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Acute Effect of Pegvisomant on Cardiovascular Risk Markers in Healthy Men: Implications for the Pathogenesis of Atherosclerosis in GH Deficiency

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Cardiovascular risk is increased in GH deficiency (GHD). GHD adults are frequently abdominally obese and display features of the metabolic syndrome. Otherwise healthy abdominally obese subjects have low GH levels and show features of the metabolic syndrome as well. We investigated in healthy nonobese males the effect of the GH receptor antagonist pegvisomant in different metabolic conditions. This is a model for acute GHD without the alterations in body composition associated with GHD. We compared the effect of pegvisomant with that of placebo before and after 3 d of fasting. In addition, we investigated the effect of pegvisomant under normal, *i.e.* fed, conditions.

Three days of fasting as well as pegvisomant alone decreased serum free IGF-I levels (1.0 ± 0.15 *vs.* 0.31 ± 0.05 ng/ml and 0.86 ± 0.23 *vs.* 0.46 ± 0.23 ng/ml, respectively). Fasting in combination with pegvisomant also decreased serum free IGF-I levels (1.0 ± 0.15 *vs.* 0.31 ± 0.07 ng/ml). Treatment with pegvisomant had no additional influence on the decline of free IGF-I induced by fasting. Pegvisomant alone had no influence on insulin sensitivity. The increase in insulin sensitivity induced by fasting was comparable to the increase in insulin sensitivity induced by fasting combined with pegvisomant. Among serum lipid concentrations, only serum triglycerides increased significantly as a result of pegvisomant alone (1.0 ± 0.2 *vs.* 1.6 ± 0.4 mmol/liter). The changes in lipid concentrations induced by fasting alone or pegvisomant were not different from those induced by pegvisomant alone. von Willebrand factor antigen levels declined significantly under the influence of pegvisomant alone (1.1 ± 0.07 *vs.* 0.8 ± 0.06 U/ml).

In conclusion, in different metabolic conditions the GH receptor antagonist pegvisomant induces no significant acute changes in the major risk markers for cardiovascular disease. These data suggest that the secondary metabolic changes, *e.g.* abdominal obesity or inflammatory factors, that develop as a result of long-standing GHD are of primary importance in the pathogenesis of atherosclerosis in patients with GHD. (*J Clin Endocrinol Metab* 86: 5165–5171, 2001)

ADULTS WITH GH deficiency (GHD) have an increased risk for cardiovascular disease (CVD) (1). Four relevant cohort studies investigating 1197 patients receiving routine replacement therapy without GH have reported a decrease in life expectancy of patients with hypopituitarism (2–5). The overall relative risk for CVD has been estimated at 1.47 (95% confidence interval, 1.27–1.70) (6). Compared with age- and sex-matched normal subjects, patients with GHD have reduced insulin sensitivity, proatherogenic hemostasis parameters, and higher serum lipid concentrations. They thus display characteristic features of the metabolic syndrome (7, 8). As central adiposity is present in GHD and because adiposity itself is associated with low GH levels, it has been postulated that low GH levels play a role in the metabolic alterations associated with the metabolic syndrome (7, 9–11).

GH acts by binding to receptors on liver and other cells. One GH molecule binds to two receptor molecules on the target cell, initiating dimerization of these receptor molecules and finally resulting in the secretion of IGF-I (12, 13). Pegvisomant is a genetically manipulated GH molecule that disables functional dimerization of the two GH receptor molecules (14, 15). In normal subjects and in patients with acromegaly, pegvisomant is an effective blocker of GH action and significantly decreases IGF-I concentrations (16, 17).

The aim of the present study was to investigate whether GH receptor blockade, as a model of GHD but without the typical alterations in body composition, influences insulin sensitivity, hemostasis parameters, and serum lipid concentrations in healthy nonobese males. Because fasting has marked effects on the GH-IGF-I axis, we performed two studies: 1) a double blind, placebo-controlled, cross-over study comparing the effects of fasting on insulin sensitivity, hemostasis parameters, and serum lipid concentrations with and without pegvisomant; and 2) a single arm study investigating the effect of pegvisomant during normal (fed) conditions.

Abbreviations: CV, Coefficient of variation; CVD, cardiovascular disease; FVIII:c, factor VIII activity; GHD, GH deficiency; HDL, high density lipoprotein; HOMA, homeostatic model assessment; LDL, low density lipoprotein; Lp(a), lipoprotein(a); PAI-I, plasminogen activator inhibitor I; t-PA, tissue plasminogen activator; vWF:ag, von Willebrand factor antigen; vWF:Cba, vWF collagen-binding activity; vWF:Rco, vWF ristocetin cofactor.

Subjects and Methods

Study subjects

Ten healthy male subjects (mean ± SD age, 23.4 ± 2.7 yr; range, 20–28) with a normal body weight (mean ± SD body mass index, 21.8 ± 1.8 kg/m^2; range, 19.7–25.8) were asked to participate. None of the subjects had a relevant medical history or used medication. All 10 subjects participated in the cross-over study, and 5 participated in the single arm

study. The local ethical committee approved the study, and all subjects gave written informed consent.

Experimental design

The study consisted of two parts (Fig. 1). The first part was a double blind, placebo-controlled, cross-over study comparing GHR blockade with placebo before and after 3 d of fasting. After an overnight fast subjects were admitted to the Clinical Research Unit on d 1 at 0730 h. Blood was drawn at 0800 h, and at 1800 h a single dose of 80 mg pegvisomant (Sensus Drug Development Corp., Austin, TX) or placebo was administered sc (Fig. 2). From midnight until the end of the study (on d 4 at 2000 h), subjects fasted (while having free access to noncaloric fluids). Each morning at 0800 h blood was drawn. Between the study periods there was a wash-out period of 3–7 wk.

The second part of the study was a single arm, open label study in which the effect of GHR blockade under nonfasted conditions (subjects received a standardized diet) was investigated. After an overnight fast subjects were admitted to the Clinical Research Unit on d 1 at 0730 h, at 0800 h blood was drawn, and at 1800 h a single dose of 80 mg pegvisomant was administered sc. After an overnight fast, blood was drawn each morning at 0800 h. In this study a detailed analysis of hemostasis was performed. Hemostasis was assessed on d 1 and 4 at 0800 h.

GH, total IGF-I, free IGF-I, glucose, insulin, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and lipoprotein(a) [Lp(a)] were determined from all blood samples. In the single arm study, fibrinogen, plasminogen, antiplasmin, factor VIII activity (FVIII:c), von Willebrand factor antigen (vWF:ag), vWF ristocetin cofactor (vWF:Rco) activity, vWF collagen-binding activity (vWF:Cba), plasminogen activator inhibitor-I (PAI-I) antigen, PAI-I activity, tissue plasminogen activator (t-PA) activity, and t-PA antigen were determined at baseline and on d 4.

Assays

All assays were performed in duplicate. Samples were measured for endogenous GH in a two-site immunoassay that does not cross-react with pegvisomant. The assay exhibits a lower detection limit of 0.02 μg/liter GH, an upper end of the working range of 50 μg/liter for 25-μl serum samples, and no cross-reaction with pegvisomant up to a concentration of 50,000 μg/liter (16). The interassay coefficients of variation (CVs) are 4.1% at 4.0 μg/liter and 3.8% at 20 μg/liter. The intraassay CVs are 3.4% at 0.25 μg/liter, 1.9% at 2.5 μg/liter, and 4.5% at 25 μg/liter (Med Klinik Innenstadt, Munich, Germany). Serum IGF-I was determined with a commercially available RIA (Biosource Technologies, Inc., Nivelles, Belgium; intra- and interassay CVs, 5.0% and 9.6%, respectively), and free IGF-I was determined with a commercially available immunoradiometric assay (Diagnostics Systems Laboratories, Inc., Webster, TX; intra- and interassay CVs, 10.3% and 10.7%, respectively). Glucose was assessed with an automatic hexokinase method (Roche, Almere, The Netherlands). Insulin was assessed by RIA (Medgenix Diagnostics, Brussels, Belgium; intra- and interassay CVs, 13.7% and 8.0%, respectively). Total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides were assessed with an enzymatic colorimetric test (Roche Diagnostics, Mannheim, Germany). Lp(a) was assessed by RIA (Mercodia AB, Uppsala, Sweden).

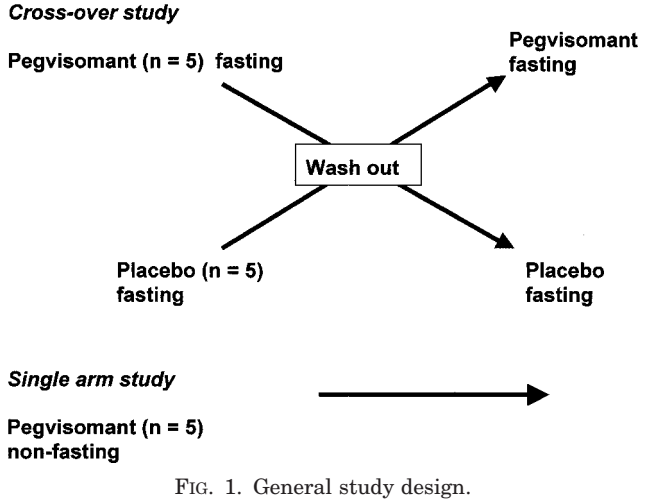

FIG. 1. General study design.

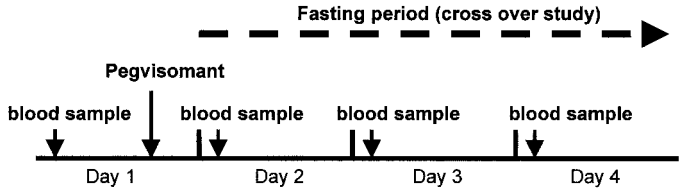

FIG. 2. Overview of the study periods.

Blood for determination of hemostasis parameters was obtained by venipuncture under standardized conditions (overnight fasting, after 15-min rest, no tourniquet use) and collected in citrate (final concentration, 0.105 M), in Stabilyte (Biopool, Umea, Sweden) for determination of t-PA and PAI-I activities, and in citric acid, theophylline, adenosine, dipyridamole (Becton Dickinson, Plymouth, UK) for determination of PAI-I and t-PA antigens. Plasma was obtained by centrifugation at 2000 $\times$ *g* for 20 min at 4 C and was stored at -80 C until use. FVIII:c was measured by a one-stage clotting assay. vWF:ag was measured by ELISA, using rabbit antihuman vWF (DAKO Corp., Glostrop, Denmark) as the primary antibody and a horseradish peroxidase-conjugated antibody as the secondary antibody. vWF:Rco was assayed with formalin-fixed platelets using the PAP-4 aggregometer (BioData). vWF:Cba was assessed by measuring the amount of plasma vWF that binds to collagen by EIA. Fibrinogen was measured using the Clauss method. PAI-1 activity was measured using the Chromolize PAI-1 bioimmunoassay (Biopool). t-PA activity was determined using a biofunctional immunosorbent assay (Chromolize, Biopool). PAI-1 antigen and t-PA antigen were measured using a TintElize PAI-1 and t-PA ELISA, also obtained from Biopool. Plasminogen and antiplasmin were determined using chromogenic substrates, S-2251 and S-2403, respectively, on an automated analyzer (Sysmex, Dade Behring, Marburg, Germany).

Homeostatic model assessment (HOMA)

β-Cell function and insulin sensitivity were analyzed with the HOMA model (provided by Dr. Jonathan Levy, Diabetes Research Laboratories, Oxford, UK). This is a structural model of glucose-insulin interaction that describes the functioning of the major effector organs. Simultaneous assessment of the glucose and insulin concentrations after an overnight fast in each person allows evaluation of the combination of β-cell function and insulin sensitivity. β-Cell function and insulin sensitivity are expressed in relation to values in a "standard individual," in which they are each accorded the value 100%. The HOMA model has been validated previously (18–21).

Assessment of body composition

Body composition was assessed with bioelectrical impedance assessment (Holtain Ltd., Croswell, UK). Total body resistance was measured with a four-terminal portable impedance analyzer. Measurements were made while the subjects lay comfortably on a bed with limbs abducted from the body. Current injector electrodes were placed just below the metacarpo-phalangeal/metacarpo-tarsal joint on the dorsal side of the right hand/foot. Detector electrodes were placed on the posterior side of the wrist. Impedance was measured after 800 μA at 50-kHz current was injected. A computer program employing empirically derived formulas was used to calculate total body water, fat-free mass, and fat mass. This method has been shown to be a reliable and valid approach for the estimation of human body composition in healthy humans (22).

Statistical analysis

All results are reported as the mean $\pm$ SEM. As the maximal effect for all parameters studied was reached on d 4, all comparisons are reported as baseline *vs.* d 4. Because all study periods were exactly the same until

1800 h on d 1 and to minimize intraindividual differences we took the means of the values of the fasting study periods as baseline values. Means from baseline and d 4 were compared with the Wilcoxon signed ranks test. Correlations were calculated with Pearson correlation coefficient. All *P* values are two-sided, $P < 0.05$ was considered significant. Analyses were performed using SPSS version 9.0 for windows (SPSS, Inc., Chicago, IL).

Results

GH and total and free IGF-I

Cross-over study. Fasting nonsignificantly increased GH levels (0.3 ± 0.09 *vs.* 0.6 ± 0.2 μg/liter; Fig. 3A). Three days of fasting combined with pegvisomant resulted in a significant increase in GH levels (0.3 ± 0.09 *vs.* 1.8 ± 0.5 μg/liter; $P = 0.005$). Serum total IGF-I levels did not change (fasting, 204.6 ± 19.2 *vs.* 214.6 ± 30.8 ng/ml; fasting and pegvisomant, 204.6 ± 19.2 *vs.* 181.5 ± 32.3 ng/ml; Fig. 3B). Three days of fasting as well as fasting in combination with pegvisomant decreased serum free IGF-I levels [1.0 ± 0.15 *vs.* 0.31 ± 0.05 ng/ml ($P = 0.005$) and 1.0 ± 0.15 *vs.* 0.31 ± 0.07 ng/ml ($P = 0.005$), respectively; Fig. 3C]. Treatment with pegvisomant had no influence on the decline of free IGF-I induced by fasting.

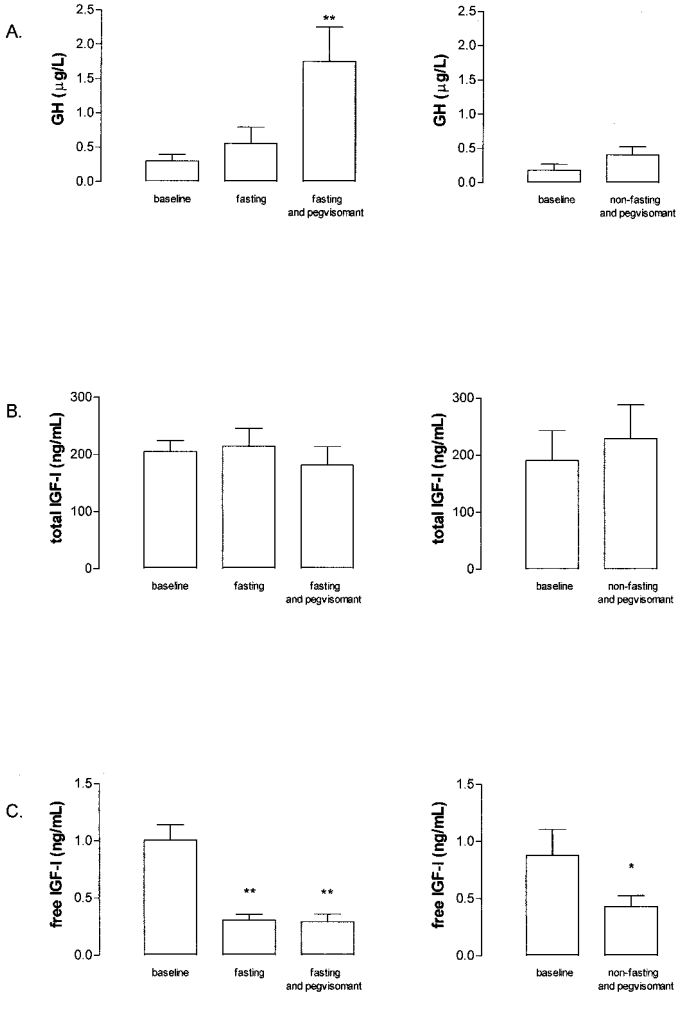

FIG. 3. Mean values (±SEM) of GH (A), total IGF-I (B), and free IGF-I (C): effects of fasting, fasting with pegvisomant, and pegvisomant alone. *, $P < 0.05$; **, $P < 0.01$ (compared with baseline, by Wilcoxon signed rank test).

Single arm study. Pegvisomant alone did not significantly increase GH levels (0.3 ± 0.2 *vs.* 0.4 ± 0.1 μg/liter; Fig. 3A). Serum total IGF-I levels did not change (190.7 ± 52.3 *vs.* 229.2 ± 59.2 ng/ml; Fig. 3B), whereas serum free IGF-I levels decreased significantly (0.86 ± 0.23 *vs.* 0.46 ± 0.08 ng/ml; $P = 0.04$; Fig. 3C).

Cross-over study vs. single arm study. Compared with pegvisomant alone fasting alone and fasting in combination with pegvisomant had similar effects on GH, total IGF-I, and free IGF-I concentrations.

Body composition

Cross-over study. Fat mass did not change (Table 1). Fasting either with or without pegvisomant resulted in a significant decrease in fat-free mass and total body water (Table 1). As the change in fat-free mass was significantly correlated with the change in total body water ($P < 0.001$ for all study periods), the change in fat-free mass during fasting was most likely a result of the expected decline in total body water.

Single arm study. Fat mass, fat-free mass, and total body water did not change (Table 1).

β-Cell function and insulin sensitivity

Cross-over study. β-Cell function, as measured with the HOMA model, remained unchanged (baseline *vs.* fasting, 139.6 ± 14.0% *vs.* 125.7 ± 20.6%; baseline *vs.* fasting and pegvisomant, 139.6 ± 14.0% *vs.* 156.0 ± 32.5%; Fig. 4A). Fasting alone or with pegvisomant resulted in a significant increase in insulin sensitivity [166.4 ± 47.7% *vs.* 637.3 ± 139.1% ($P = 0.02$) and 166.4 ± 47.7% *vs.* 450.1 ± 96.4% ($P = 0.008$), respectively; Fig. 4B]. The change in insulin sensitivity induced by fasting was not different from that induced by fasting combined with pegvisomant.

Single arm study. β-Cell function as measured with the HOMA model remained unchanged (baseline *vs.* nonfasting and pegvisomant, 145.8 ± 25% *vs.* 166.5 ± 15.2%; Fig. 4A). Pegvisomant without fasting had no significant effect on insulin sensitivity (163.3 ± 78.6% *vs.* 82.2 ± 8.0%; Fig. 4B).

Cross-over study vs. single arm study. The changes in β-cell function and insulin sensitivity induced by fasting alone or with pegvisomant were not different from those induced by pegvisomant without fasting.

Hemostasis (single arm study only)

VWF:ag changed significantly under the influence of pegvisomant alone (Table 2). A similar trend was observed for FVIII:c (baseline *vs.* 3 d of fasting, 1.7 ± 0.1 *vs.* 0.9 ± 0.2 IU/ml; $P = 0.3$) and vWF:activity [baseline *vs.* 3 d of fasting: vWF:Rco 1.0 ± 0.09 *vs.* 0.8 ± 0.1 U/ml ($P = 0.08$); vWF:Cba, 1.1 ± 0.2 *vs.* 0.9 ±1.0 U/ml ($P = 0.2$)]. Fibrinolysis parameters, including PAI-I and t-PA, did not change during treatment (Table 2).

TABLE 1. Mean ± SEM body composition values: effects of fasting, fasting with pegvisomant and pegvisonant alone

	Baseline	Day 4		
		Cross-over study		Single arm study: PegV
		Fasting	Fasting + PegV	
Fat mass (kg)	19.0 ± 2.6	19.3 ± 2.4	18.7 ± 2.1	20.0 ± 3.8
Fat-free mass (kg)	57.0 ± 2.1	53.1 ± 1.9[a]	53.8 ± 2.0[b]	57.6 ± 4.0
Total body water (liter)	41.6 ± 1.5	38.7 ± 1.4[a]	39.2 ± 1.5[b]	42.1 ± 2.9

All comparisons are pairwise. PegV, Pegvisomant.
[a] $P < 0.01$, baseline *vs.* d 4.
[b] $P < 0.05$, baseline *vs.* d 4.

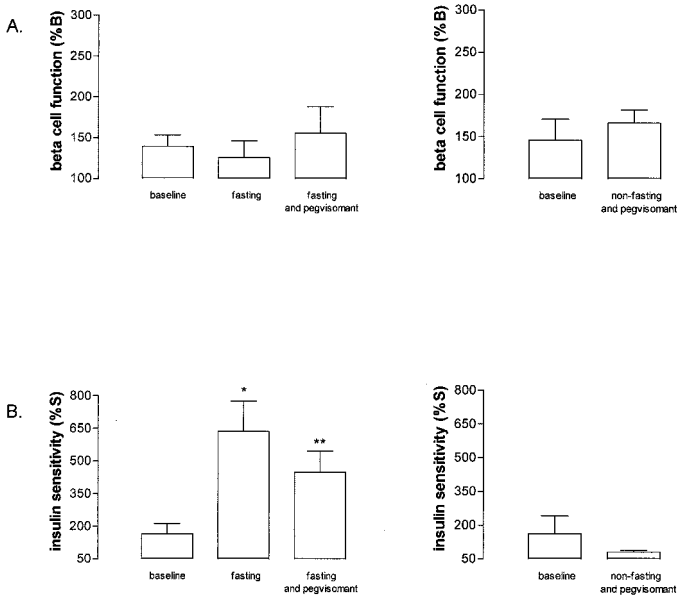

FIG. 4. Mean values (±SEM) of β-cell function (%B; A) and insulin sensitivity (%S; B): effects of fasting, fasting with pegvisomant, and pegvisomant alone. *, $P < 0.05$; **, $P < 0.01$ (compared with baseline, by Wilcoxon signed rank test).

TABLE 2. Mean ± SEM coagulation and fibrinolysis parameters: effects of pegvisomant alone

	Baseline	Pegvisomant
Fibrinogen (g/liter)	2.3 ± 0.2	2.4 ± 0.3
Plasminogen (IU/mliter)	1.0 ± 0.03	1.0 ± 0.03
Antiplasmin (IU/mliter)	0.9 ± 0.04	1.0 ± 0.08
FVIII:c (IU/mliter)	1.1 ± 0.1	0.9 ± 0.2
VWF:ag (U/mliter)	1.1 ± 0.07	0.8 ± 0.06[a]
VWF:Cba. (U/mliter)	1.1 ± 0.2	0.9 ± 1.0
VWF:Rco (U/mliter)	1.0 ± 0.09	0.8 ± 0.1
PAI-I activity (IU/mliter)	10.9 ± 3.8	19.9 ± 6.8
PAI-I antigen (ng/mliter)	19.3 ± 6.0	22.2 ± 5.5
t-PA activity (IU/mliter)	0.4 ± 0.2	0.4 ± 0.2
t-PA antigen (ng/mliter)	5.6 ± 0.5	5.9 ± 1.2

FVIII:c, Factor VIII activity; vWF:ag, von Willebrand factor antigen; Cba, collagen-binding activity; Rco, ristocetin cofactor activity; PAI-I, plasminogen activator inhibitor-I; t-PA, tissue plasminogen activator.
[a] $P < 0.05$, baseline *vs.* d 4.

Lipids

Cross-over study. Fasting alone or in combination with pegvisomant resulted in a significant increase in LDL [2.2 ± 0.2 *vs.* 2.9 ± 0.3 mmol/liter ($P = 0.01$) and 2.2 ± 0.2 *vs.* 3.1 ± 0.2 mmol/liter ($P = 0.005$)]; however, total cholesterol levels did not change (3.9 ± 0.2 *vs.* 4.4 ± 0.3 mmol/liter and 3.9 ± 0.2 *vs.* 4.7 ± 0.2 mmol/liter; Fig. 5, A and B). HDL cholesterol levels decreased as a result of fasting alone (1.2 ± 0.1 *vs.* 1.0 ± 0.1 mmol/liter). This decrease was statistically significant if fasting was combined with pegvisomant (1.2 ± 0.1 *vs.* 1.1 ± 0.07 mmol/liter; $P = 0.04$; Fig. 5C). As a result of the changes in total and HDL cholesterol, the total/HDL cholesterol ratio rose significantly as a result of fasting alone and fasting in combination with pegvisomant [3.5 ± 0.3 *vs.* 4.7 ± 0.4 ($P = 0.005$) and 3.5 ± 0.3 *vs.* 4.5 ± 0.4 ($P = 0.005$); Fig. 5D]. Triglycerides rose after fasting with pegvisomant (0.8 ± 0.1 *vs.* 1.1 ± 0.2 mmol/liter; $P = 0.02$), but not after fasting alone (0.8 ± 0.1 *vs.* 1.0 ± 0.09 mmol/liter; $P = 0.1$; Fig. 5D). Lp(a) increased significantly after fasting either without or with pegvisomant [308.2 ± 153.5 *vs.* 391.8 ± 170.7 U/liter ($P = 0.008$) and 308.2 ± 153.5 *vs.* 359.3 ± 153.0 U/liter ($P = 0.02$), respectively (Fig. 5F)]. The changes in lipid levels induced by fasting alone were not significantly different compared with the changes in lipid levels induced by fasting combined with pegvisomant.

Single arm study. Total, LDL, and HDL cholesterol levels did not change (3.9 ± 0.3 *vs.* 4.2 ± 0.3 mmol/liter, 2.2 ± 0.2 *vs.* 2.4 ± 0.2 mmol/liter, and 1.1 ± 0.2 *vs.* 1.0 ± 0.07 mmol/liter, respectively; Fig. 5, A–C). Pegvisomant alone had no influence on the total cholesterol/HDL cholesterol ratio (4.0 ± 0.5 *vs.* 4.5 ± 0.6; Fig. 5D). Lp(a) did not change (567.0 ± 313.5 *vs.* 599.8 ± 333.7 U/liter; Fig. 5F). The only lipid particle that changed significantly was triglycerides (1.0 ± 0.2 *vs.* 1.6 ± 0.4 mmol/liter; $P = 0.04$; Fig. 5E).

Cross-over study vs. single arm study

The changes in lipid concentrations induced by fasting alone or with pegvisomant were not different from those induced by pegvisomant without fasting.

Discussion

GHD is associated with central obesity, insulin resistance, proatherogenic hemostasis parameters, and elevated serum lipid concentrations (7, 8, 23). Treatment of GHD adults with GH attenuates central obesity and induces positive effects on lipid levels (24–27). Because adiposity in otherwise healthy subjects is associated with low levels of GH, and treatment of middle-aged obese males with GH resulted in a reduction of central adiposity with favorable effects on insulin sensitivity and lipid metabolism, several researchers have suggested an important role for GH in the development of cen-

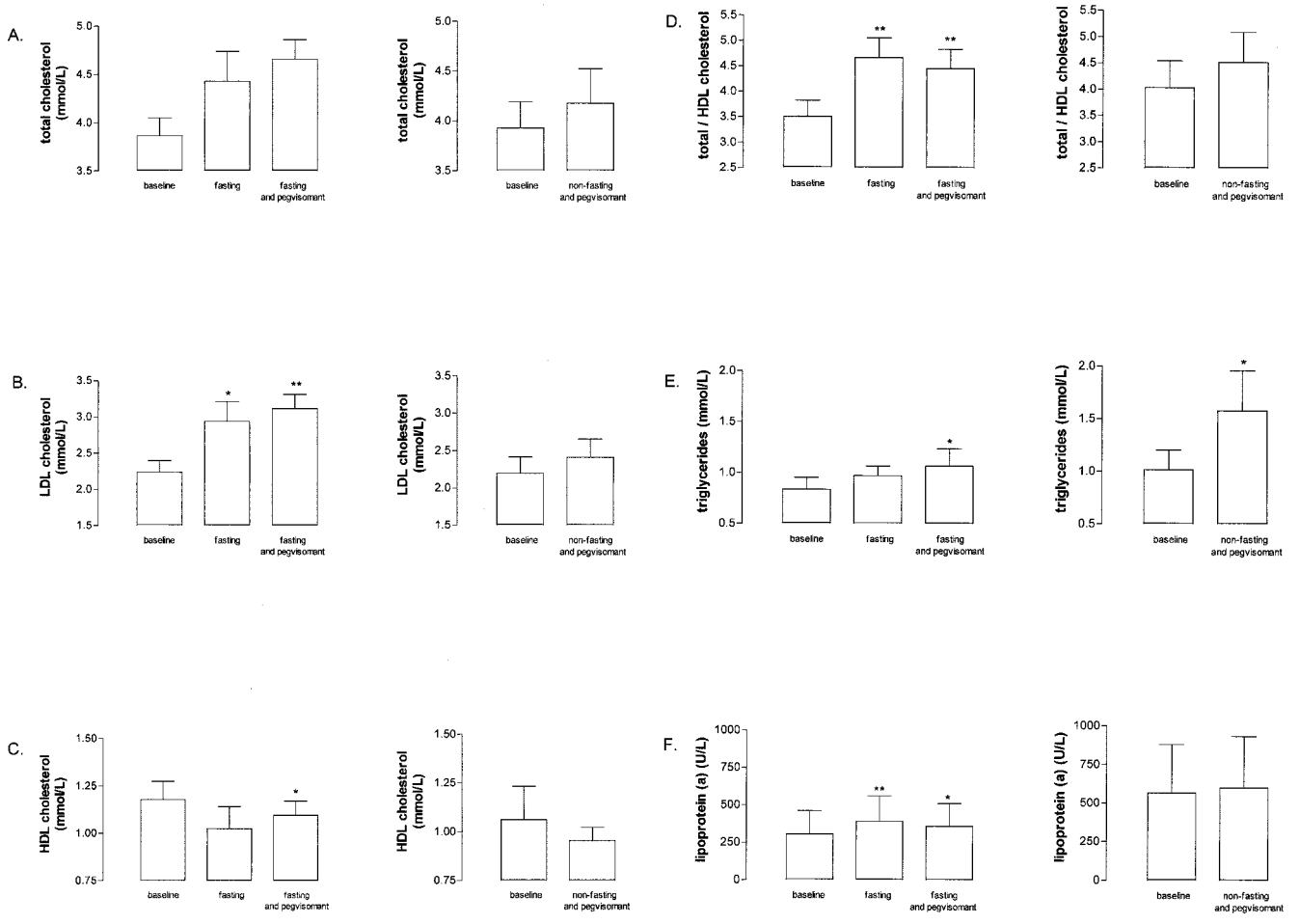

FIG. 5. Mean values (±SEM) of total cholesterol (A), LDL cholesterol (B), HDL cholesterol (C), total/HDL cholesterol ratio (D), triglycerides (E), and Lp(a) (F). *, $P < 0.05$; **, $P < 0.01$ (compared with baseline, by Wilcoxon signed rank test).

tral adiposity and the associated metabolic consequences (28, 29).

By using the GH receptor antagonist pegvisomant we set out to study the effects of functional GHD independently from the eventual alterations in body composition that are associated with long-standing GHD (7). In our study maximal pegvisomant drug levels were achieved on d 4 (fasting in combination with pegvisomant *vs.* pegvisomant only, 5228 ± 745.6 *vs.* 4164 ± 788.3 ng/ml; $P = 0.45$, by Mann-Whitney U test), these levels were more than 2000-fold higher than endogenous GH levels. We used free IGF-I as a measure for assessing the efficacy of the GH receptor blockade (30–32). Indeed, pegvisomant induced a significant reduction in free IGF-I resulting in free IGF-I levels on d 4 comparable to those observed in hypopituitarism with GHD (33). Although free IGF-I may not be a marker for all local (paracrine and/or autocrine) GH actions, the observed decline in free IGF-I levels can be taken to indicate efficient GH receptor blockade and, as we have to assume, that GH exerts its actions through the GH receptor. These data, indeed, indicate that the administration of pegvisomant creates a state of acute (functional) GHD.

Fat mass was not altered in our study; however, fat-free mass decreased as a result of fasting. This implies that lean body mass also decreased. It should be noted that this decrease in fat-free mass could be fully accounted for by the concomitant, metabolically inert decline in total body water.

Pegvisomant alone had no effect on insulin sensitivity, and although insulin sensitivity increased as a result of fasting, this increase was not influenced by additional GH receptor blockade. From these data we conclude that acute impairment of the GH signaling cascade has no immediate effect on insulin sensitivity. In a recent study Christopher *et al.* (34) described a negative correlation between insulin sensitivity and IGF-I levels. In our study no significant correlations were observed between changes in free IGF-I and insulin sensitivity.

The increased risk of cardiovascular disorders in GHD has been partially ascribed to changes in hemostasis in these patients, such as an increase in serum fibrinogen concentration and increased PAI-I activity (8, 35). High fibrinogen levels are associated with an increased incidence of stroke and myocardial infarction, and high PAI-I activity or antigen is an independent risk factor for primary and recurrent myo-

cardial infarction (36–38). Johansson *et al.* (8) have shown that GHD adults have higher fibrinogen and PAI-I activity compared with healthy controls matched for sex, age, and body mass index. In a subsequent study they observed a decrease in PAI-I after 2 yr of GH therapy (35). In addition to GHD, increased PAI-I activity has been found in abdominally obese subjects, and a reduction of PAI-I levels has been observed after weight loss (39). Probably this is due to a decrease in insulin resistance as a result of weight loss (40). So it is unclear whether the observed changes in PAI-I activity, PAI-I antigen, and t-PA antigen are due to a direct effect of GH itself or to changes in body composition. However, the fact that in our study we observed no significant changes in PAI-I levels makes a causative role of GH itself in the elevation of PAI-I observed in GHD less likely.

Interestingly, pegvisomant resulted in a significant decrease in vWF.ag, and a similar trend was seen for FVIII and vWF activity. It has long been recognized that there is a relationship between GH and vWF. A rise in GH is associated with increased vWF activity in healthy subjects (41). Our study thus supports these data. In GHD subjects, Jorgensen *et al.* (42) found only a nonsignificant increase in vWF:ag levels after 4 months of GH replacement therapy in 22 adult GHD subjects. Johansson *et al.* (35) investigated the long-term effects of GH replacement therapy on hemostasis and fibrinolysis and found a nonsignificant decrease 24 months after initiation of GH replacement therapy. However, vWF levels before the start of GH replacement were not decreased compared with those in normal subjects in this study (35). Besides vWF, all other hemostatic parameters, including fibrinogen, plasminogen, antiplasmin, and t-PA, remained unchanged by pegvisomant administration. Therefore, GH does not seem to play an important direct role in the regulation of hemostasis. The changes in coagulation factors observed in GHD are probably caused by the metabolic changes induced by long-standing GHD.

Pegvisomant alone increased the serum triglyceride concentration, indicating that GH is directly involved in the regulation of serum triglycerides. However, in a previous cross-sectional study we observed a strong negative correlation between free IGF-I and triglycerides (43). Moreover, administration of recombinant IGF-I has been reported to cause a decrease in triglyceride levels (44). Taken together these data indicate that GH and IGF-I are both involved in triglyceride metabolism. Fasting alone, fasting in combination with pegvisomant, and pegvisomant alone had similar effects on all of the assessed lipid particles. In accordance with the previously described data concerning insulin sensitivity and coagulation and fibrinolysis factors, these data also point to a primary role of changes in body composition and not of GH itself in the metabolic changes seen in GHD patients.

Several researchers have reported that GH increases and IGF-I decreases circulating Lp(a) (45–47). Surprisingly, pegvisomant induced no change in Lp(a). It could be argued that the 4-d study period is too short to induce significant changes. However, 4 d of fasting independently of GH receptor blockade was able to induce significant changes in Lp(a). Apparently GH and IGF-I are, at least in the short term, of only minor importance in the regulation of Lp(a).

In conclusion, in different metabolic conditions the GH receptor antagonist pegvisomant induces no significant changes in the major risk markers for CVD. Based on these data we hypothesize that the secondary metabolic changes, *e.g.* abdominal obesity or inflammatory factors (48), that develop as a result of long-standing GHD are of primary importance in the pathogenesis of atherosclerosis in patients with GHD.

Acknowledgments

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