Effect of vitamin K supplementation on insulin resistance in older men and women

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**Objective**: Vitamin K has a potentially beneficial role on insulin resistance, but evidence is limited in humans. We tested the hypothesis that vitamin K supplementation for 36 months will improve insulin resistance in older men and women.

**Research Design**: This was an ancillary study of a 36-month, randomized, double-blind, controlled trial designed to assess the impact of supplementation with 500 μg/d of phylloquinone on bone loss. Study participants were older, non-diabetic men and women (n = 355; 60-80 y; 60% women). The primary outcome of this study was insulin resistance, measured by the homeostasis model (HOMA-IR) assessed at 36 months. Fasting plasma insulin and glucose were examined as the secondary outcomes.

**Results**: The effect of 36-month vitamin K supplementation on HOMA-IR differed by sex (sex-by-treatment interaction: \( P = 0.02 \)). HOMA-IR was statistically significantly lower at the 36-month visit among men in the supplement group vs. the men in the control group (\( P = 0.01 \)) after adjustment for baseline HOMA-IR, BMI, and body weight change. There were no statistically significant differences in outcome measures between intervention groups in women.

**Conclusions**: Vitamin K supplementation for 36 months at doses attainable in the diet may reduce progression of insulin resistance in older men.

**Clinical Trial Registration**: This study is registered with ClinicalTrial.gov (#NCT00183001). This manuscript has not been submitted to Clinical Trials.gov.
Limited evidence from human and animal studies suggests vitamin K may be inversely associated with insulin resistance (1-4). In an observational study, higher dietary and supplemental vitamin K intakes were associated with greater insulin sensitivity and better glycemic status in a community-based cohort of men and women (3). In a small metabolic study of young men (n = 12), short-term (1-week) vitamin K supplementation improved the insulin response after an oral glucose challenge (2). Although these studies support a potential novel role for vitamin K in insulin resistance, the available human data are limited. Furthermore, biological mechanisms behind the association between vitamin K and insulin and glucose metabolism are uncertain. Vitamin K and vitamin K-dependent proteins (prothrombin and protein S) have been identified in organs important for glucose and insulin metabolism, such as liver and pancreas (5; 6). However, the function of vitamin K is not well understood beyond its role as an enzyme cofactor for γ-carboxylation of certain glutamic acid residues in vitamin K-dependent proteins (7).

The objective of this study was to assess the effect of vitamin K supplementation for 36 months on insulin resistance, defined as the homeostasis model assessment of insulin resistance (HOMA-IR), among older men and women in a randomized, double-blind, controlled trial. The primary end point for the original trial was the 36-month change in bone mineral density of the femoral neck. We hypothesized that vitamin K supplementation at intakes attainable in the diet would have beneficial effects on insulin resistance. As secondary objectives, we also examined the effect of vitamin K supplementation on fasting plasma insulin and glucose concentrations.

METHODS

Study design—Men and post-menopausal women, aged 60 to 80 years, were recruited as part of a 36-month randomized, double-blind, controlled vitamin K supplementation trial designed to test the primary hypothesis that vitamin K supplementation would reduce age-related bone loss and vascular calcification, as described elsewhere (8). Exclusion criteria included coronary heart disease, renal disease or nephrolithiasis, liver disease, osteoporosis, and use of medications known to influence bone health. Diabetes was not an exclusion and use of insulin or oral hypoglycemic medications was allowed. However for the purpose of the present study, we excluded subjects with diabetes mellitus from the analysis. Diabetes mellitus was defined as fasting glucose ≥ 126 mg/dL and/or self-reported use of hypoglycemic drugs or insulin medication during the study.

The subjects were advised to maintain their usual diets and to avoid taking dietary supplements other than those provided throughout the study. The treatment group received 500 μg/d of phylloquinone (vitamin K₃), which is approximately five times the Adequate Intake for vitamin K (9), as part of an effervescent multivitamin formulation. The control group received the multivitamin formulation without phylloquinone. All study participants also received a second daily effervescent tablet containing 600 mg of elemental calcium in the form of calcium carbonate, and 10 μg (400 IU) of vitamin D in the form of cholecalciferol. The detailed information on the supplements is provided elsewhere (8).
**Study participants**—Of the original 452 enrolled, 355 who did not have diabetes mellitus completed the study (Figure 1). All participants signed a written informed consent, and this study was approved by the Institutional Review Board at Tufts Medical Center. This study was registered with ClinicalTrials.gov (#NCT00183001).

**Biochemical measurements**—All blood samples were drawn between 7:00 and 10:00 am after a minimum 10-hour fast. Dedicated aliquots of plasma and serum were stored at -80 ºC and protected from light until the time of analysis. Plasma insulin was determined using the Human Insulin Specific RIA Kits (LINCO research St. Charles, Missouri). All assays were done in duplicate, and the measurements were repeated if the total coefficient of variation (CV) of the duplicates was greater than 15%. The within run CV of the samples was 6.4%. Plasma glucose was analyzed using the enzymatic, kinetic (hexokinase-UV/NAD) method on an Olympus AU400 instrument with Olympus agents. The intra assay CV% was 2.0% and the inter assay CV% was 3.4%. HOMA-IR was calculated as \[\text{fasting plasma glucose (mmol/L)} \times \text{fasting plasma insulin (μU/mL)}]/22.5 \ (10)\). Plasma phylloquinone and the proportion of osteocalcin that is not carboxylated (percent undercarboxylated osteocalcin [%ucOC]) are biochemical markers for vitamin K status (11; 12), and the changes of these markers were examined to assess the overall efficacy of vitamin K supplementation. Plasma concentrations of phylloquinone were measured by reverse-phase HPLC (13). The total CVs for the two control samples with an average phylloquinone result of 1.2 and 4.5 nmol/L were 7.4 and 8.0%, respectively. Serum total and undercaboxylated osteocalcin were measured by radioimmunoassay, using the method of Gundberg (11). The total CVs for the three control samples with an average total osteocalcin result of 6.4, 14.7, and 23.8 were 8.8, 8.9, and 7.6%, respectively. Since vitamin K is a cofactor for carboxylation of specific glutamate residues, lower [%ucOC] indicates high vitamin K status (11).

**Other measurements**—Total body fat was measured by a whole body dual energy X-ray absorptiometry scan using GE-Lunar model Prodigy scanner (encore 2002; version 6.10.029). Leisure, household and occupational activity was estimated with use of the Physical Activity Scale for the Elderly questionnaire (14). Tobacco and alcohol use was determined by questionnaire. Height was measured with a stadiometer and weight with a digital scale. BMI was calculated as weight (kg)/height (m²). Usual dietary intakes over the year prior to entry in the study were assessed using the Harvard food frequency questionnaire (15). Information on adherence to the supplementation (%) was created based on self-reported pill count.

**Statistical analysis**—SAS statistical software (version 9; SAS institute, Cary, NC) was used for all statistical analyses. Statistical significance was defined as a \(P\) value < 0.05.

The characteristics of the two study treatment groups were compared at baseline using Student's t-test and Fisher's exact test for continuous and categorical variables, respectively. The distributions of HOMA-IR, fasting insulin, plasma phylloquinone and phylloquinone intake were skewed to the right; thus, we analyzed these variables using the natural logarithm transformation. To assess overall efficacy of vitamin K
supplementation, we examined changes in biochemical measures of vitamin K status (i.e. plasma phylloquinone and %ucOC) between baseline and 36-month visit. Student t-test was used to compare changes of vitamin K status and other characteristics between two study groups.

The effect of vitamin K supplementation on HOMA-IR and fasting plasma insulin and glucose concentrations was assessed by using analysis of covariance at the 6 and 36-month visits. Covariates used in the analysis were baseline outcome measures and BMI, and weight change. We subsequently repeated the analysis using 36-month change in physical activity and total body fat as additional covariates. There was one potential outlier that had an extremely high plasma fasting insulin concentration (fasting insulin = 82.3 µU/mL). This participant was removed from the HOMA-IR and fasting insulin analysis, although inclusion or exclusion of this potential outlier did not significantly change the results. We also repeated analyses with two sets of data excluding participants with < 85% adherence to vitamin K supplement (n = 66), or including those with diabetes mellitus (n = 46), to assess the stability of the findings.

We tested for statistical interaction with vitamin K intervention and sex. Since we observed significant interactions between sex and treatment group in HOMA-IR and plasma fasting insulin at the 36-month visit (HOMA-IR: P = 0.02; insulin: P = 0.01), men and women were analyzed separately. With our overall sample size of 355, and significant between group difference in HOMAIR of 0.44 with a between group SD of 1.4, there was a 82% chance that the hypothesis of no supplement effect would be rejected at the 0.05 level of significance. However, upon stratification by gender, the statistical power was reduced to 44% in men and 62% in women.

To explore a possible explanation for the observed sex interaction, we examined the associations between plasma phylloquinone concentrations and BMI. We calculated plasma triglyceride-adjusted Pearson’s partial correlation coefficients (Pearson’s partial r).

RESULTS

The baseline characteristics are summarized in Table 1. Participant characteristics were comparable between the two treatment groups in both men and women, with the exception of higher prevalence of overweight or obesity among women in the vitamin K-treated group.

In the vitamin K treated group, 36-month changes in plasma phylloquinone and %ucOC were significantly different from control group in both men and women (P < 0.001). The mean 36-month changes (±SD) in plasma phylloquinone concentrations for men and women receiving the vitamin K supplement were 1.4 (±2.5) and 2.3 (±2.8) nmol/L, respectively, whereas the changes in the men and women in control group were -0.2 (±1.9) and 0.1 (±1.3) nmol/L, respectively. The 36-month changes in %ucOC were -19.0% (±23.4%) for men and -19.3% (±20.5%) for women in the treated group, and 0.4% (±17.7%) for men and 2.3% (±21.2%) for women in the control group.

HOMA-IR and plasma insulin concentrations were statistically significantly lower at the 36-month visit among men in the supplement group vs. the men in the control group after adjustment for baseline HOMA-IR, BMI
and weight change (Table 2). There was no significant difference among men in HOMA-IR and plasma insulin at the 6-month visit. When statistical analyses were repeated using 36-month change in physical activity and % body fat as additional covariates, differences among men in HOMA-IR were still statistically different (p=0.03) whereas there was attenuation in differences in the plasma insulin (p=0.07). When statistical analyses were restricted to those with ≥ 85% adherence, similar results were noted for 36-month changes in HOMA-IR and plasma insulin among men (vitamin K treated group vs. control group: HOMA-IR, -0.11 [95%CI: -0.40, 0.19] vs. 0.47 [95% CI: 0.15, 0.80], P = 0.01; insulin, -0.46 [95% CI: -1.56, 0.65] vs. 1.47 [95%CI: 0.22, 2.71], P = 0.03). Fasting plasma glucose concentrations did not differ between two study groups among men at any time point. There were no statistically significant differences in changes in HOMA-IR and fasting plasma insulin and glucose between women in the vitamin K treated groups and control group. Repeating the analyses including diabetes individuals did not affect our findings (data not shown).

In analysis performed to examine a possible reason for the discrepancy of findings between men and women, we found plasma phylloquinone concentrations were inversely associated with BMI in women, but not men, after adjustment for plasma triglycerides (Pearson’s partial r = -0.15, P = 0.02). In contrast, the % body fat was not correlated with plasma phylloquinone concentrations in men or women. Among women in our study, there was a significant increase in mean % body fat of 0.6% over the 36 months (p=0.002), while body weight did not significantly change. In men, the mean body weight decreased by 0.5 kg (p=0.02) and body fat increased by 0.5% (p=0.001), respectively. There were no statistically significant changes in physical activity for men or women.

**DISCUSSION**

The major finding of this study was that daily supplementation with 500 μg of phylloquinone for 36 months had a protective effect on progression of insulin resistance in older men.

In an animal study, rats fed a low vitamin K diet had impaired early insulin response and subsequent increased insulin secretion after intravenous administration of glucose (1). Higher vitamin K intake was cross-sectionally associated with reduced insulin resistance in both men and women in the Framingham Offspring cohort (3). A metabolic study of young men showed a significant association between vitamin K and post-glucose challenge measures, but not fasting measures (2). In our study, beneficial effect of vitamin K supplementation for 36 months was observed using fasting measures of insulin resistance. The effect of this intervention on post-glucose challenge measures of insulin resistance was not tested in our study.

Recent studies proposed that the uncarboxylated form of osteocalcin, a vitamin K-dependent bone protein, may improve insulin sensitivity and increase β-cell insulin secretion partially through the enhancement of β-cell proliferation, energy expenditure, and adiponectin expression in mice (16; 17). In our study, men receiving vitamin K supplementation had less uncarboxylated osteocalcin compared to the control group, which does not support the findings of the animal studies. Alternatively, it is plausible that vitamin K may improve insulin sensitivity through suppression of
inflammation. In vivo and in vitro studies have shown that vitamin K reduced lipopolysaccharide-induced inflammation (18; 19). More recently, it was reported that biochemical and dietary measures of vitamin K status were inversely associated with inflammatory markers in an observational study (20).

The beneficial effect of vitamin K on insulin resistance was limited to men in this study. However, in a recent observational study, the inverse association between vitamin K intake and insulin resistance was observed in men and women (3). In a previous metabolic study, only young men were studied (2), and there are no other studies from which to make a comparison. Although adjustment of BMI in the statistical model did not change our finding in women, one potential explanation for this lack of protective effect of vitamin K on insulin resistance in women is the role of adipose tissue in modulating response to vitamin K supplementation. In our study, overweight or obesity, which is major determinant of insulin resistance (21; 22) was more prevalent in women who received vitamin K supplementation compared with those did not. Furthermore, there was an inverse association between plasma phylloquinone concentrations and BMI in the women suggestive of an impaired response to vitamin K supplementation. It may be plausible that adipose tissue stores the fat-soluble vitamin K, which may render the vitamin K unavailable for peripheral organs. In the absence of data on the role of the adipose tissue in vitamin K metabolism, this is currently speculative. Alternatively, it is plausible that we were statistically underpowered to detect statistically significant differences in HOMA-IR in response to vitamin K supplementation among women.

The interpretation of these findings is limited by several factors. First, this study was based on the analyses of data obtained from a study designed to determine the effect of vitamin K supplementation on changes in bone mineral density and vascular calcification in older men and women. Our findings may not be representative of the general population due to the exclusion criteria of the parent study, and measures presented in this study were not necessarily obtained by optimal techniques. We acknowledge that use of the hyperinsulinemic euglycemic clamp would have provided a more direct measure of insulin secretion and sensitivity than HOMA-IR, which only provides an indirect estimate of insulin resistance. Likewise, our assessment of body composition was limited to BMI and % body fat, as measured by DXA, and did not provide information on regional adiposity. We also had limited statistical power to detect differences in HOMA-IR in response to vitamin K supplementation, which may explain the null findings in women. Finally, most of our participants were Caucasians, thus generalizability of our finding need to be examined in other populations.

In summary, 36 months of vitamin K supplementation had a beneficial effect on insulin resistance in older men, but not older women. As the parent study was not designed to test this hypothesis, these findings need to be replicated in a study designed specifically to test the hypothesis that vitamin K plays a protective role in insulin resistance in older adults.

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The authors responsibilities were as follows – MY: statistical analysis and draft of the manuscript; SLB: formulation of original idea, and provision of data; All authors: study design, interpretation of data, and critical revision of the manuscript.

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**Disclosure Statement:** SLB, MKS, CG, JWP, BD-H, MY, PFJ, JBM, ES have nothing to declare; GD consulted for legal matter unrelated to this study.
REFERENCES:


Figure Legend:

Figure 1: Study sample selection

Randomization (n = 452)
185 Men & 267 Women

Received vitamin K supplementation (n = 229)
95 Men & 134 Women

Excluded from the analysis
(n = 45; 15 M & 30 F)
Lost to Follow-up (n = 25)
Diabetes (n = 20)

Completed Study (n = 184)
80 Men & 104 Women

Excluded those with < 85% adherence
to multivitamin supplement (with
phyloquinone) from the subgroup
analysis (n = 34; 11 M & 23 F)

Subgroup analysis (n = 150)
69 Men and 81 Women

Received no vitamin K (n = 223)
90 Men & 133 Women

Excluded from the analysis
(n = 52; 28 M & 24 F)
Lost to Follow-up (n = 26)
Diabetes (n = 26)

Completed Study (n = 171)
62 Men & 109 Women

Excluded those with < 85% adherence
to multivitamin supplement (without
phyloquinone) from the subgroup
analysis (n = 32; 7 M & 25 F)

Subgroup analysis (n = 139)
55 Men and 84 Women
Table 1: Baseline characteristics in subjects included in the primary analysis\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
<th>P value</th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Vitamin K</td>
<td></td>
<td>Control</td>
<td>Vitamin K</td>
<td></td>
<td>Control</td>
<td>Vitamin K</td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>\textbf{N}</td>
<td>62</td>
<td>80</td>
<td></td>
<td>109</td>
<td>104</td>
<td></td>
<td>109</td>
<td>104</td>
<td></td>
<td>109</td>
</tr>
<tr>
<td>\textbf{Age (yr)}</td>
<td>69.7 [6.2]</td>
<td>68.9 [5.5]</td>
<td>0.45</td>
<td>67.5 [5.2]</td>
<td>67.3 [5.5]</td>
<td>0.77</td>
<td>67.5 [5.2]</td>
<td>67.3 [5.5]</td>
<td>0.77</td>
<td>67.5 [5.2]</td>
</tr>
<tr>
<td>\textbf{Overweight/obese (%)}(^2)</td>
<td>74</td>
<td>71</td>
<td>0.70</td>
<td>59</td>
<td>72</td>
<td>0.04</td>
<td>59</td>
<td>72</td>
<td>0.04</td>
<td>59</td>
</tr>
<tr>
<td>\textbf{Total body fat (%)}</td>
<td>36.0 [9.8]</td>
<td>37.8 [8.8]</td>
<td>0.29</td>
<td>38.3 [9.2]</td>
<td>36.7 [9.8]</td>
<td>0.23</td>
<td>38.3 [9.2]</td>
<td>36.7 [9.8]</td>
<td>0.23</td>
<td>38.3 [9.2]</td>
</tr>
<tr>
<td>\textbf{Physical activity scale}(^3)</td>
<td>136 [59]</td>
<td>130 [65]</td>
<td>0.61</td>
<td>123 [56]</td>
<td>128 [55]</td>
<td>0.56</td>
<td>123 [56]</td>
<td>128 [55]</td>
<td>0.56</td>
<td>123 [56]</td>
</tr>
<tr>
<td>\textbf{Current smoking (%)}</td>
<td>8</td>
<td>3</td>
<td>0.24</td>
<td>3</td>
<td>8</td>
<td>0.13</td>
<td>3</td>
<td>8</td>
<td>0.13</td>
<td>3</td>
</tr>
<tr>
<td>\textbf{Current alcohol drink (%)}</td>
<td>73</td>
<td>70</td>
<td>0.85</td>
<td>76</td>
<td>70</td>
<td>0.36</td>
<td>76</td>
<td>70</td>
<td>0.36</td>
<td>76</td>
</tr>
<tr>
<td>\textbf{College or greater education (%)}</td>
<td>58</td>
<td>68</td>
<td>0.29</td>
<td>47</td>
<td>45</td>
<td>0.89</td>
<td>47</td>
<td>45</td>
<td>0.89</td>
<td>47</td>
</tr>
<tr>
<td>\textbf{BP medication use (%)}</td>
<td>34</td>
<td>21</td>
<td>0.13</td>
<td>29</td>
<td>24</td>
<td>0.44</td>
<td>29</td>
<td>24</td>
<td>0.44</td>
<td>29</td>
</tr>
<tr>
<td>\textbf{Statin use (%)}</td>
<td>29</td>
<td>20</td>
<td>0.24</td>
<td>24</td>
<td>24</td>
<td>1.00</td>
<td>24</td>
<td>24</td>
<td>1.00</td>
<td>24</td>
</tr>
<tr>
<td>\textbf{Undercarboxylated Osteocalcin (%)}</td>
<td>39.9 [15.2]</td>
<td>36.7 [15.5]</td>
<td>0.23</td>
<td>42.3 [17.4]</td>
<td>43.5 [16.2]</td>
<td>0.62</td>
<td>42.3 [17.4]</td>
<td>43.5 [16.2]</td>
<td>0.62</td>
<td>42.3 [17.4]</td>
</tr>
<tr>
<td>\textbf{Phylloquinone intake (μg/d)}</td>
<td>168 [114]</td>
<td>176 [118]</td>
<td>0.68</td>
<td>180.8 [113.6]</td>
<td>170 [96.7]</td>
<td>0.81</td>
<td>180.8 [113.6]</td>
<td>170 [96.7]</td>
<td>0.81</td>
<td>180.8 [113.6]</td>
</tr>
<tr>
<td>\textbf{Plasma insulin (μU/mL)}</td>
<td>10.5 [4.8]</td>
<td>10.6 [5.7]</td>
<td>0.95</td>
<td>10.1 [4.7]</td>
<td>10.6 [5.5]</td>
<td>0.78</td>
<td>10.1 [4.7]</td>
<td>10.6 [5.5]</td>
<td>0.78</td>
<td>10.1 [4.7]</td>
</tr>
<tr>
<td>\textbf{HOMA-IR}</td>
<td>2.5 [1.2]</td>
<td>2.5 [1.5]</td>
<td>0.73</td>
<td>2.3 [1.1]</td>
<td>2.5 [1.4]</td>
<td>0.33</td>
<td>2.3 [1.1]</td>
<td>2.5 [1.4]</td>
<td>0.33</td>
<td>2.3 [1.1]</td>
</tr>
<tr>
<td>\textbf{Discontinued taking calcium supplementation (%)}(^4)</td>
<td>8</td>
<td>6</td>
<td>0.75</td>
<td>12</td>
<td>11</td>
<td>0.83</td>
<td>12</td>
<td>11</td>
<td>0.83</td>
<td>12</td>
</tr>
<tr>
<td>\textbf{Discontinued taking multivitamin supplements (%)}</td>
<td>8</td>
<td>6</td>
<td>0.75</td>
<td>8</td>
<td>9</td>
<td>1.00</td>
<td>8</td>
<td>9</td>
<td>1.00</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^1\) All values are expressed as mean [SD] or % for continuous and categorical variables, respectively. For statistical analysis, logarithm transformation was performed for plasma insulin, HOMA-IR, plasma phylloquinone, and phylloquinone intakes. Phylloquinone intake included both dietary and supplemental sources of phylloquinone.

\(^2\) Defined as BMI >25 kg/m\(^2\)

\(^3\) Physical activity scale was calculated based on the questionnaire which capture leisure, household occupational activity (14). Higher scores indicate more physically active condition.

\(^4\) Discontinued taking calcium and vitamin D supplementation.
### Table 2: Changes for HOMA-IR, fasting plasma insulin and glucose concentrations for vitamin K and non-vitamin K supplementation

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Change</th>
<th>Model</th>
<th>Men</th>
<th>Vitamin K</th>
<th>P value</th>
<th>Women</th>
<th>Vitamin K</th>
<th>P value</th>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>62</td>
<td>80</td>
<td>0.77</td>
<td>109</td>
<td>104</td>
<td>0.61</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>6 mo</td>
<td>Crude</td>
<td>0.02 (-0.22, 0.25)</td>
<td>0.06 (-0.15, 0.27)</td>
<td>0.77</td>
<td>-0.03 (-0.19, 0.14)</td>
<td>0.04 (-0.13, 0.20)</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>6 mo</td>
<td>Adjusted</td>
<td>0.03 (-0.21, 0.27)</td>
<td>0.05 (-0.16, 0.26)</td>
<td>0.91</td>
<td>-0.01 (-0.17, 0.15)</td>
<td>0.02 (-0.14, 0.19)</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>36 mo</td>
<td>Crude</td>
<td>0.35 (0.03, 0.68)</td>
<td>-0.09 (-0.37, 0.20)</td>
<td>0.05</td>
<td>0.16 (-0.04, 0.37)</td>
<td>0.31 (0.10, 0.52)</td>
<td>0.32</td>
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<tr>
<td></td>
<td>36 mo</td>
<td>Adjusted</td>
<td>0.39 (0.09, 0.69)</td>
<td>-0.12 (-0.38, 0.15)</td>
<td>0.01</td>
<td>0.19 (0.00, 0.38)</td>
<td>0.28 (0.09, 0.48)</td>
<td>0.49</td>
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<tr>
<td>Plasma insulin</td>
<td>6 mo</td>
<td>Crude</td>
<td>-0.34 (-1.27, 0.59)</td>
<td>-0.2 (-1.02, 0.63)</td>
<td>0.82</td>
<td>-0.68 (-1.34, -0.03)</td>
<td>-0.19 (-0.86, 0.48)</td>
<td>0.30</td>
</tr>
<tr>
<td>(μU/mL)</td>
<td>6 mo</td>
<td>Adjusted</td>
<td>-0.29 (-1.22, 0.65)</td>
<td>-0.23 (-1.06, 0.59)</td>
<td>0.93</td>
<td>-0.60 (-1.24, 0.04)</td>
<td>-0.28 (-0.93, 0.38)</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>36 mo</td>
<td>Crude</td>
<td>1.08 (-0.15, 2.30)</td>
<td>-0.43 (-1.51, 0.64)</td>
<td>0.07</td>
<td>0.23 (-0.50, 0.97)</td>
<td>1.08 (0.33, 1.83)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>36 mo</td>
<td>Adjusted</td>
<td>1.16 (0.02, 2.30)</td>
<td>-0.49 (-1.50, 0.51)</td>
<td>0.04</td>
<td>0.37 (-0.32, 1.06)</td>
<td>0.94 (0.23, 1.64)</td>
<td>0.26</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>6 mo</td>
<td>Crude</td>
<td>3.41 (1.79, 5.04)</td>
<td>3.52 (2.09, 4.96)</td>
<td>0.92</td>
<td>2.28 (0.80, 3.75)</td>
<td>2.31 (0.80, 3.82)</td>
<td>0.97</td>
</tr>
<tr>
<td>(mg/dL)</td>
<td>6 mo</td>
<td>Adjusted</td>
<td>3.70 (2.08, 5.32)</td>
<td>3.30 (1.87, 4.72)</td>
<td>0.72</td>
<td>2.43 (0.98, 3.88)</td>
<td>2.16 (0.67, 3.64)</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>36 mo</td>
<td>Crude</td>
<td>2.15 (0.20, 4.10)</td>
<td>0.98 (-0.75, 2.72)</td>
<td>0.38</td>
<td>1.51 (-0.04, 3.05)</td>
<td>1.33 (-0.24, 2.91)</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>36 mo</td>
<td>Adjusted</td>
<td>2.57 (0.70, 4.44)</td>
<td>0.65 (-1.01, 2.31)</td>
<td>0.14</td>
<td>1.68 (0.17, 3.18)</td>
<td>1.16 (-0.38, 2.69)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values were expressed as least squared (LS) mean (95% CI). LS means are the mean changes in the outcomes, when individual values covariates are held constant. Crude analysis was adjusted for baseline outcome measure (HOMA-IR, or plasma insulin or glucose). Adjusted analysis controlled for baseline outcome measures, baseline BMI and either 6- or 36-month weight change.