British Journal of Medicine & Medical Research
5(4): 434-443, 2015, Article no.BJMMR.2015.048
ISSN: 2231-0614

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Assessment of Cardiac Function Using Pressure-Volume Loops as Cardiac Hypertrophy Develops from Iron Deficiency

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Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, and collected data. Author HGCJ performed the data and statistical analyses, literature searching, created tables and figures, and wrote the first draft of the manuscript. Author JZ provided literature searching and editing of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/BJMMR/2015/13376
Editor(s):
(1) Anonymous
Reviewers:
(1) Enrico Giustiniano, Dept. of Anesthesia and Intensive Care, IRCCS Humanitas Clinical Institute, Rozzano, Milan, Italy.
(2) Magdy ElSharkawy, Nephrology Department, Ain-Shams University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=663&id=12&aid=6114

Original Research Article

Received 14th August 2014
Accepted 2nd September 2014
Published 16th September 2014

ABSTRACT

Aims: Cardiac hypertrophy develops from prolonged, severe iron deficiency (ID), but little is known about its function. We hypothesized that 4 weeks of ID would result in enhanced cardiac function, but would transition to loss by 9 weeks.
Study Design: 38 rats were fed either control or ID diets for 4, 6, or 9 weeks, then subjected to a pressure-volume loop protocol to assess cardiac function.
Place and Duration of Study: Biology Department, Western Wyoming College, between January 2013 and December 2013.
Methodology: Rats were anesthetized with ketamine/xylazine, catheters placed in femoral and jugular veins. A pressure-conductance microcatheter was inserted through the right carotid artery, to the left ventricle. Baseline data was collected for 10 minutes, followed by occlusion of the inferior vena cava to reduce venous return. Hypertonic saline was infused through the jugular vein to allow for parallel conductance subtraction. Rats were sacrificed, and hearts and blood collected for mass and volume calibration, respectively.

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Results: Cardiac output was increased (P = .01) with ID after 4 weeks, but was not increased after 6 or 9 weeks. The increase was due to enhanced stroke volume (P = .02), but not heart rate (P = .48). Stroke volume was increased due to enhanced contractility with ID (P = .03), along with a decrease in the pressure at which ejection begins (afterload, P = .001). By 9 weeks, contractility was decreased (P = .01), but afterload remained lower. Cardiac efficiency was enhanced after 4 weeks (P = .002), which was lost by 9 weeks of ID. No diastolic parameters of cardiac function were altered by ID.
Conclusion: An adaptive compensation of cardiac function develops within 4 weeks of severe ID, but is lost within weeks, at which time, the ID heart is in a state of pathological decompensation.

Keywords: Iron deficiency; anemia; cardiac function; cardiac hypertrophy; pressure-volume loops.

1. INTRODUCTION

Iron deficiency is a serious problem throughout the world [1,2]. The World Health Organization (WHO) reports that anemia is the most common nutritional disorder, with over 2 billion people (more than 30% of the population) affected around the world. The WHO asserts that "iron deficiency exacts its heaviest overall toll in terms of ill-health, premature death, and lost earnings" (http://www.who.int/nutrition/topics/ida/en).

It has long been known that prolonged, severe, iron deficiency results in cardiac hypertrophy, in both experimental animals [3-9] and humans [1]. Iron deficiency is commonly associated with heart failure, and mortality from heart failure and hemoglobin concentration are inversely related [10,11]. Conversely, iron supplementation has been shown to improve heart failure patients [12,13], further demonstrating the importance of this essential mineral to heart health.

Cardiac hypertrophy from iron deficiency is characterized by sarcomere and mitochondrial disruption, and the up-regulation of apoptotic enzymes, after just 12 weeks of severe iron deficiency [14]. Systolic blood pressure is decreased with a few weeks of iron deficiency, but heart rate is unchanged [15,16]. Cardiac fibrosis appears much later [15]. Ventricle chamber size is greater than normal, with both ventricle length and width increased. Wall thickness appears to be unaffected [3,14]. This has led to the consideration of iron deficiency hypertrophy as a dilated cardiomyopathy [14].

While the causal mechanism for iron deficiency cardiac hypertrophy is debated, two hypotheses have been offered. The sympathetic nervous system, with the neurotransmitter norepinephrine, has been associated with this hypertrophy for decades [7-9]. However, another study has shown that beta-adrenergic blockade does not attenuate the hypertrophy [16]. More recently, a potential role for the hormone erythropoietin has emerged [15,17]; erythropoietin has also been shown to attenuate cardiac dysfunction in diabetic rats [18]. It is unclear whether or not these two factors, either alone or in conjunction with one another, sufficiently account for development of this hypertrophy.

Relatively little research attention has been paid to the functional aspects of iron deficiency induced hypertrophy. It has been reported that cardiac output with this hypertrophy is increased [17,19]. In contrast, with sideropenic anemia from both iron and copper deficiency, systolic pressure and maximal contractility is reduced, and end diastolic pressure is increased [20]. But, given that cardiac fibrosis is not an early response to iron deficiency [15], it seems logical that cardiac function with iron deficiency may transition from an initial higher than normal contractile state, to a failing heart. This concept of adaptive compensation, but ultimately failing heart, is well accepted with other forms of cardiac hypertrophy [21]. However, we know of no studies that have examined this hypothesis in detail, with regard to iron deficiency.

We therefore hypothesized that 4 weeks of iron deficiency would result in enhanced cardiac function; in particular, increases in cardiac output and contractility would be part of an early adaptation. However, with longer periods of iron deficiency (6 and 9 weeks), ventricular dysfunction would result in a transition of cardiac function to a failing state, including decreases in cardiac output and contractility. We used the pressure-volume microcatheter technique to assess cardiac function [22-24].

2. METHODOLOGY

2.1 Experimental Design

38 male CD rats, 50-75 g (Charles River Laboratories), were randomly divided into iron deficient and control groups. Iron deficient rats were fed an AIN-93G diet with reagent grade mineral mixture without iron; control rats were fed AIN-93G diet (Dyets, Inc.). All rats were given type I purified water ad libitum, were kept in light for 12 h daily, and were weighed weekly.

Rats were kept on the respective diets for 4 (18 rats), 6 (10 rats), or 9 (10 rats) weeks, after which a pressure-volume loop protocol was performed to determine heart function. After the pressure-volume loop protocol, the rat's blood and heart were collected.

2.2 Pressure-Volume Loop Protocol

Rats were anesthetized by intramuscular injection of ketamine hydrochloride (80 $mg \bullet kg^{-1}$ body mass) and xylazine (12 $mg \bullet kg^{-1}$). PE-50 catheters were surgically placed in the right femoral and jugular veins. The femoral vein catheter was used for continuous intravenous infusion of a ketamine/xylazine mixture (40/6 $mg \bullet ml^{-1}$, respectively, at a rate of 0.12 $ml \bullet hr^{-1}$), which maintained anesthesia throughout the experiment. The jugular vein catheter was for hypertonic saline infusion.

The right carotid artery was exposed, and a pressure-volume microcatheter (Millar Model 469 for 4 and 6 week rats, Millar Model 838 for 9 week rats) was passed through the artery into the left ventricle. The catheter was placed to maximize conductance signal fidelity. The catheter was connected to a Millar UltraPV device, coupled to an AD Instruments data acquisition system (Model 8SP). An iMac computer (Apple, Inc.), utilizing Parallels software to run Windows XP Professional operating system, was used to run Millar Ultra Control Interface software to calibrate and receive data from the pressure-volume catheter. Lab Chart 7 Pro with PV Loop plug-in was used to store and visualize pressure-volume loops, perform saline and cuvette calibrations, and extract data from the recorded loops.

After catheter placement in the left ventricle, a 10 min stabilization period ensured consistent baseline pressure-volume loops. The inferior vena cava was then exposed and briefly occluded with forceps to reduce venous return to the heart. This created pressure-volume loops through a range of end-diastolic volumes. A bolus of hypertonic saline (40 µl of 30% saline) was then introduced through the jugular catheter, to allow for post-hoc parallel conductance subtraction [24]. The rat was then heparinized (1000 USP units in a 0.5 ml bolus of buffer), decapitated, and blood collected. The heart was excised, weighed, and frozen at -80ºC. Blood was used for hematocrit determination and volume cuvette calibration.

2.3 Pressure-Volume Loop and Statistical Analysis

Baseline pressure-volume loops were used to determine the following cardiac function parameters: cardiac output, heart rate, stroke volume, end-diastolic volume, ventricular pressure at beginning of ejection, Tau, and end-diastolic elastance (slope of end-diastolic pressure vs. end-diastolic volume regression line). Inferior vena cava occlusion loops were used for contractility (slope of maximal $dp \bullet dt^{-1}$ vs. end-diastolic volume regression line) and cardiac efficiency measures.

Analysis of variance was used to determine statistical significance on differences of dietary group (iron deficient vs. control), and time (4, 6, and 9 weeks of respective diets). Statview 5.1 (SAS Institute, Cary, NC) was used for all analyses; a $P = .05$ level of significance was used.

3. RESULTS AND DISCUSSION

3.1 Results

Our results indicate that transient increases in cardiac output and contractility were evident after 4 weeks of iron deficiency. However, despite a loss of contractility by 9 weeks of iron deficiency, cardiac output, while no longer enhanced, was not reduced below that of control rats. This maintenance of cardiac output was due to a persistent compensatory decrease in afterload. Therefore, despite numerous changes in cardiac function, including contractile dysfunction, the transition to an unequivocal state of cardiac failure was not evident within 9 weeks of severe iron deficiency.

Table 1 provides selected characteristics of experimental groups. Body mass was less with

iron deficiency (P < .001), and both groups increased body mass over time (P < .001). Heart mass was greater with iron deficiency (P < .001), and also increased over time (P = .002). Hematocrit was lower with iron deficiency (P < .001), but not affected by length of dietary intervention (P = .74).

Fig. 1 shows cardiac output results obtained by pressure-volume loop analysis. Cardiac output was greater with iron deficiency after 4 weeks of dietary intervention (P = .01), but was different at neither the 6 (P = .52) nor 9 (P = .32) week intervals. Time was not a significant factor overall in cardiac output (P = .17).

Table 1. Selected characteristics of experimental groups

	Weeks of diet intervention		
Parameter	**4(n = 18)**	**6(n = 10)**	**9(n = 10)**
Body mass (g)			
iron deficient	265.8±8.6*#	357.4±9.0*#	445.2±20.7*#
control	325.4±10.2	425.6±21.2	558.4±4.2
Heart mass (g)			
iron deficient	1.90±0.11*#	1.92±0.04*#	2.35± 0.21*#
control	1.49±0.08	1.63±0.09	1.88± 0.05
Hematocrit (%)			
iron deficient	23.06±1.22*	22.84±0.30*	24.32±1.46*
control	46.59±1.15	48.04±0.64	46.95±0.51

**P < .05 between iron deficient and control groups; #P < .05 across time intervals for control group; all data are mean ± standard error.*

To understand the increase in cardiac output, we analyzed its two components: heart rate and stroke volume (Figs. 2 and 3). There were no differences in heart rate across either time (P = .09) or diet (P = .48). In contrast, stroke volume was increased between dietary groups after 4 weeks (P = .02), but not after 6 weeks (P = .78) nor 9 weeks (P = .27). Stroke volume was not affected overall by time (P = .07).

To elucidate how stroke volume was altered by iron deficiency, we looked at the three main factors that affect stroke volume: preload (end-diastolic volume, Fig. 4), contractility (slope of maximal $dp \cdot dt^{-1}$ vs. end-diastolic volume regression line plot, Fig. 5), and afterload (ventricular pressure at the onset of ejection, Fig. 6). End-diastolic volume was not different between dietary groups (P = .66), although it increased over time (P = .01). There was a significant interaction of diet and time (P = .004) for contractility, with iron deficient hearts having greater contractility after 4 weeks (P = .03), no diet group differences at 6 weeks (P = .16), and iron deficient hearts having lower contractility after 9 weeks (P = .01). Ventricular pressure at ejection (afterload) was significantly reduced (P = .001) with iron deficiency, but did not change over time (P = .53).

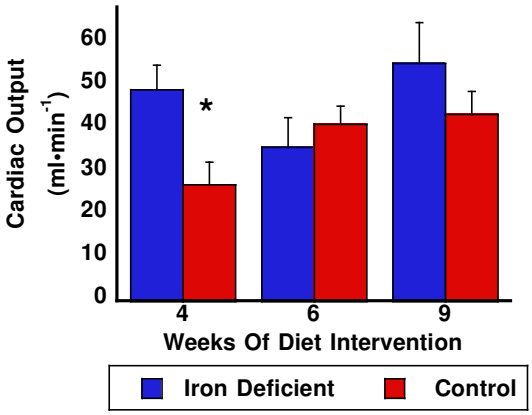

Fig. 1. Effect of iron deficiency on cardiac output at different time intervals in rats

**P < .05 between iron deficient and control groups; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet.*

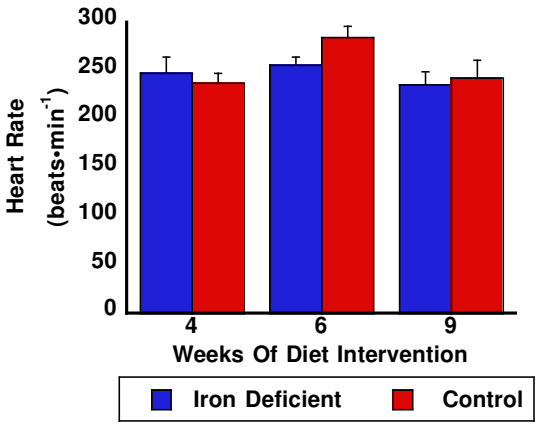

Fig. 2. Effect of iron deficiency on heart rate at different time intervals in rats

All data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet.

Fig. 7 shows cardiac efficiency for experimental groups. There was a significant interaction of diet and time (P = .01). Iron deficient hearts showed

greater efficiency at 4 (P = .002) and 6 weeks (P = .02), but not after 9 weeks (P = .88). Also, control hearts increased efficiency over time (P = .02), but iron deficient hearts did not (P = .13).

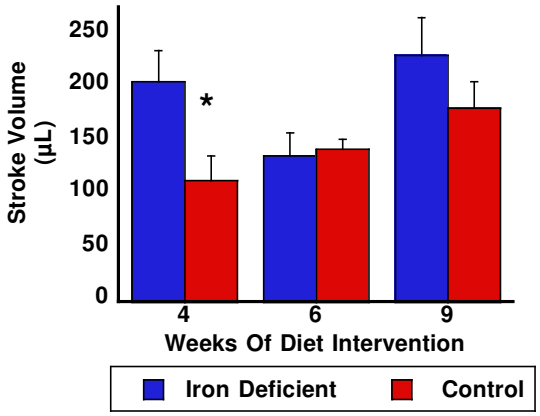

Fig. 3. Effect of iron deficiency on stroke volume at different time intervals in rats

**P < .05 between iron deficient and control groups; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet.*

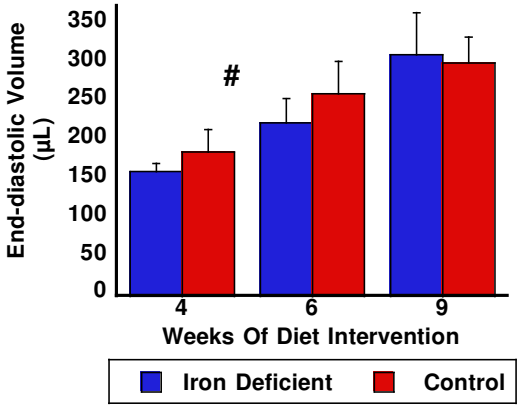

Fig. 4. Effect of iron deficiency on end-diastolic volume at different time intervals in rats

#P < .05 across time intervals; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet.

Finally, Fig. 8 and 9 examine cardiac diastolic function with iron deficiency. Fig. 8 shows the isovolumic relaxation constant, Tau. There was a decrease in Tau over time (P = .04), but no dietary group differences. Fig. 9 shows end-diastolic elastance, which revealed neither diet (P = .87) nor time (P = .54) differences.

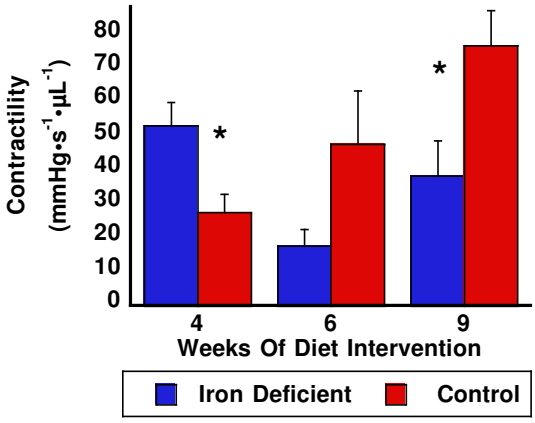

Fig. 5. Effect of iron deficiency on contractility at different time intervals in rats

**P < .05 between iron deficient and control groups; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet. Contractility determined as slope of regression plot of maximal change in pressure generated by heart vs. end-diastolic volume while inferior vena cava was occluded.*

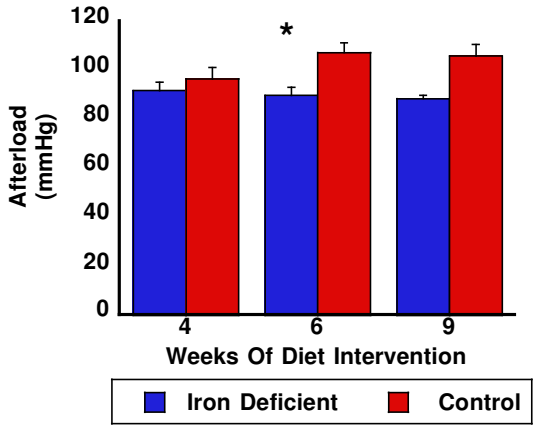

Fig. 6. Effect of iron deficiency on afterload at different time intervals in rats

**P < .05 between iron deficient and control groups; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet. Afterload determined as pressure at which ejection of blood from heart begins.*

3.2 Discussion

This study is the first to examine cardiac function with iron deficiency over time, using the pressure-volume loop technique. We hypothesized that iron deficiency would initially result in an increase in cardiac function, in particular cardiac output, but would transition to

failure by 9 weeks of severe iron deficiency. Our hypothesis was largely, but not completely, supported. The most significant finding of our study was that mean cardiac output with iron deficiency was nearly double that of control rats after 4 weeks of the respective diets; however, this difference was gone within 2 additional weeks.

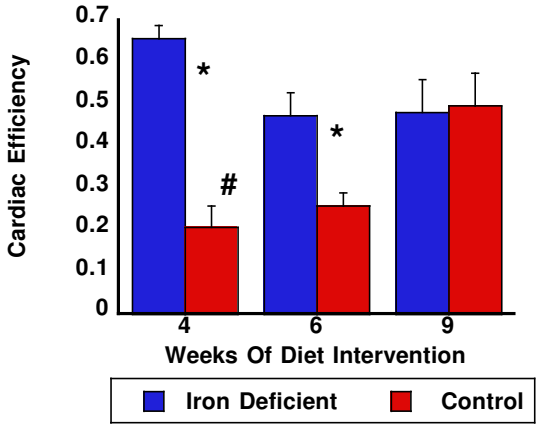

Fig. 7. Effect of iron deficiency on cardiac efficiency at different time intervals in rats

**P < .05 between iron deficient and control groups; #P < .05 across time intervals for control group; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet. Cardiac efficiency is the ratio of stroke work to pressure-volume area during pressure-volume loop.*

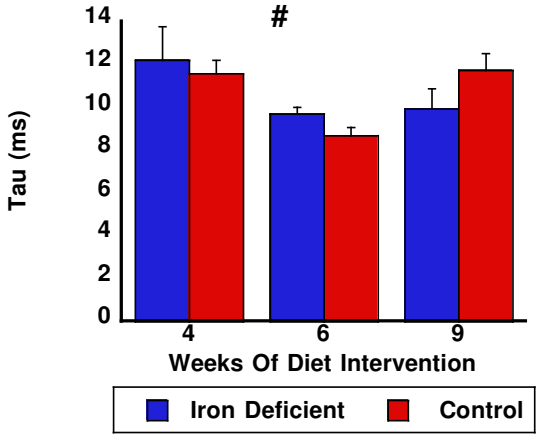

Fig. 8. Effect of iron deficiency on Tau at different time intervals in rats

#P < .05 across time intervals; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet. Tau is the isovolumic relaxation constant during a pressure-volume loop.

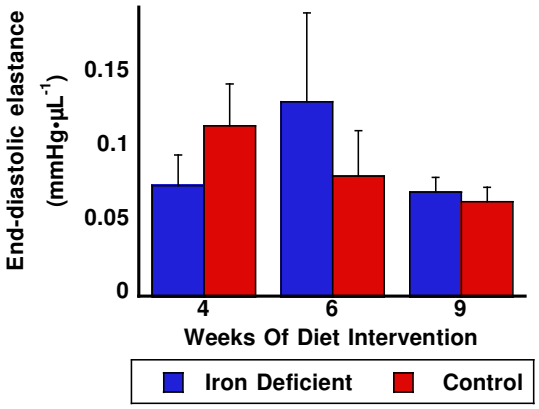

Fig. 9. Effect of iron deficiency on end-diastolic elastance at different time intervals in rats

**P < .05 between iron deficient and control groups; #P < .05 between time intervals; all data are mean ± standard error; n = 18 after 4 weeks of diet, n = 10 after 6 weeks of diet, n = 10 after 9 weeks of diet. End-diastolic elastance is the slope of regression line created by end-diastolic volume-pressure points while the inferior vena cava is occluded.*

We interpret changes in cardiac output to mean that a compensatory adaptation for reduced hematocrit, and presumably, oxygen delivery to tissues throughout the body, is quickly found with iron deficiency. This important compensation, however, cannot be maintained for long. One study has shown that, by 12 weeks of iron deficiency, significant morphological disruption of both mitochondria and sarcomere organization is present, and apoptosis has begun [14]. Our present findings are consistent with that finding, as well as the concept of an early positive adaptation to iron deficiency, which is lost as cardiomyocyte disorganization sets in [15]. We believe our study is the first to demonstrate this transient compensation in cardiac function in detail. Our study is also consistent with the concept of cardiac function with iron deficiency to be a "high output state and then the eventual transition into heart failure" [1], or at least, a state of pathological decompensation, which inevitably leads to heart failure [21]. We speculate that, had we conducted our experiments after longer periods of the respective diets, it is likely that iron deficient hearts would enter a true failing state, with irreversible loss of cardiac output.

We further examined how the components of cardiac output are altered with iron deficiency. Increases in cardiac output were explained entirely by alterations in stroke volume, with no increase in heart rate, which would reduce the

time available for ventricular filling. Stroke volume changes after 4 weeks of iron deficiency were due to an increase in contractility, combined with a modest, but significant, reduction in afterload against which the heart pumps blood. Turner et al. found that vascular remodeling to a larger arterial diameter was an adaptation to 4 weeks of iron deficiency [16], which would allow for a decrease in afterload. We believe this study, however, is the first to document the initial pressure at which blood ejects from the heart with iron deficiency, as well as direct measurement of stroke volume under these physiological conditions.

Compensatory changes in stroke volume are lost, however, as iron deficiency progresses. In particular, contractility is dramatically reduced, while the reduction in afterload is maintained. As such, stroke volume in iron deficient rats cannot remain elevated when compared to controls of similar age. This is consistent with apoptotic cardiomyocytes [14], but would appear to be in contrast to another study's finding [15] that changes in fractional shortening of cardiomyocytes did not occur over a 20 week period of iron deficiency. It should be noted, however, that in the latter study, the methodology for inducing iron deficiency may not have been as severe as ours; therefore, the timeline for changes may be different in the two studies.

One of the advantages of using the pressure-volume loop technique for assessing cardiac function is the ability to determine cardiac efficiency. Cardiac efficiency is the ratio of stroke work to pressure-volume area of the pressure-volume loop. Since pressure-volume area is correlated to myocardial oxygen consumption, cardiac efficiency quantifies how much work is done for the energy expended by the heart during contraction. Obviously, greater cardiac efficiency when aerobic metabolism is challenged, such as present with iron deficiency, would be a significant enhancement. Our results indicate that, during the early adaptive response, iron deficient hearts are highly efficient (3 times that of control hearts). However, as iron deficiency is prolonged, this adaptation is lost, while control hearts grow to become more efficient at the same time. We believe this finding is novel.

Another feature of the pressure-volume loop technique is the ability to examine diastolic heart function. Since several studies have reported an increase in ventricular fibrosis with iron deficiency [14,15], we were surprised to find that both Tau, the isovolumic relaxation constant, and end-diastolic elastance, were unchanged with iron deficiency at all three time points examined. Both Tau and end-diastolic elastance would be expected to change with changes in ventricular stiffness. Since we found no differences in these variables, we conclude that diastolic function is not altered with iron deficiency, despite changes in ventricular morphology. We speculate that changes in these variables may have been found, if we had continued our study for longer time intervals.

One limitation of this experiment is our employment of the rat model, and a severely iron deficient protocol, to induce iron deficiency rapidly. It is reasonable to wonder whether or not our results have any direct implication to human health. While many cases of iron deficiency are mild, and therefore, asymptomatic, there are human case studies in which patients have physiological circumstances comparable to our rats [1], with severe iron deficiency anemia, a slightly reduced cardiac output, and a 30% ejection fraction. There are also a number of human studies demonstrating the ties between iron status and heart failure [10-13]. We believe, therefore, that our results do have direct implications to human beings with iron deficiency, provided the iron deficiency is prolonged and severe enough. This may also suggest that early intervention, before the iron deficient heart regresses to an irreversible state of failure, will result in the best medical outcome.

We made no attempt in this study to differentiate between cardiac function changes that were the result of iron deficiency per se, anemia, or the resulting tissue hypoxia and aerobic impairment. However, it is interesting to note that both copper deficiency and pernicious anemia can result in cardiac hypertrophy [25,26]. With dietary copper deficiency, which impairs aerobic metabolism without iron deficiency, individual cardiomyocytes appear to have enhanced contractile function, although fibrosis impairs overall cardiac function [25]. With pernicious anemia, in which anemia is present without iron deficiency, clinical features of heart failure are present [26]. However, these features are reversible with correction of the condition. Combined with the knowledge that iron deficiency for 12 weeks results in cardiomyocyte apoptosis, one may reasonably conclude that the changes in our present study are attributable to iron deficiency. However, to our knowledge, there are no studies with either copper deficiency

nor pernicious anemia, in which the progression of cardiac function changes have been examined, similar to our study. It is difficult, therefore, to draw definitive conclusions among the three different physiological conditions that result in cardiac hypertrophy.

4. CONCLUSION

In conclusion, iron deficiency results in a short term increase in cardiac output, due entirely to changes in stroke volume. Stroke volume is enhanced due to both an increase in contractility, and a decrease in afterload. However, these enhancements are lost within a few weeks, and cardiac output returns to normal as contractility fails. In this way, an important adaptive compensation to a significant metabolic challenge to survival, is quickly lost, and the iron deficient heart is in a state of pathological decompensation.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the University of Wyoming Institutional Animal Care and Use Committee (protocol # 08022013BC0007, approved August 2, 2013).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge funding from the United States Department of Health and Human Services, National Institutes of Health IDeA Network for Biomedical Research Excellence (INBRE) grant number GM103432 to the University of Wyoming.

Ms. Emily Thompson is acknowledged for assistance in data collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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doi: 10.1136/bcr.01.2012.5576.

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