

# **The Effects of Weight Gain and Atorvastatin Treatment on Arterial Stiffness**

Jeb S. Orr

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Kevin P. Davy, Ph.D., Chair  
Brenda M. Davy, Ph.D., R.D.  
Matthew W. Hulver, Ph.D.  
Jose M. Rivero, M.D.  
Madlyn I. Frisard, Ph.D.

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## **ABSTRACT**

Aging is characterized by a progressive stiffening of large elastic arteries in the cardiothoracic region <sup>1,2</sup>. Importantly, large artery stiffness is an independent predictor of cardiovascular events and mortality in both healthy and diseased populations <sup>3</sup>. The results of several studies suggest that obesity <sup>4</sup>, particularly visceral adiposity <sup>5,6</sup>, is associated with the accelerated stiffening of central elastic arteries in middle-aged and older adults. Despite the widely recognized association between obesity, aging and arterial stiffness, there remains a paucity of information regarding both the initiation of arterial stiffening and effective treatment strategies. To address these issues, we tested the hypotheses that weight gain increases arterial stiffness in nonobese young males, and atorvastatin treatment reduces large artery stiffness in overweight and obese middle-aged and older adults. Consistent with our first hypothesis, weight gain increased arterial stiffness in nonobese young men. In addition, we demonstrated that, independent of total body fat, those individuals with relatively larger increases in abdominal visceral fat also experienced correspondingly larger increases in arterial stiffness. Regarding our second hypothesis, atorvastatin treatment decreased arterial stiffness in overweight and obese middle-aged and older adults. Importantly, the reduction in arterial stiffness with atorvastatin appeared to be independent of the reduction in C-reactive protein. The findings of the present studies could potentially

lead to the identification of effective strategies for the prevention and treatment of arterial stiffening in the population.

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# CHAPTER 1

## Introduction

**Arterial Stiffness.** The results of various observational studies have implicated arterial stiffness as an independent predictor of stroke, coronary events, coronary heart disease, and cardiovascular and all-cause mortality in elderly, hypertensive, diabetic, and end-stage renal disease subjects <sup>1</sup>. In addition, arterial stiffness has been shown to predict coronary heart disease and cardiovascular events and mortality in younger, healthy populations <sup>2,3</sup>. In order to better understand the pathophysiological link between arterial stiffness and cerebro- and cardiovascular disease (CVD), a brief discussion of normal arterial tree structure and function, and how perturbations in either, compromise cardiac and end-organ function is warranted.

Proper function of the arterial tree relies on the integration of its two main functions, providing a conduit for blood from the left ventricle (LV) to the capillary beds of organs according to their metabolic requirements, and the buffering of pulsatile intermittent ventricular ejections <sup>4</sup>. In this context, it is important to point out that the major conduit arteries differ with respect to their structure and function. Large elastic arteries of the cardiothoracic region (e.g., aorta, brachiocephalic, and carotid) serve as the primary buffers to pulsatile blood flow, whereas peripheral muscular arteries mainly function as conduit vessels <sup>4</sup>. This distinction is best illustrated by the fact that the aorta, brachiocephalic and carotid arteries dilate 10-15% with each pulsation, whereas the femoral and brachial arteries dilate approximately 5%, and the radial artery approximately 2% <sup>5</sup>. It is because of these differing roles that stiffening of central elastic

arteries has a much greater impact on cerebro- and cardiovascular disease risk. Arterial stiffening in the cardiothoracic region reduces buffering capacity of the arterial tree, thus, impairing functional integration which has wide ranging implications for central systolic and pulse pressure, aortic impedance and LV load, coronary perfusion, and the microcirculation <sup>4</sup>.

Under circumstances of optimal functional integration (i.e., young, healthy arteries), reflections of the forward incident wave, originating primarily at bifurcations and high-resistance arterioles, arrives back at the ascending aorta during late systole or early diastole and serves to augment pressure during coronary perfusion <sup>4</sup>. Arterial stiffness may truly be viewed as a marker of vascular age, as the cumulative effects of distending stress cycles fracture the load-bearing elastic lamellae of the media, and produce a progressive dilation and stiffening of central elastic arteries with age <sup>6</sup>. As a result, aortic pulse wave velocity is increased and wave reflections arrive back at the ascending aorta during early systole <sup>7</sup>. The early return of reflected waves combined with stiffening of the proximal aorta (i.e., increased characteristic impedance) serves to augment local systolic and pulse pressure, which increases LV load and leads to LV hypertrophy. Thus, under conditions where central elastic arteries have stiffened, we arrive at a situation where pressure is elevated throughout systole producing increased LV load, which leads to LV hypertrophy and increased myocardial oxygen demands in the setting of impaired coronary filling. As such, the functional consequences of large artery stiffening conspire to predispose to LV failure and ischemia <sup>8</sup>.

While the 'upstream' consequences of arterial stiffness are well-appreciated, the effects of reduced arterial buffering capacity on the 'downstream' microcirculation of the

brain and kidneys are no less significant. In most organs and tissues, the microcirculation (i.e., small arteries <400µm, arterioles <100µm, and capillaries) presents a high resistance to blood flow, which serves to reflect pulse waves and convert pulsatile blood flow to steady blood flow at the capillary beds <sup>4</sup>. In contrast, blood vessels immediately upstream of central and renal capillary beds are more dilated and, thus, wave reflection and resistance to blood flow are much lower than at other sites <sup>9</sup>. Consequently, microcirculation of the brain and kidneys are much more susceptible to increases in pressure pulsatility brought about by large artery stiffening, and may explain the link between aortic stiffness and impaired renal and cognitive function <sup>10</sup>.

**Aging, Obesity and AS.** In the United States, CVD has been the leading cause of death in all but 1 year since 1900 <sup>11</sup>. Aging is the primary risk factor for CVD; of the deaths attributed to CVD, over 80% occur in those over 65 years <sup>11</sup>. The risk of CVD increases sharply beginning in middle age, such that men and women free of CVD at 50 years of age have a remaining lifetime risk of 52 and 39 percent, respectively <sup>12</sup>. As previously mentioned, aging is associated with a progressive stiffening of large arteries in the cardiothoracic region, which is an independent predictor of cardiovascular events and mortality in both healthy and diseased populations <sup>1</sup>. The effects of aging are most pronounced in proximal elastic arteries. Carotid stiffness increases 40-50% between the ages of 20 and 80, whereas peripheral muscular arteries experience little to no change over the same age range <sup>13, 14</sup>.

Although inextricably linked to the aging process, there remains a great deal of interindividual variability with respect to the degree of arterial stiffening with age, which

can be explained, at least in part, by regional body fat distribution<sup>15, 16</sup>. Currently over 70 percent of middle-aged and older adults in the US are overweight or obese, and greater than 60 percent of adults 40-69 years of age present abdominal obesity<sup>17, 18</sup>. The results of several clinical and epidemiological studies suggest that obesity is associated with the stiffening of large arteries in the cardiothoracic region<sup>16, 19-24</sup>. More importantly, it appears as though abdominal visceral fat mediates this relation, as numerous cross-sectional studies report associations between surrogate (i.e., waist circumference) and direct measures of visceral adiposity and large artery stiffness across a variety of subject populations<sup>16, 19-24</sup>. Most notably, abdominal visceral fat (measured via computed tomography) was found to be the strongest predictor of aortic stiffness when adjusted for age and systolic blood pressure in a large cohort of the Health, Aging, and Body Composition (Health ABC) study<sup>16</sup>. Additionally, Lee et al.<sup>22</sup> recently reported brachial-ankle PWV, a measure correlated with aortic stiffness, to be significantly greater in normal weight, viscerally obese women compared with overweight, nonviscerally obese women of the same age. The future public health implications of aging- and obesity-associated arterial stiffening are of great concern because 1) middle-aged and older adults are disproportionately affected by obesity and abdominal adiposity, and 2) the number of persons 65 years or older will increase more than two-fold and comprise 20% of the US population by the year 2030<sup>25</sup>.

**Weight Gain and AS.** Currently, over 1.3 billion people worldwide, including 66% of the US adult population, are overweight ( $25.0 \leq \text{BMI} \leq 29.9$ ) or obese ( $\text{BMI} \geq 30.0$ )<sup>18, 26</sup>, and at increased risk of CVD<sup>27</sup>. Despite the observations of increased large artery stiffness in obesity, an important question remains as to the time course of vascular

alterations producing arterial stiffening in this setting. That is, does arterial stiffening occur as a result of weight gain *per se*, or do the functional and/or structural changes represent a 'time exposure' consequence of the obese disposition (i.e., chronic low-grade inflammation, insulin resistance, increased oxidative stress, etc.)? It appears as though long duration obesity is not prerequisite, as the relation between adiposity and arterial stiffness is evident even in young children<sup>28, 29</sup>. Unfortunately, the only two previous longitudinal studies to have explored the determinants of large artery stiffening have reported inconsistent results with respect to the role of weight gain. Wildman et al.<sup>30</sup> found weight gain over a two-year period to be associated with aortic pulse-wave velocity (aPWV) progression among healthy young adults, whereas a study by Benetos et al.<sup>31</sup>, comparing aPWV progression between normotensive and treated hypertensive groups, reported no association between baseline BMI and PWV progression over a 6-year period. Reasons for these disparate findings remain unclear, but may be explained, in part, by the inclusion of older (>50 years) subjects by Benetos et al.<sup>31</sup> Thus, the effects of weight gain on arterial stiffness remains a critical void in our current understanding of the pathophysiological mechanisms of increased CVD risk in obesity.

**Current Treatment Strategies in AS.** While the mechanisms underlying the occurrence of arterial stiffening in obesity remains an important area of future research, the great number of individuals currently overweight or obese and, thus, at increased risk of cerebro- and cardiovascular disease<sup>27</sup> necessitates that we also focus on identifying effective treatment strategies to combat arterial stiffening in this population. Current approaches include pharmacological interventions, as well as therapeutic lifestyle changes. Numerous studies have found arterial vasodilators (e.g., nitrates,

calcium-channel blockers, angiotensin converting enzyme inhibitors) to reduce wave reflection via modulation of vascular smooth muscle tone<sup>4</sup>. As such, these agents reduce aortic systolic and pulse pressure, which can improve LV function by promoting regression of LV hypertrophy. In contrast, drugs that target structural alterations to the vasculature have shown limited promise in humans<sup>32, 33</sup>. In regards to therapeutic lifestyle changes, current evidence suggests that weight loss and regular aerobic exercise impart beneficial effects on arterial stiffness<sup>34-37</sup>. Likewise, weight loss following bariatric surgery appears to reduce arterial stiffness<sup>38</sup>. However, it is important to emphasize that additional therapeutic options are needed for those individuals who are not successful in initiating or maintaining lifestyle changes, as vascular benefits have been shown to dissipate following one month of detraining<sup>39</sup>, as well as for those who require benefits beyond that which can be achieved with lifestyle modification or current pharmacological approaches.

One such therapeutic option that appears promising for improving arterial stiffness are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). In addition to their widely accepted role in the secondary prevention of coronary and cerebrovascular outcomes, statins have recently been shown to reduce the risk of major coronary and cerebrovascular events and revascularizations in patients without cardiovascular disease by ~29 and 34%, respectively<sup>40</sup>. Importantly, data regarding their effectiveness in primary prevention suggests that such benefits are not entirely explained by their ability to reduce LDL-cholesterol. Various pleiotropic effects have been advanced as potential explanations, including their capacity to reduce arterial stiffness<sup>41</sup>.

Although a number of prospective studies have observed reductions in large artery stiffness with statin therapy, the results of randomized placebo-controlled studies have been equivocal. Kool et al.<sup>42</sup> reported no changes in the distensibility or compliance of carotid, femoral or brachial arteries following 8 weeks of pravastatin treatment in hypercholesterolemic (HC) patients. Likewise, 4 weeks of simvastatin treatment was found to have no effect on systemic arterial compliance (SAC) or aPWV in HC subjects<sup>43</sup>, while 6 weeks of atorvastatin treatment increased small artery compliance, but not large artery compliance in patients with stage 3-5 chronic kidney disease<sup>44</sup>. A preliminary study by Raison and colleagues<sup>45</sup> reported a trend for increased aPWV (i.e., *increased aortic stiffness*) in subjects with combined HC and hypertension following atorvastatin treatment. In contrast, 12 weeks of atorvastatin treatment increased SAC by 24 percent in normolipidemic subjects with isolated systolic hypertension<sup>46</sup>. Additionally, Ichihara et al.<sup>47</sup> observed a 14 percent decrease in PWV after 6 months of treatment with fluvastatin in diabetic patients with end-stage renal disease.

Reasons for these disparate results are unclear, but may include differences in subject populations, duration of treatment, and/or type of statin and doses used. Additionally, interpretation of existing trials is limited by their use of blood pressure dependent measures of arterial stiffness<sup>43, 45, 47</sup>, as well as methodological flaws (e.g., failure to measure central blood pressure)<sup>42</sup>. Thus, the potential utility of statins as a destiffening therapy remains unclear. Importantly, none of these previous studies have focused on overweight and obese middle-aged and older adults, a population with accelerated arterial stiffening and at increased risk of CVD<sup>27</sup>. This is a critical void in

our understanding because 1) middle-aged and older adults are disproportionately affected by obesity, and 2) advancing aging and increasing abdominal adiposity synergistically increase arterial stiffness and its pathophysiological consequences.

**Specific Aims and Hypotheses of the Present Investigations.** Despite extensive evidence as to the influence of obesity on arterial stiffness, there remains a paucity of information regarding the effects of weight gain on large artery stiffness. Accordingly, we performed a study of experimental (diet-induced) weight gain in young, nonobese males with the **specific aim** of determining whether weight gain increased large artery stiffness in this population. We **hypothesized** that weight gain would increase large artery stiffness in young, nonobese males. Additionally, we sought to determine whether larger increases in abdominal visceral fat, independent of changes in total body fat, would be associated with greater increases in arterial stiffness.

The widespread prevalence of obesity and abdominal adiposity in middle-aged and older adults highlights the clear need to identify effective strategies to combat accelerated large artery stiffening in this population. Previous randomized controlled trials regarding the effects of statin treatment on large artery stiffness have yielded inconsistent results, which may be due to their use of blood pressure dependent measures of arterial stiffness and/or methodological flaws. Thus, we undertook a randomized placebo-controlled trial, using direct blood pressure independent measures of arterial stiffness, with the **specific aim** of determining whether atorvastatin treatment reduces large artery stiffness in overweight and obese middle-aged and older adults. We **hypothesized** that atorvastatin treatment would reduce large artery stiffness in overweight and obese middle-aged and older adults.

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## CHAPTER 2

### Large Artery Stiffening With Weight Gain in Humans: Role of Visceral Fat Accumulation

#### Abstract

We tested the hypothesis that weight gain would increase arterial stiffness in healthy nonobese adults. To address this, we overfed 14 nonobese men (age= 23 ±1 yrs) ≈1000 kcal/day for 6-8 weeks until a 5-kg weight gain was achieved. Carotid diameters (high resolution ultrasound) and pressures (applanation tonometry), body composition (dual energy x-ray absorptiometry) and abdominal fat distribution (computed tomography) were measured at baseline and following 4 weeks of weight stability at each individual's elevated body weight. Overfeeding increased body weight 5.1 ± 0.1 kg and body fat 3.4±0.4 kg (both P<0.001) in 45±7 days. Total abdominal fat increased 46±7 cm<sup>2</sup> with weight gain, due to increases in both subcutaneous (30±6 cm<sup>2</sup>) and visceral fat (15±4 cm<sup>2</sup>; all P<0.01). As hypothesized, weight gain increased arterial stiffness 13±6% and decreased arterial compliance 21±4% (both P<0.05). Furthermore, those individuals above the median increase in abdominal visceral fat demonstrated a significantly greater increase in arterial stiffness (0.97±0.29 vs. 0.06±0.36 units; P<0.05) compared with those below the median. Consistent with these observations, the only correlates of the changes in arterial stiffness with weight gain were the increases in total abdominal fat (r=0.794), abdominal visceral fat (r=0.651), and waist circumference (r=0.470; all P<0.05). Taken together, these findings suggest that modest weight gain is associated with increases in arterial stiffness in nonobese males. The degree of large artery stiffening with weight gain appears to be determined,

in part, by the amount of abdominal visceral fat gain. Importantly, this relation is independent of the amount of total body fat gained.

*Key Words: Arterial Distensibility; Adiposity; Obesity; Pulse Pressure; Hypertension*

## Introduction

Approximately 65% of the U.S. population <sup>2</sup> and >1 billion people worldwide <sup>30</sup> are overweight or obese and, thus, at increased risk for cardiovascular diseases <sup>7</sup>. There is considerable heterogeneity in the risks associated with excess adiposity; the accumulation of abdominal, particularly visceral, fat appears to be particularly deleterious <sup>31, 32</sup>. Importantly, elevated abdominal visceral fat, independent of total body adiposity, is associated with the development of cardiovascular diseases <sup>31, 32</sup>.

One mechanism by which the cardiovascular complications associated with obesity may be advanced is through remodeling of the vasculature. The results of numerous studies suggest that obesity is associated with the stiffening of arteries in the cardiothoracic region <sup>20-22, 24, 25, 33, 34</sup>. Importantly, large artery stiffness is associated with adverse cardiovascular outcomes <sup>35-38</sup> which occur more frequently in obese individuals <sup>7, 32</sup>. The relation between adiposity and arterial stiffness is evident even in young children <sup>26, 27</sup>, suggesting that long duration obesity is not a prerequisite for arterial stiffening. Unfortunately, the available data, from observational studies of weight gain, are inconsistent <sup>28, 29</sup>. In addition, measurements of body composition were not included in these previous studies. Furthermore, it is currently unknown whether increases in abdominal visceral fat with weight gain are associated with large artery stiffening, independent of increases in total body fat. Therefore, we tested the hypothesis that modest weight gain would increase arterial stiffness in healthy nonobese adults. We further sought to determine whether those individuals who demonstrate the largest increases in abdominal visceral fat, independent of total body fat gain, would also demonstrate the largest increases in arterial stiffness.

## **Materials and Methods**

### **Subjects**

Fourteen young (age =  $23 \pm 1$  years), nonobese (body mass index  $< 30 \text{ kg/m}^2$ ) males included in previous publications<sup>39, 40</sup> were studied. Subjects were normotensive, free from overt chronic disease, nonsmokers, and not taking any medications. All subjects were weight stable ( $\pm 2 \text{ kg}$ ) for at least 6 months prior to entry into the study. The Virginia Tech Institutional Review Board approved all experimental protocols. The nature, purpose, risks, and benefits of the study were explained to all subjects before obtaining informed consent.

### **Experimental Design and Protocol**

Following baseline measurements, subjects were overfed  $\approx 1000 \text{ kcal/day}$  for 6-8 weeks until a 5-kg weight gain was achieved. Subjects were provided with a liquid meal replacement supplement (Boost Plus; Novartis Nutrition Corp.; 35% fat, 50% CHO, 15% protein) to meet their excess energy requirements. To avoid the potential effects of acute energy imbalance on the primary outcome variables, baseline measurements were repeated on each subject following a 4 week period of weight stability at their elevated body weight. Subjects underwent weekly assessment by a research dietician (B.M.D) throughout weight gain and weight stability phases to ensure adequate progress and compliance. Subjects reported to the Virginia Tech Human Integrative Physiology Laboratory between 7 and 11 am following a 12-hour fast, and having refrained from caffeine and exercise for 24-hours prior to each testing session. Following post testing, subjects were provided with dietary counseling, physical activity

recommendations and, if desired, meal replacement products to facilitate return to their baseline body weight.

## **Measurements**

Body mass and height were measured with a digital scale and stadiometer (Scale-Tronix model 5002), respectively. Dual-energy x-ray absorptiometry (GE Lunar Prodigy Advance; software version 8.10e) was used to determine body composition. Computed tomography scans (HiSpeed CT/i, GE Medical) were taken between the L3-L4 vertebra, and abdominal fat distribution was quantified using commercially available analysis software (Slice-O-Matic 4.3 Rev-4, Tomovision Inc.). Maximal oxygen consumption was measured during graded treadmill exercise to volitional exhaustion using open circuit spirometry (TrueMax 2400, ParvoMedics). Standard criteria for achievement of valid maximal oxygen consumption were met<sup>41</sup>. Casual blood pressure was measured over a brachial artery via mercury sphygmomanometry following 15 minutes of seated rest in a quiet room. Measurements were repeated until within-session stability was achieved ( $\pm$  6 mmHg on 3 sequential measurements), and on at least 3 separate occasions over a 2-wk period until between-session stability was reached. Resting heart rate was determined from lead II of an electrocardiogram. Plasma lipid and lipoprotein concentrations were measured via nuclear magnetic resonance (NMR) spectroscopy (LipoScience, Inc, Raleigh, NC), as previously described<sup>42</sup>.

$\beta$ -stiffness index<sup>43</sup> and arterial compliance were assessed by combining simultaneous measurements of carotid artery diameter and blood pressure over three consecutive cardiac cycles via B-mode ultrasonography and applanation tonometry, respectively. Following 10-min of quiet rest in a supine position, arterial blood pressure

was measured over a brachial artery via automated sphygmomanometry (Pilot 9200, Colin Medical Instruments) until stability was achieved (3 consecutive readings  $\pm$  6 mmHg). Subsequently, common carotid artery diameters were obtained with an ultrasound unit (Sonos 7500, Philips Medical Systems) equipped with a high-resolution linear array transducer (3-11 MHz). Longitudinal B-mode images of the cephalic portion of the common carotid artery were obtained 1-2 cm proximal to the carotid bulb. The transducer was placed 90° to the vessel so that near and far walls were clearly visible. The images were stored to optical disk for quantification of carotid artery diameters offline using commercially available analysis software (Vascular Research Tools 5, Medical Imaging Applications, LLC). The distance between the near and far wall vessel boundaries were measured at time points that corresponded with maximal systolic and minimal diastolic diameters. Throughout the imaging process, pressure waveforms were obtained from the contralateral carotid artery using a high fidelity non-invasive pulse tonometer (SPT-301, Millar Instruments), and digitized at 500 Hz for subsequent analysis using signal processing software (Windaq, Dataq Instruments). Carotid pressure waveforms were corrected for hold down pressure by calibration to brachial diastolic and mean arterial pressures<sup>44</sup>. The reproducibility of measurements of  $\beta$ -stiffness index in our laboratory is excellent ( $r=0.90$ ,  $P<0.05$ ).

### **Statistical Analysis**

Differences in subject characteristics and dependent variables before and after weight gain were assessed with paired Student's *t*-tests. In order to gain better insight into the relation between abdominal fat distribution and arterial compliance and stiffness, subjects were subsequently divided into two groups (smaller increase in

abdominal visceral fat [SVF] and larger increase in abdominal visceral fat [LVF]) based on the median split of abdominal visceral fat change with weight gain. Repeated measures analysis of variance was used to assess changes in subject characteristics and dependent variables in the SVF and LVF groups with weight gain. Differences in the magnitude of change in subject characteristics and dependent variables with weight gain between the two groups were assessed with independent-samples *t*-tests. Comparisons performed using Wilcoxon signed rank tests yielded similar outcomes. Relations among variables of interest were assessed using simple correlation analyses. All data are expressed as means  $\pm$ SE. The significance level was set *a priori* at  $P < 0.05$ .

## Results

Subject characteristics at baseline and following weight gain are shown in Table 1. Overfeeding resulted in  $5.1 \pm 0.1$  kg (range = 4.2-5.8 kg) of body weight gain in  $45 \pm 7$  days. Body fat ( $3.4 \pm 0.4$  kg), percent body fat ( $3.2 \pm 0.6\%$ ), lean body mass ( $1.5 \pm 0.4$  kg), and waist circumference ( $5.6 \pm 0.6$  cm) increased with weight gain (all  $P < 0.001$ ). Total abdominal fat ( $46 \pm 7$  cm<sup>2</sup>) increased due to increases in both visceral ( $15 \pm 4$  cm<sup>2</sup>) and subcutaneous fat ( $30 \pm 6$  cm<sup>2</sup>) depots (all  $P < 0.01$ ). There was a small, but significant, reduction in maximal oxygen consumption expressed relative to body weight with weight gain. However, maximal oxygen consumption expressed in absolute terms and relative to fat-free mass did not change. Systolic blood pressure ( $5 \pm 1$  mmHg;  $P < 0.01$ ) but not diastolic blood pressure increased with weight gain, whereas resting heart rate tended to increase ( $3 \pm 2$  beats/min;  $P = 0.056$ ). Regarding traditional cardiovascular risk factors, only total cholesterol and triglyceride concentrations increased significantly after weight

gain ( $15\pm 5$  and  $43\pm 16$  mg/dL, respectively). As hypothesized, arterial stiffness increased ( $0.51\pm 0.26$  U;  $P<0.05$ ; Figure 1, *top panel*) and compliance decreased ( $0.43\pm 0.09$  mm<sup>2</sup>/mmHg x 10<sup>-1</sup>;  $P<0.001$ ; Figure 1, *bottom panel*) with weight gain. Relative to baseline values, this represents a change in arterial stiffness and compliance of  $+13\pm 6$  and  $-21\pm 4\%$ , respectively (both  $P<0.05$ ).

To gain further insight into the relation between abdominal fat distribution and arterial stiffness (and compliance), we divided the subjects into two groups (SVF and LVF) based on the median change in abdominal visceral fat with weight gain. Subject characteristics and arterial stiffness (and compliance) of the SVF and LVF groups at baseline and following weight gain are shown in Table 2. At baseline, the two groups did not differ with respect to age, body weight, body composition, abdominal fat distribution, maximal oxygen consumption, heart rate, blood pressure, arterial compliance, or arterial stiffness (all  $P>0.05$ ). In contrast, LVF had higher concentrations of total cholesterol, LDL-C, and triglycerides compared with SVF (all  $P<0.05$  for group effect). Despite gaining a slightly lesser amount of body weight ( $4.8\pm 0.2$  versus  $5.3\pm 0.2$  kg;  $P=0.04$ ), the LVF group experienced a significantly greater increase in total abdominal fat ( $58\pm 10$  versus  $33\pm 7$  cm<sup>2</sup>) due to greater visceral fat ( $28\pm 5$  vs.  $3\pm 2$  cm<sup>2</sup>) accumulation with weight gain (both  $P<0.05$ ). The increase in abdominal subcutaneous fat ( $30\pm 5$  versus  $30\pm 11$  cm<sup>2</sup>;  $P>0.05$ ) with weight gain was not different in the 2 groups. Consistent with our hypothesized association between abdominal visceral fat and arterial stiffness, the LVF group experienced a significantly greater increase in arterial stiffness ( $0.97\pm 0.29$  versus  $0.06\pm 0.36$  U;  $P<0.05$ ; Figure 2, *top panel*) and decrease in arterial compliance ( $-0.589\pm 0.119$  versus  $-0.280\pm 0.120$  mm<sup>2</sup>/mmHg x 10<sup>-1</sup>;  $P<0.05$ ;

Figure 2, *bottom panel*) compared with SVF. The magnitudes of change in body fat, fat-free mass, maximal oxygen consumption, systolic and diastolic blood pressure, and plasma lipid and lipoprotein concentrations did not differ between the 2 groups (all  $P>0.05$ ).

In the pooled sample, the magnitude of change in abdominal visceral fat with weight gain was correlated with the magnitude of change in arterial stiffness ( $r=0.651$ ;  $P<0.05$ ; Figure 3, *top panel*) and arterial compliance ( $r=-0.589$ ;  $P<0.05$ ; Figure 3, *bottom panel*). The magnitude of increase in total abdominal fat and waist circumference with weight gain were also correlated with the increases in arterial stiffness ( $r=0.794$  and  $0.470$ , respectively; both  $P<0.05$ ) and the decreases in arterial compliance ( $r=-0.765$  and  $-0.496$ , respectively; all  $P<0.05$ ). Furthermore, baseline values of arterial stiffness and compliance correlated with the magnitude of their respective changes with weight gain ( $r=0.683$  and  $-0.711$ , respectively, both  $P<0.05$ ). There were no other significant correlations.

## Discussion

The major new finding of the present study is that modest diet-induced weight gain results in increases in large artery stiffness in healthy young adult males. Those individuals with relatively larger increases in abdominal visceral fat demonstrated correspondingly larger increases in arterial stiffness and greater reductions in arterial compliance. Importantly, the adverse effect of abdominal visceral fat accumulation on arterial stiffness and compliance occurred independent of the amount of total body fat gained.

The results of previous prospective studies regarding the determinants of arterial stiffening are inconsistent with respect to the role of weight gain. Wildman et al. found weight gain over a two-year period to be associated with an increase in pulse-wave velocity among healthy young adults whereas Benetos et al.<sup>28, 29</sup> failed to support such an association. The reasons for these disparate findings remain unclear, but may be explained, in part, by the inclusion of older subjects (>50 years) by Benetos et al.<sup>28</sup>. Nonetheless, our current findings confirm and extend the findings of Wildman et al.<sup>29</sup> by demonstrating that experimental weight and fat gain, particularly in the abdominal visceral region, is associated with a reduction in arterial stiffness in healthy young males.

Numerous cross-sectional studies report associations between surrogate (i.e., waist circumference) and direct measures of visceral adiposity and large artery stiffness across a variety of subject populations<sup>20-22, 24, 25, 33, 34</sup>. Most notably, abdominal visceral fat (measured via computed tomography) appears to be the strongest predictor of aortic stiffness<sup>24, 34</sup>. Taken together with these previous cross-sectional studies, our findings implicate abdominal fat partitioning as an important mediator of the arterial stiffening that occurs with weight gain.

The mechanisms linking abdominal visceral fat and arterial stiffening are unclear, although numerous possibilities have been advanced<sup>45</sup>. Seals and Gates<sup>46</sup> hypothesized that the pro-inflammatory state and oxidative stress accompanying weight gain (and aging) alters vascular structure and function by disrupting the balance of key extracellular matrix proteins (i.e., elastin and collagen), vasoconstrictive and vasodilatory molecules (e.g., nitric oxide, prostacyclins, endothelin-1, and angiotensin-

II), and promoting vascular smooth muscle cell hypertrophy. Together these pathophysiological mechanisms lead to arterial stiffening. Given the short duration of the present study, it seems unlikely that structural modifications to the vasculature, as observed in age-associated arterial stiffening, can account for the increase in stiffness observed following weight gain. A more plausible explanation is that a multitude of interrelated factors sensitive to acute changes in body weight including reductions in insulin sensitivity, dyslipidemia, activation of the sympathetic nervous and renin-angiotensin systems and perhaps other factors conspire to impair endothelial function (i.e., increase vascular smooth muscle tone) and, in turn, results in arterial stiffening<sup>45</sup>. However, we found no association between the changes in carotid stiffness or compliance and changes in estimates of insulin sensitivity (i.e., fasting insulin and HOMA score), plasma renin activity, or muscle sympathetic nerve activity<sup>(39)</sup>; data not shown). We should emphasize that our study was not designed to address potential mechanisms. As such, future studies will be necessary to address this important issue.

There are some limitations of the present study that should be discussed. First, we did not include a control group and our sample size was relatively small. Thus, inclusion of a control group and/or a larger sample size may have yielded different results. However, that 11 out of the 14 subjects demonstrated an increase in carotid artery stiffness following weight gain and, as hypothesized, the subjects with the largest increase in abdominal visceral fat (manipulated variable) also demonstrated the largest increase in arterial stiffness (the outcome variable), suggests that our results are unlikely to have been the results of random deviations over time.

Second, the subjects in the present study were limited to young, nonobese males. Females tend to accumulate fat in the gluteal-femoral regional and older adults are more susceptible to visceral fat accumulation. As such, the vascular responses to weight gain may be attenuated or amplified, respectively. For these reasons, caution should be taken in extrapolating our findings to females or beyond the age-range studied.

Third, the experimental weight gain produced in the present study may not be representative of the more gradual changes that occur over time in the general population. As such, our findings should be considered with this in mind.

Finally, we should emphasize that our findings do not preclude the possibility that the expansion of other fat depots, such as perivascular adipose tissue, may play an important role in mediating the effects of weight gain on arterial stiffness<sup>47</sup>.

In conclusion, the results of the present study indicate that modest diet-induced weight gain results in large artery stiffening in young, nonobese males. Those individuals who demonstrated the largest increases in abdominal visceral fat also demonstrated the largest increases in arterial stiffness. Importantly, the increase in arterial stiffness associated with abdominal visceral fat accumulation occurred independent of the amount of total body fat gained.

### **Perspectives**

Arterial stiffening has long been regarded as an indicator of disease<sup>48</sup> and is independently associated with an increased risk for developing hypertension<sup>49</sup>. Arterial stiffening reduces the cushioning function of the aorta. As a consequence, systolic blood pressure rises, diastolic blood pressure falls and the ability to convert pulsatile

cardiac ejection to continuous flow at the level of the microcirculation is impaired<sup>48</sup>. As such, arterial stiffening may lead to left ventricular hypertrophy, cardiac dysfunction and a reduction in myocardial perfusion in the face of increased demand. In addition, microvascular damage can occur in key target organs such as the kidney and brain as a result of the reduced cushioning function<sup>17</sup>. Our current findings suggest that individuals who gain even modest amounts of weight may experience arterial stiffening even if they do not become obese. The accumulation of abdominal visceral fat appears to be particularly important in this regard. Taken together with the recent findings of an increasing prevalence of abdominal obesity<sup>50</sup>, our data highlight the importance of abdominal visceral fat as an important therapeutic target. Importantly, overfeeding-induced weight gain in humans may provide an insightful model to discern the mechanism(s) responsible for weight-gain induced arterial stiffening.

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### **Disclosures**

None

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Table 1. *Subject Characteristics at Baseline and After Weight Gain*

Variable	Baseline	Weight Gain
Age (years)	23±1	NA
Body weight (kg)	75.3±2.9	80.3±3.0*
Body mass index (kg/m <sup>2</sup> )	24.0±0.7	25.7±0.7*
Body fat (%)	21.3±1.2	24.6±1.1*
Total fat mass (kg)	15.5±1.1	18.9±1.1*
Lean body mass (kg)	56.7±2.3	58.2±2.5*
Waist circumference (cm)	84.9±1.9	90.5±1.7*
Total abdominal fat (cm <sup>2</sup> )	226±19	271±20*
Abdominal subcutaneous fat (cm <sup>2</sup> )	160±13	190±11*
Abdominal visceral fat (cm <sup>2</sup> )	66±8	81±11*
VO <sub>2max</sub> (ml/kg/min)	44.7±1.8	43.8±1.5*
VO <sub>2max</sub> (l/min)	3.4±0.2	3.5±0.2
Heart rate (beats/min)	62±2	65±3
Systolic blood pressure (mmHg)	114±2	119±2*
Diastolic blood pressure (mmHg)	75±2	75±2
Triglycerides (mg/dL)	92±17	135±31*
Total cholesterol (mg/dL)	134±6	148±8*
HDL cholesterol (mg/dL)	40±2	40±2
LDL cholesterol (mg/dL)	80±6	89±8

All values are expressed as mean ± SEs; \**P* < 0.05 vs. baseline.

Table 2. *Subject Characteristics in Individuals with SVF versus LVF*

Variable	SVF (n=7)		LVF (n=7)	
	Baseline	Weight Gain	Baseline	Weight Gain
Age (years)	21.3±1.0	NA	24.9±2.3	NA
Body weight (kg)	78.2±3.9	83.5±4.0	72.3±4.4	77.1±4.5*
Body mass index (kg/m <sup>2</sup> )	23.9±0.9	25.5±0.9	24.2±1.0	25.9±1.0*
Body fat (%)	20.0±1.4	23.0±1.6	22.7±1.9	26.1±1.4*
Total fat mass (kg)	15.1±1.5	18.4±1.7	15.9±1.8	19.4±1.6*
Lean body mass (kg)	59.8±2.9	61.5±3.2	53.5±3.3	54.8±3.5*
Waist circumference (cm)	83.8±2.4	89.3±2.4	85.9±3.0	91.8±2.5*
Total abdominal fat (cm <sup>2</sup> )	200±21	233±24	251±31	310±24*
Abdominal subcutaneous fat (cm <sup>2</sup> )	143±14	173±16	177±21	207±13*
Abdominal visceral fat (cm <sup>2</sup> )	57±9	60±10	75±12	102±15*‡
VO <sub>2max</sub> (ml/kg/min)	45.9±3.0	43.9±2.3	43.6±2.1	43.6±2.2*
VO <sub>2max</sub> (l/min)	3.6±0.3	3.7±0.3	3.2±0.3	3.3±0.3
Heart rate (beats/min)	62±3	63±5	62±3	67±4
Systolic blood pressure (mmHg)	115±3	120±3	114±4	118±4*
Diastolic blood pressure (mmHg)	73±3	74±4	76±2	77±3
Triglycerides (mg/dL)	58±8	84±14	133±26	196±56*†
Total cholesterol (mg/dL)	120±5	128±6	150±6	172±4*†
HDL cholesterol (mg/dL)	41±1	42±4	38±3	37±1

LDL cholesterol (mg/dL)	68±6	70±6	94±6	111±8†
Arterial compliance (mm <sup>2</sup> /mmHg x 10 <sup>-1</sup> )	1.85±0.12	1.57±0.09	1.98±0.12	1.39±0.09*§
β-Stiffness index (U)	5.4±0.2	5.4±0.3	5.0±0.5	5.9±0.3*§

---

All values are expressed as mean ± SEs; effect of time (\*), group (†), and time x group interaction (‡),  $P < 0.05$ . § $P = 0.07$  to  $0.10$  for interaction effect.

## Figure Legends

**Figure 1.** Top panel:  $\beta$ -Stiffness index at baseline and following weight gain. Bottom panel: Arterial compliance at baseline and following weight gain. (\* $P < 0.05$  vs. baseline)

**Figure 2.** Top panel: Change in  $\beta$ -stiffness index in subjects with smaller and larger increases in visceral fat. Bottom panel: Change in arterial compliance in subjects smaller and larger increases in visceral fat. SVF=Smaller change in visceral fat; LVF=Larger change in visceral fat. (\* $P < 0.05$  vs. SVF)

**Figure 3.** Top panel: Relation between changes in abdominal visceral fat and changes in  $\beta$ -stiffness index in the pooled sample. Bottom panel: Relation between change in abdominal visceral fat and magnitude of change in arterial compliance in the pooled sample.

Figure 1

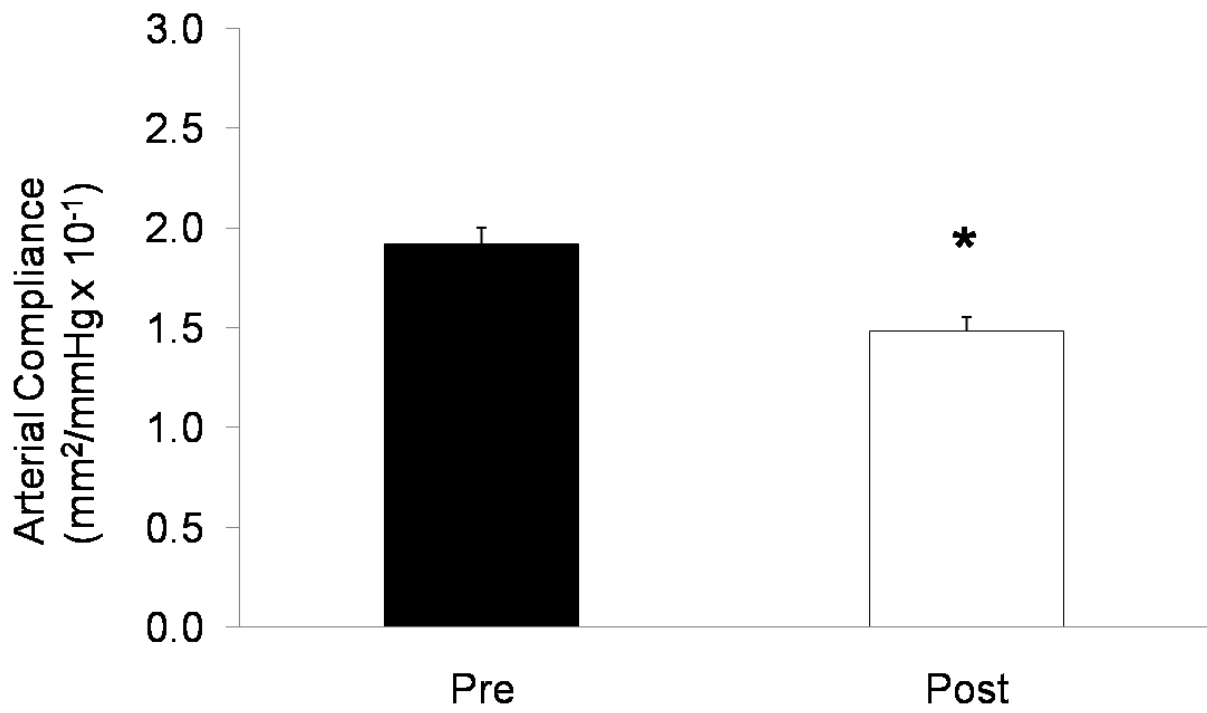
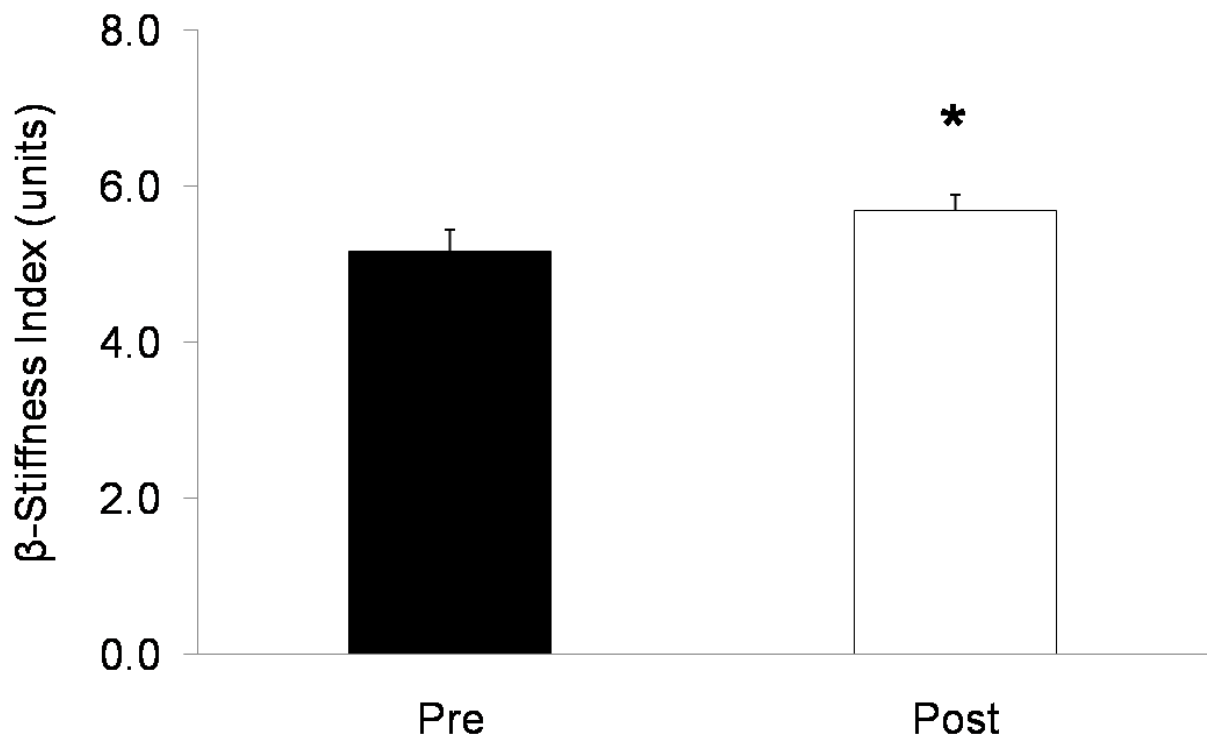


Figure 2

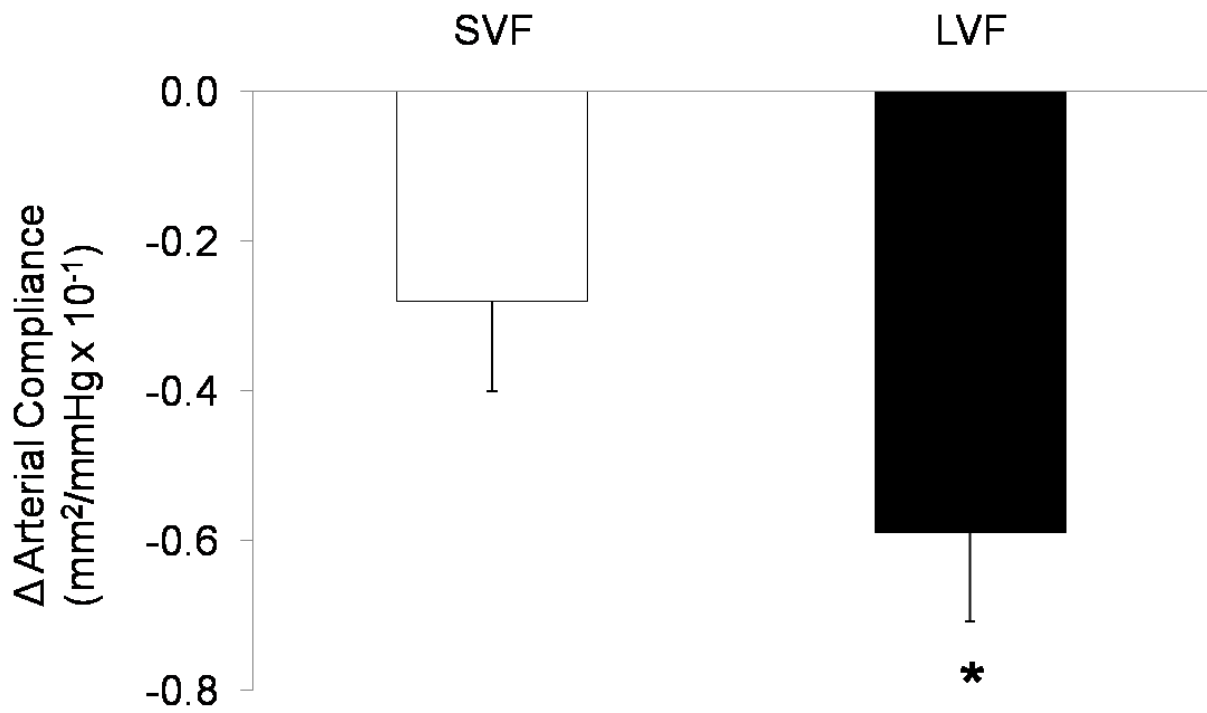
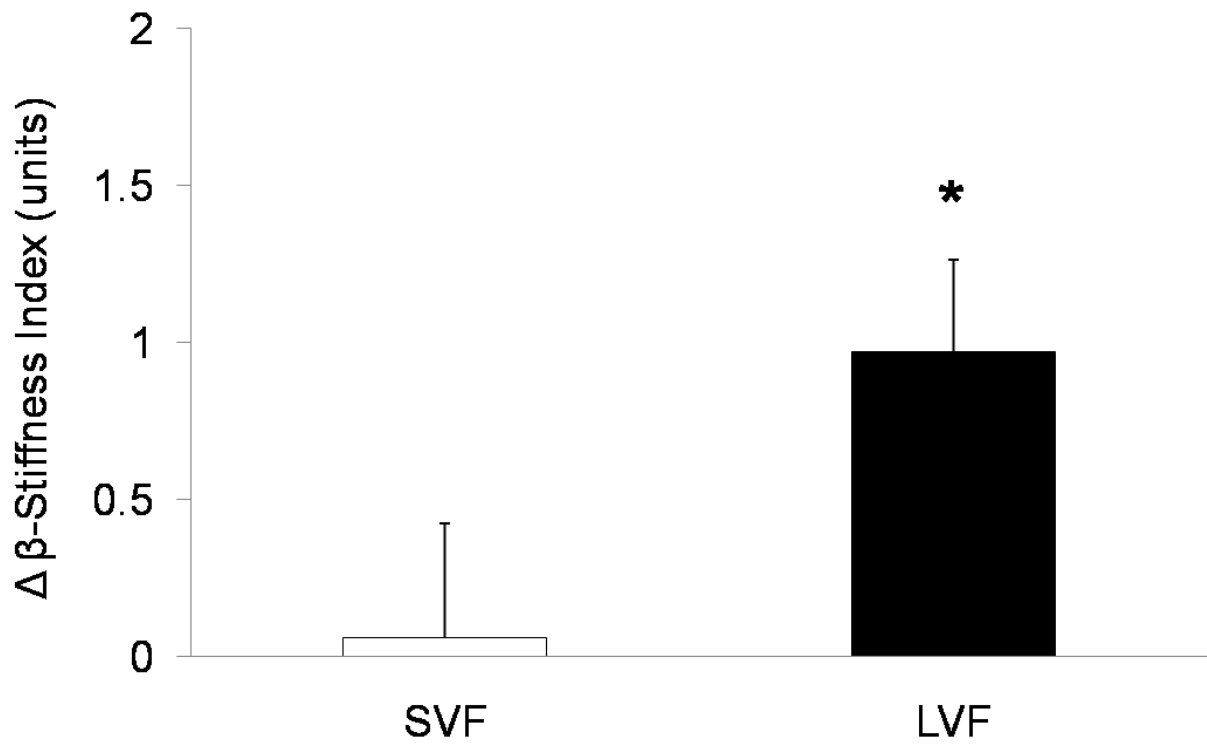
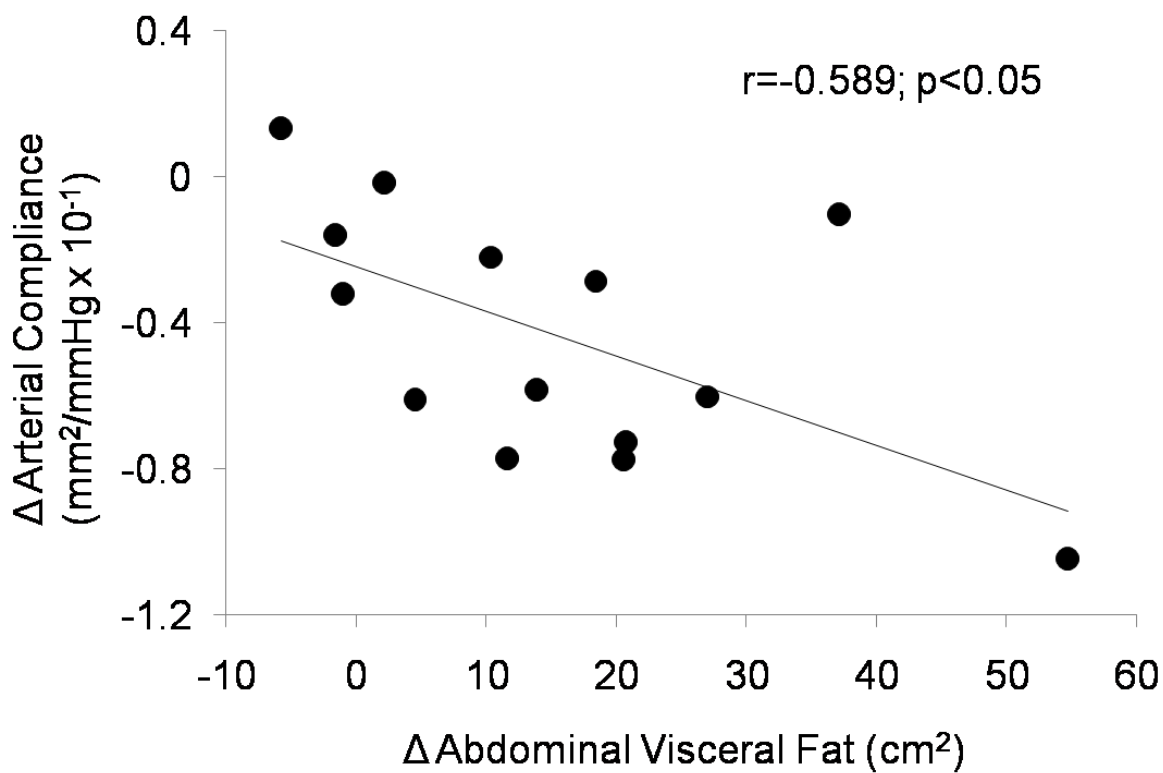
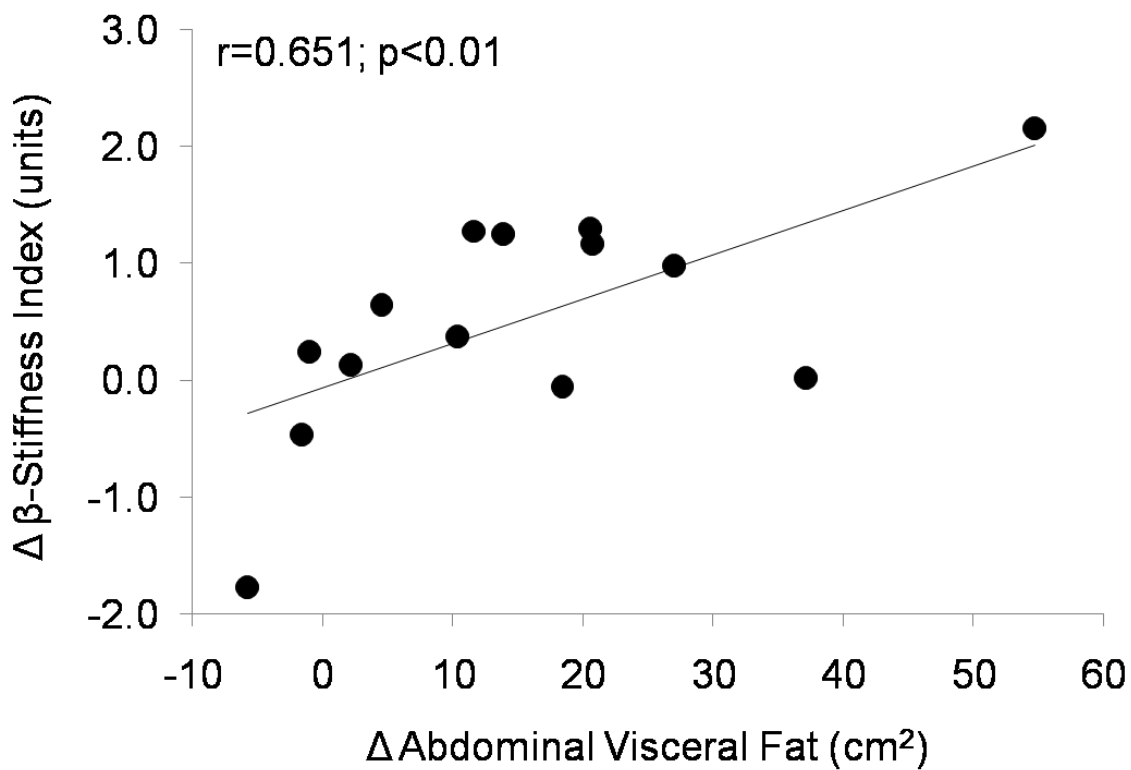


Figure 3



## Chapter 3

### Arterial Destiffening with Atorvastatin in Overweight and Obese Middle-Aged and Older Adults

#### Abstract

Arterial stiffness (AS) has been shown to be an independent predictor of cardio- and cerebrovascular events and mortality. AS increases with both advancing age and obesity. It has been suggested that the ability of statin drugs to reduce coronary and cerebrovascular event risk may be due, in part, to their effects on the vasculature. However, whether statin treatment improves AS in overweight and obese middle-aged and older adults is not known. We hypothesized that atorvastatin (ATOR) treatment would reduce AS in overweight and obese middle-aged and older adults. Twenty-six (15 females, 11 males) overweight or obese ( $BMI=31.6\pm 0.7 \text{ kg/m}^2$ ), and otherwise healthy middle-aged and older adults ( $\text{age}=54\pm 2 \text{ yrs}$ ) were randomly assigned to receive either ATOR (80mg/day) or placebo for 12 weeks. At baseline and following the 12-wk treatment period aortic and brachial pulse wave velocity (PWV) was measured, and  $\beta$ -stiffness and compliance of the left common carotid artery were determined via applanation tonometry and B-mode ultrasound. At baseline, the ATOR ( $n=16$ ) and placebo ( $n=10$ ) groups did not differ with respect to age, BMI, blood pressure, serum lipid and lipoprotein concentrations, high sensitivity C-reactive protein (hs-CRP), or indices of arterial stiffness or compliance (all  $P>0.05$ ). Following the 12-wk treatment period, the ATOR group experienced a 47% reduction in LDL-cholesterol ( $149\pm 6$  to  $80\pm 8 \text{ mg/dL}$ ) and a 42% reduction in hs-CRP ( $3.6\pm 0.8$  to  $2.1\pm 0.5 \text{ mg/L}$ ). Additionally,  $\beta$ -stiffness and aortic PWV decreased ( $9.4\pm 0.6$  to  $7.6\pm 0.5 \text{ U}$  and  $1096\pm 36$  to  $932\pm 32$

cm/sec, respectively; both  $P < 0.001$ ) and carotid compliance increased ( $0.89 \pm 0.06$  to  $1.15 \pm 0.10 \text{ mm}^2/\text{mmHg} \times 10^{-1}$ ,  $P < 0.01$ ) with ATOR. In contrast, there were no significant changes in  $\beta$ -stiffness ( $9.1 \pm 0.8$  to  $9.1 \pm 0.7$  U), aortic PWV ( $1238 \pm 89$  to  $1191 \pm 90$  cm/sec), or carotid compliance ( $0.96 \pm 0.14$  to  $0.96 \pm 0.12 \text{ mm}^2/\text{mmHg} \times 10^{-1}$ ) with placebo (all  $P > 0.05$ ). Brachial PWV did not significantly change in either group. The improvements in AS with ATOR were significantly correlated to the reduction in LDL-cholesterol, but not hs-CRP. Taken together, our findings suggest that ATOR reduces AS in overweight and obese middle-aged and older adults.

Key Words: *Statins; Arterial Stiffness; Pulse Wave Velocity; Arterial Compliance; Obesity*

## Introduction

In the United States, cardiovascular disease (CVD) has been the leading cause of death in all but 1 year since 1900 <sup>1</sup>. The risk of CVD increases sharply beginning in middle age, such that men and women free of CVD at 50 years of age have a remaining lifetime risk of 52 and 39 percent, respectively <sup>2</sup>. Aging is characterized by a progressive stiffening of large arteries in the cardiothoracic region <sup>3</sup>. Arterial stiffness reduces the buffering capacity of central elastic arteries and, in turn, serves to augment central systolic and pulse pressure, increase aortic impedance and LV load, and impair coronary perfusion <sup>3</sup>. Additionally, reduced buffering of intermittent cardiac ejections increases the pulsatility of blood flow at the level of capillary beds, which is especially detrimental to the central and renal microcirculations <sup>4</sup>. Arterial stiffness is an independent predictor of CVD events and mortality in both healthy and diseased populations <sup>5</sup>.

Although inextricably linked to the aging process, there remains a great deal of interindividual variability with respect to the degree of arterial stiffening with age, which can be explained, at least in part, by regional body fat distribution <sup>6,7</sup>. A number of cross-sectional studies suggest that obesity <sup>8-11</sup>, particularly abdominal adiposity <sup>6,7,12-16</sup>, is associated with an acceleration of large artery stiffening. Currently over 70 percent of middle-aged and older adults in the US are overweight or obese, and greater than 60 percent of adults 40-69 years of age present abdominal obesity <sup>17,18</sup>.

Current evidence suggests that weight loss and regular aerobic exercise impart beneficial effects on arterial stiffness <sup>19-23</sup>. Likewise, weight loss following bariatric

surgery appears to reduce arterial stiffness<sup>24</sup>. However, it is important to emphasize that additional therapeutic options are needed for those individuals who are not successful in initiating or maintaining lifestyle changes, as vascular benefits have been shown to dissipate following one month of detraining<sup>25</sup>.

One such therapeutic option that appears promising for improving arterial stiffness are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), which have received increased attention for their ability to reduce the risk of major cardiovascular events in patients without cardiovascular disease<sup>26</sup>. Importantly, data regarding their effectiveness in primary prevention suggests that such benefits are not entirely explained by their ability to reduce LDL-cholesterol. Various pleiotropic effects have been advanced as potential explanations, including their capacity to reduce arterial stiffness<sup>27</sup>. Unfortunately, the only randomized placebo-controlled trials to have explored the effects of statin therapy on arterial stiffness have produced equivocal results<sup>28-33</sup>. Furthermore, no studies to date have focused on overweight and obese middle-aged and older adults; a population with accelerated arterial stiffening and at increased risk for CVD<sup>34</sup>. To address this, we tested the hypothesis that atorvastatin treatment would reduce large artery stiffness in overweight and obese middle-aged and older adults.

## **Materials and Methods**

### **Subjects**

Twenty-six men (n=11) and women (n=15) 40-65 years of age participated in the study. All subjects were overweight or obese (BMI  $\geq 25$  kg/m<sup>2</sup>) with an arterial blood pressure (BP) less than or equal to 159/99 mmHg. Individuals with BP  $\geq 160/100$ , total

cholesterol  $\geq$  300 mg/dL, fasting plasma glucose  $\geq$  126 mg/dL, triglyceride concentration  $\geq$  450 mg/dL, or elevated transaminase levels were excluded from the study. All subjects were free from chronic disease (other than stage I hypertension) as assessed by medical history, physical examination, negative exercise electrocardiogram, complete blood count, blood chemistry, and urinalysis. All subjects were weight stable ( $\pm$ 2.0 kg for the previous 6 months), non-smokers, and not taking medications or dietary supplements that may influence the dependent variables or interact with statin drugs. Female participants were not receiving hormone replacement therapy. The Virginia Tech Institutional Review Board approved all of the experimental protocols. The nature, purpose, risks, and benefits of the study were explained to all of the subjects before obtaining informed consent.

### **Experimental Design and Protocol**

Following baseline measurements, subjects were randomized in a double-blind manner to receive either atorvastatin (ATOR) 80mg (n=16) or placebo (n=10) for 12 weeks. Subjects were provided with a predetermined excess number of tablets at the onset and every two weeks throughout the treatment period. Subject compliance was assessed by counting the number of tablets returned at each refill. Due to the blinded nature of the study, all subjects were continuously screened for adverse events throughout the treatment period. Additionally, subjects were instructed to maintain their current dietary intake and daily physical activity level for the duration of the study in order to ensure weight stability. After the 12-week treatment period, baseline measurements were repeated over two weeks during which subjects continued to receive either ATOR or placebo. Measurements were performed in all subjects

between 8 and 11 AM after a 12-hr fast and having performed no vigorous physical activity for 48-hours prior to each testing session.

## **Measurements**

Body mass and height were measured with a digital scale and stadiometer (Scale-Tronix model 5002), respectively. Waist circumference was measured at the level of the iliac crest, according to established guidelines<sup>35</sup>. Body composition was determined via dual-energy x-ray absorptiometry (GE Lunar Prodigy Advance, software version 8.10e). Casual blood pressure was measured over a brachial artery via automated sphygmomanometry (Pilot 9200, Colin Medical Instruments). Blood pressure measurements were obtained after 15 minutes of seated rest in a quiet room, and were repeated until within-session stability was achieved ( $\pm 5$  mmHg on 3 sequential measurements). Resting heart rate was determined from lead II of an ECG. Habitual physical activity and dietary intake were assessed via accelerometer (GT1M, Actigraph Inc.) and self-reported 4-day food intake records, respectively. Energy and macronutrient intake were assessed using nutritional analysis software (NDS-R 6.0; University of Minnesota, Minneapolis, MN). Plasma levels of total cholesterol and triglyceride were determined by conventional enzymatic methods<sup>36</sup>. High-density lipoprotein cholesterol was determined by the dextran precipitation technique<sup>37</sup>, and low-density lipoprotein cholesterol was measured using beta quantification<sup>38</sup>. High sensitivity C-reactive protein (hs-CRP) concentrations were determined via immunometric assay (IMMULITE 2000, Diagnostic Products Corp.). Standard enzymatic methods and a commercially available ELISA (Diagnostic Systems Laboratory) were used to measure fasting serum glucose and insulin concentrations,

respectively. The homeostasis model assessment (HOMA) score [(glucose, mM x insulin,  $\mu$ IU)/22.5] was used to provide an estimate of insulin resistance <sup>39</sup>.

Pulse wave velocity measurements were performed following 10 minutes of quiet rest in a supine position, and the subsequent achievement of blood pressure stability, as measured over a brachial artery by automated sphygmomanometry ( $\pm 5$  mmHg on 3 sequential measurements). Once blood pressure stability had been established, non-invasive pulse tonometers (SPT-301, Millar Instruments) were used to simultaneously obtain arterial pressure waveforms at the carotid and femoral arteries throughout ten cardiac cycles. Surface distance between the two recording sites was then measured to the nearest 0.5 cm. Subsequently, this process was repeated to measure arterial pressure waveforms at the carotid and radial arteries. All arterial pressure waveforms were digitized at 500 Hz and analyzed using signal processing software (Windaq, Dataq Instruments). Pulse wave velocity (PWV) for the carotid-femoral (aortic PWV) and carotid-radial (brachial PWV) recordings were determined by normalizing the waveform foot-to-foot time delay to the distance between recording sites (i.e.,  $PWV = D \text{ (cm)}/\Delta t \text{ (sec)}$ ).

$\beta$ -Stiffness index <sup>40</sup> and arterial compliance were assessed by combining simultaneous measurements of carotid artery diameter and blood pressure, as described by us previously <sup>41</sup>. Briefly, longitudinal B-mode images of the left common carotid artery (1-2 cm proximal to the carotid bulb) were obtained with an HP Sonos 7500 ultrasound unit (Phillips Healthcare, Bothel, WA) equipped with a high-resolution linear array transducer (3-11 MHz). Concomitant measurement of arterial blood pressure was obtained via applanation tonometry of the contralateral common carotid

artery. Carotid diameters were then quantified offline using commercially available software (Vascular Research Tools 5, Medical Imaging Applications, LLC), and tonometric pressure waveforms were calibrated to brachial diastolic and mean arterial pressures. The reproducibility of measurements of  $\beta$ -stiffness index in our laboratory is excellent ( $r=0.90$ ;  $p<0.05$ ).

### **Statistical Analysis**

Independent-sample  $t$ -tests were used to assess baseline differences in subject characteristics and dependent variables between ATOR and placebo groups. Variables displaying a non-normal distribution (as assessed by the Shapiro-Wilk  $W$  test) were log transformed prior to statistical analyses. Variables for which log-transformation was performed are indicated in the appropriate table, but the untransformed data are reported for clarity (e.g., insulin  $\mu\text{IU/ml}$ ). Repeated-measures ANOVA was used to assess changes in subject characteristics and dependent variables in the ATOR and placebo groups, and independent-sample  $t$ -tests were used to test for differences in the magnitude of change in these variables between groups. Simple correlation analyses were used to assess relations among variables of interest. All of the data are expressed as means  $\pm$ SEs. The significance level was set *a priori* at  $P<0.05$ .

### **Results**

Subject characteristics at baseline and following treatment are shown in Table 1. At baseline, the ATOR and placebo groups did not differ with respect to any of the characteristic variables (all  $P>0.05$ ). There were no changes in body weight or composition during the study. As expected, ATOR treatment resulted in significantly

greater reductions in total (-71±8 versus -14±10 mg/dL) LDL- (-69±8 versus -27±7 mg/dL) and VLDL-cholesterol (-7±2 versus +6±3 mg/dL), and plasma triglycerides (-37±8 versus +31±15 mg/dL ;all  $P<0.001$  time x group interaction) compared with placebo. There was a slight decrease in HDL-cholesterol over time in both groups (-3±1 and -2±1 mg/dL for ATOR and placebo, respectively;  $P<0.05$  for time effect). The magnitude of change in hs-CRP was greater in the ATOR group compared with placebo (-1.5±0.6 versus +0.2±1.1 mg/L;  $P<0.05$ ), but the interaction effect failed to reach significance ( $P=0.998$ ). Systolic blood pressure decreased similarly over time in both groups (-4±2 and -3±2 mmHg for ATOR and placebo, respectively;  $P<0.05$  for time effect), whereas diastolic blood pressure and heart rate remained unchanged (both  $P>0.05$ ).

No adverse events were reported during the study. Although ATOR treatment significantly increased AST (+4±1 IU/L;  $P<0.01$ ), transaminase levels remained well within the normal reference ranges in both groups (data not shown). There were no significant changes in serum creatine kinase levels during the intervention in either group ( $P>0.05$ ). Subject physical activity and dietary intake data are shown in Table 2. There were no changes in habitual physical activity during the study. Dietary intake remained largely unchanged, although sodium intake (-260±297 and -769±375 mg in ATOR and placebo, respectively) and the percent of kcal from alcohol (-0.7±0.3 and -0.5±0.3% in ATOR and placebo, respectively) decreased in both groups similarly over time (both  $P<0.05$  for time effect). There was no difference in the percentage of extra tablets returned by the two groups (97.8±0.5 versus 98.3±0.4% in ATOR and placebo, respectively).

Baseline and post-treatment values for arterial stiffness and compliance are shown in Table 3. There were no differences at baseline between the ATOR and placebo groups with respect to aortic PWV (aPWV), brachial PWV (brPWV), arterial compliance, or  $\beta$ -stiffness index (all  $P>0.05$ ). As hypothesized, reductions in central elastic arterial stiffness, as indicated by reductions in  $\beta$ -stiffness index ( $-1.8\pm 0.4$  versus  $+0.1\pm 0.1$  U;  $P<0.001$ ; Figure 1, *top panel*) and aPWV ( $-163\pm 21$  versus  $-48\pm 34$  cm/sec;  $P<0.001$ ; Figure 2, *top panel*), and increased arterial compliance ( $+0.26\pm 0.07$  versus  $0.00\pm 0.03$  mm<sup>2</sup>/mmHg x 10<sup>-1</sup>;  $P<0.001$ ; Figure 1, *bottom panel*), were greater in the ATOR compared with placebo group. Brachial PWV did not change in either group (both  $P>0.05$ ; Figure 2, *bottom panel*).

In the pooled sample, baseline aPWV and  $\beta$ -stiffness index were correlated with insulin resistance, as assessed by HOMA score ( $r=0.578$  and  $0.502$ , respectively; both  $P<0.05$ ). Additionally, baseline arterial compliance was correlated with percent body fat ( $r=-0.447$ ;  $P<0.05$ ); there was also a trend for a similar relation between percent body fat and  $\beta$ -stiffness index ( $r=0.368$ ;  $P=0.07$ ). The magnitudes of change in aPWV,  $\beta$ -stiffness index and arterial compliance following ATOR treatment were significantly correlated to the magnitude of change in LDL-cholesterol ( $r=0.515$ ,  $0.598$  and  $-0.539$ , respectively; all  $P<0.05$ ), whereas the observed changes in arterial stiffness and compliance were not correlated to the change hs-CRP ( $P>0.05$ ).

## Discussion

The major finding of the present study is that atorvastatin therapy reduces large artery stiffness in overweight and obese middle-aged and older adults. Importantly,

these vascular improvements appeared to occur independently of the hs-CRP lowering effects of atorvastatin.

Although a number of prospective studies have observed reductions in large artery stiffness with statin therapy<sup>42-46</sup>, the results of randomized placebo-controlled studies have been equivocal. The findings of such previous studies have varied greatly and suggest that statin therapy may reduce<sup>29, 30</sup>, increase<sup>32</sup> or have no effect<sup>28, 31, 33</sup> on large artery stiffness. Reasons for these disparate results are unclear, but may include differences in subject populations, duration of treatment, and/or type of statin and doses used. Interpretation of these studies is also confounded by their use of BP-dependent measures of arterial stiffness<sup>30, 32, 33</sup> and methodological flaws (i.e., failure to assess central BP)<sup>31</sup>. In an effort to reconcile these differences we utilized both indirect (aPWV) and direct BP-independent ( $\beta$ -stiffness index) measurements of large artery stiffness, and found that atorvastatin therapy reduced aPWV and  $\beta$ -stiffness index by 15 and 18 percent, respectively.

Results of the present study most closely agree with those of Ferrier et al.<sup>29</sup> who reported a 24 percent increase in systemic arterial compliance following 12 weeks of atorvastatin therapy (80 mg/day) in subjects with isolated systolic hypertension (ISH). Subjects in both studies were similar with respect to age, BMI, and cholesterol levels. It should be emphasized, however, that in our study no differences in the magnitude of vascular improvements with atorvastatin therapy between normo- and prehypertensive (n=11) subjects and those presenting ISH (n=5) were observed. Thus, our findings extend those of Ferrier and colleagues to normotensive and prehypertensive middle-aged and older adults.

There are several important limitations of the study that warrant discussion. First, our sample size was relatively small and the age range was limited to 40-65 years. Thus, larger studies are needed across a broader age range to extend the generalizability of our findings. Second, our treatment period was only 12 weeks. Therefore, we cannot speculate as to whether the observed improvements in arterial stiffness would be greater or persist with long-term treatment and/or confer any clinically relevant reductions in CVD risk. Third, our study was not designed to address the dose response effect of statin therapy on arterial stiffness. However, a previous experiment by Karter et al.<sup>42</sup> demonstrated similar improvements in carotid distensibility and compliance following 12 weeks of atorvastatin treatment using a low (20 mg/day) and high (80 mg/day) regimen. In contrast, the results of previous studies suggest 10 mg/day of atorvastatin may have no influence<sup>47</sup> or even increase arterial stiffness<sup>32</sup>. Collectively, these conflicting findings suggest additional studies are needed to determine the dose response effects of statin therapy on large artery stiffness. Finally, our study was not designed to determine the consequences of arterial destiffening with atorvastatin. Future studies will be necessary to address this important issue.

The mechanisms mediating the large artery destiffening effects of statin therapy remain unclear. The current prevailing view of arterial stiffness, as it relates to central elastic arteries, is predominantly focused on structural alterations of the arterial wall as a consequence of exposure to distending stress cycles over time<sup>3</sup>. In keeping with the progressive decrease in arterial compliance with age, this represents, at some level, an immutable consequence of the aging process. In this context, it is unlikely that any short term intervention (e.g., weight loss, exercise, pharmacological) would result in

favorable structural modifications that 'reverse' material fatigue (i.e., fracture of the load-bearing elastic lamellae). A more plausible explanation for our findings and those of other short term interventions<sup>20, 48, 49</sup> demonstrating reductions in large artery stiffness is that statin therapy improved endothelial function. Support for the direct contribution of endothelial function on large artery stiffness in humans comes from the finding that systemic nitric oxide synthase inhibition (during  $\alpha$ -adrenergic blockade) acutely increases  $\beta$ -stiffness index of the common carotid artery<sup>50</sup>. Numerous experimental and clinical studies suggest that statin therapy improves endothelial function by reducing oxidative stress and improving NO bioavailability<sup>27</sup>. Thus, it is possible that the reductions in large artery stiffness observed in the present study were the result of statin-mediated improvements in endothelial function.

The results of most<sup>51-55</sup>, but not all<sup>50, 56</sup>, studies suggest that large artery compliance is modulated by sympathetically mediated adrenergic smooth muscle tone. In this regard, statin therapy has been demonstrated to reduce sympathetic neural activity in animal models<sup>57-60</sup>. As such, it is possible that the reduction in large artery stiffness with statin therapy in the present study was the result of a reduction in sympathetic neural activity. Future studies will be necessary to address this possibility.

In conclusion, results of the present study suggest that atorvastatin may be an efficacious arterial destiffening therapy in overweight and obese middle-aged and older adults. In light of the present findings, studies regarding the mechanism(s) of arterial destiffening with statin treatment should be a focus of future research. More specifically, further studies will be needed to directly determine whether, and to what

extent, improved endothelial function and/or modulation of SNS activity contributes to large artery destiffening with statin treatment.

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### **Disclosures**

None.

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Table 1. *Subject Characteristics Before and After Treatment*

Variable	Placebo			Atorvastatin		
	n	Pre	Post	n	Pre	Post
Age, y	10	55±3	NA	16	53±2	NA
Body weight, kg	10	94.0±4.0	94.2±4.4	16	89.1±3.1	89.1±3.3
Body mass index, kg/m <sup>2</sup>	10	31.1±0.9	31.1±1.1	16	31.9±0.9	31.8±0.9
Body fat, %	10	38.8±1.8	38.9±1.9	16	42.1±2.5	42.3±2.5
Total fat mass, kg	10	34.4±0.9	34.5±1.0	16	35.8±2.3	35.9±2.3
FFM, kg	10	55.7±3.8	55.8±4.0	16	49.7±3.0	49.4±3.0
Waist circumference, cm	9	107±2	107±3	14	104±2	105±3
Brachial SBP, mmHg	10	127±4	124±4	16	129±4	124±4*
Brachial DBP, mmHg	10	75±2	75±3	16	74±2	73±3
II Triglycerides, mg/dL	10	125±17	156±23	16	123±15	87±11‡
Total cholesterol, mg/dL	10	227±11	213±12	16	211±5	140±8*†‡
II HDL cholesterol, mg/dL	10	43±5	41±5	16	44±3	41±3*
LDL cholesterol, mg/dL	10	162±8	135±10	16	149±6	80±8*†‡
II VLDL cholesterol, mg/dL	10	25±3	31±5	16	25±3	17±2‡
II C-reactive protein, mg/L	8	2.8±0.6	3.0±0.9	16	3.6±0.8	2.1±0.5*§

Glucose, mg/dL	10	101±4	102±3	16	95±3	98±4
II Insulin, µIU/ml	9	13±3	12±2	14	16±4	15±4
HOMA score	9	3.27±0.73	3.04±0.60	14	3.77±0.95	3.72±1.06

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All values are expressed as mean ± SEs. FFM indicates fat-free mass; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA, homeostasis model assessment; NA, not applicable.

Effect of time (\*), group (†); and time x group interaction (‡), P<0.05.

§P<0.10 for interaction effect.

Variables analyzed using log-transformed values are indicated by (II).

Table 2. *Physical Activity and Dietary Intake Before and After Treatment*

Variable	Placebo			Atorvastatin		
	n	Pre	Post	n	Pre	Post
Physical activity, counts/day x 10 <sup>3</sup>	10	252±35	212±29	12	204±24	225±30
Kcal	10	2293±78	2137±176	14	2027±177	1881±228
Fat, %	10	38±2	38±2	14	37±2	39±2
Carbohydrates, %	10	45±2	46±3	14	46±2	44±2
Protein, %	10	17±1	16±1	14	16±1	17±1
Alcohol, %	10	2±1	1±1	14	2±1	2±1*
Cholesterol, mg	10	368±45	380±67	14	269±23	274±30
SFA, g	10	32±3	29±3	14	28±4	26±4
MUFA, g	10	37±2	37±5	14	31±3	33±6
PUFA, g	10	19±2	20±2	14	18±2	19±3
Trans-fatty acids, g	10	6±1	8±1	14	5±1	5±1
Sodium, mg	10	4336±328	3567±463	14	3347±281	3087±341*

All values are expressed as mean ± SEs. SFA indicates saturated fatty-acids; MUFA, monounsaturated fatty-acids; PUFA, polyunsaturated fatty-acids. Effect of time (\*), group (†); and time x group interaction (‡); P<0.05.

Table 3. *Indices of Arterial Stiffness Before and After Treatment*

Variable	n	Placebo		Atorvastatin		
		Pre	Post	n	Pre	Post
Aortic PWV, cm/sec	9	1238±89	1191±90	13	1096±36	932±32*†‡
Brachial PWV, cm/sec	9	981±42	982±36	11	1020±41	1018±41
Arterial compliance, mm <sup>2</sup> /mmHg x 10 <sup>-1</sup>	10	0.96±0.14	0.96±0.12	15	0.89±0.06	1.15±0.10*‡
β-stiffness index, U	10	9.1±0.8	9.1±0.7	15	9.4±0.6	7.6±0.5*‡

All values are expressed as mean ± SEs. PWV indicates pulse wave velocity. Effect of time (\*), group (†); and time x group interaction (‡); P<0.05.

## Figure Legends

**Figure 1.** Top panel: Change in  $\beta$ -stiffness index following atorvastatin treatment.

Bottom panel: Change in arterial compliance following atorvastatin treatment. (\* $P < 0.05$  vs. placebo)

**Figure 2.** Top panel: Change in aortic pulse wave velocity following atorvastatin

treatment. Bottom panel: Change in brachial pulse wave velocity following atorvastatin treatment. (\* $P < 0.05$  vs. placebo)

Figure 1

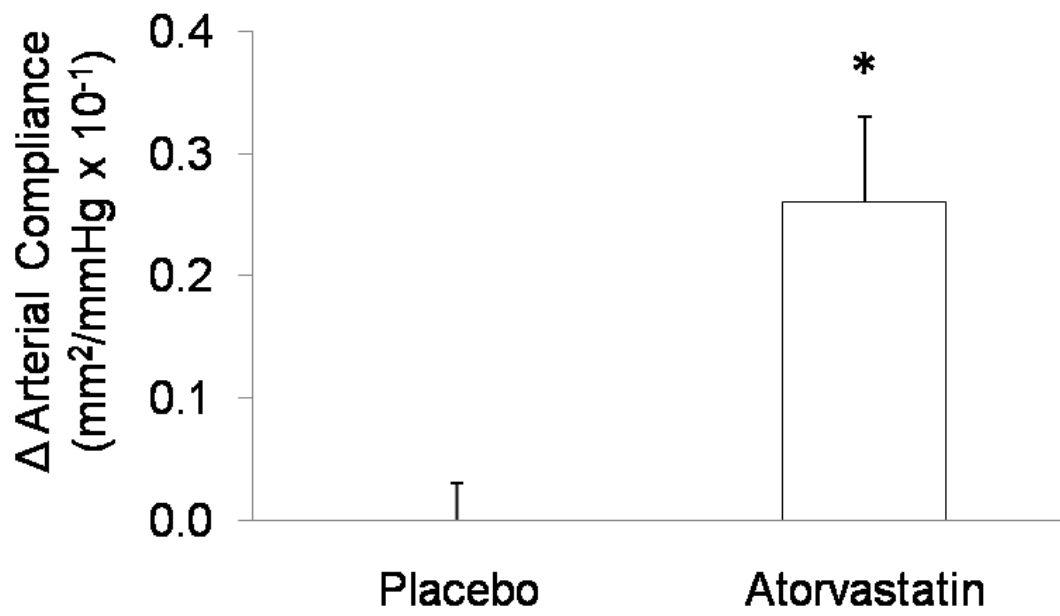
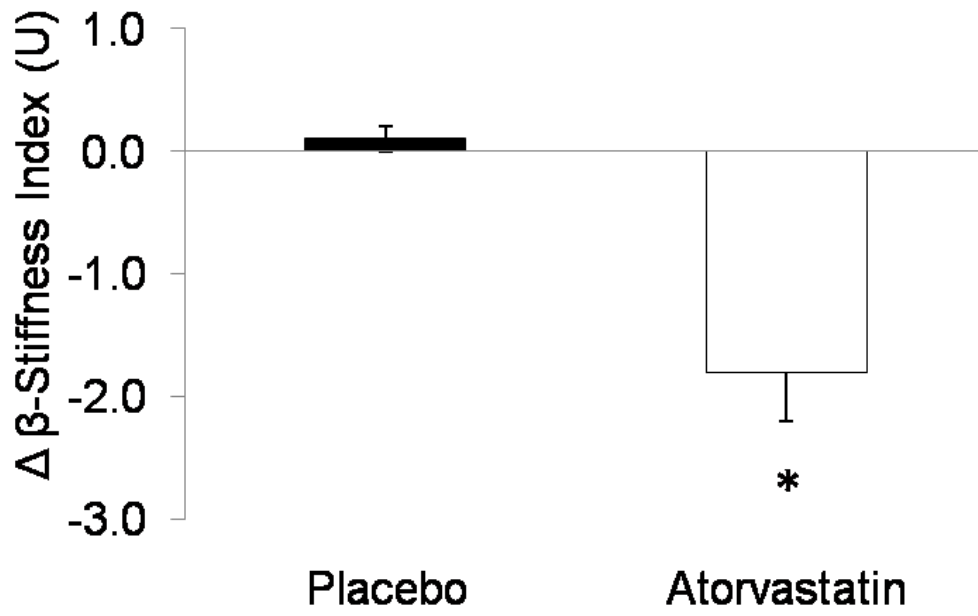
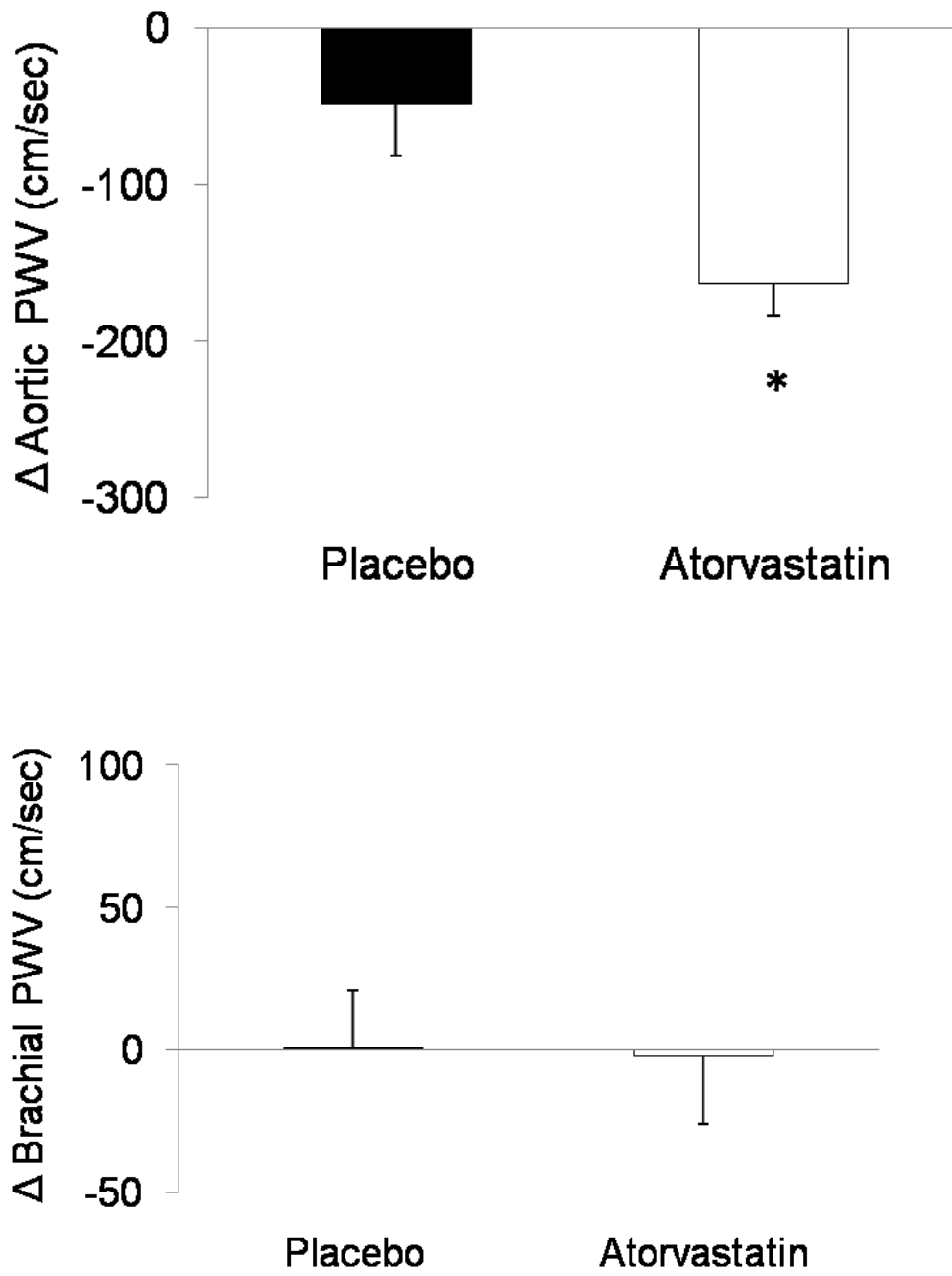


Figure 2



## Chapter 4

### Conclusions

As the US population ages and the prevalence of obesity and abdominal adiposity continue to increase, the burden of the clinical manifestations of large artery stiffening in the cardiothoracic region will undoubtedly rise. As such, the time course of vascular alterations that produce accelerated arterial stiffening in obesity is an important area of research for unraveling the complex pathophysiological mechanisms by which obesity increases CVD disease risk. Such investigations may ultimately lead to the identification of effective strategies to attenuate arterial stiffening in obesity. In light of the current lack of studies regarding the effects of weight gain on arterial stiffness, we conducted a study of the effects of experimental (diet-induced) weight gain on large artery stiffness in nonobese young men. Consistent with our hypothesis, modest weight gain (5 kg) increased arterial stiffness and decreased arterial compliance. Additionally, our study provides the first direct experimental evidence that increases in abdominal visceral fat are associated with large artery stiffening, independent of changes in total body fat.

Age is the primary determinant of stiffness in central elastic arteries, which is compounded by the disproportionate prevalence of obesity and abdominal adiposity in middle-aged and older adults. Thus, identifying effective treatment strategies to reduce large artery stiffness in this population is of clear importance. Statin therapy is effective for the primary prevention of CVD events and mortality. Importantly, these benefits are not completely explained by the LDL-lowering effects of statin therapy. The ability of

statin therapy to reduce arterial stiffness has been advanced as a potential pleiotropic mechanism by which statin therapy reduces CVD risk. Unfortunately, randomized controlled trials have produced inconsistent results regarding the influence of statin therapy on arterial stiffness. These discrepant findings may be explained by the use of blood pressure dependent measures of stiffness and/or methodological flaws. To address these issues, we conducted a randomized placebo-controlled trial, utilizing direct blood pressure independent measurement of large artery stiffness to test the hypothesis that atorvastatin reduces large artery stiffness in overweight and obese middle-aged and older adults. Consistent with our hypothesis, atorvastatin treatment reduced arterial stiffness in overweight and obese middle-aged and older adults.

Our studies provide the experimental basis for several lines of future research. First, the mechanism(s) of arterial stiffening with weight gain and the mediating influence of abdominal fat partitioning remain important areas of future research. Such research should investigate the potential contributions of physiological mediators of large artery stiffness, including endothelial function and the sympathetic nervous system, during weight gain. In addition, future studies should focus on whether aerobic exercise attenuates arterial stiffening with weight gain. Second, the mechanism(s) by which statin therapy reduces large artery stiffness should be a focus of future research. Potential mechanisms influencing large artery stiffness are likely to be similar to those affected by weight gain, as structural alterations are unlikely to result from short term interventions.

In conclusion, weight gain increases large artery stiffness in nonobese young men, and those individuals who experience relatively larger increases in abdominal visceral

fat also demonstrate larger increases in arterial stiffness, independent of total body fat. These findings suggest that the large artery stiffening is a dynamic process, beginning well before the development of obesity, and emphasizes the need for effective measures to prevent weight gain in the population. In addition, statin treatment reduces large artery stiffness in overweight and obese middle-aged and older adults. Importantly, the beneficial vascular effects of statin treatment appear to be independent of its anti-inflammatory effects, suggesting that the effectiveness of statin therapy in primary prevention may be due, in part, to their ability to reduce large artery stiffness. The latter may have important implications for future treatment strategies to reduce large artery stiffness in overweight and obese middle-aged and older adults.

## Appendix A

### Informed Consent for Participants of Investigative Projects

Department of Human Nutrition, Foods and Exercise

Virginia Tech

**TITLE:** Effect of Weight Gain on Muscle and Fat Metabolism

**INVESTIGATORS:** Kevin P. Davy, Ph.D.  
Madlyn I. Frisard, Ph.D.  
Brenda M. Davy, Ph.D., R.D.

**MEDICAL DIRECTOR:** Jose Rivero, M.D.

#### **PURPOSE:**

The amount and location of body fat can influence cardiovascular health and function. The build up of fat in the abdominal region as well as in muscle is associated with an elevated risk of developing high blood pressure, cholesterol and diabetes. The factor(s) influencing the distribution of fat in these areas with weight gain is/are unclear.

#### **METHODS:**

You are being asked to participate in all of the sessions of the study described below. If you agree to participate in this study you will first be required to complete a personal health history questionnaire. The results of your medical history and study tests may be discussed with the study medical director to determine your eligibility. Based on our evaluation, you may then be eligible to become a study subject. Eligible candidates will be non-smoking males or females between the ages of 18 and 40 years who do not have diabetes as assessed by a medical history or the diabetes test. Your body mass index must be less than 30 and your blood pressure also must be less than 135/85 mmHg. You must also be sedentary; this means you must not have been involved in any regular program (>2 days/week and >20 min/day) of aerobic exercise or weight training for the past 12 months. You will not be eligible to participate in the study if you use any medication or nutritional supplement that might influence the study variables. Forty people will be included in this study.

You are being asked to participate in a weight gain group or a weight maintenance group. Your participation in one of these groups will be determined by randomization, a procedure similar to flipping a coin. If you are placed in the weight gain group, you will be asked to increase the amount of calories you eat so that you gain 5 kg (11 lbs) over a 6-8 week period. The length of time required to gain this weight could take longer depending on your individual response. During the weight gain period, the investigators will provide some or all of the food you will need to gain weight. You will be asked to return to War Memorial Hall at Virginia Tech every 1-3 days to be weighed, receive food and return any uneaten food. You will also be asked to record any food you eat that is not provided by the investigators. For a one-month period following the weight gain phase the amount of calories you eat will be adjusted by the investigators so that you maintain the weight you have gained. After completion of the one-month weight maintenance and final testing period, you will be given instructions on how to modify your diet to then reduce the calories you eat. In addition, you will be instructed to increase the calories you burn so that you return to your initial body weight. You will have the additional option to receive a supply of weight loss shakes and meal bars (SLIMFAST or similar product) at no cost to you to assist with your weight loss efforts for the duration of the study. During the weight loss phase of the study you will be asked to come to War Memorial Hall weekly to be weighed and to discuss any problems you may be experiencing with your weight loss program with a dietician.

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If you are placed in the weight maintenance group, you will be asked to maintain your current weight for a period of approximately 10 weeks. You will be asked to report to War Memorial Hall every 1-3 days to be weighed. If your weight increases or decreases over the course of the 10 weeks, you will be instructed on how to increase or decrease the amount of food that you eat to maintain your current body weight.

If you are randomized to the weight gain group, you will be expected to gain ~1-2 lbs per week during the overfeeding phase of the study. You may be dropped from the study if by the end of the third week of the intervention you have not gained at least 4 lbs.

There will be approximately 40 visits if you choose to participate in the study. The actual number and order of visits may depend on your schedule and the availability of the study staff. The order may differ from the order of appearance in this document. You will undergo all of the testing sessions (except for medical history questionnaire) twice, at baseline and following the intervention period.

### Session 1

- **Overnight Fast:** You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require you to collect 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- **Medical History:** You will be asked to complete a medical history questionnaire. This questionnaire is used to screen for health problems or reasons you should not participate in this study. Your height and weight will also be measured at this time. Your body weight will be measured on a standard digital scale. Your height will be measured with a standard stadiometer (ruler on the wall). Your waist, hip, and neck circumference will be measured using a measuring tape.
- **Infection / Inflammation Questionnaire:** You will be asked to complete a questionnaire about any recent illnesses or infections that you may have had in the past month. You will also be asked to complete this questionnaire twice, at baseline and following the intervention period.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an blood pressure monitor.
- **Body Composition:** This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 5 minutes and there is no pain associated with the procedure. This procedure will be performed once at the beginning of the study and a second time at the end of the study. Your weight and height will also be measured at this time.
- **Fasting Blood Glucose:** To test for diabetes, we will measure your fasting blood glucose. This will involve a finger prick to draw a drop of blood. If your glucose level indicates you may have diabetes, you will not be able to continue participation in this study and you will be referred to your personal physician.
- **Physical Activity Questionnaire:** You will be asked a series of questions to estimate your usual physical activity level, which will require about 15 minutes to complete.

### Session 2

- **Overnight Fast:** You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Catheter and Blood Draw:** A small plastic tube will be inserted in your arm to draw blood (approximately 3 tablespoons). We will measure various hormones that influence your

metabolism (how your body burns calories and produces body heat) and cardiovascular system (the heart, blood vessel and lungs).

### Session 3

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours and consuming caffeine containing food or beverages for 24 hours prior to this visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require collection of 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- **Fat Biopsy:** You should not take aspirin, ibuprofen or other non-steroidal, anti-inflammatory medications (such as Advil, Motrin, Celebrex or Vioxx), or other medications or substances that may affect bleeding or bruising, for 72 hours prior and after this procedure. This procedure is used to sample a small amount of fat (about 2-4 g) from underneath the skin from the abdomen. The actual biopsy site will be on either the right or left side of the abdomen just above the level of where you would wear a belt. You will be asked to undergo this procedure twice, once at baseline and once following the intervention. A physician or a nurse will be on site, and available if needed, but may not be present during the procedure itself. This procedure will be performed by a study investigator (Kevin P. Davy, Ph.D.) or co-investigatory (Mathew Hulver, Ph.D.) who has been specifically trained to perform the biopsy. You will be lying down and your skin will be cleansed with iodine-type solution (Providine or Betadine). If you are allergic to iodine, we will use chlorhexadine which does not contain iodine. A sterile drape will be placed over the area and your skin and fat tissue will be numbed by injecting numbing medication (lidocaine/bipivacaine) into the area with a small needle. If you allergic to lidocaine or bipivacaine, you cannot participate in this study. Then, a small incision (about 1/4 of an inch) will be made in the skin and a needle (a little thinner than a pencil) will be placed under the skin to remove a small amount of fat. Some suction may be applied to the other end of the needle to help remove the fat. After the biopsy is completed, pressure will be applied and the skin will be closed with sterile tape. To ensure cleanliness, the skin will be cleaned with saline and covered with gauze and a clear adhesive dressing. The site will then be wrapped with an ACE bandage. You will be asked to keep the ACE bandage on for at least 10-15 minutes. You may take Tylenol for any discomfort you may experience following the biopsy. We will use the biopsy samples to measure factors which contribute to inflammation. The biopsy will take place at Dr. Jose Rivero's medical office in Christiansburg or the Human Integrative Physiology Laboratory (228 War Memorial Hall). Directions will be provided to you.
- **Muscle Biopsy:** You should not take aspirin, ibuprofen or other non-steroidal, anti-inflammatory medications (such as Advil, Motrin, Celebrex or Vioxx), or other medications or substances that may affect bleeding or bruising, for 72 hours prior and after this procedure. This procedure is used to sample a small amount of muscle (about 50-250 mg) from underneath the skin from the thigh. The actual biopsy site will be on the top of either the right or left leg half way between the knee and the hip. You will be asked to undergo this procedure twice, once at baseline and once following the intervention. A physician or a nurse will be on site, and available if needed, but may not be present during the procedure itself. This procedure will be performed by a study investigator (Kevin P. Davy, Ph.D.) or co-investigator (Mathew Hulver, Ph.D.) who has been specifically trained to perform the biopsy. You will be lying down and your skin will be cleansed with iodine-type solution (Providine or Betadine). If you are allergic to iodine, we will use chlorhexadine which does not contain iodine. A sterile drape will be placed over the area and your skin and fat tissue will be numbed by injecting numbing medication (lidocaine/bipivacaine) into the area with a small needle. If you allergic to lidocaine or bipivacaine, you cannot participate in this study. Then, a small incision (about 1/4 of an inch) will be made in the skin and a needle (a little thinner than a pencil) will be inserted to remove a small amount of muscle. Some suction may be applied to the other end of the needle to help remove the muscle. After the biopsy is completed,

pressure will be applied and the skin will be closed with sterile tape. To ensure cleanliness, the skin will be cleaned with saline and will be covered with gauze and a clear adhesive dressing. The site will then be wrapped with an ACE bandage. You will be asked to keep the ACE bandage on for at least 10-15 minutes. You may take Tylenol for any discomfort you may experience following the biopsy. We will use the biopsy samples to measure factors which contribute to inflammation. The biopsy will take place at Dr. Jose Rivero's medical office in Christiansburg or the Human Integrative Physiology Laboratory (228 War Memorial Hall). Directions will be provided to you.

You will be provided with instructions on how to care for the biopsy sites as well as what to look for if a problem were to occur.

### **Take-Home Tests**

- **Physical Activity Monitor:** You will be asked to wear a small monitor to measure your physical activity for one week (7 days). The monitor is slightly larger than a watch and will clip to your belt or waistband and will not interfere with your normal daily activity. You will be asked to wear this monitor 24 hours per day and only remove the monitor when showering or bathing.
- **Diet Records:** To get an idea of what and how much food you eat, you will be asked to record all of the food you eat for 4 days at 4 time points throughout the study.
- **Sodium Excretion:** You will collect all of your urine for a 24-hour period. We will give you a container to bring with you for this purpose. We will measure the amount of salt in your urine. This collection will take approximately 10 minutes of your time over the course of a day. You will be asked to return the container provided to you on the following day.

### **SUMMARY OF SUBJECT RESPONSIBILITIES**

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the investigators of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend all of the scheduled experiment.
- Follow all participant instructions for each session.
- Record any food you eat that has not been provided by the investigators.
- Return any uneaten food that has been provided by the investigators.
- Follow physical activity instructions provided by the investigators.
- Carefully read the instructions on consuming any food provided to you.
- Inform the study investigators if you are pregnant or intend to become pregnant during the study.
- You will be expected to gain ~1-2 lbs per week. You may be dropped from the study if you have not gained 4 lbs. after 3 weeks.

### **RISKS OF PARTICIPATION**

- **Catheter and Blood Draw:** Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this should persist for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.

- HIV/AIDS: Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr. Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.
- Fat and Muscle Biopsies: If you are allergic to lidocaine, you will not be allowed to participate in this study. There may be slight discomfort and burning when the local anesthetic is injected prior to the biopsy, but you are not expected to experience discomfort during the biopsy procedure. Bruising in the area of the fat biopsy for 1-2 weeks will likely occur, but local pressure and ice are applied to the site immediately after the procedure to limit this potential effect and its accompanying tenderness. There is a slight risk of infection at the biopsy site. There is a small risk that you will become lightheaded, dizzy, or anxious before or during the procedures. All of these reactions are temporary and resolve within a short time after completing or stopping the procedure. These risks are minimized by having a trained individual perform the procedure. You will be asked to return to the physiology laboratory within 5 days after the biopsy to have the site checked to ensure proper healing.

You will likely receive a scar from each of the biopsies performed but these are expected to be very small. These scars usually turn a purple color in the weeks to months following the biopsy and then fade considerably over time. The study staff will show you several pictures of examples of the scarring (greater than 1 year old) that can occur following similar biopsy procedures. It is important that you understand that these are just examples of the scarring that can occur. The actual scar you receive may be smaller or larger or differ in coloring. Individuals with darker skin (e.g., African Americans, Hispanics and Asians) tend to scar more than those with lighter skin. You should consider this before you agree to participate.

- DEXA Scan: The amount of radiation that you will receive in the DEXA exam (combined with the CT scan) is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, however the exact increase in such risk is not known.
- Pregnancy: You should not become pregnant during this study because of the exposure to x-rays. If you are capable of having a child you must have a negative pregnancy test before each session that may pose a risk to an embryo or fetus (x-ray exposure or medication injection). You must agree to use an effective method of birth control, such as abstinence, condom use, or use of an IUD to ensure that you will not get pregnant during the study. If you become pregnant during this study, you must notify your study investigator immediately. There may be unforeseen risks to the embryo or fetus in the event that you become pregnant during the study.
- Weight gain: Permanent weight gain increases your risk of developing diabetes and cardiovascular diseases in the future. Temporary weight gain, as you will experience in this study, is not expected to negatively affect your health. Participation in the weight loss intervention after weight gain should return your weight and BP to baseline levels. In addition, body weight returns to normal in most individuals as a result of normal physiological processes that occur over weeks to months. It may be necessary for you to purchase new clothes as a result of the weight gain component of the study. This will be your own financial responsibility and is a potential cost to you that is not covered by the study. You may not be able to easily

lose the weight gained during your participation in the study. You may wish to consider this before agreeing to participate in this study. In addition, if your blood pressure reaches 159/99 (i.e., the level at which blood pressure medicine is recommended), then the overfeeding phase will be terminated and you will receive necessary instructions and assistance with weight loss. You may be permitted to participate in the weight loss aspect of the study if the Medical Director approves. We should emphasize that we anticipate blood pressure increasing only 5-10 mmHg and decreasing by this amount following weight loss. Thus, the need for terminating the overfeeding for this reason is highly unlikely.

- We cannot guarantee that you will lose weight after the weight gain you experience in the present study. However, we have had excellent success in assisting subjects in our previous studies lose weight after weight gain. We will provide you with the information and support you need to modify your diet and physical activity so that you can lose weight. Those who have not lost weight in the past have purposely selected to not to lose the weight gained; these individuals were very lean. In addition, some subjects have elected not to accept our assistance with weight loss and as a result we do not know whether they were successful at losing the weight gained. If you are unable to lose the weight gained and become depressed or feel this has affected how you feel about yourself we will refer you to the Cook Counseling Center if you are student, staff or faculty at Virginia Tech. Otherwise, Dr. Rivero, the medical director of the study, will provide the appropriate referral in the community. It is important for you to know that any cost for these services will be your own responsibility. You should carefully consider this before participating.
- It is not possible to identify all potential risks in an experiential study. However, the study doctors and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory.
- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or study staff.

### **BENEFITS OF PARTICIPATION**

Your participation will provide you with:

- Information on your body composition and aerobic fitness.
- Information on your blood pressure, cholesterol and glucose tolerance

### **COMPENSATION**

You will be compensated \$50 for completing each muscle and fat biopsies at baseline (\$100 total) and \$50 for completing each muscle and fat biopsies at the end of the study (\$100 total). You will receive \$50 if you reach a target weight gain of 5kg. You will receive an additional \$50 if you complete all aspects of the study including all of the testing sessions and report for all weights and food pick up and return. You can receive up to \$ 300 for you participation in the study.

### **CONFIDENTIALITY**

The data from this study will be kept strictly confidential. No data will be released to anyone but those working on the project without your written permission. Data will be identified by subject numbers, without anything to identify you by name. In the event that your exercise test indicates you may have a heart problem, Dr. Rivero or investigators may want to share this information with your doctor but he will request your approval first.

Revised July 18, 2007

### **FREEDOM TO WITHDRAW**

You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. In addition, circumstances could arise which would lead to your exclusion from the study. For example, lack of compliance to instructions, failure to attend testing sessions, inability to gain weight, and illness could be reasons for the researchers to stop your participation in the study. Other reasons include an inability by the researchers to obtain body fat or other measurements that are necessary for the study. You may be able to participate in the study even if you choose not to participate in the muscle and fat biopsies. All of the other sessions are required components.

### **INJURY DURING PARTICIPATION IN THIS STUDY**

Neither the researchers, the University nor Montgomery Regional Hospital have money set aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

### **APPROVAL OF RESEARCH**

This research has been approved, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech. Montgomery Regional Hospital Institutional Review Board has also approved this research. You will receive a copy of this form to take with you.

### **SUBJECT PERMISSION**

I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:

- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486
- Chairman, Institutional Review Board for Research Involving Human Subjects:  
David Moore, (540) 231-4991

Name of Subject (please print)\_\_\_\_\_

Signature of Subject\_\_\_\_\_ Date\_\_\_\_\_



**Office of Research Compliance**  
 Institutional Review Board  
 2000 Kraft Drive, Suite 2000 (0497)  
 Blacksburg, Virginia 24061  
 540/231-4991 Fax 540/231-0959  
 e-mail moored@vt.edu  
 www.irb.vt.edu

FWA00000572( expires 1/20/2010)  
 IRB # is IRB00000667

DATE: July 16, 2008

MEMORANDUM

TO: Kevin P. Davy  
 Brenda M. Davy

FROM: David M. Moore 

Approval date: 7/17/2008  
 Continuing Review Due Date: 6/29/2009  
 Expiration Date: 7/16/2009

SUBJECT: **IRB Full Review Continuation 2:** "Effect of Weight Gain on Muscle and Fat Metabolism", IRB # 06-367

This memo is regarding the above referenced protocol which was previously granted approval by the IRB. The proposed research, having been previously approved at a convened IRB meeting, required full IRB review prior to granting an extension of approval, according to the specifications authorized by 45 CFR 46.110 and 21 CFR 56.110. The above referenced protocol was submitted for full review continuation and approval by the IRB at a recent meeting. Pursuant to your request, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective July 17, 2008.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in previously approved human subject research activities to the IRB, including changes to your study forms, procedures and investigators, regardless of how minor. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.
3. Report promptly to the IRB of the study's closing (i.e., data collecting and data analysis complete at Virginia Tech). If the study is to continue past the expiration date (listed above), investigators must submit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtain re-approval from the IRB before the study's expiration date.
4. If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

cc: File

*Invent the Future*

## Appendix C

Revised December 18, 2007

### **Informed Consent for Participants of Investigative Projects**

**Department of Human Nutrition, Foods and Exercise**

**Virginia Tech**

**TITLE: Arterial Destiffening with Lipitor (Atorvastatin) in Middle-Aged and Older Adults**

**INVESTIGATORS: Kevin P. Davy, Ph.D.  
Brenda M. Davy, Ph.D., R.D.  
Madlyn Frisard, Ph.D.**

**MEDICAL DIRECTOR: Jose Rivero, M.D.**

**FUNDING SOURCE: Pfizer Global Pharmaceuticals, Inc.**

#### **PURPOSE:**

Arterial stiffness, a measure of the flexibility and health of blood vessels, is a strong risk factor for high blood pressure, heart attack and stroke. Arterial stiffness is increased in overweight and obese middle-aged and older adults. Arterial stiffening is made worse in individuals with cardiovascular risk factors. High blood pressure, high blood sugar, and high cholesterol increase arterial stiffness. A low level of inflammation may also contribute to arterial stiffness and fat tissue appears to be a major site of inflammation in overweight and obese individuals.

Lipitor (also called Atorvastatin) is a medication commonly prescribed by doctors to reduce the risk of cardiovascular disease in patients with high cholesterol. The primary purpose of this study is to determine if 14 weeks of Lipitor treatment improves arterial stiffness and heart function in overweight and obese middle-aged and older adults. A second purpose is to determine if Lipitor treatment reduces inflammation in fat tissue.

#### **METHODS:**

You are being asked to participate in all of the sessions of the study described below. If you agree to participate in this study you will first be required to complete a personal health history questionnaire and undergo blood and urine tests. The results of your medical history and blood and urine tests may be discussed with the study medical director to determine your eligibility. Based on our evaluation of the questionnaire and your current health you may then be eligible to become a study subject. Eligible candidates will be non-smoking males or females between 40 and 65 years of age. You will be included in the study if you have a BMI  $\geq 25$  kg/m<sup>2</sup> and have not gained or lost more than 5 lbs in the previous 6 months. You should have a blood pressure that is less than or equal to 159/99 mmHg, glucose that is less than or equal to 125 mg/dl, total cholesterol that is less than 300 mg/dl, and a triglyceride concentration of less than 450 mg/dl. You do not need to have high cholesterol to participate in this study. You must also be sedentary; this means that you must not have been involved in any regular program (>2 days/week and >20 min/day) of aerobic or resistance training for the past 12 months. You will not be eligible to participate in the study if you have had recent surgery, have a past or current history of any diseases (including cardiovascular disease, or liver disease), or use any medication or nutritional supplement that might influence the study variables or make participation unsafe.

You are being asked to participate in either a Lipitor or a placebo group. The Lipitor group will take two 40 mg (total 80 mg, the maximum prescribed for the treatment of high cholesterol) Lipitor pills every day for 14 weeks. The placebo group will take two "sugar" pills (placebo) that look like Lipitor, but do not contain any medicine, every day for 14 weeks. Your group assignment will be determined by randomization, a process similar to flipping a coin. During the study you will not know which pills

you are taking and only one of the investigators will know which group you have been assigned to. This is to ensure that the results of the study are not biased in any way. After the treatment period, you are being asked to participate in follow-up testing, which is a repeat of the baseline testing sessions described below. Forty-eight people will be included in this study.

You are being asked to participate in all of the testing sessions (except Session 2) two times, once at baseline and again after the intervention period. There will be approximately 22 visits if you participate in this study. The actual number and order of visits may depend on your schedule and the availability of the study staff. The session order may differ from the order of appearance in this document.

### Session 1

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require collection of 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- **Medical History:** You will be asked to complete a medical history questionnaire. This procedure is used to screen for health problems or reasons you should not participate in this study.
- **Infection / Inflammation Questionnaire:** You will be asked to complete a questionnaire about any recent illnesses or infections that you may have had in the past month. You will also be asked to complete this questionnaire before the post testing sessions following the intervention period.
- **Body Mass and Height:** Your height and weight will also be measured at this time. Your body weight will be measured on a standard digital scale and will include the weight of light indoor clothing or hospital gown without your shoes. Your waist, hip, and neck circumference will be measured using a measuring tape.
- **Body Composition:** This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 5 minutes and there is no pain associated with the procedure. This procedure will be performed once at the beginning of the study and a second time at the end of the study. Your weight and height will also be measured at this time.
- **Resting Blood Pressure and Heart Rate:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor. Your heart rate, rhythm and overall heart function will be measured using an electrocardiogram.
- **Urine Test:** You will be asked to urinate in a small cup that we provide to you. We will measure the amount of sodium and other electrolytes, glucose, protein, pH and whether there are blood cells present to determine whether it is safe for you to participate in the study.
- **Catheter and Blood Draw:** A small plastic tube (catheter) will be inserted into a large forearm vein to draw blood (approximately 3 tablespoons). The blood collected will be used to measure your blood sugar, cholesterol and other hormones that influence your health.

### Session 2

- **Health and Physical Exam:** The medical director (Jose Rivero, M.D.) will listen to your heart and lungs with a stethoscope, measure your blood pressure and ask you basic questions about your health history.
- **Rest and Exercise Electrocardiogram (ECG):** The electrical activity of your heart (ECG) under resting conditions will be measured using a standard 12-lead electrocardiogram. An exercise ECG will be measured using a graded exercise test. You will be asked to walk on a treadmill at a

self selected speed while the incline of the treadmill is increased for 8-12 minutes. This test will take place at Dr. Jose Rivero's medical office in Christiansburg. Directions will be provided to you.

### Session 3

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Diet Records:** You will be asked to write down everything you eat for a 4-day period (3 consecutive weekdays and 1 weekend day) at the beginning and end of the study. This will be used to determine what and how much you eat on a daily basis.
- **Physical Activity Monitor and Questionnaire:** You will be asked a series of questions to estimate your usual physical activity level, which will require about 15 minutes to complete. You will also be asked to wear a small monitor to measure your physical activity performed during 3 consecutive weekdays (72 hrs) and 1 weekend day (24 hrs). The monitor is slightly larger than a watch and will clip to your belt or waistband and will not interfere with your normal daily activity.

### Session 4

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Ambulatory Blood Pressure:** Your blood pressure will be measured over the course of two entire days (daytime and nighttime) using a cuff placed around your arm and a small computer type device placed around your waist. Your blood pressure will be measured at 15-minute intervals during the daytime and 30-minute intervals at night. You will be asked to stop and remain still while the cuff inflates around your arm. You will wear this cuff and computer type device for an entire day and then return it to War Memorial Hall at the end of that period. You will be asked to record your physical activity and position (sitting, standing, etc.) each time the blood pressure cuff inflates.
- **Return Physical Activity Monitor and Diet Records:** You will be asked to return the diet records and physical activity monitor at this time.

### Session 5:

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. Please also avoid strenuous exercise for 36 hours prior and aspirin or other non-steroidal anti-inflammatory medications (e.g., Tylenol, Ibuprofen) for 72 hours prior to this visit. This is to make sure that the test results will not be influenced by the food you eat, recent exercise or anti-inflammatory medication use.
- **Fat Biopsy:** You should not take aspirin, ibuprofen or other non-steroidal, anti-inflammatory medications (such as Advil, Motrin, Celebrex or Vioxx), or other medications or substances that may affect bleeding or bruising, for 72 hours prior and after this procedure. This procedure is used to sample a small amount of fat (about 2-4 g) from underneath the skin from the abdomen. The actual biopsy site will be on either the right or left side of the abdomen just above the level of where you would wear a belt. You will be asked to undergo this procedure twice, once at baseline and once following the intervention. Neither a physician nor nurse will be present during the procedure. This procedure will be performed by a study investigator (Kevin P. Davy, Ph.D.) or co-investigatory (Mathew Hulver, Ph.D.) who has been specifically trained to perform the biopsy. You will be lying down and your skin will be cleansed with iodine-type solution (Providine or Betadine).

If you are allergic to iodine, we will use chlorhexadine which does not contain iodine. A sterile drape will be placed over the area and your skin and fat tissue will be numbed by injecting numbing medication (lidocaine/bipivacaine) into the area with a small needle. If you are allergic to lidocaine or bipivacaine, you cannot participate in this study. Then, a small incision (about 1/4 of an inch) will be made in the skin and a needle (a little thinner than a pencil) will be placed under the skin to remove a small amount of fat. Some suction may be applied to the other end of the needle to help remove the fat. After the biopsy is completed, pressure will be applied and the skin will be closed with sterile tape. To ensure cleanliness, the skin will be cleaned with saline and covered with gauze and a clear adhesive dressing. The site will then be wrapped with an ACE bandage. You will be asked to keep the ACE bandage on for at least 10-15 minutes. You may take Tylenol for any discomfort you may experience following the biopsy. We will use the biopsy samples to measure factors which contribute to inflammation. The biopsy will take place at Dr. Jose Rivero's medical office in Christiansburg or the Human Integrative Physiology Laboratory (228 War Memorial Hall). Directions will be provided to you.

You will be provided with instructions on how to care for the biopsy site as well as what to look for if a problem were to occur.

### Session 6:

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require collecting approximately 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study.
- **Catheter and Blood Draw:** A small plastic tube (catheter) will be inserted into a large forearm vein to draw blood (approximately 3 tablespoons) and administer drugs. The blood collected will be used to measure your blood sugar and various hormones that affect your health. The plastic tube (catheter) will be left in your arm for the procedure described below. The catheter will remain in your arm for approximately 2-3 hours. A registered nurse will check the catheter frequently over this time period.
- **Arterial Baroreflex Study:** This measures the relationship between changes in blood pressure and the change in heart rate. First, you will be injected with a small amount (100-150 micrograms) of sodium nitroprusside. This drug lowers your blood pressure by causing your blood vessels to dilate (get larger). Sixty seconds later, you will be injected with a small amount (100-150 micrograms) of phenylephrine HCl. This drug raises your blood pressure by causing your blood vessels to constrict (get smaller). The amount of each drug to be injected will begin with a small amount, and may be increased if your blood pressure does not change at least 15 points. These two drugs will be injected a total of three times. A time period of at least 15 minutes will separate each series of injections. There will be a registered nurse present during this testing session.
- **Sympathetic Nervous System Activity:** The measurement of sympathetic nervous system activity involves measuring the activity of one of your nerves on the side of your knee or arm. Two small microelectrodes (small needles) will be placed through your skin. The position of one of the electrodes will be moved back and forth through your skin while a very small electrical impulse (1-2 volts) is passed through the electrode. The needles may be inserted up to an inch below the skin of your leg or arm. This search procedure will continue until the electrode being moved causes your foot or hand to twitch. This procedure will take between 5 – 60 minutes. When a foot or hand twitch is observed, measurement of the activity of the sympathetic nervous system will begin and continue during the procedure described below.

### Session 7:

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Arterial Stiffness:** To measure arterial stiffness, the blood flow and diameter in the arteries in your neck and leg will be measured with an ultrasound machine. An ultrasonic machine is sort-of like radar – a low frequency radio wave that bounces off the tissues and sends a picture back to a “TV-like” screen. A mobile hand unit used will be pressed gently against an artery in your neck and leg. The amount of blood that your heart pumps in one beat and in one minute will be measured with another ultrasound probe. For these measurements, the probe will be pressed gently against two different places on your chest.
- **Heart Size and Function:** The ability of your heart to fill and eject blood will be measured with an ultrasound machine. This will involve placing a mobile hand held unit (probe) on your chest while you are lying on your left side.
- **Return Ambulatory Blood Pressure Monitor:** You will be asked to return the ambulatory blood pressure monitor at this time.

In addition to the above visits, you may be asked to return to have your blood pressure measured. This is to ensure that the values are consistent.

### **SUMMARY OF SUBJECT RESPONSIBILITIES**

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the experimenters of any discomfort or unusual feelings including muscle pain or aches.
- Be on time and attend all of the scheduled testing sessions.
- Follow all participant instructions for each session.
- Maintain your current diet and daily physical activity level.
- Take only the number of tablets of the medicine or placebo pills each day and return the unused portion at scheduled visits.
- Inform the study investigators if you are pregnant or intend on becoming pregnant.

### **RISKS OF PARTICIPATION**

- **Lipitor:** Lipitor is approved by the Food and Drug Administration (FDA) for reduce risk of cardiovascular disease in patients with high cholesterol. It is the most commonly prescribed medication for this purpose, but is not currently approved for reducing arterial stiffness. You should not take Lipitor if you are pregnant, intend to become pregnant, or are nursing. Common side effects of this and similar drugs include constipation, flatulence (gas), upset stomach, but abdominal pain, insomnia, joint pain, arthritis, and muscle aches and pain have also occurred in individuals taking this or similar drugs. The risk of experiencing muscle pain (myalgia) as a result of taking this type of drug is between approximately 3 (2.7%) in 100 compared with approximately 2 (1.5%) in 100 in individuals taking a placebo. In some very rare cases individuals have experienced rhabdomyolysis (severe muscle damage) or liver damage. The National Lipid Association Statin Safety Assessment Task Force recommends that individuals who take any statin should have their liver enzymes checked after 12 weeks of taking the drug. Liver enzymes will be tested before and following week 12 and any abnormal tests will be addressed by Dr. Rivero. If you experience any side effects, pain or have any concerns while participating in the study, you are to immediately contact the P.I., Kevin Davy, Ph.D. (540-231-3487; After hours: 540-230-0486). He will notify Dr. Rivero immediately with instructions for what you should do next. Any subject reporting intolerable muscle pain with or without an elevation in the muscle enzyme, creatine kinase, will be instructed to stop taking the drug and will be required to see Dr. Rivero immediately. Those with tolerable muscle pain without significant elevation in muscle creatine kinase and that can be attributed to another cause (e.g., intense physical exertion) may be allowed to continue. This will be a decision made ultimately by Dr. Rivero but subjects may

choose to discontinue at any time for any reason. You should know that the Pfizer does not provide protection or compensation for injuries or harm that might occur as a result of participation in the present study.

- **Catheter and Blood Draw:** Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200 (0.5%), while the risk of infection or significant blood loss is 1 in 1000 (0.1%). There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.
- **HIV/AIDS:** Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr. Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.
- **Exercise ECG:** Maximal exercise testing may cause fatigue, muscle strains, an irregular heart beat (dysrhythmias) and a change in blood pressure. There is a 0.01% chance of death and a 0.04% risk of heart attack requiring hospitalization.
- **DEXA Scan:** The amount of radiation that you will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however the exact increase in such risk is not known.
- **Pregnancy:** You should not become pregnant during this study because of the exposure to x-rays and study drugs. If you are capable of having a child you must have a negative pregnancy test before each session that may pose a risk to an embryo or fetus (x-ray exposure or medication injection). You must agree to use an effective method of birth control, such as abstinence, condom use, or use of an IUD to ensure that you will not get pregnant. If you become pregnant during this study, you must notify your study investigator immediately. There may be unforeseen risks to the embryo or fetus in the event that you become pregnant.
- **Ambulatory Blood Pressure:** The cuff inflation may cause some discomfort and mild bruising in your arm. The cuff inflation may cause you to have difficulty sleeping.
- **Fat Biopsy:** If you are allergic to iodine, we will use another product called chlorhexidine to clean biopsy site. If you are allergic to lidocaine/bipivacaine, you will not be allowed to participate in this procedure but you may participate in the rest of the study. There may be slight discomfort and burning when the local anesthetic is injected prior to the biopsy, but you are not expected to experience discomfort during the biopsy procedure. Bruising in the area of the fat biopsy for 1-2 weeks will likely occur, but local pressure and ice are applied to the site immediately after the procedure to limit this potential effect and its accompanying tenderness. You should not perform any strenuous exercise for 24 hours following this procedure. You should not take aspirin or ibuprofen for any discomfort you feel for at least 3 days following the procedure. You may use Tylenol as a pain reliever if necessary. You may also apply an ice pack for 20 min every 2 hours

until the pain subsides. If you have pain that lasts longer than one day, you should contact the study investigator immediately. If you have any bleeding from the biopsy site you should contact the study investigator immediately. There is a slight risk of infection at the biopsy site. There is a small risk that you will become lightheaded, dizzy, or anxious before or during the procedures. All of these reactions are temporary and resolve within a short time after completing or stopping the procedure. If you have pain, swelling, redness, pus or foul smelling drainage at the biopsy site with or without a fever, you should contact the study investigator immediately. These risks are minimized by having a trained individual perform the procedure and by using aseptic procedures and sterile instruments. You will be asked to return within 5 days after the biopsy to have the site checked to ensure proper healing.

You will likely receive a scar from each of the biopsies performed but these are expected to be very small. These scars usually turn a purple color in the weeks to months following the biopsy and then fade considerably over time. The study staff will show you several pictures of examples of the scarring (greater than 1 year old) that can occur following similar biopsy procedures. It is important that you understand that these are just examples of the scarring that can occur. The actual scar you receive may be smaller or larger or differ in coloring. Individuals with darker skin (e.g., African Americans, Hispanics and Asians) tend to scar more than those with lighter skin. You should consider this before you agree to participate.

- **Drug Infusions:** Because this procedure requires the insertion of a catheter, the risks here are identical to those described above under catheters. In addition, the infusion of nitroprusside could cause low blood pressure and nausea, sweating or a sudden elevation in heart rate. These feelings should pass within 1-2 minutes. There is an extremely low risk that your blood pressure could drop so low after the nitroprusside injection that you faint. You should know that a physician will not be on site if this were to occur. However, there will be registered nurse on site for all drug infusions. Our emergency plan would involve raising your legs to help blood flow return to your head, calling 911 to activate an emergency response from the Virginia Tech Rescue Squad, and continued monitoring of your blood pressure. The Virginia Tech Rescue Squad would provide rapid response to an emergency call at War Memorial Hall. They would initiate emergency treatment which might include intravenous fluids and additional medications to maintain your blood pressure. You would then be driven by ambulance to the emergency room at the hospital. Any costs for emergency treatment and care will be your (the participant) responsibility. Neither the researchers nor the University have money set aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. However, the amount of nitroprusside used in the present study was selected to lower blood pressure by approximately 15 points or mmHg. This is similar to how much your blood pressure falls when you rise from lying down to a standing position.

The infusion of phenylephrine may result in a headache, restlessness, a sudden decrease in heart rate, and/or rarely an irregular heart beat. These feeling or symptoms, if they occur, usually pass within a few minutes. There is also a small risk that some phenylephrine will leak out from the catheter site causing severe constriction of the surrounding small blood vessels. This may result in an inadequate blood supply to the surrounding tissues and eventual death of that tissue if untreated. If leakage of phenylephrine out of the vein occurs, a doctor may inject another drug called phentolamine into the area to prevent damage to your skin and surrounding tissue. If death of the skin and surrounding tissue does occur skin grafting may be necessary. Placing the catheter in large vein in your arm and making sure that blood can be drawn easily from the catheter reduces this risk. We have performed this procedure over 200 times and this has never occurred. However, if this problem occurs, you will be referred to a physician for immediate treatment. In the event of an emergency, the Virginia Tech Rescue Squad will be contacted.

There is a remote possibility that you may have an allergic reaction to the medications or its vehicle. If this happens during the study, we will call 911 to initiate an emergency response by the Virginia Tech Rescue Squad. They may give you another medication to treat this allergic response and then transport you to the nearest hospital emergency room. It is important to inform us if you have any known medication allergies before you participate. If you have a history of allergies to phenylephrine or nitroprusside you will be not allowed to participate in this aspect of the study. If you have an allergic reaction to the medications during the study, you will not be allowed to participate further in this aspect of the study.

These risks are slightly increased because we will repeat both drug infusions two times. It should be emphasized that the amount of these drugs you will be receiving is very small and rapidly broken down and eliminated by your body. This lowers the risk of any adverse reactions to the drugs. The principal investigator has performed over 150 of these tests with no adverse events.

- Sympathetic Nervous System Activity: Some subjects experience a temporary (seconds) pain and discomfort while the microelectrodes are being inserted into the skin. After the procedure, there is a small risk of numbness, "pins and needles"-type of sensation, or pain that may last 1-3 days. In very rare cases, numbness, pins and needles type sensations, or pain in the leg or arm has lasted several weeks or months (1 to 3 in 1000 or 0.1 to 0.3%). It is also possible that permanent nerve damage could occur. The principal investigator of this project has performed this procedure over 300 times and only one individual (0.33%) has experienced pins and needles sensations for 7 to 10 days. All of these problems can be minimized by only having experienced individuals perform this technique. In addition, by minimizing the time to find the nerve to less than 60 minutes, the risk of unpleasant after-effects is reduced even more.
- It is not possible to identify all potential risks in an experimental study; however the study investigators and staff will take all possible safeguards to minimize any known and potential risks to your well-being.
- Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or the study staff.

### **BENEFITS OF PARTICIPATION**

You will receive the following as part of your participation:

- Health and physical examination by a physician.
- Exercise stress test with electrocardiogram by a physician and trained technicians.
- Information on your blood pressure, cholesterol and other risk factors for cardiovascular disease.
- Information on your fitness level.
- Information on healthy lifestyle habits including diet and exercise.

### **COMPENSATION**

You will receive \$50 if you complete the baseline testing and another \$150 if you complete the study. In addition, you will receive an additional \$50 for completing each fat biopsy (one at the beginning and one at the end for a total of \$100). The total amount you may receive for the study is \$300.

### **CONFIDENTIALITY**

The data from this study will be kept strictly confidential. In the event that your exercise test or blood tests indicates you may have a health problem, Dr. Rivero and the investigators may want to share this information with your doctor, but he will request your approval first. The Food and Drug Administration requires that your identifying information be released to them in the event that you are injured as a result of participating in this study. This information may also be shared with Pfizer. The Food and Drug Administration and Pfizer will hold your identify in strict confidence to the fullest extent

Revised December 18, 2007

of the law. For all other situations, your study information will be identified only by a code of numbers and letters, without anything to identify you by name.

**FREEDOM TO WITHDRAW**

You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. Circumstances may come up that the researcher will determine that you should not continue as a subject in the study. For example, lack of compliance to instructions, failure to take the medication, and illness could be reasons for the researchers to stop your participation in the study.

**INJURY DURING PARTICIPATION IN THIS STUDY**

Neither the researchers nor the University have money set aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

**REVIEW OF RESEARCH**

This research protocol has been submitted to and reviewed by the Virginia Tech Institutional Review Board For Research Involving Human Subjects, and was found to meet the requirements set forth in federal laws and regulations governing the protection of human subjects. You will receive a copy of this form to take with you.

**SUBJECT PERMISSION**

I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:

- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486
- Chairman, Institutional Review Board for Research Involving Human Subjects: David Moore, (540) 231-4991

Name of Subject (please print)\_\_\_\_\_

Signature of Subject\_\_\_\_\_ Date\_\_\_\_\_



**Office of Research Compliance**  
 Institutional Review Board  
 2000 Kraft Drive, Suite 2000 (0497)  
 Blacksburg, Virginia 24061  
 540/231-4991 Fax 540/231-0959  
 e-mail moored@vt.edu  
 www.irb.vt.edu

FWA00000572( expires 1/20/2010)  
 IRB # is IRB00000667

DATE: July 16, 2008

MEMORANDUM

TO: Kevin P. Davy  
 Brenda M. Davy

FROM: David M. Moore 

Approval date: 7/17/2008  
 Continuing Review Due Date: 6/29/2009  
 Expiration Date: 7/16/2009

SUBJECT: **IRB Full Review Continuation 2: "Arterial Destiffening with Lipitor in Middle Aged and Older Adult with the Metabolic Syndrome"**, IRB # 06-368

This memo is regarding the above referenced protocol which was previously granted approval by the IRB. The proposed research, having been previously approved at a convened IRB meeting, required full IRB review prior to granting an extension of approval, according to the specifications authorized by 45 CFR 46.110 and 21 CFR 56.110. The above referenced protocol was submitted for full review continuation and approval by the IRB at a recent meeting. Pursuant to your request, I, as Chair of the Virginia Tech Institutional Review Board, have, at the direction of the IRB, granted approval for this study for a period of 12 months, effective July 17, 2008.

Approval of your research by the IRB provides the appropriate review as required by federal and state laws regarding human subject research. As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in previously approved human subject research activities to the IRB, including changes to your study forms, procedures and investigators, regardless of how minor. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.
3. Report promptly to the IRB of the study's closing (i.e., data collecting and data analysis complete at Virginia Tech). If the study is to continue past the expiration date (listed above), investigators must submit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtain re-approval from the IRB before the study's expiration date.
4. If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

cc: File

*Invent the Future*

# Large Artery Stiffening With Weight Gain in Humans

## Role of Visceral Fat Accumulation

Jeb S. Orr, Christopher L. Gentile, Brenda M. Davy, Kevin P. Davy

**Abstract**—We tested the hypothesis that weight gain would increase arterial stiffness in healthy nonobese adults. To address this, we overfed 14 nonobese men (age:  $23 \pm 1$  years)  $\approx 1000$  kcal/d for 6 to 8 weeks until a 5-kg weight gain was achieved. Carotid diameters (high-resolution ultrasound) and pressures (applanation tonometry), body composition (dual energy x-ray absorptiometry), and abdominal fat distribution (computed tomography) were measured at baseline and following 4 weeks of weight stability at each individual's elevated body weight. Overfeeding increased body weight  $5.1 \pm 0.1$  kg and body fat  $3.4 \pm 0.4$  kg (both  $P < 0.001$ ) in  $45 \pm 7$  days. Total abdominal fat increased  $46 \pm 7$  cm<sup>2</sup> with weight gain due to increases in both subcutaneous ( $30 \pm 6$  cm<sup>2</sup>) and visceral fat ( $15 \pm 4$  cm<sup>2</sup>; all  $P < 0.01$ ). As hypothesized, weight gain increased arterial stiffness  $13 \pm 6\%$  and decreased arterial compliance  $21 \pm 4\%$  (both  $P < 0.05$ ). Furthermore, those individuals above the median increase in abdominal visceral fat demonstrated a significantly greater increase in arterial stiffness ( $0.97 \pm 0.29$  versus  $0.06 \pm 0.36$  U;  $P < 0.05$ ) compared with those below the median. Consistent with these observations, the only correlates of the changes in arterial stiffness with weight gain were the increases in total abdominal fat ( $r = 0.794$ ), abdominal visceral fat ( $r = 0.651$ ), and waist circumference ( $r = 0.470$ ; all  $P < 0.05$ ). Taken together, these findings suggest that modest weight gain is associated with increases arterial stiffness in nonobese men. The degree of large artery stiffening with weight gain seems to be determined, in part, by the amount of abdominal visceral fat gain. Importantly, this relation is independent of the amount of total body fat gained. (*Hypertension*. 2008;51:1519-1524.)

**Key Words:** arterial distensibility ■ adiposity ■ obesity ■ pulse pressure ■ hypertension

Approximately 65% of the US population<sup>1</sup> and >1 billion people worldwide<sup>2</sup> are overweight or obese and, thus, at increased risk for cardiovascular diseases.<sup>3</sup> There is considerable heterogeneity in the risks associated with excess adiposity; the accumulation of abdominal visceral fat seems to be particularly deleterious.<sup>4,5</sup> Importantly, elevated abdominal visceral fat, independent of total body adiposity, is associated with the development of cardiovascular diseases.<sup>4,5</sup>

One mechanism by which the cardiovascular complications associated with obesity may be advanced is through remodeling of the vasculature. The results of numerous studies suggest that obesity is associated with the stiffening of arteries in the cardi thoracic region.<sup>6–12</sup> Importantly, large artery stiffness is associated with adverse cardiovascular outcomes,<sup>13–16</sup> which occur more frequently in obese individuals.<sup>3,4</sup> The relation between adiposity and arterial stiffness is evident even in young children,<sup>17,18</sup> suggesting that long-duration obesity is not a prerequisite for arterial stiffening. Unfortunately, the available data, from observational studies of weight gain, are inconsistent.<sup>19,20</sup> In addition, measure-

ments of body composition were not included in these previous studies. Furthermore, it is currently unknown whether increases in abdominal visceral fat with weight gain are associated with larger artery stiffening, independent of increases in total body fat. Therefore, we tested the hypothesis that modest weight gain would increase arterial stiffness in healthy nonobese adults. We further sought to determine whether those individuals who demonstrate the largest increases in abdominal visceral fat, independent of total body fat gain, would also demonstrate the largest increases in arterial stiffness.

## Materials and Methods

### Subjects

Fourteen young (age:  $23 \pm 1$  years), nonobese (body mass index:  $< 30$  kg/m<sup>2</sup>) men included in previous publications<sup>21,22</sup> were studied. Subjects were normotensive, free from overt chronic disease, non-smokers, and not taking any medications. All of the subjects were weight stable ( $\pm 2$  kg) for  $\geq 6$  months before entry into the study. The Virginia Tech Human Subjects Committee approved all of the experimental protocols. The nature, purpose, risks, and benefits of

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From the Human Integrative Physiology Laboratory, Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg.

Correspondence to Kevin P. Davy, Human Integrative Physiology Laboratory, Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. E-mail kdavy@vt.edu

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the study were explained to all of the subjects before obtaining informed consent.

### Experimental Design and Protocol

After baseline measurements, subjects were overfed  $\approx 1000$  kcal/d for 6 to 8 weeks until a 5-kg weight gain was achieved. Subjects were provided with a liquid meal replacement supplement (Boost Plus, Novartis Nutrition Corp; 35% fat, 50% carbohydrates, and 15% protein) to meet their excess energy requirements. To avoid the potential effects of acute energy imbalance on the primary outcome variables, baseline measurements were repeated on each subject after a 4-week period of weight stability at their elevated body weight. Subjects underwent weekly assessment by a research dietician (B.M.D) throughout weight gain and weight stability phases to ensure adequate progress and compliance. Subjects reported to the Virginia Tech Human Integrative Physiology Laboratory between 7 and 11 AM after a 12-hour fast and having refrained from caffeine and exercise for 24-hours before each testing session. After post testing, subjects were provided with dietary counseling, physical activity recommendations, and, if desired, meal replacement products to facilitate return to their baseline body weight.

### Measurements

Body mass and height were measured with a digital scale and stadiometer (Scale-Tronix model 5002), respectively. Dual-energy x-ray absorptiometry (GE Lunar Prodigy Advance, software version 8.10e) was used to determine body composition. Computed tomography scans (HiSpeed CT/i, GE Medical) were taken between the L3-L4 vertebra, and abdominal fat distribution was quantified using commercially available analysis software (Slice-O-Matic 4.3 Rev-4, Tomovision Inc). Maximal oxygen consumption was measured during a graded treadmill exercise to volitional exhaustion using open-circuit spirometry (TrueMax 2400, ParvoMedics). Standard criteria for the achievement of valid maximal oxygen consumption were met.<sup>23</sup> Casual blood pressure was measured over a brachial artery via mercury sphygmomanometry after 15 minutes of seated rest in a quiet room. Measurements were repeated until within-session stability was achieved ( $\pm 6$  mm Hg on 3 sequential measurements) and on  $\geq 3$  separate occasions over a 2-week period until between-session stability was reached. Resting heart rate was determined from lead II of an ECG. Plasma lipid and lipoprotein concentrations were measured via nuclear magnetic resonance spectroscopy (LipoScience, Inc), as described previously.<sup>24</sup>

$\beta$ -Stiffness index<sup>25</sup> and arterial compliance were assessed by combining simultaneous measurements of carotid artery diameter and blood pressure over 3 consecutive cardiac cycles via B-mode ultrasonography and applanation tonometry, respectively. After 10 minutes of quiet rest in a supine position, arterial blood pressure was measured over a brachial artery via automated sphygmomanometry (Pilot 9200, Colin Medical Instruments) until stability was achieved (3 consecutive readings:  $\pm 6$  mm Hg). Subsequently, common carotid artery diameters were obtained with an ultrasound unit (Sonos 7500, Philips Medical Systems) equipped with a high-resolution linear array transducer (3 to 11 MHz). Longitudinal B-mode images of the cephalic portion of the common carotid artery were obtained 1 to 2 cm proximal to the carotid bulb. The transducer was placed 90° to the vessel so that near and far walls were clearly visible. The images were stored to optical disk for quantification of carotid artery diameters offline using commercially available analysis software (Vascular Research Tools 5, Medical Imaging Applications, LLC). The distance between the near and far wall vessel boundaries were measured at time points that corresponded with the maximal systolic and minimal diastolic diameters. Throughout the imaging process, pressure waveforms were obtained from the contralateral carotid artery using a high-fidelity noninvasive pulse tonometer (SPT-301, Millar Instruments) and digitized at 500 Hz for subsequent analysis using signal processing software (Windaq, Dataq Instruments). Carotid pressure waveforms were corrected for hold-down pressure by calibration to brachial diastolic and mean arterial pressures.<sup>26</sup> The reproducibility of measurements of the  $\beta$ -stiffness index in our laboratory is excellent ( $r=0.90$ ;  $P<0.05$ ).

**Table 1. Subject Characteristics at Baseline and After Weight Gain**

Variable	Baseline	Weight Gain
Age, y	23 $\pm$ 1	NA
Body weight, kg	75.3 $\pm$ 2.9	80.3 $\pm$ 3.0*
Body mass index, kg/m <sup>2</sup>	24.0 $\pm$ 0.7	25.7 $\pm$ 0.7*
Body fat, %	21.3 $\pm$ 1.2	24.6 $\pm$ 1.1*
Total fat mass, kg	15.5 $\pm$ 1.1	18.9 $\pm$ 1.1*
FFM, kg	56.7 $\pm$ 2.3	58.2 $\pm$ 2.5*
Waist circumference, cm	84.9 $\pm$ 1.9	90.5 $\pm$ 1.7*
Total abdominal fat, cm <sup>2</sup>	226 $\pm$ 19	271 $\pm$ 20*
Abdominal subcutaneous fat, cm <sup>2</sup>	160 $\pm$ 13	190 $\pm$ 11*
Abdominal visceral fat, cm <sup>2</sup>	66 $\pm$ 8	81 $\pm$ 11*
VO <sub>2</sub> max, mL/kg/min	44.7 $\pm$ 1.8	43.8 $\pm$ 1.5*
VO <sub>2</sub> max, L/min	3.4 $\pm$ 0.2	3.5 $\pm$ 0.2
VO <sub>2</sub> max, mL/kg FFM/min	60.7 $\pm$ 2.0	60.1 $\pm$ 1.6
Heart rate, bpm	62 $\pm$ 2	65 $\pm$ 3
Systolic blood pressure, mm Hg	114 $\pm$ 2	119 $\pm$ 2*
Diastolic blood pressure, mm Hg	75 $\pm$ 2	75 $\pm$ 2
Triglycerides, mg/dL	92 $\pm$ 17	135 $\pm$ 31*
Total cholesterol, mg/dL	134 $\pm$ 6	148 $\pm$ 8*
HDL cholesterol, mg/dL	40 $\pm$ 2	40 $\pm$ 2
LDL cholesterol, mg/dL	80 $\pm$ 6	89 $\pm$ 8

All values are expressed as means $\pm$ SEs. VO<sub>2</sub>max indicates maximal oxygen consumption; FFM, fat-free mass; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable.

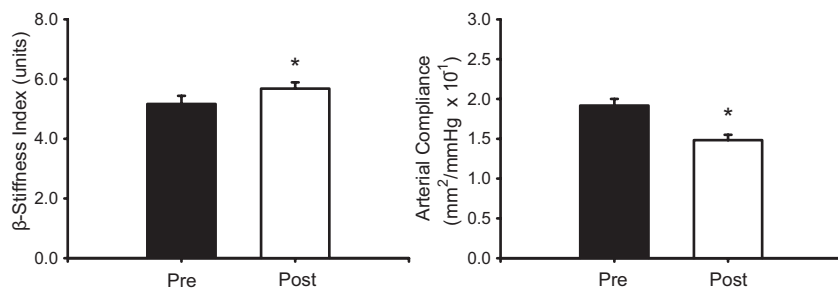
\* $P<0.05$  vs baseline.

### Statistical Analysis

Differences in subject characteristics and dependent variables before and after weight gain were assessed with paired Student *t* tests. To gain better insight into the relation between abdominal fat distribution and arterial compliance and stiffness, subjects were subsequently divided into 2 groups (smaller increase in abdominal visceral fat [SVF] and larger increase in abdominal visceral fat [LVF]) based on the median split of abdominal visceral fat change with weight gain. Repeated-measures ANOVA was used to assess changes in subject characteristics and dependent variables in the SVF and LVF groups with weight gain. Differences in the magnitude of change in subject characteristics and dependent variables with weight gain between the 2 groups were assessed with independent-sample *t* tests. Comparisons performed using Wilcoxon signed-rank tests yielded similar outcomes. Relations among variables of interest were assessed using simple correlation analyses. All of the data are expressed as means $\pm$ SEs. The significance level was set a priori at  $P<0.05$ .

### Results

Subject characteristics at baseline and after weight gain are shown in Table 1. Overfeeding resulted in 5.1 $\pm$ 0.1 kg (range: 4.2 to 5.8 kg) of body weight gain in 45 $\pm$ 7 days. Body fat (3.4 $\pm$ 0.4 kg), percent body fat (3.2 $\pm$ 0.6%), lean body mass (1.5 $\pm$ 0.4 kg), and waist circumference (5.6 $\pm$ 0.6 cm) increased with weight gain (all  $P<0.001$ ). Total abdominal fat (46 $\pm$ 7 cm<sup>2</sup>) increased due to increases in both visceral (15 $\pm$ 4 cm<sup>2</sup>) and subcutaneous fat (30 $\pm$ 6 cm<sup>2</sup>) depots (all  $P<0.01$ ). There was a small, but significant, reduction in maximal oxygen consumption expressed relative to body weight with weight gain. However, maximal oxygen consumption ex-



**Figure 1.** Left,  $\beta$ -Stiffness index at baseline and following weight gain. Right, Arterial compliance at baseline and after weight gain (\* $P < 0.05$  vs baseline).

pressed in absolute terms and relative to fat-free mass did not change. Systolic blood pressure ( $5 \pm 1$  mm Hg;  $P < 0.01$ ) but not diastolic blood pressure increased with weight gain, whereas resting heart rate tended to increase ( $3 \pm 2$  bpm;  $P = 0.056$ ). Regarding traditional cardiovascular risk factors, only total cholesterol and triglyceride concentrations increased significantly after weight gain ( $15 \pm 5$  and  $43 \pm 16$  mg/dL, respectively). As hypothesized, arterial stiffness increased ( $0.51 \pm 0.26$  U;  $P < 0.05$ ; Figure 1, left), and arterial compliance decreased ( $0.43 \pm 0.09$  mm<sup>2</sup>/mm Hg  $\times 10^{-1}$ ;  $P < 0.001$ ; Figure 1, right) with weight gain. Relative to baseline values, this represents a change in arterial stiffness and arterial compliance of  $+13 \pm 6$  and  $-21 \pm 4\%$ , respectively (both  $P < 0.05$ ).

To gain further insight into the relation between abdominal fat distribution and arterial stiffness (and compliance), we divided the subjects into 2 groups (SVF and LVF) based on the median change in abdominal visceral fat with weight gain. Subject characteristics and arterial stiffness (and compliance) of the SVF and LVF groups at baseline and following weight gain are shown in Table 2. At baseline, the 2 groups did not differ with respect to age, body weight, body composition, abdominal fat distribution, maximal oxygen consumption, heart rate, blood pressure, arterial stiffness, or arterial compliance (all  $P > 0.05$ ). In contrast, LVF had higher concentrations of total cholesterol, LDL-C, and triglycerides compared with SVF (all  $P < 0.05$  for group effect). Despite gaining a slightly lesser amount of body weight ( $4.8 \pm 0.2$  versus

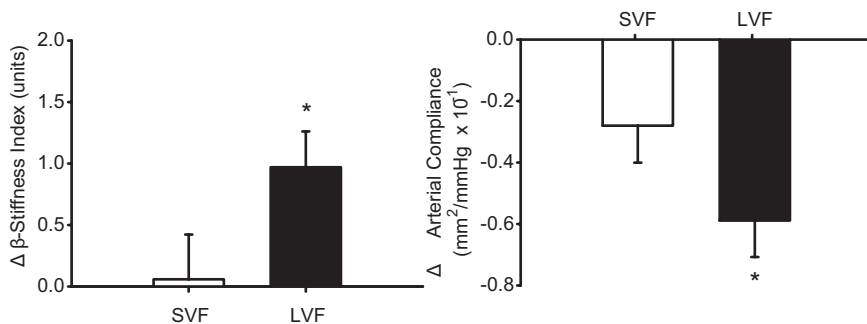
**Table 2. Subject Characteristics in Individuals With SVF Versus LVF**

Variable	SVF (n=7)		LVF (n=7)	
	Baseline	Weight Gain	Baseline	Weight Gain
Age, y	21.3 $\pm$ 1.0	NA	24.9 $\pm$ 2.3	NA
Body weight, kg	78.2 $\pm$ 3.9	83.5 $\pm$ 4.0	72.3 $\pm$ 4.4	77.1 $\pm$ 4.5*
Body mass index, kg/m <sup>2</sup>	23.9 $\pm$ 0.9	25.5 $\pm$ 0.9	24.2 $\pm$ 1.0	25.9 $\pm$ 1.0*
Body fat, %	20.0 $\pm$ 1.4	23.0 $\pm$ 1.6	22.7 $\pm$ 1.9	26.1 $\pm$ 1.4*
Fat mass, kg	15.1 $\pm$ 1.5	18.4 $\pm$ 1.7	15.9 $\pm$ 1.8	19.4 $\pm$ 1.6*
FFM, kg	59.8 $\pm$ 2.9	61.5 $\pm$ 3.2	53.5 $\pm$ 3.3	54.8 $\pm$ 3.5*
Waist circumference, cm	83.8 $\pm$ 2.4	89.3 $\pm$ 2.4	85.9 $\pm$ 3.0	91.8 $\pm$ 2.5*
Total abdominal fat, cm <sup>2</sup>	200 $\pm$ 21	233 $\pm$ 24	251 $\pm$ 31	310 $\pm$ 24*
Abdominal subcutaneous fat, cm <sup>2</sup>	143 $\pm$ 14	173 $\pm$ 16	177 $\pm$ 21	207 $\pm$ 13*
Abdominal visceral fat, cm <sup>2</sup>	57 $\pm$ 9	60 $\pm$ 10	75 $\pm$ 12	102 $\pm$ 15*‡
VO <sub>2</sub> max, mL/kg/min	45.9 $\pm$ 3.0	43.9 $\pm$ 2.3	43.6 $\pm$ 2.1	43.6 $\pm$ 2.2*
VO <sub>2</sub> max, L/min	3.6 $\pm$ 0.3	3.7 $\pm$ 0.3	3.2 $\pm$ 0.3	3.3 $\pm$ 0.3
VO <sub>2</sub> max, mL/kg FFM/min	61.3 $\pm$ 3.9	59.4 $\pm$ 2.5	60.3 $\pm$ 1.7	60.7 $\pm$ 2.4
Heart rate, bpm	62 $\pm$ 3	63 $\pm$ 5	62 $\pm$ 3	67 $\pm$ 4
Systolic blood pressure, mm Hg	115 $\pm$ 3	120 $\pm$ 3	114 $\pm$ 4	118 $\pm$ 4*
Diastolic blood pressure, mm Hg	73 $\pm$ 3	74 $\pm$ 4	76 $\pm$ 2	77 $\pm$ 3
Triglycerides, mg/dL	58 $\pm$ 8	84 $\pm$ 14	133 $\pm$ 26	196 $\pm$ 56*†
Total cholesterol, mg/dL	120 $\pm$ 5	128 $\pm$ 6	150 $\pm$ 6	172 $\pm$ 4*†
HDL cholesterol, mg/dL	41 $\pm$ 1	42 $\pm$ 4	38 $\pm$ 3	37 $\pm$ 1
LDL cholesterol, mg/dL	68 $\pm$ 6	70 $\pm$ 6	94 $\pm$ 6	111 $\pm$ 8†
Arterial compliance, mm <sup>2</sup> /mm Hg $\times 10^{-1}$	1.85 $\pm$ 0.12	1.57 $\pm$ 0.09	1.98 $\pm$ 0.12	1.39 $\pm$ 0.09*§
$\beta$ -Stiffness index, U	5.4 $\pm$ 0.2	5.4 $\pm$ 0.3	5.0 $\pm$ 0.5	5.9 $\pm$ 0.3*§

All values are expressed as means $\pm$ SEs. VO<sub>2</sub>max indicates maximal oxygen consumption; FFM, fat-free mass; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable.

Effect of time (\*), group (†), and time $\times$ group interaction (‡),  $P < 0.05$ .

§ $P = 0.07$  to  $0.10$  for interaction effect.



**Figure 2.** Left, Change in  $\beta$ -stiffness index following weight gain in subjects with smaller and larger increases in visceral fat. Right, Change in arterial compliance following weight gain in subjects with smaller and larger increases in visceral fat (\* $P < 0.05$  vs SVF).

$5.3 \pm 0.2$  kg,  $P = 0.04$ ), the LVF group experienced a significantly greater increase in total abdominal fat ( $58 \pm 10$  versus  $33 \pm 7$   $\text{cm}^2$ ) due to greater visceral fat ( $28 \pm 5$  versus  $3 \pm 2$   $\text{cm}^2$ ) accumulation with weight gain (both  $P < 0.05$ ). The increase in abdominal subcutaneous fat ( $30 \pm 5$  versus  $30 \pm 11$   $\text{cm}^2$ ;  $P > 0.05$ ) with weight gain was not different in the 2 groups. Consistent with our hypothesized association between abdominal visceral fat and arterial stiffness, the LVF group experienced a significantly greater increase in arterial stiffness ( $0.97 \pm 0.29$  versus  $0.06 \pm 0.36$  U;  $P < 0.05$ ; Figure 2, left) and decrease in arterial compliance ( $-0.589 \pm 0.119$  versus  $-0.280 \pm 0.120$   $\text{mm}^2/\text{mmHg} \times 10^{-1}$ ;  $P < 0.05$ ; Figure 2, right) compared with SVF. The magnitudes of change in body fat, fat-free mass, maximal oxygen consumption, systolic and diastolic blood pressure, and plasma lipid and lipoprotein concentrations did not differ between the 2 groups (all  $P > 0.05$ ).

In the pooled sample, the magnitude of change in abdominal visceral fat with weight gain was correlated with the magnitude of change in arterial stiffness ( $r = 0.651$ ;  $P < 0.05$ ) and arterial compliance ( $r = -0.589$ ;  $P < 0.05$ ; Figure 3). The magnitude of increase in total abdominal fat and waist circumference with weight gain were also correlated with the increases in arterial stiffness ( $r = 0.794$  and  $0.470$ , respectively; both  $P < 0.05$ ) and the decreases in arterial compliance ( $r = -0.765$  and  $-0.496$ , respectively; all  $P < 0.05$ ). Furthermore, baseline values of arterial stiffness and compliance correlated with the magnitude of their respective changes with weight gain ( $r = 0.683$  and  $r = -0.711$ , respectively; both  $P < 0.05$ ). There were no other significant correlations.

## Discussion

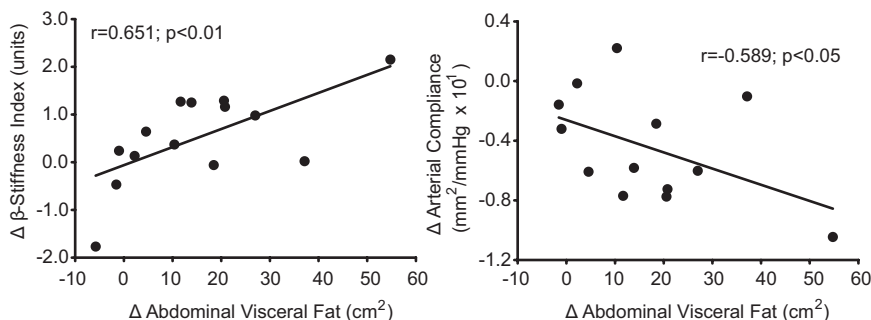
The major new finding of the present study is that modest diet-induced weight gain results in increases in large artery stiffness in healthy young adult men. Those individuals with

relatively larger increases in abdominal visceral fat demonstrated correspondingly larger increases in arterial stiffness and greater reductions in arterial compliance. Importantly, the adverse effect of abdominal visceral fat accumulation on arterial stiffness and compliance occurred independent of the amount of total body fat gained.

The results of previous prospective studies regarding the determinants of arterial stiffening are inconsistent with respect to the role of weight gain. Wildman et al<sup>20</sup> found weight gain over a 2-year period to be associated with an increase in pulse-wave velocity among healthy young adults whereas Benetos et al<sup>19</sup> failed to support such an association. The reasons for these disparate findings remain unclear, but may be explained, in part, by the inclusion of older subjects ( $> 50$  years) by Benetos et al.<sup>19</sup> Nonetheless, our current findings confirm and extend the findings of Wildman et al<sup>20</sup> by demonstrating that experimental weight and fat gain, particularly in the abdominal visceral region, are associated with an increase in arterial stiffness in healthy young men.

Numerous cross-sectional studies report associations between surrogate (ie, waist circumference) and direct measures of visceral adiposity and large artery stiffness across a variety of subject populations.<sup>6–12</sup> Most notably, abdominal visceral fat (measured via computed tomography) appears to be the strongest predictor of aortic stiffness.<sup>10,11</sup> Taken together with these previous cross-sectional studies, our findings implicate abdominal fat partitioning as an important mediator of the arterial stiffening that occurs with weight gain.

The mechanisms linking abdominal visceral fat and arterial stiffening are unclear, although numerous possibilities have been advanced.<sup>27</sup> Seals and Gates<sup>28</sup> hypothesized that the proinflammatory state and oxidative stress accompanying weight gain (and aging) alters vascular structure and function by disrupting the balance of key extracellular matrix proteins (ie, elastin and collagen) and vasoconstrictive and vasodila-



**Figure 3.** Left, Relation between changes in abdominal visceral fat and changes in  $\beta$ -stiffness index in the pooled sample. Right, Relation between change in abdominal visceral fat and magnitude of change in arterial compliance in the pooled sample.

tory molecules (eg, NO, prostacyclins, endothelin-1, and angiotensin II), and promoting vascular smooth muscle cell hypertrophy. Together these pathophysiological mechanisms lead to arterial stiffening. Given the short duration of the present study, it seems unlikely that structural modifications to the vasculature, as observed in age-associated arterial stiffening, can account for the increase in stiffness observed following weight gain. A more plausible explanation is that a multitude of interrelated factors sensitive to acute changes in body weight, including reductions in insulin sensitivity, dyslipidemia, activation of the sympathetic nervous and renin-angiotensin systems, and perhaps other factors conspire to impair endothelial function (ie, increase vascular smooth muscle tone) and, in turn, result in arterial stiffening.<sup>27</sup> However, we found no association between the changes in carotid stiffness or compliance and changes in estimates of insulin sensitivity (ie, fasting insulin and HOMA score), plasma renin activity, or muscle sympathetic nerve activity (data not shown).<sup>22</sup> We should emphasize that our study was not designed to address potential mechanisms. As such, future studies will be necessary to address this important issue.

There are some limitations of the present study that should be discussed. First, we did not include a control group, and our sample size was relatively small. Thus, inclusion of a control group and/or a larger sample size may have yielded different results. However, that 11 of the 14 subjects demonstrated an increase in carotid artery stiffness after weight gain and, as hypothesized, the subjects with the largest increase in abdominal visceral fat (manipulated variable) also demonstrated the largest increase in arterial stiffness (the outcome variable) suggest that our results are unlikely to have been the result of random deviations over time.

Second, the subjects in the present study were limited to young, nonobese men. Women tend to accumulate fat in the gluteal-femoral region, and older adults are more susceptible to visceral fat accumulation. As such, the vascular responses to weight gain may be attenuated or amplified, respectively. For these reasons, caution should be taken in extrapolating our findings to women or beyond the age-range studied.

Third, the experimental weight gain produced in the present study may not be representative of the more gradual changes that occur over time in the general population. As such, our findings should be considered with this in mind.

Finally, we should emphasize that our findings do not preclude the possibility that the expansion of other fat depots, such as perivascular adipose tissue, may play an important role in mediating the effects of weight gain on arterial stiffness.<sup>29</sup>

In conclusion, the results of the present study indicate that modest diet-induced weight gain results in large artery stiffening in young, nonobese men. Those individuals who demonstrated the largest increases in abdominal visceral fat also demonstrated the largest increases in arterial stiffness. Importantly, the increase in arterial stiffness associated with abdominal visceral fat accumulation occurred independent of the amount of total body fat gained.

## Perspectives

Arterial stiffening has long been regarded as an indicator of disease<sup>30</sup> and is independently associated with an increased risk for developing hypertension.<sup>31</sup> Arterial stiffening reduces the cushioning function of the aorta. As a consequence, systolic blood pressure rises, diastolic blood pressure falls, and the ability to convert pulsatile cardiac ejection to continuous flow at the level of the microcirculation is impaired.<sup>30</sup> As such, arterial stiffening may lead to left ventricular hypertrophy, cardiac dysfunction, and a reduction in myocardial perfusion in the face of increased demand. In addition, microvascular damage can occur in key target organs, such as the kidney and brain, as a result of the reduced cushioning function.<sup>32</sup> Our current findings suggest that individuals who gain even modest amounts of weight may experience arterial stiffening even if they do not become obese. The accumulation of abdominal visceral fat appears to be particularly important in this regard. Taken together with the recent findings of an increasing prevalence of abdominal obesity,<sup>33</sup> our data highlight the importance of abdominal visceral fat as an important therapeutic target. Importantly, overfeeding-induced weight gain in humans may provide an insightful model to discern the mechanism(s) responsible for weight gain-induced arterial stiffening.

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## Disclosures

None.

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