Characterization of Herpes Virus Entry Mediator as a Factor Linked to Obesity

Judit Bassols[1], Jose M. Moreno[1], Francisco Ortega[1], Wifredo Ricart[1] and Jose M. Fernandez-Real[1]

Herpes virus entry mediator (HVEM) is a member of the tumor necrosis factor (TNF) receptor superfamily (TNFRSF14), which serves as a receptor for herpes viruses and cytokines such as lymphotoxin-α (LT-α) and LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells). We aimed to explore the associations of HVEM with human obesity. HVEM gene expression and protein levels were studied in total adipose tissue and in their fractions (isolated adipocytes and stromovascular cells (SVCs)) obtained from 81 subjects during elective surgical procedures. HVEM −241GA and −14AG gene polymorphisms were also studied and associated with obesity measures in 840 subjects. Visceral adipose tissue had significantly higher expression of HVEM than subcutaneous adipose tissue ($P < 0.0001$). Obese patients had significantly higher subcutaneous HVEM gene expression ($P = 0.03$) and protein levels ($P = 0.01$) than lean subjects. HVEM gene expression and protein levels were found in both isolated adipocytes and SVCs. These findings were confirmed in primary cultures from human preadipocytes, in which a significant increase in HVEM was observed during the differentiation process. HVEM −241GA and −14AG gene polymorphisms were associated with obesity, diastolic pressure, several inflammatory parameters (C-reactive protein and interleukin 18 (IL-18)), and circulating LIGHT concentrations. A sample of men with the G241A gene polymorphism also showed an increased serum titer of IgG antiherpes virus 1. These results provide evidences of an existing relationship between HVEM and obesity, which suggest that this TNF superfamily receptor could be involved in the pathogenesis of obesity and inflammation-related activity.

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INTRODUCTION

Obesity is a chronic disease affecting a significant part of the population worldwide. Although the etiology involves factors related to the diet and physical activity, the accumulation of activated T cells and macrophages in the adipose tissue during weight increase, and the presence of inflammatory cytokines, suggests that a significant deregulation of the inflammatory pathways occurs in these circumstances. It has become increasingly clear in recent years that proinflammatory cytokines of the tumor necrosis factor (TNF) superfamily produced mainly by macrophages and T cells play a major role in the development of obesity.

Lymphotoxins (LTs) and TNF-α are members of the TNF superfamily, a diversified family of ligands and corresponding family of receptors defined by a cysteine-rich ectodomain that control signaling pathways which initiate cell death, survival, and cellular differentiation (1). These factors are powerful modulators of immune functions and participate in pathogenic mechanisms. As an example, it has been found that altered regulation of several of these factors may contribute to a breakdown in immune tolerance and the development of autoimmune diseases (2).

One of the most recently defined members of this network, LTB-related ligand LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells), binds to the herpes virus entry mediator (HVEM), which was discovered as an entry route for herpes simplex viruses (HSVs) (3). HVEM is encoded by a single gene which maps to chromosome 1p36.22-36.3, in the same region as several other members of the TNF-α receptors (TNFRs) superfamily. The most abundant HVEM open reading frame encodes a 283-amino acid single transmembrane protein with a 36-residue signal sequence, two perfect and two imperfect TNFR-like cysteine-rich domains, and a short cytoplasmic tail. Glycoprotein D of HSV is a virokine encoded by HSV that blocks LIGHT binding to HVEM (4). HVEM may also serve as a third receptor for secreted LT-α (4).

HVEM, as well as other TNF family receptors including LTBR and TNFR2, induces the nuclear factor–κB pathway, which is a major control point for the expression of inducible genes regulating inflammation (1). Changes in the expression of the LT/TNF ligands and receptors among various cell types will determine the dynamics of the intracellular pathways.

[1]Department of Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomédica de Girona, CIBEROBN Fisiopatología de la Obesidad y Nutrición, Girona, Spain. Correspondence: Jose M. Fernandez-Real (jmfernandezreal.girona.ics@gencat.cat)

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HVEM is expressed in multiple human tissues and cell lines and shows constitutive and relatively high expression in peripheral blood T cells, B cells, and monocytes. HVEM mRNA was also detected in several tissues with a relatively high level in the lung, spleen, and thymus, but was not found in the brain, liver, or skeletal muscle. Only a weak expression of HVEM was found in bone marrow and endothelial cells (5). This wide distribution seems to be unique to HVEM, because expression of most TNF receptor superfamily members is restricted to particular immune cells (6,7).

A massive recruitment of leukocytes and macrophages to selected organs and tissues occurs with inflammation and tissue destruction. This recruitment contributes to the production of inflammatory cytokines and chemokines (8,9). It has long been recognized that viruses interfere with cytokine communication pathways. Several families including herpes-, adeno-, papova-, and poxivi-radae have evolved strategies specifically targeting the LT/TNF network.

The present study aimed to examine whether HVEM was expressed in human subcutaneous and visceral adipose tissue and their potential associations with obesity measures. We also studied the HVEM gene expression and protein levels in stromovascular cells (SVCs) and isolated mature adipocytes, and HVEM changes during the adipocyte differentiation process in primary cultures. Finally, we also evaluated whether two HVEM gene polymorphisms were associated with obesity and related inflammatory activity.

METHODS AND PROCEDURES

Study of HVEM in adipose tissue

Subcutaneous and visceral adipose tissues were obtained from 81 subjects (23 men and 58 women, mean age 48.7 years) during elective surgical procedures (cholecystectomy, surgery of abdominal hernia, and stomach reduction surgery) performed at the Dr Josep Trueta Hospital in Girona (Spain). The anthropometrical and clinical characteristics are shown in Table 1. The study was approved by the local ethical committee and the subjects gave informed written consent.

Isolation and culture of primary adipose cells. Human preadipocytes were isolated and cultured following standard protocols (10). Briefly, minced adipose tissue ($n = 8$) was digested by collagenase treatment. The digested samples were filtered and centrifuged to obtain the pellet and the floating cells (SVCs and adipocytes, respectively). The stromovascular fraction was incubated in erythrocyte lysis buffer (0.154 mol/l NH_4Cl, 10 mmol/l $KHCO_3$, and 0.1 mmol/l EDTA) for 5 min and seeded in DME/Ham's F-12 medium (1:1, vol/vol) supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland, UK), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Gibco). After 16–20 h, cells were carefully washed with phosphate-buffered saline to remove nonadhering material, mainly white blood cells and cell debris, and used for cell culture at passage 2 in order to eliminate nonpreadipocyte cell contamination as confirmed by negative staining for macrophage markers (Ham56 and Mac-1).

At the same time, human visceral preadipocytes (40.625 cells/cm^2) from nonobese (BMI < 30) and obese (BMI > 30) nondiabetic patients aged >40 (Zen-Bio, Research Triangle Park, NC) were cultured in accordance with the manufacturer's instructions. Two weeks after the initiation of differentiation, cells were considered mature adipocytes. During the differentiation process (days 0, 7, and 14), cells were harvested and stored at −80 °C for future analysis.

To explore the effects of an inflammatory environment on HVEM expression, mature adipocytes were incubated with 10 ng/ml lipopolysaccharide for 24 h.

RNA extraction and reverse transcription. Total RNA from isolated SVCs, adipocytes, and human cultured adipocytes was extracted using RNeasy Lipid Mini Kit (Qiagen, Courtaboeuf, France) in accordance with the manufacturer's instructions. RNA quantity and integrity were determined using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). One microgram of RNA was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The cDNA reaction was incubated for 10 min at 25 °C followed by 120 min at 37 °C and heated for 5 min at 85 °C.

Real-time PCR. Quantification of the mRNA coding for HVEM and cyclophilin A were performed using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) with Hs00187058m1 for

Table 1 Study of HVEM expression in adipose tissue: anthropometric and biochemical characteristics of the study subjects

Variables	Lean	Obese	*P*
N	34	47	
Sex (M/F)	12/22	11/36	0.4
Age (years)	51.91 ± 17.30	46.38 ± 11.16	0.085
BMI (kg/m^2)	23.74 ± 2.26	37.60 ± 10.38	**<0.0001**
Waist-to-hip ratio	0.87 ± 0.06	0.94 ± 0.06	**0.003**
Glucose (mg/dl)	97.26 ± 27.97	98.85 ± 31.92	0.824
Cholesterol (mg/dl)	193.47 ± 41.69	196.90 ± 36.59	0.723
Triglycerides (mg/dl)	111.28 (78.75–164.20)	116 (78.75–159.50)	0.442
HDL cholesterol (mg/dl)	68.60 ± 12.96	54.94 ± 21.56	**0.044**
LDL cholesterol (mg/dl)	99.73 ± 30.22	117.14 ± 37.12	0.223
Systolic blood pressure (mm Hg)	132.34 ± 16.24	131.30 ± 17.73	0.800
Diastolic blood pressure (mm Hg)	75.54 ± 8.29	76.67 ± 11.88	0.655
HVEM subcutaneous ($2^{-\Delta C_T}$)	0.04 (0.036–0.059)	0.056 (0.039–0.079)	**0.031**
HVEM visceral ($2^{-\Delta C_T}$)	0.065 (0.046–0.097)	0.076 (0.061–0.093)	0.092

Data are means ± s.d. unless otherwise indicated. Parameters not giving a normal distribution are shown as medians (interquartile range). Boldface values represent $P < 0.05$. $2^{-\Delta C_T}$, relative expression; HDL, high-density lipoprotein; HVEM, herpes virus entry mediator; LDL, low-density lipoprotein.

HVEM and Hs99999904m1 for cyclophilin A. Reactions were performed as a mixture containing 4 ng/µl cDNA, 1.25 µl assay, and 12.5 µl TaqMan Universal PCR Master Mix 2× (Applied Biosystems), in a final volume of 25 µl. Relative expression was determined by the comparative threshold method and data were expressed as $2^{-\Delta C_T}$.

Western blot. Cell lysates from human cultured adipocytes and adipose tissues from lean and obese subjects ($n = 12$) were washed in ice-cold phosphate-buffered saline followed by homogenization with RIPA lysis buffer (Upstate Biotechnology, Lake Placid, NY) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) at 4 °C for 30 min. Protein concentration was determined using a Lowry assay. RIPA protein extracts (40 µg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes by conventional procedures. Membranes were immunoblotted with antihuman HVEM (Proteintech, Chicago, IL) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). IgG secondary antibodies coupled to horseradish peroxidase were used and horseradish peroxidase activity was detected by chemiluminescence.

Study of HVEM gene polymorphism

Subjects. Eight-hundred and forty white patients (503 men and 337 women, mean age 48.2 years) were recruited in the endocrinology unit of the Dr Josep Trueta Hospital in Girona. Participants were randomly selected from the census and invited to participate. All individuals with a nonpathologic glucose tolerance ($n = 396$) had a fasting plasma glucose concentration of <7.0 mmol/l and 2-h postload plasma glucose concentration of <7.8 mmol/l. Impaired glucose tolerance was diagnosed in 231 participants. Previously undiagnosed type 2 diabetes was found in 80 participants (postload glucose concentration >11.1 mmol/l).

All subjects reported that their body weight had been stable for at least 3 months before the study. Inclusion criteria were (i) absence of any systemic disease and (ii) absence of clinical symptoms and signs of infection in the previous month by structured questionnaire to the patient.

To increase the statistical power of the group of patients with type 2 diabetes, we prospectively recruited 133 patients from diabetes outpatient clinics who demonstrated stable metabolic control in the previous 6 months as defined by stable values for glycated hemoglobin A_{1c}. Data from these patients were merged with those patients with recently diagnosed type 2 diabetes. The study was approved by the local ethics committee. and informed consent was obtained from all subjects.

Anthropometric measurements. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Fat mass was calculated using the Deurenberg formula (11) (fat mass = 1.20 (BMI) + 0.23 (age) − 10.80 (sex) − 5.40, where sex is 1 for men and 0 for women).

Biochemical assays. Glucose concentrations in serum samples were measured in duplicate with a Beckman Glucose Analyzer II (Beckman Instruments) by the glucose oxidase method. Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase using a Roche/Hitachi 747 instrument. High-density lipoprotein cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Low-density lipoprotein was calculated using the Friedewald formula. Total serum triglycerides were measured by monitoring the reaction of glycerol-phosphate-oxidase and peroxidase.

Serum LIGHT concentration was measured using a commercial human LIGHT ELISA kit (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions. Analytical intra-assay sensitivity was 5.5 pg/ml. The intra- and interassay coefficients of variation were 3.6 and 7.5%, respectively. No cross-reactivity with other cytokines was evident. Serum C-reactive protein was determined by an immunoturbidimetric assay (Beckman Coulter, Brea, CA), with an intra- and interassay coefficient of variation <4% and sensitivity of 1.0 mg/l. Serum interleukin-6 (IL-6) concentrations were measured using a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (IMMULITE 2000; DPC DIPESA, Madrid, Spain). Analytical intra-assay sensitivity was 0.5 pg/ml. The intra- and interassay coefficients of variation were 11.6 and 5.1%, respectively. Serum IL-18 concentrations were measured by a sandwich enzyme-linked immunosorbent assay (human IL-18 ELISA Kit; Medical and Biological Laboratories, Nagoya, Japan). Assay sensitivity was 12.5 ng/l, and intra-assay and interassay coefficients of variation were 7.3 and 7.5%, respectively.

Genotyping of the HVEM polymorphisms. Genomic DNA was extracted from peripheral blood leukocytes following standard procedures (QIAamp DNA Blood Mini Kit; Qiagen). The *G241A* (rs2234167) and *A17G* (rs4870) polymorphisms (National Center for Biotechnology Information) were analyzed by sequence analysis using a TaqMan-based technology suitable for distinguishing alleles (ABI Prism 7000 Sequence Detection System; Applied Biosystems). PCR conditions were set as recommended by the manufacturer (Applied Biosystems).

Statistical analysis. Variables that were not normally distributed were logarithmically transformed for subsequent analyses. The transformation of C-reactive protein was performed as log (PCR+1). Anti-log transformed values are reported in the tables. Two groups of quantitative variables were compared using the Student's *t*-test. The χ^2-test was used to compare genotype and allele frequencies of HVEM polymorphisms. Simple correlation (Pearson test) and general linear models were used to analyze relationships between HVEM and clinical variables.

RESULTS

HVEM gene expression in adipose tissue

We evaluated HVEM gene expression in visceral and subcutaneous adipose tissue of 81 subjects whose biochemical and anthropometrical characteristics are shown in **Table 1**. Higher HVEM gene expression was observed in visceral adipose tissue than in subcutaneous tissue ($P < 0.0001$) (**Figure 1a**). The HVEM gene expression of these two fat depots correlated significantly with each other ($r = 0.375$, $P = 0.014$) (**Table 2**).

HVEM gene expression of subcutaneous adipose tissue correlated significantly with BMI ($P = 0.010$), % fat mass (0.013), and low-density lipoprotein cholesterol ($P = 0.011$) (**Table 2**). No significant correlations were observed for HVEM gene

Table 2 Correlation between subcutaneous adipose tissue HVEM gene expression and selected variables

	HVEM subcutaneous ($2^{-\Delta C_T}$) ($n = 81$)	
	Pearson correlation	*P*
Sex	0.108	0.337
Age (years)	–0.072	0.521
BMI (kg/m^2)	0.286	**0.010**
Systolic pressure (mm Hg)	0.080	0.508
Diastolic pressure (mm Hg)	0.034	0.776
Cholesterol (mg/dl)	0.101	0.414
HDL cholesterol (mg/dl)	–0.166	0.248
LDL cholesterol (mg/dl) ($n = 67$)	0.377	**0.011**
Fasting triglycerides (mg/dl)	–0.222	0.099
HVEM visceral ($2^{-\Delta C_T}$)	0.375	**0.014**

Boldface values represent $P < 0.05$.
$2^{-\Delta C_T}$, relative expression; HDL, high-density lipoprotein; HVEM, herpes virus entry mediator; LDL, low-density lipoprotein.

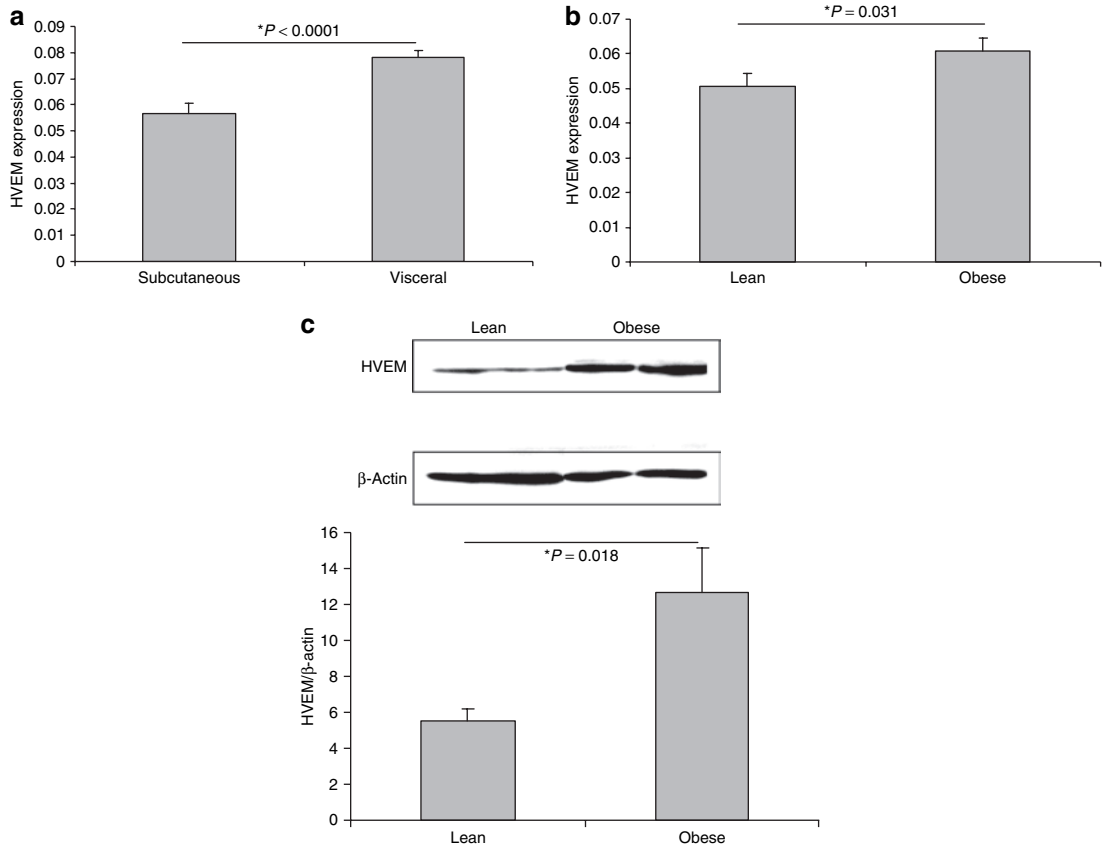

Figure 1 Data from real-time reverse transcription–PCR showing herpes virus entry mediator (HVEM) gene expression in (**a**) subcutaneous and visceral human adipose tissue and (**b**) subcutaneous adipose tissue of lean vs. obese subjects. (**c**) Western blot analysis of HVEM in subcutaneous human adipose tissue in lean and obese subjects. Relative mRNA levels were normalized to the levels of cyclophilin A and densitometric analyses of HVEM protein were normalized to β-actin. Western blot bands are representative of $n = 12$ subjects. *$P < 0.05$.

expression of visceral adipose tissue. Obese patients had significantly higher subcutaneous HVEM gene expression ($P = 0.031$) (Figure 1b) and protein levels ($P = 0.018$, $n = 12$) (Figure 1c) than nonobese subjects. This tendency was also observed in visceral adipose tissue but statistical significance was not reached.

We also measured the HSV-1 titer in a subsample of subjects in relation with HVEM expression in adipose tissue, whose characteristics did not differ significantly from the remaining group ($n = 56$). HSV-1 titer was not significantly associated with HVEM expression in subcutaneous or visceral adipose tissue ($r < 0.12$, $P > 0.2$).

HVEM gene expression in adipocytes

In order to find out which cells were responsible for HVEM gene expression in adipose tissue, we separated SVCs and mature adipocytes from adipose tissue samples ($n = 8$). Both cellular types expressed HVEM mRNA, but SVCs had significantly higher expression of HVEM than mature adipocytes ($P = 0.001$) (Figure 2a).

We then studied HVEM gene expression during the adipocyte differentiation process using primary cultures of the isolated preadipocytes. Preadipocytes obtained from Zen-Bio were subjected to same process. HVEM mRNA increased significantly during the differentiation process (Figure 2b). These results were corroborated at the protein level (Figure 2c). No differences were observed between preadipocytes obtained from lean and obese subjects. Lipopolysaccharide treatment had no effect on HVEM gene expression.

HVEM gene polymorphisms

We examined two nonsynonymous single-nucleotide polymorphisms of the HVEM gene located in the 1p36 interval. The rs2234167 is situated in exon 7 (a change of G into A, which leads to the substitution of a valine for isoleucine in the AA 241 of the mature protein). The rs4870 is located in exon 1 (a change of A into G, which leads to the substitution of a lysine for an arginine in the AA 17 of the mature protein). The anthropometric and biochemical characteristics of subjects whose polymorphisms were studied are shown in Table 3.

For the G241A HVEM polymorphism (rs2234167), 71.5% (606 subjects) were GG homozygotes, 25.3% (214 subjects) AG heterozygotes, and 3.2% (20 subjects) were AA homozygotes.

A carriers (AG/AA) had significantly increased weight ($P < 0.0001$), BMI ($P = 0.001$) (Figure 3a), fat mass ($P = 0.038$), diastolic pressure ($P = 0.037$), C-reactive protein ($P = 0.05$), and also increased LIGHT levels ($P = 0.034$) (Table 4).

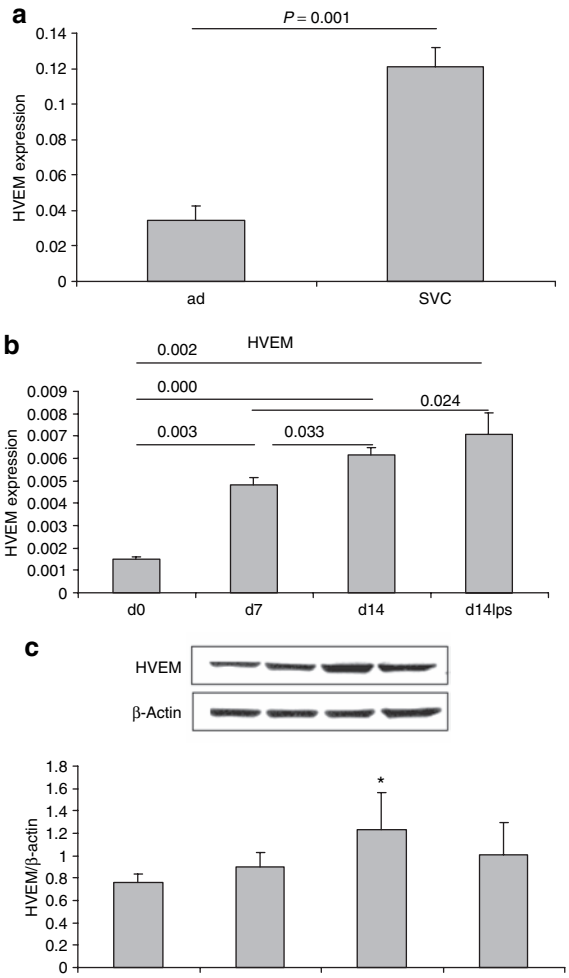

Figure 2 Herpes virus entry mediator (HVEM) gene expression in adipocytes. (**a**) Data from real-time reverse transcription–PCR (RT-PCR) showing HVEM gene expression in stromovascular cells (SVCs) and mature adipocytes (ad) separated from human adipose tissue. Data are means ± s.e.m. ($n = 8$). (**b**) Data from real-time RT-PCR and (**c**) western blot analysis of HVEM in primary culture of human preadipocytes during the differentiation process. Relative mRNA levels were normalized to the levels of cyclophilin A and densitometric analyses of HVEM protein were normalized to β-actin. Data are representative of three different experiments. *$P < 0.05$.

Circulating IL-6 and IL-18 levels were not statistically different according to this gene polymorphism. These results were even more significant after controlling for age, given that A carriers were significantly younger, and persisted when obese subjects were studied separately.

When men and women were analyzed separately, female A carriers showed significantly increased weight ($P < 0.0001$), BMI ($P < 0.001$), and fat mass ($P = 0.001$) and were significantly younger ($P = 0.016$), whereas male A carriers had significantly increased diastolic pressure ($P = 0.019$) and HSV-1 titer ($P = 0.006$) (Table 4). BMI correlated significantly and positively with HSV-1 titer ($r = 0.21$, $P = 0.006$, $n = 158$). When the analysis was restricted to nondiabetic subjects, A carriers had significantly increased weight ($P = 0.048$), fat mass ($P = 0.008$), low-density lipoprotein cholesterol ($P = 0.048$), and LIGHT levels ($P = 0.010$).

Table 3 Anthropometric and biochemical characteristics of subjects whose polymorphisms were studied

Variables	Mean
n	840
Sex (M/F)	503/337
Age (years)	48.22 ± 13.23
BMI (kg/m^2)	32.25 ± 9.10
Glucose (mg/dl)	120.74 ± 62.57
Cholesterol (mg/dl)	198.39 ± 38.56
Triglycerides (mg/dl)	140.25 (71–170)
HDL cholesterol (mg/dl)	51.93 ± 14.69
LDL cholesterol (mg/dl)	118.11 ± 36.68
Systolic blood pressure (mm Hg)	130.71 ± 20.54
Diastolic blood pressure (mm Hg)	78.85 ± 11.71
LIGHT (pg/ml) ($n = 255$)	43.75 (21.61–59.65)

Data are means ± s.d. unless otherwise indicated. Parameters not giving a normal distribution are shown as medians (interquartile range).
HDL, high-density lipoprotein; LDL, low-density lipoprotein; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells.

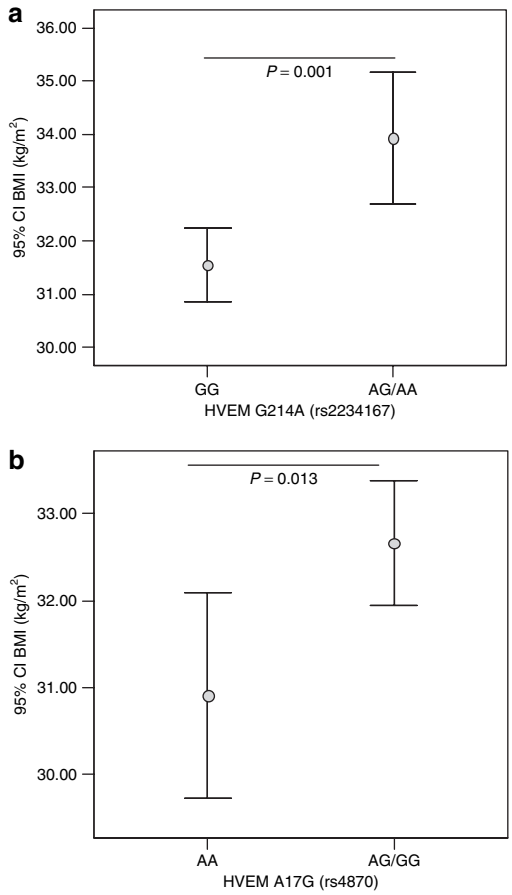

Figure 3 Error bar plot of BMI according to herpes virus entry mediator (HVEM) single-nucleotide polymorphisms (**a**) G241A (rs2234167) and (**b**) A17G (rs4870) in all subjects. Plots are means and 95% confidence interval (CI). *$P < 0.05$ from linear-trend ANOVA.

Table 4 Comparison of clinical variables according to G241A HVEM and A17G HVEM polymorphisms

	All subjects			Men			Women		
	GG	A/-	*P*	GG	A/-	*P*	GG	A/-	*P*
G241A (rs2234167)									
n	606	234	—	366	137	—	240	97	—
Age (years)	48.90 ± 13.26	46.50 ± 12.99	**0.017**	49.64 ± 13.04	48.30 ± 13.21	0.300	47.76 ± 13.53	43.96 ± 12.31	**0.016**
Weight (kg)	87.25 ± 25.62	94.67 ± 27.23	**<0.0001**	89.77 ± 25.78	92.87 ± 26.69	0.230	83.46 ± 24.96	97.22 ± 27.91	**<0.0001**
BMI (kg/m^2)	31.54 ± 8.75	33.93 ± 9.71	**0.001**	30.69 ± 8.09	31.36 ± 8.09	0.404	32.83 ± 9.55	37.54 ± 10.66	**<0.0001**
Glucose (mg/dl)	121.79 ± 61.81	118.43 ± 64.91	0.512	118.36 ± 55.68	119.77 ± 67.31	0.816	127.98 ± 71.33	116.18 ± 61.03	0.200
Cholesterol (mg/dl)	197.86 ± 38.50	199.74 ± 38.47	0.545	201.05 ± 40.05	203.65 ± 37.80	0.518	192.12 ± 34.93	193.35 ± 38.94	0.798
HDL (mg/dl)	52.63 ± 15.26	50.18 ± 13.21	**0.042**	50.15 ± 13.28	48.59 ± 12.44	0.244	57.07 ± 17.45	52.88 ± 14.10	0.062
LDL (mmol/l)	116.87 ± 36.13	121.41 ± 37.83	0.133	122.00 ± 37.18	125.03 ± 38.68	0.436	107.75 ± 32.33	115.16 ± 35.70	0.104
Triglycerides (mg/dl)	106 (71–173)	111 (72–161.5)	0.583	108 (72.25–179.75)	116 (75.75–164.25)	0.829	99.5 (65.5–163.75)	100.5 (69–154)	0.559
SBP (mm Hg)	130.61 ± 20.09	130.86 ± 21.57	0.884	130.50 ± 18.20	132.84 ± 19.89	0.237	130.78 ± 23.01	127.52 ± 23.90	0.308
DBP (mm Hg)	78.26 ± 11.83	80.30 ± 11.29	**0.037**	79.09 ± 11.40	81.93 ± 11.60	**0.019**	76.84 ± 12.41	77.54 ± 10.24	0.667
% Fat mass (Deurenberg)	37.18 ± 12.33	39.69 ± 14.05	**0.010**	32.05 ± 9.67	32.54 ± 9.55	0.603	44.98 ± 11.84	49.76 ± 13.20	**0.001**
IL-18 (pg/ml)	290.4 (229.5–367.4)	301.9 (250.6–393.1)	0.338	290.4 (229.5–367.4)	301.9 (250.6–393.1)	0.338	—	—	—
n	103	38		103	38				
LIGHT (pg/ml)	33.68 (20.27–56.45)	40.83 (25.37–60.86)	**0.034**	35.11 (21.61–56.45)	41.74 (28.38–58.97)	0.061	32.21 (15.89–57.38)	39.92 (25.10–80.34)	0.318
n	173	81		129	59		44	22	
HSV-1 titer (relative units/ml)	130 (97–149)	143 (114–168)	**0.006**	130 (97–149)	143 (114–168)	**0.006**	—	—	—
n	115	43		115	43				
A17G (rs4870)									
n	223	625	—	138	371	—	85	254	
Age (years)	48.69 ± 13.37	48.05 ± 13.18	0.537	48.49 ± 13.09	49.47 ± 13.14	0.454	49.00 ± 14.00	45.82 ± 12.95	0.056
Weight (kg)	85.28 ± 25.25	90.94 ± 26.55	**0.005**	87.59 ± 24.62	91.69 ± 26.45	0.115	81.04 ± 25.60	89.62 ± 26.48	**0.010**
BMI (kg/m^2)	30.95 ± 8.95	32.71 ± 9.11	**0.013**	30.14 ± 8.30	31.13 ± 7.98	0.221	32.13 ± 9.74	34.89 ± 10.12	**0.029**
Glucose (mg/dl)	117.66 ± 54.62	121.83 ± 65.18	0.430	120.11 ± 60.05	117.88 ± 58.03	0.717	113.90 ± 43.46	128.16 ± 75.35	0.139
Cholesterol (mg/dl)	199.33 ± 37.08	198.05 ± 39.09	0.701	202.72 ± 39.65	201.75 ± 39.32	0.814	192.06 ± 30.53	192.16 ± 37.92	0.985
HDL cholesterol (mg/dl)	53.14 ± 14.80	51.51 ± 14.65	0.192	51.04 ± 14.48	49.27 ± 12.46	0.196	57.18 ± 14.68	55.52 ± 17.12	0.480
LDL cholesterol (mmol/l)	117.15 ± 35.65	118.43 ± 37.06	0.684	122.29 ± 37.09	123.18 ± 37.67	0.824	106.73 ± 30.38	110.60 ± 34.49	0.418
Triglycerides (mg/dl)	105 (71–185.75)	107 (71–164.5)	0.526	109 (74.2–189.5)	111 (71.25–171)	0.673	96 (61.50–181.25)	101 (69–155)	0.578
SBP (mm Hg)	130.05 ± 20.98	130.95 ± 20.39	0.603	129.43 ± 18.85	131.57 ± 18.60	0.275	130.54 ± 24.38	129.42 ± 23.06	0.730

Table 4 Continued on next page

Table 4 (Continued)

	All subjects			Men			Women		
	GG	A/-	*P*	GG	A/-	*P*	GG	A/-	*P*
DBP (mm Hg)	77.32 ± 12.02	79.42 ± 11.56	**0.030**	78.20 ± 12.02	80.36 ± 11.30	0.074	75.45 ± 11.82	77.57 ± 11.85	0.198
% Fat mass (Deurenberg)	36.21 ± 12.62	38.42 ± 12.92	**0.028**	31.11 ± 10.04	32.54 ± 9.47	0.140	44.42 ± 12.06	47.00 ± 12.48	0.097
IL-18 (pg/ml)	272.9 (225.7–319.1)	308.6 (252.1–389.2)	**0.020**	272.9 (225.7–319.1)	308.6 (252.1–389.2)	**0.020**	—	—	—
n	40	93		40	93				
LIGHT (pg/ml)	31.19 (20.02–52.45)	38.63 (23.54–60.86)	0.075	36.89 (21.79–58.97)	36.68 (23.51–58.68)	0.420	20.72 (14.60–40.45)	40.69 (23.35–78.31)	**0.041**
n	74	181		56	132		18	49	
HSV-1 titer (relative units/ml)	128 (91–153.5)	137 (107.5–155)	0.083	128 (91–153.5)	137 (107.5–155)	0.083	—	—	—
n	45	113		45	113				

Data are means ± s.d. unless otherwise indicated. Parameters not giving a normal distribution are shown as medians (interquartile range). Boldface values represent $P < 0.05$.
DBP, diastolic blood pressure; HDL, high-density lipoprotein; HSV-1, herpes simplex virus 1; HVEM, herpes virus entry mediator; IL-18, interleukin 18; LDL, low-density lipoprotein; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells; SBP, systolic blood pressure.

For the A17G HVEM polymorphism (rs4870), 26.3% (223 subjects) were AA homozygotes, 49.6% (421 subjects) AG heterozygotes, and 24.1% (196 subjects) were GG homozygotes.

G allele carriers had significantly increased weight ($P = 0.005$), BMI ($P = 0.013$) (**Figure 3b**), fat mass ($P = 0.028$), diastolic pressure ($P = 0.030$), IL-18 concentration ($P = 0.015$), and a tendency toward increased C-reactive protein ($P = 0.08$) and LIGHT ($P = 0.07$) (**Table 4**). Circulating IL-6 levels were not statistically different according to this gene polymorphism. Women who were G carriers showed significantly increased weight ($P = 0.010$), BMI ($P = 0.029$), waist-to-hip ratio ($P = 0.040$), and LIGHT ($P = 0.041$) (**Table 4**). When the analysis was restricted to nondiabetic subjects, G carriers had significantly increased weight ($P = 0.050$), BMI ($P = 0.050$), IL-18 concentration ($P = 0.033$), and LIGHT ($P = 0.029$), and lower high-density lipoprotein cholesterol ($P = 0.029$).

DISCUSSION

The evidence is now overwhelming that TNF receptor members participate in the process of inflammatory and metabolic diseases. Evidence is presented here of an existing relationship between HVEM and obesity. HVEM gene expression was present in isolated adipocytes and increased during the differentiation process of preadipocytes as they become mature adipocytes. The presence of HVEM was confirmed in SVCs and mature adipocytes separated from adipose tissue samples by collagenase digestion. Real-time PCR analyses showed that both cellular types expressed HVEM, but higher levels were found in the SVC fraction. Given the known constitutive expression of HVEM in peripheral blood T cells, B cells, and monocytes, the increased expression of HVEM in SVCs probably corresponds to these blood-derived cell types. In addition, real-time PCR analysis showed that visceral adipose tissue expressed higher levels of HVEM than subcutaneous adipose tissue. On the other hand, obese patients had significantly higher HVEM gene expression and protein levels than nonobese subjects. However, this finding was only significant in the subcutaneous adipose tissue, although it tended toward significance in the visceral adipose tissue. Finally, different HVEM gene polymorphisms (−241GA and −14AG) were associated with obesity parameters.

These findings suggest that this TNF superfamily receptor could be involved in the progression of inflammation in association with obesity. We cannot exclude that increased HVEM is simply a part of the inflammatory process present in obesity. However, the association with HVEM gene polymorphisms hints at this protein being an important modulator of inflammation in obesity. These pathogenic effects could involve interaction between T cells and macrophages with adipocytes, as well as inflammatory-related cytokines.

As differentiated macrophages express HVEM constitutively, the higher levels of HVEM in obese compared to lean adipose tissue could be due to the increased number of macrophages present in the adipose tissue of obese subjects. However, HVEM gene expression was not only present in isolated adipocytes, but was also found to increase during the differentiation process of preadipocytes as they became mature adipocytes of human primary cultures. Fradette *et al.* (12) demonstrated using flow cytometry and indirect immunocytochemistry in their research into HSV-mediated transduction that HVEM was present on the surface of both human preadipocytes and mature adipocytes.

It was interesting to find an association between subcutaneous tissue HVEM gene expression and low-density lipoprotein cholesterol. One of the known HVEM ligands, LIGHT, has been associated with other inflammatory diseases, such as atherosclerosis (13,14), and has also been involved in the regulation of lipid metabolism (15). In fact, T cells and macrophages

play a key role in maintaining/perpetuating immune-mediated vascular inflammation during the progression of atherosclerosis, and altered cholesterol exerts an immunomodulatory effect locally (13,14).

To the best of our knowledge, this is the first study to associate two HVEM gene polymorphisms (−241GA and −14AG) to obesity. The frequencies of the alleles in the control group were similar to those described in other populations (16). Our results showed that homozygous subjects for both mutant alleles were over-represented among obese subjects. A-carrier subjects for the G241A HVEM polymorphism had significantly increased weight, BMI, fat mass, and diastolic blood pressure. Similar results were obtained for the A17G polymorphism, where G-carrier subjects had significantly increased weight, BMI, and diastolic pressure, as well as increased circulating levels of inflammatory cytokines such as IL-18. In addition, both polymorphisms were associated with higher levels of the HVEM ligand, LIGHT. Therefore, our data suggest that carrying any of these mutant alleles is significantly associated with inflammation and the possibility of suffering from obesity.

Interestingly, IL-18 is a proinflammatory cytokine that plays an important role in both innate and adaptive immune responses against viruses and intracellular pathogens. It is mainly expressed by dendritic cells and also by a variety of other cells including epithelial cells, adipocytes, and cells of the adrenal gland and kidneys (17). IL-18 induces interferon-γ production by helper T cells and natural killer cells and stimulates T-cell proliferation and natural killer activation (18). IL-18 has been described as playing a key role in the control of HSV-1 (19), the influenza virus (20), human immunodeficiency virus (21), and hepatitis C virus (22) infections. Increased levels of circulating IL-18 were reported in infected patients (21,22). Several studies have also shown increased levels of IL-18 in human immunodeficiency virus–seronegative subjects with obesity, insulin resistance, and type 2 diabetes (21).

Both LIGHT and HSV glycoprotein D compete to bind HVEM (4), and their binding causes a change in the orientation of the domains enabling the other to bind (23). The binding of herpes virus leads to acute inflammation and increased expression of several proinflammatory cytokines (24). Given this, we speculate that the mutant alleles of the studied polymorphisms may cause a change in the conformation of the receptor, perhaps with higher probabilities of being infected by herpes virus. This could explain the increased levels of unbound LIGHT in these subjects, as well as the increased inflammatory cytokine milieu due to herpes virus infection. We have previously reported that the IgG titer against HSV-1 is associated with fat mass in the general population (25). In this study, we found no significant relationship between HSV-1 titer and HVEM expression suggesting that other inflammatory factors mediate the increased expression of HVEM in obese subjects.

In summary, we have described increased HVEM gene expression in obese subjects and HVEM gene polymorphisms in association with obesity measures. However, further studies are needed to delineate more precisely the role of LIGHT/HVEM interactions in human obesity.

DISCLOSURE

The authors declared no conflict of interest.

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