Online Supplement

TITLE: Arterial load and ventricular-arterial coupling: physiologic relations with body size and effect of obesity

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Supplemental Methods

Anthropometric and biochemical measurements

Body size parameters (height, weight and waist circumference) were measured with participants wearing underwear and without shoes. Body surface area (BSA) was estimated with the Gehan method (BSA= 0.0235 x height in $cm^{0.42246}$ x weight in $kg^{0.51456}$).[1] Fasting serum glucose was assayed by a standard hexokinase enzymatic method. Diabetes mellitus was defined as fasting serum glucose ≥126 mg/dL or pharmacologic treatment for diabetes. Total serum cholesterol was assayed by the enzymatic colorimetric CHOD–PAP method of Allain *et al*.[2] Serum high-density lipoprotein (HDL) cholesterol was determined by the homogenous enzymatic method that uses dextran sulfate and polyethylene glycol-modified cholesterol esterase and cholesterol oxidase. For serum triglycerides the lipase kinetic colorimetric reaction without glycerol correction was used. Low-density lipoprotein (LDL)-cholesterol was calculated by the Friedewald method.[3]

Doppler echocardiography and arterial tonometry

An echocardiographic examination was performed using a Vivid-7 ultrasound platform (Vingmed Ultrasound; Horten, Norway). Pulsed wave Doppler measurements of flow velocities in the left ventricular outflow tract were performed and recorded as previously described[4, 5]. Arterial applanation tonometry was performed with a Millar pentype tonometer (SPT 301; Millar Instruments, Houston, Texas, USA) and a dedicated acquisition platform.[4, 5] Data was digitally recorded and processed using custom-designed software written in Matlab (The Mathworks, Natick, MA) as previously described.[4, 5] Briefly, arterial tonometry was first performed at the level of the left brachial artery, and the tonometric recording was calibrated with brachial systolic and diastolic blood pressure. Mean brachial arterial pressure was then computed by numerical integration of the brachial pressure wave form. Subsequently, carotid artery waveforms and left ventricular outflow tract (LVOT) pulsed wave Doppler flow velocities were simultaneously acquired. Carotid pressure wave forms were calibrated according to brachial mean and diastolic pressure. [4, 6]

Assessment of arterial load and ventricular-vascular coupling

Time-resolved numerical values of an ensemble averaged carotid pressure waveform and DICOM images containing flow velocities were used for pressure-flow analyses using custom-designed software written in Matlab (The Mathworks, Natick, MA) as previously described.[4] For each cardiac cycle, the onset and end of ejection were delineated on the DICOM images, after which the contours in the systolic phase were automatically traced using the transition in pixel intensity above a user-defined threshold value.[4] We computed the LVOT cross sectional area using the LVOT radius (r=diameter/2) measured in the parasternal long axis view (area=$\pi r2$). Instantaneous flow velocities were multiplied by LV outflow tract cross-sectional area to obtain volumetric flow. After appropriate time-alignment of pressure and flow wave forms , characteristic impedance (Zc) of the proximal aorta was calculated in the time domain as the ratio of early systolic pulsatile pressure/flow as previously described [4, 7]. Reflection magnitude was computed using wave separation analysis.[4, 6] Augmentation index was calculated as the amplitude of the second systolic peak divided by the amplitude of the first systolic peak (P2/P1) multiplied by 100. We chose this method as opposed to more widely used method proposed by Murgo (where augmentation index equals augmented pressure expressed as a proportion of pulse

pressure) because Murgo's method frequently results in negative values of augmentation index, which are incompatible with the general allometric equation.

Stroke volume was computed by multiplying the LVOT area by the LVOT pulsed-wave Doppler flow velocity-time integral. An alternative computation was performed using ventricular cavity size in end-systole and end-diastole, measured with the area-length method.[8] Allometric exponents obtained with the area-length method or using an averaged value are not shown but were very similar to those obtained with the pulsed wave Doppler method. Left ventricular ejection fraction was computed with the area-length method.[8] Central end-systolic pressure was defined as the pressure value at the dicrotic notch in the calibrated central pressure wave form. Total arterial compliance was calculated with the pulse-pressure method.[4, 9] Total arterial elastance (Ea) was calculated as the ratio of central end-systolic pressure to stroke volume. Ea is an integrated index of arterial load that is sensitive to resistive load, pulsatile load and heart rate.[10] The modified single-beat method was used to estimate end-systolic left ventricular elastance (Ees) from central end-systolic pressure, stroke volume, pre-ejection period and total systolic period measured from pulsed wave Doppler tracings of the left ventricular outflow tract, left ventricular ejection fraction and an estimated normalized ventricular elastance at arterial end diastole, as previously described and validated.[11-13] The ratio of Ea/Ees ratio was computed as an index of ventricular-arterial coupling.[14-16] Finally, because in many epidemiologic studies the ratio of pulse pressure to stroke volume is used as a crude estimate of total arterial compliance, we include data regarding this ratio as well. Systemic vascular resistance was estimated as the ratio of mean arterial pressure (derived from integration of the pressure wave form) and mean volumetric flow (measured by pulsed wave Doppler interrogation of the LVOT as described above).

Statistical Analysis

Continuous data are described as mean±standard deviation or median and interquartile range (IQR) as appropriate. Proportions are described as counts and percentages. To test for allometric relations between hemodynamic indices and body size in the reference sample, the following general allometric equation was used: $y = ax^b + \varepsilon$, where x is a measure of body size, a and b are parameters and ε is a random additive error term. Tested measures of body size in our study include height (in meters), weight (in kilograms) and BSA (in meters^2). Estimating parameters a and b while satisfying the assumptions of regression analysis can be achieved by using: (1) non-linear least squares estimation or (2) ordinary least squares estimation after logarithmic transformation to a linear model. In the latter approach, the allometric equation is linearized by taking natural logarithms of both sides of the allometric equation: $ln\ y = ln\ a + b\ ln\ x$. The exponent b is the slope of the log-log plot, and a is derived from the antilog of the y-intercept. Although this approach enables the interaction between predictors to be analyzed, the logarithmic equation above is not the exact logarithmic transformation of the original allometric equation due to the additive nature of the error term. Therefore, we used this approach only to detect significant interactions between gender and measures of body size (in order to assess for differences in the examined allometric relationships between men and women and establish the presence of a common exponent applicable to both genders).[17] In addition, at this initial stage of analysis, we examined the strength of the relationship between measures of body size and arterial load. Given our large sample size, even weak relationships can prove to be statistically significant, although not important for clinical and epidemiologic purposes. Therefore, we

further analyzed the metrics of only those relationships associated with an unadjusted R value of at least 0.25.

Definitive estimation of allometric powers was performed using non-linear regression. Allometric equations were generated by an iterative technique seeking to estimate the parameters (a and b) in a way that the sum over all the observations of the squared differences between the observed and predicted values of the dependent variable was minimized, producing the highest possible data fit. All non-linear models also included a gender term to satisfy the group difference principle.[17] Given the marked differences in body size between men and women, failure to account for gender may result in estimated allometric exponents that are influenced by both gender and body size. Of note, this may occur even in the presence of a common gender exponent (no significant gender differences in allometric exponents).[17]

After determining the allometric powers for appropriate indexing of hemodynamic variables, comparisons of indexed variables between subjects in pre-specified body mass index (BMI) categories (<25, 25-29.9 and ≥30 kg/m^2) were done with analysis of covariance, adjusting for age and gender differences between the groups. Similar comparisons were made between subjects with and without abdominal obesity, defined as a waist circumference >102 cm in men and >88 cm in women.[18] All probability values are 2-tailed. Statistical significance was defined as $\alpha<0.05$. Statistical analyses were performed using SPSS for Windows v17 (SPSS Inc., Chicago, IL).

Rationale for our statistical approach

In this study, we selected an anthropometric and metabolically normal population in order to assess the normal (physiologic) relationships between arterial load and body size. This normal sample spanned a wide range of body height, but (by design) was restricted to subjects with normal body weight. We believe this approach is advantageous for assessing physiologic relationships compared to an approach that uses a sample that spans all ranges of body weight and therefore includes subjects who have a pathologic state (obesity) which is well known to be associated with arterial disease independently of body size *per se*. Developing indexation systems that aim to effectively normalize for body size on the basis of relationships observed in a population that includes obese individuals would impair our ability to assess the effects of obesity itself (and the neurohormonal abnormalities that accompany the obese state and exert deleterious effects on the arterial tree).

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