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肥胖症心臟凋亡途徑與治療的探討：肥胖症心臟 Type I 和 Type II 的凋亡; ACE inhibitor 治療對肥胖心臟凋亡的保護 ;運動治療對肥胖心臟凋亡的改善 (1/2)

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MORE ACTIVATED CARDIAC FAS RECEPTOR-DEPENDENT APOPTOTIC PATHWAY IN OBESE ZUCKER RATS

Chih-Yang Huang and Shin-Da Lee

Abstract

Background. Obesity is often associated with the development of heart failure but the precise mechanisms remain uncertain. The purpose of this study was to evaluate the key components of Fas receptor-dependent apoptotic pathway in excised hearts from obese Zucker rats. ***Methods.*** Twelve obese Zucker rats were studied at 5~6 months of age and twelve age-matched lean Zucker rats served as control. The myocardial architecture, key components of Fas receptor-dependent apoptotic pathway, apoptotic activity, and fibrosis in the excised left ventricle from rats were measured by Hematoxylin-eosin staining, Western blotting, RT-PCR, TUNEL assay and Masson trichrome staining. ***Results.*** The ratios of whole heart weight to tibia length were significantly increased in the obese group. Cardiomyocyte disarray, increased interstitial space, TUNEL-positive cardiac myocytes and minor cardiac fibrosis were observed in obese rat hearts. The Fas ligand, Fas death receptors, and FADD were all significantly increased in obese rat hearts. In addition, pro-caspase-8 and pro-caspase-3 were significantly decreased whereas activated caspase-8 and activated caspase-3 were significantly increased in obese rat hearts, compared with lean rat heart, imply pro-forms of caspase-8 and caspase-3 were cleaved into active-forms caspase-8 and caspase-3. ***Conclusions.*** The cardiac Fas receptor-dependent apoptotic pathways were more activated in obese rat hearts, which may provide one of possible apoptotic mechanism for developing heart failure in obesity.

Key words: heart, Fas receptor-dependent pathway, Bcl2 family, cytochrome *c*,

caspases

Introduction:

The obese Zucker rat, a genetic model of morbid obesity, presents many of the same cardiopulmonary deficits as noted in obese humans, including respiratory control dysfunction (1-3), chest wall limitations (4), upper airway narrowing (5), hypertension (6), myocardial hypertrophy (7), and poor exercise capacity (8, 9). Severe obesity in human has long been recognized as causing a form of cardiomyopathy characterized by increased rates of hypertension, chronic volume overload, left ventricular hypertrophy and the development of heart failure (10-13). However, the precise mechanisms of cardiac abnormality in sever obesity remain uncertain.

Apoptosis, a physiological program of cellular death, may contribute to many cardiac disorders (14-16). The occurrence of apoptosis has been reported to contribute to the loss of cardiomyocytes in cardiomyopathy, and is recognized as a predictor of adverse outcomes in subjects with cardiac diseases or heart failure (16). The 'extrinsic' Fas receptor-dependent apoptotic pathway was believed to be one of the major pathway directly to trigger cardiac apoptosis (14, 17). This pathway was initiated by binding of Fas ligand to the Fas receptor, which results in clustering of receptors and initiates the extrinsic pathway (17). Fas ligand binding followed by Fas-receptor oligomerisation led to formation of a death-inducing signal complex starting with recruitment of the the Fas-associated death domain (FADD) of the adaptor protein (17). Fas receptor oligomerization recruits FADD and pro-caspase 8 to the complex and results in the activation of caspase 8. The activated caspase 8 cleaves pro-caspase 3, which then undergoes autocatalysis to form active caspase 3, a principle effector caspase of apoptosis (18, 19). After the occurrence of apoptosis or in the end-stage heart failure, the remodeling of the failing myocardium was

associated with excessive collagen deposition and fibrosis (20, 21). Besides, the collagen synthesis appears to replace the space of damaged cardiomyocytes in cardiomyopathy (20, 21). Hence, the cardiac fibrosis following myocardial apoptosis is recognized as a predictor of adverse outcomes in subjects with cardiomyopathy (21, 22). Therefore, the evaluation of apoptosis and fibrosis should be an important issue of predicting the development of obesity-related cardiac abnormality.

The role of cardiac apoptosis in obesity is not understood. In the current study, to understand whether cardiac abnormality in obesity is associated with more activated Fas receptor-dependent apoptotic pathway, the myocardial morphology and key components of Fas receptor-dependent apoptotic pathway were determined by histopathological analysis, Western blotting, and RT-PCR from the exercised cardiac tissue in lean and obese Zucker rats. We hypothesized that cardiac abnormality in obesity may predispose to more activated Fas receptor mediated cardiac apoptosis.

Materials and Methods

Animal model.

The studies were performed on 12 lean (Fa/Fa or Fa/fa) and 12 obese (fa/fa) age matched 5~6 month old male Zucker rats. Animals were born by Zucker breeders purchased from Charles River Lab in France. One lean and one obese rats were obtained from the same breeder and were housed per cage. Ambient temperature was maintained at 25°C and the animals were kept on an artificial 12-h light-dark cycle. The light period began at 7:00 A.M. Rats were provided with standard laboratory chow (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO, USA) and water *ad libitum*. All protocols were approved by the Institutional Animal Care and Use Committee of Chung Shan Medical University, Taichung, Taiwan and the principles of laboratory animal care (NIH publication) were followed.

Cardiac characteristics.

All rats were weighed and decapitated. The hearts of eight lean and eight obese animals were excised and cleaned with dd H_2O. The left and right atrium and ventricle were separated and weighed. The right tibias were also separated and measured tibia length by the electronic digital vernier caliper for correcting the whole heart weight. The ratios of the total heart weight to body weight, the left ventricle weight to the whole heart weight, and the whole heart weight to tibia length was calculated.

Hematoxylin-eosin and Masson trichrome staining

The hearts of six lean and six obese animals were excised and were soaked in formalin and covered with wax. Slides were prepared by first soaking for dehydration. They were passed through a series of graded alcohols (100%, 95% and 75%), 15 minutes of each. The slides were then dyed with hematoxylin and eosin or Masson trichrome. After gently rinsing with water, each slide was then soaked with 85%

alcohol, 100% alcohol I and II for 15 minutes each. At the end, they were soaked with Xylene I– Xylene II. Photomicrographs were obtained using Zeiss Axiophot microscopes.

Terminal Deoxynucleotide Transferase-mediated dUTP Nick End Labeling (TUNEL)

In heart tissues, the 3-μm thick paraffin sections were deparaffinized by immersing in xylene, rehydrated, and incubated in phosphate-buffered saline with 2% H_2O_2 to inactivate endogenous peroxidases. Next, the sections were incubated with proteinase K (20 μg/ml), washed in phosphate-buffered saline, and incubated with terminal deoxynucleotidyl transferase for 90 min and fluorescein isothiocyanate-dUTP for 30 min at 37 °C using an apoptosis detection kit (Roche Applied Science, Indianapolis, IN, USA). Then, the sections were stained with DAPI to detect cell nucleus by UV light microscopic observations (blue). Samples were analyzed in a drop of PBS under a fluorescence and UV light microscope at this state, respectively, by using an excitation wavelength in the range of 450~500 nm and detection in the range of 515~565 nm (green). The number of TUNEL-positive cardiac myocytes was determined by counting 3×10^5 cardiac myocytes. All morphometric measurements were performed by at least two independent individuals in a blinded manner.

Tissue Extraction

Cardiac tissue extracts were obtained by homogenizing the left ventricle samples in a PBS buffer (0.14 M NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 14 mM K_2HPO_4) at a ratio of 100 mg tissue/0.5 ml PBS for 5 min. The homogenates were placed on ice for 10 min and then centrifuged at 12,000 rpm for 30 min. The supernatant was collected and stored at -70°C for further experiments.

Electrophoresis and Western Blot

The tissue extract samples were prepared as described by homogenizing with buffer. Sodiumdodecyl sulfate-polyacrylamide gel electrophoresis was done with 10% polyacrylamide gels. The samples were electrophoresed at 140 V for 3.5 hours and equilibrated for 15 min in 25 mM Tris-HCl, pH 8.3, containing 192 mM glycine and 20% (V/V) methanol. Electrophoresed proteins were transferred to nitrocellulose membranes (Amersham, Hybond-C Extra Supported, 0.45 Micro) using a Bio-rad Scientific Instruments Transphor Unit at 100 mA for 14 h. Nitrocellulose membranes were incubated at room temperature for 2 hours in blocking buffer containing 100 mM Tris-HCl, pH 7.5, 0.9% (w/v) NaCl, 0.1% (v/v) fetal bovine serum. Monoclonal antibodies including Fas ligand, Fas receptor, FADD, caspase 8, caspase 3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and α-tubulin (Neo Markers, Fremont, CA, USA) were diluted 1:200 in antibody binding buffer containing 100 mM Tris-HCL, pH 7.5, 0.9% (w/v) NaCl, 0.1% (v/v) Tween-20 and 1% (v/v) fetal bovine serum. Incubations were performed at room temperature for 3.5 hours. The immunoblots were washed three times in 50 ml blotting buffer for 10 min and then immersed in the second antibody solution containing alkaline phosphatase goat anti-rat IgG (Promega Corp., Madison, WI, USA) for 1 hour and diluted 1000-fold in binding buffer. The immunoblots were then washed in blotting buffer for 10min three times. Color development was presented in a 20 ml mixture consisting of 7 mg nitro blue tetrazolium, 5 mg 5-bromo-4-chloro-3-indolyl-phosphate, 100 mM NaCl and 5 mM $MgCl_2$ in 100 mM Tris-HCl, pH9.5. Densitometric analysis of immunoblots was performed using AlphaImager 2200 digital imaging system (Digital Imaging System, San Leandro,. CA, USA).

Experimental Protocols

Protocol 1: To understand the cardiac characteristics in obesity, the body weight

(BW), the whole heart weight (WHW), left ventricular weight (LVW), WHW/BW, WHW/ Tibia length, LVW/BW, LVW/WHW were weighed and measured (Table 1).

Protocol 2: To understand the myocardial architecture, we did a histopathological analysis of ventricular tissue stained with hematoxylin and eosin in the hearts excised from 6 lean and 6 obese rats (Fig 1).

Protocol 3: To further understand the Fas receptor-dependent apoptotic pathway, the protein products of Fas ligands, Fas receptors, and FADDs extracted from the left ventricles of excised hearts in the 6 lean and 6 obese rats were measured by western blotting. (Fig 2, Fig 3).

Protocol 4: In order to further investigate the downstream signal components of Fas receptor-dependent signaling pathways, pro-caspase-8 levels, activated caspase-8, pro-caspase-3 levels, activated caspase-3 extracted from the left ventricles of excised hearts in the 6 lean and 6 obese rats were measured by Western Blotting. (fig 4).

Protocol 5: In order to understand whether the cardiac apoptosis and cardiac fibrosis occurs in obese rat hearts, we did a TUNEL assay and Masson trichrome staining analysis of ventricular tissue (Fig 5, Fig 6).

Statistical Analysis

The data were compared between lean and obese groups using Student's t-test for two independent samples. In all cases, a difference at $P<0.05$ was considered statistically significant.

Results

Body weight and cardiac characteristics.(OK)

Obese rats weighed about 40% more than age-matched lean animals (362±30 g versus 514±62 g, $p < 0.01$). Whole heart weight (WHW), Left ventricular weight (LVW), and the ratio of whole heart weight to tibia length were significantly increased in the the obese group, compared with the lean group (Table 1). The absolute whole heart weight or the whole heart weight to tibia length was significantly increased in the the obese group, compared with the lean group whereas as the ratio of whole heart weight to body weight, traditionally regarding as an index of cardiac hypertrophy, was not changed in the obese group, compared with the lean group (Table 1).

Cardiac histopathological changes in obese rats (OK)

We found that the ventricular myocardium in the lean group showed normal architecture with normal interstitial space. In contrast, the abnormal myocardial architecture such as cardiomyocyte disarray and the increased interstitial space were observed in the obese group, in 100 X and 400 X magnification images (Fig 2).

Changes of Bcl2 family components in the excised hearts of obese rats(OK)

The protein products of Fas ligands, Fas receptors (Fig 3), and FADDs (Fig 4) extracted from the left ventricles of excised hearts in the obese group were significantly increased, compared with lean group.

Changes of caspase-8 and caspase-3 protein levels in the cardiac tissues(OK)

Pro-caspase-8 levels and pro-caspase-3 levels were significantly decreased in obese group, but activated caspase-8 levels and activated caspase-3 levels were significantly increased in the obese group, compared with the lean group. (Fig 5).

Cardiac apoptotic and fibrosis changes in obese rats (OK)

We found that hearts stained with TUNEL assay showed increased TUNEL-postive cardiac cells in obese rats in x 200 magnification images (Fig 5) as well as that hearts stained with Masson trichrome showed minor fibrosis, increased collagen deposition, and myofibril disarray in obese in x 200 magnification images (Fig 6).

Discussion

Major findings

Our main findings can be summarized as follows: (1) the increased whole heart weight, the increased ratio of whole heart weight to tibia length, the abnormal myocardial architecture, the increased myocardial disarray, and minor cardiac fibrosis were observed in obese group; (2) A decrease in anti-apoptotic protein Bcl2 level and increases in pro-apoptotic Bad, BNIP3, cytosolic cytochrome *c*, activated caspase-8, and activated caspase-3 in obese rat hearts imply that the activity of cardiac Fas receptor-dependent apoptotic pathway was significantly increased in obese Zucker rats. After integrating our current findings into previously proposed apoptotic theory, our proposed hypothesis that cardiac Fas receptor-dependent apoptotic pathway might be more activated in obesity (Fig 8).

Cardiomyopathic changes in obesity

Obesity is often associated with hemodynamic overload, ventricular remodeling, and higher cardiac output due to an augmented stroke volume and an increase in heart rate (10, 27). Obesity cardiomyopathy typically occurs in persons with severe and long-standing obesity, which may progressively develop congestive heart failure and sudden cardiac death (10). In the current study, 5~6 month old obese Zucker rats appear to increase the relative cardiomyopathic changes, such as myocardial disarray and minor cardiac fibrosis. We speculate obese rats progressively develop deleterious cardiomyopathic changes in the age of earlier than 5~6 month old. In obese Zucker rats, cardiac hypertrophic effect will be underestimated if only using the index of the ratio of whole heart weight to the whole body weight, traditionally regarding as an index of cardiac hypertrophy. Potential inducers of cardiac hypertrophy and cardiac apoptosis include various factors, such as hypertension, volume overload, hypoxia

and oxidative stress (14, 16, 17). Therefore, we have to add a note of caution that any effect on cardiomyopathic changes noted in the present investigation cannot be isolated to any specific factors, such as volume overload, nocturnal hypoxemia, oxidative stress, or other unclear factors.

There is an association between the turnover of collagens and remodeling of the rat ventricles (20, 21). The remodeling progresses immediately after myocardial damage with an increased level of collagenases (28). The collagens synthesized by the fibroblasts will invade and replace the apoptotic myocytes (20-22, 29). The myocardial interstitial changes resulted from increased collagen deposition lead to cardiac stiffness and cardiac dysfunction (29). Accordingly, the accumulated collagens will further contribute to the development of ventricular fibrosis and heart failure (22). In our findings, abnormal myocardial architecture, increased interstitial space, minor cardiac fibrosis in obese rat hearts suggest the development of cardiomyocyte death characterized by the distortion in myocardium architecture and minor cardiac fibrosis in obesity.

Cardiac Fas receptor- dependent apoptotic pathways in obesity

The type I or 'extrinsic' apoptotic pathway is mediated by external factors that bind to members of the death receptor superfamily, such as Fas. In type I apoptosis, Fas death receptors respond to specific ligand binding by Fas ligand or TNF-α by recruiting specific intercellular adaptor proteins (e.g. FADD) through homologous 'death domains'. FADD couples directly aggregates procaspase 8, leading to the cleavage and activation of caspase 8, which in turn induces cell apoptosis [4].

BNIP3 expression is often linked with hypoxia-regulated protein expression and play as a key regulator of ventricular myocyte apoptosis during hypoxia (17, 30). Respiratory deficits were often noted in obesity, including impaired respiratory control

(1-3), chest wall limitations (4), upper airway narrowing (5), and sleep-related breathing disorders, all of which may potentially contribute to alveolar hypoxia or noturnal hypoxemia. Hypoxia or noturnal hypoxemia might be one of possible factor to link the increased BNIP3 expression in obese rat hearts and to aggravate apoptotic pathway in obesity. Of course, further hypoxia-related studies are required to clarify how hypoxic stress or sleep apnea in obesity impact on BNIP3 and apoptotic pathways.

Shifting the balance of Bcl2 family members toward pro-apoptotic effects will enhance cytochrome *c* release from mitochondria. Cytochrome *c* release will form a complex with pro-caspase-8 and its cofactor Apaf-1 (apoptotic protease-activating factor-1). It is responsible for activating caspase-8, which further activates caspase-3 and executes the apoptotic program (26). In the current study, Fas receptor-dependent apoptotic pathway was significantly increased in cardiac tissues in obesity from a series of evidences, decrease in Bcl2 and increases in Bad, BNIP3, cytosolic cytochrome *c*, activated caspase-8, and activated caspase-3 in obese rat hearts. All key components of Fas receptor-dependent apoptotic pathway from upstream cascade to downstream cascade consistently show toward pro-apoptotic effects in obesity. Therefore, our findings strongly suggest that cardiac Fas receptor-dependent apoptotic pathway in obese Zucker rats become more activated, which might more potential to develop cardiac apoptosis and further to develop heart failure.

Clinical Application and significance

Obesity was considered as a major risk factor for the development of heart failure in the relative risk ranging from 1.8 to 5.6 depending on the degree of obesity, even when other known risk factors for heart failure are excluded (12, 13). Elevated body-mass index was associated with an increased risk of heart failure, even in less

obese people (31). However, the mechanism why obesity resulted in heart failure had not yet been recognized. Our current findings indicating “more activated cardiac Fas receptor-dependent apoptotic pathway in obesity” might provide one of possible mechanisms to explain the development of heart failure in obesity. Besides, it might be beneficial to block cardiac Fas receptor-dependent apoptotic pathway when considering possible therapeutic agents to control or prevent the development of apoptosis and/or fibrosis-related cardiac diseases in obesity. Of course, further clinical studies are required to clarify the apoptotic pathways or possible mechanisms in obesity-related heart failure.

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References

1. Lee SD, Magalang UJ, Krasney JA, Farkas GA. Opioidergic modulation of ventilatory response to sustained hypoxia in obese Zucker rats. *Obes Res.* 2001;9:407-13.
2. Lee SD, Nakano H, Farkas GA. NMDA receptor-mediated modulation of ventilation in obese Zucker rats. *Int J Obes Relat Metab Disord.* 2001;25:997-1004.
3. Lee SD, Nakano H, Farkas GA. Adenosinergic modulation of ventilation in obese zucker rats. *Obes Res.* 2005;13:545-55.
4. Farkas GA, Schlenker EH. Pulmonary ventilation and mechanics in morbidly obese Zucker rats. *Am J Respir Crit Care Med.* 1994;150:356-62.
5. Nakano H, Magalang UJ, Lee SD, Krasney JA, Farkas GA. Serotonergic modulation of ventilation and upper airway stability in obese Zucker rats. *Am J Respir Crit Care Med.* 2001;163:1191-7.
6. Alonso-Galicia M, Brands MW, Zappe DH, Hall JE. Hypertension in obese Zucker rats. Role of angiotensin II and adrenergic activity. *Hypertension.* 1996;28:1047-54.
7. Fredersdorf S, Thumann C, Ulucan C, et al. Myocardial hypertrophy and enhanced left ventricular contractility in Zucker diabetic fatty rats. *Cardiovasc Pathol.* 2004;13:11-9.
8. Lee SD, Nakano H, Farkas GA. GABAergic modulation of ventilation and peak oxygen consumption in obese Zucker rats. *J Appl Physiol.* 2001;90:1707-13.
9. Lee SD, Nakano H, Gosselin LE, et al. Endogenous opioids modulate ventilation and peak oxygen consumption in obese Zucker rats. *Am J Respir Crit Care Med.* 2000;162:1009-15.
10. Alpert MA. Obesity cardiomyopathy: pathophysiology and evolution of the clinical syndrome. *Am J Med Sci.* 2001;321:225-36.
11. Massie BM. Obesity and heart failure--risk factor or mechanism? *N Engl J Med.* 2002;347:358-9.
12. Clark K. Obesity and the risk of heart failure. *J Insur Med.* 2003;35:59-60.
13. Coviello JS, Nystrom KV. Obesity and heart failure. *J Cardiovasc Nurs.* 2003;18:360-6; quiz 67-8.
14. Haunstetter A, Izumo S. Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ Res.* 1998;82:1111-29.
15. Biondi-Zoccai GG, Abbate A, Vasaturo F, et al. Increased apoptosis in remote non-infarcted myocardium in multivessel coronary disease. *Int J Cardiol.* 2004;94:105-10.

16. Narula J, Pandey P, Arbustini E, et al. Apoptosis in heart failure: release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. *Proc Natl Acad Sci USA.* 1999;96:8144-9.
17. Bishopric NH, Andreka P, Slepak T, Webster KA. Molecular mechanisms of apoptosis in the cardiac myocyte. *Curr Opin Pharmacol.* 2001;1:141-50.
18. Reddy MB, Clark L. Iron, oxidative stress, and disease risk. *Nutr Rev.* 2004;62:120-4.
19. Oudit GY, Trivieri MG, Khaper N, et al. Taurine supplementation reduces oxidative stress and improves cardiovascular function in an iron-overload murine model. *Circulation.* 2004;109:1877-85.
20. Sorescu D, Griendling KK. Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. *Congest Heart Fail.* 2002;8:132-40.
21. Narula J, Haider N, Virmani R, et al. Apoptosis in myocytes in end-stage heart failure. *N Engl J Med.* 1996;335:1182-9.
22. Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev.* 2000;52:11-34.
23. Adams JM, Cory S. Life-or-death decisions by the Bcl-2 protein family. *Trends Biochem Sci.* 2001;26:61-6.
24. Kubasiak LA, Hernandez OM, Bishopric NH, Webster KA. Hypoxia and acidosis activate cardiac myocyte death through the Bcl-2 family protein BNIP3. *Proc Natl Acad Sci USA.* 2002;99:12825-30.
25. Sen P, Mukherjee S, Ray D, Raha S. Involvement of the Akt/PKB signaling pathway with disease processes. *Mol Cell Biochem.* 2003;253:241-6.
26. Brown GC, Borutaite V. Nitric oxide, cytochrome c and mitochondria. *Biochem Soc Symp.* 1999;66:17-25.
27. Alexander JK. Obesity and the heart. *Heart Dis Stroke.* 1993;2:317-21.
28. D'Armiento J. Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. *Trends Cardiovasc Med.* 2002;12:97-101.
29. Anversa P, Nadal-Ginard B. Myocyte renewal and ventricular remodelling. *Nature.* 2002;415:240-3.
30. Regula KM, Ens K, Kirshenbaum LA. Inducible expression of BNIP3 provokes mitochondrial defects and hypoxia-mediated cell death of ventricular myocytes. *Circ Res.* 2002;91:226-31.
31. Kenchaiah S, Evans JC, Levy D, et al. Obesity and the risk of heart failure. *N Engl J Med.* 2002;347:305-13.

Tabel 1. Characteristics of lean and obese rat hearts

Characteristics	Lean	Obese
Body weight, g	362±30	514±62 **
Whole heart weight (WHW),g	0.85±0.05	1.23±0.13 **
Left ventricular weight (LVW),g	0.55±0.07	0.83±0.10 **
WHW/BW	24.7±1.4 ($\times 10^{-4}$)	24.1±2.2 ($\times 10^{-4}$)
WHW/ Tibia length, g/m	21.6±1.2	33.5±3.5 **
LVW/BW	15.3±1.6 ($\times 10^{-4}$)	16.3±1.5 ($\times 10^{-4}$)
LVW/WHW	0.65±0.05	0.68±0.05

Values are means ± SD (n=8). BW, body weight; WHW, whole heart weight; LVW, left ventricular weight. ** $P<0.01$ Significant difference between lean and obese Zucker rats.

Figure Legend

Fig 1. Histopathological analysis of cardiac tissue sections from left ventricles with Hematoxylin and eosin staining in lean and obese Zucker rats. The images of myocardial architecture were magnified by 100 times and 400 times.

Fig 2. (A) the protein products of Fas ligands and (B) the protein products of Fas receptors extracted from the left ventricles of excised hearts in the 3 lean and 3 obese age-matched Zucker rats were measure by Western Blotting analysis, respectively. (C) and (D) Bars represent the relative protein quantification of Fas ligands and Fas receptors on the basis of α-tubulin, respectively, and indicate mean values±SD. *P<0.05, **P<0.01, lean group (n=6) vs. obese group (n=6).

Fig 3. (A) The protein products of Fas-associated death domain (FADD) extracted from the left ventricles of excised hearts in the 3 lean and 3 obese age-matched Zucker rats were measure by Western Blotting analysis. (B) Bars represent the relative protein quantification of FADD on the basis of α-tubulin and indicate mean values±SD. **P<0.01, lean group (n=6) vs. obese group (n=6).

Fig 4. (A) The protein products pro-caspase-8, activated (cleaved) caspase-8, (B) pro-caspase-3, and activated (cleaved)-caspase-3 extracted from the left ventricles of excised hearts in the 3 lean and 3 obese age-matched Zucker rats were measure by Western Blotting analysis. Bars represent the relative caspase-8 (C) and caspase-3 (D) quantification on the basis of α-tubulin and indicate mean values±SD. ** P<0.01, significant differences between lean group (n=6) and obese group (n=6).

Fig 5. Stained apoptotic cells of of excised hearts sections from left ventricles in lean and obese Zucker rats were measured by stained with TUNEL assay with dark background (upper panel, green spots) with background tissues (lower panel). The images of myocardial architecture were magnified by 100 times.

Fig 6 Histopathological analysis of cardiac tissue sections with Masson trichrome staining (fibrosis: blue color) in lean and obese Zucker rats. The images of myocardial architecture were magnified by 200 times.

Fig 7. More activated Fas receptor-dependent apoptotic pathway in obese rat hearts. Our proposed hypothesis that cardiac Fas receptor-dependent apoptotic pathway will be more activated in obesity due to increased Fas ligand, increased Fas receptors, increased FADD, decreased pro-caspase-8, increased activated caspase-8, decreased pro-caspase-3, increased caspase-3 which may contribute to cardiac cells apoptosis in obese rat hearts. Up arrows and down arrows on the right side represent increases and decreases, respectively.

Figures

Fig 1

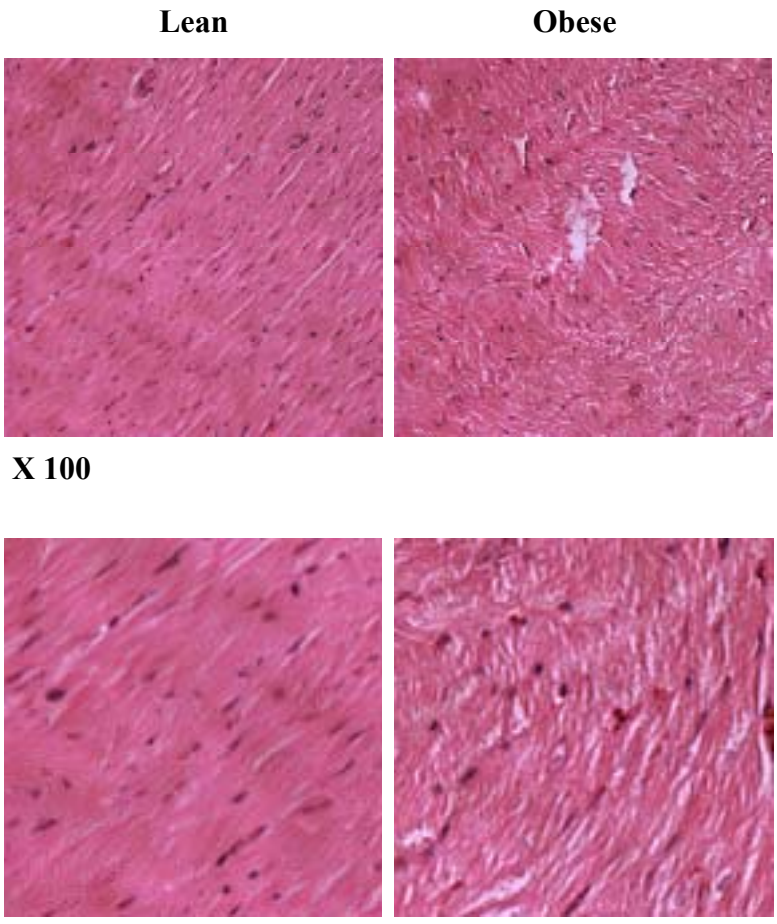
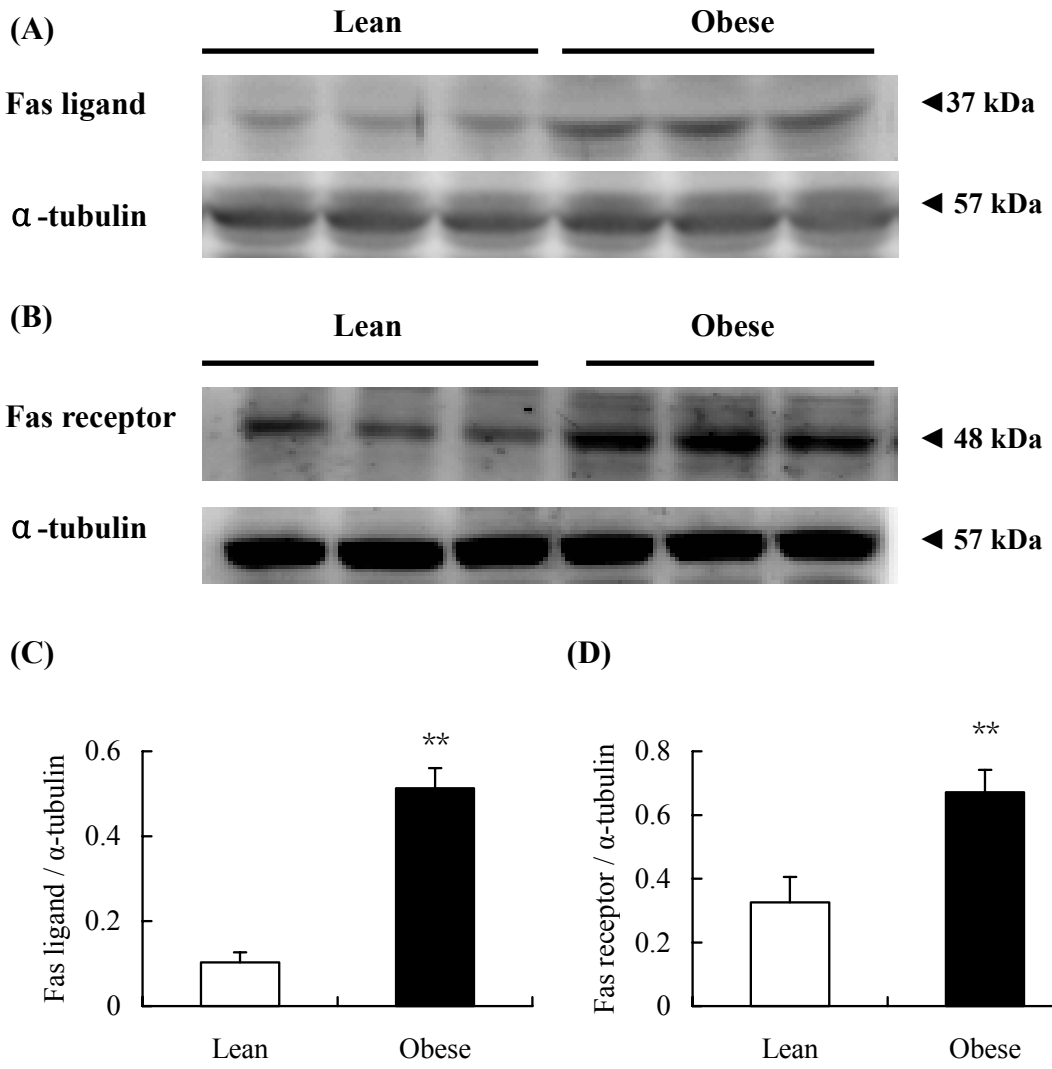


Fig 2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Obese
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α-tubulin
◀ 57 kDa
(B)
Lean
Obese
Fas receptor
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α-tubulin
◀ 57 kDa
(C)
Fas ligand / α-tubulin
0.6
0.4
0.2
0
**
Lean
Obese
(D)
Fas receptor / α-tubulin
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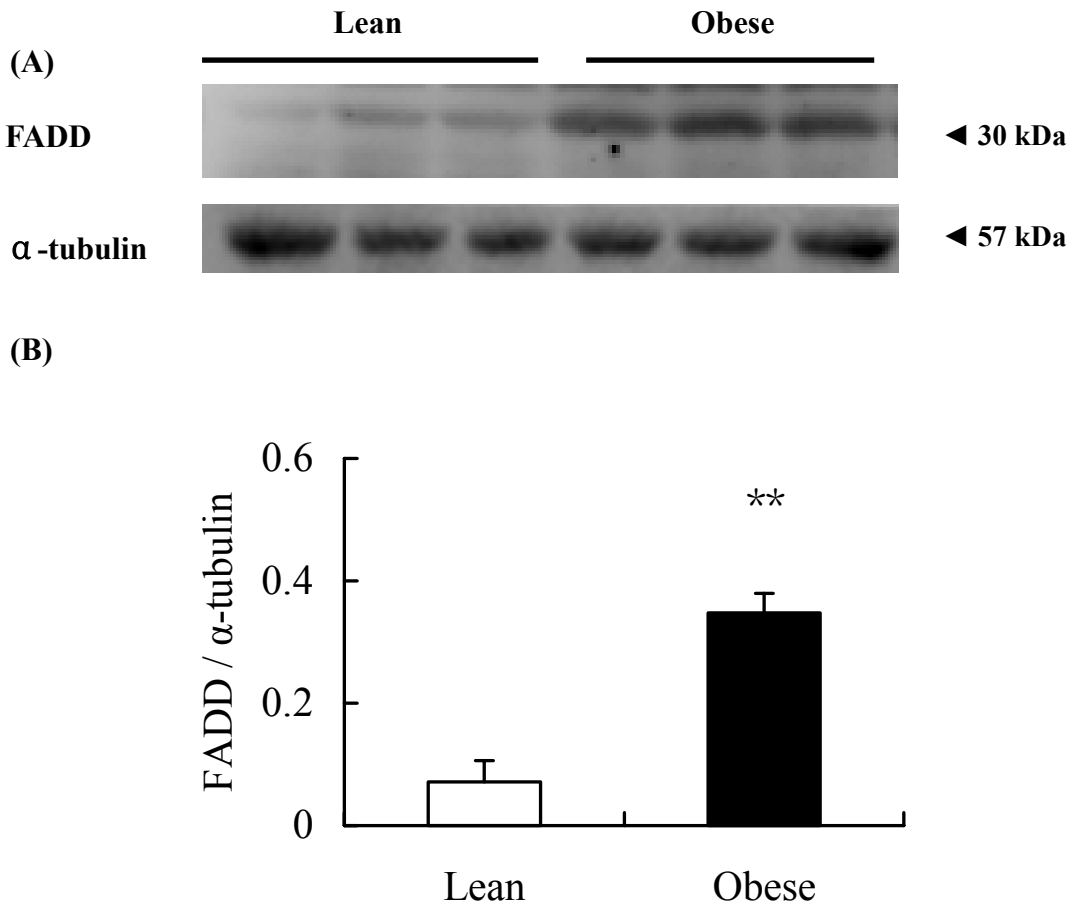
0.6
0.4
0.2
0
**
Lean
Obese

Fig 3.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Obese
FADD
◄ 30 kDa
α -tubulin
◄ 57 kDa
(B)
FADD / α-tubulin
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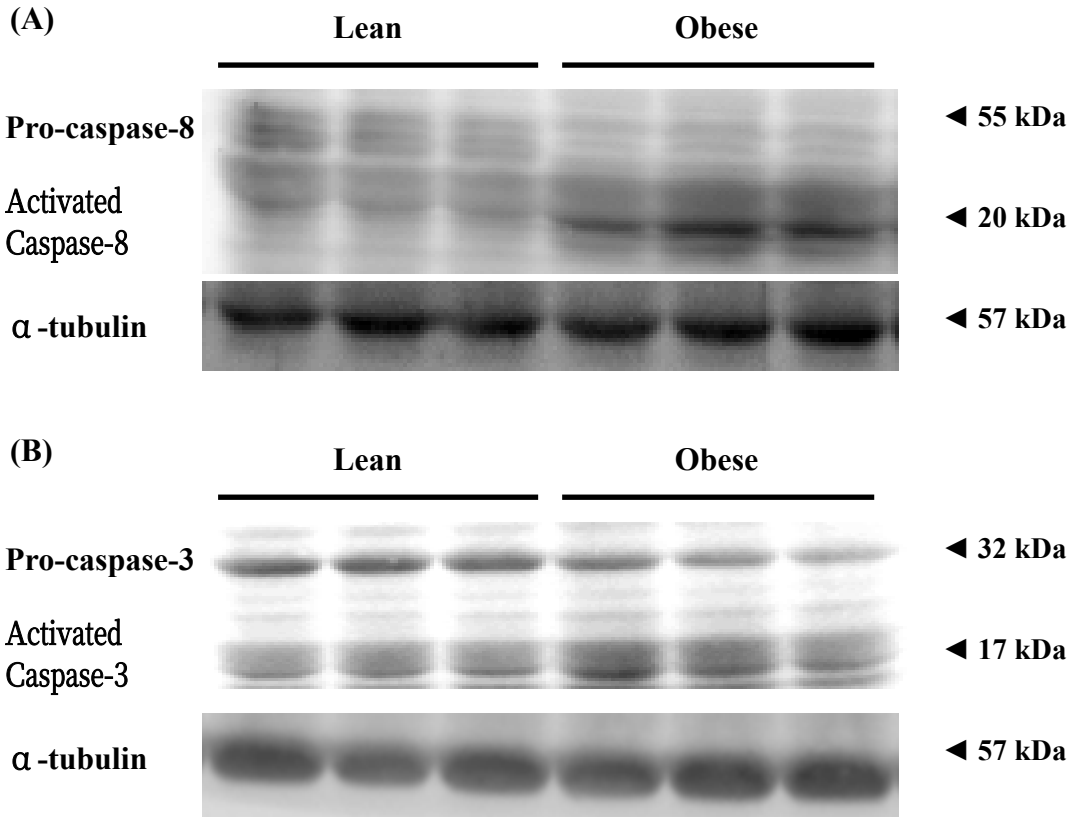
0.2
0
**
Lean
Obese

Fig 4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◀ 20 kDa
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◀ 57 kDa

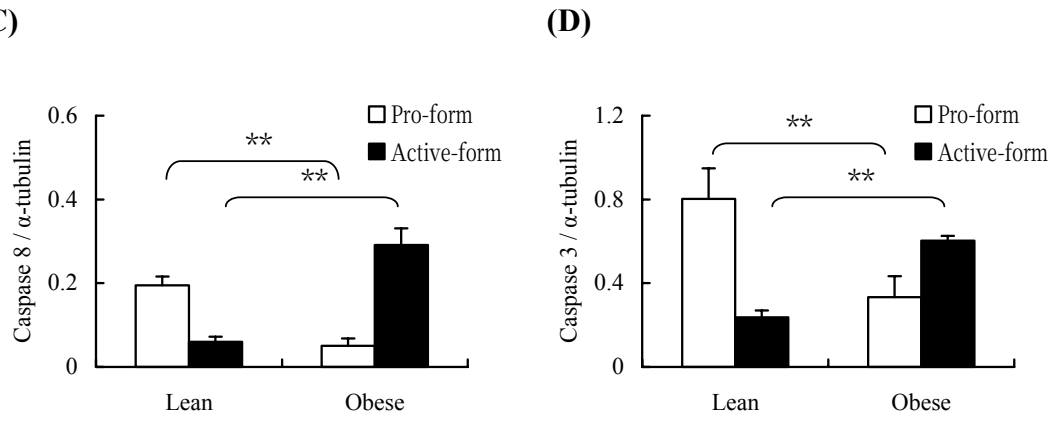

(C)
Caspase 8 / α-tubulin
0.6
0.4
0.2
0
Pro-form
Active-form
**
**
Lean
Obese
(D)
Caspase 3 / α-tubulin
1.2
0.8
0.4
0
Pro-form
Active-form
**
**
Lean
Obese

Fig 5.

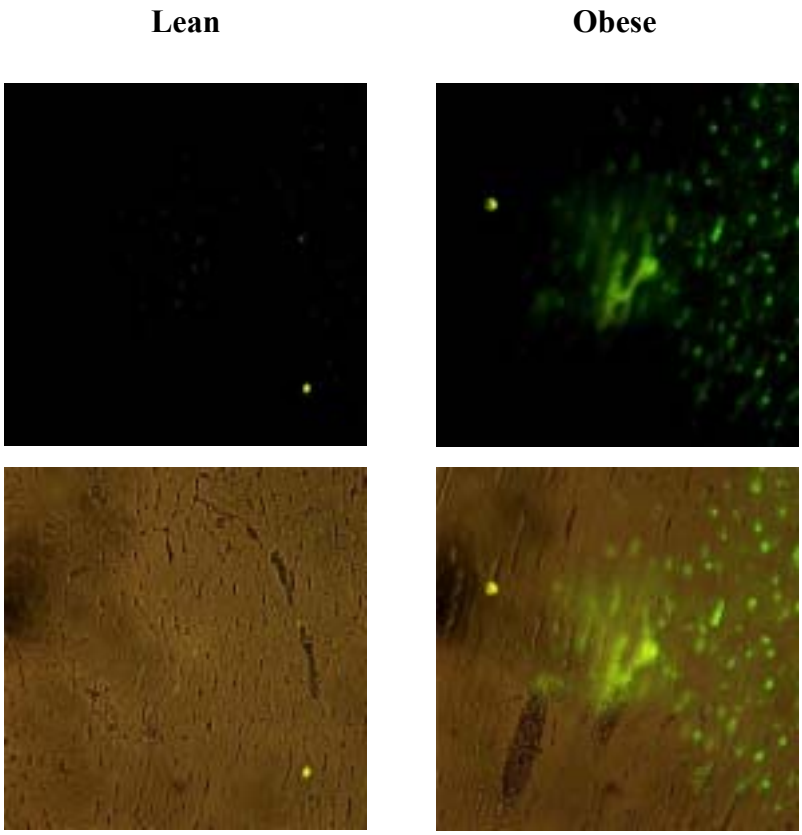


Fig 6. Masson trichrome staining

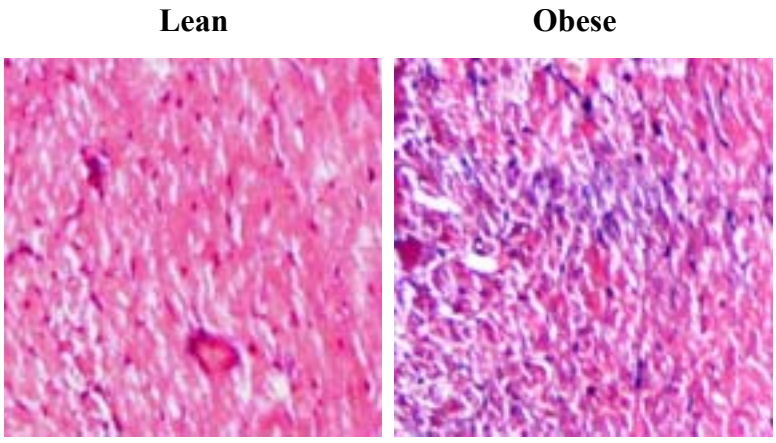
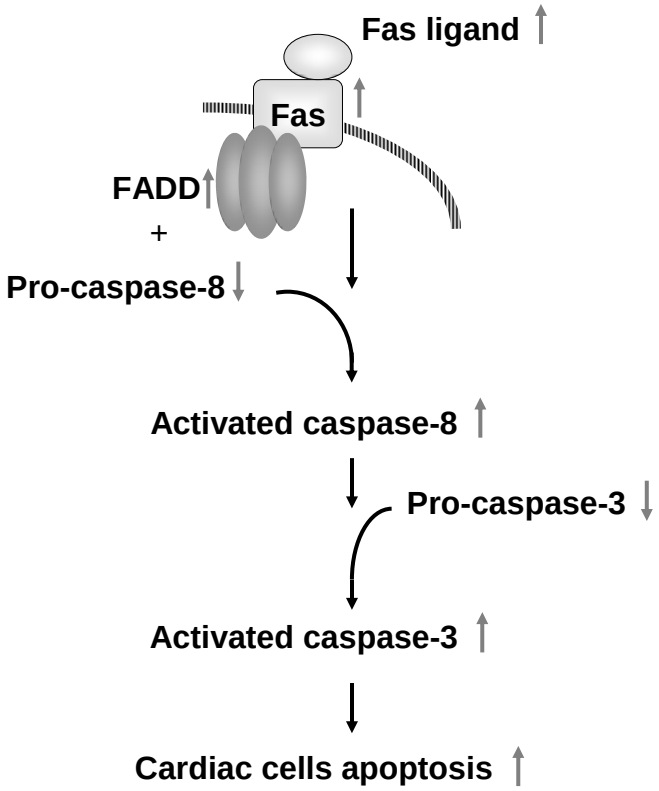


Fig 7


Fas ligand
Fas
FADD
+
Pro-caspase-8
Activated caspase-8
Pro-caspase-3
Activated caspase-3
Cardiac cells apoptosis