0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(3):1009–1018

doi: 10.1210/en.2006-0975

A Growth Hormone-Releasing Peptide Promotes Mitochondrial Biogenesis and a Fat Burning-Like Phenotype through Scavenger Receptor CD36 in White Adipocytes

Amélie Rodrigue-Way, Annie Demers, Huy Ong, and André Tremblay

Research Center (A.R.-W., A.D., A.T.), Ste-Justine Hospital, and Departments of Biochemistry (A.R.-W., A.T.) and Obstetrics and Gynecology (A.T.), Faculty of Medicine, University of Montréal, Montréal, Québec, Canada H3T 1C5; and Faculty of Pharmacy (A.D., H.O.), Pavillon Jean-Coutu, University of Montréal, Montréal, Québec, Canada H3C 3J7

Whereas the uptake of oxidized lipoproteins by scavenger receptor CD36 in macrophages has been associated with foam cell formation and atherogenesis, little is known about the role of CD36 in regulating lipid metabolism in adipocytes. Here we report that treatment of 3T3-L1 adipocytes with hexarelin, a GH-releasing peptide that interacts with CD36, resulted in a depletion of intracellular lipid content with no significant change in CD36 expression. Microarray analysis revealed an increased pattern in several genes involved in fatty acid mobilization toward the mitochondrial oxidative phosphorylation process in response to hexarelin. Interestingly, many of these up-regulated genes are known targets of peroxisomal proliferator-activated receptor (PPAR)-γ, such as FATP, CPT-1, and F_1-ATPase, suggesting that adipocyte response to hexarelin may involve PPARγ activation. Expression studies also indicate an increase in thermogenic markers PPARγ coactivator 1α and uncoupling protein-1, which are normally expressed in brown adipocytes. Electron microscopy of hexarelin-treated 3T3-L1 adipocytes showed an intense and highly organized cristae formation that spans the entire width of mitochondria, compared with untreated cells, and cytochrome c oxidase activity was enhanced by hexarelin, two features characteristic of highly oxidative tissues. A similar mitochondrial phenotype was detected in epididymal white fat of mice treated with hexarelin, along with an increased expression of thermogenic markers that was lost in treated CD36-null mice, suggesting that the ability of hexarelin to promote a brown fat-like phenotype also occurs *in vivo* and is dependent on CD36. These results provide a potential role for CD36 to impact the overall metabolic activity of fat usage and mitochondrial biogenesis in adipocytes. (*Endocrinology* 148: 1009–1018, 2007)

THE MAJOR ROLE of the adipose tissue is to store energy in the form of triglycerides and release it as fatty acids in response to an increase in energy demand, such as during fasting or exercise. Peripheral tissues such as skeletal muscle and heart oxidize fatty acids in mitochondria to produce ATP. However, when energy storage is in excess, such lipid accumulation in adipose tissue can result in many pathological states associated with the metabolic syndrome, including central obesity, type 2 diabetes, and insulin resistance (1, 2).

The scavenger receptor CD36, also known as fatty acid translocase, is expressed in adipocytes to mediate the uptake of long chain fatty acids (3), but much of the characterization of the role of CD36 has focused on its scavenging ability to interact and mediate the internalization of oxidized low-density lipoproteins (oxLDL) in macrophages. The selective uptake of oxLDL by CD36 is considered a critical step in the atherogenic formation of foam cells in the extracellular matrix of lesion-prone sites of the arterial wall (4). In addition to initiating a proinflammatory response by monocytes/macrophages, such internalization of oxLDL by CD36 provides a source of oxidized fatty acids and oxysterols that serve as endogenous ligands for the activation of the nuclear receptors peroxisomal proliferator-activated receptor (PPAR)-γ and liver X receptor (LXR)-α, and subsequent up-regulation of downstream targets involved in reverse cholesterol transport, such as ATP-binding cassette transporters ABCA1 and ABCG1, and apolipoprotein E (5, 6).

Our recent work has identified hexarelin and other analogs of the GH-releasing peptide (GHRP) family as high affinity ligands of CD36 (7, 8). GHRPs were originally described to stimulate central GH release through binding of the GH secretagogue-receptor-1a, a G protein-coupled receptor later defined as the receptor for ghrelin and expressed predominantly in the hypothalamic-pituitary region (9, 10). In recent studies, we observed that GHRPs markedly decreased plaque formation in a mouse model of atherosclerosis, an effect that was shown to require CD36 expression (11, 12). These beneficial effects of GHRPs on cholesterol metabolism were dependent on PPARγ and the activation of the PPARγ-LXRα-ABC metabolic cascade in macrophages,

First Published Online November 30, 2006

Abbreviations: ABC, ATP-binding cassette transporter; aP2, adipocyte-specific fatty acid binding protein; COX, cytochrome c oxidase; CPT, carnitine palmitoyltransferase; FATP, fatty acid transport protein; FBS, fetal bovine serum; GPAT, glycerol-3-phosphate acyltransferase; GHRP, GH-releasing peptide; LXR, liver X receptor; oxLDL, oxidized low-density lipoproteins; PGC-1, PPAR coactivator 1; PPAR, peroxisome proliferator-activated receptor; TIM, translocase of the inner membrane; UCP, uncoupling protein.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

leading to cholesterol efflux into the high-density lipoprotein reverse pathway (12).

Whereas the role of CD36 in mediating cholesterol and fatty acid uptake by macrophages is well characterized, little is known about how CD36 may impact the overall metabolic activity of fat storage and mobilization by adipocytes. Here we report on the changes in expression of genes related to fatty acid import and oxidation as well as in morphological changes of mitochondria in adipocytes induced by GHRP hexarelin. The resulting increase in expression of F_1-ATP synthase, coactivator PPARγ coactivator (PGC)-1α and uncoupling protein (UCP)-1, all normally found in brown adipocytes, suggests that fatty acids are ushered toward mitochondria oxidative phosphorylation and biogenesis, rather than being converted to triglycerides for their subsequent storage in lipid vesicles. The overall effect is a decrease in total lipid content in fat cells, which provides a functional role of CD36 to modulate fatty acid metabolism and mitochondrial functions.

Materials and Methods

Cell culture and treatments

Mouse 3T3-L1 preadipocytes were grown in DMEM supplemented with 10% fetal calf serum. Two days after confluence (d 0), adipocyte differentiation was initiated with the addition of 115 μg/ml 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 0.167 μM insulin in DMEM supplemented with 10% fetal bovine serum (FBS) for 2 d. On d 2, the media was replaced with DMEM/10% FBS containing insulin for 2 more d and then maintained in DMEM/10% FBS until d 8. Treatments with hexarelin (10^{-7} to 10^{-5} M) and troglitazone (8 μM) were done for 48–72 h with fresh medium replacement at intervals of 24 h.

Lipid staining

3T3-L1 cells were fixed with 3.7% formaldehyde/PBS and stained with oil red O (Sigma, St. Louis, MO). Quantification of lipid accumulation was achieved by extracting oil red O from stained cells with isopropyl alcohol and measuring the OD of the extracts at 510 nm.

Triglyceride measurement

Lipids from differentiated 3T3-L1 adipocytes were extracted with Folch solution consisting of a mixture of 2:1 (vol/vol) chloroform/methanol and resuspended in 20% Thesit (Sigma) in Folch solution before evaporation under nitrogen gas. Triglyceride content was determined using a colorimetric assay kit (Zen-bio, Research Triangle Park, NC) and normalized against total protein from each sample determined by Bradford reagent (Sigma).

Microarray analysis

Differentiated 3T3-L1 adipocytes were treated with 10^{-5} M hexarelin or 8 μM troglitazone for 48 h. Total RNA was isolated from 3T3-L1 cells using TRIzol reagent (Invitrogen, Burlington, Ontario, Canada), according to the manufacturer's protocol. Biotinylated cRNA was generated from 10 μg of total RNA, and hybridized onto mouse 430.2 oligonucleotide arrays. All procedures were followed according to Affymetrix protocols (Santa Clara, CA). Data were analyzed and compared with a second set of hybridization experiments using the Gene-Chip analysis suite software (Affymetrix) and representative results were generated with TM4 software (TiGR, The Institute for Genomic Research, Rockville, MD).

RT-PCR analysis

3T3-L1 cells were treated as above and cDNA was synthesized from 400 ng of total RNA using oligo(dT) primers and RevertAid H minus M-MuLV reverse transcriptase (Fermentas, Burlington, Ontario, Canada). PCR amplification was usually performed in a volume of 20 μl with 0.5–1 μl of reverse transcription reaction for 25–35 cycles. Sequences of the murine primers used in PCR are available upon request. The PCR products were separated on a 2% agarose gel, stained with ethidium bromide, and the relative signal intensity was analyzed (Alpha Innotech, San Leandro, CA) from at least three separate experiments.

Cytochrome c oxidase (COX) activity

COX activity was measured on isolated mitochondria from treated and untreated 3T3-L1 adipocytes. Briefly, adipocytes were collected and resuspended in mitochondrial buffer [0.2 mM EDTA, 0.25 M sucrose, and 0.1 mg/ml digitonin in 10 mM Tris (pH 7.8)]. Cells were ruptured using a glass-Teflon Potter-Elvehjem homogenizer, and the homogenates were centrifuged at 1000 × *g* for 10 min. Mitochondria were then pelleted at 12,000 × *g* spin for 15 min and resuspended in mitochondrial buffer supplemented with protease inhibitor cocktail (Roche, Laval, Québec, Canada). Protein content was determined by the Bradford method (Bio-Rad, Mississauga, Ontario, Canada). COX activity was determined from 10 μg of mitochondrial proteins from each treatment according to the manufacturer's protocol (Sigma). The activity was calculated from the rate of decrease in absorbance of ferrocytochrome c at 550 nm ($\varepsilon = 21.84$ $mM^{-1}cm^{-1}$), added to the assay at a final concentration of 10 μM, and represented as milliunits per milligram of protein per minute where 1 U is the amount of enzyme needed to oxidize 1 μmol of ferrocytochrome c per minute (pH 7.0) at room temperature. To assure total permeabilization of mitochondrial membrane, the assay was performed in the presence of 2.5 mM n-dodecyl β-D-maltoside (Sigma). No significant COX activity was detected in the 12,000 × *g* spin supernatants.

Fluorescence microscopy

Eight-day differentiated adipocytes seeded in Lab-Tek coverglass chambers (Nalge Nunc, Rochester, NY) were treated for 72 h with either hexarelin or troglitazone. Live cells are then rinsed with PBS and labeled at 37 C for 15 min with 1 mg/ml rhodamine-123, a mitochondrial-specific fluorochrome (Sigma), as described by the manufacturer. Mitochondria are visualized by fluorescence microscopy (TE-2000; Nikon, Melville, NY) with an excitation at 488 nm and emission at 525 nm. Photobleaching is reduced with 1 mg/ml ascorbic acid.

Antibodies and immunoblotting analysis

Antibodies to PPARγ, ATP synthase (F_1 subunit), PGC-1α, adipocyte-specific fatty acid binding protein (aP2) and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and the anti-UCP1 and -UCP2 antibodies were purchased from Calbiochem (San Diego, CA). The antibody against CD36 has been described (8). Immunoblotting analysis was performed as described (13). Briefly, cells were lysed in PBS buffer containing 1% Triton X-100, 0.5% deoxycholate acid, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors (Roche). Proteins were then resolved by SDS-PAGE and transferred to nitrocellulose for immunoblotting. Membranes were blocked at 4 C with blocking reagent (Roche) in Tris-buffered saline, probed with selected antibodies, and signals revealed by enhanced chemiluminescence using appropriate horseradish peroxidase-conjuguated secondary antibodies. For fat tissue, proteins were isolated using Trizol standard procedure and resuspended in 1% sodium dodecyl sulfate for immunoblot analysis.

In vivo experiments

Wild-type C57BL/6 and CD36-deficient mice were previously described (11) and maintained in a 12-h dark, 12-h light cycle with a standard pelleted diet and water *ad libitum*. At 12 wk of age, male mice were fed a 60% kcal/60% fat diet (Research Diets Inc., New Brunswick, NJ) and treated with sc injection of 100 μg/kg·d hexarelin, a dose known not to promote GH release (14), or 0.9% NaCl (control) for 12 wk, as previously described (12). Fat tissues were collected from the epididymal fat pads of control and treated mice and rapidly frozen at −80 C. All experimental procedures were done in accordance with the Institutional Animal Ethics Committee of the University of Montreal and the

Canadian Council on Animal Care guidelines for use of experimental animals.

Electron microscopy

3T3-L1 cells and mouse fat tissue were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). Samples were postfixed with 4% OsO_4 and dehydrated with ethanol. Before sectioning, tissues were embedded in epoxide resin (Epon 812; Sigma). Ultrathin cryosections were collected on metal grids and poststained with electron-dense uranyl acetate and lead citrate solutions and electron micrographs were recorded with an electron microscope (model 208S; Philips Medical Systems, Andover, MA). Mitochondria size and cristae formation were determined using an image analyzer (Alpha Innotech).

Results

CD36 ligand hexarelin decreases total lipid content in mature 3T3-L1 adipocytes

Our recent studies using cultured THP-1 macrophages have shown that hexarelin caused a significant decrease in total lipid accumulation via CD36, resulting in an augmentation of cholesterol efflux from cells (11, 12). Because adipocytes are known to express CD36 and not the other known hexarelin receptor-1a (Refs. 15 and 16 and data not shown), we evaluated the effect of hexarelin on lipid content in cultured 3T3-L1 adipocytes. 3T3-L1 cells were differentiated to mature adipocytes for 8 d with insulin/dexamethasone/3-isobutyl-1-methylxanthine, and treated with 10^{-7} and 10^{-5} M hexarelin for 48 h with a media change at 24 h. After treatment with hexarelin, a marked decrease in total cellular lipid and in the size of the lipid droplets was observed, compared with untreated cells (Fig. 1A). Whereas differentiation of 3T3-L1 preadipocytes into adipocytes resulted in a strong accumulation of lipids in vesicles, mature adipocytes treated with hexarelin showed a significant decrease in total lipid amount, compared with untreated cells (Fig. 1B). Such decrease was comparable with cells treated with troglitazone, a specific PPARγ ligand known to deplete lipid content in adipocytes (17). The decrease in lipid staining is associated with a significant reduction in intracellular triglyceride levels in adipocytes treated with hexarelin (Fig. 1C).

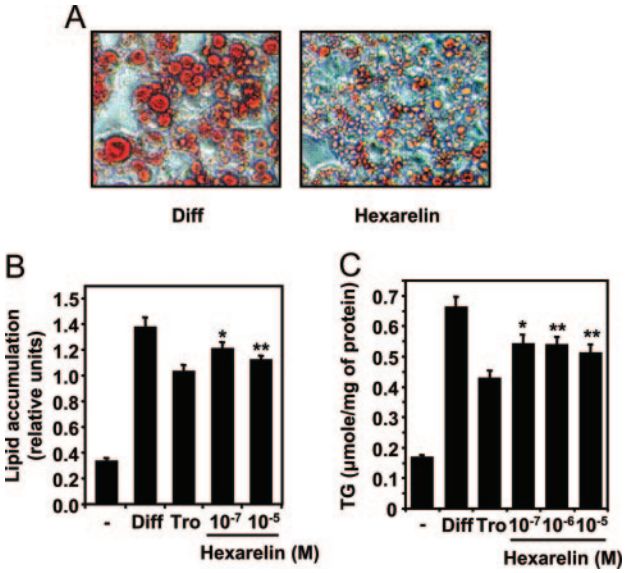

FIG. 1. Hexarelin reduced lipid content in mouse 3T3-L1 adipocytes. A, Representative images of differentiated 3T3-L1 adipocytes untreated (Diff) or treated with 10^{-5} M hexarelin for 48 h. Lipids were stained with oil red O and examined by microscopy. B, Photometric measurement of lipids stained with oil red O from undifferentiated (−) or differentiated 3T3-L1 cells treated with hexarelin or troglitazone (Tro) or left untreated (Diff). Data are presented as mean ± SEM of at least six separate experiments. C, Intracellular triglyceride content in 3T3-L1 adipocytes treated with hexarelin or troglitazone, compared with untreated cells as in A. Data are presented as mean ± SEM of at least six separate experiments. *, $P < 0.05$ and **, $P < 0.01$ *vs.* untreated differentiated cells.

Microarray analysis of genes regulated by hexarelin in 3T3-L1 adipocytes

To address the overall effect of hexarelin on genes involved in lipid metabolism in adipocytes, we performed microarray analysis on differentiated 3T3-L1 adipocytes treated with hexarelin and compared the expression profile with cells treated with troglitazone, relative to untreated cells. Total RNA was harvested from each sample treatment and probed against Affymetrix mouse 430.2 oligonucleotide chip. Probe sets that were identified as absent calls across all samples and experiments were removed from analysis. The relative gene expression levels in each treated sample were compared with untreated controls to determine significant changes. Selected genes were listed according to their known function (Fig. 2A).

Of the entire probe sets analyzed, 1119 were up-regulated in cells treated with hexarelin, suggesting that interaction with CD36 induces profound changes in the expression profile of adipocytes. Interestingly, many of these genes were shared with troglitazone treatment, indicating that PPARγ may be considered as a common regulator in both responses. Consistent with this, among the genes up-regulated by hexarelin, we found many established PPARγ targets, such as nuclear receptor LXRα, fatty acid transport protein (FATP)-1, and ATP synthase (Fig. 2A). However, the response to hexarelin was not totally mimicked by troglitazone as other described PPARγ targets, such as adipocyte fatty acid binding protein-4 (also referred to as aP2), and lipid droplet-associated protein adipophilin remained mostly unchanged upon treatment with hexarelin (Fig. 2, A–C). In addition, troglitazone treatment led to a decrease in PPARγ expression in adipocytes (0.6-fold in protein levels, compared with untreated cells), a finding also observed by others (18), whereas hexarelin did not significantly modify PPARγ expression (Fig. 2). We also reported a similar response in PPARγ expression in macrophages, indicating that this regulation is not cell specific (12).

Given the decrease in triglyceride content in cells treated with hexarelin (Fig. 1), we looked at several genes involved in various aspects of fatty acid metabolism including those involved in entry, transport, synthesis, and mobilization. Of those, hormone-sensitive lipase, GDSL motif-containing lipase, fatty acid synthase, acetyl-CoA synthase, and FATP1 were all up-regulated by hexarelin (Fig. 2A). In contrast, glycerol-3-phosphate acyltransferase (GPAT), which catalyzes the initial and committing step in glycerolipid biosynthesis, was down-regulated by hexarelin. This type of profile

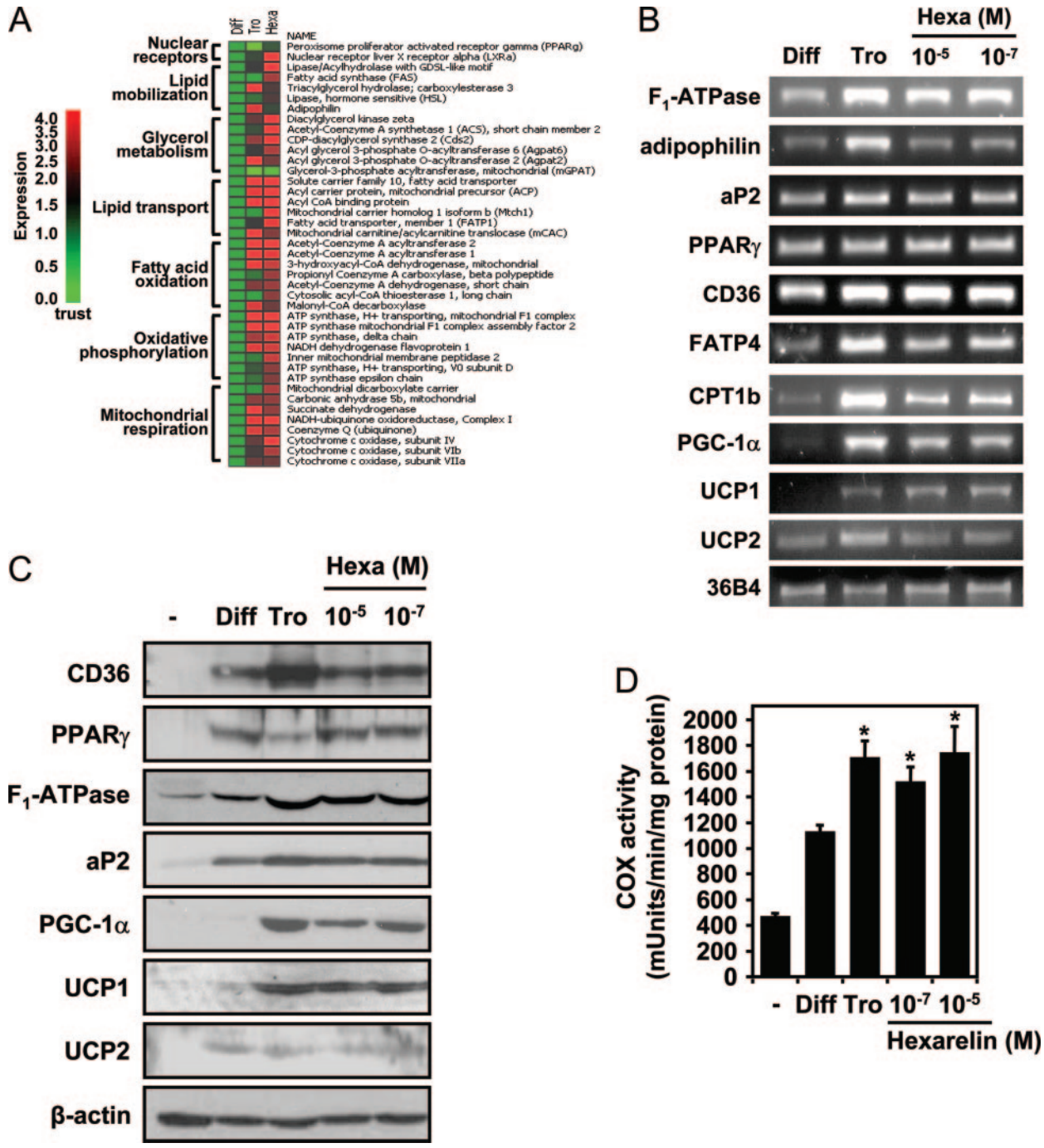

FIG. 2. Hexarelin induced the expression of genes associated with fatty acid oxidation and brown adipocyte phenotype. A, DNA microarray analysis of differentiated 3T3-L1 adipocytes treated with troglitazone, or hexarelin. Shown are selected PPARγ target genes and genes associated with fatty acid metabolism. Results are presented as fold changes compared with control cells set at 1.0. B, RT-PCR analysis of selected markers from differentiated 3T3-L1 cells treated with troglitazone (Tro) or hexarelin (Hexa) or left untreated for 48 h before RNA isolation. Representative images are shown from at least three separate experiments. 36B4 expression was used to normalize samples. C, Western analysis of 3T3-L1 adipocytes treated as above. Samples were normalized for protein loading with β-actin. D, Hexarelin (Hexa) induces COX activity in 3T3-L1 adipocytes. Differentiated adipocytes were treated with hexarelin or troglitazone (Tro) or left untreated (Diff) for 72 h, and COX activity was measured on isolated mitochondria and normalized to protein content. Data are presented as mean ± SEM of at least six separate experiments. *, $P < 0.005$ *vs.* untreated differentiated cells.

is strongly suggestive of an increase in the cellular mobilization of free fatty acids in response to hexarelin.

Hexarelin up-regulates genes involved in fatty acid oxidation and oxidative phosphorylation

What seemed more striking from the microarray experiments was the expression changes of mitochondrial genes involved in fatty acid metabolism. Several genes required for fatty acid transport into mitochondria, such as mitochondrial acyl carrier protein, acyl-CoA binding protein, and carnitine/acylcarnitine carrier protein were up-regulated by hexarelin (Fig. 2A). Similarly, many genes involved in fatty acid oxidation and oxidative phosphorylation were also up-regulated by hexarelin, such as acetyl-CoA acyltransferase 1 and 2, hydroxyacyl-CoA dehydrogenase, and several subunits of the ATP synthase complex (Fig. 2, A and B). These changes may reflect an increased activity of mitochondrial processes toward oxidation of fatty acids and oxidative phosphorylation, two features closely related to mitochondrial thermogenic activity and biogenesis.

One of the key enzymes involved in β-oxidation of long-chain fatty acids for energy production is the carnitine palmitoyltransferase (CPT). The muscle isoform M-CPT I, also known as CPT1b, is not normally expressed in mouse adipose tissue (19). Interestingly, we observed by RT-PCR a strong induction of the expression of CPT1b in 3T3-L1 adipocytes treated with hexarelin and with troglitazone (Fig. 2B). Because mitochondrial proteins that process fatty acids through entry and oxidation in mitochondria are often associated with energy production, we next analyzed the expression of genes involved in ATP production. Mitochondrial F_1-ATPase is responsible for the synthesis of ATP during oxidative phosphorylation to generate energy. Interestingly, the expression of F_1-ATP synthase was increased by hexarelin to levels slightly lower then those obtained with troglitazone when compared with untreated cells (Fig. 2, B and C). Protein levels of F_1-ATPase were increased by 3.1- and 3.4-fold in response to, respectively, 10^{-7} and 10^{-5} M hexarelin, whereas troglitazone induced a 4.4-fold increase, compared with controls (Fig. 2C). These results correlate with the microarray data showing many of the catalytic subunits of F_1-ATPase up-regulated in response to hexarelin. These results therefore link the response of adipocytes to hexarelin with the production of energy.

Hexarelin promotes the expression of thermogenic markers in 3T3-L1 adipocytes

The increase we observed in the expression of genes involved in β-oxidation of fatty acids and oxidative phosphorylation in response to hexarelin suggests that these cells may generate more ATP. Several studies have shown that such metabolic needs for energy requires the PPARγ coactivator PGC-1, which by inducing the expression of UCP1, a biological uncoupler of mitochondrial oxidative phosphorylation, initiates a broad program of thermogenesis in brown fat and muscle tissues (20–24). Although both proteins are poorly expressed, if not absent in white adipocytes, we found a remarkable increase in the expression of PGC-1α and UCP1 in 3T3-L1 adipocytes treated with increasing doses of hexarelin (Fig. 2, B and C). Protein levels of PGC-1α and UCP1 reached, respectively, a 5.1- and 4.2-fold increase in response to 10^{-5} M hexarelin. Similar increases were also observed in cells treated with troglitazone, suggesting that the response to troglitazone and hexarelin may converge at some point with PPARγ activation. UCP2 was detected in 3T3-L1 adipocytes but was not substantially modulated by hexarelin.

Mitochondrial cytochrome c oxidase activity is increased by hexarelin in 3T3-L1 adipocytes

In view of the above results indicating a marked increase in genes related to energy production, we measured the activity of COX, which catalyzes the terminal and rate-limiting step of the energy-transducing respiratory chain in mitochondria leading to ATP production. We found that treatment of 3T3-L1 adipocytes with hexarelin for 72 h significantly induced COX activity in isolated mitochondria, compared with untreated cells (Fig. 2D). Similarly, COX activity was also augmented in response to troglitazone using the same conditions. These changes in COX activity are consistent with the increases in expression levels of subunits forming COX enzymatic complex and other components of the respiratory chain in cells treated with hexarelin (Fig. 2A) and therefore support the ability of hexarelin to induce mitochondrial activity in adipocytes.

Hexarelin induces ultrastructural changes indicative of increased mitochondrial activity and biogenesis

Expression of PGC-1α is known to stimulate mitochondrial energy-producing capacity and biogenesis in tissues with high oxidative potential, such as heart, muscle, and brown fat (24, 25). First, we determined whether the changes in mitochondrial gene expression correlated with changes in mitochondrial morphology by staining differentiated 3T3-L1 adipocytes with rhodamine-123, a nontoxic mitochondrial fluorescent dye. Mitochondria of untreated cells were seen as a dense interconnected reticular motif (Fig. 3A), a pattern also reported by others (26). However, when treated with hexarelin for 72 h, the mitochondrial appearance was remodeled into individual densely packed structures, highly similar to the mitochondrial shape observed in cells treated with troglitazone (Fig. 3A).

The ultrastructure of the mitochondria was further defined using electron microscopy. Mitochondria of 3T3-L1 adipocytes treated with hexarelin were characterized by an increase in size and intense formation of lamellar cristae, compared with untreated cells (Fig. 3B). In addition, the cristae membrane of mitochondria from cells treated with hexarelin was highly organized and linearly displayed across the entire width of the organelle, compared with controls. The average mitochondrial size and percentage of mitochondrial matrix occupied by cristae were calculated and showed that mitochondrial size was increased by more than 2-fold ($P < 0.001$), and the percentage of surface within the mitochondrial matrix occupied by cristae membrane increased from 32% to almost 45% ($P < 0.001$) in adipocytes treated with hexarelin, compared with control cells (Fig. 3, C and D). This particular phenotype depicts a condition to maximize the intramitochondrial spanning of cristae, a pattern highly characteristic

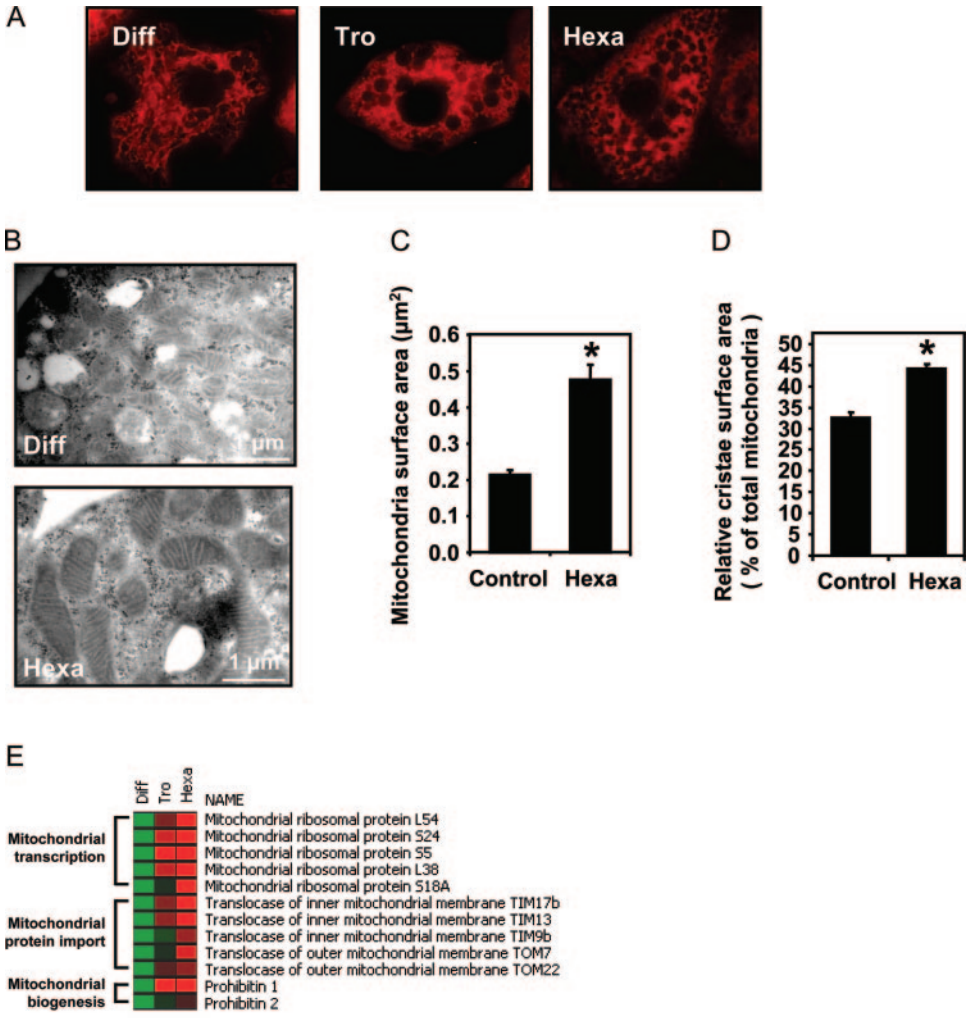

FIG. 3. Hexarelin induces morphological changes in mitochondrial ultrastructure. A, Representative images of 3T3-L1 adipocytes stained with mitochondria-specific rhodamine-123 dye. Cells were untreated (Diff) or treated with 10^{-5} M hexarelin (Hexa) or 8 μM troglitazone (Tro) for 72 h before staining and microscopic analysis. Magnification, ×100. B, 3T3-L1 cells were treated with 10^{-5} M hexarelin for 72 h or left untreated and visualized by electron microscopy. Representative images show an increase in mitochondrial size and cristae formation in response to hexarelin. C, Quantification of the average mitochondrial size in hexarelin-treated 3T3-L1 adipocytes, compared with untreated cells. Sizes are depicted as the mean of calculated surface area ± SEM of more than 70 mitochondria per group. *, $P < 0.001$. D, Relative surface area occupied by the cristae membrane within mitochondria expressed as % of total surface area. Data are presented as mean ± SEM of more than 25 mitochondria per group. *, $P < 0.001$. E, DNA microarray analysis of selected genes involved in mitochondrial biogenesis and found to be up-regulated by hexarelin, compared with untreated 3T3-L1 cells. Results with troglitazone are also shown. Fold changes are presented as in Fig. 2A.

of mitochondria from tissues with high energy production rate, such as brown adipose tissue, heart, and skeletal muscle (27).

Consistent with enhanced *de novo* mitochondrial synthesis, we found that hexarelin increased the expression of several translocases of the outer and inner membrane (TIM) of mitochondria (Fig. 3E). The translocases of the outer membrane and TIMs are responsible for the import of mitochondrial proteins encoded by the nuclear genome into the matrix and the intermembrane space of mitochondria (28). Among these family translocases, TIM17b reached a 3.8-fold increase in response to hexarelin, compared with control cells. Interestingly, TIM17b is ubiquitously expressed in humans and mice with a higher expression pattern in tissues with high oxidative potential, such as heart and skeletal muscle (29). Also up-regulated by hexarelin were several of the mitochondrial ribosomal proteins or MRPs involved in the translation of many proteins of the respiratory chain (30), indicating that mitochondrial transcription and translation was increased in response to hexarelin (Fig. 3E). In addition, both subunits of prohibitin, which form a large complex in the mitochondrial inner membrane to stabilize newly synthesized subunits of the respiratory chain (31), were up-regulated by hexarelin (Fig. 3E). It was reported that impaired function of these subunits resulted in a decreased number and mass of mitochondria and was associated with deficient mitochondrial biogenesis (32).

Induction of thermogenic markers and mitochondrial biogenesis by hexarelin occurs in vivo and is dependent on CD36

To address whether the phenotypic changes we observed in cultured adipocytes in response to hexarelin could also occur *in vivo*, we treated C57BL/6 mice with saline (control) or 100 μg/kg·d hexarelin for 12 wk. The concentration of hexarelin used in this study was reported not to elicit GH release and therefore prevented any undesired effects of GH (11, 12, 14). No adverse health problems were noticed throughout the treatment. The epididymal white fat was collected from treated mice and saline controls and analyzed by electron microscopy. Electronic images of fat tissues of hexarelin-treated mice showed an intense cristae formation in mitochondria, compared with controls, and more noticeably, the size in mitochondria was increased by 55% in these conditions (Fig. 4, A and B). In addition, we performed Western blot analysis on epididymal tissue that showed that protein levels of F_1-ATPase and thermogenic markers PGC-1α

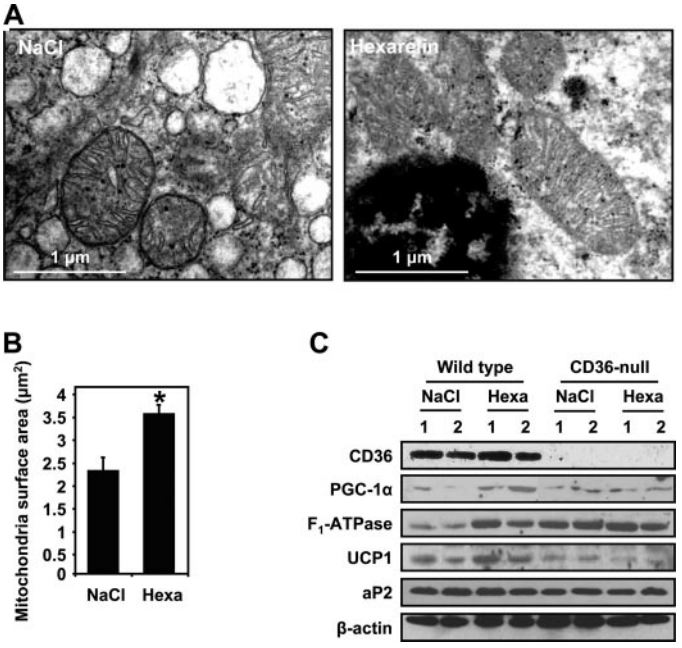

FIG. 4. Hexarelin induced expression of thermogenic markers and mitochondrial biogenesis *in vivo*. A, C57BL/6 mice were treated with hexarelin or saline (control) for 12 wk, and epididymal adipose tissue was analyzed by electron microscopy. Representative images from both samples are shown. B, Quantification of the average mitochondrial size in adipose tissue from mice treated as in A. Sizes are depicted as the mean of calculated surface area ± SEM of more than 50 mitochondria per group. *, $P < 0.001$. C, Western analysis of epididymal fat isolated from C57BL/6 wild-type and CD36-null mice treated with hexarelin (Hexa) or saline for 12 wk. Shown are samples obtained for each treatment from two separate experiments.

and UCP1 were increased in response to hexarelin (Fig. 4C). In contrast, there was no apparent changes in steady-state levels of these proteins in epididymal fat tissue derived from CD36-null mice treated as above with hexarelin, compared with saline-treated CD36-null mice (Fig. 4C). Invalidation of CD36 also induced an increase in F_1-ATPase levels in epididymal fat, whereas those of PGC-1α and UCP1 remained mostly unchanged. These results therefore suggest that the ability of hexarelin to promote mitochondrial metabolic changes in cultured adipocytes can be transposed *in vivo* and are dependent on CD36.

Discussion

One of the critical regulators of fatty acid metabolism in fat is PPARγ, which controls the expression of a broad range of genes involved in fatty acid and glucose uptake, β-oxidation, and lipid storage (33). Based on our previous reports that ligands of the GHRP family interact with scavenger receptor CD36 to promote PPARγ activation and downstream effects on cholesterol metabolism in macrophages (11, 12), we hypothesized that GHRP hexarelin might have an impact on adipocytes that express CD36. In this article, we described profound changes in the gene expression profile and mitochondria morphology in white fat cells treated with hexarelin correlating with a fat burning-like phenotype characteristic of brown adipocytes.

Interestingly, many of the genes up-regulated by hexarelin were shared with troglitazone treatment, indicating that PPARγ activation is likely to be involved in the response of adipocytes to hexarelin. Among the PPARγ target genes up-regulated by hexarelin, we found nuclear receptor LXRα, FATP1, FATP4, CPT1b, and F_1-ATP synthase. Otherwise, not all established PPARγ genes were regulated in the same manner as with troglitazone. Genes such as aP2 and adipophilin remained unaffected in hexarelin-treated cells, suggesting that the response to hexarelin is likely to be more complex than the sole activation of the PPARγ pathway. Consistent with this, CD36 gene expression was modestly increased by hexarelin with no change in protein levels, whereas troglitazone significantly induced both in treated adipocytes. Similar results were found in macrophages in which CD36 expression remains mostly unaffected by GHRPs, whereas troglitazone significantly up-regulated CD36 (11, 12). Such regulation was associated with a differential CD36 promoter occupancy by PPARγ as determined by chromatin immunoprecipitation assay (12). Additionally, PPARγ expression seems to be regulated differently in response to GHRPs than PPARγ ligands. We found that treating adipocytes with troglitazone lead to a decrease in PPARγ expression, a finding that was not associated with a decrease in target gene expression in mature adipocytes (18, 34) and that is generally observed for many nuclear receptors in response to ligands. However, the PPARγ mRNA level was slightly increased in response to hexarelin, whereas no obvious change was noticed for its protein level. A similar observation was also obtained in macrophages, suggesting that GHRPs contribute to maintain steady-state levels of PPARγ (11, 12). The mechanism by which hexarelin stimulates PPARγ activity but not down-regulating its expression deserves further investigation.

The white adipose tissue is the major site for triglyceride storage in the body and plays a critical role in maintaining homeostatic levels of circulating fatty acids and energy balance by promoting triglyceride breakdown and fatty acid release. Our results indicate that adipocytes respond to hexarelin with an increased mobilization of fatty acids rather than triglyceride synthesis. The depletion in lipid content in cells treated with hexarelin correlates with an increase in expression level of hormone-sensitive lipase, the enzyme involved in lipolysis. Genes involved in fatty acid synthesis and import were also augmented, such as fatty acid synthase and transporters FATP1 and FATP4. Interestingly, the expression of mitochondrial GPAT was decreased in adipocytes treated with hexarelin. It was recently shown that mitochondrial GPAT1, which catalyzes the initial and rate-controlling step in glycerolipid synthesis, partitions acyl-CoAs toward triacylglycerol synthesis and its deficiency in mice resulted in a redirection of fatty acids into the oxidation pathway in liver (35).

Such apparent mobilization of fatty acids induced by hexarelin seems to be unexpectedly directed toward the β-oxidation pathway in treated mature white adipocytes. Adipose tissue functions normally to release fatty acids in the circulation to be used by peripheral tissues of high oxidative potential, such as heart and muscle to produce ATP in response to energy expenditure. Brown adipocytes also use fatty acid oxidation to burn fat necessary for adaptive ther-

mogenesis. We found that cultured 3T3-L1 adipocytes treated with hexarelin exhibit an increased expression profile of mitochondrial genes related to long-chain fatty acid oxidation. The expression of CPT1b, a key enzyme for fatty acid oxidation in the heart, skeletal muscle and brown adipose tissue in human and rat, but normally absent in mouse white adipocytes or in 3T3-L1 cells (19), was strongly induced by hexarelin. Induction in CPT1b was described to be responsible for the dramatic increase in fatty acid oxidation that occurs in the heart after birth in which energy production switches from glucose to fatty acid use (36). Compared with its related isoform CPT1a, mainly expressed in liver, kidney, and intestine, CPT1b is more sensitive to the inhibition by malonyl-CoA (37). The expression of malonyl-CoA decarboxylase, which catalyzes the conversion of malonyl-CoA to acetyl-CoA, was up-regulated by hexarelin. Such an increase would potentially result in CPT1 activation by relieving the inhibitory effect of malonyl-CoA, and therefore increasing fatty acid oxidation.

Studies using genetic approaches and PPAR ligands have described the gene for CPT1 as regulated by PPAR isoforms, including PPARα and PPARβ (38–40). Although the precise role of PPARβ on adipocyte functions remains to be determined, PPARα plays a pivotal role in fatty acid metabolism by regulating the expression of genes involved in mitochondrial and peroxisomal β-oxidation pathways (41, 42). This raises the possibility that, in response to hexarelin, the increase of the expression of genes related to fatty acid oxidation in adipocytes might also depend on PPARα activation. Consistent with this, we found that hexarelin contributed to activate all three PPAR subtypes using a cell reporter assay, suggesting that cellular signaling induced by CD36 might influence PPAR activity (12). However, because the PPARs can all be activated to various degrees by low micromolar concentrations of unsaturated fatty acids (43), we cannot exclude the possibility that the mobilization of free fatty acids in cells due to hexarelin could provide endogenous ligands to selectively activate the PPARs and therefore fatty acid oxidation. Nevertheless, using genetically ablated PPAR$\gamma\pm$ macrophages, we showed that the activation of PPARγ target genes such as LXRα in response to hexarelin was impaired, suggesting that PPARγ activation is a major determinant of the response to GHRPs (12). Further studies are required to determine the exact contribution of hexarelin to PPAR activation in adipocytes.

Genes involved in oxidative phosphorylation and ATP synthesis were also strongly up-regulated by hexarelin, supporting the redirection of fatty acids toward mitochondrial oxidation rather than their release or their conversion into triacylglycerol. This profile was supported by a significant increase in F_1-ATP synthase expression and mitochondrial COX activity and a noticeable change in mitochondrial morphology in either treated adipocytes or mouse white adipose tissue. Electronic microscopy showed a significant increase in the intramitochondrial matrix surface and an intense cristae formation that spans the entire width of the organelle in response to hexarelin. Microarray analysis indicated an increase in the expression of many catalytic subunits of the ATPase and COX multimeric complexes, which both reside within the cristae membrane. Such phenotypic organization of mitochondria is typical of tissues with high oxidative potential, including muscle and brown fat, to support an enhanced activity in ATP production by the ATP synthase complex and mitochondrial respiration process (27). Most strikingly were the enhanced mRNA and protein levels of PGC-1α and UCP1 in response to hexarelin, which rose from low detectable levels normally found in white adipocytes to those mainly characteristic of brown fat. Under the same conditions, UCP2, a more ubiquitously expressed protein than UCP1 but for which its role is normally less related to the thermogenic response (44–46), appeared not to be regulated by hexarelin, indicating that the effects of hexarelin in promoting mitochondrial metabolic activity are more dependent on UCP1 up-regulation.

PGC1α is highly expressed in brown fat and plays a critical role in initiating a broad program of thermogenesis that includes enhanced oxidative metabolism and mitochondrial biogenesis (22). Interestingly, the transgenic expression of PGC-1α in white fat cells was shown to induce UCP1 expression and mitochondrial biogenesis, indicative that uncoupling of mitochondrial respiration is an important component of energy expenditure *in vivo* (47). Such metabolic need for energy supported by PGC-1α and UCP1 expression and mitochondrial morphological changes also occurred in white fat of treated mice, indicating that the ability of hexarelin to promote a fat burning-like phenotype was maintained *in vivo*. PGC-1α also controls critical aspects of energy metabolism in other tissues such as heart and muscle and largely contributes to the expression of genes of gluconeogenesis in liver (21, 23, 48, 49). Thus, modulating the relative activity of PGC-1 within a particular tissue may lead to fine-tuning of mitochondrial function in fatty acid oxidation and energy balance. Whether hexarelin may promote similar effects in other tissues expressing PGC-1α remains to be determined. In addition, with the propensity of PGC-1α to coactivate other nuclear receptors besides PPARγ, such as thyroid hormone receptor-α, retinoic acid receptor-α, estrogen-related receptor, and PPARα, and to result in enhanced UCP1 expression (47), it is expected that these pathways may also be affected by hexarelin.

Although the exact mechanisms by which GHRPs exert their effects through CD36 are not fully understood, it becomes clear that interacting with CD36 induces profound changes in metabolic activities of target tissues, especially regarding PPARγ-regulated events. In macrophages, GHRPs induced the PPARγ-LXRα-ABC pathway, leading to cholesterol efflux and reduction of atherosclerosis (11, 12). Here we report that hexarelin promotes the expression of key regulatory genes in fat metabolism, many of which are controlled by PPARγ, resulting in the mobilization of fatty acids toward mitochondria oxidative phosphorylation and biogenesis in white fat cells. These results therefore implicate CD36 in the regulation of the overall metabolic activity of mitochondria in adipocytes. With the emerging evidence that mitochondria dysfunction is associated with metabolic defects such as insulin resistance and type 2 diabetes (50), one can expect that modulating CD36 function might be potentially beneficial.

Acknowledgments

We acknowledge the expert assistance of Hugues Bernard for microarray analysis. We are grateful to Ksenia Scoulsky for skillful technical assistance in electron microscopy and Lynda Abdel for expert tissue analysis. We also acknowledge the technical assistance of Eve-Marie Charbonneau, Carole Garofalo, and Petra Pohankova. We are grateful to Susan Acton and Grant Mitchell for critical examination of the manuscript.

Received July 20, 2006. Accepted November 25, 2006.

Address all correspondence and requests for reprints to: André Tremblay, Research Center, Ste-Justine Hospital, 3175 Côte Ste-Catherine, Montréal (Québec), Canada H3T 1C5. E-mail: andre.tremblay@recherche-ste-justine.qc.ca.

This work was supported by the Canadian Institutes of Health Research (CIHR) and the Canadian Foundation for Innovation. A.R.-W. holds a doctoral award from the Natural Sciences and Engineering Research Council of Canada. A.D. holds a doctoral award from the CIHR. A.T. is a New Investigator of the CIHR.

Disclosure Statement: The authors have nothing to disclose.

References

1. **Eckel RH, Grundy SM, Zimmet PZ** 2005 The metabolic syndrome. Lancet 365:1415–1428
2. **Moller DE, Kaufman KD** 2005 Metabolic syndrome: a clinical and molecular perspective. Annu Rev Med 56:45–62
3. **Ibrahimi A, Abumrad NA** 2002 Role of CD36 in membrane transport of long-chain fatty acids. Curr Opin Clin Nutr Metab Care 5:139–145
4. **Li AC, Glass CK** 2002 The macrophage foam cell as a target for therapeutic intervention. Nat Med 8:1235–1242
5. **Castrillo A, Tontonoz P** 2004 Nuclear receptors in macrophage biology: at the crossroads of lipid metabolism and inflammation. Annu Rev Cell Dev Biol 20:455–480
6. **Ricote M, Valledor AF, Glass CK** 2004 Decoding transcriptional programs regulated by PPARs and LXRs in the macrophage: effects on lipid homeostasis, inflammation, and atherosclerosis. Arterioscler Thromb Vasc Biol 24:230–239
7. **Bodart V, Febbraio M, Demers A, McNicoll N, Pohankova P, Perreault A, Sejlitz T, Escher E, Silverstein RL, Lamontagne D, Ong H** 2002 CD36 mediates the cardiovascular action of growth hormone-releasing peptides in the heart. Circ Res 90:844–849
8. **Demers A, McNicoll N, Febbraio M, Servant M, Marleau S, Silverstein R, Ong H** 2004 Identification of the growth hormone-releasing peptide binding site in CD36: a photoaffinity cross-linking study. Biochem J 382:417–424
9. **Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Van der Ploeg LH** 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977
10. **Kojima M, Hosoda H, Matsuo H, Kangawa K** 2001 Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 12:118–122
11. **Marleau S, Harb D, Bujold K, Avallone R, Iken K, Wang Y, Demers A, Sirois M, Febbraio M, Silverstein M, Tremblay A, Ong H** 2005 EP80317, a ligand of the CD36 scavenger receptor, protects apolipoprotein E-deficient mice from developing atherosclerotic lesions. FASEB J 19:1869–1871
12. **Avallone R, Demers A, Rodrigue-Way A, Bujold K, Harb D, Anghel S, Wahli W, Marleau S, Ong H, Tremblay A** 2006 A growth hormone-releasing peptide that binds scavenger receptor CD36 and ghrelin receptor upregulates ABC sterol transporters and cholesterol efflux in macrophages through a PPARγ-dependent pathway. Mol Endocrinol 20:3165–3178
13. **St. Laurent V, Sanchez M, Charbonneau C, Tremblay A** 2005 Selective hormone-dependent repression of estrogen receptor β by a p38-activated ErbB2/ErbB3 pathway. J Steroid Biochem Mol Biol 94:23–37
14. **Locatelli V, Rossoni G, Schweiger F, Torsello A, De Gennaro Colonna V, Bernareggi M, Deghenghi R, Muller EE, Berti F** 1999 Growth hormone-independent cardioprotective effects of hexarelin in the rat. Endocrinology 140:4024–4031
15. **Zhang W, Zhao L, Lin TR, Chai B, Fan Y, Gantz I, Mulholland MW** 2004 Inhibition of adipogenesis by ghrelin. Mol Biol Cell 15:2484–2491
16. **Thompson NM, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, Wells T** 2004 Ghrelin and des-octanoyl ghrelin promote adipogenesis directly *in vivo* by a mechanism independent of the type 1a growth hormone secretagogue receptor. Endocrinology 145:234–242
17. **Tamori Y, Masugi J, Nishino N, Kasuga M** 2002 Role of peroxisome proliferator-activated receptor-γ in maintenance of the characteristics of mature 3T3–L1 adipocytes. Diabetes 51:2045–2055
18. **Perrey S, Ishibashi S, Yahagi N, Osuga J, Tozawa R, Yagyu H, Ohashi K, Gotoda T, Harada K, Chen Z, Iizuka Y, Shionoiri F, Yamada N** 2001 Thiazolidinedione- and tumor necrosis factor alpha-induced downregulation of peroxisome proliferator-activated receptor γ mRNA in differentiated 3T3–L1 adipocytes. Metabolism 50:36–40
19. **Brown NF, Hill JK, Esser V, Kirkland JL, Corkey BE, Foster DW, McGarry JD** 1997 Mouse white adipocytes and 3T3–L1 cells display an anomalous pattern of carnitine palmitoyltransferase (CPT) I isoform expression during differentiation. Inter-tissue and inter-species expression of CPT I and CPT II enzymes. Biochem J 327(Pt 1):225–231
20. **Lin J, Wu PH, Tarr PT, Lindenberg KS, St. Pierre J, Zhang CY, Mootha VK, Jager S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM** 2004 Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. Cell 119:121–135
21. **Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, Ahmad F, Matsui T, Chin S, Wu PH, Rybkin II, Shelton JM, Manieri M, Cinti S, Schoen FJ, Bassel-Duby R, Rosenzweig A, Ingwall JS, Spiegelman BM** 2005 Transcriptional coactivator PGC-1α controls the energy state and contractile function of cardiac muscle. Cell Metab 1:259–271
22. **Spiegelman BM, Heinrich R** 2004 Biological control through regulated transcriptional coactivators. Cell 119:157–167
23. **St. Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM** 2003 Bioenergetic analysis of peroxisome proliferator-activated receptor γ coactivators 1α and 1β (PGC-1α and PGC-1β) in muscle cells. J Biol Chem 278:26597–26603
24. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM** 1999 Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115–124
25. **Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP** 2000 Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Invest 106:847–856
26. **Wilson-Fritch L, Burkart A, Bell G, Mendelson K, Leszyk J, Nicoloro S, Czech M, Corvera S** 2003 Mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone. Mol Cell Biol 23:1085–1094
27. **Gilkerson RW, Selker JM, Capaldi RA** 2003 The cristal membrane of mitochondria is the principal site of oxidative phosphorylation. FEBS Lett 546:355–358
28. **Rehling P, Brandner K, Pfanner N** 2004 Mitochondrial import and the twin-pore translocase. Nat Rev Mol Cell Biol 5:519–530
29. **Bauer MF, Gempel K, Reichert AS, Rappold GA, Lichtner P, Gerbitz KD, Neupert W, Brunner M, Hofmann S** 1999 Genetic and structural characterization of the human mitochondrial inner membrane translocase. J Mol Biol 289:69–82
30. **Sylvester JE, Fischel-Ghodsian N, Mougey EB, O'Brien TW** 2004 Mitochondrial ribosomal proteins: candidate genes for mitochondrial disease. Genet Med 6:73–80
31. **Nijtmans LG, de Jong L, Artal SM, Coates PJ, Berden JA, Back JW, Muijsers AO, van der Spek H, Grivell LA** 2000 Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J 19:2444–2451
32. **Mishra S, Murphy LC, Nyomba BL, Murphy LJ** 2005 Prohibitin: a potential target for new therapeutics. Trends Mol Med 11:192–197
33. **Shulman AI, Mangelsdorf DJ** 2005 Retinoid X receptor heterodimers in the metabolic syndrome. N Engl J Med 353:604–615
34. **Takamura T, Nohara E, Nagai Y, Kobayashi K** 2001 Stage-specific effects of a thiazolidinedione on proliferation, differentiation and PPARγ mRNA expression in 3T3–L1 adipocytes. Eur J Pharmacol 422:23–29
35. **Hammond LE, Neschen S, Romanelli AJ, Cline GW, Ilkayeva OR, Shulman GI, Muoio DM, Coleman RA** 2005 Mitochondrial glycerol-3-phosphate acyltransferase-1 is essential in liver for the metabolism of excess acyl-CoAs. J Biol Chem 280:25629–25636
36. **Onay-Besikci A, Campbell FM, Hopkins TA, Dyck JR, Lopaschuk GD** 2003 Relative importance of malonyl CoA and carnitine in maturation of fatty acid oxidation in newborn rabbit heart. Am J Physiol Heart Circ Physiol 284:H283–H289
37. **McGarry JD, Brown NF** 1997 The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 244:1–14
38. **Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK** 2003 PPARγ and PPARΔ negatively regulate specific subsets of lipopolysaccharide and IFN-γ target genes in macrophages. Proc Natl Acad Sci USA 100:6712–6717
39. **Brandt JM, Djouadi F, Kelly DP** 1998 Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor α. J Biol Chem 273:23786–23792
40. **Yu GS, Lu YC, Gulick T** 1998 Co-regulation of tissue-specific alternative human carnitine palmitoyltransferase Iβ gene promoters by fatty acid enzyme substrate. J Biol Chem 273:32901–32909
41. **Djouadi F, Brandt JM, Weinheimer CJ, Leone TC, Gonzalez FJ, Kelly DP** 1999 The role of the peroxisome proliferator-activated receptor α (PPARα) in the

control of cardiac lipid metabolism. Prostaglandins Leukot Essent Fatty Acids 60:339–343

42. **Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W** 1999 Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. J Clin Invest 103:1489–1498
43. **Lee CH, Olson P, Evans RM** 2003 Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. Endocrinology 144: 2201–2207
44. **Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D** 2000 Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nat Genet 26:435–439
45. **Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB** 2001 Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes. Cell 105:745–755
46. **Brand MD, Esteves TC** 2005 Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2:85–93
47. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM** 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829–839
48. **Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM** 2001 Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 413:131–138
49. **Russell LK, Mansfield CM, Lehman JJ, Kovacs A, Courtois M, Saffitz JE, Medeiros DM, Valencik ML, McDonald JA, Kelly DP** 2004 Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. Circ Res 94: 525–533
50. **Lowell BB, Shulman GI** 2005 Mitochondrial dysfunction and type 2 diabetes. Science 307:384–387

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**