Hepatocyte Growth Factor and Clinical Diabetes in Postmenopausal Women

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Objective: To investigate the association between circulating levels of hepatocyte growth factor (HGF), a mesenchymal-derived pleiotrophic factor that is elevated in obesity, and the prevalence of type 2 diabetes.

Research design and methods: A cross-sectional analysis among 892 postmenopausal women within the Women’s Health Initiative Observational Study (WHI-OS).

Results: HGF levels positively correlated with body mass index (BMI) and homeostatic assessment model for insulin resistance (HOMA-IR). In multivariable analysis comparing highest tertile to the lowest tertile of HGF, the odds ratio (OR) for prevalent diabetes was 2.47 (95% CI: 1.12, 5.47; p for trend: 0.014) after accounting for age, race, BMI and other risk factors for diabetes.

Conclusion: HGF levels are associated with presence of type 2 diabetes in postmenopausal women. Future studies should consider prospective evaluation of the association of HGF with the development of type 2 diabetes.
Hepatocyte growth factor (HGF) is a mesenchymal-derived pleiotropic factor that regulates growth, motility and morphogenesis of various cells(1). HGF is highly expressed in white adipose tissue (2; 3) and it stimulates glucose uptake in cultured adipocytes(4). In humans, circulating HGF positively correlates with insulin and glucose (5) and is reported to be elevated in obesity(6), metabolic syndrome(5), hypertension(7) and coronary heart disease(8). Although HGF levels are observed to be elevated in these diabetes-associated conditions, the specific HGF-diabetes association has not been yet investigated. Hence, we conducted a study to examine the cross-sectional relationship between HGF and diabetes in a representative sample of postmenopausal women (n=892) within the Women’s Health Initiative Observational Study (WHI-OS).

**RESEARCH DESIGN AND METHODS**

The WHI-OS is an ongoing prospective study of 93,676 postmenopausal women aged 50-79 years (9). At baseline, women were queried about lifestyle factors, medical history, and personal habits and a physical examination was performed to obtain height, weight, and blood pressure. Fasting blood samples were collected, centrifuged, frozen on site at –70°C and stored in the specimen repository.

We conducted a study using data from a case-cohort study within the WHI-OS that aimed to evaluate the association of several adipokines and risk of cancers of the breast, colorectum, and endometrium(10). The study population for the current analysis included 892 women selected in the subcohort, a representative sample of WHI-OS women without cancer at baseline. Diabetes was defined as history of treated diabetes or fasting glucose ≥126 mg/dl. Plasma HGF levels were measured by a multiplex assay (Human Adipokine Panel B, Millipore, Billerica, MA) based on Luminex xMAP technology (http://www.luminexcorp.com). The inter-assay coefficient of variation for HGF assay was 11.7%. We performed unconditional logistic regression analysis to evaluate the association between HGF tertiles and prevalent diabetes while accounting for potential confounders. Tests of linear trend across HGF tertiles were conducted by assigning a score for each tertile and including this variable as a continuous variable in the model. All statistical analyses were performed using SAS® version 9.1 (Cary, NC), and p-values <0.05 were considered statistically significant.

**RESULTS**

In this population, HGF levels showed modest correlation with age (r=0.20; p<0.0001), body mass index (BMI; r=0.18; p<0.0001), waist circumference (r=0.19; p<0.0001) and insulin resistance as measured by homeostasis model assessment for insulin resistance (HOMA-IR; r=0.21; p<0.0001). In addition, current postmenopausal hormone use and alcohol intake were associated with lower HGF levels (data not shown). In age- and race adjusted logistic regression model, the odds ratio (OR) for prevalent diabetes comparing women in the highest tertile of HGF to those in the lowest tertile was 3.63 (95%CI:1.83-7.19; p-trend<0.0001) which was attenuated to 2.78 (95%CI:1.36-5.69; p-trend=0.003) after additional adjustment for BMI (Table 1). This association remained significant when we further accounted for smoking, physical activity, family history of diabetes, alcohol intake, postmenopausal hormone use and plasma levels of C-reactive protein (CRP) (OR:2.47; 95%CI:1.12-5.47; p-trend=0.014). To control for potential residual confounding by adiposity, we evaluated the effect of additional inclusion of waist in the model. The results, however, were similar
HGF and diabetes

(OR:2.34; 95%CI:1.04-5.28; p-trend=0.024). Further, additional adjustment for circulating insulin levels in the final multivariable model attenuated the results to borderline significance (OR:1.95; 95%CI:0.87-4.40; p-trend=0.078). There was no effect modification of the association by age, race, BMI, hormone use or CRP levels.

DISCUSSION

We found that high HGF levels were associated with prevalence of type 2 diabetes. HGF is a mesenchymal-derived pleiotropic factor that regulates growth, motility and morphogenesis of various cells(1). Although HGF was known initially as a potent mitogen for hepatocytes, it has been recently shown to have effects on other cells including epithelial and endothelial cells. It is expressed in several tissues including lung, kidney, heart, brain and especially fat(11). Circulating levels of HGF are up to 3-fold elevated in obese individuals, demonstrate strong correlation with BMI ($r=0.68; \ p<0.0001$) and substantially decline following weight loss(2; 6).

In addition to obesity, several studies have linked HGF to other diabetes-associated disease conditions. HGF levels are elevated in patients with acute myocardial infarction and predict mortality following coronary intervention(8). Furthermore, circulating HGF level are also associated with the metabolic syndrome(5) and hypertension(7), reinforcing its potential role in cardiometabolic disease. We recently reported that circulating HGF levels predicted the development of ischemic stroke among postmenopausal women in a large nested case-control study within the WHI-OS(12).

All these observations support the notion that HGF may also be involved in the pathogenesis of diabetes. The biological mechanisms linking HGF to the development of diabetes, however, are not well understood. It has been shown that HGF is highly expressed in adipose tissue where it exerts insulin-like effects and stimulates glucose uptake by augmenting the activity of phosphatidylinositol (PI) 3-kinase-dependent protein kinase B (PKB)(4). It is possible that obese individuals exhibit HGF resistance, much like insulin resistance, which then affects efficiency of glucose metabolism as well as leads to endothelial dysfunction, a known risk factor for diabetes. Alternatively, HGF may not be directly associated with diabetes risk, but it could be merely a bystander correlated with or induced by diabetes risk factors. Circulating HGF levels may rise in obesity as a compensatory mechanism for the increased insulin resistance. The elevated HGF levels in obesity may be secondary to fatty liver as reported in a study of patients with non-alcoholic steatohepatitis(13); however, another study suggests that the high HGF levels in obesity occur even in the absence of any apparent liver dysfunction(5). In our study, the HGF-diabetes association was significant even after control of both BMI and waist, suggesting that it is independent of obesity. The attenuation of the association after controlling for insulin possibly suggests that HGF may increase diabetes risk by increasing insulin resistance. However, only prospective studies can confirm this possibility. Our study is limited by its cross-sectional design, and we cannot determine the cause and effect between HGF and diabetes. Additional studies, especially prospective investigations, are warranted to further explore the role of HGF in the development of type 2 diabetes.

AUTHOR CONTRIBUTION: Author contributions: SR researched data, contributed to discussion, wrote/reviewed/edited manuscript; GH researched data, contributed to discussion and reviewed/edited manuscript; JC contributed to discussion and reviewed/edited manuscript, SL reviewed/edited manuscript and SWS
contributed to discussion and reviewed/edited manuscript.

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Conflict of interest: None

REFERENCES

**Table 1**: Odds Ratio (95% Confidence Intervals) from Logistic Regression Analysis for the Association of HGF with Diabetes.

<table>
<thead>
<tr>
<th>TERTILES OF HGF</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>P-Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 298</td>
<td>N = 298</td>
<td>N = 296</td>
<td></td>
</tr>
<tr>
<td>Mean (Range), pg/mL</td>
<td>307.0 (2.6 - 474.7)</td>
<td>622.1 (474.8 - 779.8)</td>
<td>1,369.4 (779.9 – 31,535.5)</td>
<td></td>
</tr>
<tr>
<td>Number of Cases</td>
<td>12</td>
<td>17</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>Reference</td>
<td>1.40 (0.65-3.02)</td>
<td>3.63 (1.83-7.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>Reference</td>
<td>1.38 (0.62-3.03)</td>
<td>2.78 (1.36-5.69)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 3</td>
<td>Reference</td>
<td>1.27 (0.54-2.98)</td>
<td>2.47 (1.12-5.47)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and race
Model 2: Model 1 + adjusted for body mass index
Model 3: Model 2 + adjusted for smoking, physical activity, family history of diabetes, hormone use, alcohol intake and CRP