General Discussion
INTRODUCTION

The general purpose of this thesis was to gain more insight in the longitudinal associations between body fatness, fat distribution and arterial stiffness. In addition, we were interested in whether the associations were explained by adipokines, biomarkers of low-grade inflammation and/or biomarkers of endothelial dysfunction. Also, after we found that the adipokine profile mediated the relationship between body fat (distribution) and arterial stiffness to a large extent, we were interested in the longitudinal development of fatness parameters from the age of 13 to the age of 36 years in relation to favourable or unfavourable levels of adipokines at the age of 36 years.

Main findings

Chapter 2 addressed the associations between changes in central (i.e. trunk) fat versus peripheral (i.e. limbs) fat and lean masses with changes in arterial stiffness over a 6-year follow-up period between 36 and 42 years of age. First, throughout the 6-yr longitudinal study, greater levels of total body fatness, particularly of trunk body fat, were adversely whereas peripheral lean mass was favourably associated with carotid and femoral stiffness. Trunk and peripheral fat also exhibited opposite associations with aortic stiffness. Second, changes in trunk fat were adversely whereas changes in peripheral fat and lean masses were favourably associated with changes in the carotid and aortic, but not femoral, stiffness. Finally, the detrimental and additive ‘effects’ of increases in trunk and decreases in peripheral fat masses on arterial stiffness were independent of one another and of concomitant changes in lean mass and other risk factors, and were accompanied by only minor increases in body weight. Importantly, this pattern of changes in body fat distribution was observed for individuals who were on average in the normal-weight range, who made up about one third of the study population, and who exhibited the steepest increases in arterial stiffness.

Chapter 3 evaluated the relationship between the development of an extensive array of biomarkers of endothelial dysfunction and low-grade inflammation on the one hand and arterial stiffness on the other over a 6-year period. First, endothelial
dysfunction and low-grade inflammation were associated with greater arterial stiffness. Endothelial dysfunction was associated with greater femoral artery stiffness, whereas low-grade inflammation was associated with both greater carotid and femoral artery stiffness. However, both endothelial dysfunction and low-grade inflammation were not associated with aortic stiffness. Second, mutual adjustment for low-grade inflammation or endothelial dysfunction showed that the associations between endothelial dysfunction and low-grade inflammation with femoral artery stiffness were interdependent.

Chapter 4 focussed on the associations between leptin, adiponectin, and the leptin-to-adiponectin ratio (LAR) with carotid, femoral and aortic stiffness throughout the 6-year period. We found that lower levels of adiponectin and higher levels of leptin and of the LAR were adversely associated with carotid and femoral stiffness. However, neither the adipokines nor their ratio was associated with aortic stiffness (as measured by cfPWV). Thus, the adipokines may affect arterial stiffening in a site-specific way, exerting their effect on first and second-generation branches of the aorta, but sparing the aorta itself.

Chapter 5 examined the potential mediating role of adipokines, low-grade inflammation and endothelial dysfunction in the relationship between total fat and trunk fat mass on the one hand and arterial stiffness on the other hand (research question 2 and 3, outlined in Introduction). Adipokines, rather than low-grade inflammation and endothelial dysfunction, to a large extent explained the relationship between overall fatness and arterial stiffness, and also between central fatness and carotid stiffness.

Chapter 6 addressed to what extent the longitudinal development of fatness parameters (BMI, sum of skinfolds and skinfold ratio) from the age of 13 to 36 years precede favourable or unfavourable levels of adipokines at the age of 36 years. It appeared that higher levels of total fatness (as estimated by sum of skinfolds) and BMI during adolescence were adversely associated with unfavourable levels of leptin, but to a lesser extent with adiponectin, in adulthood.
Methodological considerations

All studies included in this thesis were conducted within the ongoing AGAHLS, an observational longitudinal study. It should be noted that the AGAHLS is not a representative sample of the Dutch population. First, the participants were selected from two secondary schools with relatively high education levels (i.e., the school of higher general secondary education (HAVO) or secondary education (VWO). Second, the socio-economic status of the parents of the participants were above average of the total Dutch population at that time [1]. Therefore, relatively unhealthy people will be underrepresented, as they are usually overrepresented in groups with lower socio-economic status. Further, the secondary schools were located in the north of the Netherlands (i.e., Amsterdam and Purmerend) and only few participants have a non-Caucasian ethnic background.

Main strengths of the AGAHLS are its longitudinal design, and during the last two measurement rounds; the robust assessment of body composition (by DXA) and arterial properties (by ultrasound), and the extensive characterization of biomarkers reflecting underlying pathobiological mechanisms of interest.

Selection bias

Loss to follow-up (i.e., dropout) is a major cause of selection bias in longitudinal studies [2], in the sense that subjects still attending the study could differ considerably from the ones who have dropped-out. During the 30 years of follow-up, about 40% of the participants of the AGAHLS dropped out. We analysed if there were differences between the participants included in the present studies and the drop-outs with regard to levels of anthropometric measures, blood pressure, blood lipids and physical activity at the beginning of the study in 1976 when the subjects were 13 years of age (Table 1).
Table 1. Differences in characteristics at age 13 between drop-outs (n=294) and non-drop-outs (n=361)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-drop-outs (i.e., present at age 36)</th>
<th>Drop-outs (not present at age 36)</th>
<th>P-value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>17.4 ± 1.8</td>
<td>17.9 ± 2.3</td>
<td>0.005</td>
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<tr>
<td>Sum of 4 skinfolds, mm</td>
<td>31.7 ± 12.1</td>
<td>35.2 ± 16.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Bodyfat, %</td>
<td>19.9 ± 5.3</td>
<td>20.8 ± 6.2</td>
<td>0.051</td>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>124.6 ± 9.3</td>
<td>123.8 ± 8.7</td>
<td>0.428</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>75.4 ± 7.8</td>
<td>75.4 ± 7.8</td>
<td>0.926</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.47 ± 0.73</td>
<td>4.48 ± 0.74</td>
<td>0.866</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.45 ±0.29</td>
<td>1.43 ± 0.30</td>
<td>0.474</td>
</tr>
<tr>
<td>Physical activity, 10³ METS/wk</td>
<td>4.40 ± 1.83</td>
<td>3.98 ± 2.00</td>
<td>0.025</td>
</tr>
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</table>

Data are means ± SD.

These results showed that levels of BMI, sum of skinfolds and bodyfat percentage were significantly lower, whereas levels of physical activity were slightly higher, in the non-drop-outs versus the drop-outs. This suggests that the associations found between fatness parameters, on the one hand, and arterial stiffness, adipokines, endothelial dysfunction and/or low-grade inflammation, on the other, could possibly be an underestimation of the real associations.

Information bias

Information bias is caused by measurement errors. Data on arterial properties were measured with reasonable or good reproducibility (Chapter 2). However, the use of different DXA whole-body scanners and BP devices at both time points may have caused seemingly unexpected phenomena, such as an increase in peripheral lean mass and a decrease in PP (Chapter 2). We attempted to circumvent the problem of systematic overestimation of body lean (and underestimation of body fat) mass with the more recent QDR 4500-A device by calibrating these data, but this may have not been optimal. In addition, the decreases in PP observed herein resulted from an increase (~6 mmHg) in DBP and the practically unchanged levels of SBP between the ages of 36 and 42. Although this may seem unexpected and measurement errors can indeed not be excluded, increases in PP with ageing are often observed after the 5th or 6th decades of life only as a consequence of lifelong arterial stiffening [3].
data may thus reflect real changes among young adults. As a consequence, at the population level and in absolute levels, PP and all arterial stiffness estimates could have been underestimated in the 2006 vs. 2000 measurement period. It was unclear whether the potential measurement bias was systematic or random; if systematic, it would not impact on the relationships found. If (part of) the differences could be explained by random measurement error, the strength of the associations was most likely underestimated.

**Mediation and confounding**

In this thesis all research questions were answered with linear regression analyses (including GEE). In regression analyses, the methods to examine confounding and mediation are the same. Our main interest was identifying mediators, especially in Chapter 5. Both confounding and mediation are quantified by measuring the change in the relationship between an independent and a dependent variable (i.e., the attenuation of the regression coefficient) after adding a third variable to the analysis. A potential confounder is associated both with the outcome and the determinant, but is not involved in the causal pathway, i.e. is not a mediator. The latter, however, is often difficult to determine or unknown, because, as already mentioned, mediation and confounding are statistically identical and can be distinguished only on conceptual grounds [4]. Adjustment for variables that are involved in the causal pathway is only justified if one is interested in identifying mediators.

**Multicollinearity**

Another potential problem in regression analyses is multicollinearity. If independent variables are highly correlated with each other, it is difficult to separate the effects of these variables statistically. This is certainly true for components of body fat distribution. It could be argued that the opposite associations of trunk and peripheral fat mass with arterial stiffness are, to some extent, statistical artefacts. There are some diagnostic checks (i.e., tolerance test) in statistical packages to test multicollinearity, but these criteria or cut-off values are rather arbitrary. However, as there is no real alternative, we performed all tests (i.e., correlation, tolerance testing, looking at possible disturbances in the models such as remarkable large
standard errors) and observed no sign of multicollinearity. In addition, when putting our results in pathophysiological perspective (Chapter 1), we were convinced of the plausibility of our findings.

**Implications for public health**

From the results presented in this thesis, it is difficult to estimate the direct implications for public health because the outcome used in the present thesis, arterial stiffness, is a pre-clinical measure. However, stiffness of mainly elastic arteries is predictive of incident CVD and mortality [5]. A recent meta-analysis [6] estimated a 14-15% increased CVD and mortality risk per 1m/s increase in cfPWV. On the basis of these data, our estimates may translate to comparable or even greater increases or decreases in risk per 10 kg increase in trunk fat mass or decreases in peripheral fat mass in young adults over the course of 6 years, respectively. All together, our data support the view that adiposity-related increases in central stiffness may explain, at least in part, the increased CVD and mortality attributed to a central patterning of fat distribution.

Furthermore, the results of Chapter 2 suggest that, irrespective of the underlying mechanisms, both trunk and peripheral fat are important determinants of arterial stiffness, and thus of cardiovascular disease. In Chapter 2 we divided the subjects into 3 groups (ie, the so-called phenotypes):

1. Those whose absolute levels of both trunk and peripheral fat decreased, 22% of the study participants;

2. Those whose levels of both trunk and peripheral fat increased, 47%;

3. Those whose levels of trunk fat increased but peripheral fat decreased; 30%.

After we performed this classification, the additive ‘effects’ of increases in trunk and decreases in peripheral fat on arterial stiffness were accompanied by only minor increases in body weight and were within or around the limits of a normal-weight
range (i.e., BMI<25 or close to a BMI-value of 25). The additive ‘effects’ of *increases* in trunk and *decreases* in peripheral fat on arterial stiffness occurred in about one third of the study population, and identified a group of individuals exhibiting the steepest increases in arterial stiffness. This phenotype of (changes in) body fat distribution shows directionally similar perturbations as can be observed in a relatively prevalent (5 to 45%) subgroup of ‘metabolically obese but normal weight’ (MONW) individuals at the population level [7,8]. In addition to elevated abdominal/visceral adiposity despite a BMI<25 kg/m², MONW individuals are generally characterized by reduced insulin sensitivity, a more atherogenic lipid profile and/or higher levels of BP, all of which are known determinants of arterial stiffness. Our data were consistent with some of these characteristics [i.e. more adverse changes in total-to-HDL cholesterol ratio [+0.29 mmol/L (0.08 to 0.51)] and HbA1c [+0.19% (0.04 to 0.33)] in the individuals who displayed this critical phenotype vs. those whose absolute levels of both trunk and peripheral fat decreased], but their comparatively steeper increases in levels of carotid and aortic stiffness were independent of changes in these risk factors. This suggests that other adiposity-related factors may be involved. In order to investigate this issue more thoroughly, we analysed if this ‘critical’ phenotype differed in other adiposity-related factors, like decreases in adiponectin and increases in leptin, circulating proinflammatory cytokines and related endothelial dysfunction (Table 2).

**Table 2.** Changes in adiposity-related factors in the ‘critical’ phenotype versus those whose absolute body fat levels decreased

<table>
<thead>
<tr>
<th>Changes in</th>
<th>Β</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Leptin, ng/L</td>
<td>+5.60</td>
<td>2.03 to 9.16</td>
</tr>
<tr>
<td>Adiponectin, mg/L</td>
<td>-0.56</td>
<td>-1.27 to 0.15</td>
</tr>
<tr>
<td>Low-grade inflammation</td>
<td>0.01</td>
<td>-0.16 to 0.19</td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>-0.02</td>
<td>-0.18 to 0.14</td>
</tr>
</tbody>
</table>

Consistent with our earlier results in Chapter 5 and 6, these results showed that leptin levels were significantly increased in the individuals who displayed this phenotype versus those whose absolute levels of both trunk and peripheral fat decreased, whereas changes in adiponectin or changes in biomarkers of low-grade inflammation or endothelial dysfunction were not significantly different between the two groups.
With regard to the opposite changes in trunk and peripheral fat mass, these findings emphasize the importance of assessment of regional changes in body composition as it may enable identification of individuals with an unrecognized increased cardiovascular risk. From a primary prevention point of view, monitoring changes in fat distribution should be considered for the well-known groups with increased risk (i.e., individuals with obesity or type 2 diabetes), but also for other (normal weight) individuals who may also be in need for diet and/or exercise weight-loss programs and could also benefit from such interventions. To monitor such interventions, as well as for clinical applications and large epidemiological studies, a simple and easy to perform way of assessing regional changes in body composition could be the combination of measuring BMI, waist and hip circumferences [9,10].

The question remains how to trace these ‘normal weight individuals’ who may also be in need for health programs. Campaigns for measurement of BMI and circumferences of waist and/or hip for the general population could be initiated by (local) governments and implemented by trained personnel, as the measurement of the circumferences should be performed precisely. In order to detect changes in BMI and fat distribution, it would be best if these interventions were carried out with regularity. Whether such interventions are cost-effective should be investigated further.

In Chapter 6 we concluded that higher and increasing levels of total fatness and BMI, starting at early adolescence and throughout the course of life, were associated with adverse levels of leptin in adulthood. This confirms the importance of promoting healthy lifestyles and healthy weight in children and adolescents. This is nowadays of great importance, since there is a worldwide increase in the incidence of obesity due to the increased availability of food and the greater energy density of food in combination with a more sedentary lifestyle [11]. Interventions of healthy lifestyles early in life, a period in which lifetime eating and physical activity are commonly developed, may have positive health consequences for prevention of cardiovascular risk factors and obesity-related disorders in adulthood. In our opinion, these interventions should involve the whole environment of children and adolescents, meaning that this is a shared responsibility of parents, other family members like grand-parents, teachers at schools, health care providers, policy makers and governments.
Suggestions for future research

We have two distinct suggestions for further research. First, despite the fact that trunk fat as assessed by DXA correlates highly with intra-abdominal fat [12], it does not distinguish between subcutaneous and visceral fat in the trunk/abdominal region. We could thus not ascertain whether, and the extent to which, the associations of changes in arterial stiffness with abdominal visceral fat differed from those with abdominal subcutaneous fat. These may need to be further examined as some studies have suggested that the former are stronger than the latter [13-15]. These different fat depots can be distinguished by the use of sophisticated imaging techniques, such as magnetic resonance imaging (MRI) and computed tomography (CT).

Second, although we found interesting results during the 6-year follow-up between changes in trunk fat and peripheral fat on the one hand, and changes in carotid and aortic stiffness on the other (Chapter 2), we did not find opposite effects of changes in these fat depots on changes in femoral stiffness. Furthermore, in Chapter 3 and 4, we showed that changes in biomarkers of endothelial dysfunction or low-grade inflammation or adipokines did not parallel changes in arterial stiffness. The timeframe in which changes in adipokines and changes in biomarkers of endothelial dysfunction and low-grade inflammation have their possible impacts on arterial stiffness is unknown and might differ from the six-year period of the present investigation. The question remains for what period a person should have unfavourable levels of endothelial dysfunction or low-grade inflammation before it affects the vasculature. Therefore it would be challenging to analyse these relationships again within the AGAHLS when the subjects are 5-10 years older in order to see if a larger timeframe impacts on these associations. It might be quite reasonable to expect clearer results in such analyses, as also increases in pulse pressure are often observed after the 5th or 6th decades of life as a consequence of arterial stiffening [3]. In fact, in order to distinguish the possible combined effects of a larger timeframe and the older age of the participants, it would even be better to investigate aforementioned relationships in subjects around the same age but with a larger follow-up period (e.g., 10-year changes between 35 and 45 years of age).
REFERENCES