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ORIGINAL ARTICLE

LIGHT is associated with hypertriglyceridemia in obese subjects and increased cytokine secretion from cultured human adipocytes

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Background: LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells) is a member of the tumor necrosis factor (TNF) family, primarily expressed in lymphocytes, which was associated with the induction of pro-inflammatory cytokines and alterations of lipid homeostasis in animal models. We aimed to analyze whether LIGHT has a role in the human obesity-associated inflammatory status.
Methods: The association between circulating LIGHT concentrations and clinical variables was studied in 190 subjects with different degrees of obesity and glucose tolerance. The expression and release of 21 different cytokines, and the expression of genes involved in lipid metabolism were also evaluated after stimulation with LIGHT in cultured human differentiated adipocytes.
Results: Serum LIGHT concentrations positively associated with body mass index (BMI), fat mass, glycated hemoglobin and fasting triglycerides, and negatively with high-density lipoprotein cholesterol. Circulating LIGHT concentrations were significantly increased in morbidly obese subjects and in patients with type 2 diabetes. LIGHT induced the secretion of several cytokines and upregulated the expression and secretion of interleukin-6 (IL-6), IL-8, Growth Regulated Oncogene (GRO) and monocyte chemotactic protein-1 (MCP-1). These observations were concomitant with the activation of nuclear factor (NF)-κB signalling in human differentiated adipocytes. LIGHT also upregulated the expression and synthesis of its own receptor (herpesvirus entry mediator (HVEM)) and decreased the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) and fatty acid synthase.
Conclusion: These data suggest that LIGHT may have a role in mediating chronic inflammation and alterations of lipid metabolism in obese subjects.
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Keywords: LIGHT; inflammation; lipids; cytokines

Introduction

Obesity is associated with a chronic low-grade inflammatory state as adipose tissue secretes a large number of inflammatory markers and thrombogenic factors, including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and fibrinogen. These factors, which can be reduced by weight loss, are likely to modulate vascular, metabolic, inflammatory and other functional aspects of the cardiovascular system that directly contribute to cardiovascular risk.[1]

Many cytokines have been implicated in the regulation of inflammation, such as the members of TNF cytokine superfamily, which serve as an intricate extracellular communication network of ligands and receptors that regulate multiple facets of development. Their major roles in maintaining effector functions of the immune system, often through the induction of programmed cell death or promotion of cell survival, have been extensively analyzed.[2]

LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells) is a cytokine in the TNF ligand superfamily that is expressed on activated T cells, monocytes, granulocytes and immature dendritic cells.[3–5] LIGHT binds three distinct members of

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the TNF receptor family, including the lymphotoxin β receptor,[4,6] herpesvirus entry mediator (HVEM)[7,8] and decoy receptor 3.[9] The functions of the signalling pathways associated with these receptors are often cell-context specific because of their expression by a variety of cell types and make LIGHT to have multiple biological activities, including stimulation of either cell growth[10] or cell death[11,12] and/or induction of inflammatory cytokines.[10,13,14]

HVEM is a receptor of the TNF receptor superfamily and has two ligands: herpes simplex virus surface envelope glycoprotein D and lymphotoxin-α.[3] The cytoplasmic region of HVEM bounds to several members of the TNF receptor-associated factor family[15] and induces marked activation of nuclear factor (NF)-κB,[16] a transcriptional regulator of multiple immunomodulatory and inflammatory genes. HVEM has a wide tissue distribution[17] and its expression has been found in different cell types including adipocytes.[17,18]

LIGHT-HVEM signalling results in T-cell activation and expansion, and modulates a number of T-cell responses,[19] promoting the development of T-cell-associated autoimmune diseases.[20] This cytokine has also been suggested to promote atherogenesis by inducing matrix metalloproteinase activity and inflammation in macrophages.[21,22] Recently, LIGHT signalling pathway has been related with the progression of chronic heart failure involving IL-6-related mechanisms.[23]

Tumor necrosis factor is also described as a molecule that causes hypertriglyceridemia and wasting of muscle and fat tissue.[24] In these sense, several studies have implicated the TNF superfamily of pro-inflammatory cytokines in lipid metabolism. Recently, Lo *et al*.[25] observed that transgenic mice that overexpressed LIGHT on T cells develop hyperlipidemia and show elevated cholesterol and triglyceride concentrations in the blood. This is explained by a reduction in the activity of hepatic lipase, which promotes the receptor-mediated uptake of plasma lipoproteins that harbor triglycerides and cholesterol and specifically catalyzes hydrolysis of the triglycerides.[26]

LIGHT produced by lymphocytes could interact with adipocyte metabolism. In fact, cross-talk between lymphocytes and adipocytes is increasingly recognized.[27]

Given this background, we hypothesize that LIGHT and its receptor HVEM could be involved in the inflammatory status of human obesity and the development of cardiovascular disease. In this work we analyze this hypothesis using various approaches, including clinical studies in subjects with different degrees of obesity, as well as *in vitro* experiments using human differentiated adipocytes.

Materials and methods

Subjects

This study was performed with a group of 190 Caucasian men recruited at the Endocrinology Service of the Hospital of Girona, Spain. Participants were randomly identified from census and invited to participate. All participants underwent a 75 g oral glucose tolerance test and, in accordance with American Diabetes Association Criteria, were diagnosed as individuals with normal glucose tolerance ($n=116$; fasting plasma glucose concentration of $<7.0\,\mathrm{mmol\,l^{-1}}$ and 2-h post-load plasma glucose concentration of $<7.8\,\mathrm{mmol\,l^{-1}}$), impaired glucose tolerance ($n=43$; post-load plasma glucose concentration of 7.8–11.1 $\mathrm{mmol\,l^{-1}}$) and type 2 diabetes ($n=31$; post-load glucose concentration $>11.1\,\mathrm{mmol\,l^{-1}}$).

All subjects reported that their body weight had been stable for at least 3 months before the study. None of the subjects was taking any medication. Inclusion criteria were: (1) absence of systemic disease and (2) absence of clinical symptoms and signs of infection in the previous month, determined on the basis of a structured questionnaire to the patient. Informed consent was obtained from all subjects. The local ethics committee approved the study.

Anthropometric measurements

Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared. Blood pressure was measured in the right arm after the participant had rested for 10 min in the supine position by using a standard sphygmomanometer of appropriate cuff size, and the first and fifth phases were recorded. The values used in analyses were the means of three readings taken at 5-min intervals. Fat mass and percent fat mass were calculated using bioelectric impedance (Holtain BC Analyzer, Cambridge, UK).

Biochemical assays

The glucose concentrations in serum samples were measured in duplicate using a Beckman Glucose Analyzer II (Beckman Instruments, Fullertone, CA, USA) by the glucose oxidase method. Total serum cholesterol was measured through the reaction of cholesterol estearase/cholesterol oxidase/peroxidase using a Roche Hitachi 747 instrument (Roche Diagnostics, Indianapolis, IN, USA). 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 insulin was measured in duplicate using monoclonal immunora-diometric assay (Medgenix Diagnostics, Fleunes, Belgium). The intra-assay coefficient of variation was 5.2 and 3.4% at a concentration of 10 and 130 $\mathrm{mU\,l^{-1}}$, respectively. The interassay coefficients of variation were 6.9 and 4.5% at 14 and 89 $\mathrm{mU\,l^{-1}}$, respectively.

Serum LIGHT concentration was measured using a commercial human LIGHT enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The analytical intra-assay sensitivity was 0.5 $\mathrm{pg\,ml^{-1}}$. The intra- and interassay coefficients of variation were 11.6 and 5.1%, respectively. No cross-reactivity with other cytokines was evident.

Cell cultures

Human visceral pre-adipocytes (40.625 cells cm^{-2}) from non-diabetic male patients with BMI >30 and age >40 (Zen-Bio, Research Triangle Park, NC, USA) were cultured in 12-well plates with pre-adipocyte medium, provided by Zen-Bio, in a humidified 37 °C incubator with 5% CO_2. At 24 h after plating, cells were checked for complete confluence and differentiation was induced using the differentiation medium provided (Zen-Bio, Inc.) and following the manufacturer's instructions. At 2 weeks after the initiation of differentiation, cells appeared rounded with large lipid droplets apparent in the cytoplasm. Cells were then considered mature adipocytes and were incubated with different doses of human recombinant LIGHT (0, 10, 100 and 1000 ng ml^{-1}) in serum-free media for 24 h. The supernatants were centrifuged at 400 *g* for 5 min, the cells were harvested and both were stored at −80 °C for future analysis.

Six additional samples of adipose tissue were obtained from obese subjects undergoing open abdominal surgery under anesthesia after an overnight fast at the hospital of Girona. The subjects had a mean age of 54 ± 5.5 years, BMI of 38.2 ± 6.2 kg m^{-2}, fasting glucose of 4.38 ± 0.32 mmol l^{-1}, and homeostatic model assessment value of 2.7 ± 1.6. The medical histories, physical examination, electrocardiogram and analytical tests showed that all patients were in good health.

Adipose tissue biopsies were cut into small explants and incubated in cultured media for 24 h or digested with 1.5 mg ml^{-1} collagenase (CLS type 1, Worthing Biochemical Corp., Freehold, NJ, USA) to obtain the stromal-vascular cells and adipocytes fractions. The supernatants of explants, the tissue explants and both fractions of stromal-vascular cells and mature adipocytes were stored at −80 °C for studying LIGHT secretion and expression.

Measurement of cell cytokine secretion

The levels of 21 cytokines (granulocyte-macrophage colony-stimulating factor (GM-CSF), GRO, GRO-α, IL-1α, IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, interferon-δ, monocyte chemotactic protein-1 (MCP-1), MCP-2, MCP-3, Monokine Induced by Gamma-Interferon (MIG), regulated upon activation, normal T-cell expressed, and presumably secreted (RANTES), transforming growth factor-β1, TNF-α and TNF-β) in all culture supernatants were determined using commercially available human cytokine antibody array 1 for Bionova (RayBiotech, Inc., Norcross, GA, USA). The assay was followed precisely as stated in the directions from the manufacturer. In brief, membranes were blocked with a blocking buffer, and then were incubated with 1 ml of medium from adipocytes with different LIGHT concentrations, at 4 °C for overnight. The membranes were washed and 1 ml of primary biotin-conjugated antibody was added and incubated at room temperature for 2 h. After washing, 2 ml of horseradish peroxidise-conjugated streptavidin was added and incubated for 30 min at room temperature. The membranes were developed by using the detection buffer, exposed to X-ray film and processed by autoradiography. Detectable spots were scanned and analyzed for densitometry with Scion Image software (Scion Corporation, Frederick, MD, USA).

Adiponectin secretion was assessed by using a commercial human adiponectin enzyme-linked immunosorbent assay kit (Linco Research, MO, USA) according to the manufacturer's instructions. Analytical intra-assay sensitivity was 0.78 ng ml^{-1}. The intra- and interassay coefficients of variation were 7.4 and 6.2% respectively. No crossreactivity with other cytokines was evident.

RNA extraction and reverse transcription

Total RNA from cultured human adipocytes treated with different LIGHT concentrations was extracted using RNeasy Lipid Mini Kit (Qiagen, Courtaboeuf, France) according to manufacturer's instructions. The RNA quantity and integrity was determined using 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA, USA). One microgram of RNA was reverse transcribed using high-capacity complementary DNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The complementary DNA 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, IL-6, MCP-1, IL-8, adiponectin, peroxisome proliferator-activated receptor-γ (PPAR-γ), fatty acid synthase (FASN), LIGHT and cyclophilin A were performed using ABI Prism 7000 Sequence Detection System (Applied Biosystems) using the following assays: Hs00187058m1 for HVEM, Hs00174103m1 for IL-8, Hs00234140m1 for MCP-1, Hs00985639m1 for IL-6, Hs00605917m1 for adiponectin, Hs00234592m1 for PPAR-γ, Hs00188012m1 for FASN, Hs01006800m1 for LIGHT and Hs99999904m1 for cyclophilin A. The reactions were performed as a mixture containing 4 ng μl^{-1} complementary DNA, 1.25 μl of assay and 12.5 μl of TaqMan Universal PCR Master Mix 2 × (Applied Biosystems), in a final volume of 25 μl. The relative expression was determined using the comparative threshold method.

Western blotting

Cell lysates from cultured human adipocytes treated with different LIGHT concentrations were washed in ice-cold phosphate buffered saline followed by homogenization with radioimmunoprecipitation assay lysis buffer (Upstate) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) at 4 °C for 30 min. Cellular debris were eliminated by centrifugation of the solubilizated samples at 20 000 *g* for 15 min (4 °C). Protein concentration was determined using Lowry assay. Radioimmunoprecipitation assay protein extracts (40 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to

Table 1 Anthropometric and biochemical characteristics of subjects according to (A) glucose tolerance and (B) obesity status

Variables	*NFG*	*IGT*	*DM2*	P-*value*
(A)				
N	116	43	31	
Age (years)	48.03 ± 11.71	56.09 ± 10.23	50.35 ± 11.74	0.001
BMI (kg m^{-2})	29.07 ± 6.27	29.12 ± 4.51	31.54 ± 7.62	0.138
Glucose (mg per 100 ml)	92.96 ± 7.40	103.09 ± 11.47	156.76 ± 105.71	<0.001
Fasting insulin (mU l^{-1})	8.57 ± 4.86	11.68 ± 6.90	15.63 ± 9.67	<0.001
Glycated hemoglobin	4.81 ± 0.39	4.96 ± 0.40	6.43 ± 2.33	<0.001
Cholesterol (mg per 100 ml)	204.85 ± 39.60	218.58 ± 35.95	200.89 ± 48.83	0.109
Triglycerides (mg per 100 ml)	108.99 (61–128)	118.44 (70–146)	209.21 (100.5–248)	<0.001
HDL cholesterol (mg per 100 ml)	52.13 ± 14.14	52.15 ± 10.26	44.30 ± 10.82	0.014
LDL cholesterol (mg per 100 ml)	131.42 ± 37.22	141.31 ± 32.83	119.60 ± 44.35	0.062
Systolic blood pressure (mm Hg)	125.58 ± 15.97	129.61 ± 14.00	137.42 ± 21.76	0.002
Diastolic blood pressure (mm Hg)	79.99 ± 10.57	82.35 ± 8.98	82.66 ± 18.53	0.380
LIGHT (pg ml^{-1})	40.77 (22.59–54.27)	32.44 (16.98–42.87)	52.07 (31.15–70.49)	0.001

(B)	*Lean*	*Overweight*	*Obese*	*Morbidly obese*	
N	40	80	50	20	
Age (years)	44.58 ± 10.97	52.18 ± 11.30	52.51 ± 12.28	44.45 ± 7.35	0.001
BMI (kg m^{-2})	23.02 ± 1.30	27.33 ± 1.43	33.41 ± 2.89	46.88 ± 5.18	<0.001
Glucose (mg per 100 ml)	102.62 ± 32.57	103.38 ± 58.47	105.07 ± 30.58	119.90 ± 59.39	0.748
Fasting insulin (mU l^{-1})	7.07 ± 5.87	8.95 ± 4.32	14.41 ± 7.62	18.50 ± 8.74	<0.001
Glycated hemoglobin	5.08 ± 1.12	5.00 ± 1.33	5.19 ± 0.87	5.57 ± 1.15	0.451
Cholesterol (mg per 100 ml)	195.70 ± 30.39	206.57 ± 46.51	214.95 ± 37.24	217.40 ± 38.47	0.113
Triglycerides (mg per 100 ml)	108.00 (52–103.5)	106.00 (59.50–131.50)	145.45 (86.50–183.25)	246.10 (112.5–257.25)	0.001
HDL cholesterol (mg per 100 ml)	53.99 ± 16.08	52.18 ± 11.86	48.90 ± 11.78	41.14 ± 12.17	0.019
LDL cholesterol (mg per 100 ml)	124.06 ± 28.71	131.70 ± 42.95	135.97 ± 34.53	143.40 ± 43.33	0.365
Systolic blood pressure (mm Hg)	120.50 ± 11.79	127.00 ± 17.17	135.89 ± 16.86	130.28 ± 22.76	<0.001
Diastolic blood pressure (mm Hg)	78.64 ± 9.62	77.95 ± 12.25	86.07 ± 10.95	87.42 ± 12.83	<0.001
LIGHT (pg ml^{-1})	41.21 (19.64–61.28)	38.83 (24.88–53.74)	39.31 (22.25–49.96)	60.38 (42.19–83.48)	0.024

Abbreviations: BMI, body mass index; DM2, type 2 diabetic patients; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells; NFG, normal fasting glucose. Data are the means ± s.d. unless otherwise indicated. Parameters that did not fulfill normal distribution are shown as median (interquartile range).

nitrocellulose membranes by conventional procedures. Membranes were immunoblotted with phospho-NF-κB p65 (Ser536)(93H1), NF-κB p65 antibodies (Cell Signaling Technology, Inc., Danvers, MA, USA), HVEM (N-19) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). IgG secondary antibodies, coupled to horseradish peroxidase, were used. The horseradish peroxidase activity was detected by chemiluminescence and quantification of protein expression was performed using Scion Image software. The quantitative levels were normalized to that of β-actin and expressed as optical density.

Statistical analysis

Statistical analyses were performed using SPSS statistical package (version 13.0 for windows; SPSS Inc., Chicago, IL, USA). Descriptive results of continuous variables were expressed as means ± s.d. or median (first–third quartile). Before statistical analyses, normal distribution and homogeneity of the variables were tested. Parameters that did not fulfill normal distribution were log transformed for subsequent analyses. The relation between variables were tested using Pearson's test and stepwise multiple linear regression analysis. Levels of statistical significance were set at $P<0.05$.

For culture experiments, the values were expressed as the mean ± standard error of mean (s.e.m.) of three independent experiments. Each experiment was carried out in triplicate using adipocytes from different human subjects. Student's *t*-tests were used to assess the statistical significance of the differences, with *P*-values of less than 0.05 considered statistically significant.

Results

LIGHT was increased in morbidly obese and type 2 diabetic patients

The anthropometric and clinical characteristics of subjects are shown in Table 1 and the correlation of LIGHT with clinical and biochemical variables are shown in Table 2. LIGHT was statistically increased in morbidly obese subjects (BMI >40, $P=0.025$, $n=20$, Table 1B, Figure 1a) and in type 2 diabetic subjects ($P=0.045$, $n=31$, Figure 1b).

In obese subjects (Table 2B), serum LIGHT correlated positively with BMI ($r=0.423$, $P=0.001$, Figure 1c), triglycerides ($r=0.319$, $P=0.008$, Figure 1d), fat mass ($r=0.307$, $P=0.017$) and glycated hemoglobin ($r=0.257$, $P=0.034$) and negatively with age ($r=-0.384$, $P=0.001$) and high-density lipoprotein cholesterol ($r=-0.333$, $P=0.006$), whereas no significant associations were found in non-obese subjects.

Table 2 Correlations between LIGHT concentrations and selected variables

	r	P-*value*
Lean subjects (BMI < 30) (n = 120)		
Age	−0.071	0.441
Weight (kg)	0.055	0.554
BMI (kg m^{-2})	0.014	0.880
Fat mass (%)	−0.050	0.620
Cholesterol (mg per 100 ml)	−0.105	0.260
HDL cholesterol (mg per 100 ml)	−0.142	0.125
LDL cholesterol (mg per 100 ml)	−0.072	0.443
Triglycerides (mg per 100 ml)	0.011	0.906
Glycated hemoglobin (mg per 100 ml)	0.103	0.267
Obese subjects (BMI > 30) (n = 70)		
Age	−0.384	**0.001**
Weight (kg)	0.358	**0.002**
BMI (kg m^{-2})	0.423	**0.001**
Fat mass (%)	0.307	**0.017**
Cholesterol (mg per 100 ml)	0.003	0.984
HDL cholesterol (mg per 100 ml)	−0.333	**0.006**
LDL cholesterol (mg per 100 ml)	0.017	0.894
Triglycerides (mg per 100 ml)	0.319	**0.008**
Glycated hemoglobin (mg per 100 ml)	0.257	**0.034**

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells. Bold values signify $P<0.05$

We performed a multiple linear regression analysis to predict circulating LIGHT (Table 3). BMI ($P=0.001$) and age ($P=0.003$) contributed to 31% of LIGHT variance among obese subjects. In addition, in obese subjects, LIGHT contributed to 10% of the variance in fasting triglycerides, even after adjusting for BMI and age.

Pro-inflammatory effect of LIGHT on human differentiated adipocytes

In order to analyze whether LIGHT was implicated in the inflammatory status of obesity, human differentiated adipocytes were treated with growing concentrations of LIGHT for 24 h and the levels of secreted cytokines and their expression patterns were studied.

GRO, GRO-α, IL-8, MCP-1, IL-6, GM-CSF, MCP-2 and RANTES were spontaneously secreted from human visceral differentiated adipocytes. After LIGHT treatment, IL-3, IL-1α, IL-2, IL-7, IL-13, IL-15, MCP-3, MIG, TNF-α, TNF-β, IL-10, transforming growth factor-β1 and interferon-γ increased significantly. The secretions of IL-5 and GCSF were not detected (Table 4 and Supplementary Figure 1).

It should be noted that the pro-inflammatory cytokines, GRO, IL-8 and MCP-1, increased in a dose-dependent manner after LIGHT treatment, whereas IL-6 and GRO-α were significantly higher only after 1000 ng ml^{-1}. In contrast, adiponectin secretion, tested using enzyme-linked immunosorbent assay, tended to decrease gradually (Table 4 and Supplementary Figure 1).

To further confirm the results obtained, IL-6, IL-8, MCP-1 and adiponectin gene expressions were also analyzed using real-time PCR. The data obtained showed that the expression of the studied genes showed a behavior similar to their secretion. MCP-1 mRNA increased in a dose-dependent manner and IL-6 and IL-8 expressions were statistically higher only after 1000 ng ml^{-1} of LIGHT. Again, adiponectin expression tended to decrease (Figure 2a).

LIGHT affects PPAR-γ and FASN gene expression in human differentiated adipocytes

We analyzed the expression of PPAR-γ and FASN in human differentiated adipocytes treated with growing concentrations of LIGHT for 24 h. LIGHT downregulated the expression of both genes in a dose-dependent manner and their levels were significantly reduced after 1000 ng ml^{-1} of LIGHT (Figure 2b).

Study of LIGHT in human adipose tissue and isolated cells

LIGHT protein and mRNA were analyzed in human adipose tissue explants, in isolated human stromal-vascular cells and mature adipocytes and in pre-adipocytes during differentiation. Secretion and expression of LIGHT was detected in human adipose tissue explants and in the stromal-vascular cell fraction but not in human pre-adipocytes during differentiation or mature adipocytes (Figure 3a).

LIGHT upregulated HVEM expression in human differentiated adipocytes

After observing that cultured human adipocytes expressed HVEM, we analyzed their expression under different concentrations of LIGHT. The results showed that LIGHT increased the expression of its own receptor HVEM, in a statistically significant dose-dependent manner (Figure 3b). In addition, the relatively low levels of HVEM receptor in cultured adipocytes increased after LIGHT treatment (Figure 3c).

LIGHT activates NF-κB signalling

To test whether LIGHT pro-inflammatory effects were due to NF-κB signalling, we studied the phosphorylation of NF-κBp65 on Ser536. As expected, LIGHT induced the phosphorylation of NF-κB at all tested concentrations (Figure 3d).

Discussion

Current findings suggest a possible role of LIGHT on hypertriglyceridemia of obese subjects and increased cytokine secretion by human adipose tissue. LIGHT was associated with hyperlipidemia, showing a positive association with triglycerides and a negative correlation with high-density lipoprotein cholesterol. Importantly, LIGHT contributed to 10% of the variance in fasting triglycerides, even after adjusting for BMI and age. These results are in

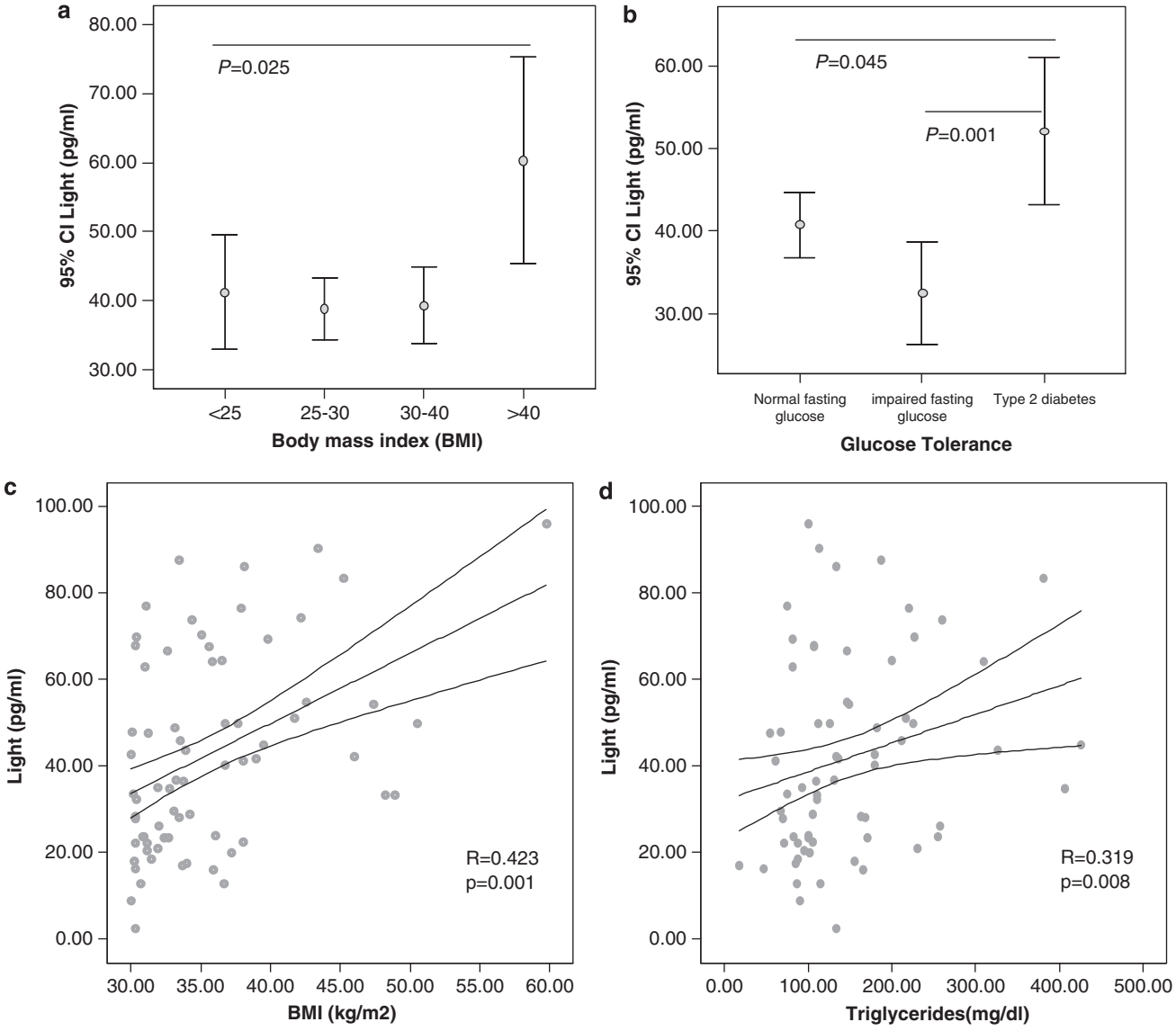

Figure 1 Serum LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells) concentrations. Error-bar plots of LIGHT according to (**a**) body mass index (BMI) and (**b**) glucose tolerance. Linear correlation association between (**c**) LIGHT and BMI and between (**d**) LIGHT and triglycerides in obese subjects. *$P<0.05$ from linear-trend analysis of variance (ANOVA).

agreement with Lo *et al*.[25] who, using transgenic mice overexpressing LIGHT, showed that this cytokine directly influenced lipid metabolism by increasing triglycerides and cholesterol.

It is widely known that lipid homeostasis is mainly regulated by the liver, and a complex interaction between LIGHT and liver cells has been previously suggested.[25,28] However, according to current findings, LIGHT could also play a role within the environment of the adipose tissue, according to current findings: (1) The association of LIGHT with adiposity measures (BMI and percent fat mass). These positive correlations suggest an upregulation of LIGHT with obesity. (2) LIGHT was produced within adipose tissue by the non-adipocyte component, probably by activated T cells, monocytes, granulocytes and immature dendritic cells as previously described by others.[3–5] LIGHT was not expressed or secreted by human adipose cells. (3) The presence of the LIGHT receptor (HVEM) in adipose tissue. (4) The LIGHT-induced downregulated expression of the adipogenic genes PPAR-γ and FASN in human differentiated adipocytes.

Given these results, it is tempting to speculate that LIGHT may affect lipogenesis, leading to decreased storage of fatty acids and increased circulating fatty acid concentrations. These fatty acids would have direct access to the liver through the portal venous system, interfering with liver metabolism and finally leading to hypertriglyceridemia.

Unfortunately, the effects of LIGHT on lipolytic genes or lipolytic parameters were not evaluated and further studies are needed to corroborate the proposed function. It is interesting to note that both adipose tissue and the liver have an architectural organization in which metabolic cells (adipocytes or hepatocytes) are in close proximity to immune cells (Kupffer cells or macrophages) and both have immediate access to a vast network of blood vessels. Thereby, all these data suggest that LIGHT might contribute directly to the complex regulation of lipid homeostasis. In fact, it is well known that TNF superfamily, in addition to being involved in a wide array of biological processes, particularly immune responses and tumor apoptosis,[29] is also implicated in lipid metabolism.[24]

Table 3 Multiple linear regression analysis with circulating LIGHT concentration and fasting triglycerides as dependent variables

	Non-obese subjects		*Obese subjects*	
	β	P-*value*	β	P-*value*
Circulating light				
BMI	−0.03	0.60	0.36	0.001
Age	−0.04	0.60	−0.33	0.003
Log triglycerides	−0.15	0.10	0.19	0.081
Adjusted R^2			**0.31**	
Fasting triglycerides				
BMI	0.06	0.50	0.06	0.6
Age	−0.02	0.89	−0.15	0.2
Light	−0.14	0.10	0.33	0.005
Adjusted R^2			**0.10**	

Abbreviations: BMI, body mass index; LIGHT, lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells. Bold values signify $P<0.05$

HVEM is the LIGHT receptor necessary to transduce the pro-inflammatory activity in adipocytes. A high expression of HVEM was detected in human pre-adipocytes and adipocytes in culture from obese subjects.[18] Both HVEM gene expression and protein levels were positively associated with LIGHT concentrations and increased in a dose-dependent manner in cultured differentiated adipocytes. Morel *et al*. provided evidence that HVEM expression was down-regulated by LIGHT stimulation on activated T cells.[30] However, increased expression of HVEM was observed in T cells from the LIGHT-transgenic mice,[10] indicating that the balanced expression of LIGHT and its receptor may be disrupted in several situations.

Among the multiple biological activities described, LIGHT is known to stimulate the expression and/or secretion of various molecules involved in the inflammatory response.[14,22,29,31] Given this background, we studied the secretion of 21 cytokines by human differentiated adipocytes under different LIGHT concentrations. The results showed that differentiated adipocytes from obese subjects spontaneously released pro-inflammatory cytokines

Table 4 Diagram showing the changes of cytokine secretion by human differentiated adipocytes incubated with 0, 10, 100 and 1000 ng ml^{-1} human LIGHT for 24 h

	LIGHT (ng/ml)			
	0	10	100	1000
Acrp30				
GM-CSF				
GRO				
GRO-α				
IL-1α				
IL-2				
IL-3				
IL-6				
IL-7				
IL-8				
IL-10				
IL-13				
IL-15				
IFN-δ				
MCP-1				
MCP-2				
MCP-3				
MIG				
RANTES				
TGF-β1				
TNF-α				
TNF-β				

Proteins present on cell-free supernatants of differentiated adipocytes are shown in light gray, and proteins in which secretion was not detected are in white. The increase in darkness show that the level of these cytokines was increased compared with the previous concentration. The results are the average of three independent experiments.

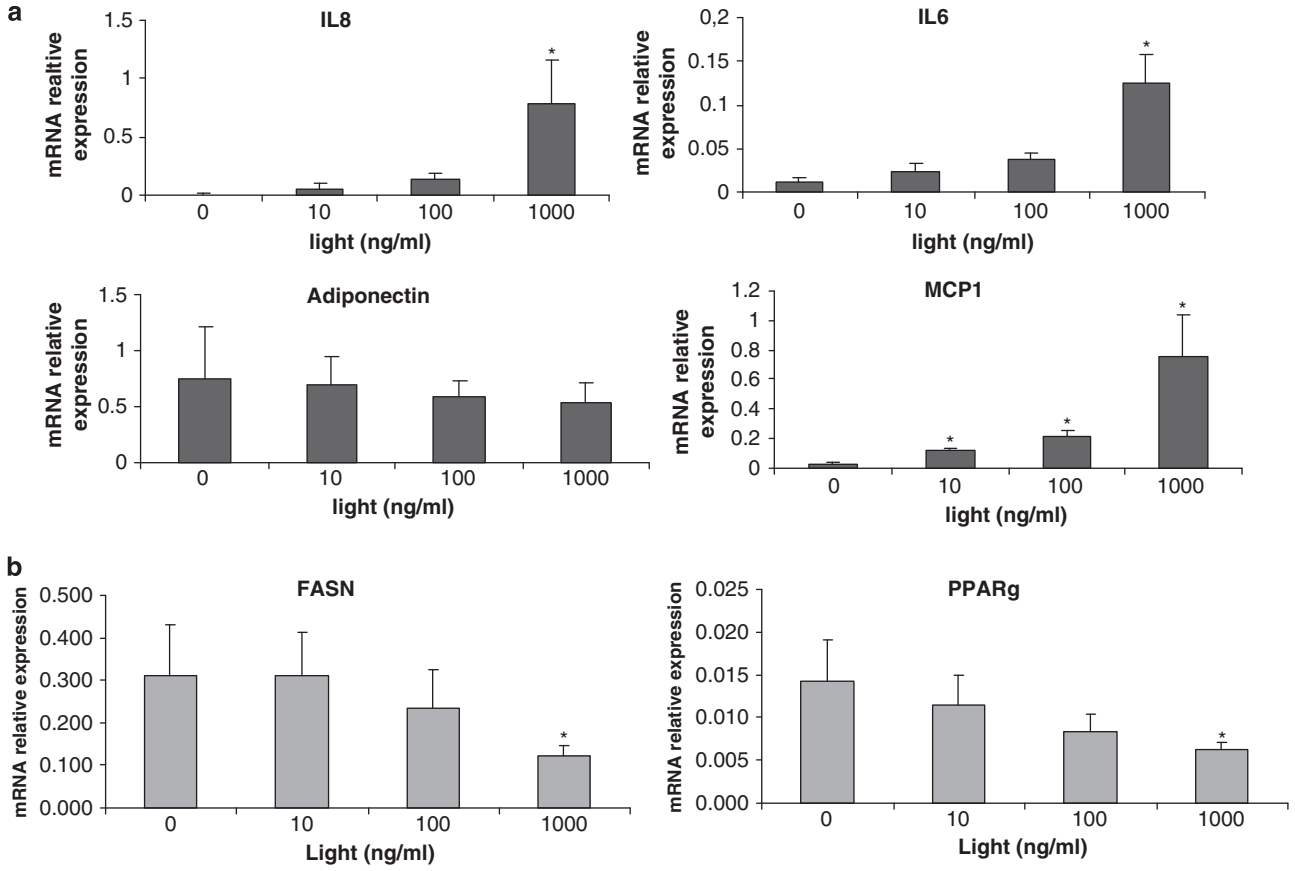

Figure 2 Effect of LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells) on mRNA expression. Real-time reverse transcriptase PCR (RT-PCR) of (**a**) genes coding for the cytokines, interleukin-8 (IL-8), IL-6, monocyte chemotactic protein-1 (MCP-1) and adiponectin, and (**b**) the genes involved in lipid metabolism peroxisome proliferator-activated receptor-γ (PPAR-γ) and fatty acid synthase (FASN) in human differentiated adipocytes treated with 0, 10, 100 and 1000 ng ml^{-1} of human LIGHT for 24 h. Relative mRNA levels were normalized to the levels of cyclophilin A. Data are means ± s.e.m. of three independent experiments. *$P<0.05$ versus 0 ng ml^{-1}.

(IL-6 and GM-CSF) and chemokines (GRO, GRO-α, IL-8, RANTES, MCP-1 and MCP-2) whose levels were increased after LIGHT (mainly GRO, GRO-α, IL-8, MCP-1 and IL-6). LIGHT also induced *de novo* secretion of some other cytokines, including IL-3, IL-1α, IL-2, IL-7, IL-13, IL-15, MCP-3, MIG, TNF-β, IL-10, transforming growth factor-β1, interferon-γ and TNF-α. Adiponectin expression and secretion tended to decrease.

LIGHT effects have been evaluated in other cell types. In activated monocytes, macrophages and neutrophils, LIGHT led to an increased expression of cytokines, such as IL-8 and TNF-α and matrix metalloproteinases, such as matrix metalloproteinase-9.[22,29,31] In Human umbilical vein endothelial cells, LIGHT induced the secretion of chemokines (IL-8 and GRO) and cell-surface expression of adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1).[14]

A few of the cytokines were significantly upregulated by LIGHT in adipocytes, and often 100–1000 ng ml^{-1} of LIGHT was required to produce a significant response. However, the adipocyte-derived cytokine production might still be relevant to LIGHT-induced pro-inflammatory status, given the upregulation of the LIGHT receptor (HVEM) with obesity. In addition, the magnitude of adipocyte response to LIGHT was only slightly lower compared with the other cell types present in adipose tissue, which could also contribute to the LIGHT-induced pro-inflammatory status.[22,32]

The potential role of LIGHT-HVEM in adipose tissue is unknown, and no data about the effect of LIGHT on isolated adipose cells have been previously reported. Our results showed that LIGHT led to phosphorylation of NF-κB in human differentiated adipocytes, which may lead to the transcription and secretion of some pro-inflammatory cytokines. In fact, HVEM has been described to interact with TNF receptor-associated factors 2 and 5, leading to activation of the NF-κB or the c-Jun N-terminal kinase/activator protein 1 pathway, and resulting in the transcription genes that promote cell survival, cytokine production or cell proliferation.[16]

Obesity affects both humoral and cellular immunity,[33] and is associated with an elevated leukocyte and lymphocyte subset counts, higher monocyte and granulocyte phagocytosis and oxidative burst activity.[28] The higher

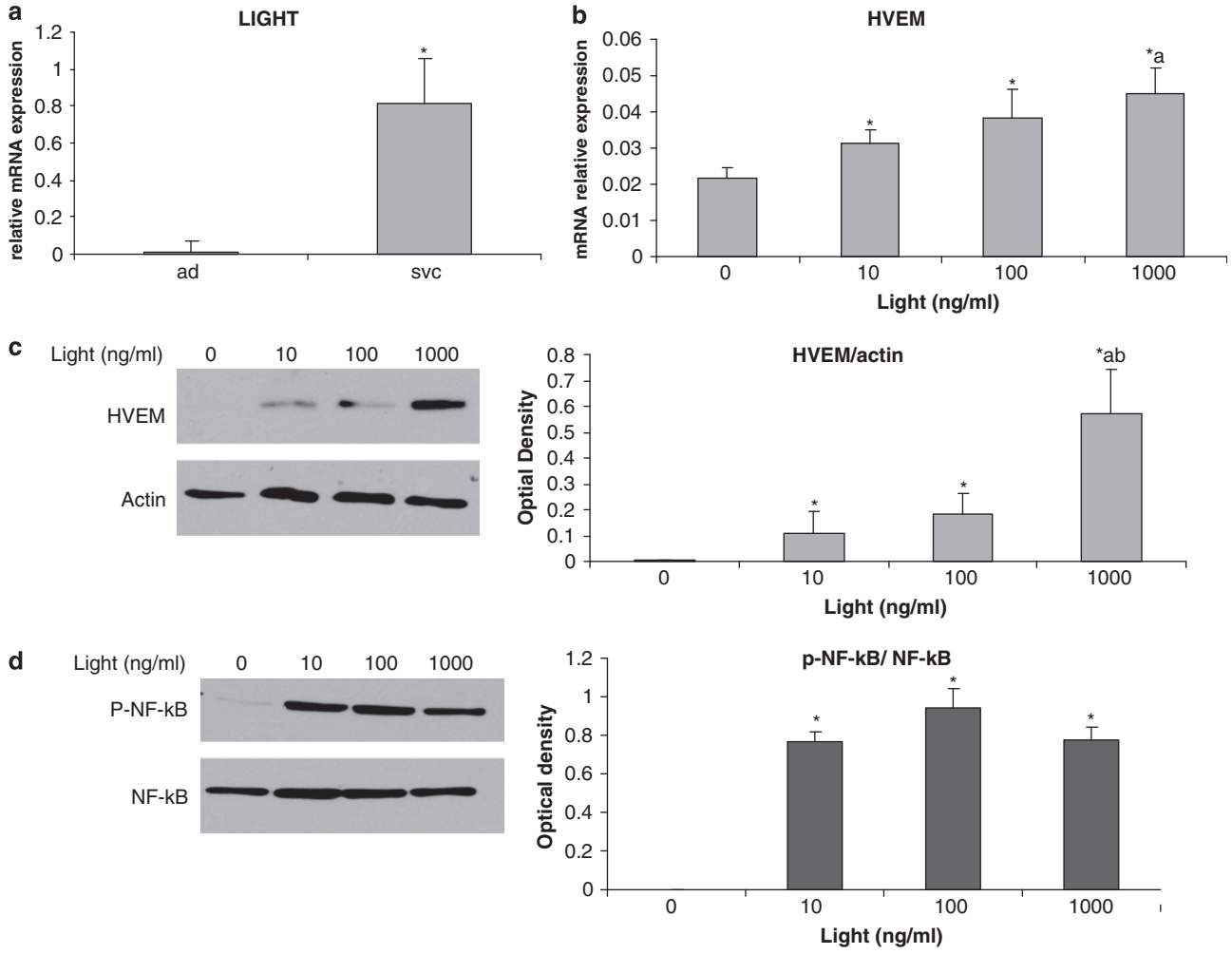

Figure 3 LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells) upregulates its own receptor, herpesvirus entry mediator (HVEM), and activates NF-κB signalling on human differentiated adipocytes. (**a**) Real-time reverse transcriptase PCR (RT-PCR) of LIGHT in mature adipocytes (AD) and stromal-vascular cells (SVCs) obtained by collagenase digestion of six human adipose tissue biopsies. (**b**) Real-time RT-PCR (**c**) and western blot analysis of HVEM in human differentiated adipocytes treated with 0, 10, 100 and 1000 ng ml^{-1} of human LIGHT for 24 h. Relative mRNA levels were normalized to the levels of cyclophilin A and densitometry analyses of protein were normalized to B-actin. (**d**) Western blot analysis of phospho-NF-κB p65 (Ser536) and total NF-κB p65. Data are means ± s.e.m. of three independent experiments. *$P<0.05$ versus 0 ng ml^{-1}, $^{a}P<0.05$ versus 10 ng ml^{-1} and $^{b}P<0.05$ versus 100 ng ml^{-1}.

number of lymphocytes in obese subjects could explain the increased LIGHT levels in these patients, which in turn could act upon adipocytes and have a role in the modulation of lipid metabolism. Similar links between cellular immunity and lipid homeostasis have been highlighted in atherosclerosis, in which T cells expressing LIGHT inhibited hepatic lipase expression in hepatocytes, leading to increased plasma lipoprotein concentrations, and contributing to the progression of the disease.[34]

The potent inflammatory effect of LIGHT expression may also reveal its importance for viral immunity. In particular, the presence of HVEM in adipose cells and their use by herpesviruses may not be fortuitous, and could point to adipose tissue as a target for tissue infection. The high levels of LIGHT in obese and type 2 diabetic patients, and the upregulation of HVEM in adipose cells, suggest that these subjects would be more vulnerable to chronic infection, as has been previously suggested.[35]

Finally, it is well known that obesity is a risk factor for the development of cardiovascular disease. Even a modest increase in body weight (BMI > 30 kg m^{-2}) results in a fourfold increase in the risk of cardiovascular disease in both men and women.[36] Obesity contributes to heart disease primarily through obesity-related covariates such as hypertension, dyslipidemia and impaired glucose tolerance or type 2 diabetes. Given the high levels of LIGHT in morbidly obese and type 2 diabetic patients, as well as the positive correlations of LIGHT with serum lipid levels, it is tempting to speculate a relationship between LIGHT and increased cardiovascular risk.

In summary, this study points to a complex interaction between LIGHT, mainly secreted by T cells, and adipose

tissue. Our results give evidence that, in addition to the pro-inflammatory effect of LIGHT described in monocytes, macrophages and neutrophils,[22,29,31] LIGHT also induces several adipose-driven pro-inflammatory cytokines and downregulates the expression of several genes involved in lipid metabolism. The combined presence of high levels of LIGHT in morbidly obese and type 2 diabetic patients, the positive correlations with serum lipid levels and the presence of HVEM receptor in adipose tissue of obese subjects may constitute additional factors that can contribute to aggravate the inflammatory state and dyslipidemia observed in these diseases and suggest a possible relationship between LIGHT and increased cardiovascular risk.

Conflict of interest

The authors declare no conflict of interest.

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