Insulin Sensitivity and Carotid Intima-Media Thickness
Relationship Between Insulin Sensitivity and Cardiovascular Risk Study

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Objective—Despite a wealth of experimental data in animal models, the independent association of insulin resistance with early carotid atherosclerosis in man has not been demonstrated.

Approach and Results—We studied a European cohort of 525 men and 655 women (mean age, 44±8 years) free of conditions known to affect carotid wall (diabetes mellitus, hypertension, and dyslipidemia). All subjects received an oral glucose tolerance test, a euglycemic hyperinsulinemic clamp (M/I as a measure of insulin sensitivity), and B-mode carotid ultrasound. In 833 participants (380 men), the carotid ultrasound was repeated after 3 years. In men, baseline intima-media thickness in the common carotid artery (CCA-IMT) was significantly higher (P<0.05) in the lowest M/I tertile, whereas in women CCA-IMT was higher (P<0.0005) in the highest fasting plasma glucose tertile (after adjustment for established risk factors). In multiple regression models, with CCA-IMT as the dependent variable and with risk factors and univariate metabolic correlates as independent variables, circulating free fatty acids and the leptin/adiponectin ratio replaced M/I as independent metabolic determinants of CCA-IMT in men. The strongest metabolic determinant of CCA-IMT in women was fasting plasma glucose. Three-year CCA-IMT changes were not associated with any cardiometabolic risk factor.

Conclusions—In young-to-middle aged apparently healthy people, the association of CCA-IMT with insulin sensitivity and its metabolic correlates differs between men and women. Lower insulin sensitivity is associated with higher IMT only in men; this association seems to be mediated by circulating free fatty acids and adipocytokines. In women, CCA-IMT is independently associated with fasting plasma glucose. (Arterioscler Thromb Vascl Biol. 2013;33:1409-1417.)

Key Words: adipokine ■ atherosclerosis ■ carotid intima-media thickness ■ fatty acids ■ glucose

Reduced insulin sensitivity (IS) is considered a primary pathophysiologic mechanism linking together several metabolic and hemodynamic abnormalities that may result in accelerated atherosclerosis and increased risk for cardiovascular diseases. Whether the blunted response to insulin has a direct role in the development of atherosclerotic changes within the arterial wall or whether its proatherogenic effect is mediated by the associated systemic abnormalities, such as hyperglycemia, hypertension, dyslipidemia, or chronic inflammation, is not clear. Early atherosclerotic processes, including subendothelial retention of apolipoprotein B–containing lipoproteins, activation of endothelial cells, recruitment of monocytes, foam cell formation, and migration of smooth muscle cells to the intima, might be directly or indirectly influenced by insulin resistance alone or in combination with hyperinsulinemia and hyperglycemia.1-6

Carotid intima-media thickness (IMT) is a generally accepted marker of early subclinical atherosclerosis, and several clinical studies have evaluated the effect of IS and fasting plasma insulin levels on the carotid wall.7-14 However, the results of these studies have not been conclusive, probably because of differences in the methods used to estimate IS, characteristics of the study populations (including people with other conditions possibly directly influencing carotid wall thickness), and also because of the lack of adequate adjustment for established risk factors.

The aim of the Relationship Between Insulin Sensitivity and Cardiovascular Risk (RISC) study15 was to test the association among IS, carotid IMT, and the presence of early carotid plaques, cross-sectionally and prospectively, in a relatively large and apparently healthy European population free of confounding morbidities, such as hypertension, diabetes mellitus, dyslipidemia, chronic inflammatory, or cardiovascular diseases (Figure 1). IS was measured by the gold-standard method of the euglycemic hyperinsulinemic clamp, and all associations were evaluated separately for
men and women and further controlled for conventional cardiovascular risk factors and habitual physical activity (PA). Carotid IMT was measured at the level of common carotid artery (CCA) because previous studies have demonstrated that traditional risk factors and metabolic variables account for a greater proportion of IMT variability in the CCA than in the carotid bulb or internal carotid artery, and that the reproducibility of measurements is better for CCA than for other carotid segments. The presence of plaques, however, was evaluated in the entire extracranial carotid tree as one of the first and the most common site of early atherosclerotic plaque formation is carotid bifurcation. In addition, we assessed the association of carotid wall thickness and plaque presence with recognized metabolic correlates of blunted insulin signaling, such as an increase in triglycerides, circulating free fatty acids (FFA), apolipoprotein B level, inflammatory markers, and adipocytokines.

**Materials and Methods**

Materials and Methods are available in the online-only Supplement.

**Results**

At baseline, men and women differed in insulin sensitivity (M/I) and in all cardio-metabolic risk factors (Table 1), except for smoking habits, high-sensitive C-reactive protein, and 2-hour plasma glucose and insulin levels. Average daily PA, as assessed by accelerometer monitoring, was also comparable between the 2 sexes (mean monitoring time, 5.7±1.5 days). One hundred sixty-one women (24%) were post-menopausal. During the 3-year period, there were increases in body mass index (+0.3±1.6 kg/m²; P<0.0001), fat mass (+1.0±4.7 kg; P<0.0001), waist circumference (+1.1±6.3 cm; P<0.0001), systolic blood pressure (BP; +2.4±12.1 mm Hg; P<0.00001), and plasma glucose concentrations (+0.12±0.59 mmol/L; P=0.001) and decreases in heart rate (−2.2±9.2 bpm; P<0.0001) and high-density lipoprotein–cholesterol (−0.03±0.31 mmol/L; P=0.001), whereas low-density lipoprotein (LDL)–cholesterol (+0.03±0.63 mmol/L; P=0.18), triglycerides (+0.04±0.58 mmol/L; P=0.14), and plasma insulin concentrations (+2.1±19.4 pmol/L; P=0.11) did not change significantly. These changes did not differ between men and women. Thirty-two men and 25 women started pharmacological treatment for high BP, dyslipidemia, or glucose intolerance/diabetes mellitus during the follow-up period.

CCA-IMT values showed a positively skewed distribution. At baseline, CCA-IMT was higher in men than women (Table 2). Carotid plaques, if present, were localized in carotid bulb or origin of internal carotid artery, and their prevalence and thickness were comparable between the 2 sexes. Over the 3-year period, CCA-IMT changes were small, if significantly different from zero (P<0.0001), and they did not differ between men and women. In men, plaque presence after 3 years was higher than at baseline (P<0.0001) and higher than in women.

We next examined baseline CCA-IMT, 3-year IMT changes, and carotid plaque presence across tertiles of IS (M/I), fasting, and 2-hour plasma glucose and insulin levels (Table 3) and homeostasis model assessment of insulin resistance (not shown), separately in men and women, and after adjustment for recruitment center and established atherosclerotic risk factors (age, systolic BP, LDL-cholesterol, current smoking, and menopausal status). Three-year IMT changes were also adjusted for baseline IMT and for therapeutic interventions if started during the follow-up period. In men, baseline CCA-IMT was significantly higher in the lowest M/I tertile (P<0.05); in women, baseline CCA-IMT was higher in the highest tertile of fasting glucose (P<0.0005; Figure 2). CCA-IMT did not differ across tertiles of 2-hour plasma glucose or insulin in either sex or across tertiles of homeostasis model assessment of insulin resistance. Three-year CCA-IMT changes (Table 3) did not differ across tertiles of tested metabolic measures.
Plaque presence in men was comparable across tertiles of the tested parameters, either at baseline or at 3 years; in women, plaque presence was higher in the highest glucose tertile at both time points (Table 1).

Impaired fasting glucose (IFG), impaired glucose tolerance, or both during baseline oral glucose tolerance test were detected, respectively, in 117, 33, or 17 men and in 61, 46, or 16 women. In men, there were no differences in carotid parameters between individuals with normal glucose tolerance and those with IFG or impaired glucose tolerance (Table 1 in the online-only Data Supplement). In contrast, women with IFG, but not those with impaired glucose tolerance, had higher baseline CCA-IMT ($P=0.005$) and carotid plaque presence, both at baseline ($P<0.01$) and at 3 years ($P<0.05$), as compared with women with normal glucose tolerance. The subgroups did not differ for 3-year IMT changes.

A complete correlation matrix was generated, including baseline CCA-IMT, 3-year CCA-IMT changes, and those with IFG or impaired glucose tolerance (Table 1 in the online-only Data Supplement). In contrast, women with IFG, but not those with impaired glucose tolerance, had higher baseline CCA-IMT ($P=0.005$) and carotid plaque presence, both at baseline ($P<0.01$) and at 3 years ($P<0.05$), as compared with women with normal glucose tolerance. The subgroups did not differ for 3-year IMT changes.

### Table 1. Clinical and Metabolic Characteristics, Mean±SD, Median (Interquartile Range), Percentages of Participants in the RISC Study

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>$P$ Value</th>
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<tbody>
<tr>
<td>n</td>
<td>525</td>
<td>655</td>
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</tr>
<tr>
<td>Age, y</td>
<td>43±9</td>
<td>44±8</td>
<td>0.01</td>
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<tr>
<td>Waist circumference, cm</td>
<td>93±10</td>
<td>81±12</td>
<td>&lt;0.0001</td>
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<tr>
<td>BMI, kg/m²</td>
<td>26.2±3.4</td>
<td>24.6±4.1</td>
<td>&lt;0.0001</td>
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<tr>
<td>Fat mass, kg</td>
<td>17.6 (9.7)</td>
<td>21.0 (11.1)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Systolic BP, mmHg</td>
<td>122±11</td>
<td>114±13</td>
<td>&lt;0.0001</td>
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<tr>
<td>Pulse pressure, mmHg</td>
<td>46±8</td>
<td>41±8</td>
<td>&lt;0.0001</td>
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<tr>
<td>Heart rate, bpm</td>
<td>66±10</td>
<td>70±10</td>
<td>&lt;0.0001</td>
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<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>3.1±0.8</td>
<td>2.8±0.8</td>
<td>&lt;0.0001</td>
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<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.2±0.3</td>
<td>1.6±0.4</td>
<td>&lt;0.0001</td>
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<tr>
<td>Apolipoprotein B, mg/dL*</td>
<td>137±57</td>
<td>122±50</td>
<td>&lt;0.001</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.1 (0.7)</td>
<td>0.8 (0.5)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Leptin:adiponectin ratio</td>
<td>0.8 (1.1)</td>
<td>1.5 (2.3)</td>
<td>&lt;0.0001</td>
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<tr>
<td>hsCRP, mg/L</td>
<td>0.5 (0.8)</td>
<td>0.4 (0.9)</td>
<td>0.95</td>
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<tr>
<td>FFA, mmol/L</td>
<td>0.44 (0.25)</td>
<td>0.56 (0.28)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.2±0.5</td>
<td>4.9±0.6</td>
<td>&lt;0.0001</td>
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<tr>
<td>2-hour glucose, mmol/L</td>
<td>5.7±1.5</td>
<td>5.8±1.5</td>
<td>0.26</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>33 (25)</td>
<td>29 (20)</td>
<td>0.01</td>
</tr>
<tr>
<td>2-hour insulin, pmol/L</td>
<td>129 (160)</td>
<td>149 (148)</td>
<td>0.17</td>
</tr>
<tr>
<td>M/I, µmol·min⁻¹·kg⁻¹·(nmol/L)⁻¹</td>
<td>113 (71)</td>
<td>146 (82)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>28.9</td>
<td>25.9</td>
<td>0.27</td>
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<tr>
<td>Average daily PA, counts/min†</td>
<td>342 (198)</td>
<td>352 (177)</td>
<td>0.65</td>
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</table>

**BMI** indicates body mass index; **BP**, blood pressure; **FFA**, free fatty acids; **HDL**, high-density lipoprotein; **hsCRP**, high-sensitive C-reactive protein; **LDL**, low-density lipoprotein; **M/I**, insulin sensitivity; **PA**, physical activity; and **RISC**, the Relationship Between Insulin Sensitivity and Cardiovascular Risk.

*in 384 men and 484 women; and †in 328 men and 456 women.

### Table 2. Baseline Values and 3-Year Changes ($\Delta$) in CCA-IMT, Median (Interquartile Range), and Carotid Plaque Presence, Percentages, in Men and Women of the Study

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>$P$ Value</th>
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<tbody>
<tr>
<td>Baseline IMT and plaque presence</td>
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<tr>
<td>CCA-IMT, µm</td>
<td>610 (120)</td>
<td>580 (110)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Plaques present, %</td>
<td>8.0</td>
<td>7.0</td>
<td>0.52</td>
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<tr>
<td>Maximal plaque thickness, mm</td>
<td>1.9±0.5</td>
<td>1.8±0.3</td>
<td>0.10</td>
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<tr>
<td>3-year IMT changes and plaque presence*</td>
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<tr>
<td>$\Delta$CCA-IMT, µm</td>
<td>+12 (55)</td>
<td>+20 (58)</td>
<td>0.30</td>
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<tr>
<td>Plaques present, %</td>
<td>11.5§</td>
<td>7.1</td>
<td>&lt;0.05</td>
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<tr>
<td>Maximal plaque thickness, mm</td>
<td>2.1±0.8</td>
<td>1.8±0.3</td>
<td>&lt;0.05</td>
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</table>

**CCA-IMT** indicates baseline intima-media thickness in the common carotid artery.

*measurements available in 833 (71%), 380 men, and 453 women; and §$P<0.0001$ as compared with baseline.
Table 3. Baseline Values and 3-Year Changes (Δ) in CCA-IMT, Median (Interquartile Range), and Carotid Plaques Presence, Percentages, by Tertiles of M/I, Fasting and 2-Hour Plasma Insulin and Glucose Concentrations

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<th>Men</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>P Value*</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>P Value*</td>
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<tr>
<td>M/I</td>
<td>16–91</td>
<td>91.1–135</td>
<td>135.1–454</td>
<td>&lt;0.05</td>
<td>21–122</td>
<td>122.1–176</td>
<td>176.1–657</td>
<td>0.57</td>
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<tr>
<td>Baseline CCA-IMT, μm</td>
<td>630 (110)</td>
<td>600 (108)</td>
<td>590 (120)</td>
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<td>600 (110)</td>
<td>580 (120)</td>
<td>580 (100)</td>
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<tr>
<td>ΔCCA-IMT, μm</td>
<td>13 (54)</td>
<td>17 (53)</td>
<td>9 (72)</td>
<td>0.86</td>
<td>15 (60)</td>
<td>20 (54)</td>
<td>20 (62)</td>
<td>0.88</td>
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<tr>
<td>Plaques baseline, %</td>
<td>8.0</td>
<td>8.4</td>
<td>7.7</td>
<td>0.73</td>
<td>6.4</td>
<td>6.0</td>
<td>8.7</td>
<td>0.43</td>
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<tr>
<td>Plaques 3 years, %</td>
<td>12.4</td>
<td>10.2</td>
<td>12.2</td>
<td>0.88</td>
<td>7.3</td>
<td>4.3</td>
<td>9.1</td>
<td>0.26</td>
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<tr>
<td>Fasting insulin</td>
<td>3–25</td>
<td>26–41</td>
<td>42–136</td>
<td>0.08</td>
<td>7–22</td>
<td>23–35</td>
<td>36–118</td>
<td>0.51</td>
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<tr>
<td>Baseline CCA-IMT, μm</td>
<td>590 (120)</td>
<td>605 (110)</td>
<td>620 (110)</td>
<td></td>
<td>570 (100)</td>
<td>585 (127)</td>
<td>592 (120)</td>
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<tr>
<td>ΔCCA-IMT, μm</td>
<td>20 (52)</td>
<td>10 (67)</td>
<td>9 (50)</td>
<td>0.27</td>
<td>20 (60)</td>
<td>23 (60)</td>
<td>15 (62)</td>
<td>0.49</td>
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<tr>
<td>Plaques, %</td>
<td>7.9</td>
<td>5.8</td>
<td>11.4</td>
<td>0.22</td>
<td>4.2</td>
<td>9.4</td>
<td>7.3</td>
<td>0.38</td>
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<tr>
<td>Plaques 3 y, %</td>
<td>9.4</td>
<td>8.3</td>
<td>16.3</td>
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<td>4.3</td>
<td>9.5</td>
<td>7.7</td>
<td>0.62</td>
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<td>Fasting glucose</td>
<td>3.1–5.0</td>
<td>5.1–5.4</td>
<td>5.5–6.9</td>
<td>0.08</td>
<td>2.7–4.6</td>
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<td>5.2–6.8</td>
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<td>Baseline CCA-IMT, μm</td>
<td>600 (120)</td>
<td>600 (110)</td>
<td>625 (120)</td>
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<td>570 (90)</td>
<td>570 (110)</td>
<td>610 (120)</td>
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<tr>
<td>ΔCCA-IMT, μm</td>
<td>13 (64)</td>
<td>10 (57)</td>
<td>10 (59)</td>
<td>0.05</td>
<td>20 (53)</td>
<td>20 (55)</td>
<td>13 (60)</td>
<td>&lt;0.01</td>
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<tr>
<td>Plaques, %</td>
<td>7.6</td>
<td>7.2</td>
<td>9.4</td>
<td>0.67</td>
<td>4.0</td>
<td>3.9</td>
<td>4.0</td>
<td>0.01</td>
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<tr>
<td>Plaques 3 y, %</td>
<td>8.2</td>
<td>9.4</td>
<td>16.6</td>
<td>0.68</td>
<td>4.4</td>
<td>2.9</td>
<td>13.3</td>
<td>0.01</td>
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<tr>
<td>2-hour insulin</td>
<td>10–90</td>
<td>91–200</td>
<td>201–1391</td>
<td>0.11</td>
<td>12–115</td>
<td>116–211</td>
<td>212–1750</td>
<td>0.33</td>
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<tr>
<td>Baseline CCA-IMT, μm</td>
<td>600 (120)</td>
<td>600 (120)</td>
<td>630 (110)</td>
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<td>590 (110)</td>
<td>570 (90)</td>
<td>595 (130)</td>
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<tr>
<td>ΔCCA-IMT, μm</td>
<td>8 (65)</td>
<td>14 (62)</td>
<td>15 (53)</td>
<td>0.64</td>
<td>20 (61)</td>
<td>23 (56)</td>
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<td>Plaques baseline, %</td>
<td>8.8</td>
<td>5.9</td>
<td>9.0</td>
<td>0.14</td>
<td>6.7</td>
<td>8.8</td>
<td>5.2</td>
<td>0.11</td>
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<tr>
<td>Plaques 3 y, %</td>
<td>12.6</td>
<td>6.3</td>
<td>14.4</td>
<td>0.16</td>
<td>7.1</td>
<td>7.5</td>
<td>7.1</td>
<td>0.82</td>
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<tr>
<td>2-hour glucose</td>
<td>2.3–4.9</td>
<td>5.0–6.2</td>
<td>6.3–11.0</td>
<td>0.57</td>
<td>2.2–5.0</td>
<td>5.1–6.2</td>
<td>6.3–11.0</td>
<td>0.48</td>
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<tr>
<td>Baseline CCA-IMT, μm</td>
<td>600 (120)</td>
<td>600 (120)</td>
<td>620 (110)</td>
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<td>580 (100)</td>
<td>580 (110)</td>
<td>590 (130)</td>
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<tr>
<td>ΔCCA-IMT, μm</td>
<td>8 (57)</td>
<td>12 (57)</td>
<td>18 (59)</td>
<td>0.45</td>
<td>21 (57)</td>
<td>20 (60)</td>
<td>13 (56)</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaques baseline, %</td>
<td>9.1</td>
<td>9.6</td>
<td>5.3</td>
<td>0.08</td>
<td>8.7</td>
<td>4.7</td>
<td>7.6</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaques 3 y, %</td>
<td>12.9</td>
<td>11.6</td>
<td>10.3</td>
<td>0.57</td>
<td>6.5</td>
<td>4.2</td>
<td>10.3</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CCA-IMT indicates baseline intima-media thickness in the common carotid artery; and M/I, insulin sensitivity.

*P value for the differences in baseline CCA-IMT, ΔCCA-IMT, or plaque presence across tertiles of the variables in bold type adjusted for recruiting center, age, systolic blood pressure, low-density lipoprotein–cholesterol, current smoking, menopausal status and, in the case of ΔCCA-IMT, also for baseline CCA-IMT and 3-year therapeutic interventions.

anthropometric parameters, and metabolic variables (Table II in the online-only Data Supplement). In men, baseline CCA-IMT was inversely related to M/I and directly to fat mass, waist circumference, fasting insulin, triglycerides, FFA, and leptin:adiponectin ratio. In women, baseline CCA-IMT was directly related to fat mass, waist circumference, triglycerides, fasting glucose, insulin, apolipoprotein B, and leptin:adiponectin ratio. The 3-year IMT changes did not correlate with any anthropometric or metabolic factor or with their 3-year changes, either in men or women. In both sexes, CCA-IMT changes were inversely related to baseline IMT (p=-0.26 and -0.36; P<0.0001 for both) and directly to age (p=0.11 and 0.10; P<0.05 for both). M/I was inversely related to expected anthropometric (waist circumference and fat mass) and metabolic (insulin, triglycerides, FFA, high-sensitive C-reactive protein, and leptin:adiponectin ratio) parameters in both sexes (Table II in the online-only Data Supplement).
In the subgroups of men (n=328) and women (n=456) undergoing accelerometer monitoring, average daily PA was directly related to M/I (ρ=0.17 and 0.21; P<0.005 and <0.0001) and inversely to waist circumference (ρ=−0.15 and −0.17; P<0.01 and <0.001), fat mass (ρ=−0.21 and −0.19; P<0.0005 and <0.0001), fasting insulin (ρ=−0.23 and −0.19; P<0.0001 for both), and the leptin:adiponectin ratio (ρ=−0.17 and −0.23; P<0.005 and <0.0001). In men only, PA was inversely related to LDL-cholesterol and triglycerides (ρ=−0.16 and −0.21; P<0.005 and <0.0005) and in women to systolic BP and baseline CCA-IMT (ρ=−0.14 and −0.14; P<0.005 for both). Habitual PA was comparable in men and women with and without carotid plaques (data not shown).

Multiple regression models, with standardized baseline CCA-IMT as the dependent variable and established risk factors (age, systolic BP, LDL-cholesterol, current smoking, and menopause) as independent variables, were run separately for men and women. After backward stepwise removal, independent determinants of baseline CCA-IMT were age, systolic BP, and LDL-cholesterol in men, and age, systolic BP, and current smoking in women. M/I, when added into the models (Table 4, model A), was independently related to baseline CCA-IMT only in men. When other univariate anthropometric and metabolic correlates were included (Table 4, model B), FFA and the leptin:adiponectin ratio replaced M/I in men, whereas in women, fat mass, fasting glucose, and triglycerides entered as additional independent determinants of baseline CCA-IMT. When daily PA was also added into the model, it did not influence the independent association between CCA-IMT and FFA in men nor the association between CCA-IMT and fasting plasma glucose in women (Table III in the online-only Data Supplement).

Models A and B were also run separately for lean and overweight/obese (body mass index=25–39.9 kg/m²) men and women (Table IV in the online-only Data Supplement). M/I or FFA and the leptin:adiponectin ratio were independently related to baseline CCA-IMT only in overweight/obese men, whereas in lean men the only metabolic determinant of

Table 4. Independent Determinants of Baseline CCA-IMT in Men and Women

<table>
<thead>
<tr>
<th>Baseline CCA-IMT</th>
<th>β±SE</th>
<th>P Value</th>
<th>β±SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>0.37±04</td>
<td>&lt;0.0001</td>
<td>Age, y</td>
<td>0.44±04</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>0.13±04</td>
<td>&lt;0.005</td>
<td>Systolic BP, mmHg</td>
<td>0.16±04</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>0.20±04</td>
<td>&lt;0.0001</td>
<td>Current smoking (yes)</td>
<td>0.10±04</td>
</tr>
<tr>
<td>M/I, µmol.min⁻¹.kg⁻¹.mol⁻¹.L⁻¹</td>
<td>-0.09±04</td>
<td>&lt;0.05</td>
<td>M/I, µmol.min⁻¹.kg⁻¹.mol⁻¹.L⁻¹</td>
<td>-0.06±04</td>
</tr>
<tr>
<td>Total model R²</td>
<td>0.29</td>
<td>&lt;0.0001</td>
<td>Total model R²</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>0.37±04</td>
<td>&lt;0.0001</td>
<td>Age, y</td>
<td>0.40±04</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>0.09±04</td>
<td>&lt;0.05</td>
<td>Systolic BP, mmHg</td>
<td>0.11±04</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>0.19±04</td>
<td>&lt;0.0001</td>
<td>Current smoking (yes)</td>
<td>0.09±04</td>
</tr>
<tr>
<td>Leptin:adiponectin ratio</td>
<td>0.11±04</td>
<td>&lt;0.01</td>
<td>Fat mass, kg</td>
<td>0.09±04</td>
</tr>
<tr>
<td>FFA, mmol/L</td>
<td>0.10±04</td>
<td>0.01</td>
<td>Fasting glucose, mmol/L</td>
<td>0.11±04</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.09±04</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total model R²</td>
<td>0.30</td>
<td>&lt;0.0001</td>
<td>Total model R²</td>
<td>0.39</td>
</tr>
</tbody>
</table>

All analyses adjusted for recruiting center. β indicates standardized regression coefficient; BP, blood pressure; FFA, free fatty acids; LDL, low-density lipoprotein; and M/I, insulin sensitivity.
CCA-IMT was LDL-cholesterol. Fasting plasma glucose was independently related to baseline CCA-IMT only in overweight/obese women, in whom LDL-cholesterol also entered as additional IMT determinant (Table IV in the online-only Data Supplement).

Independent determinants of carotid plaque presence in men were age and LDL-cholesterol both at baseline and at 3 years ($R^2=0.15$ and 0.17; $P<0.005$ for both). In women, independent determinants of plaque presence were age, current smoking, and fasting glucose at baseline ($R^2=0.19$; $P<0.0001$), and age, menopausal status, and fasting glucose at 3 years ($R^2=0.24$; $P<0.0001$). The percentage with plaques did not differ between lean and overweight/obese men either at baseline (5.6% versus 9.5%; $P=0.10$) or at 3 years (8.1% versus 13.9%; $P=0.08$), nor in women at baseline (6.9% versus 7.0%; $P=0.93$) or at 3 years (6.5% versus 7.9%; $P=0.57$).

**Discussion**

To our knowledge, this is the first study in which the association between IS, measured directly by the euglycemic hyperinsulinemic clamp technique, and carotid IMT has been explored in a relatively large cohort of individuals free of hypertension, diabetes mellitus, or dyslipidemia, and in which results have been analyzed separately for men and women and controlled for established atherosclerotic risk factors. It is important to emphasize that in people with hypertension, diabetes mellitus, or dyslipidemia, it may not be possible to separate the potential atherogenic effect of an impaired tissue response to insulin from the known impact of clinically manifest disease. It is also firmly established that IS, some cardiovascular risk factors, and carotid IMT exhibit definite sex-related differences.24,25

In our healthy men, in particular in overweight and obese men, lower IS was associated with higher baseline CCA-IMT independently of established risk factors. However, the association between CCA-IMT and MI was eliminated on introducing FFA and the leptin:adiponectin ratio into the prediction model. This result suggests that in men, plasma FFA and adipocytokines might link insulin resistance with increase in carotid IMT, especially in the presence of increased body fat. Abnormal insulin signaling in adipose tissue results in inappropriate lipolysis and elevations in circulating FFA,21 as well as an increase in proatherogenic leptin22 and a decrease in antiatherogenic adiponectin.23 Indeed, in the present data set, FFA levels and the leptin:adiponectin ratio were related to MI and adiposity, inversely and directly, respectively (Table II in the online-only Data Supplement).

Circulating FFA and adipocytokines may induce structural changes in the vascular wall through different pathways. Circulating FFA have been shown to stimulate vascular smooth muscle cell proliferation and migration24 and to modify the expression of genes controlling extracellular matrix formation25; at the endothelial level, FFA may induce oxidative stress, apoptosis, and an inflammatory response.28 Leptin exerts its proatherogenic effect through stimulation of an inflammatory reaction and vascular smooth muscle cell proliferation,29 whereas adiponectin is supposed to attenuate inflammation and to inhibit proliferation of smooth muscle cells induced by growth factors.30 The role of insulin resistance and FFA or adipocytokines in carotid atherosclerosis has also been suggested by other studies. In renal transplant recipients with a high prevalence of insulin resistance, carotid IMT was independently associated with plasma FFA.31 In patients with type 2 diabetes mellitus, FFA-rich areas were demonstrated within carotid plaques,32 and the degree of carotid stenosis was related with plasma FFA.33 An association between carotid IMT and the leptin:adiponectin ratio has been reported both in patients with type 2 diabetes mellitus34 and healthy men.35

Interestingly, the independent association between baseline CCA-IMT and FFA was detected in men but not women, who, despite being more insulin sensitive, had higher levels of circulating FFA, probably because of higher body adiposity (Table I). This discrepancy might reflect the differences in endogenous antioxidant capacity, which seemingly is higher in females than in males.36,37

In women, fasting plasma glucose was an important determinant of carotid wall thickness and early plaque presence, which were both higher in women in the top tertile of fasting plasma glucose, with fasting glucose levels ranging from 5.2 to 6.8 mmol/L (ie, well below the diabetic range). The independent association between fasting plasma glucose and baseline CCA-IMT was also confirmed in a multiple regression models, although only for overweight/obese women. Finally, both baseline CCA-IMT and plaque presence were higher in women with IFG. In contrast, no association was demonstrated between CCA-IMT or plaque presence and 2-hour glucose, and women with impaired glucose tolerance had carotid measures comparable with those of women with normal glucose metabolism.

These data contribute to the still open discussion on the role of fasting and postchallenge glucose levels in macrovascular disease and cardiovascular risk38 and are in agreement with the suggestion of the American Diabetes Association to lower the IFG diagnostic threshold to 5.6 mmol/L,39 as well as with the results of a meta-analysis, demonstrating that the relative cardiovascular risk associated with plasma glucose level is higher for nondiabetic women than men.40 The rationale for the sex-specific relationship between plasma glucose and markers of atherosclerosis or cardiovascular risk is still unclear, but it is supposed to reflect the differences in other cardiovascular risk factors.40 In the men of this study, the strongest metabolic determinant of carotid wall thickness and plaque presence was LDL-cholesterol, which might overrule the proatherogenic effect of glucose.2

Plasma glucose can directly provoke structural changes in the vascular wall by a variety of mechanisms: endothelial dysfunction, vascular smooth muscle cell proliferation, and inflammatory phenotype change in macrophages.1,2,6 In addition, in the current data, women in the highest tertile of fasting glucose had a significantly higher leptin:adiponectin ratio as compared with women in the lower tertiles (2.0 [2.7] versus 1.2 [1.7] and 1.5 [2.1]; $P<0.0001$ for both); similarly, women with IFG had a higher leptin:adiponectin ratio as compared with those with normal glucose metabolism (2.6 [3.4] versus 1.4 [1.0]; $P<0.01$). This observation suggests that circulating adipocytokines, whose secretion seems to be influenced by
glucose levels\textsuperscript{41} may participate in the proatherogenic action of plasma glucose.

CCA-IMT progression did not correlate with any cardio-metabolic risk factors or with their changes but only with age and baseline IMT values, directly and inversely, respectively. The lack of correlations between IMT progression rate and risk factors may be related to a lower precision of IMT progression measurements, which show substantially higher within-subject variance than baseline IMT measures\textsuperscript{19,42} However, it must be considered that the use of progression data in a healthy population probably requires a longer follow-up period, during which significant changes in risk load can develop and consequently influence the carotid wall.\textsuperscript{42} In our population, the main determinants of baseline CCA-IMT did not change substantially during a 3-year period. For this reason, a subset of the the RISC population is being followed up, and additional evaluations of carotid IMT and risk load are planned at 10 years. The inverse relationship between IMT progression and baseline IMT values was apparent also in a healthy population of Young Finns study and was explained by regression to the mean\textsuperscript{43}; however, it might reflect a physiological vascular remodeling aimed to maintain low wall tensile stress of the artery by increasing the thickness of its wall.\textsuperscript{44}

**Study Limitations**

Conventional ultrasound scanners and not radiofrequency-based wall-tracking systems were used; therefore, indices of carotid stiffness representing a very early marker of vascular damage could not be measured. Information about menopause, smoking habit, and therapy was obtained by self-reported questionnaires and not verified. Another limitation of our study is the loss of 29% of participants during the follow-up. However, cross-sectional and longitudinal cohorts were comparable for sex distribution, body mass, and IS, and the difference in age was <1 year (Figure 1). As discussed above, the 3-year follow-up period was probably too short for evaluating the impact of cardio-metabolic risk factors on IMT progression rate in healthy people. Finally, the population of the present study consists of healthy young-to-middle aged whites, and we cannot rule out that a different pattern of associations may be found in older populations, different ethnic groups, or in selected cohorts of subjects with advanced atherosclerotic disease.

**Conclusions**

In young-to-middle aged European participants without confounding comorbidities, the association of carotid IMT or plaque presence with IS and its metabolic correlates differs between men and women. In men, especially if overweight/obese, a lower IS is associated with higher carotid IMT. This association seems to be mediated by higher circulating FFA and by the mutual relation between adipoktyones with proatherogenic and antiatherogenic properties. In women, especially if overweight/obese, both CCA-IMT and plaque presence are independently associated with fasting plasma glucose levels. Collectively, these data provide new insight into the role of sex, adiposity, and IS in the development of early atherosclerotic changes.

**Acknowledgments**

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Disclosures

None.

References


Significance

This is the first study in which the association between insulin sensitivity, measured directly by the euglycemic hyperinsulinemic clamp technique, and carotid intima-media thickness has been explored in a relatively large cohort of individuals free of hypertension, diabetes mellitus, or dyslipidemia, and in which all analyses have been performed separately for men and women and controlled for established atherosclerotic risk factors and habitual physical activity. Our results suggest that the association of carotid intima-media thickness with insulin sensitivity and its metabolic correlates differs between men and women. In men, especially if overweight/obese, a lower insulin sensitivity is associated with higher carotid intima-media thickness. This association seems to be mediated by higher circulating free fatty acids and by the mutual relation between adipocytokines with proatherogenic (leptin) and antiatherogenic (adiponectin) properties. In women, especially if overweight/obese, baseline intima-media thickness in the common carotid artery is independently associated with fasting but not postchallenge plasma glucose levels.
Insulin Sensitivity and Carotid Intima-Media Thickness: Relationship Between Insulin Sensitivity and Cardiovascular Risk Study
Michaela Kozakova, Andrea Natali, Jacqueline Dekker, Henning Beck-Nielsen, Markku Laakso, Peter Nilsson, Beverley Balkau and Ele Ferrannini
on behalf of the RISC Investigators*

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Materials and Methods

Study Population  The Relationship between Insulin Sensitivity and Cardiovascular Risk (RISC) Study (www.egir.org) recruited apparently healthy Caucasians in 19 centers in 14 European countries between June 2002 and July 2004 [1]. Study participants were aged between 30 and 60 years and blood pressure (BP; <140/<90 mmHg), serum cholesterol (<7.8 mmol/L), plasma triglycerides (<4.6 mmol/L), fasting and 2-hour plasma glucose concentrations (<7.0 and 11.1 mmol/L) were within established limits. Exclusion criteria were the presence of overt cardiovascular disease, chronic diseases (hypertension, diabetes, dyslipidemia, inflammatory disease), class III obesity (body mass index [BMI] ≥40 kg/m²), the presence of carotid stenosis >40% or calcified carotid plaques, drug treatment for hypertension, diabetes, dyslipidemia or obesity, steroid use. Our objective was to study a healthy population free of therapeutic interventions, in which, however, the relationships between IMT and established risk factors were still detectable.

In the RISC study, 1,566 participants were originally recruited; 356 were excluded for not meeting inclusion criteria or for an incomplete baseline examination (Figure 1). For the purpose of this study, we further excluded 30 persons in whom only the common carotid artery (CCA) segment was recorded and the presence/absence of carotid plaques in the bulb or proximal internal carotid artery could not be verified. Therefore, the final cross-sectional cohort included 1,180 persons (525 men and 655 women) with complete baseline data and adequate carotid US. In 784 out of 1,180 participants of the cross-sectional cohort (66%) habitual physical activity (PA) was also measured (Figure 1); this subgroup was comparable to the entire cross-sectional cohort for gender distribution, age, CCA-IMT, BMI and IS. The 3-year follow-up included 380 men and 453 women (longitudinal cohort). The subgroup of 347 persons who were not available for follow-up evaluation was younger and had lower CCA-IMT as compared to the longitudinal cohort (Figure 1).

Study Protocol  A standardized examination protocol included anthropometry, brachial BP measurements, resting ECG, an oral glucose tolerance test (OGTT), a euglycemic hyperinsulinemic clamp, a high-resolution ultrasound of the extracranial carotid arteries, and accelerometer monitoring of habitual PA. Information regarding medical history, drug use, alcohol and cigarette consumption was collected using standardized self-reported questionnaires. For smoking habits, participants were categorized as never smoker, current smoker, and ex-smoker (if smoking had ceased ≥1 year prior to the study). All examinations, except the clamp and accelerometric monitoring, were repeated after an observational period of 3 years±1 month. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the local ethics committee in each recruitment center. Written consent was obtained from all participants.

Body Composition Assessment and Blood Pressure Measurement  Body weight and fat-free mass (FFM) were measured by electrical bioimpedance using a Body Composition Analyzer Model TB-300 (TANITA, Tokyo, Japan) and following standardized protocol; fat mass was calculated as the difference between body weight and FFM. Waist circumference was measured as the narrowest circumference between the lower rib margin and anterior superior iliac crest. Brachial BP was measured by a digital electronic tensiometer (Omron, model 705cp, Kyoto, Japan, regular or large adult cuffs according to the arm circumference) in participants seated for at least 10 min.

Oral Glucose Tolerance Test (OGTT) and Insulin Clamp  A standard 75-g OGTT was performed, with blood samples taken before and 30, 60, 90, and 120 min after glucose ingestion. Impaired fasting plasma glucose (IFG) was defined as fasting plasma glucose levels between 5.6 and 7.0 mmol/L and impaired glucose tolerance (IGT) as 2-hour glucose levels between 7.8 and 11.1 mmol/L on the 75-g OGTT.

On a separate day within one month of the OGTT, a euglycemic hyperinsulinemic clamp was performed. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 pmol/min·m² simultaneously with a variable 20% dextrose infusion adjusted every 5–10 min to maintain plasma glucose level within 0.8 mmol/L (±15%) of the target glucose level (4.5–5.5 mmol/L). Additional blood samples were obtained at 20-min intervals for insulin determination. The clamp procedure was standardized across centers [1]. IS was expressed as the ratio of the M value – averaged over the final 40 min of the 2-hour clamp and normalized by the FFM - to the mean plasma insulin concentration measured during the same interval (M/I, in units of µmol·min⁻¹·m⁻²).
**Carotid Artery Ultrasound Imaging and Analysis**  High resolution B-mode ultrasound of the extracranial carotid arteries was performed in each recruiting center by technicians. Each technician underwent training at the centralized reading center, and was certified upon revision of 10 complete carotid ultrasound scans performed in his/her own laboratory according to the standardized protocol. Longitudinal B-mode images of the left and right CCA, carotid bifurcation and internal carotid artery were recorded from anterior, lateral and posterior angle. In addition, a continuous short-axis scan, along the course of the CCA and origins of internal and external carotid arteries, was taken in order to detect plaques that might be missed in longitudinal views. All carotid ultrasounds were analyzed in a centralized reading center (Pisa) by a single reader (M.K.), using the computer-driven image analysis system MIP (Medical Image Processing; Institute of Clinical Physiology, CNR, Pisa, Italy). CCA far-wall IMT was measured bilaterally in digitized end-diastolic frames, in a segment ~10 mm before the flow divider, and the value reported represents the average of the left and right side. Carotid plaque was defined as IMT >1.5 mm in any carotid segment [4]. In the RISC study, intra-observer variability of IMT measurements was tested in 140 randomly chosen scans; the correlations between the two readings was \( r=0.95 \), and the absolute mean difference was 4.8±2.8%. Inter-test IMT variability was evaluated in 75 examinations repeated 1-2 weeks apart; examinations for inter-test variability were obtained in all centers, both at baseline and at 3 years. The correlation between the two exams was \( r=0.91 \), and the absolute mean difference was 7.2±4.6%.

**Physical Activity Assessment**  Habitual PA was estimated by accelerometer monitoring; a single-axis accelerometer (Computer Science Applications Model AM7164, Manufacturing Technology, Inc., FL, USA) was used to monitor ambulatory movements [5]. The accelerometer was secured by a belt at the small of the back from waking up until going to sleep. Subjects were asked to wear the monitor for 7 days if possible, weekend included, and to behave in their usual manner. In the final analysis, only those days when the accelerometer was worn for at least 10 hours were included. Non-wearing periods were identified as 60 min or more of continuous zero counts. Accelerometer data were processed with custom software developed for this project and were checked for spurious recording: high counts >20,000 counts/min or repeated recording of the same number of counts; the days with spurious data were excluded. The average intensity of daily PA was expressed as the average number of accelerometer counts per 1 min of monitoring time.

**Analytical Procedures**  Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (Roche Method for Modular System, Basel, Switzerland), ApoB (multiplexed immunoassay, LINCOplex, Millipore, Billerica MA), glucose (Cobas Integra Roche, Basel, Switzerland), insulin (a specific time-resolved fluoro-immunoassay; AutoDELFIA Insulin kit; Wallac Oy, Turku, Finland), total plasma adiponectin (in-house time-resolved immunofluometric assay TRIFMA [6]), leptin (in-house DELFIA assay on AutoDELFIA autoanalyzer Wallac Oy, Turku, Finland), FFA (Randox enzymatic kit, Hitachi Modular P unit, Hitachi, Tokyo, Japan), and high-sensitive C-reactive protein (hsCRP; monoclonal antibodies from R&D System; Abingdon, UK) were measured centrally.

**Statistical analysis**  Data are expressed as mean±SD, categorical data as percentages. Variables with a skewed distribution (fat mass, triglycerides, leptin/adiponectin ratio, hsCRP, FFA, fasting and 2-hour insulin, M/I, HOMA-IR, PA, CCA-IMT) are summarized as median and [interquartile range], and were logarithmically transformed for parametric statistical analyses (except for IMT changes, which included negative values). ANCOVA and Kruskal-Wallis tests were used to compare continuous variables, Wilcoxon signed rank test to compare paired values and chi\(^2\) test to compare binary variables. Relations between the outcome variables and continuous variables were assessed by Spearman correlation coefficient (\( \rho \)). Multiple linear regression and logistic regression analyses (adjusted for center) were used to test the independent association of outcome variables (baseline CCA-IMT, 3-year CCA-IMT changes and plaque presence) with their significant univariate correlates. Statistical tests were two-sided, and significance was set at a value of \( P<0.05 \). Statistical analysis was performed by JMP software, version 3.1 (SAS Institute Inc., Cary, NC, USA).
References


Supplemental Table I – Baseline values and 3-year changes (Δ) in common carotid artery (CCA) IMT, median[interquartile range], and carotid plaques presence, percentages, in men and women with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) as compared to those with normal glucose tolerance (NGT).

<table>
<thead>
<tr>
<th></th>
<th>MEN</th>
<th>WOMEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGT n=358</td>
<td>IFG n=117</td>
</tr>
<tr>
<td>Baseline CCA-IMT (µm)</td>
<td>600 [110]</td>
<td>620 [120]</td>
</tr>
<tr>
<td>Plaque baseline (%)</td>
<td>7.3</td>
<td>10.3</td>
</tr>
<tr>
<td>ΔCCA-IMT (µm)*</td>
<td>10 [57]</td>
<td>15 [54]</td>
</tr>
<tr>
<td>Plaque 3 years (%)*</td>
<td>9.0</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>NGT n=532</td>
<td>IFG n=61</td>
</tr>
<tr>
<td>Baseline CCA-IMT (µm)</td>
<td>570 [110]</td>
<td>640 [110]†</td>
</tr>
<tr>
<td>Plaque baseline (%)</td>
<td>5.5</td>
<td>21.3†</td>
</tr>
<tr>
<td>ΔCCA-IMT (µm)*</td>
<td>20 [58]</td>
<td>30 [65]</td>
</tr>
<tr>
<td>Plaque 3 years (%)*</td>
<td>4.9</td>
<td>20.9§</td>
</tr>
</tbody>
</table>

*: at 3 years, data on carotid artery structure available only in 252, 100 and 23 men, and in 364, 45 and 34 women with NGT, IFG and IGT, respectively; †: p<0.01 as compared to NGT; §: p<0.05 as compared to NGT; reported significance adjusted for recruiting center, age, systolic BP, LDL-cholesterol, current smoking, menopausal status and, in the case of ΔCCA-IMT, also for baseline CCA-IMT and 3-year therapeutic interventions.
Supplemental Table II– Univariate Spearman correlation coefficients between baseline values and 3-year changes (Δ) in common carotid artery (CCA) IMT, anthropometric and metabolic variables.

<table>
<thead>
<tr>
<th>Baseline CCA-IMT</th>
<th>Δ CCA-IMT*</th>
<th>Fat mass</th>
<th>Waist</th>
<th>M/I</th>
<th>Insulin</th>
<th>Glucose</th>
<th>Triglycerides</th>
<th>FFA</th>
<th>ApoB**</th>
<th>hsCRP</th>
<th>L/A ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.36</td>
<td>--</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.03</td>
<td>-0.07</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.04</td>
<td>-0.05</td>
<td>-0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>0.29</td>
<td>-0.01</td>
<td></td>
<td>0.83</td>
<td>-0.47</td>
<td>0.55</td>
<td>0.23</td>
<td>0.40</td>
<td>0.19</td>
<td>0.29</td>
<td>0.20</td>
<td>0.74</td>
</tr>
<tr>
<td>0.23</td>
<td>0.05</td>
<td>0.80</td>
<td>--</td>
<td>-0.45</td>
<td>0.56</td>
<td>0.24</td>
<td>0.40</td>
<td>0.19</td>
<td>0.31</td>
<td>0.23</td>
<td>0.71</td>
</tr>
<tr>
<td>-0.09</td>
<td>0.04</td>
<td>-0.31</td>
<td>-0.31</td>
<td>--</td>
<td>-0.53</td>
<td>-0.08</td>
<td>-0.31</td>
<td>-0.27</td>
<td>-0.22</td>
<td>-0.14</td>
<td>-0.56</td>
</tr>
<tr>
<td>0.10</td>
<td>-0.03</td>
<td>0.50</td>
<td>0.49</td>
<td>-0.45</td>
<td>--</td>
<td>0.30</td>
<td>0.39</td>
<td>0.09</td>
<td>0.25</td>
<td>0.19</td>
<td>0.70</td>
</tr>
<tr>
<td>0.24</td>
<td>-0.01</td>
<td>0.33</td>
<td>0.32</td>
<td>0.02</td>
<td>0.37</td>
<td>--</td>
<td>0.22</td>
<td>0.01</td>
<td>0.09</td>
<td>0.07</td>
<td>0.20</td>
</tr>
<tr>
<td>0.24</td>
<td>0.07</td>
<td>0.32</td>
<td>0.34</td>
<td>-0.23</td>
<td>0.32</td>
<td>0.22</td>
<td>--</td>
<td>0.09</td>
<td>0.57</td>
<td>0.14</td>
<td>0.41</td>
</tr>
<tr>
<td>0.09</td>
<td>-0.04</td>
<td>0.11</td>
<td>0.05</td>
<td>-0.24</td>
<td>0.05</td>
<td>0.01</td>
<td>0.09</td>
<td>--</td>
<td>0.08</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>0.22</td>
<td>0.03</td>
<td>0.15</td>
<td>0.16</td>
<td>-0.06</td>
<td>0.16</td>
<td>0.10</td>
<td>0.48</td>
<td>0.01</td>
<td>--</td>
<td>0.11</td>
<td>0.27</td>
</tr>
<tr>
<td>0.05</td>
<td>0.06</td>
<td>0.24</td>
<td>0.23</td>
<td>-0.13</td>
<td>0.19</td>
<td>0.06</td>
<td>0.15</td>
<td>0.01</td>
<td>0.09</td>
<td>--</td>
<td>0.23</td>
</tr>
<tr>
<td>0.17</td>
<td>-0.09</td>
<td>0.72</td>
<td>0.66</td>
<td>-0.48</td>
<td>0.61</td>
<td>0.25</td>
<td>0.30</td>
<td>0.06</td>
<td>0.13</td>
<td>0.27</td>
<td>--</td>
</tr>
</tbody>
</table>

Upper-right side of the Table reports the values of Spearman coefficients for men; lower-left side of the Table reports the values of Spearman coefficients for women (in italics); L/A ratio: leptin:adiponectin ratio; *: in 380 men and 453 women; **: in 384 men and 484 women.
Supplemental Table III – Independent determinants of baseline common carotid artery (CCA) IMT in men (N=328) and women (N=456) undergoing the monitoring of physical activity ($\beta$=standardized regression coefficient).

<table>
<thead>
<tr>
<th></th>
<th>Men ($\beta \pm SE$)</th>
<th></th>
<th>Women ($\beta \pm SE$)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P$</td>
<td></td>
<td>$P$</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline CCA-IMT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.37 ± 0.05</td>
<td>&lt;0.0001</td>
<td>0.44 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.08 ± 0.06</td>
<td>0.19</td>
<td>0.15 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.18 ± 0.05</td>
<td>0.001</td>
<td>Current smoking (yes)</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td>Leptin:adiponectin ratio</td>
<td>0.08 ± 0.05</td>
<td>0.10</td>
<td>Fat mass (kg)</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.11 ± 0.05</td>
<td>&lt;0.05</td>
<td>Fasting glucose (mmol/L)</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>PA (counts/min)</td>
<td>-0.05 ± 0.05</td>
<td>0.37</td>
<td>Triglycerides (mmol/L)</td>
<td>0.03 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PA (counts/min)</td>
<td>-0.04 ± 0.04</td>
</tr>
<tr>
<td>Total model $R^2$</td>
<td>0.32</td>
<td>&lt;0.0001</td>
<td>Total model $R^2$</td>
<td>0.43</td>
</tr>
</tbody>
</table>

All analyses adjusted for recruiting center.
Supplemental Table IV – Independent determinants of baseline common carotid artery (CCA) IMT in lean and overweight/obese men and women ($\beta$=standardized regression coefficient).

<table>
<thead>
<tr>
<th>Baseline CCA-IMT</th>
<th>$\beta \pm SE$</th>
<th>$P$</th>
<th>$\beta \pm SE$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MEN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lean (N=198)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.42 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.34 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.23 ± 0.07</td>
<td>&lt;0.001</td>
<td>0.03 ± 0.05</td>
<td>0.62</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.18 ± 0.07</td>
<td>0.01</td>
<td>0.20 ± 0.05</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>M/I</td>
<td>0.07 ± 0.07</td>
<td>0.29</td>
<td>-0.16 ± 0.05</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>0.38 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.26 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Overweight/Obese (N=327)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.40 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.34 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.21 ± 0.07</td>
<td>&lt;0.005</td>
<td>0.01 ± 0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.16 ± 0.07</td>
<td>&lt;0.05</td>
<td>0.21± 0.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leptin:adiponectin ratio</td>
<td>0.02 ± 0.07</td>
<td>0.79</td>
<td>0.13± 0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.06 ± 0.07</td>
<td>0.41</td>
<td>0.12 ± 0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total model $R^2$</td>
<td>0.36 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.28 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Model B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.40 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.34 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.21 ± 0.07</td>
<td>&lt;0.005</td>
<td>0.01 ± 0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.16 ± 0.07</td>
<td>&lt;0.05</td>
<td>0.21± 0.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leptin:adiponectin ratio</td>
<td>0.02 ± 0.07</td>
<td>0.79</td>
<td>0.13± 0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.06 ± 0.07</td>
<td>0.41</td>
<td>0.12 ± 0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total model $R^2$</td>
<td>0.36 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.28 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>WOMEN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lean (N=401)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.48 ± 0.06</td>
<td>&lt;0.0001</td>
<td>0.29 ± 0.07</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.16 ± 0.05</td>
<td>&lt;0.0005</td>
<td>0.14 ± 0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.14 ± 0.06</td>
<td>0.01</td>
<td>0.14 ± 0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Current smoking (yes)</td>
<td>0.08 ± 0.05</td>
<td>0.09</td>
<td>0.14 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Current smoking (yes)</td>
<td>0.04 ± 0.05</td>
<td>0.50</td>
<td>0.05 ± 0.06</td>
<td>0.41</td>
</tr>
<tr>
<td>M/I</td>
<td>0.37 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.37 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total model $R^2$</td>
<td>0.37 ± 0.07</td>
<td>&lt;0.0001</td>
<td>0.37 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
**Model B**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>P-value</th>
<th>Mean ± SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.43 ± 0.06</td>
<td>&lt;0.0001</td>
<td>0.27 ± 0.07</td>
<td>0.0005</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.13 ± 0.05</td>
<td>&lt;0.01</td>
<td>0.11 ± 0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.14 ± 0.06</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking (yes)</td>
<td>0.06 ± 0.05</td>
<td>0.19</td>
<td>0.16 ± 0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.10 ± 0.04</td>
<td>&lt;0.05</td>
<td>0.08 ± 0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>0.08 ± 0.05</td>
<td>0.10</td>
<td>0.14 ± 0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.09 ± 0.05</td>
<td>0.06</td>
<td>0.03 ± 0.06</td>
<td>0.67</td>
</tr>
<tr>
<td>Total model $R^2$</td>
<td>0.39</td>
<td>&lt;0.0001</td>
<td>0.40</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

All analyses adjusted for recruiting center.