Pathways Leading to Atherosclerosis
A Structural Equation Modeling Approach in Young Adults

Gerthe F. Kerkhof, Hugo J. Duivenvoorden, Ralph W.J. Leunissen, Anita C.S. Hokken-Koelega

Abstract—Several risk factors of cardiovascular diseases have been studied using direct association measures. Because the incidence of obesity and cardiovascular diseases is rising, it is important to correctly model these risk factors involved in development of cardiovascular diseases. Until now, statistical methods lacked to achieve this goal because of complex interrelationships involved. Structural Equation Modeling (SEM) is an advanced statistical technique that enables solving this issue. The aims of this study were to investigate whether SEM could unravel pathways involved in cardiovascular diseases and to visualize these pathways in a model. In 322 healthy participants of the PROGRAM (PROgramming factors for GRowth And Metabolism) study, 18 to 24 years of age, we explored pathways leading to atherosclerosis measured by carotid intima-media thickness. Using SEM, we were able to model these pathways for males and females using body fat percentage, serum lipid levels, and blood pressure. We are the first to present a model of complex direct and indirect effects of fat mass leading to atherosclerosis using SEM. Both male and female path-model had an excellent fit. Fat mass had a significant effect on carotid intima-media thickness through various pathways, with the largest effect size on carotid intima-media thickness via blood pressure. SEM showed that the pathways differed between males and females, with a larger effect of serum lipids on carotid intima-media thickness in males. In conclusion, SEM is suitable in identifying models to unravel potential causal pathways in complex origins of diseases. We present a model involving several pathways, showing that fat mass has an influence on risk factors for atherosclerosis, already at 21 years of age. (Hypertension. 2011;57:255-260.) • Online Data Supplement

Key Words: atherosclerosis ■ blood pressure ■ lipids ■ obesity ■ young adults

The World Health Organization estimates a rise in mortality of cardiovascular diseases (CVD) from 17.1 million in 2004 to 23.4 million in 2030.1 These statistics explain the emerging interest of clinical researchers to determine risk factors for CVD. Atherosclerosis is an important etiologic element of CVD, and although causes of atherosclerosis have been explored previously, it remained difficult to investigate several atherosclerosis risk factors simultaneously. Cohort studies have been used to determine associations between risk factors of atherosclerosis.2,3 However, these studies did not take into account indirect effects of risk factors because only direct effects between 2 variables were analyzed. Thus, such studies lacked to provide a statistical method that could unravel the pathways simultaneously in one path analysis.

Structural Equation Modeling (SEM) is an advanced statistical technique that enables solving these issues. SEM has been applied in several research fields but is still rarely used in clinical research, despite its ability to identify, test, and estimate pathways in a non–hypothesis-driven manner.4

We hypothesized that SEM is a suitable method to unravel multidirectional associations and potential causal pathways in complex origins of diseases such as CVD. This approach, in which the interdependency of risk factors is unraveled simultaneously, is innovative in this field. Our objective was to explore several pathways, leading to vascular changes in early adulthood, using SEM. We examined direct and indirect effects of fat mass in particular because fat mass accumulation during childhood is an important risk factor for CVD in adulthood.5–7 Prevalence of obesity in children and young adults is rising, and this is likely to induce future problems in public health.8,9 We aimed to study pathways through several determinants of atherosclerosis, including lipid levels and blood pressure. As far as we know, this is the first study using SEM to explore the pathways between fat mass and vascular changes in early adulthood.

Our study population consisted of 322 healthy subjects 18 to 24 years of age who participated in the PROgramming factors for GRowth And Metabolism (PROGRAM) study cohort. Several parameters were measured to determine metabolic and cardiovascular status of the participants.

Methods

Study Participants
The PROGRAM study cohort comprises 322 healthy subjects 18 to 24 years of age. The PROGRAM study was performed in one medical center in The Netherlands between August 2004 and September 2007. Participants were recruited randomly from several...
hospitals in The Netherlands, where they had been registered because of small size at birth or short stature. Also randomly, healthy subjects from schools of various educational levels were asked to participate. Only those born singleton, at $\geq 36$ weeks of gestation and white, were invited to participate. The study population has been described previously in detail.

The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, The Netherlands, approved the study. Signed informed consent was obtained from all participants.

**Measurements**

All participants were invited to visit Erasmus Medical Centre and were reimbursed for travel expenses. Before the taking of measurements, participants fasted for 12 hours and had abstained from smoking and alcohol for 16 hours. Fasting blood samples were drawn and centrifuged between 8 AM and 1 PM and were kept frozen until assayed ($\sim 8^\circ$C).

Fat mass was measured on a dual-energy X-ray absorptiometry machine (Lunar Prodigy; GE Healthcare). The intra-assay coefficient of variation for fat tissue was 0.41% to 0.88%. Brachial blood pressure was measured after 10 minutes at rest, in the supine position, using the nondominant arm with an automatic device (Accutorr Plus; Datascope Corp.) every 5 minutes for 1 hour, and the mean values of these 13 measurements were taken to reflect resting blood pressure. A standard cuff size was used unless a large cuff was necessary.

Carotid intima-media thickness (cIMT) was measured by recording ultrasonographic images of both left and right carotid artery, when subjects were supine, using a 7.5-MHz linear array transducer (ATL Ultramark IV; Advanced Tech Laboratories). On the R wave of the ECG, 3 longitudinal images of the near and far wall of the common carotid artery were frozen and stored on videotape. These images were digitized and displayed on the screen of a computer using a frame grabber (VP 1400-KIT-512-E-AT; Imaging Technology). The common cIMT was determined as the mean of the mean near-wall and far-wall measurements of both the left and right side common carotid artery.

**Laboratory Measurements**

Lipid concentrations were analyzed in the same laboratory. Free fatty acids (FFA) and triglycerides (TG) were measured using an enzymatic colorimetric method (WAKO Chemicals), an automated enzymatic method, with the GPO-PAP reagent kit (Roche Diagnostics). HDL cholesterol was measured using a homogenous enzymatic colorimetric assay (Roche Diagnostics). LDL cholesterol was calculated using the Friedewald formula: LDL cholesterol (mmol/L) = total cholesterol - HDL cholesterol - 0.45$\times$TG. Apolipoprotein A-I (apoA-1) and apolipoprotein B (apoB) were determined by rate nephelometry on the Image Immunochemistry System according to manufacturer instructions (Beckman Coulter). Plasma acylation stimulating protein (ASP) concentrations were measured using a sandwich ELISA. The intra-assay variations of measurements of TG, HDL cholesterol, and ASP were 2.9, 3.9%, and $<4\%$, respectively. Between-run coefficients of variation for apoA-1 and apoB were 4.2% and 2.8% at levels of 0.94 and 0.53 g/L, respectively.

**Statistical Analysis**

Body fat percentage was calculated as $[\text{body fat (kg)/weight (kg)}]\times100\%$. Differences between males and females and between oral contraceptive (OC) users and non-OC users were determined using ANOVA. The difference between males and females regarding the percentage of smokers was determined using a Pearson $\chi^2$ test. We used the Pearson correlations to estimate intercorrelations, and the Fisher $Z$-transformation was used to explore differences between male and female correlation coefficients.

To unravel the interrelationships among atherosclerosis risk factors, we used SEM.13 A powerful statistical tool for path analysis using maximum likelihood estimation. SEM has been used in psychological, social, educational, and management fields14 and is applicable in clinical research, specifically to visualize pathways and calculate the magnitudes of direct and indirect effects on human diseases. Using SEM, we explored several path models to identify, test, and estimate models.

Although there are no absolute standards for the relationship between sample size and model complexity, a desirable goal is to have a minimal subject/parameter ratio of 10:1.13 The models generated in this study consist of 8 parameters. Because the female model was based on data from 197 subjects and the male model on those from 125 subjects, both models had a subject/parameter ratio clearly larger than 10:1, indicating a sufficient sample size.

Because of statistical collinearity of the variables HDL and apoA1 (males, $r=0.83$; females, $r=0.70$), LDL and apoB (males, $r=0.92$; females, $r=0.87$), and diastolic and systolic blood pressure (males, $r=0.78$; females, $r=0.78$), each of these pairs of variables was combined as one variable using Z scores for standardization. These variables are strongly related because apoA1 and apoB are structural proteins for HDL and LDL, respectively. The variable with the most unfavorable Z score of the 2 was used in analysis. The combined variables were called HDL&apoA1, LDL&apoB, and blood pressure.

Using the model-generating approach in SEM, we first explored relationships between exogenous (independent) and endogenous (dependent) variables in a model starting with fat mass percentage and ending with cIMT. Secondly, for each nonsignificant path, we determined whether it was acceptable to remove the path while maintaining an acceptable fit. Models were tested until no meaningful improvements were found on models that had been tested previously. All models were constructed for males and females, separately. Regression-based imputation was used for missing data using full information matrix. The number of missing data for blood pressure was 103, and for cIMT, 79; for all other parameters, the number of missing data were $<20$. The generated SEM model was also tested using a complete case analysis without imputed data. Bootstrapping was applied for internal validation. The 95% confidence intervals after bootstrapping are shown in Table I, available in an online supplement at http://hyper.ahajournals.org. Because not all variables were characterized by a normal distribution, robust maximum likelihood was used to test the generated model. This showed similar results.

We used standardized path coefficients as effect estimate (range, −1.0 to 1.0). The effect size of these coefficients can be determined using this classification: $<0.10$ as a small effect, $0.30$ as a medium effect, and $>0.50$ as a large effect. These values are recommendations. Effect sizes can be reasonably estimated in combination with tests of significance, which also take account of sample size and intercorrelations among variables.18 Supplemental Figure II provides more information regarding path diagrams.

**Model Fit**

For each model, we evaluated the fit by measures of overall fit and detailed assessment of fit (fitted and standardized residuals and modification indices) and by examining the individual parameter estimates. The following performance measures were used: (1) $x^2$ for model fit (low and nonsignificant values of the $x^2$ are desired); (2) $x^2/\text{degrees of freedom ratio}$ (a value $<2.0$ was considered acceptable); (3) Comparative Fit Index; (4) Tucker–Lewis Index (Comparative Fit Index and Tucker–Lewis Index, where values of 1.0 suggest a perfect fit, and high values are desired, but where values $>1.0$ indicate an overidentification); (5) root mean square error of approximation (a value $<0.05$ indicates a close fit); and (6) standardized root mean squares of residuals (where a value of $<0.08$ indicates a good fit).18

Statistical package SPSS version 15.0 (SPSS, Inc.) was used for the Pearson $r$, test and ANOVA. M-plus version 5.2.1 (Muthén and Muthén) was used for SEM. Results were regarded as statistically significant if 2-sided $p$ was $<0.05$.

**Results**

**Study Population**

Table I shows unadjusted clinical characteristics of the 322 participants and males and females separately. There were no
Male and Female Model by SEM

The implementation of SEM as statistical approach to identify pathways from fat mass leading to changes in cIMT resulted in a model for males and females with an adequate model fit (Figures 1 and 2): χ² was 41.1 with 34 degrees of freedom (ratio 1.2) and a P value of 0.188. Low, nonsignificant values of the χ² are desired, and the ratio has to be <2.0. The Comparative Fit Index was 0.96 and the Tucker–Lewis Index was 0.93. Both Comparative Fit Index and Tucker–Lewis Index need to be high for a good fit, but values >1.0 indicate an overfit. The root mean square error of approximation and standardized root mean squares of residuals had values of 0.036 and 0.050, respectively. Root mean square error of approximation and standardized root mean squares of residuals need to be <0.05 and <0.08, respectively, for a good fit. Also after using bootstrapping for internal validation, the model fit remained good.

The complete case analysis resulted in a similar model with a good fit. The directionality and magnitude of the path coefficients also resembled those of the original model.

SEM analyses resulted in a good model for both males (Figure 1) and females (Figure 2). The largest effect in both males and females was that of fat mass on cIMT, via blood pressure. In contrast, the pathways regarding the serum lipid levels differed between males and females. The effect of LDL&apoB on cIMT was present in the male model but absent in the female model, whereas the effect of FFA on TG in the female model was absent in the male model. Further, the effect sizes of the pathways via serum lipids were higher in males than in females.

Table 3 shows the direct, indirect, and total effects of fat mass on endogenous variables used in path analyses. Both models show a relatively large effect of fat mass on blood pressure. For males, the total effect of fat mass on blood pressure was 0.34 (P<0.001; range, −1.0 to 1.0). For females, this effect was 0.35 (P<0.001). The total effect of

Table 1. Clinical Characteristics of Males and Females

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male (n=125)</th>
<th>Female (n=197)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>20.9 (1.66)</td>
<td>20.9 (1.69)</td>
<td>0.756</td>
</tr>
<tr>
<td>%FM</td>
<td>16.3 (7.91)</td>
<td>29.5 (8.55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.91 (0.07)</td>
<td>0.87 (0.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>27.0</td>
<td>27.5</td>
<td>0.863</td>
</tr>
<tr>
<td>OC use (%)</td>
<td>—</td>
<td>76.7</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>114.1 (7.96)</td>
<td>107.6 (7.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>66.6 (5.39)</td>
<td>65.8 (5.26)</td>
<td>0.231</td>
</tr>
<tr>
<td>cIMT (mm)</td>
<td>0.53 (0.05)</td>
<td>0.51 (0.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.55 (0.24)</td>
<td>0.66 (0.23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.90 (0.44)</td>
<td>1.11 (0.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASP (nmol/L)</td>
<td>14.3 (7.17)</td>
<td>19.6 (11.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.49 (0.66)</td>
<td>2.83 (0.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.24 (0.29)</td>
<td>1.47 (0.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>0.73 (0.18)</td>
<td>0.88 (0.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoA1 (g/L)</td>
<td>1.19 (0.17)</td>
<td>1.38 (0.22)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values given are mean (SD).
%FM indicates percentage of body fat; BP, blood pressure.

Table 2. Linear Correlation Coefficients Between Atherosclerosis Parameters Used in SEM, for Males and Females Separately

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%FM</th>
<th>FFA</th>
<th>TG</th>
<th>ASP</th>
<th>HDL&amp;apoA1</th>
<th>LDL&amp;apoB</th>
<th>BP</th>
<th>cIMT</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>%FM</td>
<td>-</td>
<td>0.00</td>
<td>0.02</td>
<td>0.16‡</td>
<td>0.06</td>
<td>0.13</td>
<td>0.35*</td>
<td>-0.06</td>
<td>29.5</td>
<td>8.55</td>
</tr>
<tr>
<td>FFA</td>
<td>0.30†</td>
<td>-</td>
<td>0.09</td>
<td>0.02</td>
<td>-0.02</td>
<td>0.10</td>
<td>-0.04</td>
<td>0.09</td>
<td>0.66</td>
<td>0.23</td>
</tr>
<tr>
<td>ASP</td>
<td>0.49†</td>
<td>0.04</td>
<td>-</td>
<td>0.19†</td>
<td>0.06</td>
<td>0.29*</td>
<td>0.14</td>
<td>-0.05</td>
<td>1.11</td>
<td>0.52</td>
</tr>
<tr>
<td>HDL&amp;apoA1</td>
<td>0.01</td>
<td>-0.08</td>
<td>0.21‡</td>
<td>-</td>
<td>0.02</td>
<td>0.10</td>
<td>0.15‡</td>
<td>0.00</td>
<td>19.6</td>
<td>11.1</td>
</tr>
<tr>
<td>LDL&amp;apoB</td>
<td>0.24‡</td>
<td>0.01</td>
<td>0.31†</td>
<td>0.18‡</td>
<td>-0.01</td>
<td>-</td>
<td>0.12</td>
<td>0.03</td>
<td>-0.02</td>
<td>1.00</td>
</tr>
<tr>
<td>BP</td>
<td>0.34*</td>
<td>0.10</td>
<td>0.26‡</td>
<td>-0.07</td>
<td>-0.07</td>
<td>0.18‡</td>
<td>-</td>
<td>0.17‡</td>
<td>0.15</td>
<td>0.83</td>
</tr>
<tr>
<td>cIMT</td>
<td>0.07</td>
<td>0.10</td>
<td>0.14</td>
<td>0.03</td>
<td>-0.30‡</td>
<td>0.24†</td>
<td>0.22‡</td>
<td>-</td>
<td>0.51</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Mean                  | 16.3      | 0.55      | 0.90      | 14.3      | -0.53     | -0.51    | 0.82      | 0.53      |        |      |
SD                     | 1.66      | 0.24      | 0.44      | 7.17      | 0.78      | 0.77     | 0.93      | 0.05      |        |      |

Dark grey: males, light grey: females
Significant Pearson correlation coefficients are given in bold.
p-value of the correlation coefficients: *: p<0.001, †: p<0.01, ‡: p<0.05
Significant difference between correlations of males and females: §: p<0.001, ||: p<0.01, ¶: p<0.05
%FM= percentage body-fat, FFA= free fatty acids, TG= triglycerides, ASP= acyl stimulation protein, HDL= high-density lipoprotein cholesterol, apoA1= apolipoprotein A-I, LDL= low-density lipoprotein cholesterol, apoB= apolipoprotein B, BP = blood-pressure, cIMT= carotid intima-media thickness
fat mass on cIMT was significant for both males (P=0.002) and females (P=0.013).

Of the females, 76.7% used OCs. The parameters (means) that differed between OC users and non-OC users were, respectively, TG (1.21 versus 0.81; P<0.001), FFA (0.70 versus 0.54; P<0.001), LDL (2.92 versus 2.58; P=0.024), apoB (0.92 versus 0.76; P<0.001), and systolic blood pressure (108.2 versus 105.0; P=0.008). The body fat percentage did not differ between OC users and non-OC users (29.7 versus 28.4; P=0.39). We tested the same female model in OC users and non-OC users separately to test whether the model was applicable to both groups. Despite the small number of participants without OC use (n=44), the female models both showed a good fit, thus, we decided to combine the data for OC users and non-OC users in the final model.

**Discussion**

This is the first study to show that SEM is an innovative statistical method to unravel multidirectional associations and potential causal pathways in complex origins of diseases like CVD. SEM can analyze complex interrelationships among variables in a non–hypothesis-driven manner. By using SEM, we could visualize the direct and indirect effects of fat mass on cIMT via various pathways such as blood pressure and serum lipids.

SEM has been used in other fields, such as genetic epidemiology and psychology, but remains very rarely used in medical research. Path analysis is an appropriate method to assess the causal contribution of one variable to another. It assumes that causality is not a 1-to-1 correspondence between cause and effect but that each dependent variable has an unexplained variance. However, to determine causal relationships with certainty, there has to be a time course. The promising results of the present study, applying path analysis in clinical research, might motivate the use of SEM in complex origins of disease to assess causal relationships.

Many of the estimated effects in the present study were substantial and statistically significant, which is remarkable, especially when taking into account the young age of the healthy study population. The effect sizes on cIMT remained low, but we can conclude that even at such a young age, a higher fat mass already has a negative influence on the cardiovascular status. This finding is alarming because the effects are likely to be larger in subjects of an older age.

As was expected, the pathways differed between males and females. The relatively large effect of fat mass on ASP in

![Figure 1. Path diagram for males with standardized coefficients of direct effects.](http://hyper.ahajournals.org/)

**Figure 1.** Path diagram for males with standardized coefficients of direct effects. Bold (red) arrows indicate P<0.05, and dashed arrows indicate P>0.10. Standardized path coefficients (presented next to all arrows) are used as effect estimate (range, −1.0 to 1.0): <0.10 is considered a small effect, 0.30 as a medium effect, and >0.50 as a large effect.

![Figure 2. Path diagram for females with standardized coefficients of direct effects.](http://hyper.ahajournals.org/)

**Figure 2.** Path diagram for females with standardized coefficients of direct effects. Bold (red) arrows indicate P<0.05, red arrows indicate P<0.10, and dashed arrows indicate P>0.10. Standardized path coefficients (presented next to all arrows) are used as effect estimate (range, −1.0 to 1.0): <0.10 is considered a small effect, 0.30 as a medium effect, and >0.50 as a large effect.
females might be attributable to their higher percentage of fat mass compared with males. Fat mass had an indirect effect on TG via ASP in the female model, in contrast to the direct effect shown in the male model. It was shown previously that females have higher lipolytic rates than males, independently of the percentage of fat mass. This induces higher levels of fatty acids, and consequently, the effect of FFA on TG might mask the relatively small direct effect of fat mass on fat mass on TG in the female model. The sexual dimorphism in effects of serum lipid levels on cIMT is likely to be affected by sex hormones as well. It is well established that premenopausal females have a lower risk of developing atherosclerosis than age-matched males. Because estrogens have hypolipidemic properties, these are also likely to attribute to the differences between the male and female models.

In contrast to the female model, the male model showed a direct effect of LDL&apoB on cIMT, a measure of atherosclerosis. An explanation of this finding might be that LDL particles in males are smaller than those in females, which was shown previously to be predictive of increased CVD risk.

Both models show a significant total effect of fat mass on cIMT. The indirect effects are, for a considerable part, ascribed to the effect of fat mass on blood pressure, both in males and females. This indicates that the effect of fat mass on blood pressure and cIMT is not exerted only via serum lipids. Other pathophysiological processes might also play a role, such as inflammatory effects. The present results warrant further investigations using SEM to expand the models, including more variables.

When interpreting the female model for clinical practice, it should be taken into account that a large percentage of the females used OC. Because the lipid profile of OC users differs from that of non-OC users, we tested the model fit of the female model in OC users and non-OC users separately. Despite the small number of participants without OC use, the same female model did show a good fit in both OC users and non-OC users. Thus, it is very likely that the pathways from fat mass to cIMT are similar for OC users and non-OC users.

A desirable goal of SEM is to have a minimal subject/parameter ratio of 10:1 to achieve sufficient power. Although the present study meets this requirement, this rule remains arbitrary. The present study used a model-generating rather than a confirmatory approach. The strength of this approach is that it is non–hypothesis driven, although the measured variables have to be preselected. However, one weakness is that this is inevitably accompanied by multiple testing. In addition, this approach is exploratory, and for definitive conclusions, external validation of the models in another large group of young adults is desirable, using SEM in a confirmatory approach taking into account multiple testing.

In conclusion, this is the first study using SEM to present a model of complex direct and indirect effects of fat mass leading to changes in cIMT. This study resulted in a path model with a good fit. It showed that vascular status, measured by cIMT, is influenced by fat mass via blood pressure and serum lipids, even in young healthy adults. Further, the model showed that the lipid profile has a larger effect in the development of atherosclerosis in males than in premenopausal females.

### Perspectives

We introduce an accessible method for analyzing complex origins of diseases. The promising results of the present study, applying path analysis in clinical research, might motivate the use of SEM to assess causal relationships in future research. In a public health perspective, our data indicate that higher fat mass in young adulthood should be prevented because it is associated with vascular changes through various pathways, even at such a young age. Because the prevalence of fat accumulation in childhood and adulthood is increasing rapidly, this is likely to induce future problems in public health.

### Acknowledgments

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### Table 3. Direct, Indirect, and Total Effects of Fat Mass on Endogenous Variables in Males and Females

<table>
<thead>
<tr>
<th>Endogenous Variables</th>
<th>Coeff</th>
<th>P Value</th>
<th>Coeff</th>
<th>P Value</th>
<th>Coef</th>
<th>P Value</th>
<th>Coeff</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>0.006</td>
<td>n.s.</td>
<td>0.006</td>
<td>n.s.</td>
<td>0.163</td>
<td>0.019</td>
<td>0.163</td>
<td>0.019</td>
</tr>
<tr>
<td>FFA</td>
<td>0.295</td>
<td>0.008</td>
<td>0.295</td>
<td>0.008</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TG</td>
<td>0.485</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>n.s.</td>
<td>0.486</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LDL&amp;apoB</td>
<td>0.118</td>
<td>n.s.</td>
<td>0.125</td>
<td>0.012</td>
<td>0.242</td>
<td>0.004</td>
<td>0.125</td>
<td>0.067</td>
</tr>
<tr>
<td>HDL&amp;apoA1</td>
<td>−0.131</td>
<td>n.s.</td>
<td>−0.113</td>
<td>0.022</td>
<td>−0.244</td>
<td>0.004</td>
<td>−0.086</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BP</td>
<td>0.277</td>
<td>0.003</td>
<td>0.064</td>
<td>n.s.</td>
<td>0.341</td>
<td>&lt;0.001</td>
<td>0.337</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cIMT</td>
<td>—</td>
<td>—</td>
<td>0.127</td>
<td>0.002</td>
<td>0.127</td>
<td>0.002</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The direct, indirect, and total effects of fat mass on the endogenous variables included in the models. We used standardized path coefficients as effect estimate (range, −1.0 to 1.0); <0.10 is considered a small effect, 0.30 a medium effect, and >0.50 a large effect.

Coeff indicates path coefficient; BP, blood pressure.

Significant P values (P<0.05) are given in bold; n.s., not shown (P>0.10).
the dual-energy x-ray absorptiometry results. None of the persons mentioned here received any compensation.

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**Disclosures**

All authors read and approved submission of the article. The article has not been published and is not being considered for publication previously, in whole or in part, in any language, except as an abstract. A.C.S.H.-K. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**References**

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PATHWAYS LEADING TO ATHEROSCLEROSIS – A STRUCTURAL EQUATION MODELING APPROACH IN YOUNG ADULTS

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Table S1. Standardized path coefficients of the males and females, respectively

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Males Coeff</th>
<th>95% CI</th>
<th>Bootstrapped 95% CI</th>
<th>Females Coeff</th>
<th>95% CI</th>
<th>Bootstrapped 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>%FM → ASP</td>
<td>0.006</td>
<td>-0.169 to 0.181</td>
<td>-0.172 to 0.184</td>
<td>0.163</td>
<td>0.027 to 0.299</td>
<td>0.038 to 0.288</td>
</tr>
<tr>
<td>%FM → FFA</td>
<td>0.295</td>
<td>0.135 to 0.455</td>
<td>0.115 to 0.475</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>%FM → TG</td>
<td>0.485</td>
<td>0.353 to 0.616</td>
<td>0.295 to 0.672</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>%FM → LDL&amp;apoB</td>
<td>0.118</td>
<td>-0.071 to 0.306</td>
<td>-0.105 to 0.341</td>
<td>0.125</td>
<td>-0.007 to 0.257</td>
<td>-0.004 to 0.254</td>
</tr>
<tr>
<td>%FM → HDL&amp;apoA</td>
<td>-0.131</td>
<td>-0.321 to 0.058</td>
<td>-0.320 to 0.058</td>
<td>-0.086</td>
<td>-0.098 to -0.074</td>
<td>-</td>
</tr>
<tr>
<td>%FM → BP</td>
<td>0.277</td>
<td>0.095 to 0.459</td>
<td>0.094 to 0.460</td>
<td>0.337</td>
<td>0.214 to 0.460</td>
<td>0.203 to 0.471</td>
</tr>
<tr>
<td>ASP → TG</td>
<td>0.206</td>
<td>0.059 to 0.353</td>
<td>0.071 to 0.341</td>
<td>0.188</td>
<td>0.055 to 0.322</td>
<td>0.048 to 0.328</td>
</tr>
<tr>
<td>FFA → TG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.121</td>
<td>-0.014 to 0.256</td>
<td>-0.012 to 0.254</td>
</tr>
<tr>
<td>TG → LDL&amp;apoB</td>
<td>0.257</td>
<td>0.073 to 0.442</td>
<td>-0.006 to 0.520</td>
<td>0.276</td>
<td>0.148 to 0.403</td>
<td>0.135 to 0.417</td>
</tr>
<tr>
<td>TG → HDL&amp;apoA</td>
<td>-0.233</td>
<td>-0.419 to -0.046</td>
<td>-0.428 to -0.038</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TG → BP</td>
<td>0.110</td>
<td>-0.078 to 0.297</td>
<td>-0.110 to 0.330</td>
<td>0.113</td>
<td>-0.019 to 0.246</td>
<td>-0.012 to 0.238</td>
</tr>
<tr>
<td>LDL&amp;apoB → BP</td>
<td>0.043</td>
<td>-0.040 to 0.126</td>
<td>-0.053 to 0.139</td>
<td>0.062</td>
<td>-0.058 to 0.181</td>
<td>-0.076 to 0.200</td>
</tr>
<tr>
<td>LDL&amp;apoB → IMT</td>
<td>0.205</td>
<td>0.039 to 0.372</td>
<td>0.033 to 0.377</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HDL&amp;apoA → IMT</td>
<td>-0.061</td>
<td>-0.148 to 0.027</td>
<td>-0.161 to 0.039</td>
<td>-0.084</td>
<td>-0.202 to 0.035</td>
<td>-0.222 to 0.054</td>
</tr>
<tr>
<td>BP → IMT</td>
<td>0.182</td>
<td>0.015 to 0.349</td>
<td>0.005 to 0.359</td>
<td>0.177</td>
<td>0.041 to 0.312</td>
<td>0.037 to 0.317</td>
</tr>
</tbody>
</table>

Coeff= Standardized path coefficients, 95% CI= 95% confidence interval, %FM= percentage body-fat, FFA= free fatty acids, TG= triglycerides, ASP= acyl stimulation protein, HDL= high-density lipoprotein cholesterol, apoA1= apolipoprotein A-I, LDL= low-density lipoprotein cholesterol, apoB= apolipoprotein B, BP= blood-pressure, cIMT= carotid intima-media thickness

* %FM → ASP stands for: effect of fat mass percentage on ASP.
† Because the effect of fat mass percentage on HDL&apoA was fixed at -0.10, it was not possible to calculate the 95% confidence interval for this effect after bootstrapping.
Figure S1 Path Analysis

Path analysis is used to study interrelationships between variables. The path ways that are determined using path analysis, can be displayed using a path diagram.

Path Diagrams
The following diagram is an example of a path analysis.

In this diagram;
- Latent, unmeasured, or unobserved variables are denoted in path analysis by a circle. Manifest, measured or observed variables enclosed in squares.
- Variables 1 and 2 are exogenous variables. Exogenous variables are variables whose causes are not represented in the model. These variables are causally prior to all dependent variables in the model. Any variable without a single-headed arrow going into it is termed an exogenous variable. One exogenous variable can be joined to another by a double headed arrow; this denotes a correlation between the two exogenous variables.
- Variables 3 and 4 are endogenous variables. The causes of endogenous variables are specified in the model.
- Exogenous variables must always be independent variables. Endogenous variables can be either dependent or independent.
- U3 and U4 are disturbances/residual terms.
- The arrows represent direct causal effects of the model, also known as the structural effects.
- Path coefficients are represented by p12, p23, p24, and p34 in the model

References:
http://ibgwww.colorado.edu/~carey/p4102dir/handouts/path_analysis/pathnew.htm