

Testosterone deprivation accelerates cardiac dysfunction in obese male rats

Wanpitak Pongkan^{1,2,3}, Hiranya Pintana^{1,2,3}, Sivaporn Sivasinprasasn^{1,2,3},
Thidarat Jaiwongkam^{1,2,3}, Siriporn C Chattipakorn^{1,2,4} and Nipon Chattipakorn^{1,2,3}

¹Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

²Cardiac Electrophysiology Unit, Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

³Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, Thailand

⁴Department of Oral Biology and Diagnostic Science, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

Correspondence
should be addressed
to N Chattipakorn
Email
nchattip@gmail.com

Abstract

Low testosterone level is associated with increased risks of cardiovascular diseases. As obese-insulin-resistant condition could impair cardiac function and that the incidence of obesity is increased in aging men, a condition of testosterone deprivation could aggravate the cardiac dysfunction in obese-insulin-resistant subjects. However, the mechanism underlying this adverse effect is unclear. This study investigated the effects of obesity on metabolic parameters, heart rate variability (HRV), left ventricular (LV) function, and cardiac mitochondrial function in testosterone-deprived rats. Orchiectomized or sham-operated male Wistar rats ($n=36$ per group) were randomly divided into groups and were given either a normal diet (ND, 19.77% of energy fat) or a high-fat diet (HFD, 57.60% of energy fat) for 12 weeks. Metabolic parameters, HRV, LV function, and cardiac mitochondrial function were determined at 4, 8, and 12 weeks after starting each feeding program. We found that insulin resistance was observed after 8 weeks of the consumption of a HFD in both sham (HFS) and orchiectomized (HFO) rats. Neither the ND sham (NDS) group nor ND orchiectomized (NDO) rats developed insulin resistance. The development of depressed HRV, LV contractile dysfunction, and increased cardiac mitochondrial reactive oxygen species production was observed earlier in orchiectomized (NDO and HFO) rats at week 4, whereas HFS rats exhibited these impairments later at week 8. These findings suggest that testosterone deprivation accelerates the impairment of cardiac autonomic regulation and LV function via increased oxidative stress and impaired cardiac mitochondrial function in obese-orchiectomized male rats.

Key Words

- ▶ testosterone deprivation
- ▶ obese-insulin resistance
- ▶ cardiac function
- ▶ cardiac mitochondrial function

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Introduction

Testosterone deprivation is a condition in which the concentration of testosterone is lower than the normal physiological level (Handelsman & Zajac 2004). This state can be found in aging male individuals. The prevalence

of testosterone deficiency increases substantially with age (Araujo *et al.* 2007) and is associated with the development of several disorders including obesity, dyslipidemia, insulin resistance, metabolic syndrome,

and cardiovascular disease (Grossmann *et al.* 2008, Oskui *et al.* 2013). Moreover, testosterone deprivation alone significantly induces left ventricular (LV) contractile dysfunction and cardiac sympathovagal imbalance in orchietomized rats (Pongkan *et al.* 2015).

Obesity is one of the most important risk factors contributing to the overall burden of disease worldwide, and is known as the initial main sign of metabolic syndrome, which can manifest itself as many diseases, such as cardiovascular disease, type 2 diabetes mellitus, and several types of cancer (McMillen *et al.* 2009, WHO 2011). In men, there has been a significant increase in clinical obesity, rising from 31.8 to 37.1% in the past decade (Flegal *et al.* 2010). In animal studies, it has been shown that rats fed on a HFD for at least 8 weeks developed obese-insulin resistance (Pratchayasakul *et al.* 2011), leading to cardiac sympathovagal imbalance, LV contractile dysfunction, increased oxidative stress, and cardiac mitochondrial dysfunction (Apaijai *et al.* 2014). Although either testosterone deprivation or obese-insulin resistance affected cardiac function, the temporal effects of combined obese-insulin resistance and the loss of testosterone on metabolic parameters, cardiac function, heart rate variability (HRV), and cardiac mitochondrial function have never been investigated. We tested the hypothesis that testosterone deprivation in rats, together with obese-insulin resistance, accelerates and aggravates the impairment of metabolic parameters, cardiac function, HRV, and cardiac mitochondrial function.

Materials and methods

Animal preparation

All experiments were approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. Male Wistar rats weighing ~200 g (aged 5–6 weeks) were obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. All animals were housed in a climate-controlled room (22–25°C, with a ratio of 12h light:12h darkness cycle) and fed with standard laboratory chow (normal diet (ND)) or a high-fat diet (HFD) and water. Both food and water were given *ad libitum*.

Orchiectomy procedure

Rats were anesthetized and maintained using isoflurane 2–3% and the scrotal area was shaved and scrubbed using a sterile technique. The orchiectomy was performed

using the scrotal approach technique (Foley 2005). The blood vessels and vas deferens were ligated with an absorbable suture. Then, both testes and epididymal fat pads were removed. The skin incision was closed using a nonabsorbable suture. Rats were monitored carefully to prevent them from chewing of sutures and also any other complications. Analgesic drugs and antibiotics were injected subcutaneously for 3 days after the operation.

ND and HFD preparation

Standard laboratory chow (Mouse Feed Food No. 082, C.P. Company, Bangkok, Thailand) was used as a ND (19.77% of energy fat) and fed to control rats. In addition, the HFD (57.60% of energy fat) was mixed with the following ingredients: standard rat diet, casein, lard, cholesterol, vitamins, DL-methionine, yeast powder, and sodium chloride (Pratchayasakul *et al.* 2011). All ingredients were mixed and molded into a spherical shape. Rats were fed with these diets for 12 weeks.

Experimental protocol

Rats were randomly divided into two groups: a sham-operated group and an orchietomized (ORX) group. One week after surgery, rats in each group were divided into two subgroups and were given either a ND or a HFD for 4, 8, and 12 weeks ($n=6$ per group for each duration in each dietary group). Animals in the ND group were fed on standard laboratory chow (Mouse Feed Food No. 082, C.P. Company, Bangkok, Thailand), which had an energy content of 4.02 kcal/g and 19.77% of total energy (%E from fat). Animals in the HFD group were fed a HFD, which had an energy content of 5.35 kcal/g and 59.60% E from fat. Body weight and food intake were recorded daily, and blood samples were collected at baseline and then at 4th, 8th, and 12th week after fasting for 5–6 h. Plasma of blood samples were kept at -80°C and used for the determination of metabolic parameters. At the end of the study, the heart was removed and used to study cardiac mitochondrial function.

HRV determination

HRV is a noninvasive assessment of cardiac autonomic function, which is used to determine cardiac sympathovagal balance (Chattipakorn *et al.* 2007). HRV was analyzed before the operation and then at 4th, 8th, and 12th week after being fed a ND or HFD. An ECG was

conducted on each rat using PowerLab (PowerLab 4/25T, AD Instruments, Bella Vista, NSW, Australia) with chart 5.0 continuously for 20 min, while the animals were still conscious. Data from the ECG recording was analyzed using MATLAB program (Glos *et al.* 2007). A high frequency (HF, 0.6–0.3 Hz) was taken as parasympathetic activity, while a low frequency (LF, 0.2–0.6 Hz) was taken as a combination of sympathetic and parasympathetic activities (Chattipakorn *et al.* 2007). The LF/HF ratio was used as an index of cardiac sympathovagal balance (Latchman *et al.* 2011). An increased LF/HF ratio is indicative of cardiac sympathovagal imbalance (Apaijai *et al.* 2014).

Cardiac function determination

LV function was determined using an ECG (SONOS4500, Philips) and analyzed before the operation and then at 4-, 8-, and 12-week feeding program. Rats were stabilized in a supine position, and the probe was gently placed on the chest and moved to collect data along the short and long axes of the heart. Signals from an M-mode ECG at the level of the papillary muscles were recorded. Parameters obtained included right ventricular dimension during diastole, interventricular septum during systole and diastole, LV internal dimension during systole (LVIDs) and diastole (LVIDd), and LV posterior wall thickness during systole and diastole. Fractional shortening (FS) was calculated using the formula: %FS = (LVIDd – LVIDs) × 100 / LVIDd.

At the end of the experiment (week 12), LV pressure was evaluated using a pressure catheter cannulated via the right carotid artery into the LV chamber. The LV pressure was recorded using LabScribe2 program (iWorx Systems Inc., Dover, NH, USA) (Chinda *et al.* 2014). Systolic and diastolic blood pressure, heart rate (HR), LV end-systolic pressure, and LV end-diastolic pressure (LVEDP) were assessed.

Cardiac mitochondrial function study

At the end of the experiment, cardiac mitochondria were isolated from the whole heart in each rat. Rats were deeply anesthetized and the hearts were removed and homogenized in ice-cold buffer. The tissues were homogenized by the homogenizer and then the homogenate was centrifuged at 800 g for 5 min. The supernatant was collected and centrifuged at 8800 g for 5 min. Mitochondrial pellets were resuspended in an ice-cold buffer and centrifuged one more time at 8800 g for 5 min. Protein concentration was determined by bicinchoninic acid assay as described previously (Thummasorn *et al.* 2011).

Cardiac mitochondrial function was determined by measuring mitochondrial reactive oxygen species (ROS) production, mitochondrial membrane potential change ($\Delta\Psi_m$), and mitochondrial swelling. Transmission electron microscopy (TEM) was also used to determine the morphology of the isolated cardiac mitochondria (Chinda *et al.* 2014).

Measurement of cardiac mitochondrial ROS production

Mitochondrial ROS production was determined by using dichlorodihydrofluorescein diacetate dye (DCFDA) (Thummasorn *et al.* 2011). Cardiac mitochondrial proteins (0.4 mg/mL) were incubated at 25°C with 2 μ M DCFDA for 20 min. The DCFDA diffuses through the mitochondrial membrane and is deacetylated by intracellular esterases to a nonfluorescent compound, which is later oxidized by ROS into dichlorofluorescein (DCF). DCF is a highly fluorescent compound, which can be detected by fluorescence spectroscopy with maximum excitation. An increase in DCF intensity indicates an increase in ROS production (Chinda *et al.* 2014). Fluorescent intensity of the DCF was measured at excitation wavelength (λ_{ex}) 485 nm (bandwidth 5 nm) and emission wavelength (λ_{em}) 530 nm (bandwidth 10 nm), using a fluorescent microplate reader, and represented as arbitrary units of fluorescent intensity.

Measurement of cardiac mitochondrial membrane potential change ($\Delta\Psi_m$)

Mitochondrial membrane potential change was determined using the dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1). The isolated cardiac mitochondria (0.4 mg/mL) were stained with JC-1 at 37°C for 30 min. The fluorescence intensity was determined by using a fluorescent microplate reader (Thummasorn *et al.* 2011).

In the mitochondrial membrane interaction, JC-1 could be changed to either a monomer form or an aggregate form, which have different fluorescent properties. The monomer form of JC-1 was a cation and caused a display of green fluorescence, whereas the aggregate form of JC-1 was a reaction between JC-1 (cation) and O_2^- (anion) within a mitochondrial matrix resulting in a display of red fluorescence. Inside unhealthy mitochondria, the levels of anions are lower, which leads to the reduction in the red (due to JC-1 (cation))/green (due to O_2^- (anion)) fluorescence ratio in the mitochondrial matrix; this represents mitochondrial depolarization. JC-1 monomer and aggregate form were excited at wavelengths 485 and 585 nm, whereas the emission of JC-1 monomer

and aggregate form was detected at wavelengths 530 and 590 nm, respectively. The change in the mitochondrial membrane potential was calculated from the ratio of the intensity of red to green fluorescence. A decrease in the red/green fluorescence intensity ratio indicates mitochondrial membrane depolarization (Chinda *et al.* 2014).

Measurement of cardiac mitochondrial swelling The isolated mitochondrial suspension was prepared for the determination of the level of mitochondrial swelling. Mitochondria (0.4 mg/mL) were incubated in respiration buffer as described previously (Thummasorn *et al.* 2011). Mitochondrial swelling was determined by measuring the change in the absorbance of the suspension at 540 nm (A540) using a microplate reader (Synergy HT, BioTek). Cardiac mitochondrial swelling was indicated by a decrease in the absorbance by the mitochondrial suspension (Chinda *et al.* 2014). TEM was also used to determine the morphology of the isolated cardiac mitochondria (Chinda *et al.* 2014).

Metabolic parameters, insulin resistance, and testosterone level determination

Fasting plasma glucose, total cholesterol, and triglyceride concentrations were determined by an enzymatic colorimetric assay from a commercially available kit (Biotech, Bangkok, Thailand). Fasting plasma HDL was determined using a sensitive, colorimetric and fluorometric assay from a commercially available kit (BioVision), whereas plasma LDL was determined using Friedewald's equation (Friedewald *et al.* 1972). Fasting plasma insulin level was determined by Sandwich ELISA (Millipore).

Insulin resistance was assessed using the homeostasis model assessment (HOMA) (Appleton *et al.* 2005), which was calculated from fasting plasma insulin and fasting plasma glucose concentrations. A higher HOMA index indicates a higher degree of insulin resistance (Pratchayasakul *et al.* 2011). Total plasma testosterone (ng/dL) was analyzed by ELISA technique at the Central Laboratory Service of Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University.

Determination of malondialdehyde in plasma and cardiac tissues

Plasma and tissue malondialdehyde (MDA) levels were measured using a HPLC system (Thermo Scientific) (Apaijai *et al.* 2012). Plasma or tissue MDA was mixed

with 0.44 M H₃PO₄ and 0.6% thiobabutaric acid solution, resulting in the generation of pink-colored products called thiobarbituric acid reactive substances (TBARS). Plasma or tissue TBARS concentrations were determined directly from a standard curve, and reported as an MDA equivalent concentration using an HPLC-based assay (Mateos *et al.* 2005).

Western blot analysis

Heart tissue was frozen in liquid nitrogen and stored at -80°C until use (Chinda *et al.* 2014). Myocardial protein extract was prepared by homogenization of the nitrogen-frozen heart tissues in a lysis buffer (containing 1% Nonidet P-40 (Merck Millipore), 0.5% sodium deoxycholate, 0.1% SDS in 1×PBS), and subsequently homogenized at 4°C. The homogenate was then centrifuged at 16,707g for 15 min. The total protein from tissue pellets was mixed with a loading buffer (10% mercaptoethanol, 0.05% bromophenol blue, 75 mM Tris-HCl, pH 6.8, 2% SDS, and 10% glycerol) and heated at 95°C for 10 min. The protein samples were then subjected to 10 or 15% SDS-PAGE, and transferred to polyvinylidene difluoride membranes using a semi-dry transfer system (Trans-Blot SD; Bio-Rad). The membranes were blocked with 5% nonfat skimmed milk in TBST (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) for 1 h. The membranes were subsequently exposed to anti-BAX (Santa Cruz Biotechnology), anti-BCL2 (Cell Signaling Technology), or β-actin (Santa Cruz Biotechnology) overnight at 4°C. On the following day, the membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology) for 1 h at room temperature. The signals were developed by incubating with an enhanced chemiluminescence reagent and subjected to autoradiography. The immunoblot films were scanned, and the band density was analyzed using ImageJ software (NIH, Bethesda, MD, USA).

Statistical analysis

Data were expressed as mean ± s.e.m. Statistical comparisons at the different time points in the feeding program for HRV, cardiac function, and cardiac mitochondrial function were analyzed using a one-way ANOVA, followed by *post hoc* least significant difference analysis. Statistical comparisons of the biochemical parameters were calculated using the Mann-Whitney U test. All statistical analyses were performed using SPSS (version 16; SPSS). The statistical significance was accepted at a *P* value <0.05.

Results

Obesity, but not testosterone deficiency, led to insulin resistance

At week 4, testosterone deprivation was confirmed by decreased plasma testosterone levels and the ratio of seminal vesicle per body weight (seminal vesicle ratio). Orchiectomized rats in both dietary groups (NDO and HFO) had significantly decreased plasma testosterone levels and seminal vesicle ratio, when compared with sham rats at the same time points. Animals fed on a HFD for 4 weeks had significantly increased body weight compared with ND rats. In addition, orchiectomized rats in both dietary groups (NDO and HFO) had decreased body weight, food intake, and visceral fat when compared with sham rats in the same dietary groups. However, there was no significant difference in the metabolic parameters (the level of plasma insulin, plasma glucose, HOMA index, plasma total cholesterol, plasma triglyceride, plasma HDL and LDL in the blood) among the dietary groups at 4 weeks (Table 1).

At week 8, the body weight, food intake, and visceral fat mass were similar to the results at week 4. Peripheral insulin resistance developed only in the HFD groups (HFS and HFO) at 8 weeks, as shown by both an increased insulin level and HOMA index. The metabolic parameters did not differ between HFS and HFO, but they were significantly higher than those in ND rats (NDS and NDO) (Table 2).

At week 12, HF rats (HFS and HFO) still demonstrated consistent impairments in metabolic parameters, as shown by increased levels of all metabolic parameters,

except triglyceride level, compared with NDS and NDO rats (Table 3). Moreover, HFS rats still showed a significant increase in body weight compared with NDS rats, whereas orchiectomized rats from both dietary groups (NDO and HFO) had decreased body weight, food intake, and visceral fat mass compared with the sham rats in the same dietary groups (Table 3).

Testosterone deprivation led to oxidative stress earlier than high-fat-induced obesity

Orchiectomized rats (NDO and HFO) had increased plasma MDA from week 4 onward, when compared with sham rats in the same dietary groups, whereas cardiac tissue MDA was increased only in the HFO group (Table 1). Plasma and cardiac tissue levels of MDA in orchiectomized rats (NDO and HFO) continued to show an increase at week 8 and 12. Plasma and cardiac tissue levels of MDA in the HFS group in week 8 (Table 2) and 12 (Table 3) were also elevated, showing the same levels of plasma MDA observed in the NDO and HFO groups, and was significantly different when compared with the NDS group.

Testosterone deprivation led to cardiac dysfunction and HRV impairment earlier than obesity

In ORX rats fed on either a normal diet (NDO) or a high-fat diet (HFO), both FS and ejection fraction (EF) showed a significant decrease at week 4, and continued to decrease by week 8 and 12, when compared with sham rats in the

Table 1 Effect of obese-insulin resistance in testosterone-deprived rats on metabolic parameters investigated at 4 weeks ($n=6$ per group).

Parameters	Normal diet		High-fat diet	
	NDS	NDO	HFS	HFO
Body weight (g)	448 ± 12	420 ± 9*	493 ± 15* [†]	460 ± 10 ^{†,‡}
Food intake (g/day)	20.7 ± 0.2	18.8 ± 0.4* [‡]	21.1 ± 0.1	20.4 ± 0.3 ^{†,‡}
Visceral fat (g)	22.8 ± 2.07	15.8 ± 2.16* [‡]	35.2 ± 2.22*	29 ± 0.6* ^{†,‡}
Plasma testosterone (ng/dL)	1.5 ± 0.4	0.1 ± 0.01* [‡]	1.0 ± 0.01	0.1 ± 0.01* [‡]
Seminal vesicle ratio (×10 ³)	3.3 ± 0.26	0.37 ± 0.1* [‡]	3.4 ± 0.41	0.3 ± 0.12* [‡]
Plasma insulin (ng/mL)	2.1 ± 0.2	2.0 ± 0.2	2.2 ± 0.3	2.2 ± 0.3
Plasma glucose (mg/dL)	116 ± 11	127 ± 7	125 ± 8	125 ± 7
HOMA index	11 ± 2	11.2 ± 1	12.2 ± 1	12.5 ± 2
Plasma total cholesterol (mg/dL)	90 ± 3	91 ± 5	95 ± 4	102 ± 7
Plasma triglyceride (mg/dL)	134 ± 12	139 ± 8	138 ± 16	142 ± 7
Plasma HDL (mg/dL)	32 ± 1	31 ± 3	29 ± 2	28 ± 2
Plasma LDL (mg/dL)	62 ± 3	68 ± 3	67 ± 5	69 ± 8
Plasma MDA (mmol/mL)	4.7 ± 0.08	5.6 ± 0.19* [‡]	5.0 ± 0.06	5.5 ± 0.17* [‡]
Cardiac MDA (µmol/mL)	1.53 ± 0.26	3.7 ± 0.76	1.57 ± 0.15	5.2 ± 0.79*

* $P < 0.05$ vs NDS; [†] $P < 0.05$ vs NDO; [‡] $P < 0.05$ vs HFS.

Table 2 Effect of obese insulin resistance in testosterone-deprived rats on metabolic parameters investigated at 8 weeks ($n=6$ per group).

Parameters	Normal diet		High-fat diet	
	NDS	NDO	HFS	HFO
Body weight (g)	460±10	443±8 ^{*,‡}	535±15 [*]	497±10 ^{*,†,‡}
Food intake (g/day)	19.2±0.7	17.7±0.6 ^{*,‡}	21.9±0.1	19.8±0.3 ^{†,‡}
Visceral fat (g)	30.3±0.8	18.3±1.8 ^{*,‡}	48.5±3.1 [*]	37.2±2.38 ^{*,†,‡}
Plasma testosterone (ng/dL)	2.1±0.7	0.03±0.01 ^{*,‡}	1.2±0.31 [*]	0.08±0.01 ^{*,‡}
Seminal vesicle ratio (×10 ³)	3.8±0.22	0.21±0.01 ^{*,‡}	3.1±0.1 [*]	0.34±0.04 ^{*,‡}
Plasma insulin (ng/mL)	1.95±0.1	2.0±0.1	2.9±0.3 ^{*,†}	2.8±0.3 ^{*,†}
Plasma glucose (mg/dL)	123±7	133±7	128±4	130±9
HOMA index	11.0±1	11.2±1	16.6±1 ^{*,†}	16.7±3 ^{*,†}
Plasma total cholesterol (mg/dL)	91±4	93±4	108±6 ^{*,†}	111±5 ^{*,†}
Plasma triglyceride (mg/dL)	129±10	130±14	124±17	123±24
Plasma HDL (mg/dL)	31±2	33±2	23±2 ^{*,†}	21±3 ^{*,†}
Plasma LDL (mg/dL)	61±4	68±5	83±6 [*]	89±5 ^{*,†}
Plasma MDA (mmol/mL)	5.1±0.06	5.7±0.13 [*]	5.9±0.1 [*]	5.6±0.12 [*]
Cardiac MDA (µmol/mL)	1.98±0.26	4.7±1.02 [*]	4.39±0.79 [*]	4.53±0.71 [*]

* $P<0.05$ vs NDS; [†] $P<0.05$ vs NDO; [‡] $P<0.05$ vs HFS.

same dietary groups (NDS and HFS). Unlike ORX rats, HFS rats exhibited a significant reduction in both %FS and %EF later at week 8 and 12 of the study, when compared with rats in the NDS group (Fig. 1A and B).

Consistent with the %FS and %EF data, ORX rats (NDO and HFO) were the first groups to present as having impaired sympathovagal balance. The LF/HF ratio in ORX rats showed a significant increase from week 4 onward, and still showed this impairment at week 8 and 12, when compared with the NDS group. The HFS group exhibited sympathovagal imbalance later, by week 8 and 12 (Fig. 1C). No significant difference in the LF/HF ratio was found in the NDO, HFS, and HFO groups at weeks 8 and 12.

The changes in the LV pressure at week 12 in all groups are given in Table 4. Rats in the NDO, HFS, and HFO groups had increased systolic and diastolic blood pressure when compared with rats in the NDS group. Moreover, these groups exhibited diastolic dysfunction at week 12, as shown by increased LVEDP, when compared with the NDS group.

Testosterone deprivation led to cardiac mitochondrial dysfunction earlier than obesity

In ORX rats (NDO and HFO), cardiac mitochondrial dysfunction had developed by week 4 and dysfunction

Table 3 Effect of obese-insulin resistance in testosterone-deprived rats on metabolic parameters investigated at 12 weeks ($n=6$ per group).

Parameters	Normal diet		High-fat diet	
	NDS	NDO	HFS	HFO
Body weight (g)	485±15	447±16 ^{*,‡}	575±21 [*]	508±17 ^{†,‡}
Food intake (g/day)	20.7±0.2	16.4±1 ^{*,‡}	20.3±0.4	18.4±0.3 ^{†,‡}
Visceral fat (g)	30.2±2.6	21.8±0.6 ^{*,‡}	51±4.6 [*]	28.5±3.4 ^{†,‡}
Plasma testosterone (ng/dL)	1.2±0.4	0.07±0.01 ^{*,‡}	0.5±0.06 [*]	0.1±0.06 ^{*,‡}
Seminal vesicle ratio (×10 ³)	3.82±0.28	0.51±0.33 ^{*,‡}	3.1±0.07 [*]	0.18±0.02 ^{*,‡}
Plasma insulin (ng/mL)	1.9±0.2	2.2±0.3	3.3±0.2 ^{*,†}	3.0±0.3 ^{*,†}
Plasma glucose (mg/dL)	140±5	136±4	132±4	136±6
HOMA index	13.5±1	12.5±1	20.1±1 ^{*,†}	19.8±2 ^{*,†}
Plasma total cholesterol (mg/dL)	89±5	100±2	109±6 ^{*,†}	130±7 ^{*,†,‡}
Plasma triglyceride (mg/dL)	133±32	135±10	146±9	133±4
Plasma HDL (mg/dL)	33±2	35±2	25±2 ^{*,†}	25±3 ^{*,†}
Plasma LDL (mg/dL)	63±5	73±4	82±8 [*]	95±8 ^{*,†}
Plasma MDA (mmol/mL)	4.04±0.4	5.6±0.27 [*]	5.8±0.5 [*]	5.4±0.15 [*]
Cardiac MDA (µmol/mL)	2.48±0.63	6.08±0.75 [*]	6.37±1.30 [*]	7.07±0.67 [*]

* $P<0.05$ vs NDS; [†] $P<0.05$ vs NDO; [‡] $P<0.05$ vs HFS.

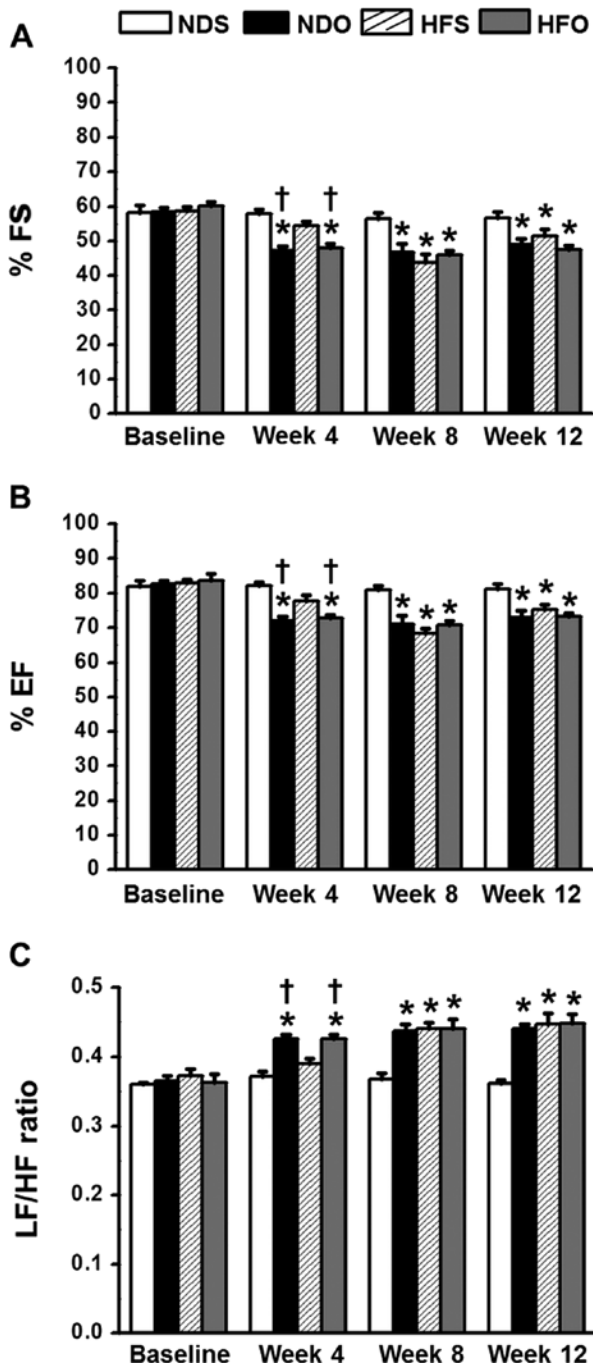


Figure 1
The effect of obese-insulin resistance in testosterone-deprived rats on FS, EF, and HRV (represented as LF/HF ratio) at different time courses (4, 8, and 12 weeks). (A) %FS and (B) %EF were decreased in NDO and HFO groups at any time following week 4, whereas %FS in the HFS group was decreased at week 8 and onward, compared with the NDS group. (C) LF/HF ratio was significantly increased in NDO and HFO groups from week 4, whereas the LF/HF ratio in the HFS group was increased at week 8, compared with the NDS group. * $P < 0.05$ vs NDS, † $P < 0.05$ vs HFS, $n = 5-6$ per group. NDS, normal diet + sham surgery; NDO, normal diet + orchietomy; HFS, high-fat diet + sham surgery; HFO, high-fat diet + orchietomy.

continued to week 8 and 12, as indicated by increased mitochondrial ROS production (Fig. 2A), mitochondrial depolarization as indicated by a decrease in the red/green fluorescent intensity ratio (Fig. 2B) and mitochondrial swelling as indicated by a decrease in the absorbance at 540nm (Fig. 2C) when compared with sham rats at the same point in time. In HFS rats, it was not until week 8 that cardiac mitochondrial dysfunction developed (Fig. 2). No significant difference in these cardiac mitochondrial parameters was found between NDO, HFS, and HFO groups at week 8 and 12. Representative TEM pictures of cardiac mitochondria are shown in Fig. 2D. Cardiac mitochondrial swelling could be observed in ORX rats (NDO and HFO) at week 4, 8, and 12, whereas in the HFS group was observed later, at week 8 and 12.

Increased cardiac apoptotic protein expression in testosterone-deprived rats with and without obese-insulin resistance

In the heart, the expression of BAX and BCL2 showed no significant difference between all groups at week 4. At week 8, when compared with the NDS group, the pro-apoptotic (BAX) level was increased in NDO, HFS, and HFO groups (Fig. 3A,D), whereas the anti-apoptotic (BCL2) levels showed no significant difference between the groups (Fig. 3B,D). At week 12, the BAX level in NDO, HFS, and HFO groups was continuously increasing (Fig. 3A,D), whereas the BCL2 level in NDO, HFS, and HFO groups was lower at this time point, compared with the NDS group (Fig. 3B,D). The BAX/BCL2 ratio increased earlier in the HFO group (week 4), whereas this ratio increased later (week 8) in the NDO and HFS groups (Fig. 3C).

Discussion

This study has demonstrated the adverse effects of testosterone deprivation and obese-insulin resistance on the heart. The major findings of this study are as follows. First, testosterone deprivation alone does not cause insulin resistance, but leads to earlier development of LV dysfunction, cardiac sympathovagal imbalance, cardiac mitochondrial dysfunction, and increased circulating oxidative stress. Secondly, long-term HFD consumption alone enhances the development of obese-insulin resistance, LV dysfunction, cardiac sympathovagal imbalance, cardiac mitochondrial dysfunction, and increased circulating and cardiac tissue oxidative stress. These adverse effects, however, developed later than

Table 4 Effect of obese insulin resistance in testosterone-deprived rats on cardiac function at 12 weeks ($n=6$ per group).

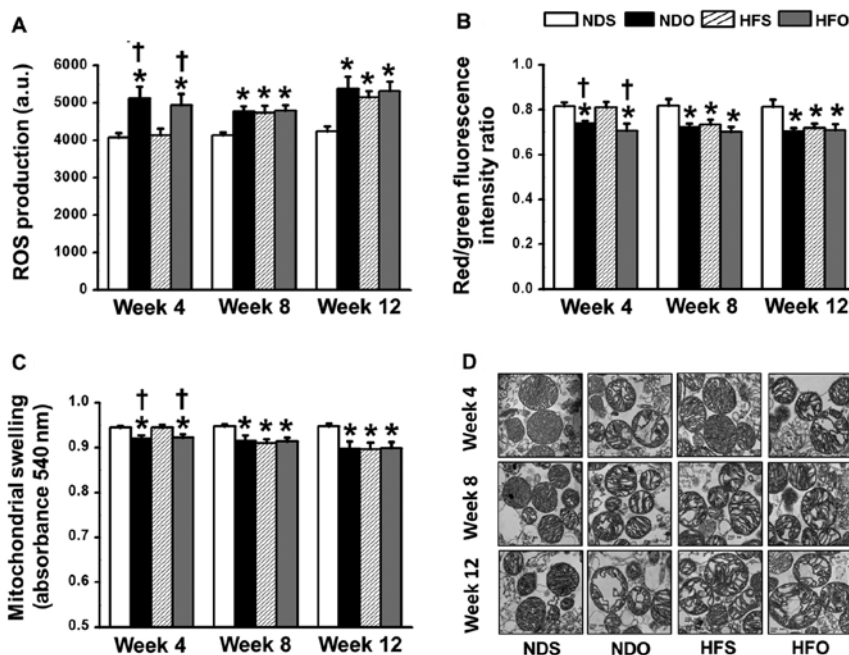
Cardiac function parameters	Normal diet		High-fat diet	
	NDS	NDO	HFS	HFO
Heart rate (bpm)	285 ± 5	284 ± 15	276 ± 11	284 ± 15
Systolic BP (mmHg)	109 ± 2	128 ± 7*	127 ± 6*	129 ± 6*
Diastolic BP (mmHg)	86 ± 1	106 ± 7*	99 ± 7*	103 ± 5*
LVESP (mmHg)	103 ± 6	96 ± 5	109 ± 2	101 ± 4
LVEDP (mmHg)	5 ± 1	9 ± 1*	10 ± 1*	10 ± 1*
P _{max} (mmHg)	106 ± 6	104 ± 6	115 ± 3	106 ± 4
P _{min} (mmHg)	3 ± 1	6 ± 3	7 ± 1	8 ± 1
dP/dt _{max} (mmHg/s)	7056 ± 522	6757 ± 410	7057 ± 502	6264 ± 286
dP/dt _{min} (mmHg/s)	-5458 ± 461	-4433 ± 449	-6463 ± 662†	-5950 ± 1001

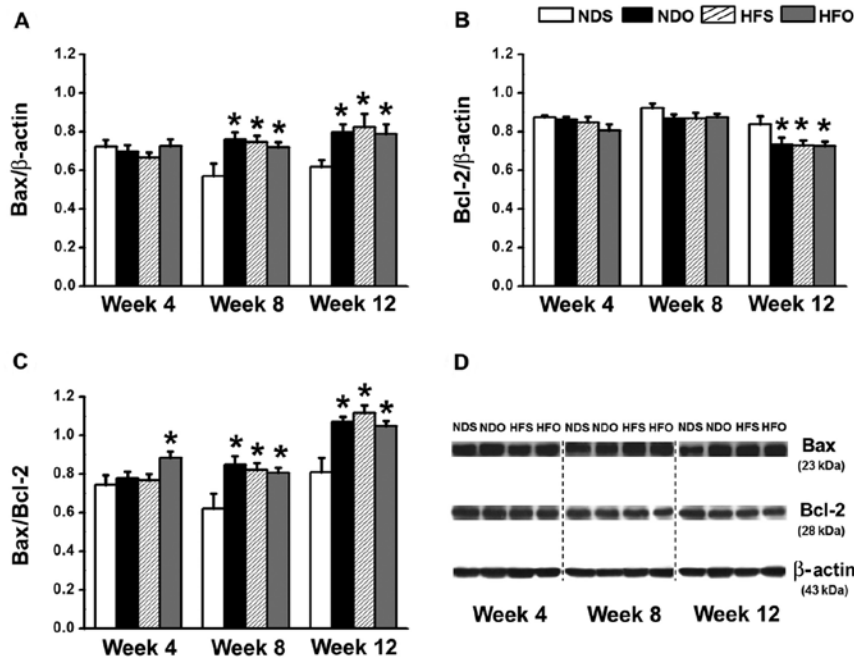
BP, blood pressure; LVESP, left ventricular end-systolic pressure. * $P < 0.05$ vs NDS; † $P < 0.05$ vs NDO.

those observed in the testosterone-deprived rats. Thirdly, although obese-insulin resistance does not aggravate these adverse effects of testosterone deprivation, it accelerates the apoptotic process in the heart of testosterone-deprived rats. A summary of these findings is given in Table 5.

In this study, testosterone deprivation did not have adverse effects on metabolic parameters, except for weight loss, at all measured time points. The weight loss found in NDO rats could be due to decreased food intake. This finding is consistent with previous studies, which reported that an orchietomy could cause permanent hypophagia (Gentry & Wade 1976), an increase in oxidative stress (Pongkan *et al.* 2015), and an increase in leptin (Baumgartner *et al.* 1999). The possible mechanism for this could be due to the induction of proinflammatory

cytokines (via nuclear factor- κ -B signaling molecules) causing oxidative stress (Vlantis & Pasparakis 2010). This may result in an increased concentration of circulating leptin via activation of the leptin gene through the p38-MAPK pathway (Finck & Johnson 2002) and lead to decreased food intake and a resulting drop in body weight (Aoyama *et al.* 2015). Although testosterone deprivation did not appear to have any measurable effects on metabolic parameters, it caused LV dysfunction and cardiac autonomic imbalance as observed from week 4 onward. Cardiac autonomic control has been shown to be mainly influenced by oxidative stress (Brook *et al.* 2010). It has been shown that increased oxidative stress is associated with cardiac autonomic imbalance (Sivasinprasasn *et al.* 2015). Moreover, cardiac mitochondrial function has been



**Figure 3**

The effects of obese-insulin resistance in testosterone-deprived rats on myocardial apoptosis at different time courses (4, 8, and 12 weeks). Anti-apoptotic (BCL2) expression (A), pro-apoptotic (BAX) expression (B), BAX/BCL2 ratio (C), and representative bands from western blot analysis (D). BCL2 expression was reduced in NDO, HFS, and HFO groups at week 12, whereas BAX expression was increased in NDO, HFS, and HFO groups at week 8 and 12. BAX/BCL2 ratio showed an increase earlier in the HFO group (week 4), whereas this ratio increased later (week 8) in NDO, HFS, and HFO groups, compared with the NDS group at the same point in the feeding program. * $P < 0.05$ vs NDS at the same point in the feeding program, $n = 5-6$ per group. NDS, normal diet+sham surgery; NDO, normal diet+orchietomy; HFS, high-fat diet+sham surgery; HFO, high-fat diet+orchietomy.

shown to play an important role on cardiac contractile function (Marchini *et al.* 2013). Therefore, the adverse effects observed in testosterone-deprived rats could be due to the increased oxidative stress and impaired cardiac mitochondrial function, thus resulting in cardiac autonomic imbalance and LV dysfunction, respectively.

Long-term consumption of a HFD has been shown to cause obese-insulin resistance (Pratchayasakul *et al.* 2011), which is characterized by increased plasma insulin, HOMA index, and plasma total cholesterol levels (Pratchayasakul *et al.* 2011). Although body weight, food intake, and visceral fat were decreased in obese-testosterone deficiency (HFO) rats, the insulin resistance index (HOMA index) was not improved in this study. Since a previous report showed that the reduction of food intake and body weight could improve insulin sensitivity (Schenk *et al.* 2009), our finding emphasized the importance of testosterone deficiency condition, which might aggravate insulin resistance as observed in this study.

Supporting this hypothesis is due to the fact that testosterone deficiency has been shown to cause insulin resistance (Dubois *et al.* 2015, Pintana *et al.* 2015a).

In this study, insulin resistance was not observed until after 8 weeks of consumption of a HFD, despite the fact that weight gain had been observed in these rats since week 4. However, the levels of metabolic impairment, LV dysfunction, and cardiac autonomic imbalance in HFS and HFO rats at weeks 8 and 12 were no different, suggesting that the obese-insulin-resistant condition did not aggravate these adverse effects caused by testosterone deprivation as observed in NDO rats. This is also supported by the findings that both HFO and HFS rats had similar levels of oxidative stress and cardiac mitochondrial dysfunction. However, these findings indicate that testosterone deprivation accelerated the development of cardiac autonomic disturbance and LV dysfunction as HFO rats had already developed these impairments by week 4, whereas HFS rats showed no signs of this at this period.

Table 5 A summary of the cardiometabolic impairment of the experimental groups at different points in the feeding program (4, 8, and 12 weeks).

Impairment	4 week				8 week				12 week			
	NDS	NDO	HFS	HFO	NDS	NDO	HFS	HFO	NDS	NDO	HFS	HFO
Metabolic disturbance							✓	✓			✓	✓
LV dysfunction		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Cardiac autonomic imbalance		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Oxidative stress		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Mitochondrial impairment		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓

Therefore, the dysfunctions in week 4 could be due to the influence of testosterone deprivation alone. Moreover, HFO rats at week 8 have testosterone deprivation combined with obese-insulin resistance and did not show any additive adverse effect, compared with the same group at 4 weeks. In addition, these abnormalities were similar between 8 and 12 weeks. These findings suggest an unclear relationship between the effects produced by testosterone deprivation on cardiac function and those caused by obesity and insulin resistance since no additive adverse effect of these two abnormal conditions was observed. This is consistent with the previous study demonstrating the effects of testosterone deprivation on the cognitive function (Pintana *et al.* 2016). However, whether additive adverse effects from these two conditions can be observed at a longer duration of obese-insulin-resistant condition are not known and need further investigation in the future. Nevertheless, at this point our study demonstrated that testosterone deprivation did not aggravate these impairments, but accelerated the development of these impairments in obese-insulin-resistant rats.

Regarding apoptotic markers, the HFO rats demonstrated an increased ratio of BAX/BCL2 from week 4, which was earlier than that observed in the HFS rats. The possible mechanism could be due to the increasing level of plasma and cardiac MDA, and increased cardiac mitochondrial ROS production in HFO rats. The increase in the level of oxidative stress in the heart may have aggravated an apoptotic pathway (Zorov *et al.* 2006) and led to a decrease in anti-apoptotic (BCL2) levels, and an increase in pro-apoptotic protein (BAX) levels (Pongkan *et al.* 2015), therefore, leading to cardiomyocyte apoptosis and finally LV dysfunction (Aon *et al.* 2009).

Our findings are consistent with previous clinical studies. The cohort clinical study demonstrated that the prevalence of cardiovascular morbidity and mortality increased in patients suffering from prostate cancer (PCa) who have undergone to androgen deprivation therapy (ADT), suggesting that testosterone deficiency was the risk factor of cardiovascular diseases (Gandaglia *et al.* 2015). Moreover, diastolic dysfunction was found in patients with type 2 diabetes with low testosterone level compared with similar patients with normal testosterone levels (Tinetti *et al.* 2015). Furthermore, patients suffering from congestive heart failure (Jankowska *et al.* 2006) and coronary artery disease (Li *et al.* 2012) have been shown to have lower levels of testosterone compared with healthy men, and the severity of these conditions had a strong correlation with the degree of testosterone deficiency (Li *et al.* 2012, Oskui *et al.* 2013). Recent clinical studies

demonstrated that testosterone replacement could improve insulin sensitivity, increase lean mass, and decrease subcutaneous fat (Dhindsa *et al.* 2016) and also improve cardiometabolic parameters in type 2 diabetes men with testosterone deprivation (Hackett *et al.* 2014) and in hypogonadal men with metabolic syndrome (Saad *et al.* 2016).

Although low testosterone level may be a risk factor for diabetes or insulin resistance (Muraleedharan & Jones 2010), the effects of testosterone replacement on insulin resistance and metabolic parameters may depend on its dose. Physiological level of testosterone exhibited the beneficial effect on insulin resistance and metabolic parameters (Pintana *et al.* 2015b). However, supraphysiological level of testosterone had been shown to provide adverse effect on insulin resistance (Yasui *et al.* 2012, Garrido *et al.* 2014). In healthy and menopausal women, supraphysiological level of testosterone could induce the development of breast cancer, heart disease, and insulin resistance (Polderman *et al.* 1994, Yasui *et al.* 2012). Moreover, high-dose testosterone significantly decreased plasma HDL, increased LDL cholesterol in healthy men and postmenopausal women (Taggart *et al.* 1982, Hurley *et al.* 1984, Thompson *et al.* 1989), and induced insulin resistance in a rat model (Holmang & Bjornorp 1992). High-dose testosterone also increased oxidative stress levels by decreasing the expression of endothelial nitric oxide synthase and consequently the formation of NO, resulting in endothelial dysfunction (Skogastierna *et al.* 2014). Moreover, in an ischemia and reperfusion (I/R) injury rabbit model, supraphysiological level of testosterone increased fibrosis and oxidative stress and exacerbated such detrimental effects of I/R (Chuang *et al.* 2013), whereas testosterone replacement at the physiological level exerted antioxidative effects by reducing mitochondrial oxidative stress (Pongkan *et al.* 2015, Pintana *et al.* 2015b) and serum MDA levels (Pintana *et al.* 2015b).

In conclusion, testosterone deprivation did not cause metabolic disturbance, but caused cardiac autonomic imbalance and LV dysfunction due to increased oxidative stress and impaired cardiac mitochondrial function. Obese-insulin resistance caused by long-term consumption of a HFD could lead to metabolic disorders, cardiac autonomic imbalance, and LV dysfunction due to increased oxidative stress and impaired cardiac mitochondrial function. Despite these similar adverse effects on the heart, the findings of this study demonstrated that testosterone deprivation did not aggravate these impairments, but accelerated the development of these impairments in

obese-insulin-resistant rats, the obesity and resulting insulin resistance being caused by consumption of a HFD. These findings may suggest the necessity for early testosterone replacement to ensure cardioprotection in obese-insulin-resistant conditions linked with testosterone deprivation. Further studies are needed to investigate the roles of testosterone replacement therapy under these conditions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Aon MA, Cortassa S, Akar FG, Brown DA, Zhou L & O'Rourke B 2009 From mitochondrial dynamics to arrhythmias. *International Journal of Biochemistry and Cell Biology* **41** 1940–1948. (doi:10.1016/j.biocel.2009.02.016)
- Aoyama N, Asano S, Shingu M, Ueno K, Miyashita M, Hamada H, Aikata H & Kataoka T 2015 Changes in appetite, nutrition, and oxidative stress reaction of cancer patients during chemotherapy. *Gan To Kagaku Ryoho* **42** 961–965.
- Apaijai N, Pintana H, Chattipakorn SC & Chattipakorn N 2012 Cardioprotective effects of metformin and vildagliptin in adult rats with insulin resistance induced by a high-fat diet. *Endocrinology* **153** 3878–3885. (doi:10.1210/en.2012-1262)
- Apaijai N, Chinda K, Palee S, Chattipakorn S & Chattipakorn N 2014 Combined vildagliptin and metformin exert better cardioprotection than monotherapy against ischemia-reperfusion injury in obese-insulin resistant rats. *PLoS ONE* **9** e102374. (doi:10.1371/journal.pone.0102374)
- Appleton DJ, Rand JS & Sunvold GD 2005 Basal plasma insulin and homeostasis model assessment (HOMA) are indicators of insulin sensitivity in cats. *Journal of Feline Medicine and Surgery* **7** 183–193. (doi:10.1016/j.jfms.2004.12.002)
- Araujo AB, Esche GR, Kupelian V, O'Donnell AB, Travison TG, Williams RE, Clark RV & McKinlay JB 2007 Prevalence of symptomatic androgen deficiency in men. *Journal of Clinical Endocrinology and Metabolism* **92** 4241–4247. (doi:10.1210/jc.2007-1245)
- Baumgartner RN, Waters DL, Morley JE, Patrick P, Montoya GD & Garry PJ 1999 Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. *Metabolism* **48** 378–384. (doi:10.1016/S0026-0495(99)90089-6)
- Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, *et al.* 2010 Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation* **121** 2331–2378. (doi:10.1161/CIR.0b013e3181d8bec1)
- Chattipakorn N, Incharoen T, Kanlop N & Chattipakorn S 2007 Heart rate variability in myocardial infarction and heart failure. *International Journal of Cardiology* **120** 289–296. (doi:10.1016/j.ijcard.2006.11.221)
- Chinda K, Sanit J, Chattipakorn S & Chattipakorn N 2014 Dipeptidyl peptidase-4 inhibitor reduces infarct size and preserves cardiac function via mitochondrial protection in ischaemia-reperfusion rat heart. *Diabetes & Vascular Disease Research* **11** 75–83. (doi:10.1177/1479164113516134)
- Chuang SM, Liu KM, Lee YC, Lin RJ, Chang CY, Wu WJ, Chang WC, Levin RM & Juan YS 2013 Effects of supraphysiological testosterone treatment and orchiectomy on ischemia/reperfusion-induced bladder dysfunction in male rabbits. *Journal of Sexual Medicine* **10** 1278–1290. (doi:10.1111/jsm.12104)
- Dhindsa S, Ghanim H, Batra M, Kuhadiya ND, Abuaysheh S, Sandhu S, Green K, Makdissi A, Hejna J, Chaudhuri A, *et al.* 2016 Insulin resistance and inflammation in hypogonadotropic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes. *Diabetes Care* **39** 82–91. (doi:10.2337/dc15-1518)
- Dubois V, Laurent MR, Jardi F, Antonio L, Lemaire K, Goyvaerts L, Deldicque L, Carmeliet G, Decallonne B, Vanderschueren D, *et al.* 2015 Androgen deficiency exacerbates high fat diet-induced metabolic alterations in male mice. *Endocrinology* **157** 648–665. (doi:10.1210/en.2015-1713)
- Finck BN & Johnson RW 2002 Anti-inflammatory agents inhibit the induction of leptin by tumor necrosis factor-alpha. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **282** R1429–R1435. (doi:10.1152/ajpregu.00569.2001)
- Flegal KM, Carroll MD, Ogden CL & Curtin LR 2010 Prevalence and trends in obesity among US adults, 1999–2008. *JAMA* **303** 235–241. (doi:10.1001/jama.2009.2014)
- Foley PL 2005 Common surgical procedures in rodents. In *Laboratory Animal Medicine and Management*. Eds JD Reuter & MA Suckow. Office of Animal Research Education and Compliance, University of Virginia, Charlottesville, VA, USA. Ithaca, NY, USA: International Veterinary Information Service. (available at: www.ivia.org)
- Friedewald WT, Levy RI & Fredrickson DS 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18** 499–502.
- Gandaglia G, Sun M, Popa I, Schiffmann J, Trudeau V, Shariat SF, Trinh QD, Graefen M, Widmer H, Saad F, *et al.* 2015 Cardiovascular mortality in patients with metastatic prostate cancer exposed to androgen deprivation therapy: a population-based study. *Clinical Genitourinary Cancer* **13** e123–e130. (doi:10.1016/j.clgc.2014.12.003)
- Garrido P, Salehzadeh F, Duque-Guimaraes DE & Al-Khalili L 2014 Negative regulation of glucose metabolism in human myotubes by supraphysiological doses of 17beta-estradiol or testosterone. *Metabolism* **63** 1178–1187. (doi:10.1016/j.metabol.2014.06.003)
- Gentry RT & Wade GN 1976 Androgenic control of food intake and body weight in male rats. *Journal of Comparative and Physiological Psychology* **90** 18–25. (doi:10.1037/h0077264)
- Glos M, Romberg D, Fietze I & Penzel T 2007 Heart rate and systolic blood pressure variability before and during obstructive sleep apnea episodes. *Conference Proceedings of the IEEE Engineering in Medicine and Biology Society* 263–266. (doi:10.1109/ieems.2007.4352274)
- Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, Zajac JD & Jerums G 2008 Low testosterone levels are common and associated with insulin resistance in men with diabetes. *Journal of Clinical Endocrinology and Metabolism* **93** 1834–1840. (doi:10.1210/jc.2007-2177)
- Hackett G, Cole N, Bhartia M, Kennedy D, Raju J & Wilkinson P 2014 Testosterone replacement therapy improves metabolic parameters

- in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. *Journal of Sexual Medicine* **11** 840–856. (doi:10.1111/jsm.12404)
- Handelsman DJ & Zajac JD 2004 Androgen deficiency and replacement therapy in men. *Medical Journal of Australia* **180** 529–535.
- Holmang A & Bjornorp P 1992 The effects of testosterone on insulin sensitivity in male rats. *Acta Physiologica Scandinavica* **146** 505–510. (doi:10.1111/j.1748-1716.1992.tb09452.x)
- Hurley BF, Seals DR, Hagberg JM, Goldberg AC, Ostrove SM, Holloszy JO, Wiest WG & Goldberg AP 1984 High-density-lipoprotein cholesterol in bodybuilders v powerlifters. Negative effects of androgen use. *JAMA* **252** 507–513. (doi:10.1001/jama.1984.03350040037019)
- Jankowska EA, Biel B, Majda J, Szklarska A, Lopuszanska M, Medras M, Anker SD, Banasiak W, Poole-Wilson PA & Ponikowski P 2006 Anabolic deficiency in men with chronic heart failure: prevalence and detrimental impact on survival. *Circulation* **114** 1829–1837. (doi:10.1161/CIRCULATIONAHA.106.649426)
- Latchman PL, Mathur M, Bartels MN, Axtell RS & De Meersman RE 2011 Impaired autonomic function in normotensive obese children. *Clinical Autonomic Research* **21** 319–323. (doi:10.1007/s10286-011-0116-8)
- Li L, Guo CY, Jia EZ, Zhu TB, Wang LS, Cao KJ, Ma WZ & Yang ZJ 2012 Testosterone is negatively associated with the severity of coronary atherosclerosis in men. *Asian Journal of Andrology* **14** 875–878. (doi:10.1038/aja.2012.95)
- Marchini T, Magnani N, D'Annunzio V, Tasat D, Gelpi RJ, Alvarez S & Evelson P 2013 Impaired cardiac mitochondrial function and contractile reserve following an acute exposure to environmental particulate matter. *Biochimica et Biophysica Acta* **1830** 2545–2552. (doi:10.1016/j.bbagen.2012.11.012)
- Mateos R, Lecumberri E, Ramos S, Goya L & Bravo L 2005 Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress. Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* **827** 76–82. (doi:10.1016/j.jchromb.2005.06.035)
- McMillen IC, Rattanaray L, Duffield JA, Morrison JL, MacLaughlin SM, Gentili S & Muhlhauser BS 2009 The early origins of later obesity: pathways and mechanisms. *Advances in Experimental Medicine and Biology* **646** 71–81. (doi:10.1007/978-1-4020-9173-5)
- Muraleedharan V & Jones TH 2010 Testosterone and the metabolic syndrome. *Therapeutic Advances in Endocrinology and Metabolism* **1** 207–223. (doi:10.1177/2042018810390258)
- Oskui PM, French WJ, Herring MJ, Mayeda GS, Burstein S & Kloner RA 2013 Testosterone and the cardiovascular system: a comprehensive review of the clinical literature. *Journal of the American Heart Association* **2** e000272. (doi:10.1161/JAHA.113.000272)
- Pintana H, Chattipakorn N & Chattipakorn S 2015a Testosterone deficiency, insulin-resistant obesity and cognitive function. *Metabolic Brain Disease* **30** 853–876. (doi:10.1007/s11011-015-9655-3)
- Pintana H, Pongkan W, Prachayasakul W, Chattipakorn N & Chattipakorn SC 2015b Testosterone replacement attenuates cognitive decline in testosterone-deprived lean rats, but not in obese rats, by mitigating brain oxidative stress. *Age* **37** 84. (doi:10.1007/s11357-015-9827-4)
- Pintana H, Prachayasakul W, Sa-Nguanmoo P, Pongkan W, Tawinvisan R, Chattipakorn N & Chattipakorn SC 2016 Testosterone deprivation has neither additive nor synergistic effects with obesity on the cognitive impairment in orchietomized and/or obese male rats. *Metabolism* **65** 54–67. (doi:10.1016/j.metabol.2015.10.015)
- Polderman KH, Gooren LJ, Asscheman H, Bakker A & Heine RJ 1994 Induction of insulin resistance by androgens and estrogens. *Journal of Clinical Endocrinology and Metabolism* **79** 265–271. (doi:10.1210/jc.79.1.265)
- Pongkan W, Chattipakorn SC & Chattipakorn N 2015 Chronic testosterone replacement exerts cardioprotection against cardiac ischemia-reperfusion injury by attenuating mitochondrial dysfunction in testosterone-deprived rats. *PLoS ONE* **10** e0122503. (doi:10.1371/journal.pone.0122503)
- Prachayasakul W, Kerdphoo S, Petsophonakul P, Pongchaidecha A, Chattipakorn N & Chattipakorn SC 2011 Effects of high-fat diet on insulin receptor function in rat hippocampus and the level of neuronal corticosterone. *Life Sciences* **88** 619–627. (doi:10.1016/j.lfs.2011.02.003)
- Saad F, Yassin A, Doros G & Haider A 2016 Effects of long-term treatment with testosterone on weight and waist size in 411 hypogonadal men with obesity classes I-III: observational data from two registry studies. *International Journal of Obesity* **40** 162–170. (doi:10.1038/ijo.2015.139)
- Schenk S, Harber MP, Shrivastava CR, Burant CF & Horowitz JF 2009 Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. *Journal of Physiology* **587** 4949–4961. (doi:10.1113/jphysiol.2009.175489)
- Sivasinprasasn S, Sa-Nguanmoo P, Prachayasakul W, Kumfu S, Chattipakorn SC & Chattipakorn N 2015 Obese-insulin resistance accelerates and aggravates cardiometabolic disorders and cardiac mitochondrial dysfunction in estrogen-deprived female rats. *Age* **37** 28. (doi:10.1007/s11357-015-9766-0)
- Skogastierna C, Hotzen M, Rane A & Ekstrom L 2014 A supraphysiological dose of testosterone induces nitric oxide production and oxidative stress. *European Journal of Preventive Cardiology* **21** 1049–1054. (doi:10.1177/2047487313481755)
- Taggart HM, Applebaum-Bowden D, Haffner S, Warnick GR, Cheung MC, Albers JJ, Chestnut CH III & Hazzard WR 1982 Reduction in high density lipoproteins by anabolic steroid (stanozolol) therapy for postmenopausal osteoporosis. *Metabolism* **31** 1147–1152. (doi:10.1016/0026-0495(82)90166-4)
- Thompson PD, Cullinane EM, Sady SP, Chenevert C, Saritelli AL, Sady MA & Herbert PN 1989 Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. *JAMA* **261** 1165–1168. (doi:10.1001/jama.1989.03420080085036)
- Thummasorn S, Kumfu S, Chattipakorn S & Chattipakorn N 2011 Granulocyte-colony stimulating factor attenuates mitochondrial dysfunction induced by oxidative stress in cardiac mitochondria. *Mitochondrion* **11** 457–466. (doi:10.1016/j.mito.2011.01.008)
- Tinetti M, Gysel M, Farias J, Ferrer M, Lombardero M & Baranchuk A 2015 Left ventricular filling pressure in male patients with type 2 diabetes and normal versus low total testosterone levels. *Cardiology Journal* **22** 206–211. (doi:10.5603/CJ.a2014.0056)
- Vlantis K & Pasparakis M 2010 Role of TNF in pathologies induced by nuclear factor kappaB deficiency. *Current Directions in Autoimmunity* **11** 80–93. (doi:10.1159/000289198)
- WHO 2011 *Obesity and Overweight*. Fact sheet N°311. Geneva, Switzerland: World Health Organization. (available at: <http://www.who.int/mediacentre/factsheets/fs311/en>)
- Yasui T, Matsui S, Tani A, Kunimi K, Yamamoto S & Irahara M 2012 Androgen in postmenopausal women. *Journal of Medical Investigation* **59** 12–27. (doi:10.2152/jmi.59.12)
- Zorov DB, Juhaszova M & Sollott SJ 2006 Mitochondrial ROS-induced ROS release: an update and review. *Biochimica et Biophysica Acta* **1757** 509–517. (doi:10.1016/j.bbabi.2006.04.029)

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