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Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity

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Bell, Lauren N., Jennifer L. Ward, Mikako Degawa-Yamauchi, Jason E. Bovenkerk, RoseMarie Jones, Brenda M. Cacucci, Christine E. Gupta, Carol Sheridan, Kevin Sheridan, Sudha S. Shankar, Helmut O. Steinberg, Keith L. March, and Robert V. Considine. Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity. *Am J Physiol Endocrinol Metab* 291: E843–E848, 2006. First published June 6, 2006; doi:10.1152/ajpendo.00174.2006.—Serum HGF is elevated in obese individuals. This study examined the contribution of excess adipose tissue to increased circulating HGF levels in obesity. Serum HGF was measured by ELISA before and after weight loss due to bariatric surgery or a 24-h fast. At 6.1 ± 0.1 mo following surgery, BMI (50.6 ± 1.6 vs. 35.1 ± 1.3 kg/m²; *P* < 0.0001) and serum HGF were significantly decreased (1,164 ± 116 vs. 529 ± 39 pg/ml, *P* < 0.001). A 24-h fast did not change serum HGF, but serum leptin was significantly reduced (67.7 ± 7.1 vs. 50.3 ± 8.3 ng/ml, *P* = 0.02). HGF secretion in vitro from adipocytes of obese (BMI 40.3 ± 2.8 kg/m²) subjects was significantly greater (80.9 ± 10.4 vs. 21.5 ± 4.0 pg/10⁵ cells, *P* = 0.008) than release from adipocytes of lean (BMI 23.3 ± 1.4 kg/m²) subjects. HGF mRNA levels determined by real-time RT-PCR were not different in adipocytes from lean (BMI 24.0 ± 0.8 kg/m²) and obese (45.7 ± 3.0 kg/m²) subjects, but serum HGF was significantly elevated in the obese individuals studied (787 ± 61 vs. 489 ± 49 pg/ml, *P* = 0.001). TNF-α (24 h treatment) significantly increased HGF release from subcutaneous adipocytes 23.6 ± 8.3% over control (*P* = 0.02). These data suggest that elevated serum HGF in obesity is in part attributable to excess adipose tissue and that this effect can be reversed by reducing adipose tissue mass through weight loss. Increased HGF secretion from adipocytes of obese subjects may be due to posttranscriptional events possibly related to adipocyte size and stimulation by elevated TNF-α in the adipose tissue of obese individuals.

adipocytes; obesity; tumor necrosis factor-α; adiponectin; leptin; weight loss; hepatocyte growth factor

A LARGE BODY OF WORK has documented the synthesis of hormones such as leptin and adiponectin and cytokines such as TNF-α and IL-6 by adipose tissue and the significant effect of these hormones and cytokines on metabolism in health and disease (1, 7, 9, 13, 32). Recent work (25, 26) from our laboratory documenting that hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are synthesized in human adipose tissue, and that

serum HGF is elevated more than threefold in obese individuals, has focused study on the production of angiogenic growth factors by human adipose tissue and the role of such factors both systemically and within adipose tissue itself. Thus Miyazawa-Hoshimoto et al. (19) observed that serum VEGF is highly correlated with body mass index (BMI) and visceral fat area in overweight and obese subjects, and Silha et al. (28) found that the angiogenic factors VEGF-C, VEGF-D, angiopoietin-2, and angiogenin are elevated in obese men and women.

HGF is a pleiotropic factor with potent angiogenic and mitogenic effects (20). A paracrine role in promoting tumorigenesis has been suggested for HGF synthesized by adipocytes in breast tissue (33). More recently, the role of HGF in cardiovascular disease has been reemphasized by the fact that high circulating levels of HGF in patients with coronary artery disease predict a greater long-term risk of atherothrombotic events following percutaneous coronary revascularization (29). Furthermore, Hiratsuka et al. (10) found that serum HGF levels are strongly associated with components of the metabolic syndrome in individuals free of liver and kidney disease. Taken together, these observations and others implicate HGF in the development of cardiovascular disease and cancer. Because elevated serum HGF levels are found in obesity, and obesity is strongly associated with cardiovascular disease and progression of certain cancers (1), it is important to investigate the mechanisms that result in elevated serum HGF in obese humans.

In the present study, the relationship between excess adipose tissue and serum HGF was examined in subjects before and after significant weight loss following bariatric surgery. The effect of acute caloric restriction on serum HGF was also tested. Secretion of HGF by isolated adipocytes from lean and obese subjects was quantitated in vitro, as was the effect of tumor necrosis factor-α (TNF-α) to regulate HGF secretion. The findings of these studies extend our original observation that serum HGF is elevated in obese humans and demonstrate that weight loss results in reduced serum HGF. We also show that HGF secretion from adipocytes in obese subjects is increased. These observations suggest that excess adipose tissue results in elevated serum HGF, which may contribute to the increased prevalence of cardiovascular disease and cancer in obese humans.

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MATERIALS AND METHODS

Subjects. A total of 89 subjects participated in these studies. After an explanation of the research that was to be conducted, all subjects gave informed consent for the collection of blood and tissue samples. The protocols were approved by the Institutional Review Board at Indiana University-Purdue University (Indianapolis, IN) and St. Vincent Hospital (Indianapolis, IN).

To study the effect of weight loss on serum HGF, 29 subjects (14 women and 15 men; BMI 50.6 ± 1.6 kg/m², age 47 ± 2 yr) undergoing laparoscopic Roux-en-Y surgery were recruited. At the time of surgery, 16 subjects (6 women and 10 men) had type 2 diabetes and 19 subjects (8 women and 11 men) had hypertension. Serum samples were obtained immediately before and 6 mo after surgery.

Four healthy obese women (BMI 47.9 ± 4.3 kg/m², age 35 ± 6 yr) fasted for 24 h, beginning at 0800 after a standard overnight fast and ending at 0800 the following day. Serum samples were obtained at the beginning and end of the fasting period.

To study the release of HGF from subcutaneous adipocytes, cells were obtained from 17 healthy subjects (10 women and 7 men; age 36 ± 2 yr). In this group, two men and one woman were lean (BMI ≤ 25 kg/m²), one man and two women were overweight ($25 < \text{BMI} \leq 30$ kg/m²), and four men and seven women were obese (BMI > 30 kg/m²).

Subcutaneous adipocyte HGF mRNA expression was examined in 33 subjects (16 women and 17 men, age 40 ± 2 yr). Within this group, three women and six men were lean (BMI ≤ 25 kg/m²), five women and one man were overweight ($25 < \text{BMI} \leq 30$ kg/m²), and eight women and 10 men were obese (BMI > 30 kg/m²). Of the obese subjects, two women and five men had type 2 diabetes and two women and five men had hypertension. Serum samples were also obtained from all subjects who provided adipose tissue samples.

To study the effect of TNF- α on HGF release in vitro, subcutaneous adipocytes were obtained from three lean (3 women; BMI 23.0 ± 1.7 kg/m², age 42 ± 4 yr) and three obese (2 women and 1 man; BMI 40.0 ± 6.1 kg/m², age 46 ± 5 yr) subjects.

Isolation and culture of adipocytes. Adipocytes were isolated by collagenase digestion of adipose tissue biopsies obtained from bariatric surgical procedures or needle liposuction (15). For the in vitro experiments, freshly isolated adipocytes were suspension cultured in DMEM-Ham's F-12 plus 10% fetal bovine serum (FBS) in 50 ml of sterile conical tubes at 37°C for 24 h. To examine the effect of TNF- α on HGF release, adipocytes were cultured for 24 h in the presence or absence of recombinant human TNF- α (R&D Systems, Minneapolis, MN). Culture medium was purchased from Sigma Chemical (St. Louis, MO), and FBS was obtained from Invitrogen (Carlsbad, CA).

Assays. HGF in serum samples and culture medium was quantitated by ELISA (R&D Systems). Serum adiponectin was measured by ELISA (Linco Research, St. Charles, MO) and serum leptin determined by radioimmunoassay (Linco Research). Blood chemistry was quantitated using a Roche COBAS MIRA clinical analyzer.

Determination of mRNA levels using real-time RT-PCR. Total RNA was isolated from adipocytes by standard techniques. HGF and leptin mRNA were quantitated using iTaq SYBR Green Supermix with ROX (Bio-Rad, Hercules, CA) in an Applied Biosystems 7900 thermocycler. Total RNA (0.5 μ g) was reverse transcribed in a 100 μ l reaction. Five microliters of cDNA (25 ng of total RNA) were amplified in a reaction volume of 25 μ l. Cycling conditions were one cycle for 2 min at 50°C and one cycle for 3 min at 95°C, followed by 40 cycles of 15 s of denaturation at 95°C and 1 min of annealing/extension at 60°C. Primers (200 nM; Invitrogen) were as follows: HGF forward 5'-ATGTGGGTGACCAAACCTCCTG-3', HGF reverse 5'-CTATTGAAGGGGAACAGAGG-3'; leptin forward 5'-TTTG-GCCCTATCTTTTCTATGTCC-3', leptin reverse 5'-TGGAG-GAGACTGACTGCGTG-3'. Expression of β -actin (12) was used to normalize HGF and leptin expression using the $\Delta\Delta C_T$ method. There

was no difference in β -actin expression between adipocytes from lean and obese subjects.

Statistical analyses. All data are expressed as means \pm SE. Statistical comparisons of data before and after weight loss were made by paired *t*-test. Comparisons between lean and obese were made using unpaired Student's *t*-test. Pearson's correlation coefficient was used to describe simple relationships between variables. The significance of the effect of TNF- α on HGF release from cultured cells was determined by paired *t*-test. A *P* value of < 0.05 was considered significant. All statistical analyses were done using Statview for Macintosh.

RESULTS

Serum HGF is reduced with weight loss. To more fully evaluate the relationship between serum HGF and obesity, HGF was quantitated before and after bariatric surgery-induced weight loss in 29 subjects (14 women and 15 men). There was no difference between the women and men in either BMI (48.2 ± 2.1 vs. 52.9 ± 2.4 kg/m², respectively) or serum HGF ($1,202 \pm 217$ vs. $1,130 \pm 105$ pg/ml, respectively) at the time of surgery. At 6.1 ± 0.1 mo (range 5.5–7.25) after surgery there was a significant $30.6 \pm 1.3\%$ decrease in BMI for the entire group ($32.0 \pm 1.5\%$ for the women and $29.3 \pm 2.2\%$ for the men). Weight loss also resulted in significant reductions in serum glucose, triglycerides, total cholesterol, and LDL cholesterol (Table 1). There was no change in serum HDL cholesterol. As shown in Fig. 1, *top*, serum HGF was reduced $50.0 \pm 3.4\%$ with weight loss ($51.5 \pm 6.2\%$ for the women and $48.5 \pm 3.2\%$ for the men). As a positive control, serum adiponectin was significantly increased $43.3 \pm 7.3\%$ with weight loss in these subjects (Fig. 1, *bottom*). These observations demonstrate that elevated serum HGF in obese subjects can be reversed with weight loss.

To investigate whether a significant reduction in caloric intake, such as that which occurs following bariatric surgery, can alter serum HGF levels, four healthy obese women with BMI comparable to that of those undergoing surgery (BMI 47.9 ± 4.3 kg/m²) fasted for 24 h. Fasting had no effect on serum HGF levels ($1,064.6 \pm 201.4$ vs. $1,142.4 \pm 261.6$ pg/ml). In contrast, serum leptin was significantly reduced with fasting in these subjects (67.7 ± 7.1 vs. 50.3 ± 8.3 ng/ml, *P* = 0.02). These findings suggest that extreme fluctuations in caloric intake, such as fasting, can alter serum leptin but not serum HGF levels.

HGF secretion is greater from adipocytes of obese subjects. To study the release of HGF from adipocytes of lean and obese subjects, cells were isolated from 17 subjects (10 women and 7 men) and cultured for 24 h in vitro. As shown in Fig. 2, there is a significant positive correlation between HGF secretion and

Table 1. Subject characteristics before and 6 mo after bariatric surgery

	Baseline	6 mo	<i>P</i> Value
Men/women	15/14		
Age, yr	46.7 ± 1.8		
BMI, kg/m ²	50.6 ± 1.6	35.1 ± 1.3	< 0.0001
Glucose, mg/dl	113.0 ± 6.3	87.4 ± 3.1	0.0003
Cholesterol, mg/dl	194.0 ± 7.6	170.2 ± 6.0	0.005
HDL, mg/dl	46.8 ± 2.2	45.7 ± 10.0	0.47
LDL, mg/dl	132.2 ± 6.5	114.7 ± 6.3	0.019
Triglyceride, mg/dl	209.7 ± 20.5	114.3 ± 9.8	< 0.0001

Values represent means \pm SE. BMI, body mass index.

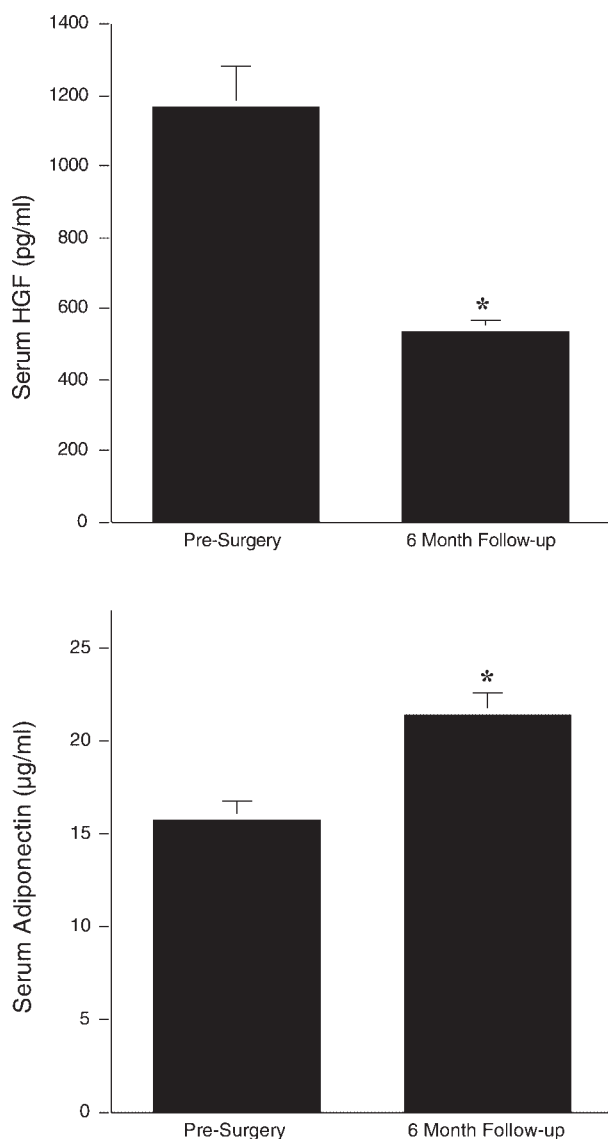


Fig. 1. Serum hepatocyte growth factor (HGF) is reduced (*top*) and serum adiponectin increased (*bottom*) with weight loss after bariatric surgery. Serum was obtained from 29 subjects (14 women and 15 men) before and after bariatric surgery. Body mass index (BMI) was significantly reduced (50.6 ± 1.6 vs. 35.1 ± 1.3 kg/m², $P < 0.0001$) at 6.1 ± 0.1 mo (range 5.5–7.25) after surgery. * $P < 0.0001$.

BMI, demonstrating that adipocytes from obese subjects secrete more HGF than adipocytes from lean individuals.

To examine the mechanism leading to greater HGF release from adipocytes of obese subjects, HGF mRNA was quantitated in subcutaneous adipocyte preparations from 33 subjects (16 women and 17 men). Serum HGF in these subjects was positively correlated with BMI (Fig. 3) and significantly increased in the obese subjects (787 ± 61 vs. 489 ± 49 pg/ml, $P = 0.001$). As shown in Fig. 4, *top*, there was no difference in HGF mRNA expression in subcutaneous adipocytes from lean (BMI 24.0 ± 0.8 kg/m², range 20–28, $n = 14$) and obese (BMI 45.7 ± 3.0 kg/m², range 30–79.2, $n = 19$) subjects. As a positive control for the experiment, *LEP* mRNA was analyzed using the same reverse-transcribed cDNA. There was a significant positive correlation ($r = 0.386$, $P = 0.03$) between

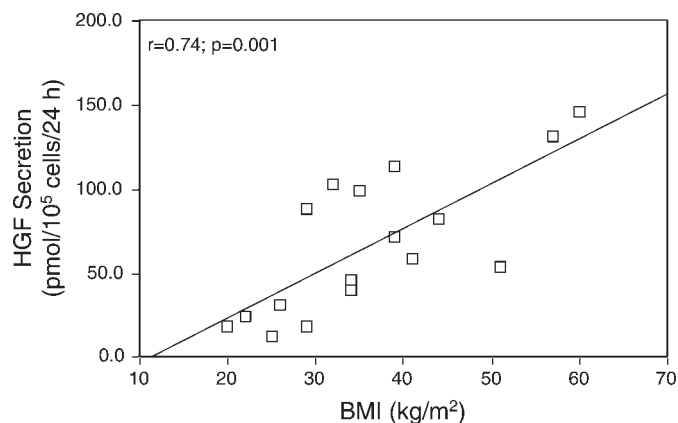


Fig. 2. Secretion of HGF from subcutaneous adipocytes in vitro is significantly correlated with adiposity. Isolated adipocytes were cultured in DMEM-Ham's F-12 + 10% FBS for 24 h and HGF secretion to the medium quantitated by ELISA. Values obtained for isolated adipocytes from 17 subjects (10 women and 7 men).

LEP mRNA and BMI, and *LEP* mRNA was significantly greater in the adipocytes obtained from obese subjects (Fig. 4, *bottom*). As a final analysis, HGF mRNA level in the eight leanest subjects (BMI 22.1 ± 0.9 kg/m², range 20–25) was compared with that in the eight most obese subjects (BMI 57.4 ± 3.8 kg/m², range 47–79.2). There was no difference in HGF mRNA expression (0.24 ± 0.7 vs. 0.27 ± 0.1 relative units) in the two extreme subgroups. *LEP* mRNA was significantly increased in the obese subjects of this subgroup analysis (231 ± 34 vs. 551 ± 75 relative units). These data suggest that the increase in HGF release from adipocytes of obese subjects is mediated through a nontranscriptional mechanism.

TNF-α stimulates HGF secretion from adipocytes. *TNF-α* stimulates HGF release from fibroblasts and monocytes. Therefore, we tested whether *TNF-α* could regulate HGF secretion from adipocytes in vitro. Subcutaneous adipocytes were obtained from three lean (BMI 23.0 ± 1.7 kg/m²) and three obese (BMI 40.0 ± 6.1 kg/m²) subjects. At a concentration of 10 ng/ml, *TNF-α* significantly increased HGF release $23.6 \pm$

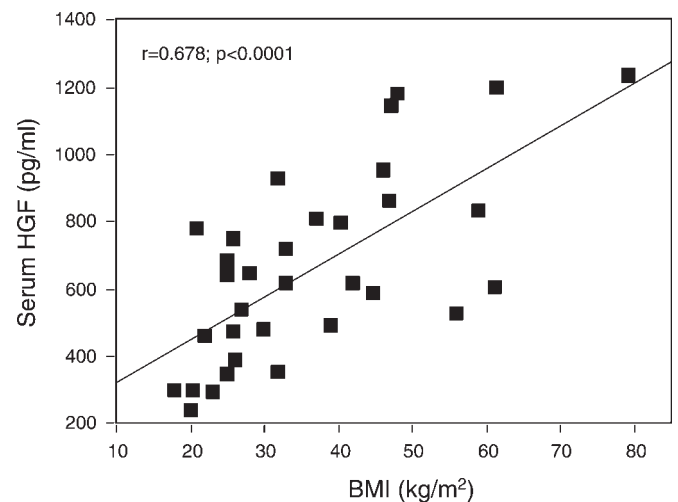


Fig. 3. Serum HGF is positively correlated with BMI. HGF was quantitated in 33 subjects (16 women and 17 men) from whom adipose tissue was obtained for quantitation of HGF and *LEP* mRNA levels.

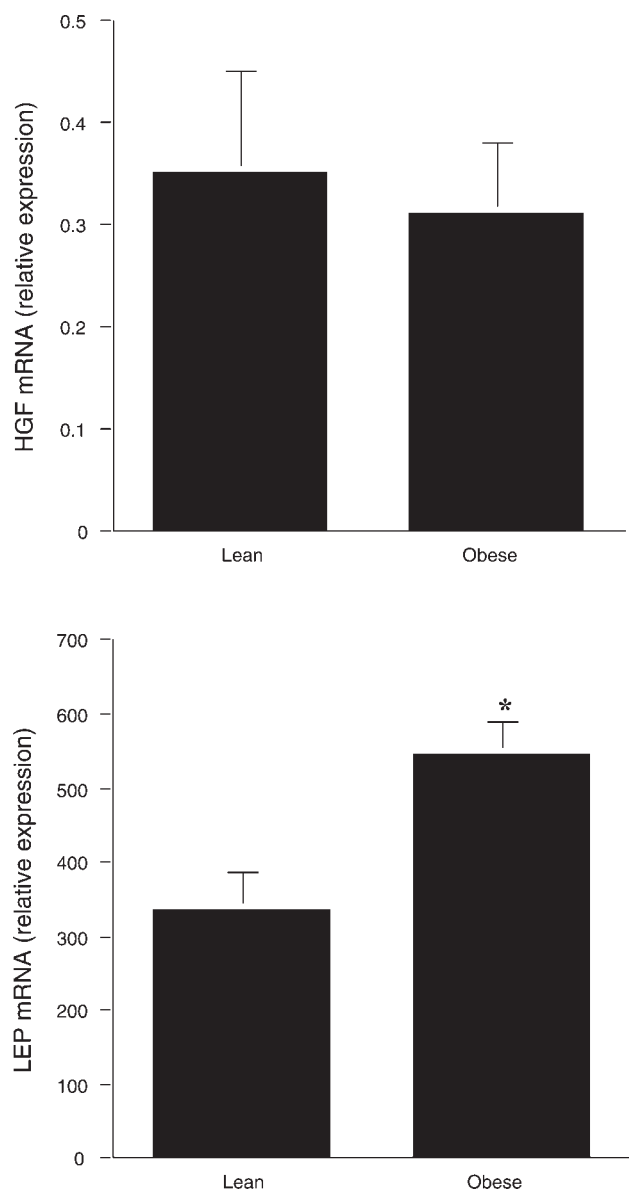


Fig. 4. HGF and *LEP* mRNA expression in subcutaneous adipocytes. HGF mRNA expression is not increased in adipocytes of obese subjects (top). *Lep* mRNA is significantly increased in the same samples using the same reverse-transcribed cDNA (bottom). Values represent means \pm SE for isolated adipocytes from 14 lean (BMI 24.0 ± 0.8 kg/m², range 20–28) and 19 obese (BMI 45.7 ± 3.0 kg/m², range 30–79.2) subjects. * $P = 0.0047$.

8.3% over control ($P = 0.02$; Fig. 5) during a 24-h incubation period (213 ± 35 vs. 257 ± 41 pg/ml). No difference in the response between the lean and obese could be determined in this small sample size. This observation suggests that an increase in TNF- α synthesis in adipose tissue of obese subjects may stimulate HGF synthesis and secretion from adipocytes.

DISCUSSION

In this study, we demonstrate that elevated serum HGF in obese subjects is reduced with weight loss and that the extent of this reduction is similar in men and women. We further show that adipocytes obtained from obese subjects secrete more HGF in vitro than do adipocytes of lean subjects and that

exposure to TNF- α has a modest stimulatory effect on HGF secretion from human adipocytes. These observations extend our previous finding that obesity is associated with elevated serum HGF (25) and provide evidence that an increase in HGF secretion from adipocytes of obese subjects contributes to the increase in serum HGF in obesity.

Adipose tissue produces a large array of different hormones and factors that influence metabolism, including leptin, adiponectin, cortisol, TNF- α , and IL-6 (4). Adipose tissue also produces a number of angiogenic growth factors that act in an autocrine or paracrine manner within adipose tissue but may also have endocrine effects throughout the body. Early work demonstrated that basic fibroblast growth factor and VEGF were synthesized in adipose tissue and that these factors potentially had a role in regulating preadipocyte differentiation and adipose tissue metabolism (8). HGF was first detected by in situ hybridization in mammary adipocytes from normal and malignant breast tissue (33) and was subsequently demonstrated to be a secretory product of 3T3-L1 adipocytes (23). More recently, our group (25, 26) has shown that both adipocytes and the stromal vascular cells in human adipose tissue secrete HGF and that serum HGF is significantly elevated in obese humans.

Serum HGF has been linked to vascular complications in a number of different studies. Serum HGF is increased in hypertensive individuals and is positively associated with hypertension-mediated organ damage (21). Diabetic patients with proliferative retinopathy have greater serum HGF concentrations than diabetics without retinopathy (22). Serum HGF is elevated in patients with peripheral arterial occlusive disease (35) and is positively associated with measures of carotid artery remodeling (34). HGF is related to inflammation and intima-media thickness in end-stage renal disease (18) and is found within atherosclerotic lesions in the human carotid artery (17). Elevated serum HGF has been shown to be an independent predictor of subsequent death or myocardial infarction following percutaneous coronary revascularization (29). Finally, in a

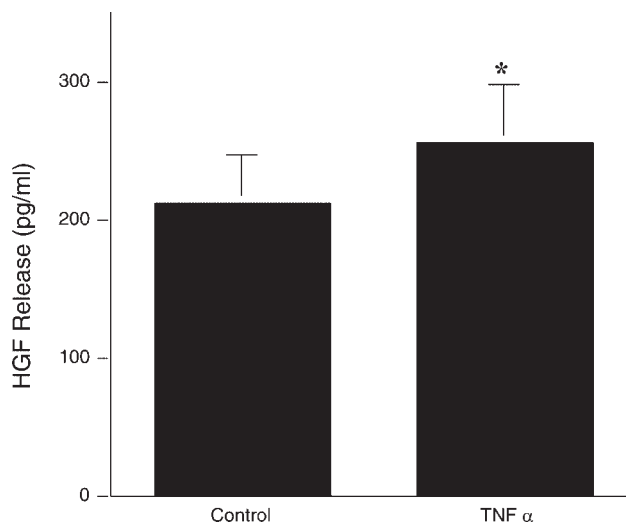


Fig. 5. TNF- α (10 ng/ml, 24 h) significantly increases HGF release $23.6 \pm 8.3\%$ from isolated human adipocytes in vitro. Subcutaneous adipocytes were isolated from 3 lean (BMI 23.0 ± 1.7 kg/m²) and 3 obese (BMI 40.0 ± 6.1 kg/m²) subjects and cultured in the presence and absence of TNF- α . HGF release into the culture medium was quantitated by ELISA. * $P = 0.02$.

recent large study of Japanese men and women, serum HGF was strongly associated with metabolic syndrome independently from liver function (10). Taken together, these studies suggest that elevated serum HGF in obesity may contribute to the development of cardiovascular disease.

We (25) have previously reported and confirm in this study that serum HGF is positively correlated with BMI. One simple interpretation, given the finding that adipocytes secrete HGF, is that the greater number of adipocytes in obesity results in greater release of HGF to the bloodstream. Our observation that serum HGF is significantly decreased following bariatric surgery-induced weight loss supports such an interpretation and is in agreement with the recent report of Swierczynski et al. (30) that serum levels of HGF are significantly reduced following vertical-banded gastroplasty in obese women. It is important to note that Silha et al. (28) did not observe significantly greater serum HGF in obese subjects despite finding significant elevations in other angiogenic factors, including VEGF, angiopoietin-2, and angiogenin. This discrepancy may be due to the fact that the subjects in the study of Silha et al. were not as obese (mean BMI ~ 39 kg/m²) as those in the present study (mean BMI 50.6 ± 1.6 kg/m²).

To examine the possible contribution of caloric restriction in bariatric subjects to the reduction in serum HGF, we examined the effect of a 24-h fast on serum HGF levels in obese women. Short-term fasting has been shown to reduce serum leptin levels in the absence of changes in adipose tissue content (14). Although fasting for 24 h resulted in a significant reduction in serum leptin in the present study, there was no change in serum HGF. This observation suggests that HGF release from adipocytes is not regulated by acute changes in caloric intake, strengthening the interpretation that the reduction in number of adipocytes with weight loss results in lower serum HGF.

HGF secretion *in vitro* from isolated adipocytes of obese subjects was greater than that from adipocytes of lean subjects. This observation suggests that elevated serum HGF in obesity results from both a greater amount of adipose tissue and increased HGF release from adipocytes of obese subjects. The elevation in serum leptin in obesity is also due to greater fat mass and increased synthesis and release of leptin by the adipocytes of obese subjects (3, 16). Interestingly, we did not detect an increase in HGF mRNA in adipocytes from obese subjects despite the finding that serum HGF was significantly elevated in these same individuals. In contrast, *LEP* mRNA levels were significantly greater in adipocytes from obese subjects, confirming previous observations (3, 16) and the quality of our RNA preparations. The lack of a difference in HGF mRNA in adipocytes of lean and obese subjects suggests that greater HGF release from adipocytes of obese subjects is due to posttranscriptional events, as has been previously reported (24) for regulation of lipoprotein lipase synthesis in adipocytes. Bluher et al. (2) recently utilized a combined proteomics and genomics approach to demonstrate that adipocyte size determines the synthesis of several proteins involved in key steps of lipid and energy metabolism. Expression of eight different proteins, including aP2, was increased in larger adipocytes, but message levels for these proteins were unchanged. Thus increased HGF protein synthesis and secretion from adipocytes of obese humans may be due to posttranscriptional regulation linked to adipocyte size. Future experiments will be necessary to fully investigate this possibility.

TNF- α had a modest but significant stimulatory effect on HGF release from human adipocytes. TNF- α has previously been shown (11) to increase HGF expression in fibroblasts and monocytes. Because TNF- α is increased in adipose tissue of obese subjects (1, 9, 13, 32) and regulates the synthesis of other adipokines, such as leptin and adiponectin (4, 5), TNF- α -stimulated HGF release from adipose tissue is likely one of several mechanisms contributing to increased serum HGF in obesity.

It is important to note that adipose tissue may not be the only source contributing to the elevated serum HGF in obesity. Serum HGF is increased in humans with various liver diseases, including hepatitis, cirrhosis, and hepatocellular carcinoma (27). However, it has not been established whether the elevation in serum HGF is due to increased hepatic synthesis or decreased clearance by the damaged liver. Furthermore, there have been no studies directly examining HGF secretion from fatty liver in obese animals or humans, nor have any studies been done to determine whether HGF clearance by the fatty liver is impaired. IL-6-activated monocytes have been shown to secrete HGF, and HGF in turn results in increased IL-6 release from these cells (6). Because obesity is a low-grade inflammatory state (31), activated monocyte/macrophage release of HGF may contribute to the increase in serum HGF. Arteriovenous measurements of HGF across the liver and adipose tissue in obese animal models or humans should provide greater insight into the relative contribution of these tissues to elevated serum HGF in obesity. However, no matter the source, weight loss was associated with decreased HGF, which should reduce the contribution of this angiogenic and mitogenic factor to cardiovascular disease and cancer.

In summary, we show that serum HGF is regulated by changes in adipose tissue mass and that adipocytes from obese subjects secrete greater amounts of HGF than adipocytes from lean subjects. We also show that HGF secretion from adipocytes of obese subjects is increased at a posttranscriptional level and that TNF- α is one stimulus for increased HGF release from human adipocytes. A better understanding of HGF synthesis by adipose tissue will provide important information relevant to vascular complications in obesity.

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