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Leptin Increases Cardiomyocyte Hyperplasia via Extracellular Signal-Regulated Kinase- and Phosphatidylinositol 3-Kinase-Dependent Signaling Pathways

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Obesity is a major risk factor for the development of heart failure. Importantly, it is now appreciated that a change in the number of myocytes is one of multiple structural and functional alterations (remodeling) leading to heart failure. Here we investigate the effect of leptin, the product of the obese (*ob*) gene, on proliferation of human and murine cardiomyocytes. Leptin caused a time- and dose-dependent significant increase in proliferation of HL-1 cells that was inhibited by preincubation with PD98059 and LY294002, suggesting that leptin mediated proliferation via extracellular signal-regulated kinase-1/2- and phosphatidylinositol-3-kinase-dependent signaling pathways. We confirmed that leptin activates both extracellular signal-regulated kinase-1/2 phosphorylation and association of phosphatidylinositol-3-kinase (regulatory p85 subunit) with phosphotyrosine immunoprecipitates. We also examined bromodeoxyuridine incorporation as a measure of new DNA synthesis and demonstrated a stimulatory effect of leptin in both HL-1 cells and human cardiomyocytes. Bromodeoxyuridine incorporation in HL-1 cells was inhibited by PD98059 and LY294002. Our results establish a mitogenic effect of leptin in cardiomyocytes and provide additional evidence for a potential direct link between leptin and cardiac remodeling in obesity. (*Endocrinology* 145: 1550–1555, 2004)

THE EMERGING OBESITY epidemic (1) is recognized as an independent risk factor for congestive heart failure, coronary artery disease, hypertension, diabetes, and other diseases (2–4). Indeed, obesity is now classified by the American Heart Association as a major modifiable risk factor for cardiovascular complications (3). The Framingham heart study found that the risk of heart failure was increased 34% in overweight [body mass index (BMI) of 25.0–29.9) and by 104% in obese (BMI $>$ 30.0) individuals (5). However, the pathogenesis of heart failure in obese individuals is still unclear.

Heart failure is believed to manifest due to multiple structural and functional alterations (remodeling) that interact over a sustained period of time (6). Broadly speaking, the changes that occur include those affecting size or number of myocytes and those altering the composition and structure of the extracellular matrix (7). Importantly, the longstanding concept that ventricular myocytes are terminally differentiated cells has recently been revised to incorporate myocyte replication as a significant component of remodeling (8, 9). Whether cardiac remodeling leading to left ventricular hypertrophy occurs as a result of an increased hemodynamic load or via direct neurohumoral signaling has generated much debate.

Adipose tissue, once considered simply a lipid storage depot, is now known to be a dynamic endocrine organ secreting factors such as leptin, the product of the obese (*ob*) gene (4, 10). Fasting plasma leptin concentrations are associated with increased myocardial wall thickness, independent of body weight or blood pressure (11), and the role of leptin as a link between obesity and cardiovascular disease is rapidly emerging as an important research focus (12). In recent years it has become clear that leptin mediates many direct effects on peripheral tissues (13). Leptin has been shown to directly attenuate contraction (14) and induce (15) or prevent (16) hypertrophy in isolated myocytes. The effect of leptin on proliferation of cardiomyocytes has not yet been examined. Here we provide additional evidence for a potential direct link between leptin and cardiac remodeling in obesity by demonstrating a mitogenic effect of leptin on primary human pediatric cardiomyocytes and a stable murine cardiomyocyte cell line.

Materials and Methods

Culture of HL-1 cells

HL-1 cells (17) were maintained as previously described (18) using Claycomb medium (JRH Biosciences, Lenexa, KS), supplemented with 10% (vol/vol) fetal bovine serum (Wisent, Quebec, Canada), 4 mM L-glutamine (Wisent), 10 μM noradrenaline (norepinephrine) (Sigma Chemical Co., St. Louis, MO), and 1% (vol/vol) penicillin-streptomycin (Wisent). During leptin treatment periods, the cells were cultured in media containing 1% fetal bovine serum. Cells were grown at 37 C in an atmosphere of 5% CO_2. During continued growth, the medium was changed routinely every 48 h.

Isolation of human ventricular cardiomyocytes

The method used here for isolation of human ventricular cardiomyocytes has been described many times (19–21). Biopsy samples were

Abbreviations: BMI, Body mass index; BrdU, bromodeoxyuridine; ERK, extracellular signal-regulated kinase; PI, phosphatidylinositol.

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obtained from children undergoing elective surgical repair of tetralogy of Fallot. All patients gave written informed consent to obtain biopsies through a protocol approved by the University of Toronto Human Experimentation Committee. Briefly, myocardial ventricular resection biopsies (~20 mg) from children (6 months or older) were immediately washed in PBS and then digested by 0.1% collagenase/0.2% trypsin in PBS. Cardiomyocytes were subsequently purified by dilution cloning and maintained at 37 C (95% air/5% CO_2) in Iscove's modified Dulbecco's medium (Canada Life Technologies Inc., Burlington, Ontario, Canada) containing 10% fetal calf serum, 0.1 mM β-mercaptoethanol, 0.4 mM L-arginine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Purification of a cardiomyocyte population can be confirmed by fluorescent staining with a monoclonal antibody for human ventricular myosin heavy chain (20, 22). During the leptin treatment period, cells were cultured in media containing 1% fetal bovine serum. Based on previous experience, cells were used for experiments only at passage 3 or 4 (23).

Cell counting

To measure cell number, 0.5×10^6 cells were added per well of a six-well culture dish and allowed to grow for up to 96 h in the presence or absence of leptin (mouse recombinant, endotoxin: $\leq$1.0 EU/μg) (Calbiochem, La Jolla, CA) at the concentrations indicated in figure legends. Where indicated, cells were also pretreated for 1 h with PD98059 (20 μM) or LY294002 (20 μM). PD98059 and LY294002 were from Bioshop Canada Inc. (Burlington, Ontario, Canada). At each time point, cells from each well were harvested by trypsinization and counted using a hemocytometer. Eight fields of view were analyzed in a blinded manner for each of three experiments. Trypan blue dye exclusion was used to confirm viability of cells. To reinforce the accuracy of this approach, cell number was also determined using a Coulter counter.

Bromodeoxyuridine (BrdU) uptake assay

The effect of leptin on DNA synthesis was assessed by measuring BrdU incorporation into newly synthesized DNA using a colorimetric ELISA-based assay kit according to the manufacturer's instructions (Oncogene Research Products, San Diego, CA). Briefly, HL-1 or human pediatric cardiomyocytes cultured in 96-well plates were stimulated with leptin (6–60 nM) for 12 h and then BrdU was added with subsequent incubation for an additional 12 h. Where indicated in HL-1 cell experiments, cells were also pretreated for 1 h with PD98059 (20 μM) or LY294002 (20 μM) before the addition of leptin. Thereafter, binding of anti-BrdU antibody to BrdU incorporated in DNA was detected by measuring absorbance at dual wavelengths of 450–540 nm using a spectrophotometer.

Analysis of extracellular signal-regulated kinase (ERK) and phosphatidylinositol (PI) 3-kinase activation

Cells grown on six-well plates were treated with leptin (6 nM, up to 15 min) and then washed quickly with ice-cold PBS. Lysates to assess ERK phosphorylation were prepared as previously described (24). Equal amounts of protein (~40 μg) were then immunoblotted using a phospho-ERK antibody that specifically recognizes the kinase phosphorylated on Thr202/Tyr204 (Cell Signaling Technology, Beverly, MA). To determine association of p85 with phosphotyrosine, cell lysates were prepared and immunoprecipitation using antiphosphotyrosine antibody performed essentially as described previously (25). Immunoprecipitated proteins were immunoblotted with an antibody specifically recognizing the p85 regulatory subunit of PI 3-kinase (Santa Cruz Biotechnology, Santa Cruz, CA). Higher association of this subunit with phosphotyrosine immunoprecipitates is indicative of PI 3-kinase activation.

Statistical analysis

Statistical analysis was performed using either one- or two-way ANOVA with Tukey-Kramer multiple comparison test or Bonferroni *post hoc* tests where appropriate. The level of significance was set at $P < 0.05$.

Results

To investigate the effect of leptin on cardiomyocyte hyperplasia we have used both isolated pediatric human cardiomyocytes and an established murine cardiomyocyte cell line (17, 19). The cultured human cardiomyocytes isolated by clonal dilution measured approximately 40×80 μm, proliferate *in vitro*, and have been used extensively for analysis of myocyte function (19–22). Characteristics of the murine cardiomyocytes used in this study include a mature cardiomyocyte phenotype, an ultrastructure similar to primary cultures of cardiomyocytes, cytoplasmic reorganization and myofibrillogenesis similar to that observed in mitotic cardiomyocytes in the developing heart, presence of highly ordered myofibrils, the ability to undergo spontaneous contraction, expression of cardiac-specific genes, and the presence of several cardiac-specific voltage-dependent characteristics (17). Furthermore, transplantation studies suggest HL-1 cells act as a functional equivalent of endogenous adult cardiomyocytes (26). A previous study using mouse heart suggests that both the short and long leptin receptor isoform transcripts are expressed and in a ratio of approximately 11:1, respectively (27). Before using HL-1 cells for this study we demonstrated expression of long and short isoforms in a similar ratio (data not shown). Hence, these cells represent an excellent model (28) to begin to elucidate the effects of leptin on the heart.

Effect of leptin on cardiomyocyte hyperplasia

To determine whether leptin altered cardiomyocyte growth, we treated cells with leptin (6 nM, 0–96 h) and then analyzed cell number using a hemocytometer. Leptin caused a time-dependent increase in proliferation of these cells (Fig. 1A). A small increase was evident after 4 h of leptin treatment and a statistically significant increase observed after 24 h. The magnitude of response observed is significant and typical of growth responses by other cells in response to leptin (29, 30). We also investigated the sensitivity of HL-1 cell growth to leptin, and Fig. 1B shows that when cells were incubated for 24 h in varying concentrations of leptin, the hormone caused a statistically significant increase in cell growth at all concentrations tested (0.3–60 nM). The magnitude of response elicited reached approximately 2.3-fold above control at 60 nM leptin. To further support our observations, results presented in Fig. 2 were confirmed using a Coulter counter (data not shown). Subsequent experiments were performed with 6 nM leptin.

Intracellular signaling mechanisms responsible for the effect of leptin on hyperplasia of cardiomyocytes

It is known that sustained activation of ERK is sufficient to induce the proliferation of several cell types (31), and several studies have shown that the proliferative effect of leptin was reduced by the MAPK kinase 1 inhibitor PD98059 (30, 32, 33). We therefore examined the effect of PD98059 (20 μM and 1-h pretreatment) and found it significantly reduced the ability of leptin (6 nM for 24 h) to increase HL-1 cell proliferation (Fig. 2A). We also demonstrated that leptin induces ERK (p42/p44 MAPK) phosphorylation in these

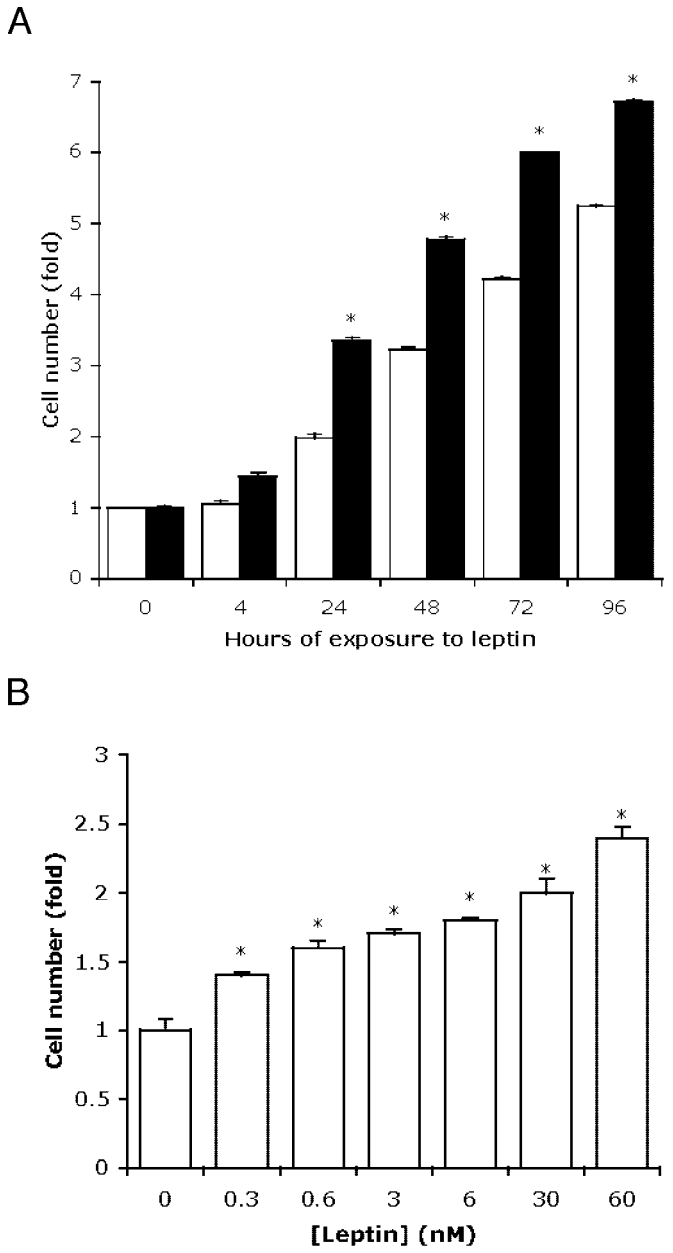

FIG. 1. Effect of leptin on proliferation of HL-1 cells. Cells were grown in the presence or absence of leptin and then cell number was determined. A, Time course showing untreated cells (*white bars*) and cells treated with 6 nM leptin for up to 4 d (*black bars*); B, dose response, varying leptin concentrations for 24 h. Results are expressed relative to initial number at time zero. Three independent experiments were conducted; *, $P < 0.05$ with respect to control at same time point.

cells (Fig. 2C), by immunoblotting cell lysates with phospho-ERK antibody, which specifically recognizes the kinase phosphorylated on Thr202/Tyr204. Many growth factors promote proliferation via PI 3-kinase-mediated signaling pathways (34), and it has been shown that this kinase can be activated by leptin in several cell types (35). We confirm here that leptin increased PI 3-kinase activation in HL-1 cells by measuring the association of the regulatory (p85) subunit with phosphotyrosine immunoprecipitates (Fig. 2D). We also demonstrated that the PI 3-kinase inhibitor LY294002 (20 μM and 1-h pretreatment) significantly reduced the ability of leptin to increase HL-1 cell proliferation (Fig. 2B).

Effect of leptin on BrdU incorporation in HL-1 and human cardiomyocytes

To further investigate the effect of leptin on cardiomyocyte growth we have also measured incorporation of BrdU into DNA as an index of cell proliferation. Results shown in Fig. 3 confirm that leptin caused an approximately 2.3-fold increase in BrdU uptake in HL-1 cells. This effect was sensitive to inhibition by both PD98059 and LY294002 (Fig. 3). Importantly, we also used primary human cardiomyocytes and demonstrate that treatment with leptin for up to 48 h significantly increased BrdU uptake approximately 1.4-fold above control (Fig. 4). These results further support the conclusion that leptin directly stimulates cardiomyocyte cell division and thus increases cell number.

Discussion

Although still controversial, it is likely that endocrine effects of factors secreted by fat tissue play an important role in the pathogenesis of heart failure associated with obesity (2, 12). Indeed, current treatment of heart failure is largely based on inhibition of neuroendocrine activation to limit progression of heart failure (36). Many studies have suggested that leptin plays a role in the pathogenesis of cardiovascular disease. Elevated plasma leptin levels are found in patients with congestive heart failure (37). Fasting plasma leptin concentrations in hypertensive men were significantly associated with myocardial wall thickness, independent of hypertension (11, 38). Plasma leptin levels correlate with heart rate, independently of plasma insulin, BMI, waist:hip ratio, and percentage body fat in men (39). The ability of leptin to attenuate cardiac muscle contraction has been proposed to explain the decrease in diastolic compliance and prolonged relaxation observed in obese individuals (14). Activation of fatty acid oxidation, and thus decreased triglyceride content, or altered adenylate cyclase function by leptin have been suggested to contribute to reduced cardiac function in obese individuals (40, 41). Intriguingly, a direct impact of leptin on remodeling was recently demonstrated by correction of ventricular hypertrophy in *ob/ob* mice upon injection of leptin (16). Whereas this implies an antihypertrophic effect of leptin, another study in cultured myocytes suggested that leptin induces hypertrophy via a p38MAPK-dependent mechanism (15).

Only recently was the longstanding paradigm of the adult heart as a postmitotic organ challenged and the importance of myocyte death and regeneration realized (42). However, many studies have now noted an ability of fetal human cardiomyocytes (19, 21, 22), cells isolated from 30- to 65-yr-old adults with hypertrophic cardiomyopathy (43), and rodent cardiomyocytes (15, 17) to proliferate in culture. It is interesting to note that transplantation of fetal rat cardiomyocytes, which demonstrate hyperplasia *in vitro*, to sc tissue of an adult rat lose their ability to proliferate (44). This suggests that non-cardiomyocyte-derived factors act *in vivo* to regulate cardiomyocyte hyperplasia. Thus, investigations of the effects of adipose-derived factors on cardiomyocyte

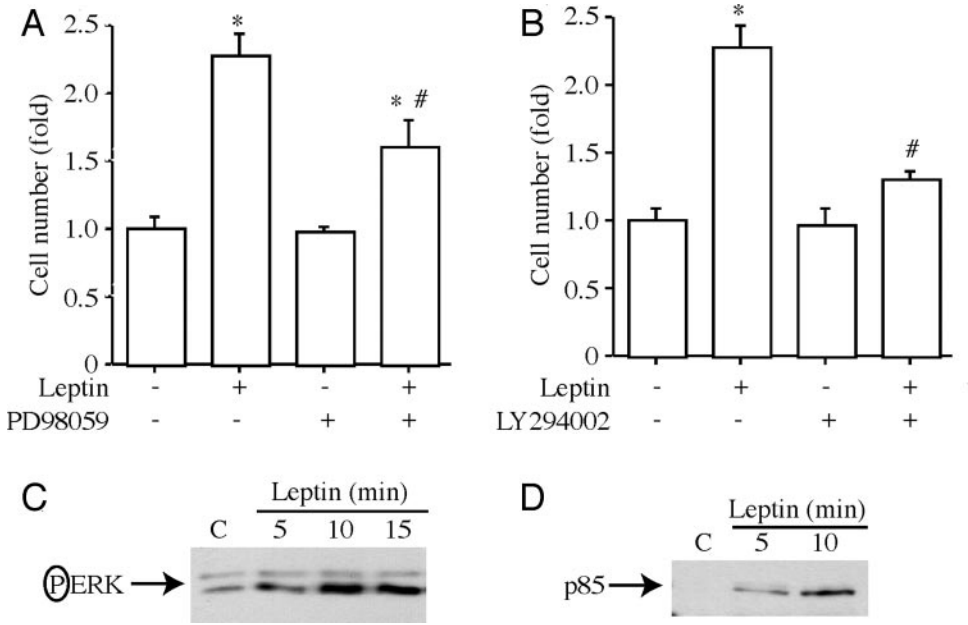

FIG. 2. Role of ERK and PI 3-kinase in stimulation of HL-1 cell growth by leptin. The mitogenic effect of leptin was examined in cells pretreated for 1 h with 20 μM PD98059 (A) or 20 μM LY294002 (B) before stimulation with 6 nM leptin for 24 h in the continued presence of inhibitor. Results are expressed relative to cell growth seen in control (C) samples. Results shown are mean ± SEM of three independent experiments; *, $P < 0.05$ with respect to control; #, $P < 0.05$ with respect to leptin alone. Western blots to determine the effect of leptin (6 nM) on ERK phosphorylation (C) and association of p85 with phosphotyrosine (D) are shown.

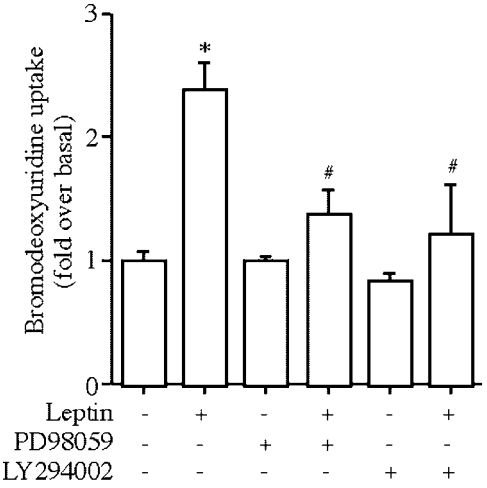

FIG. 3. Effect of leptin on BrdU incorporation in HL-1 cells. HL-1 cells were treated with or without leptin (6 nM) for 24 h before analysis of BrdU incorporation into newly synthesized DNA. The inhibitors PD98059 (20 μM) and LY294002 (20 μM) were added 1 h before leptin and their presence maintained during subsequent incubation. Values are expressed relative to control, and the results represent mean ± SEM of three independent experiments. *, $P < 0.05$ with respect to control; #, $P < 0.05$ with respect to leptin alone.

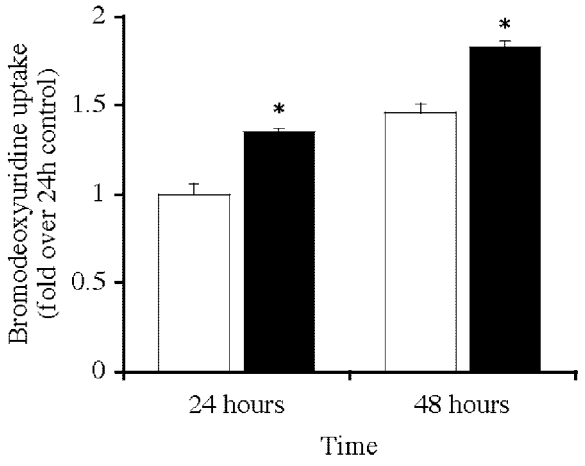

FIG. 4. Effect of leptin on BrdU incorporation in human cardiomyocytes. Primary human cardiomyocytes were incubated in the absence (*white bars*) or presence (*black bars*) of leptin (60 nM) for up to 48 h before analysis of BrdU incorporation into newly synthesized DNA. Values are expressed relative to BrdU uptake in untreated cells after 24 h. Data presented represent mean ± SEM of four independent experiments; *, $P < 0.05$ with respect to control at the corresponding time.

proliferation are timely and may enhance our understanding of the mechanisms responsible for the association between obesity and heart failure. The ability of leptin to regulate cell growth was demonstrated recently in a surprisingly wide variety of cell types including vascular smooth muscle cells (29), glomerular endothelial cells (45), tracheal epithelial cells (33), monocytes (46), and β-cells (30). Here we make the novel observation that leptin increases proliferation of both human and murine cardiomyocytes. Importantly, our observations are made over a range of leptin concentrations that are similar to those found in human plasma of obese individuals: 0.06 nM (~1 ng/ml) in normal to 6.1 nM (~100 ng/ml) in obese (47, 48).

The signaling pathways activated by leptin (49) to mediate effects in cardiomyocytes are only beginning to be characterized. We show here that leptin activated both ERK1/2 and PI 3-kinase in cardiomocytes. It is known that sustained activation of ERK is sufficient to induce the proliferation of several cell types (31) and in fact three studies have demonstrated that the proliferative effect of leptin was reduced by the MAPK kinase1 inhibitor, PD98059 (30, 32, 33). Many growth factors also promote proliferation via phosphatidylinositol (PI) 3-kinase-mediated signaling pathways (34). Here we show that inhibitors of ERK1/2 activation and PI 3-kinase action reduced leptin-stimulated cardiomyocyte proliferation, implicating a role for these kinases in mediating this effect of leptin.

BrdU labeling has recently been used to demonstrate the existence of cycling myocytes in the adult myocardium (50). Thus, whereas many myocytes in the adult heart may be terminally differentiated there is a continuously renewing pool of myocytes that do enter the cell cycle in response to appropriate physiological stimuli (8, 9). It is likely that cell proliferation declines over time or with risk factors such as obesity, contributing to heart failure (51). Here we measured incorporation of BrdU into DNA as an index of cell proliferation and found that, in keeping with cell number analysis, leptin caused an increase in BrdU uptake in HL-1 cells, an effect that was sensitive to inhibition by both PD98059 and LY294002. Importantly, we also demonstrated the ability of leptin to increase BrdU uptake in human cardiomyocytes. This suggests that our studies are likely to be of direct physiological relevance in humans.

In summary, we demonstrate that leptin increased DNA synthesis and proliferation of human and murine cardiomyocytes. This likely occurs via ERK- and PI 3-kinase-dependent signaling pathways. The implications of our results are that leptin may promote myocardial growth and thus help repair the failing myocardium. However, as recently discussed (12), obese individuals are predominantly hyperleptinemic and leptin resistant. Under such circumstances we may speculate from our results that the contribution made by leptin to myocyte regeneration would be lost in obese individuals, thus causing or exacerbating heart failure. Although it is tempting to extrapolate observations made in isolated myocytes to *in vivo* physiology, care must be extended and appropriate *in vivo* experiments performed before definite conclusions are drawn. Hopefully our results will provide impetus for such studies and help establish a direct role of leptin on cardiac remodeling and thus in the pathogenesis of heart failure.

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References

1. **Manson JE, Bassuk SS** 2003 Obesity in the United States: a fresh look at its high toll. JAMA 289:229–230
2. **Sharma AM** 2003 Obesity and cardiovascular risk. Growth Horm IGF Res 13(Suppl A):S10–S17
3. **Eckel RH, Barouch WW, Ershow AG** 2002 Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on the pathophysiology of obesity-associated cardiovascular disease. Circulation 105:2923–2928
4. **Ahima RS, Flier JS** 2000 Adipose tissue as an endocrine organ. Trends Endocrinol Metab 11:327–332
5. **Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, Kannel WB, Vasan RS** 2002 Obesity and the risk of heart failure. N Engl J Med 347:305–313
6. **Mann DL** 1999 Mechanisms and models in heart failure: a combinatorial approach. Circulation 100:999–1008
7. **Lorell BH** 1997 Transition from hypertrophy to failure. Circulation 96:3824–3827
8. **Anversa P, Nadal-Ginard B** 2002 Myocyte renewal and ventricular remodelling. Nature 415:240–243
9. **Anversa P, Leri A, Kajstura J, Nadal-Ginard B** 2002 Myocyte growth and cardiac repair. J Mol Cell Cardiol 34:91–105
10. **Rajala MW, Scherer PE** 2003 The adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144:3765–3773
11. **Paolisso G, Tagliamonte MR, Galderisi M, Zito GA, Petrocelli A, Carella C, de Divitiis O, Varricchio M** 1999 Plasma leptin level is associated with myocardial wall thickness in hypertensive insulin-resistant men. Hypertension 34:1047–1052
12. **Sader S, Nian M, Liu P** 2003 Leptin: a novel link between obesity, diabetes, cardiovascular risk, and ventricular hypertrophy. Circulation 108:644–646
13. **Ahima RS, Flier JS** 2000 Leptin. Annu Rev Physiol 62:413–437
14. **Nickola MW, Wold LE, Colligan PB, Wang GJ, Samson WK, Ren J** 2000 Leptin attenuates cardiac contraction in rat ventricular myocytes. Role of NO. Hypertension 36:501–505
15. **Rajapurohitam V, Gan XT, Kirshenbaum LA, Karmazyn M** 2003 The obesity-associated peptide leptin induces hypertrophy in neonatal rat ventricular myocytes. Circ Res 93:277–279
16. **Barouch LA, Berkowitz DE, Harrison RW, O'Donnell CP, Hare JM** 2003 Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. Circulation 108:754–759
17. **Claycomb WC, Lanson Jr NA, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A, Izzo Jr NJ** 1998 HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc Natl Acad Sci USA 95:2979–2984
18. **Chaudary N, Shuralyova I, Liron T, Sweeney G, Coe IR** 2002 Transport characteristics of HL-1 cells: a new model for the study of adenosine physiology in cardiomyocytes. Biochem Cell Biol 80:655–665
19. **Li RK, Weisel RD, Williams WG, Mickle DAG** 1992 Methods of culturing cardiomyocytes from human pediatric ventricular myocardium. J Tissue Culture Methods 32:362–373
20. **Merante F, Mickle DA, Weisel RD, Li RK, Tumiati LC, Rao V, Williams WG, Robinson BH** 1998 Myocardial aerobic metabolism is impaired in a cell culture model of cyanotic heart disease. Am J Physiol 275:H1673–H1681
21. **Shiono N, Rao V, Weisel RD, Kawasaki M, Li RK, Mickle DA, Fedak PW, Tumiati LC, Ko L, Verma S** 2002 L-Arginine protects human heart cells from low-volume anoxia and reoxygenation. Am J Physiol Heart Circ Physiol 282: H805–H815
22. **Li RK, Mickle DA, Weisel RD, Carson S, Omar SA, Tumiati LC, Wilson GJ, Williams WG** 1996 Human pediatric and adult ventricular cardiomyocytes in culture: assessment of phenotypic changes with passaging. Cardiovasc Res 32:362–373
23. **Luss H, Li RK, Shapiro RA, Tzeng E, McGowan FX, Yoneyama T, Hatakeyama K, Geller DA, Mickle DA, Simmons RL, Billiar TR** 1997 Dedifferentiated human ventricular cardiac myocytes express inducible nitric oxide synthase mRNA but not protein in response to IL-1, TNF, IFNγ, and LPS. J Mol Cell Cardiol 29:1153–1165
24. **Tajmir P, Kwan JJ, Kessas M, Mozammel S, Sweeney G** 2003 Acute and chronic leptin treatment mediate contrasting effects on signaling, glucose uptake, and GLUT4 translocation in L6-GLUT4myc myotubes. J Cell Physiol 197:122–130
25. **Sweeney G, Niu W, Canfield VA, Levenson R, Klip A** 2001 Insulin increases plasma membrane content and reduces phosphorylation of Na(+)-K(+) pump α(1)-subunit in HEK-293 cells. Am J Physiol Cell Physiol 281:C1797–C1803
26. **Watanabe E, Smith Jr DM, Delcarpio JB, Sun J, Smart FW, Van Meter Jr CH, Claycomb WC** 1998 Cardiomyocyte transplantation in a porcine myocardial infarction model. Cell Transplant 7:239–246
27. **Lollmann B, Gruninger S, Stricker-Krongrad A, Chiesi M** 1997 Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and, e in different mouse tissues. Biochem Biophys Res Commun 238:648–652
28. **Luo H, Zhang J, Cheung C, Suarez A, McManus BM, Yang D** 2003 Proteasome inhibition reduces coxsackievirus B3 replication in murine cardiomyocytes. Am J Pathol 163:381–385
29. **Oda A, Taniguchi T, Yokoyama M** 2001 Leptin stimulates rat aortic smooth muscle cell proliferation and migration. Kobe J Med Sci 47:141–150
30. **Tanabe K, Okuya S, Tanizawa Y, Matsutani A, Oka Y** 1997 Leptin induces proliferation of pancreatic β cell line MIN6 through activation of mitogen-activated protein kinase. Biochem Biophys Res Commun 241:765–768
31. **Marshall CJ** 1995 Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80: 179–185
32. **Takahashi Y, Okimura Y, Mizuno I, Iida K, Takahashi T, Kaji H, Abe H, Chihara K** 1997 Leptin induces mitogen-activated protein kinase-dependent proliferation of C3H10T1/2 cells. J Biol Chem 272:12897–12900
33. **Tsuchiya T, Shimizu H, Horie T, Mori M** 1999 Expression of leptin receptor in lung: leptin as a growth factor. Eur J Pharmacol 365:273–279
34. **Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD** 2001 Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 17:615–675

35. **Martin-Romero C, Sanchez-Margalet V** 2001 Human leptin activates PI3K and MAPK pathways in human peripheral blood mononuclear cells: possible role of Sam68. Cell Immunol 212:83–91
36. **Francis GS** 2001 Pathophysiology of chronic heart failure. Am J Med 110(Suppl 7A):37S–46S
37. **Leyva F, Anker SD, Egerer K, Stevenson JC, Kox WJ, Coats AJ** 1998 Hyperleptinaemia in chronic heart failure. Relationships with insulin. Eur Heart J 19:1547–1551
38. **Paolisso G, Tagliamonte MR, Galderisi M, Zito GA, D'Errico A, Marfella R, Carella C, de Divitiis O, Varricchio M** 2001 Plasma leptin concentration, insulin sensitivity, and 24-hour ambulatory blood pressure and left ventricular geometry. Am J Hypertens 14:114–120
39. **Narkiewicz K, Kato M, Phillips BG, Pesek CA, Choe I, Winnicki M, Palatini P, Sivitz WI, Somers VK** 2001 Leptin interacts with heart rate but not sympathetic nerve traffic in healthy male subjects. J Hypertens 19:1089–1094
40. **Atkinson LL, Fischer MA, Lopaschuk GD** 2002 Leptin activates cardiac fatty acid oxidation independent of changes in the AMP-activated protein kinase-acetyl-CoA carboxylase-malonyl-CoA axis. J Biol Chem 277:29424–29430
41. **Illiano G, Naviglio S, Pagano M, Spina A, Chiosi E, Barbieri M, Paolisso G** 2002 Leptin affects adenylate cyclase activity in H9c2 cardiac cell line: effects of short- and long-term exposure. Am J Hypertens 15:638–643
42. **Nadal-Ginard B, Kajstura J, Leri A, Anversa P** 2003 Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. Circ Res 92:139–150
43. **Tumiati LC, Li RK, Weisel RD, Williams WG** 1993 An in vitro model of hypertrophic cardiomyopathy (HCM). J Mol Cell Cardiol 25:A233
44. **Li RK, Mickle DA, Weisel RD, Zhang J, Mohabeer MK** 1996 In vivo survival and function of transplanted rat cardiomyocytes. Circ Res 78:283–288
45. **Wolf G, Hamann A, Han DC, Helmchen U, Thaiss F, Ziyadeh FN, Stahl RA** 1999 Leptin stimulates proliferation and TGF-β expression in renal glomerular endothelial cells: potential role in glomerulosclerosis. Kidney Int 56:860–872
46. **Santos-Alvarez J, Goberna R, Sanchez-Margalet V** 1999 Human leptin stimulates proliferation and activation of human circulating monocytes. Cell Immunol 194:6–11
47. **Licinio J, Negrao AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB, Mulla A, Cearnal L, Veldhuis JD, Flier JS, McCann SM, Gold PW** 1998 Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proc Natl Acad Sci USA 95:2541–2546
48. **Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern P, Friedman JM** 1995 Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155–1161
49. **Sweeney G** 2002 Leptin signalling. Cell Signal 14:655–663
50. **Anversa P, Kajstura J** 1998 Ventricular myocytes are not terminally differentiated in the adult mammalian heart. Circ Res 83:1–14
51. **Kajstura J, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P** 1998 Myocyte proliferation in end-stage cardiac failure in humans. Proc Natl Acad Sci USA 95:8801–8805

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