Basic Science and Experimental Studies

Dependence of Cardiac Systolic Function on Elevated Fatty Acid Availability in Obese, Insulin-Resistant Rats

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ABSTRACT

Background: Clinical data advocating an adverse effect of obesity on left ventricular (LV) systolic function independent of comorbidities is controversial. We hypothesized that in obesity with prediabetic insulin resistance, circulating fatty acids (FAs) become a valuable fuel source in the maintenance of normal systolic function.

Methods: Male Wistar rats were fed a high caloric diet for 32 weeks to induce obesity. Myocardial LV systolic function was assessed using echocardiography and isolated heart preparations.

Results: Aortic output was reduced in obese rat hearts over a range of filling pressures (for example: 15 cmH_2O, obese: 32.6 ± 1.2 ml/min vs control: 46.2 ± 0.9 ml/min, $P < .05$) when perfused with glucose alone. Similarly, the slope of the LV end-systolic pressure-volume relationship decreased, and there was a right shift in the LV end-systolic stress-strain relationship as determined in Langendorff perfused, isovolumic rat heart preparations in the presence of isoproterenol (10^{-8}M) (LV systolic stress-strain relationship and a reduced load-independent intrinsic systolic myocardial function, obese: 791 ± 62 g/cm^2 vs control: 1186 ± 74 g/cm^2, $P < .01$). The addition of insulin to the perfusion buffer improved aortic output, whereas the addition of FAs completely normalized aortic output. LV function was maintained in obese animals in vivo during an inotropic challenge.

Conclusions: Elevated circulating FA levels may be important to maintain myocardial systolic function in the initial stages of obesity and insulin resistance. (*J Cardiac Fail 2016;22:560–568*)

Key Words: Obesity, fatty acids, cardiac systolic function.

Obesity is an independent risk factor for heart failure.[1] However, whether obesity promotes cardiac pump dysfunction through mechanisms unrelated to hypertension and diabetes is still uncertain. In this regard, clinical studies conducted with overweight to severely obese patients have demonstrated either augmented[2,3] preserved[4–8] or reduced[9] left ventricular (LV) systolic function independent of diabetes and hypertension. Other authors additionally report reduced load-independent measures of systolic function (subclinical systolic dysfunction) with preserved ejection fraction in similar participants.[10,11] Data from animal studies also provide inconclusive observations regarding the effect of diet induced obesity on LV systolic function.[12–19]

One potential explanation for the conflicting data regarding the independent effect of obesity on cardiac systolic function is that obesity may indeed be associated with intrinsic myocardial alterations that impair contraction, but that at least in the early stages of obesity, compensatory myocardial changes may occur that enable myocardial function to be preserved. This is supported by the observation that the duration of especially morbid obesity is associated with a reduction in LV systolic function (LV fractional shortening).[20] In line with this hypothesis, obesity is associated with

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increased circulating fatty acid (FA) concentrations[21] and in most instances elevated rates of myocardial FA oxidation.[16,22] Because the myocardium relies less on glucose as a fuel source in the setting of obesity and insulin resistance,[16,22] it is plausible that increased FA availability combined with enhanced FA oxidation becomes vital to help maintain myocardial mechanical function in hearts that are metabolically compromised. We therefore tested the hypothesis that elevated circulating FAs and insulin associated with obesity and insulin resistance may compensate for an impaired myocardial systolic function noted in obese, insulin-resistant (prediabetic) rats when glucose is present as the only fuel source. To test this hypothesis, we studied cardiac mechanical function in a previously described animal model of obesity and insulin resistance.[12,18,23,24] In the present study cardiac systolic function was determined both in vivo and ex vivo. In the ex vivo studies, we assessed cardiac mechanical performance in the absence and in the presence of FAs and insulin at concentrations found in vivo in lean and obese rats.

Methods

Animals

This study was conducted in accordance with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health publication no 80-23, revised 1985). Sixty male Wistar rats weighing 200 ± 10 g received a standard rodent chow supplemented with sucrose and condensed milk for 32 weeks. The experimental diet is designed to induce obesity through hyperphagia with the experimental group (n = 31) consuming 570 ± 23 kJ/day compared with the control group (n = 29) that consumed 371 ± 18 kJ/day.[12,25] The compositions of the standard rat chow and the experimental diet used in this study have previously been reported.[24] We have previously demonstrated that this experimental diet induces both obesity and insulin resistance after 16 weeks of consumption.[23,26] Blood pressures were determined using a tail cuff method.[27]

Isolated, Perfused Heart Preparations

To assess whether the absence of circulating FAs and insulin resulted in cardiac pump dysfunction, left ventricular systolic function was assessed ex vivo in 1) the isolated working heart model (as modified by Opie et al[28]) to ensure that systolic function was assessed in a heart preparation performing work and 2) in the isolated retrograde perfused, isovolumic, constant coronary flow heart preparations[29] to account for the effects of load on systolic function; to assess intrinsic myocardial systolic function (see the following section); and to account for potential effects of obesity on coronary flow.

Rats were anesthetized with an intraperitoneal injection of pentobarbitone-sodium (60 mg/kg) and the hearts rapidly excised. The hearts were placed in ice-cold buffer before being transferred to a heart perfusion apparatus where they were perfused with a Krebs-Henseleit buffer equilibrated with 95%O_2 and 5%CO_2 at 37°C (pH 7.4) (in mmol/L: NaCl 118.0, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10 [Merck Pty. Ltd., Darmstadt, Germany]) at a pressure of 100 cmH_2O. Retrograde perfusion was initiated within 45 seconds of excision of the heart.

The influence of FAs or insulin (or the combination of both) on LV systolic function was only assessed in working heart preparations. FAs (final concentration: 0.7 mM for control and 1.5 mM for obese rats) were prebound to 3% bovine serum albumin (Fraction V, Roche, Germany) and added to the perfusion buffer as previously described.[30] FAs in the bovine serum albumin contributed 0.3 mM to the FA concentration in the buffer and the rest of the FAs were added in the form of palmitate (Sigma-Aldrich, St. Louis, MO). Insulin (Humulin-N, Eli Lilly, South Africa) was added to the perfusion buffer to achieve concentrations of 30 μIU/ml for control hearts and 50 μIU/ml in obese rat hearts. These FA and insulin concentrations closely resemble the plasma concentrations we previously reported in control and obese rats following 16 weeks of feeding.[23]

Working Heart Model. For working heart perfusions, the left atrium was cannulated and hearts were perfused in the working heart mode initially at a preload of 15 cmH_2O. The preload was subsequently increased at five minute intervals from 15 to 17.5 cmH_2O and then to 20 cmH_2O. Afterload was maintained at 100 cmH_2O. Hearts perfused with glucose or glucose and insulin as the only substrate were paced at 330 beats/min with the stimulation voltage 10% above threshold via platinum wire electrodes attached to the left atrium and the apex of the heart. Hearts perfused in the presence of glucose and FAs were not paced. The heart rates from these unpaced hearts were, however, comparable between control and obese animals (see the Results section). Hearts with a rate below 200 beats/min were excluded from the study (n = 1). During FA and insulin perfusion, the perfusion buffer (200 ml) was recirculated for the duration of the experiment. This was not the case when glucose was used as the sole substrate. Coronary flow and aortic output were documented. Systolic performance was assessed from aortic output determined at incremental filling pressures.

Isovolumic, Retrograde Perfusion Model. These studies were performed in a separate group of rats from those used for the working heart preparations, using an approach previously described.[31] Once hearts were mounted on the perfusion apparatus, the coronary flow rate was determined volumetrically and adjusted to achieve a constant flow of 12 ml/min/g heart weight. The hearts were paced at 300 beats/min as described previously. LV-developed pressure was determined by use of a water-filled balloon-tipped cannula coupled to a pressure transducer inserted via the left atrium into the LV cavity. A thin-walled latex balloon with a 0 pressure filling volume beyond maximum LV lumen capacities was selected for this study to avoid the stiffness of the balloon wall contributing to LV pressure at higher filling volumes. The volume of the balloon wall was assessed with a water-displacement technique, and the same balloon was used