 11 to 20% (smaller degree of stenosis) shows a frequent stenosis in the arteriosclerotic arteria tibialis anterior and dorsalis pedis and in the case of diabetics, in the arteria poplitea and arteria tibialis posterior (Table 6).

Table 6. The lumen of the arteries of the lower limb. Frequency polygon. The values of one artery are equal to 100%. The percentage of the measured values related to % lumen of the total area of the vascular wall (lumen + intima + media) is given

Lumen (%)	<10%		11–20%		31–40%		41–50%	
	ascl.	d.m.	ascl.	d.m.	ascl.	d.m.	ascl.	d.m.
Arteria femoralis	12.5	5.5	30.0	11.0	20.0	27.3	5.0	22.0
Arteria poplitea	11.0	6.3	50.0	43.8	5.0	12.5	5.0	12.5
Arteria tibialis anterior	0	8.7	46.2	4.4	30.8	13.0	15.3	21.7
Arteria tibialis posterior	0	10.8	12.5	32.1	12.5	25.0	25.0	10.0
Arteria dorsalis pedis	0	9.5	50.0	14.3	14.3	19.0	7.1	9.5

ascl. = banal arteriosclerotic gangrene
d.m. = diabetic gangrene

4. Discussion

The life expectancy of adult diabetics is 70% of the norm, mainly due to cardiovascular diseases and only seldom (1%) to coma (Bibergeil and Michaelis 1983). The increased predeliction for macrovascular complications is not restricted to any one type of diabetes (Ganda 1980). The diabetic patient, in addition to being susceptible to any, or a combination, of the pathogenetic factors operative in the non-diabetic, is at a particular disadvantage, because of at least four additional factors unique to the diabetic state (microangiopathy, hyperglycaemia, hormonal aberration, neuropathy) and because of a multiplicity of other factors occuring with an increased frequency in the diabetics, e.g. hypertension, hyperlipidaemia and hypercoagulability (Ganda 1980; Jarrett et al. 1982). On the other side widespread arteriosclerotic disease may be absent in special groups of diabetics affected with microangiopathy suggesting that small vessel disease could be at best only a contributary factor in large vessel disease (Jarrett et al. 1982). Diabetes may develop in individuals who already possess characteristics which increase the risk of coronary disease in addition to the risk of developing diabetes (Jarrett 1984). Possibly the concept of common antecedents for both type 2 diabetes and arteriosclerosis may have a genetic background (Jarrett 1984). Possibly some diabetics are prone to arterial disease and others not (Jarrett et al. 1982). The considerable geographical variation in the prevalence of large vessel disease in diabetics runs broadly parallel with the prevalence in the population from which they were derived (Jarrett et al. 1982). The variability of the disease in time and along the artery and the changing geometry of arteries removed from organism are the difficulties of morphologic analysis. Therefore we have studied some arteries of two groups of patients and have used independent techniques.

The finding of more roentgenologically linear, histologically medial calcification in diabetics confirms the findings reported by Ferrier (1964) and Lundbaek (1977). Medial calcification is the characteristic arterial lesion in diabetics (Ferrier 1964), seen here in the gangrene and in the non-gangrene

group. Whereas Neubauer (1971) observes no difference between roentgenologically spotty, histologically intimal calcification in the diabetic and in the non-diabetic population, we additionally find more spotty calcification in the diabetics without gangrene. In patients with advanced disease, i.e. with gangrene, the figures for the intimal calcification are higher in cases of normal metabolism, but this is statistically proven in the arteria femoralis and poplitea only. So spotty calcification is no sure marker either of diabetic or banal arteriosclerosis. Indeed the intima plaques of diabetics and non-diabetics have a similar calcium content (Diedzic-Goclawska et al. 1984). Nevertheless, because of the medial sclerosis and the more widespread changes in the intima of diabetics, the calcium content of the vascular wall is increased. This is shown by Hevelke (1959). He reported findings on the arteria femoralis of diabetics and non-diabetics in the age group 61 to 80 years. Related to 1 g wet weight, he found approximately 23 and 11 mg calcium and 8.6 and 3.5% ash content in diabetics and non-diabetics, respectively, whereas Lundbaek and Petersen (1953) find only 13.4 ± 2.1 and 18.8 ± 1.3 mg calcium per 1 g dry weight in coronary arteries of diabetics and non-diabetics, respectively. In contrast to these low values in diabetics, Ledet (1968) found more calcium in the proximal and in the distal part of the extramural coronary arteries in the diabetics than in the non-diabetics. Similar differences were not obtained with the aorta of diabetics and non-diabetics (Hevelke 1959).

The functional importance of linear calcification is that it can limit maximal blood flow (Christensen 1968; Lundbaek 1977). In azotemic arteriopathy marked luminal compression is also due mainly to heavy calcific infiltration of the media and only partially to swelling and hyperplasia of the intima, which is clearly different from arteriosclerosis (Rosen et al. 1972). In diabetics the most important mechanism of the reduction of blood flow, however, is luminal stenosis (Lundbaek 1977). It is often more proximally localized in patients with banal arteriosclerosis. Some 10% amputations in diabetic patients are performed in the presence of palpabel peripheral pulses (Ellenberg 1973). Strandness jr., Priest and Gibbons (1964) have shown that the diabetic has the same incidence of occlusion in the femoropopliteal system but a higher incidence below the knee. Haimovici (1968) found femoral-popliteal occlusions more often in non-diabetics and popliteal-tibial occlusions more often in diabetics (non-diabetics/diabetics 9%/1.4% and 12.0%/18.8%). In his femoral-popliteal-tibial group of occlusion the arteriosclerotic group often had only one artery of the distal artery tree occluded (51.5%), while in the diabetic group 78.9% had 2 or 3 arteries occluded. We also find multiple stenosis in the distal arterial tree of diabetics.

It is rather difficult to account for the increased medial sclerosis of diabetics. More calcium binding structures, calciphylactic arteriopathy, changed calcium metabolism, medial dystrophy and calcification due to elevated and changing catecholamine levels are potential factors. Electron microscopically calcification of elastin and collagen is seen (Gendre and Tingaud 1974). A correlation between radiological medial calcification and glucose tolerance in diabetics has been found, whereas no correlation with

intimal calcification was evident (Neubauer 1971). Hyperglycaemia, insulinaemia, changed lipoprotein spectrum, disturbed mineral levels and acid-base equilibrium are all important risk factors (Bibergeil and Michaelis 1982).

References

Barth D, Uhlmann M (1979) Verkalkte Arterien der unteren Extremitäten bei diabetischer und arteriosklerotischer Gangrän. Med Dipl Arbeit, Leipzig

Bibergeil H, Michaelis D (1983) Zur Epidermologie und Ätiopathogenese der Arteriosklerose (Makroangiopathie) bei Diabetes mellitus. Dtsch. Ges Wesen 38:89–95

Bloodworth JMB (1968) Diabetes mellitus. Extrapancreatic pathology. In: Bloodworth JMB jr., (ed) Endocrine Pathology, Baltimore: Williams and Wilkins 1968

Christensen NJ (1968) Muscle blood flow, measured by xenon 133 and vascular calcification in diabetics. Acta Med Scand 183:449–454

Dziedzic-Goclawska A, Fuchs U, Krautschick I, Ostrowski K, Stachowicz W, Michalik J (1984) Crystallinity of mineral deposited in arterial walls in the course of arteriosclerosis in diabetics and in patients with normal carbohydrate metabolism. Bas Appl Histochem 28:21–28

Ellenberg M (1973) Diabetic foot. New York State J Med 73:2778–2785

Ferrier TM (1964) Radiologically demonstrable arterial calcification in diabetes mellitus. Aust Ann Med 13:222–228

Fleischhauer I, Frank T, Hollmann P, Weber A (1979) Die Verkalkung der arteriellen Gefäßwand bei Diabetikern und Stoffwechselgesunden: Quantitative radiologische und chemische Analyse. Med Dipl Arbeit Leipzig

Ganda OMP (1980) Pathogenesis of macrovascular disease in the human diabetic. Diabetes 29:931–942

Glagolev AA (1976) Zit n Gröbler, Kleinteich, Schickedanz (1976)

Gröbler B, B Kleinteich, H Schickedanz (1976) Über die Bestimmung des statistischen Fehlers bei der Treffermethode. Z mikroskop anat Forsch 90:1137–1144

Gendre P, Tingaud R (1974) Ultrastructure of the human arterial wall in diabetic patients. J Cardiovasc Surg 15:21–32

Haimovici H (1968) Patterns of arteriosclerotic lesions of the lower extremity. Ann NY Acad Sci 149:997–1021

Hevelke G (1959) Angiochemische Gefäßveränderungen bei Diabetes mellitus. In: Oberdisse K, Jahnke K (Hrsg) Diabetes mellitus, Thieme, Stuttgart pp 93–98

Jarrett RJ, Keen R, Chakrabarti (1982) Diabetes, hyperglycaemia and arterial disease. In: Keen H, Jarrett J Complications of diabetes. 2nd ed., Arnhold, London pp 179–203

Jarrett RJ (1984) Type 2 (non insulin dependent) diabetes mellitus and coronary heart disease – chicken, egg or neither? Diabetologia 26:99–102

Kunz J (1975) Pathologie der Arterienwand mit besonderer Berücksichtigung der Glykosaminoglykane. Berlin Volk u. Gesundheit

Ledet T (1968) Histological and histochemical changes in the coronary arteries of old diabetic patients. Diabetologia 4:268–272

Ledet T, Fischer-Dzoga K, Wissler RW (1974) Diabetic macroangiopathy: in vitro study of the proliferation of the aortic smooth muscle cells. Am J Pathol 74:50a

Ledet T, Fischer-Dzoga K, Wissler RW (1976) Growth of rabbit aortic smooth muscle cells cultured in media containing diabetic and hyperlipidemic serum. Diabetes 25:207–215

Ledet T, Vuust J (1980) Arterial procollagen type I, type III and Fibronectin. Diabetes 29:964–970

Lieberwirth A (1981) Quantitative morphometrische Untersuchungen an Arterien der unteren Extremitäten bei diabetischer und arteriosklerotischer Gangrän. Med Dipl Arbeit Leipzig

Lindbom Å (1950) Arteriosclerosis and arterial thrombosis in the lower limbs. Acta Radiol Suppl 80:38–48

Lundbaek K (1977) Diabetische Angiopathie. Münch Med Wochenschr 119:647–654

Lundbaek K, Posborg-Petersen V (1953) Lipid composition of diabetic and non diabetic coronary arteries. Acta Med Scand 144:354–359

Meyer WW (1974) Einführung in die Pathomorphologie der Arterien In: Heberer G, Rau G u.W. Schoop (Hrsg) Angiologie. 2. Aufl. Thieme, Stuttgart pp 54–103
Neubauer B (1971) A quantitative study of peripheral calcification and glucose tolerance in elderly diabetics and non diabetics. Diabetologia 7:409–413
Schadt DC, Hines jr., EA, Juergens J, Barker NW (1961) Chronic arteriosclerotic occlusion of the femoral artery. J Am Med Ass 175:937–940
Randerath E, Diezel PB (1959) Morphologische Pathologie der extrarenalen Angiopathie beim Diabetes mellitus. In: Oberdisse K, Jahnke K: Diabetes mellitus, Thieme, Stuttgart pp 54–60
Reinknecht C (1981) Untersuchungen an Arterien bei Diabetikern und Stoffwechselgesunden mit der Glagolev'schen Treffermethode. Med Dipl Arbeit, Leipzig
Rosen H, Friedmann SA, Raizner AE, Gerstmann K (1972) Azotemic arteriopathy. Am Heart J 84:250–255
Seldinger SJ (1964) Arteries of the extremities. In: Handbuch der medizinischen Radiologie, pp. 400–472 Bd. X., Tl 3, Springer, Berlin, Göttingen, Heidelberg, New York
Strandness DE jr., Priest RE, Gibbons GE (1964) Combined clinical and pathologic study of diabetic and non-diabetic peripheral arterial disease. Diabetes 13:366–372
Weibel ER, Kistler GS, Scherle WF (1966) Practical stereological methods for morphometric cytology. J Cell Biol 30:23–38
Weibel ER, Elias H (1967) Quantitative methods in morphology. Springer, Berlin, Heidelberg, New York
Wissler RW (1976) Abnormalities of the arterial wall and its metabolism in atherogenesis. Progr Cardiovasc Dis 18:341–369

Accepted May 28, 1985