The coexistence of nocturnal sustained hypoxia and obesity additively increases cardiac apoptosis

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Lee S-D, Kuo W-W, Bau D-T, Ko F-Y, Wu F-L, Kuo C-H, Tsai F-J, Wang PS, Lu M-C, Huang C-Y. The coexistence of nocturnal sustained hypoxia and obesity additively increases cardiac apoptosis. J Appl Physiol 104: 1144–1153, 2008. First published January 17, 2008; doi:10.1152/japplphysiol.00152.2007.—Background: nocturnal sustained hypoxia during sleeping time has been reported in severe obesity, but no information regarding the cardiac molecular mechanism in the coexistence of nocturnal sustained hypoxia and obesity is available. This study evaluates whether the coexistence of nocturnal sustained hypoxia and obesity will increase cardiac Fas death receptor and mitochondrial-dependent apoptotic pathway. Methods: 32 lean and 32 obese 5- to 6-mo-old rats with or without nocturnal sustained hypoxia were studied and assigned to one of four subgroups: normoxia lean (NL), normoxia obese (NO), hypoxia lean (HL, 12% O2 for 8 h and 21% O2 16 h/day, 1 wk), and hypoxia obese (HO). The heart weight index, tail cuff plethysmography, echocardiography, hematoxylin-eosin staining, TUNEL assays, Western blotting, and RT-PCR were performed. Results: systolic and diastolic blood pressures in HO were higher than those in NL, and fractional shortening in HO was reduced compared with others. The whole heart weight, the left ventricular weight, the abnormal myocardial architecture, and TUNEL-positive apoptotic cells, as well as the activity of cardiac Fas-dependent and mitochondrial-dependent apoptotic pathway, were significantly increased in obese group or nocturnal sustained hypoxia group and were further increased when obesity and nocturnal sustained hypoxia coexisted, the evidence for which is based on decreases in an anti-apoptotic protein Bcl2 level and Bid and increases in Fas, FADD, pro-apoptotic Bad, BNIP3, cytosolic cytochrome c, activated caspase-8, activated caspase-9, and activated caspase-3. Conclusions: The cardiac Fas receptor- and mitochondrial-dependent apoptotic pathways were more activated in obesity with coexistent nocturnal sustained hypoxia, which may represent one possible apoptotic mechanism for the development of heart failure in obesity with nocturnal sustained hypoxia.

Fas receptor; mitochondrial; caspases; cell death; obese

cardiac hypertrophy, and poor exercise capacity (3, 15, 22, 23). Severe obesity has long been recognized as the cause of a form of cardiomyopathy characterized by increased rates of hypertension, chronic volume overload, left ventricular hypertrophy, and the development of heart failure (4, 11, 12, 26). Left ventricular dysfunction or biventricular failure was found in morbidly obese patients with respiratory deficiency, severe sleep apnea, or hypoponpnea syndrome (2, 8). Nocturnal desaturation or nocturnal sustained hypoxia has often been reported in severely obese patients affected by various respiratory deficiencies such as restrictive lung, increased chest wall loading, ventilation-perfusion mismatch, blunted hypoxic response, nocturnal hypoponpnea, sleep hypopnea, and/or sleep apnea during sleep (5, 17, 18, 33).

Apoptosis, a physiological program of cellular death, may contribute to many cardiac disorders (16, 20). The occurrence of apoptosis has been reported to contribute to the loss of cardiomyocytes in cardiomyopathies and is recognized as a predictor of adverse outcomes in subjects with cardiac diseases or heart failure (27). The “extrinsic” Fas receptor-dependent (type I) apoptotic pathway is believed to be one of the major pathways directly triggering cardiac apoptosis (7, 16). This pathway is initiated by binding the Fas ligand to the Fas receptor, which results in the clustering of receptors and initiating an extrinsic pathway (7). Fas ligand binding followed by Fas-receptor oligomerization is known to lead to the formation of a death-inducing signal complex starting with recruitment of the Fas-associated death domain (FADD) of the adaptor protein (7). 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 principal effector caspase of apoptosis (28, 29). Additionally, activated caspase-8 can cleave Bcl-2 homology domain 3 (BH3)-interfering domain death agonist (Bid), and the cleaved Bid causes the release of mitochondrial cytochrome c, leading to the activation of pro-caspase-9, which can then activate pro-caspase-3 (1, 7). Bid is one of the key components involved in the intracellular molecule signaling.
from Fas-dependent apoptotic pathway to the mitochondrial-dependent apoptotic pathway (1, 7).

The “intrinsic” mitochondrial-dependent (type II) apoptotic pathway is mediated by internal factors, especially in mitochondria (7). The mitochondria is the main site of action for members of the apoptosis-regulating protein family exemplified by Bcl-2 family, such as Bcl-2, BNIP3, and Bad (7). Commitment to apoptosis is typically governed by opposing factions of the Bcl-2 family, including pro-apoptotic vs. anti-apoptotic family members (13). Pro-apoptotic and anti-apoptotic Bcl2 family members can homodimerize or heterodimerize to each other and appear to interact with and neutralize each other, so that the relative balance of these effectors strongly influences cytochrome c release and cell fate (19). Bcl-2, an anti-apoptotic protein, prevents cytochrome c release, whereas BNIP3 and Bad, pro-apoptotic proteins, enhance cytochrome c release from mitochondria (7). BNIP3 (Bcl-2/adenovirus E1B 19-kDa interacting protein) has been reported to be a key regulator of mitochondrial function and cell death of ventricular myocytes during hypoxia (32). When cytochrome c is released from mitochondria into cytosol, it is responsible for activating caspase-9, which further activates caspase-3 and executes the apoptotic program (9). One of our previous studies showed that longer duration of nocturnal sustained hypoxia at 0-, 4-, and 8-wk periods appeared to exert more deleterious effects on Fas and mitochondrial-dependent apoptotic pathways in Sprague-Dawley rats (20, 21). The increased cardiac apoptosis was found in leptin-deficient and leptin-resistant mice (6), implying that leptin-signaling impairments may lead to cardiac apoptosis. In addition, our previous studies indicate that Fas receptor-dependent and mitochondrial-dependent apoptotic pathways are more activated in obese Zucker rats than those in lean littermates (24, 25).

The role of cardiac apoptosis in the coexistence of nocturnal sustained hypoxia and obesity is not understood. In the current study, we investigated whether cardiac abnormalities in obesity with coexistent nocturnal sustained hypoxia are associated with more activated Fas-dependent and mitochondrial-dependent apoptotic pathways. The heart weight index, blood pressure, left ventricular function, myocardial morphology, and key components of Fas-dependent and mitochondrial-dependent apoptotic pathways and apoptotic activity were determined by heart weighing, tail-cuff plethysmography, echocardiography, histopathological analysis, Western blotting, and RT-PCR in lean and obese Zucker rats under exposure of normoxia or nocturnal hypoxia. We hypothesized that cardiac abnormality in the coexistence of nocturnal sustained hypoxia and obesity may predispose to more activated Fas and mitochondrial-mediated cardiac apoptosis.

MATERIALS AND METHODS

Animal model. The studies in the part I were performed on 18 lean (Fa/Fa or Fa/fa) and 18 obese (fa/fa) age-matched (5- to 6-mo old) male Zucker rats and another 14 lean and 14 obese were studied in part II. The animals were born by Zucker breeders purchased from Charles River Lab in France. One obese rat and one age-matched lean rat were housed per cage. There were a total of 18 cages in part I and 14 cages in part II. Ambient temperature was maintained at 25°C, and the animals were kept on an artificial 12:12-h light/dark cycle. The light period began at 7:00 AM. Rats were provided with standard laboratory chow (Lab Diet 5001; PMI Nutrition International, Brentwood, MO) and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee of China Medical University, Taichung, Taiwan, and the protocols of laboratory animal care (NIH publication) were followed.

Hypoxia exposures. Eighteen lean and eighteen obese rats in part I and another fourteen lean and fourteen obese in part II were randomly divided into normoxic and hypoxic groups, i.e., normoxic lean group (NL), normoxic obese group (NO), hypoxic lean group (HL), and hypoxic obese group (HO). In part I, 36 rats were transferred to the hypoxia laboratory and were habituated in the environment for 3 wk. The hypoxia group of nine lean and nine obese rats was placed in the hypoxic chamber (1 × 1 × 1.2 m³) filled with daily cycle of hypoxia (12% O₂ balanced with 88% N₂) during the animals’ diurnal sleep period for 8 h and was exposed to room air (21% O₂ and 79% N₂) for 16 h/day for 1 wk. The normoxia group of nine lean and nine obese rats were exposed to room air for 16 h/day for 1 wk. CO₂ in both chambers was absorbed with soda lime. After normoxic or hypoxic exposure, the rats were weighed and decapitated. In addition, another age-matched 14 lean and 14 obese rats were studied in part II following the same protocol for measuring blood pressure and echocardiography.

Cardiac characteristics. The hearts of eight lean and eight obese animals were excised and cleaned with PBS. The left ventricle was separated and weighed. The right tibias were also separated and tibia lengths were measured by the electronic digital vernier caliper to adjust 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 were calculated.

Blood pressure and echocardiography. The animals were loosely restrained. Systolic, diastolic, and mean arterial blood pressures were determined with an automated tail-cuff system (29SSP; HIC/Life Science Instruments) 16 h after the completion of all exposures to hypoxia. Transthoracic echocardiographic images of rats were performed using Philips M2424A ultrasound systems (Andover, MA) under anesthesia with 1% isoflurane via a nose cone. M-mode echocardiographic examination was performed using a 6- to 15-MHz linear transducer (15-6L) via parasternal long axis approach. Left ventricular M-mode measurements at the level of the papillary muscles included left ventricular internal end-diastolic dimensions (LVIDd), left ventricular internal end-systolic dimensions (LVIDs), interventricular septum (IVS), and posterior wall thicknesses (LVPW), and fractional shortening (FS). FS% was calculated according to the following equation: FS% = [(LVIDd – LVIDs)/LVIDd] × 100.

Tissue extraction. Cardiac tissue extracts were obtained by homogenizing the left ventricle samples in a lysis buffer at a ratio of 100 mg tissue/1 ml buffer for 1 min. The homogenates were placed on ice for 10 min and then centrifuged at 12,000 g for 40 min twice. The supernatant was collected and stored at −70°C for further experiments.

Separation of cytosolic and mitochondrial fractions. To detect cytosolic cytochrome c, cardiac tissue extracts were suspended in a buffer [Tris, EDTA, and proteinase inhibitor cocktail tablet (Roche)] for 1 min on ice, homogenized by Polytron, and centrifuged at 1,200 g for 10 min. The supernatant was centrifuged at 10,000 g for 15 min to collect the mitochondrion-enriched pellet and the supernatant as the cytosolic fraction. The pellet was resuspended in lysis buffer as the mitochondrial fraction.

Electrophoresis and Western blot. Protein concentration of cardiac tissue extracts was determined by the Bradford method (Bio-Rad Protein Assay, Hercules, CA). Protein samples (50 μg/lane) were separated on a 10% SDS-PAGE with a constant voltage of 75 V. Electrophoresed proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, 0.45 μm pore size) with a transfer apparatus (Bio-Rad). PVDF membranes were incubated in 5% milk in TBS buffer. Primary antibodies including Fas ligand, Fas receptor, FADD, Bcl2, BNIP3, Bad, cytochrome c, caspase-8,
RNA extraction. Total RNA (8 μg) was reverse transcribed and then amplified by the PCR using a Super Script Preamplification System for first-strand cDNA synthesis and Taq DNA polymerase (Life Technologies, Rockville, MD). RT-PCR products were separated on a 1.5% agarose gel (Life Technologies). Amplimers were synthesized by MdBio, based on cDNA sequences from GenBank. The rat GAPDH was used as an internal standard. The following rat primers and rat GAPDH reverse primer-CCACA GTCTT CTGAG TGGCA. Den-
slide was dehydrated through graded alcohols. Finally, they were soaked in Xylene twice. Photomicrographs were obtained using Zeiss Axiophot microscopes. For TUNEL assay, the sections were incubated with proteinase K, washed in PBS, incubated with permeabilization solution, blocking buffer, and then washed two times with PBS. The terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP was determined for 60 min at 37°C using an apoptosis detection kit (Roche Applied Science, Indianapolis, IN) for detection. TUNEL-positive nuclei (fragmented DNA) fluoresced bright green at 450–500 nm. The mean number of TUNEL-positive cells was counted for at least five to six separate fields /H11003 2 slices /H11003 3 regions of the left ventricle (upper, middle, lower) excised from six rat hearts in each group. All counts were performed by at least two independent individuals in a blinded manner.

Statistical analysis. The weight index, protein levels, mRNA levels, and the percentage of TUNEL-positive cells were compared among the NL, NO, HL, and HO groups using one-way ANOVA with preplanned contrast comparison. In all cases, P < 0.05 was considered significant.

RESULTS

Body weight and cardiac characteristics. The obese rats weighed ~48–50% more than the age-matched lean rats regardless of normoxic (NL: 326 ± 65 g vs. NO: 485 ± 58 g, P < 0.01) or hypoxic (HL: 324 ± 16 g vs. HO: 468 ± 56 g, P < 0.01) exposure (Table 1). Although there were no differences in body weight between the NL and HL groups or between the NO and HO groups at the beginning and at the end of the study, lean or obese rats gained significantly more weight during the 1 wk normoxic exposure compared with the hypoxic rats based on a statistical analysis of repeated measures.

Fig. 2. A: The protein products of Fas receptor and 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 measured by Western blotting analysis. B: bars represent the relative protein quantification of Fas receptor and FADD on the basis of α-tubulin and indicate mean values ± SD (n = 6 in each group). **P < 0.01, significant differences between normoxia and hypoxia in lean or obese group. ‡P <0.01 significant differences between lean and obese group in normoxia or hypoxia. ##P < 0.01 significant differences between normoxic lean group and hypoxic obese group.

Fig. 3. A: The protein products of Bid extracted from the left ventricles of excised hearts in the 3 lean and 3 obese age-matched Zucker rats were measured by Western blotting analysis. B: bars represent the relative protein quantification of Bid on the basis of α-tubulin and indicate mean values ± SD (n = 6 in each group). **P < 0.01, significant differences between normoxia and hypoxia in lean or obese group. ‡P < 0.01 significant differences between lean and obese group in normoxia or hypoxia. ##P < 0.01 significant differences between normoxic lean group and hypoxic obese group.
Whole heart weight, left ventricular weight, the ratio of whole heart weight to tibia length, and the ratio of left ventricular weight to tibia length were significantly higher in the NO and HL groups than those in the NL group; these parameters became more obvious in the HO group than those in the NL group (Table 1). Systolic blood pressure (SBP) in the NO, HL, and HO groups was significantly higher than those in the NL group whereas diastolic blood pressure (DBP) was similar among four groups. Mean blood pressure (MBP) in the HL and HO groups was significantly higher than those in the NL group. The interventricular septum and posterior wall thickness in the NO and HO groups were thicker than those in the NL group, whereas those measurements in the NL and HL groups were similar. Fractional shortening (FS) in the NL, NO, and HL groups were similar, whereas FS in the HO group was significantly reduced compared with the other three groups (Table 1).

To further confirm whether there were changes in cardiac architecture, we did a histopathological analysis of ventricular tissue stained with H&E. After viewing ×400 magnified images, we found that the ventricular myocardium in the NL group showed normal architecture with normal interstitial space, but the abnormal myocardial architecture and the increased interstitial space were observed in the NO and HL groups. These abnormalities became more obvious in the HO group (Fig. 1).

**Upstream components of cardiac Fas receptor-dependent apoptotic pathways.** To investigate the upstream components of cardiac Fas receptor-dependent apoptotic signaling pathways in obese rats with coexistent nocturnal sustained hypoxia, we measured the protein levels of Fas and FADD in hearts excised from all four groups. Compared with the NL group, Fas protein levels rose in the NO and HL group and further increased in the HO group (Fig. 2).
Main intracellular molecule signaling mediator from Fas to mitochondrial pathway. To investigate the cardiac Bid cleavage, a mediator that connects the Fas receptor-dependent to the mitochondrial-dependent apoptotic pathway in the coexistence of nocturnal sustained hypoxia and obesity, we examined the protein levels of Bid in the hearts excised from the NL, NO, HL, and HO groups. The protein level of Bid was significantly decreased in the NO and HL groups and further decreased in the HO group, compared with the NL group (Fig. 3).

Upstream components of cardiac mitochondrial-dependent apoptotic pathways. To further understand the cardiac Bcl-2 family in mitochondrial-dependent apoptotic pathway in obese rats with coexistent nocturnal sustained hypoxia, we examined the levels of the Bcl2 family (Bcl2, BNIP3, and Bad) and cytosolic cytochrome c in the hearts excised from the NL, NO, HL, and HO groups. The anti-apoptotic proteins and gene expressions of Bcl2 were significantly decreased in the NO and HL groups and even further decreased in the HO group, compared with the NL group, while the pro-apoptotic proteins and gene expression of Bad were significantly increased in the NO and HL groups and even further increased in the HO group (Fig. 4). Furthermore, the gene expression of pro-apoptotic BNIP3 was significantly increased in the NO and HL groups and further increased in the HO group, compared with the NL group (Fig. 5). Compared with levels found in the NL group, the protein level of cytochrome c in the cytosolic fraction was significantly increased in the NO and HL groups and further increased in the HO group (Fig. 6).

Downstream components of cardiac Fas receptor and mitochondrial-dependent apoptotic pathways. To identify the downstream components of cardiac Fas receptor (caspase-8 and -3) and mitochondrial (caspase-9 and -3)-dependent apoptotic pathways, the caspase-8, -9, and -3 was measured by Western blotting. Western blot analysis revealed that, compared with the NL group, the activated forms of caspase-8, -9, and -3 protein products were increased in the NO and HL groups and further increased in the HO group (Fig. 7).

TUNEL-positive apoptotic cells of cardiac tissues. Viewing images magnified ×400, we observed that the left ventricles of the NO and HL groups stained with TUNEL assay had a greater number of TUNEL-positive cardiac cells than those in the NL group. Further increases in number of TUNEL-positive cardiac cells were found in the HO group (Fig. 8).

DISCUSSION

Major findings. Our main findings can be summarized as follows. 1) The increased whole heart weight, the increased left ventricular weight, the increased ratio of whole heart weight to tibia length, the increased ratio of left ventricular weight to tibia length, the abnormal myocardial architecture, the increased myocardial disarray, and TUNEL-positive apoptotic cells were observed in obese or nocturnal sustained hypoxia groups compared with lean groups, and these increases became more obvious when obesity and nocturnal sustained hypoxia coexisted. 2) Blood pressure was significantly increased and the fractional shortening of the left ventricle was significantly decreased in obese rats with coexistent nocturnal sustained hypoxia. 3) The activity of the cardiac Fas receptor-dependent apoptotic pathway was significantly increased in the obese group and the nocturnal sustained hypoxia group and further increased in obese rats with coexistent nocturnal sustained hypoxia, the evidence for which is based on increases in Fas, FADD, activated caspase-8, and activated caspase-3. 4) The activity of the cardiac mitochondrial-dependent apoptotic pathway was significantly increased in the obese group and the nocturnal sustained hypoxia group and further increased in obese rats with coexistent nocturnal sustained hypoxia, the evidence for which is based on a decrease in an anti-apoptotic protein Bcl2 level and increases in pro-apoptotic Bad, BNIP3, cytosolic...
cytochrome c, activated caspase-9, and activated caspase-3. After integrating our current findings into previously proposed apoptotic theories, we hypothesize that cardiac Fas receptor-dependent and mitochondrial-dependent apoptotic pathways might be more activated in the coexistence of nocturnal sustained hypoxia and obesity.

**Experimental limitation.** Obese rats exposed to 8 h of sustained hypoxia can be used as an animal model of sustained nocturnal hypoxic stress during sleep in morbidly obese humans. Nocturnal desaturation or nocturnal sustained hypoxia in severe obese patients is often affected by multiple factors such as restrictive lung, increased chest wall loading, blunted hypoxic response, hypoventilation syndrome, hypopnea, and/or sleep apnea during sleeping time (5, 17, 18, 33). Morbidly obese humans are susceptible to sustained nocturnal hypoxia that fluctuates from mild to severe degrees (5, 17, 18). The
Cardiac apoptosis, appear to be increased in 5- to 6-mo-old obese Zucker rats or after nocturnal sustained hypoxic challenge and appear to be more severe in the simultaneous presence of both obesity and nocturnal sustained hypoxia. One previous study suggests that impaired leptin signalings lead to obesity-related cardiac apoptosis, DNA damage, and premature mortality (6). Additionally, chronic intermittent hypoxia may result in mild to moderate systemic and pulmonary hypertension with resultant left and right ventricular hypertrophy (10). Cardiac apoptosis in the coexistence of obesity and nocturnal sustained hypoxia may be induced directly or indirectly via various possible factors, such as impaired leptin signalings, lipotoxicity, systemic hypertension, pulmonary hypertension, volume overload, hypoxia, and oxidative stress (6, 7, 10, 16, 27). Therefore, any effect on cardiomyopathic changes noted in obesity cannot be isolated and attributed to any specific factor, such as volume overload, nocturnal hypoxemia, oxidative stress, blood pressure changes, or other unclear factors. However, the current study can differentiate effects of normoxia, obesity, nocturnal sustained hypoxia, and the coexistence of obesity and nocturnal sustained hypoxia. This study is the first to report that the coexistence of nocturnal sustained hypoxia and obesity additively increase myocardial disarray, cardiac hypertrophy, and cardiac apoptosis. The balance between cell death and cell survival is a tightly controlled process, especially in terminally differentiated cells, such as the cardiomyocytes (14). Therefore, the coexistence of nocturnal sustained hypoxia and obesity, which additively induces cardiac apoptosis, might help to explain the pathophysiology of heart failure or obesity-associated heart diseases in severely obese humans with severe sleep apnea or nocturnal sustained hypoxemia.

Cardiac Fas receptor- and mitochondrial-dependent apoptotic pathways. This study also represents the first to report an increase in cardiac activity of Fas receptor-dependent apoptotic pathway in the coexistence of nocturnal sustained hypoxia and obesity. The Fas apoptotic pathway is mediated by Fas, Fas death receptors, FADD, and activation of caspase-8 and -3, which in turn induces cell apoptosis (7). In the current study, Fas receptor-dependent apoptotic pathway was found to be significantly increased in cardiac tissues in obesity or after nocturnal sustained hypoxia and further increased in the coexistence of nocturnal sustained hypoxia and obesity, as evidenced by increases in cardiac Fas ligands, Fas receptors, FADDs, activated caspase-8 levels, and activated caspase-3 levels in the hearts of obese rats.

The mitochondrial-dependent apoptotic pathway is mediated by internal factors, especially in mitochondria (7). To our knowledge, no previous studies have reported on the cardiac activity of mitochondrial-dependent apoptotic pathway in obese subjects with coexistent nocturnal hypoxia. Pro-apoptotic and anti-apoptotic members of the Bcl2 family appear to interact with and neutralize each other, so that the relative balance of these effectors strongly influences cell fate (19). In other words, the downregulation of anti-apoptotic Bcl2 family members (Bcl2) in obesity with coexistent nocturnal sustained hypoxia or the upregulation of pro-apoptotic Bcl2 family members (BNIP3 and Bad) will shift the balance between cell death and cell survival is a tightly controlled process, especially in terminally differentiated cells, such as the cardiomyocytes (14). Therefore, the coexistence of nocturnal sustained hypoxia and obesity, which additively induces cardiac apoptosis, might help to explain the pathophysiology of heart failure or obesity-associated heart diseases in severely obese humans with severe sleep apnea or nocturnal sustained hypoxemia.

Cardiac changes. Obesity is often associated with hemodynamic overload, ventricular remodeling, and higher cardiac output (4) as well as, at the same time, associated with respiratory insufficiency, nocturnal hypoxemia, and sleep apnea (34). Obesity-associated cardiomyopathies may progressively develop in congestive heart failure or may result in sudden cardiac death (4). Our previous studies show that more activated Fas receptor-dependent and mitochondrial-dependent apoptotic pathways in obese Zucker rats (24, 25). In the current study, cardiomyopathic changes, such as cardiac hypertrophy, abnormal myocardial architecture, myocardial disarray, and current experimental design of daily cycle of nocturnal 8-h sustained hypoxia and 16-h room air exposure is likely to mimic the phenomenon. Physiological and pathophysiological responses to chronic vs. intermittent hypoxia are known to be different (31). For example, sustained hypoxia appears to preferentially activate transcription factors such as hypoxia-inducible factor-1 (HIF-1), whereas shorter cycles of intermittent hypoxia do not (31). Pathophysiological adaptations between sustained hypoxia (high mountain sickness), daily cycle of hypoxia-reoxygenation (nocturnal hypoxia), and intermittent hypoxia (sleep apnea) are different; therefore the nocturnal sustained hypoxic model in the current study is not an exact match for sleep apnea but it adds important information regarding the pathophysiological relationship between obesity and hypoxia.

Cardiac changes. Obesity is often associated with hemodynamic overload, ventricular remodeling, and higher cardiac output (4) as well as, at the same time, associated with respiratory insufficiency, nocturnal hypoxemia, and sleep apnea (34). Obesity-associated cardiomyopathies may progressively develop in congestive heart failure or may result in sudden cardiac death (4). Our previous studies show that more activated Fas receptor-dependent and mitochondrial-dependent apoptotic pathways in obese Zucker rats (24, 25). In the current study, cardiomyopathic changes, such as cardiac hypertrophy, abnormal myocardial architecture, myocardial disarray, and
finding in the current study is that the coexistence of obesity and nocturnal sustained hypoxia additively shifted the balance of Bcl2 family members toward pro-apoptotic effects in cardiac tissues.

Shifting the balance of Bcl2 family members toward pro-apoptotic effects will enhance cytochrome c release from mitochondria. Additionally, caspase-8 from Fas-dependent apoptotic pathway can cleave Bid and then cause the release of mitochondrial cytochrome c (1, 7). Cytochrome c release will form a complex with pro-caspase-9 and its cofactor Apaf-1 (apoptotic protease-activating factor-1). It is responsible for activating caspase-9, which further activates caspase-3 and executes the apoptotic program (9). In the current study, mitochondrial-dependent apoptotic pathway was significantly increased in cardiac tissues in obese rats with coexistent nocturnal sustained hypoxia, as evidenced based on measurement of key components of mitochondrial-dependent apoptotic pathway, including decreases in Bcl2 and Bid and increases in Bad, BNIP3, cytosolic cytochrome c, activated caspase-9, and activated caspase-3. Therefore, our findings strongly suggest that cardiac Fas receptor-dependent and mitochondrial-dependent apoptotic pathways in obesity with coexistent nocturnal sustained hypoxia become more activated, which might increase potential for the development of cardiac apoptosis or cardiac diseases.

Hypothesized clinical application. Since cardiac tissues are difficult to extract from obese human hearts, the current obese animal model under nocturnal sustained hypoxia should provide an important explanation of apoptosis-related cardiac diseases in obese humans with nocturnal sustained hypoxia. Obese-related cardiomyopathies may progressively develop in congestive heart failure and sudden cardiac death typically in persons with severe and long-standing obesity (4). Since the coexistence of nocturnal sustained hypoxia and obesity will additively enhance cardiac apoptosis, obesity with severe sleep apnea or obesity with nocturnal hypoxemia are conditions with possibility of progressive development in cardiac abnormality. Obesity is considered as a major risk factor for the development of heart failure, with a 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 (11, 12).

Our current findings that cardiac Fas-dependent and mitochondrial-dependent apoptotic pathway are more activated in obese rats with coexistent nocturnal sustained hypoxia might provide one possible mechanism behind the development of heart failure in obesity with nocturnal hypoxemia. Additionally, a further question is raised whether it might be beneficial to block cardiac Fas-dependent and mitochondrial-dependent apoptotic pathways when considering possible therapeutic agents to control or prevent the development of apoptosis-related cardiac diseases in obese patients with nocturnal hypoxemia. Of course, further studies are required to elucidate specific mechanisms responsible for this phenomenon in obesity with coexistent nocturnal hypoxia and further clinical studies are required to clarify the apoptotic pathways or possible mechanisms in obesity-related heart failure.

GRANTS

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