Eccentric May Differ From Concentric Left Ventricular Hypertrophy Because of Variations in Cardiomyocyte Numbers☆

RICHARD E. TRACY, MD, PhD

New Orleans, Louisiana

ABSTRACT

Background: When overweight or hypertension is present, some patients at first examination exhibit left ventricular (LV) dilation and others concentric hypertrophy that persists indefinitely. Perhaps the ventricles with the fewest myocytes could enter systolic dysfunction with dilation and those with more myocytes may sustain persistent concentric hypertrophy.

Methods and Results: Cardiomyocyte numbers, MyN, were counted in paraffin sections of left ventricles from 99 forensic autopsies, excluding instances of coronary heart disease. MyN lacked statistically significant relationships with age, race, sex, height, weight, LV mass, and mean arterial pressure estimated from renal histology. Specimens with the fewest myocytes, however, did manifest significant dilation and thinner chamber walls.

Conclusions: The tendency toward an eccentric pattern of hypertrophy in ventricles with the fewest myocytes is a clear conclusion, but has an ambiguous interpretation. This is the pattern expected from the initial hypothesis, but it also has other possible explanations. This ambiguity arises chiefly from limitations in the methods used for estimating MyN. These limitations are accessible to control in future studies. Our findings failed to contradict the hypothesis introduced here. These conclusions are implicit in the underlying observations of MyN constancy across such groupings as sex, height, weight, and LV mass, which provide the less speculative findings. (*J Cardiac Fail 2013;19:517–522*)

Key Words: Cardiomyopathy, heart failure, human, hypertension, obesity.

Hypertensive subjects may or may not manifest left ventricular (LV) hypertrophy when first observed.[1–3] Those with hypertrophy may reveal concentric or eccentric patterns.[2–4] The long-held view that the process begins as concentric and evolves to eccentric hypertrophy is now subject to some doubt, because observations taken over a period of years seldom encountered such an evolution in the absence of intervening myocardial infarction.[4–7] Likewise, newly assessed obese subjects may or may not have hypertrophy,[1,8] and those with hypertrophy can display a broad range of chamber sizes from concentric to dilated eccentric patterns.[5–9] Although retrospective data on morbid obesity in place for up to 30 years found the duration of the condition to correlate strongly with progression to heart failure, this was usually diastolic, implying an infrequent evolution to systolic failure.[8] Prospective studies offering follow-up of >7.5 years found systolic dysfunction to evolve from concentric hypertrophy in $\sim$20% of the cases in the absence of ischemic events.[7] Therefore, the frequent finding of eccentric hypertrophy on first examination of hypertensive and overweight subjects raises the question of why some subjects exhibit dilation and others remain in a state of concentric hypertrophy without subsequent dilation.[6] Recent reports from our laboratory,[10,11] using data derived from forensic autopsies, reiterate these clinically derived findings.

A readily developed hypothesis to reconcile these paradoxic findings is that variations in the number of cardiomyocytes might underlie these disparate responses to cardiac work load. Ventricles with the fewest myocytes could be the first ones to enter systolic dysfunction and undergo dilatation. Ventricles with more myocytes could be the ones that use concentric hypertrophy to compensate for the work load. Uncommon ventricles with exceptionally many myocytes might even adapt to the load without enlarging the ventricular mass across pathologic boundary

From the Department of Pathology, Louisiana State University Health Sciences Center, New Orleans, Louisiana.

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Reprint requests: Dr Richard E. Tracy, MD, PhD, Louisiana State University Health Science Center, Department of Pathology, 1901 Perdido St, P5-1, New Orleans, LA 70112. Tel: 1-504-568-6072; Fax: 1-504-568-6037. E-mail: rtracy@lsuhsc.edu

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lines. Unlike the situation for animal models, where evolution from concentric to eccentric hypertrophy is readily induced, the human situation likely includes a wider range of variation in the number of cardiomyocytes endowed during infancy than is expected in laboratory animals. In the human setting, the proposed hypothesis would predict that an evolution from one form of hypertrophy to the other ought to be uncommon and delayed, as it is observed to be.

The series of forensic autopsies previously reported is expanded here by inclusion of newly assembled cases. With the enlarged sample size, data on cardiomyocyte sizes determined histologically are used to explore the topic of myocyte numbers (MyN) in relation to left ventricular dimensions. The findings are the first of their kind and are offered as provisional to further the proposed hypothesis.

Methods

Subjects

The 99 cases reported here were derived from a series of forensic autopsies (Appendix). They included few women and had little clinical information, as is usual for a forensic service. All subjects with cardiomegaly were retained with the exception of those with coronary heart disease, which were omitted. Comparison cases without cardiomegaly were retained when time best permitted this activity. The Institutional Review Board declared this autopsy study to be exempt from their review.

Left Ventricular Measurements

Left ventricular chamber diameter (LVD), free wall thickness (LVT), and mass (LVM) were measured during the autopsy on the high chordal equatorial plane and expressed in millimeters and grams (Appendix).

Preparations for Histology

A slice of myocardium presenting the high chordal plane was formalin fixed for 1−3 weeks. Samples were excised perpendicular to this plane from lateral and posterior ventricular walls. Paraffin-embedded samples were sectioned at 6 μm and stained with hematoxylin and eosin. To adjust for shrinkage in paraffin sections, a factor of 1.21 was applied to subsequent histologic measurements,[10] although this was not needed for the relative comparisons of myocyte characteristics in the specimens reported here.

Myocyte Profiles

Myocyte profiles in cross-section (Fig. 1) were evaluated by imposing an ellipse upon each profile and measuring the minor axis of the idealized outline. These measurements were made on black-and-white photographic prints with the use of a digital caliper accurate to 0.01 mm. Measurements were taken in 60 anucleate profiles from LV sites showing the best cross-sectional profiles (4 sites in the first 78 cases and 2 sites in the 21 supplemental cases). Figure 1A represents an exceptionally small ventricle and 1B a greatly hypertrophied specimen, exemplifying the full range of myocyte sizes in this data set. The irregular forms of these cross-sections revealed especially great variation in the major axis of the imposed ellipse, thus precluding confidence in its measurement. For this reason, measurements were confined only to the minor axis with no attempt to determine major axis.

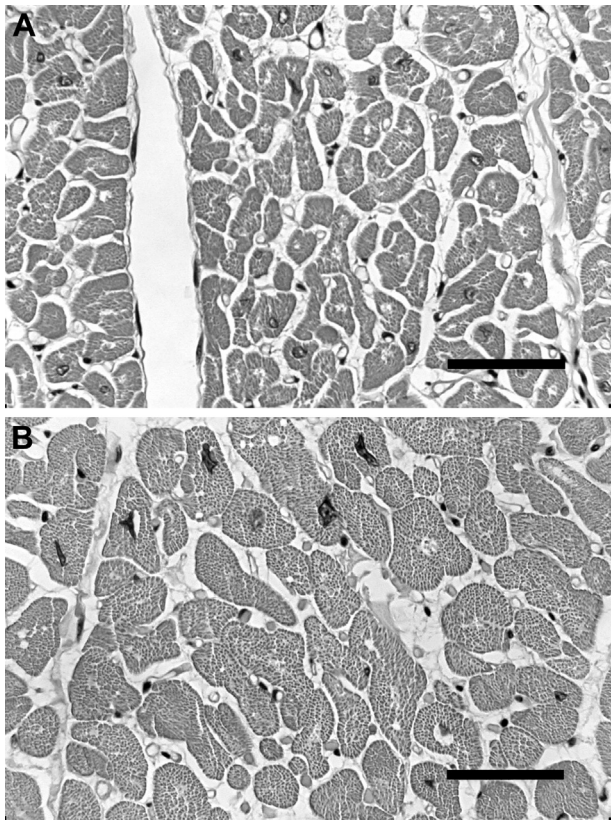

Fig. 1. Cross-sectional planes of cut representing (A) smallest and (B) largest sizes encountered in this study. Hematoxylin and eosin; bars = 50 μm.

Separation of cells in these views is a shrinkage artifact from paraffin embedding.

Myocyte Breadth (MyB), Cross-Sectional Area (MyA), Length (MyL), Volume (MyV), and Number (MyN)

These quantities were all derived from measurements on cross-sectional profiles like those in Figure 1, using LVM for computing MyN. Details are provided in the Appendix.

Overweight

Cutoff for overweight was retained as used before, ie, weight >104 kg, as described in the Appendix.

Hypertension

Intimal thickness of interlobular arteries in the renal cortex was measured and mean arterial pressure (MAP) calculated with the use of a previously derived regression equation[10−13]: $MAP = (S + 2 \times D)/3$. The cutoff point of 106.7 mm Hg (eg, 140/90) was used to specify hypertension in keeping with previous practice.

Statistical Methods

We used commonly used techniques available in the SAS programs (SAS Institute, Cary, North Carolina). Some of the linear regression equations yielded insignificant intercept terms, so the appropriate equations were forced through the origin; the squared correlation coefficients reported with these equations are those retaining the intercept terms.

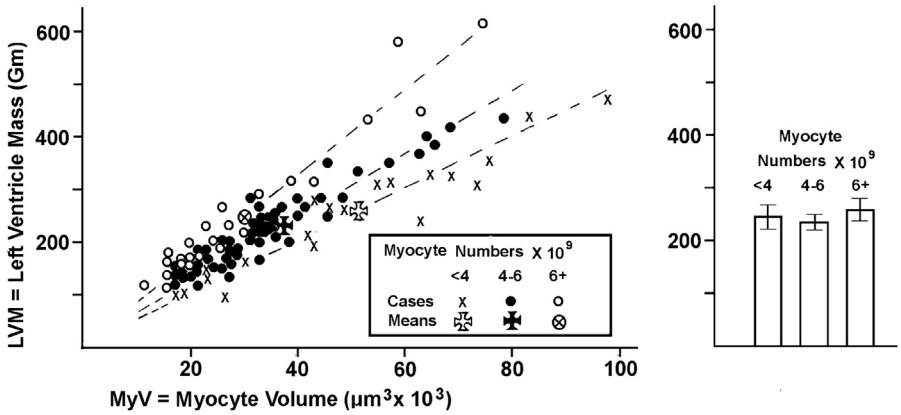

Fig. 2. Left: Each symbol presents 2-dimensional means of a single left ventricle; means of the grouped means are also plotted. The sloping dashed lines plot LVM calculated from equation 1 (see text) in 3 groupings by myocyte numbers. Right: Means on the vertical axis in the left panel are emphasized by bar graphs with SEM.

Results

Relationship of LVM to MyV

Three regression equations for this relationship were derived, 1 for each of the groupings by LV MyN <4, $4-6$, and $>6 \times 10^9$ myocytes per ventricle (Fig. 2). In each of these 3 equations, a nonzero intercept term was found to lack significance, so the appropriate equations were forced through the origin (P values .85, .80, and .35 for the respective intercepts). Also lacking significance were the 3 quadratic terms, MyV^2, so LVM emerged as proportional to MyV in all 3 myocyte groupings (P values .67, .14, and .90 for the respective quadratic terms). Therefore

$$\text{LVM} = \text{b} \times \text{MyV} \qquad (1)$$

with b = 0.0051, 0.0064, and 0.0083 and $R^2 = 0.92, 0.91$, and 0.94 for the respective myocyte groupings. In the relationship $\text{MyN} = 0.75 \times \text{LVM/MyV}$, the variations in MyN are, in these data, governed solely by the denominator with no significant contribution by the numerator. This is seen in Figure 2 by the significant segregation of the MyN groupings on the horizontal but not on the vertical axis (analysis of variance [ANOVA] in the table), emphasized in the bar graph panel.

Relationship of MyA to LVD

Figure 3 shows a scatter plot of these 2 variables. The fitted equation rejects an intercept term, so the appropriate equation was forced through the origin:

$$\text{MyA} = 11.0 \times \text{LVD} - 0.066 \times \text{LVD}^2 \qquad (2)$$

$$\left(R^2 = 0.44;\ \text{SE of } 11.0 \text{ is } 0.66 \text{ and of } -0.066 \text{ is } 0.015\right)$$

This equation is not plotted in Figure 3. In its place, an arbitrary line of proportionality is introduced to aid discussion, and this is chosen to pass near the means of the 3 MyN groupings. The segregation of MyN groupings on the vertical axis (MyA) is a stepwise progression, emphasized in the bar graph panel, whereas on the horizontal axis (LVD) the segregation is large from group <4 to $4-6$ but small from group $4-6$ to >6, yielding a curvilinear pattern in keeping with equation 2 (ANOVA in the table).

Relationship of Selected Variables to MyN

Groupings of cases on MyN did not significantly differ in age, race, sex, height, weight, LVM, or MAP determined from renal histology (Table 1). LVD had a weak positive

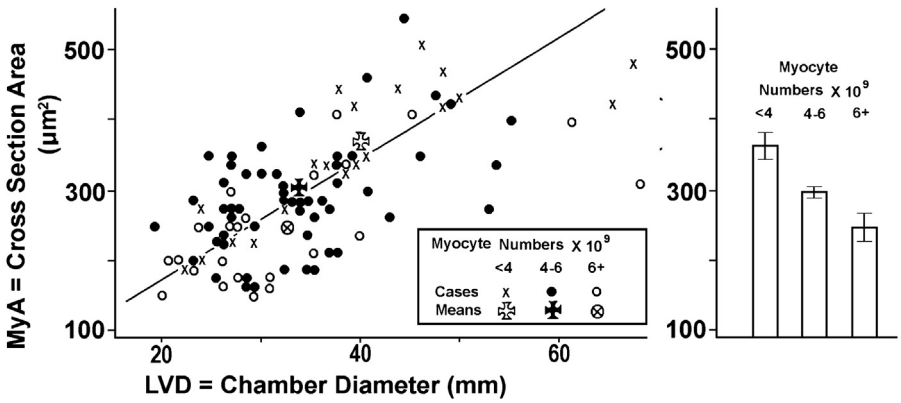

Fig. 3. Left: An arbitrary line of proportionality is chosen to pass near the 3 instances of 2-dimensional means to aid discussion. Right: Means on the vertical axis in the left panel are emphasized by bar graphs with SEM.

association ($P = .04$) and LVT a weak inverse association ($P = .03$) with MyN. The ventricles with the fewest myocytes tended to be somewhat dilated and with thinner free walls compared with the ventricles with many myocytes.

Discussion

Myocyte numbers, as estimated here, display some provocative patterns in this data set. These patterns are not offered as definitive conclusions, but simply as provisional observations to propose working hypotheses with potentially useful consequences.

MyN did not differ significantly between men and women nor between blacks and whites (Table 1; $P = .64$ for interactions). This would seem to imply that female infants receive the same number of myocytes as male infants on average, and that blacks receive the same numbers as whites. This comparison of sex groupings extends the same concept introduced by de Simone et al using different methodology.[14] Therefore, the larger size of hearts in men could be due to exaggerated hypertrophy of normal growth and not to greater physiologic endowment with myocytes, as detailed in an earlier report from this series of cases.[10] Similar conclusions apply to height, a proxy for lean body mass, as detailed before.[10] The endowment of myocytes in infancy appears not to anticipate the adult body stature. Therefore, the larger heart sizes in taller men and women may be due to exaggerated hypertrophy of normal growth, which would signal accelerating progression toward pathologic boundaries in persons of greater stature.

Body weight, a reflection in large part of fat body mass,[10] did not correlate with MyN, nor did MAP, calculated from renal histology (Table 1). Simplistically this would seem to indicate that these sources of enhanced work load, acquired as adults, may not propel changes in myocyte numbers, ie, hyperplasia, necrosis, or apoptosis, in this data set which excludes recognizable instances of coronary heart disease. This interpretation is reinforced by the lack of correlation of MyN with LVM (Table 1), indicating an equality of MyN across groupings by LVM. That MyN might diminish by necrosis or apoptosis in the most deformed ventricles was examined in detail earlier, with the conclusion that the data were inconsistent with those suggestions.[10] In specimens of all masses and chamber sizes, the frequency distributions of myocyte sizes did not reveal any truncation of the largest myocytes, whereas the distributions continued to fit closely to a log normal even in the most hypertrophied and dilated ventricles. An earlier comparison of hypertensive and overweight subjects found no significant differences between them in mean ventricular mass or diameter or their ranges of distribution.[11] Pressure and volume overloads may vary in response to overweight and hypertension in ways that are more nearly similar than is often presumed. Grossman et al[15] emphasized the variations between pressure and volume overloads as determinants of LV dimensions. Those principles were derived from patients with valve defects where the types of overload can be easily distinguished, whereas this is not so easily done for overweight and hypertension.

Differences across groupings by MyN did emerge as significant for LVD and LVT (Table 1). The ventricles with the fewest myocytes were somewhat dilated and had slightly thinner walls. The interpretation of this outcome is ambiguous, because it carries a risk of circular reasoning. The mean MyV, when used to calculate MyN, should appropriately be calculated from the product of mean MyA times mean MyL. The mean MyL was not accessible to measurement in this study, and an approximation of this quantity was estimated from LVD, giving $MyV = 3.654 \times MyA \times LVD \times 10^{-3}$ μm (Appendix). This calls for a judgement about how to account for the variations in LVD. In the formula $MyN = 0.75 \times LVM/(MyA \times 3.654 \times LVD \times 10^{-3}) \times 10^9$, the values for MyN calculated here were fewer when LVD increased with dilation. Whatever may account for the dilation, this dilation does, in part, govern the calculated values of MyN. An appropriate decision about the underlying causes of the dilation must be deferred until better data are in hand.

Table 1. Means of Selected Variables by Number of Left Ventricular Myocytes

	Number of Myocytes $\times 10^9$			ANOVA	
	<4	4–6	>6	F	*P* Value
MyB (μm)	21.1^{C}	19.1^{B}	17.4^{A}	10.1	<.001
MyA (μm^2)	360.3^{B}	291.7^{A}	245.4^{A}	10.1	<.001
MyV ($\mu m^3 \times 10^3$)	49.7^{B}	34.7^{A}	29.5^{A}	8.6	<.001
LVD (mm)	39.9^{B}	33.7^{A}	32.9^{A}	3.2	.04
LVT (mm)	13.6^{A}	14.7^{AB}	15.5^{B}	3.7	.03
LVM (G)	239.3^{A}	223.2^{A}	262.3^{A}	1.4	.33
Weight (kg)	85.4^{A}	95.3^{A}	99.8^{A}	1.4	.26
Height (m)	1.71^{A}	1.74^{A}	1.76^{A}	1.6	.21
MAP (mm Hg)	103.6^{AB}	104.2^{A}	102.3^{A}	0.2	.78
Age (y)	49.6^{A}	46.1^{A}	42.2^{A}	1.2	.30
Race (% black)	50.0^{A}	50.1^{A}	60.0^{A}	0.3	.76
Sex (% male)	75.0^{A}	74.6^{A}	83.3^{A}	0.4	.69
MyN ($\times 10^9$)	3.6^{A}	4.9^{B}	6.8^{C}	213.8	<.001
No of cases	24	55	20		

LV, left ventricular; LVD, LV chamber diameter; LVM, LV mass; LVT, free wall thickness; MAP, mean arterial pressure = (systolic + 2 × diastolic)/3; MyA, myocyte cross-sectional area; MyB, myocyte breadth; MyN, total LV myocyte number; MyV, myocyte volume.

^{A,B,C}Means within a row that fail to share a symbol A, B, or C differ significantly by the Tukey multiple range test.

Conclusion

The hypothesis under exploration here offers one possible explanation for the variations in LVD across MyN groupings: when MyN is small the ventricle seems vulnerable to systolic dysfunction and subsequent dilatation. Another possibility is the dilatation that often happens during a terminal course of progressive deterioration toward demise. In future studies, these and other possibilities could be sorted out by direct measurement of mean myocyte length, thereby avoiding the potential for circular reasoning. For now it seems fair to conclude that the patterns in the data do not contradict the proposed hypothesis and

that the hypothesis therefore emerges as meriting further exploration. These conclusions are implicit in the underlying observations of myocyte number constancy across such groupings as sex, height, weight, and LVMs, which provide the less speculative findings.

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Disclosures

None.

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Appendix

Most of the methods used in this report are already published[10] and are presented here in brief.

Subjects

From November 2007 to May 2009, the Orleans Parish Coroner's Office supplied 78 specimens, as detailed previously.[10] From January to September 2012, 21 more cases were added to the series, yielding a total of 99 cases. All cases with cardiomegaly (whole heart weight >399 g in women and >449 g in men), other than coronary heart disease (CHD), were included. CHD was recognized by finding scars or grossly visible evidence of ongoing necrosis, often with chamber wall thinning, in company with coronary artery narrowing, usually by calcific plaques, with or without thrombosis. Noncardiovascular cases without cardiomegaly were chosen to have <12 hours' postmortem interval, no immediately preceding hospitalization, and cause of death unrelated to cardiovascular disease, emphasizing tall women and short men. Later classification identified 21 subjects as hypertensive, 26 as overweight, and 10 as both.

Left Ventricular Chamber Diameter (LVD), Free Wall Thickness (LVT), and Mass (LVM)

Chamber diameter (LVD) and thicknesses of the posterior (P), lateral (L), and septal (S) walls, all in millimeters, were measured in the formalin-fixed heart slices with a caliper on the ventricular high chordal plane (touching the edges of the opened mitral leaflets). LVT is the mean of posterior and lateral wall thicknesses. The mass of the left ventricle in grams was calculated with the use of a slight modification of the American Society of Echocardiology formula[16]: $\mathrm{LVM} = 0.8 \times (1.04 \times [\mathrm{S} + \mathrm{LVD} + \mathrm{L}]^3 - \mathrm{LVD}^3) + 35$. This differs from the formula preferred by Devereux et al[16] by replacing their constant term 0.6 with the term 35. This change is apt to be a result of methodologic variations between the 2 studies (eg, the series used here placed the trabeculae carneae on the right side of the interventricular septum with the right ventricle rather than with the left). The term 35 is added to every specimen alike and can not affect the correlations of interest reported here. An equation relating LVM to weights of isolated ventricles derived from a subset of 66 cases had regression coefficient not significantly different from $b = 1$ and rejecting nonzero intercept ($r = 0.93$), showing the calculated value to agree precisely with the weight of the isolated ventricle with gratifying precision.

Myocyte Breadth (MyB) and Cross-Sectional Area (MyA)

In the idealized model of a myocyte as an elliptic cylinder, the dimensions of its cross-section being major axis A and minor axis B. The name ''width'' is often given to the quantity 2r in the imaginary circle of radius r with area = $\pi AB/4$. However, the name ''width'' is also often used for the major axis or for the minor axis, making the name ''width'' ambiguous. To avoid this ambiguity, the name ''breadth'' is given to the quantity 2r. For calculating the mean of MyB needed here, r is calculated from the minor axis as $r = 1.048 \times B$, as derived previously.[10] Mean MyA is πr^2. It was previously found in specimens of this study that sarcomere lengths averaged closely $\sim$1.65 μm in all specimens, indicating that the formalin-fixed postmortem state displays the myocytes in fully contracted systole thus removing this possible source of variation across specimens.[10]

Myocyte Length (MyL), Volume (MyV), and Number (MyN)

This study required only mean values for MyL and MyV in each specimen and had no need for individual measurements of each myocyte. In a study of maturing spontaneously hypertensive rats from birth to 24 months, many with left ventricle hypertrophy, Tamura et al[17] found a regression equation relating mean MyL, determined with enzymatically isolated cells in suspension, to chamber circumference. Adaptation of that formula to the human data proved to be surprisingly consistent with derived outcomes reported here: $MyL = (7 \times 0.84 \times 17.4 \times LVD/28.0) \times 10^{-3} = 3.654 \times LVD \times 10^{-3}$ μm.[10] From this, $MyV = \pi \times r^2 \times MyL = MyA \times MyL$ is then derived. Total number of myocytes (MyN) in a ventricle then follows as $MyN = 0.75 \times LVM/MyV$, ignoring the negligible effect of specific gravity. The term 0.75 is a commonly reported estimate[10] of myocytes as a proportion of total LVM (myocyte volume fraction), an estimate that should be measured for each specimen in future studies. The grouping of cases into ranges of MyN of <4, 4–6, and $>6 \times 10^9$ myocytes is arbitrary to generate ample numbers of cases within each interval.

Overweight

Body weight is the sum of lean plus fat masses with proxies of height and body mass index (BMI; kg/m^2), respectively. It was found elsewhere[10] that MyB had multiple correlation with height[2,7] and BMI that was indistinguishable from the correlation with total body weight, and with equal importance for lean and fat masses in the relationship. Therefore, within this data set body weight was better than BMI as a measure of overweight for the limited purposes of this report.

Hypertension

Intimal thickness of interlobular arteries in the renal cortex was measured, and mean arterial pressure (MAP) calculated with the use of a previously derived regression equation[10–13] ($MAP = [S + 2 \times D]/3$). For the equation, the correlation coefficient was $r = 0.702$, showing a relatively crude quantification of the desired blood pressure levels. These are of limited worth for specifying values for individuals but can be useful when comparing group averages.