(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 February 2007 (15.02.2007)

PCT

(10) International Publication Number
WO 2007/019302 A2

(51) International Patent Classification:
H04N 7/14 (2006.01)

(21) International Application Number:
PCT/US2006/030427

(22) International Filing Date: 3 August 2006 (03.08.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/705,926 3 August 2005 (03.08.2005) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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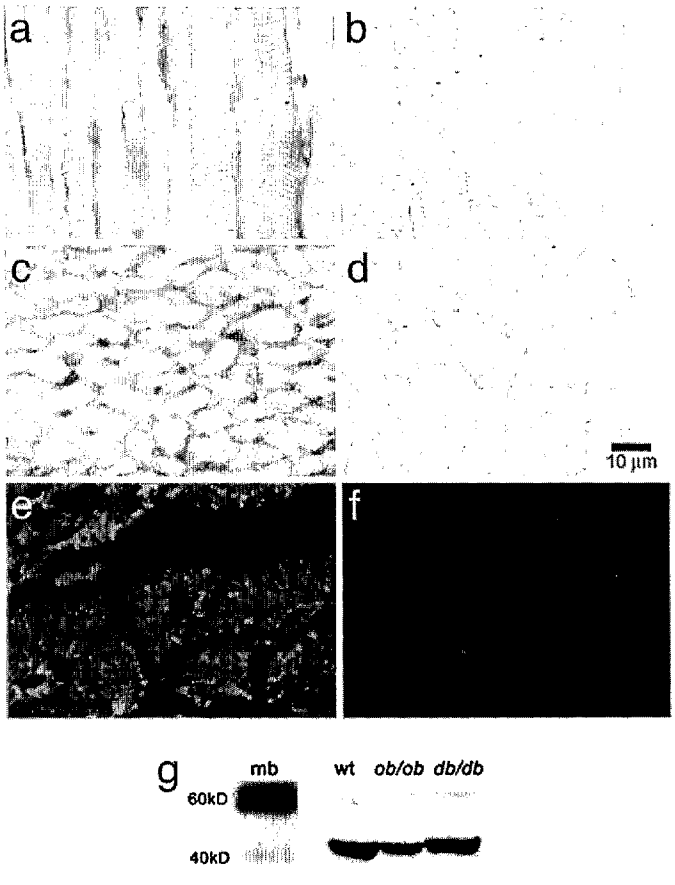
(54) Title: TREATMENT OF CARDIAC HYPERTROPHY BY ACTIVATION OF CILIARY NEURTROPHIC FACTOR RECEPTOR



(57) Abstract: Methods are provided to treat and prevent cardiac hypertrophy and obesity-related cardiovascular diseases such as left ventricular hypertropy, hypertension, myocardial infarction, and heart failure. The methods involve administration of ciliary neurotrophic factor (CNTF), or another agonist of CNTF receptors in myocardium.

WO 2007/019302 A2

Published:

— *without international search report and to be republished upon receipt of that report*

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TREATMENT OF CARDIAC HYPERTROPHY BY ACTIVATION OF CILIARY NEUROTROPHIC FACTOR RECEPTOR

[0001] This application claims priority from U.S. Provisional Application No: 60/705,296, filed August 3, 2005, which is incorporated herein by reference.

TECHNICAL FIELD

[0002] The invention relates to the use of agonists of cilliary neurotrophic factor in the treatment and prevention of cardiac hypertrophy and related disorders.

BACKGROUND

[0003] Left ventricular hypertrophy (LVH) and its subsequent progression to congestive heart failure represents a major cause of morbidity and mortality (1). Obesity, an important mediator of LVH (2), results from either deficiency of or receptor insensitivity to leptin (3–5), a hormone that regulates appetite and energy metabolism (6). Both leptin deficiency and resistance contribute to LVH in murine models (7). Additionally, regression of LVH can be achieved with leptin repletion in leptin-deficient *ob/ob* mice. However, because the majority of human obesity is associated with hyperleptinemia and leptin resistance (4, 8, 9) that is unresponsive to leptin treatment, an alternate signaling axis could regulate cardiac architecture.

[0004] Recent epidemiologic studies support a role for leptin in regression of cardiac hypertrophy. In a study conducted in healthy individuals free from cardiovascular disease, Pladevall et al. (28) showed that leptin deficiency was associated with increased left ventricular mass index, thus demonstrating that leptin has an antihypertrophic effect in the presence of an intact signaling pathway. Although some older studies showed a positive correlation between leptin and LVH when adjusting for body mass index (BMI) (29), others

have highlighted that this positive correlation is completely abrogated after adjusting for BMI (30). Nonetheless, it is well established that obesity in clinical populations is associated with elevated leptin primarily due to leptin resistance, implying depressed downstream signaling despite increased leptin levels (4, 8, 9).

[0005] Ciliary neurotrophic factor (CNTF) activates a related signaling pathway to leptin and has similar effects on body weight and metabolism (10–12). The CNTF receptor (CNTFR) complex closely resembles the leptin receptor (ObR) structurally and has a similar distribution in the hypothalamic nuclei associated with regulation of feeding and body weight (13, 14). Both receptors are members of the gp130 cytokine family of receptors. The CNTF receptor is a trimeric receptor complex with a CNTF binding component (CNTFRα), a leukemia inhibitory factor β subunit, and the signal transducer of IL-6 (gp130) (15). Leptin and CNTF signaling pathways share structural homology between ObR and the gp130 subunit of the CNTFR (16) as well as activation of similar signal transduction pathways such as the Janus kinase-signal transducer and activator of transcription pathway (17–19).

SUMMARY OF THE INVENTION

[0006] The invention is related to novel methods of treating or preventing cardiac hypertrophy and several related cardiac disorders. The methods involve activation of ciliary neurotrophic factor receptors in myocardium.

[0007] Generally, the methods of treating or preventing cardiac hypertrophy according to the invention comprise administering an effective amount of an agonist of CNTF to a subject who has cardiac hypertrophy or is at risk of developing cardiac hypertrophy. In one embodiment, the agonist is either human CNTF or an analog or variant thereof capable of activating CNTF. For example, the agonist can be a polypeptide comprising amino acids 1 – 200 of SEQ ID NO:1, a polypeptide comprising amino acids 1 – 185 of SEQ ID NO:1, a polypeptide having the amino acid sequence of SEQ ID NO:2, a S166D/Q167H mutant of human CNTF, a T169I/H174A mutant of human CNTF, a D30Q mutant of human CNTF, a F152A mutant of human CNTF, a D30Q/S166D/Q167H mutant of human CNTF, a F152A/S166D/Q167H mutant of human CNTF, or a D30Q/F152A/S166D/Q167H mutant of human CNTF.

[0008] In one aspect, the method of the invention is practised on a subject having a disease or disorder which is associated with cardiac hypertrophy. For example, the disease or disorder can be diabetes, obesity, hypertension, heart failure, dilated cardiomyopathy, ischemic cardiomyopathy, hypertrophic cardiomyopathy, or myocardial infarction.

[0009] In another aspect, the method further comprises the step of performing echocardiography on the subject. The echocardiography can be performed either before or after administering the CNTF agonist. The echocardiography is used to assess the extent of or risk of developing cardiac hypertrophy, for example by determining left ventricular mass, left ventricular dimensions, left ventricular end diastolic diameter, interventricular septal thickness in diastole, or posterior wall thickness. In a related embodiment, the method comprises performing myocardial biopsy. The width of cardiac myocytes obtained from the biopsy sample can be used to assess the extent of or risk of developing cardiac hypertrophy.

[0010] In yet another aspect, the invention provides the opportunity to treat cardiac hypertrophy in patients who are either leptin resistant or leptin deficient. Such patients are especially at risk for developing cardiac hypertrophy, which cannot be addressed by leptin supplementation.

[0011] Further features and advantages of the invention and further embodiments will become more fully apparent in the following description of the embodiments and drawings thereof, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0012] Figures 1a – 1f demonstrate the expression of CNTFR in the heart. Immunohistochemistry with peroxidase staining performed on longitudinal (a and b) and transverse (c and d) sections of the heart show the presence of CNTFR in the heart. Double immunofluorescence staining for desmin (green) and CNTFR (red) localized its presence to the myocyte (e). Figs. 1b, 1d, and 1f are negative controls omitting the primary antibody. Fig. 1g is a Western blot on isolated cardiomyocytes from C57BL/6 (wt), *ob/ob* and *db/db* mice confirming the presence of CNTFR in the myocyte. Bands corresponding to the 60-kDa glycosylated and 40-kDa nonglycosylated portions of the receptor can be seen. Mouse brain (mb) was used as a positive control.

[0013] Figures 2a – 2g show the regression of LVH with CNTF treatment in *ob/ob* and *db/db* mice. M-mode echocardiograms after 4 weeks of intervention of 5–6 month old *ob/ob* mice fed ad libitum (a), treated with CNTFAx15 (b), or calorie-restricted (c); and *db/db* mice fed ad libitum (d), treated with CNTFAx15 (e), calorie restricted (f), or treated with leptin (g). Notable findings include reduction in both septal and posterior wall thickness in the CNTFAx15-treated animals compared with other groups. Figures 2h and 2i are graphical demonstrations of significant decreases in posterior wall thickness in diastole (PWTd) in *ob/ob* mice (h; *, $P = 0.000022$) and *db/db* mice (i; *, $P = 0.029$; †, $P = 0.00019$) with CNTFAx15 treatment. AL, ad libitum; LEP, leptin; CR, calorie restriction. Arrows denote endo- and epicardial borders of heart wall.

[0014] Figures 3a – 3g depict CNTFAx15-mediated reduction of myocyte width in *ob/ob* and *db/db* mice. Histology sections stained with hematoxylin/eosin from *ob/ob* mice fed ad libitum (a), treated with CNTFAx15 (b), or calorie-restricted (c); and *db/db* mice fed ad libitum (d), treated with CNTFAx15 (e), calorie-restricted (f), or treated with leptin (g). Figures 3h and 3i are graphical demonstrations that myocytes from CNTFAx15-treated *ob/ob* (h) and *db/db* (i) mice had decreased cell width compared to the control groups (*, $P < 0.0001$). AL, ad libitum; LEP, leptin; CR, calorie restriction. (Scale bar: 5 microns.) Arrows denote borders of cardiac myocytes.

[0015] Figures 4a and 4b show that leptin and CNTFAx15 activate STAT3 and ERK pathways. (a) Western blot analysis demonstrates time-dependent STAT3 phosphorylation in isolated adult mouse cardiomyocytes after exposure to leptin (squares; 50 ng/μl) or CNTFAx15. (circles; 50 ng/μl). STAT3 phosphorylation occurred within 15 min and subsided over time. (b) Similar activation occurred in the ERK1/2 pathway, with the effect of CNTFAx15 being more prolonged. [*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ vs. time 0; §, $P < 0.05$ between groups (two-way ANOVA).] Figures 4c and 4d show immunoblots from *ob/ob* mice (filled bars) and *db/db* mice (hatched bars) demonstrating increased abundance in both phosphorylated STAT3 and STAT3 expression with 4 weeks of CNTFAx15 (CNTF) treatment. Leptin (LEP) increased STAT3 abundance in *ob/ob* but not *db/db* mice. CR, calorie restriction. [*, $P < 0.05$; †, $P < 0.01$ vs. ad libitum (AL).]

[0016] Figures 5a and 5b show the impact of leptin and $CNTF_{Ax15}$ on pJNK and CaN signaling in cultured cardiomyocytes, *ob/ob*, and *db/db* mice. Western blot analysis showed no induction of JNK phosphorylation (*a*) or altered calcineurin (CaN) protein expression (*b*) in isolated adult mouse cardiomyocytes with leptin (*Upper*) or $CNTF_{Ax15}$ (*Lower*) treatment.

[0017] Figures 6a and 6b show the impact of CNTFAx15 on ERK and JNK signal transduction pathways in *ob/ob* and *db/db* mice. Western blot analysis depicts increased ERK phosphorylation and ERK protein expression in cardiac tissue of *ob/ob* (filled bars) and *db/db* mice (hatched bars) with CNTFAx15 (CNTF) treatment but not with leptin (LEP) in db/db mice. Figures 6c and 6d show that CNTFAx15 increased JNK protein abundance in heart tissue in *ob/ob* and *db/db* mice. However, calorie restriction (CR) does not affect JNK protein expression in cardiac tissue of *ob/ob* and *db/db* mice. (*, $P < 0.05$.)

DETAILED DESCRIPTION OF THE INVENTION

[0018] Definitions

[0019] As used herein, the term "an agonist of ciliary neurotrophic factor" or "an agonist of CNTF" refers to a ligand which activates a cardiac CNTF receptor. CNTF agonists include CNTF polypeptides from humans and other mammalian species, both in their native form and analogs or derivatives thereof, including polypeptide fragments and polypeptides having amino acid substitutions compared with a naturally ocurring form of CNTF. CNTF analogs include CNTF polypeptides possessing one or more naturally occuring variations (e.g., polymorphisms) of a CNTF amino acid sequence, and also include synthetic or recombinant forms. CNTF agonists include truncations of naturally occuring CNTFs, fragments resulting from enzymatic or chemical cleavage, and fragments of a naturally occuring CNTF produced using recombinant DNA technology. CNTFR agonists include recombinant forms derived from one or more naturally occuring CNTFs by inclusion of conservative amino acid substitutions, deletion of one or more amino acids, and amino acid substitutions that improve the stability, storage, or pharmacokinetics of CNTF, for example by improving its absorption into the body or its resistance to proteases. Examples of CNTFR agonists include human CNTF, mouse CNTF, polypeptides comprising an amino acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2, and a polypeptide comprising amino acids 1 – 185 of SEQ ID NO:1.

The polypeptide of SEQ ID NO:2 is also known as CNTFAx15, which is available from Regeneron Pharmaceuticals, Tarrytown, NY, and is sold under the name "Axokine". See also Lambert, et al., Proc. Natl. Acad. Sci. USA 98:4652-4657 (2001), hereby incorporated by reference. Further examples of CNTFR agonists also include mutants described in Di Marco et al. (J. Biol. Chem. 272:23069-23075 (1997), which is hereby incorporated by reference. See in particular Table I of Di Marco et al., which lists several mutants of CNTF, including S166D/Q167H, T169I/H174A, D30Q, F152A, D30Q/S166D/Q167H, F152A/S166D/Q167H, and D30Q/F152A/S166D/Q167H.

[0020] The amino acid sequence of human CNTF (SEQ ID NO:1) is as follows:

```
  1 maftehsplt phrrdlcsrs iwlarkirsd ltaltesyvk hqglnkninl dsadgmpvas
 61 tdqwseltea erlqenlqay rtfhvllarl ledqqvhftp tegdfhqaih tlllqvaafa
121 yqieelmill eykiprnead gmpinvgdgg lfekklwglk vlqelsqwtv rsihdlrfis
181 shqtgiparg shyiannkkm
```

[0021] The amino acid sequence of human CNTFAx15 (SEQ ID NO:2) is as follows:

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  1 maftehsplt phrrdlasrs iwlarkirsd ltaltesyvk hqglnkninl dsadgmpvas
 61 tdrwseltea erlqenlqay rtfhvllarl ledqqvhftp tegdfhqaih tlllqvaafa
121 yqieelmill eykiprnead gmpinvgdgg lfekklwglk vlqelsqwtv rsihdlrfis
181 shqtg
```

[0022] As used herein, an "effective amount" of a CNTFR agonist is an amount which, when administered to a subject, produces a demonstrable effect of activation of a cardiac CNTFR. Assays which can be employed to assess whether a particular ligand activates a cardiac CNTF receptor include measurement of heart morphology (e.g., by echocardiogram or other imaging method) or measurement of cardiac myocyte width in *ob/ob* or *db/db* mice, as well as determination of activation of the STAT3 or ERK1/2 pathways, as shown in the Examples. Furthermore, the activation of CNTFR can be demonstrated by measuring activation of either the beta subunit (LIFR) or the gp130 subunit of the CNTFR.

[0023] As used herein, "cardiac hypertrophy" refers to an increase in size, usually a thickening of the myocardium. Cardiac hypertrophy can be assessed by imaging techniques, including echocardiography, NMR, and CT scan, and by determining myocyte width from tissue obtained through myocardial biopsy. For the purposes of the invention, any type of cardiac hypertrophy can be treated by administration of a CNTFR agonist, including left ventricular hypertrophy, and cardiac hypertrophy associated with diabetes, obesity, hypertension, heart failure, dilated cardiomyopathy, ischemic cardiomyopathy, hypertrophic cardiomyopathy, and myocardial infarction. Especially preferred is any cardiac hypertrophy associated with obesity caused by either leptin deficiency or leptin resistance.

[0024] A "subject"according to the invention can be any mammal. Preferably, the mammal is a human.

[0025] Description

[0026] The inventors have discovered that the CNTF signaling pathway, which is known to activate a signal transduction pathway which is parallel to that of leptin in the hypothalamus, has important cardiac bioactivity. The CNTF receptor is present in the cardiac sarcolemma, and its activation regresses established LVH in leptin-deficient mice. Signalling by CNTFAx15, a CNTF agonist, has similar cardiac effects in mice with leptin receptor dysfunction, proving that the effects are mediated by CNTFR, not by the leptin receptor. While not intending to limit the invention to any particular mechanism, it was observed that CNTFAx15 influences signal transduction in isolated cardiac myocytes, supporting a role for direct effects on the heart through activation of the phosphorylation of both STAT3 and ERK1/2. The invention has important therapeutic implications, especially given that the majority of human obesity is due to leptin resistance rather than leptin deficiency (4, 8, 9). In leptin-resistant individuals, who would not respond to treatment with leptin or a leptin agonist, a CNTF agonist will regress or prevent cardiac hypertrophy.

[0027] Leptin deficiency contributes to LVH in vivo in intact *ob/ob* mice with established LVH (7). Yet, in normal neonatal myocytes, exposure to leptin in vitro stimulates growth or hypertrophy (21, 22). The apparent dicotomy can be explained because of differences in milieu. Neonatal cells physiologically undergo rapid growth under the influence of normal

trophic factors, unlike adult myocytes. Syed et al. (23) recently provided proof of principle for this concept and showed that although certain genes produced changes in ventricular structure and function in neonatal cells, they had no impact on the adult myocardium. Physiologic differences in characteristics between neonatal and adult myocytes play an important role in their response to external stimuli and the net result to a given treatment may even be the opposite in neonatal vs. adult myocytes (23).

[0028] Both CNTFAx15 and leptin acutely stimulate the phosphorylation of STAT3 in cardiac myocytes and augment total STAT3 levels in association with regression of hypertrophy in *ob/ob* and *db/db* mice. STAT3 phosphorylation is associated with a cardioprotective antiremodeling effect (26, 27). The data obtained by the present inventors demonstrate that CNTFAx15 directly activates signal transduction cascades within cardiac myocytes, and support the dominance of the cardioprotective effects.

[0029] Although the present inventors have demonstrated direct actions of CNTF agonists within the heart (such as STAT3 and ERK1/2 activation), systemic effects may also play a role in the regression of cardiac hypertrophy by CNTF and CNTF agonists. For example, central nervous system effects of CNTFAx15 may directly influence the heart. The leptin/leptin-receptor axis is known to activate the central nervous system (31, 32) and to mediate metabolic actions by acting centrally. Additionally, it is possible that cardiac CNTF receptors are not present solely on cardiac myocytes but also on other tissues, such as cardiac ganglia, raising the possibility of paracrine signaling effects within the heart. Nonetheless, the inventors' finding that CNTFAx15 causes a regression of established LVH, not only in leptin-deficient animals but also in those lacking a functional leptin receptor in the context of stimulating signal transduction within cardiac myocytes, establishes the existence of a previously unrecognized pathway in the regulation of LVH.

[0030] *Dosing and Administration*

[0031] The use of an agonist of CNTFR in treatment or prevention of cardiac hypertrophy involves dosing the patient to produce a serum concentration that is at least 50 percent of the EC50 for activation of cardiac CNTFR, and preferably from 1 to 10 times the EC50 for activating CNTFR in myocardium. In a preferred embodiment, the dose is not more than 5

micrograms per kg body weight per day for a human subject. For example, a CNTFR agonist such as Axokine (consisting of the amino acid sequence shown in SEQ ID NO:2) can be administered by a single daily subcutaneous injection in the range of 0.1 to 1.0 micrograms per kg body weight. Administration can be by any route, such as oral or parenteral, but subcutaneous, intravenous, or intramuscular injection is preferred for CNTFR agonists which are polypeptides.

[0032] *Evaluation of Cardiac Hypertrophy*

[0033] Any method known in the art can be used to evaluate the extent of or risk for developing cardiac hypertrophy. Preferred methods are echocardiography, cardiac magnetic resonance (CMR) imaging, and multidetector cardiac computerized tomography (MDCT) scan. Each of these methods is well known in the art. See for example Foppa et al., Cardiovasc. Ultrasound 3:17 (2005); Sechtem et al., Ernst Schering Res. Found. Workshop 55:261 (2006); and Woodard et al., Semin. Ultrasound CT MR, 27:56 (2006); each of these publications is hereby incorporated by reference.

[0034] EXAMPLES

[0035] The examples below are non-limiting and are merely representative of various aspects and features of the subject invention. All documents mentioned herein are incorporated herein by reference in their entirety.

[0036] Example 1

[0037] Presence of CNTFR in Cardiac Tissue.

[0038] *General Methods*

[0039] *Histology*. Mouse hearts were harvested, washed with ice-cold PBS, sectioned, and either fixed in 10% formalin or in Streck's tissue fixative (Streck Laboratories, Omaha, NE) overnight and were paraffin embedded the next morning. Histochemical and immunohistochemical studies were carried out on 4-μm-thick sections.

[0040] *Immunohistochemistry.* For antigen retrieval, the sections were steamed with Target Retrieval System (DAKO) for 20 min, before blocking with avidin and biotin blocking solutions (DAKO) and 10% normal goat serum. The CNTF receptor was stained with a monoclonal mouse anti-CNTFR-α (BD Pharmingen, No. 558783; 1:100 dilution). After washing, the secondary antibody (biotinylated goat anti-mouse Ig; E0433, DAKO; 1:400 dilution) and streptavidin peroxidase (Vectastain, Vector Laboratories) were applied.

[0041] The peroxidase activity was developed 5 min with 0.066% diaminobenzidine/0.01% H_2O_2/2.5% $NiSO_4$. Slides were cleared in xylene, coverslipped, and viewed on a Zeiss Axiovert 200 microscope equipped with 510-Meta confocal laser scanning module.

[0042] *Fluorescence.* Tissue sections were rinsed with ice-cold PBS twice for 2 min and were blocked with 10% goat serum for 30 min. The slides were incubated with rabbit anti-human desmin antibody (Accurate Chemical & Scientific, no. YMPS31) and monoclonal mouse anti-CNTFR-α antibody (BD Pharmingen, no. 558783) at 4°C overnight. After washing, FITC and rhodamine coupled secondary antibodies (Vector Laboratories) were applied for 45 min at room temperature. Tissue slides were washed and coverslipped with mounting medium and viewed.

[0043] *Western Blots.* Hearts from *ob/ob*, *db/db*, and WT mice were harvested after cervical dislocation and rinsed in isolation buffer to remove excess blood. Cells were isolated as described by Minhas et al. (34). Isolated myocytes were homogenized in cold cell lysis buffer (Cell Signaling Technology, Beverly, MA) with one tablet of complete protease inhibitors (Roche Diagnostics) per 10 ml of buffer. The protein lysate was quantitated with the bicinchonic assay (Pierce), and 30–50 μg of total protein was separated in 4–12% Bis-Tris•HCl polyacrylamide gels (Invitrogen). The proteins were transferred onto nitrocellulose membranes and blocked for 1 h with 5% nonfat milk. After blocking, the membranes were incubated with monoclonal anti-CNTFR antibody (1:1,000, BD Pharmingen), polyclonal anti-phospho-STAT3 antibody, polyclonal anti-phospho-ERK1/2 antibodies, polyclonal anti-phospho-JNK antibodies (all 1:1,000, Cell Signaling Technology), or monoclonal anti-calcineurin antibodies (1:2,000, Chemicon International) overnight at 4°C. Immunoblots were detected by using enhanced chemiluminescent kits (SuperSignal, Pierce) and analyzed with a densitometer (Bio-Rad). The membranes were then stripped and reprobed with polyclonal

anti-STAT3 antibody, polyclonal anti-ERK1/2 antibodies, polyclonal anti-JNK antibodies (all 1:1,000, Cell Signaling Technology), or monoclonal anti-GAPDH antibodies (1:10,000, Research Diagnostics). Mouse brain was used as a positive control for calcineurin.

[0044] *Results.* To determine whether CNTFRs were present in the heart, CNTFRα was visualized using peroxidase staining of myocytes in both longitudinal and transverse sections of C57bl/6 wt mouse hearts (Fig. 1a and 1c). Double immunofluorescence staining for desmin and CNTFR localized the presence of CNTFR to the sarcolemma (Fig. 1e), which was further confirmed by immunoblots (60-kDa glycosylated and 40-kDa nonglycosylated bands) on isolated myocytes (Fig. 1g). Mouse brain (mb) was used as a positive control.

[0045] Example 2

[0046] Regression of LVH in *ob/ob* Mice with CNTFAx15.

[0047] *Ob/ob* mice were obtained from The Jackson Laboratory. All animals were housed under diurnal lighting conditions and allowed food and tap water ad libitum except the calorie-restricted groups, where each mouse received 1 g of food per day.

[0048] *Sample Size and Statistical Analysis.* Sample size was estimated as five to seven animals in each group for statistical power >0.80 to determine regression of hypertrophy and LVM. Data are reported as mean ± SEM. Statistical significance was determined by paired t tests (GraphPad (San Diego) INSTAT statistical software), or by two-way ANOVA with Bonferroni correction [STATA (StataCorp LP, College Station, TX) statistical software]. P values <0.05 were considered significant.

[0049] *Treatment by Administration of Exogenous Leptin, CNTFAx15, and Calorie Restriction.* Five- to 6-month-old *ob/ob* and *db/db* mice were randomly assigned to three or four groups, respectively, and treated for a period of 4 weeks. The first group received CNTFAx15, the second group was calorie-restricted so as to lose similar weight as treated animals, and the third group was fed ad libitum as controls. Additional *db/db* mice were assigned to a fourth group to receive leptin. Mice in the CNTFAx15 group were injected daily with recombinant mouse CNTFAx15 (Regeneron Pharmaceuticals, Tarrytown, NY, 0.1 mg•kg–1 per day), whereas the leptin group were injected with recombinant mouse leptin (R

& D Systems, 1 mg•kg–1 per day). The calorie restriction regimen consisted of 1 g of food per day, which was previously determined to result in a similar rate of weight loss to leptin or CNTFAx15 administration at these doses (7). The calorie-restricted groups and the ad libitum controls were injected with Dulbecco's PBS.

[0050] *Echocardiography.* To examine the impact of CNTFAx15 and leptin on established LVH (Table 1) in leptin-deficient *ob/ob* mice (7), echocardiograms were performed on mice randomized to one of three treatment groups: CNTFAx15 (n = 11, 5–6 months old, weight: 70 ± 2 g), calorie restriction (n = 9, 5–6 months old, weight: 71 ± 2 g), and controls fed ad libitum (n = 11, 5–6 months old, weight: 70 ± 1 g). The effects of CNTFAx15 (n = 8, 5–6 months old, weight: 60 ± 2 g), calorie restriction (n = 8, 5–6 months old, weight: 60 ± 2 g), leptin (n = 7, 5–6 months old, weight: 58 ± 2 g), and controls fed ad libitum (n = 7, 5–6 months old, weight: 59 ± 1 g) on LVH after a 4-week treatment period in *db/db* mice were also investigated. Echocardiography was performed on conscious unanesthetized mice at baseline and repeated at the end of the treatment period. The individual performing the echocardiograms was blinded to the group assignments. Mice were trained before each study until they were relaxed for the procedure. Studies were performed by using a Sequoia C256 (Siemens, Mountain View, CA) echocardiogram with a 15 MHz linear array transducer. Interventricular septal and posterior wall thicknesses, as well as diastolic and systolic LV dimensions were recorded from M-mode images by using averaged measurements from three to five consecutive cardiac cycles. LVM was calculated by using LVM = 1.055 x (interventricular septal thickness in diastole thickness + posterior wall thickness + left ventricular end diastolic diameter)^3 – (left ventricular end diastolic diameter)^3.

[0051] *Results.* CNTFAx15 regressed cardiac hypertrophy, decreasing interventricular septal thickness by 20 ± 3% (P = 0.000035) posterior wall thickness by 29 ± 8%, (P = 0.000022), (Fig. 2b and 2h), and calculated left ventricular (LV) mass by 21 ± 9%, (P = 0.0077). In contrast, calorie restriction did not reduce LVH in these mice (Table 2). The degree of regression with CNTFAx15 restored wall thickness and left ventricular mass (LVM) essentially to normal (Table 2). A reduction in LVM was noted in the calorie restricted group, but this decrease was due entirely to a smaller chamber size in these animals and, in the absence of reduced wall thickness, is unlikely to represent true regression of LVH.

Table 1. Baseline body weights and echocardiographic parameters in WT, *ob/ob,* and *db/db* mice prior to the start of intervention

Parameter	WT	*ob/ob*	*db/db*
No. of mice	15	31	30
Age, months	5	5–6	5–6
BW, g	22 ± 0	70 ± 1*	59 ± 1*†
IVSd, mm	0.76 ± 0.03	1.10 ± 0.02*	1.03 ± 0.03*
PWTd, mm	0.85 ± 0.02	1.03 ± 0.02*	0.96 ± 0.02*
LVEDd, mm	3.26 ± 0.10	3.51 ± 0.07	3.59 ± 0.07‡
LVEDs, mm	1.63 ± 0.06	1.51 ± 0.07	1.51 ± 0.07
LVM, mg	87 ± 6	143 ± 4*	138 ± 5*

BW, body weight; IVSd, interventricular septal thickness in diastole; PWTd, posterior wall thickness in diastole; LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter.

*, $P < 0.01$ vs. WT;

†, $P < 0.01$ vs. *ob/ob*; and

‡, $P < 0.05$ vs. WT by one-way ANOVA.

Table 2. Changes in echocardiographic parameters after intervention in *ob/ob* mice

Parameter	Fed ad libitum ($n = 11$)		$CNTF_{Ax15}$-treated ($n = 11$)		Calorie-restricted ($n = 9$)	
	Before	After	Before	After	Before	After
BW, g	70 ± 1	72 ± 1	70 ± 2	43 ± 2*	71 ± 2	46 ± 2*
IVSd, mm	1.11 ± 0.04	1.15 ± 0.04	1.13 ± 0.04	0.90 ± 0.03*	1.05 ± 0.04	0.95 ± 0.04
PWTd, mm	1.02 ± 0.03	1.12 ± 0.03	1.10 ± 0.03	0.86 ± 0.03*	0.97 ± 0.02	0.90 ± 0.04
LVEDd, mm	3.60 ± 0.14	3.29 ± 0.10	3.38 ± 0.09	3.20 ± 0.09	3.56 ± 0.16	3.22 ± 0.08
LVEDs, mm	1.49 ± 0.10	1.19 ± 0.06†	1.50 ± 0.11	1.12 ± 0.03†	1.54 ± 0.15	1.23 ± 0.05†
LVM, mg	149 ± 7	144 ± 6	147 ± 10	106 ± 14*	135 ± 5	100 ± 5*

*, $P < 0.01$ vs. preintervention baseline and

†, $P < 0.05$ vs. preintervention baseline by paired t tests. Abbreviations are as per Table 1.

[0052] Example 3

[0053] Regression of Myocyte Width in *ob/ob* Mice with CNTFAx15.

[0054] *Histochemical Staining.* Hematoxylin/eosin staining was performed to evaluate cell sizes. Myocyte diameters were measured in regions of myocardium with parallel myocyte fascicles in longitudinal sections by using IMAGEJ (National Institutes of Health). Representative high-powered-fields distributed around the myocardium were used to measure 7–29 cells from each heart. The individual analyzing histology data were blinded to the group assignments.

[0055] *Results.* To determine whether the observed impact of CNTFAx15 on LVH could be attributed to a regression of myocyte hypertrophy, histological studies were performed with hematoxylin/eosin staining on *ob/ob* mouse hearts (n = 3–5) from each of the three groups (controls fed ad libitum, calorie restricted, and CNTFAx15 treated), at the end of their treatment period. Myoycte width with CNTFAx15 in the *ob/ob* mice was reduced to 9.0 ± 0.3 μm compared to 11.3 ± 0.5 μm in those fed ad libitum ($P < 0.001$), and, in the calorie-restricted mice, 12.0 ± 0.4 μm ($P < 0.001$). There was no significant difference in cell size between the calorie restricted and ad libitum groups (Fig. 3b and 3h). For comparison, myocyte width in age-matched C57bl/6 wt mice is 8.2 ± 0.2 μm (20).

[0056] Example 4

[0057] Regression of LVH in *db/db* Mice with CNTFAx15.

[0058] *Db/db* mice were obtained from The Jackson Laboratory. All animals were housed under diurnal lighting conditions and allowed food and tap water ad libitum except the calorie-restricted groups, where each mouse received 1 g of food per day.

[0059] *Results.* The impact of CNTFAx15 on LVH was investigated in leptin-receptor deficient *db/db* mice. CNTFAx15 reduced septal thickness by 27 ± 6% ($P = 0.0068$), posterior wall thickness by 21 ± 3% ($P = 0.00019$) (Fig. 2e and 2i), and calculated LV mass by 41 ± 6% ($P = 0.00064$), restoring cardiac architecture toward normal. CNTFAx15 also significantly reduced LV end-systolic and -diastolic dimensions ($P = 0.042$ and 0.013, respectively) (Table 3). Importantly, as predicted on the basis of an absent leptin receptor,

leptin did not change LV wall thickness or LVM in *db/db* mice (Table 3). LVM was decreased in the calorie-restricted group; however, as discussed earlier, this reduction is most likely attributable to a decrease in chamber size.

Table 3. Changes in echocardiographic parameters post intervention in *db/db* mice.

	Fed *Ad libitum* (n=7)		$CNTF_{Ax15}$ Treated (n=8)		Calorie Restricted (n=8)		Leptin Treated (n=7)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BW, gm	59±1	62±2	60±2	43±1*	60±2	41±1*	58±2	61±2
IVSd, mm	0.94±0.06	1.08±0.06	1.11±0.07	0.79±0.05*	1.02±0.02	0.93±0.06	1.05±0.06	1.11±0.02
PWTd, mm	0.90±0.04	1.04±0.05†	1.02±0.02	0.80±0.04*	0.94±0.02	0.94±0.05	1.02±0.04	1.08±1.02
LVEDd, mm	3.29±0.17	3.33±0.15	3.65±0.13	3.31±0.08*	3.80±0.12	3.22±0.08*	3.56±0.12	3.31±0.08
LVEDs, mm	1.21±0.11	1.09±0.18	1.50±0.13	1.20±0.02†	1.72±0.14	1.25±0.05†	1.53±0.06	1.23±0.04*
LVM, mg	107±11	135±6	150±7	87±8 *	148±7	103±8 *	143±5	137±4

*$P<0.01$ vs. pre-intervention baseline and †$P<0.05$ vs. pre-intervention baseline by paired t-tests. Abbreviations as per table 1.

[0060] Example 5

[0061] Regression of Myocyte Width in *db/db* Mice with CNTFAx15.

[0062] Assessment of myocyte width in *db/db* mice by hematoxylin/eosin staining showed that CNTFAx15 regressed toward normal (9.3 ± 0.3 microns), while leptin-treated (12.6 ± 0.6 microns; $P < 0.001$), calorie-restricted (10.8 ± 0.4 microns; $P < 0.05$), and ad libitum controls (11.4 ± 0.5 microns; $P < 0.01$) (Fig. 3e and 3i) did not regress.

[0063] Example 6

[0064] CNTFAx15 Signal Transduction.

[0065] To determine whether CNTFAx15 and leptin activated similar signal transduction pathways in isolated cardiac myocytes, the activation of STAT3, ERK1/2 and JNK pathways, and calcineurin (CaN) signaling was determined.

[0066] *Isolated Myocyte Preparation.* Cardiac myocytes were isolated and prepared from mouse hearts as described in detail by Khan et al. (33). Myocytes were plated with 80% confluency into 35-mm dishes (Falcon) containing a serum-free 500 μM Ca^{2+} containing isolation solution for 30 min. Myocytes were then treated with 50 ng/μl leptin and 50 ng/μl CNTFAx15 for various periods of time.

[0067] *Results.* CNTFAx15 and leptin produced nearly identical phosphorylation of STAT3 (Fig. 4a). Similarly, CNTFAx15 and leptin both led to the phosphorylation of ERK1/2, although the potency of this effect was greater with CNTFAx15 (Fig. 4b). Neither CNTFAx15 nor leptin affected JNK phosphorylation or CaN levels in isolated myocytes (Fig. 5). In terms of chronic 4-week therapy, the predominant effect was an increase in STAT3 phosphorylation and protein expression after CNTFAx15 treatment. Although the p-STAT3/STAT3 ratio was not increased with CNTFAx15, the overall STAT3 phosphorylation and total STAT3 abundance were higher in cardiac tissue from CNTFAx15-treated *ob/ob* (Fig. 4c) and *db/db* mice (Fig. 4d). CNTFAx15 treatment resulted in a similar effect on the ERK1/2 pathway (Fig. 6a and 6b). As expected, leptin did not produce this effect in *db/db* mice lacking the leptin receptor. Consistent with the isolated cardiomyocyte data, JNK

phosphorylation and CaN protein level were not increased in the cardiac tissue. However, total JNK protein expression was augmented (Fig. 6c and 6d).

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CLAIMS

1. A method of treating or preventing cardiac hypertrophy, comprising administering an effective amount of an agonist of ciliary neurotrophic factor receptor (CNTFR) to a subject who has cardiac hypertrophy or is at risk of developing cardiac hypertrophy.

2. The method of claim 1, wherein the agonist is selected from the group consisting of: human CNTF, a polypeptide comprising amino acids 1 – 200 of SEQ ID NO:1, a polypeptide comprising amino acids 1 – 185 of SEQ ID NO:1, a polypeptide having the amino acid sequence of SEQ ID NO:2, a S166D/Q167H mutant of human CNTF, a T169I/H174A mutant of human CNTF, a D30Q mutant of human CNTF, a F152A mutant of human CNTF, a D30Q/S166D/Q167H mutant of human CNTF, a F152A/S166D/Q167H mutant of human CNTF, and a D30Q/F152A/S166D/Q167H mutant of human CNTF.

3. The method of claim 1, wherein the cardiac hypertrophy is left ventricular hypertrophy.

4. The method of claim 1, wherein the subject has a disease or disorder selected from the group consisting of diabetes, obesity, hypertension, heart failure, dilated cardiomyopathy, ischemic cardiomyopathy, hypertrophic cardiomyopathy, and myocardial infarction.

5. The method of claim 1, further comprising the step of performing a cardiac imaging based assessment on the subject.

6. The method of claim 5, wherein the imaging based assessment is performed prior to administering the agonist.

7. The method of claim 5, wherein the imaging based assessment is performed after administering the agonist.

8. The method of claim 5, wherein the imaging based assessment is used to determine left ventricular mass, left ventricular dimensions, left ventricular end diastolic diameter, interventricular septal thickness in diastole, or posterior wall thickness.

9. The method of claim 5, wherein the imaging based assessment is selected from the group consisting of echocardiography, NMR, and CT scan.

10. The method of claim 1, further comprising the step of performing myocardial biopsy on the subject.

11. The method of claim 10, wherein myocardial biopsy is performed prior to administering the agonist.

12. The method of claim 10, wherein myocardial biopsy is performed after administering the agonist.

13. The method of claim 10, wherein cardiac myocyte width is determined from the biopsy.

14. The method of claim 1, wherein the CNTFR agonist is administered at a dose of not more than 5 micrograms per kg body weight and the subject is human.

15. The method of claim 14, wherein the CNTFR agonist is a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 and the dose is in the range of 0.1 to 1.0 micrograms per kg body weight.

16. The method of claim 1, wherein the subject is leptin resistant.

17. The method of claim 1, wherein the subject is leptin deficient.

18. The method of claim 1, wherein the administration of CNTFR agonist is by subcutaneous injection.

19. The method of claim 1, wherein the administration of CNTFR agonist is performed daily.

20. The method of claim 1, wherein the administration of CNTFR agonist is performed over a period of at least four months.

21. The method of claim 1, wherein the CNTFR agonist is synthetic.

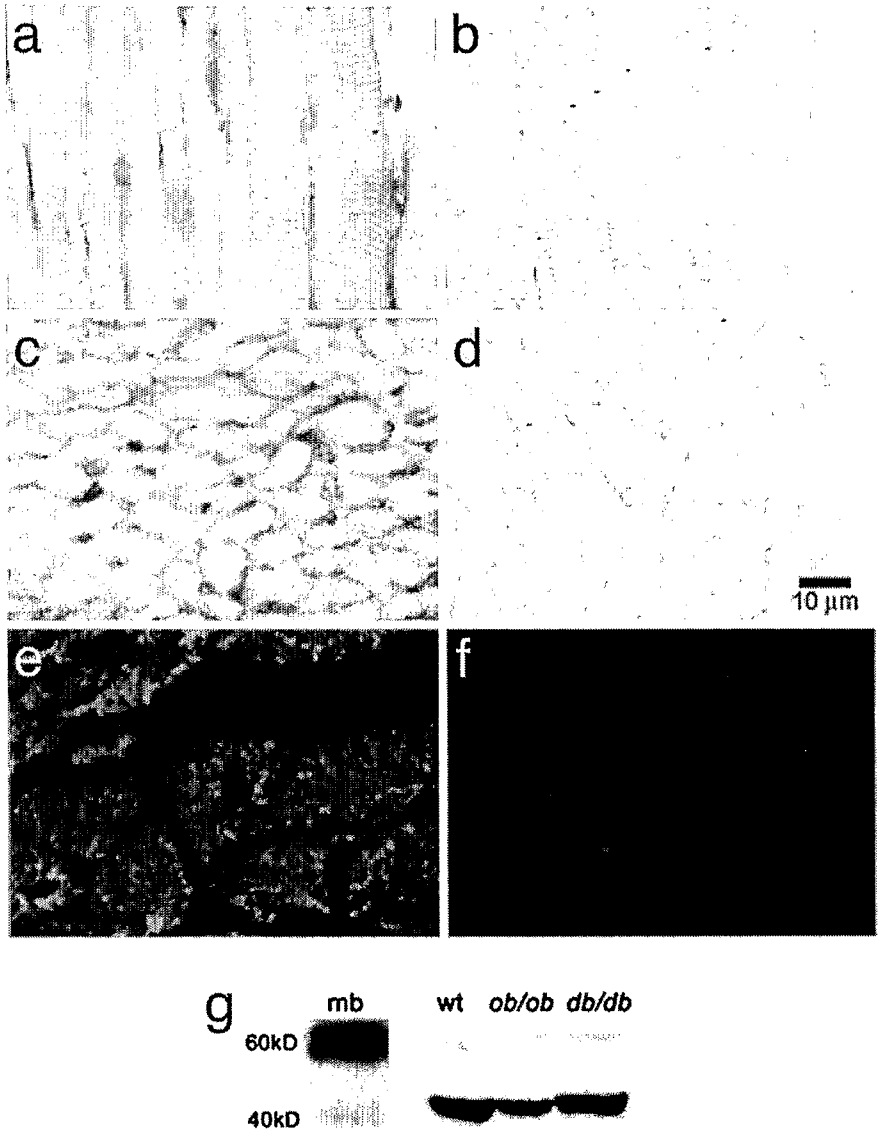
22. The method of claim 1, wherein the CNTFR agonist is a naturally occuring polypeptide.

23. The method of claim 1, wherein the CNTFR agonist activates leukemia inhibitory factor receptor (LIFR).

24. The method of claim 1, wherein the CNTFR agonist activates gp130.

25. The method of claim 1, wherein the CNTFR agonist increases phosphorylation of STAT3.

27. The method of claim 1, wherein the CNTFR agonist increases phosphorylation of ERK.



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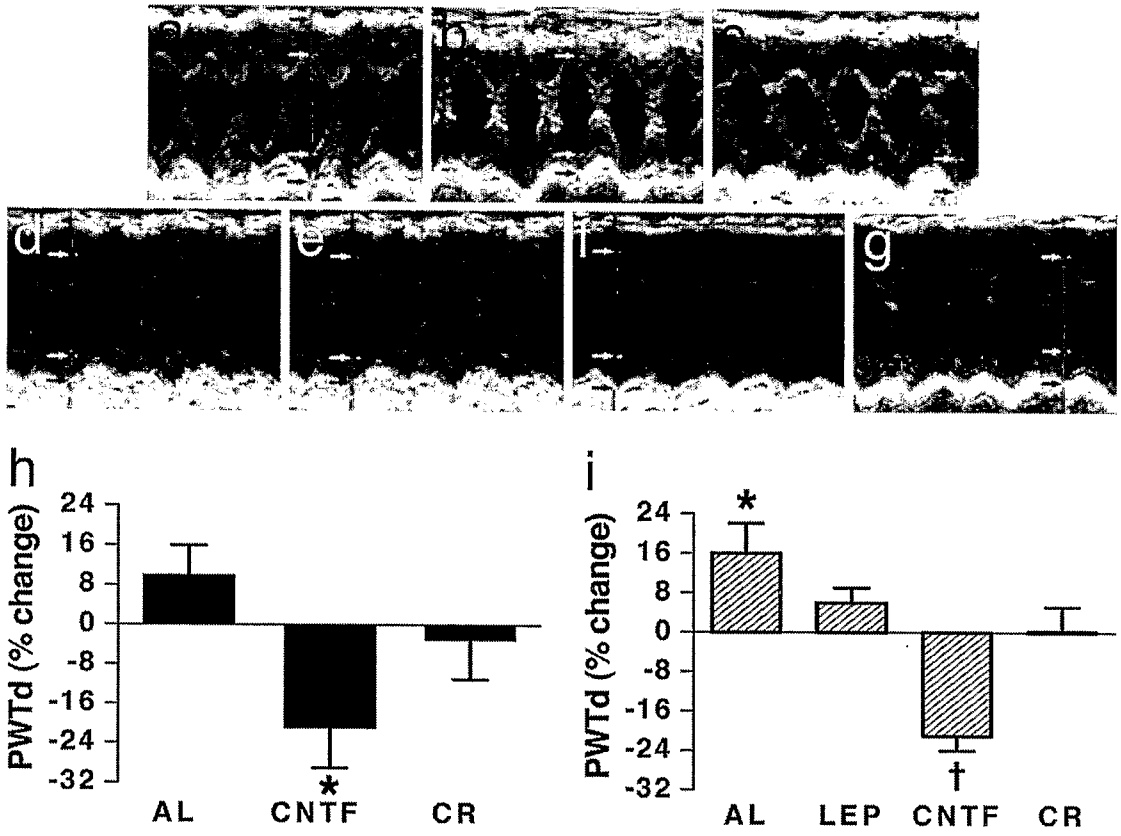
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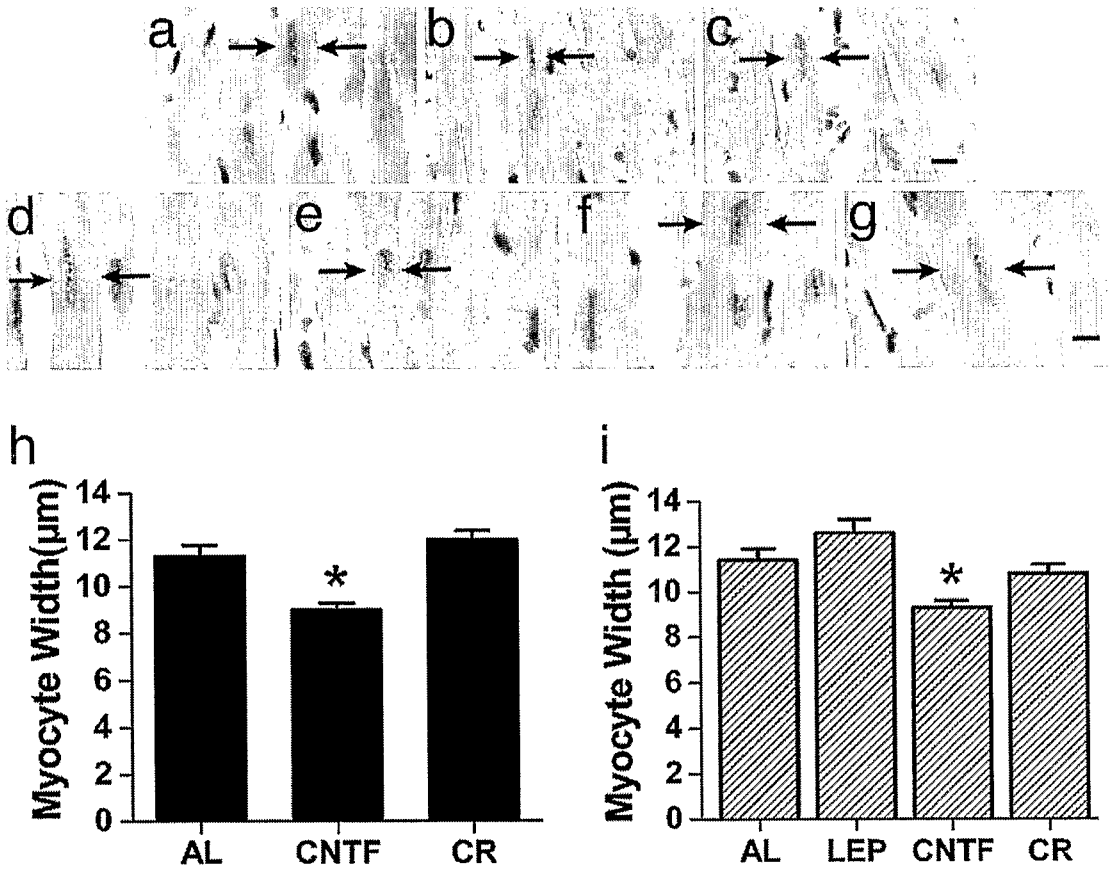
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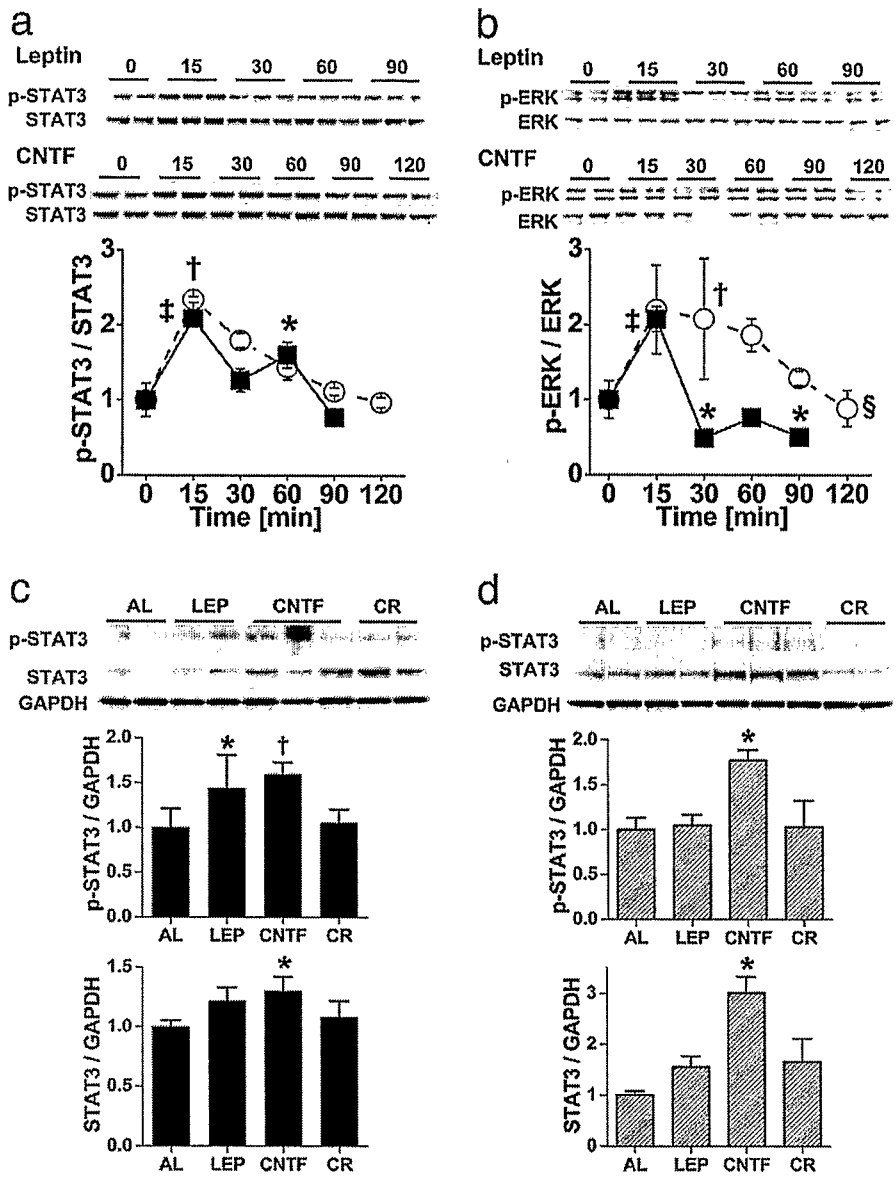
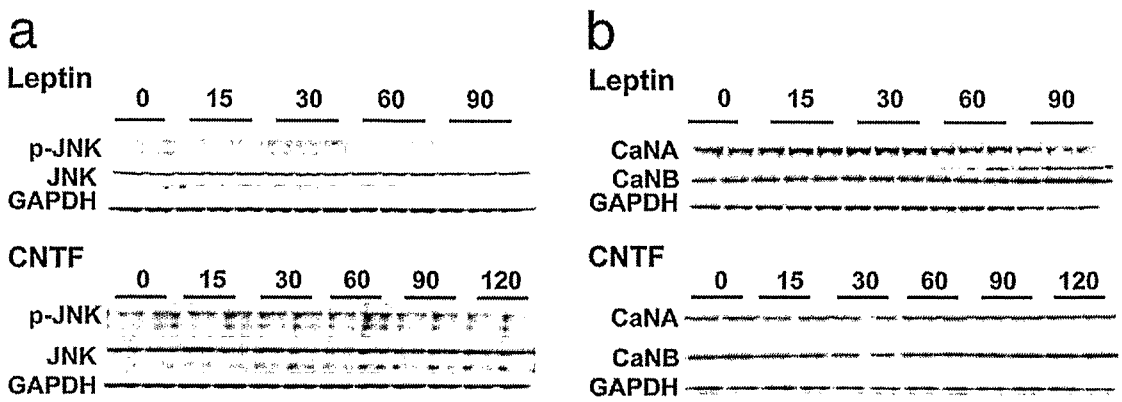
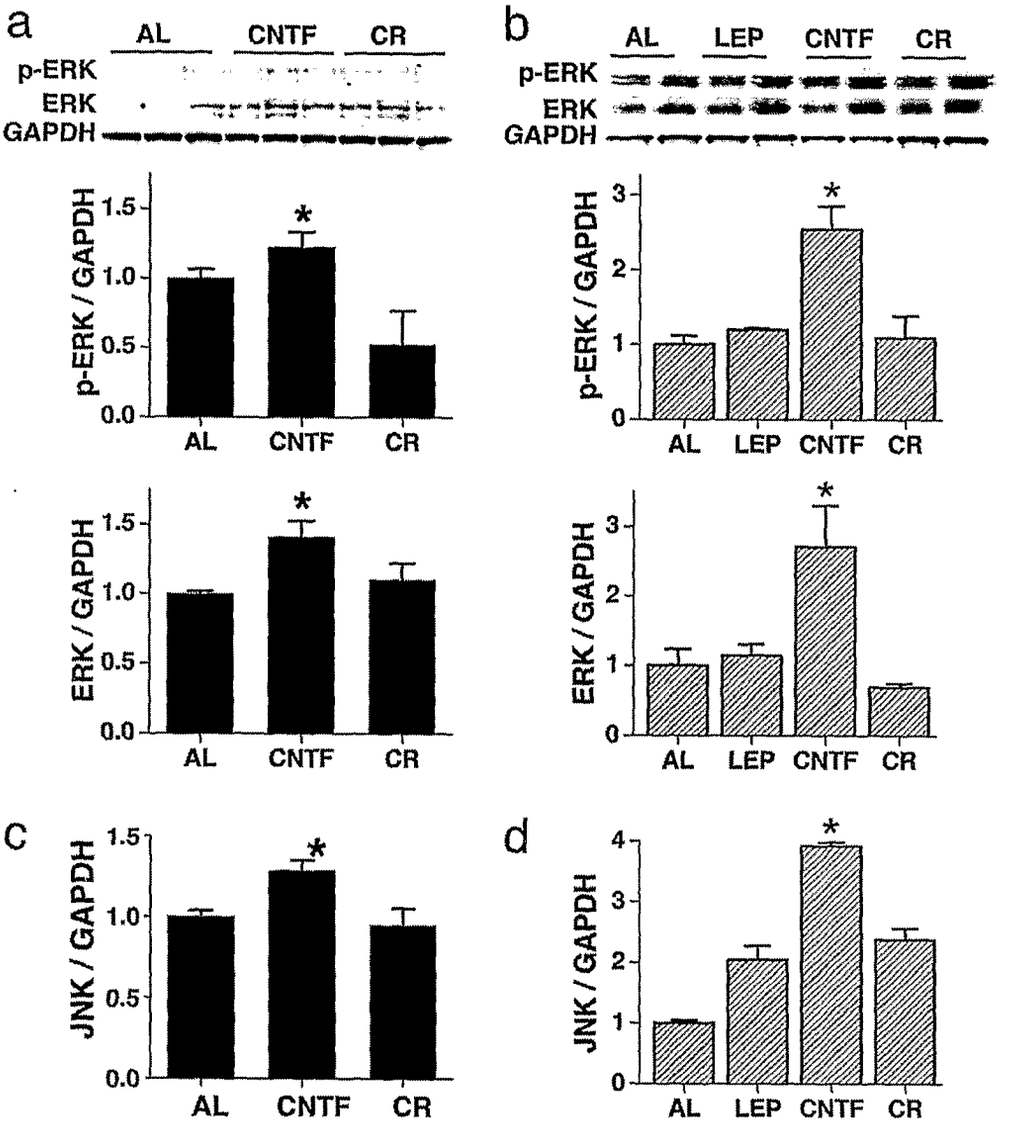


Figures 4A and 4B



Figures 5A and 5B



Figures 6A and 6B

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