Multiphasic Regulation of Systemic and Peripheral Organ Metabolic Responses to Cardiac Hypertrophy

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Background—Reduced fat oxidation in hypertrophied hearts coincides with a shift of carnitine palmitoyl transferase I from muscle to increased liver isoforms. Acutely increased carnitine palmitoyl transferase I in normal rodent hearts has been shown to recapitulate the reduced fat oxidation and elevated atrial natriuretic peptide message of cardiac hypertrophy.

Methods and Results—Because of the potential for reduced fat oxidation to affect cardiac atrial natriuretic peptide, we studied peripheral and systemic metabolism in male C57BL/6 mice model of transverse aortic constriction in which left ventricular hypertrophy occurred by 2 weeks without functional decline until 16 weeks (ejection fraction, −45.6%; fractional shortening, −22.6%). We report the first evidence for initially improved glucose tolerance and insulin sensitivity in response to 2 weeks transverse aortic constriction versus sham, linked to enhanced insulin signaling in liver and visceral adipose tissue (epididymal white adipose tissue [WAT]), reduced WAT inflammation, elevated adiponectin, multilocular subcutaneous adipose tissue (inguinal WAT) with upregulated oxidative/thermogenic gene expression, and downregulated lipolysis and lipogenesis genes in epididymal WAT. By 6 weeks transverse aortic constriction, the metabolic profile reversed with impaired insulin sensitivity and glucose tolerance, reduced insulin signaling in liver, epididymal WAT and heart, and downregulation of oxidative enzymes in brown adipose tissue and oxidative and lipogenic genes in inguinal WAT.

Conclusions—Changes in insulin signaling, circulating natriuretic peptides and adipokines, and varied expression of adipose genes associated with altered insulin response/glucose handling and thermogenesis occurred prior to any functional decline in transverse aortic constriction hearts. The findings demonstrate multiphasic responses in extracardiac metabolism to pathogenic cardiac stress, with early iWAT browning providing potential metabolic benefits. (Circ Heart Fail. 2017;10:e003864. DOI: 10.1161/CIRCHEARTFAILURE.117.003864.)

Key Words: adipose tissue ■ hypertension ■ insulin resistance ■ metabolism ■ pressure overload

Insulin resistance is a common comorbidity of diabetic cardiomyopathy and congestive heart failure, and the link between whole body metabolism and the metabolism of the pathological heart is an emerging area of investigation, with links to the metabolic basis of complex disease. Indeed, in animal models of heart failure and, importantly, congestive heart failure patients without diabetes mellitus, evidence exists for impaired insulin signaling within the cardiomyocytes of the failing heart. With the well-documented reduction of fat oxidation by pathologically hypertrophied hearts, we hypothesized a general systemic and a specific adipose metabolic shift to occur in response to chronic pressure overload of the heart. The pathogenesis of cardiac decompensation, as explored here in an animal model of chronic pressure overload, is distinct from heart failure with preserved ejection fraction that can be either associated with obesity-associated adipose dysfunction and nutrient overload or induced by volume overload, as have recently been associated with beiging of adipose tissue. With this model of chronic pressure overload alone, the findings of this current study demonstrate for the first time a biphasic metabolic response across peripheral organs to pathological stress on the heart.

See Clinical Perspective

While the heart responds to pathological stress with a well recognized, but poorly understood metabolic remodeling, the consequences of this shift on systemic metabolism and peripheral organs remain unknown. The demands on energy production to support cardiac function within the heart are long known to be primarily fueled by long-chain fatty acids (LCFA). The heart is the primary organ for LCFA oxidation, and disruption of LCFA utilization, as occurs in the
pathologically stressed heart, can then be expected to create a shift in the systemic metabolism of LCFA, potentially involving peripheral tissues and organs. While systemic metabolic stress, such as diabetes mellitus, induces disruption of cardiac metabolism and cardiomyopathy, much less is known about the reverse processes associated with the systemic response to altered cardiac metabolism as a consequence of pathological stress on the heart.

We have previously found that acute expression of an exogenous gene for the fetal carnitine palmitoyl transferase isoform (CPT1a) in healthy hearts not only recapitulates the shift in the cardiac CPT1 isoform distribution of the hyper trophyed heart but also induces a similar reduction in LCFA oxidation that is characteristic of cardiac hypertrophy. Surprisingly, the CPT1a isoform and metabolic shifts coincided with elevated myocardial natriuretic peptide expression, particularly ANP (atrial natriuretic peptide). This finding is consistent with hypertrophic signaling in the heart but in this case is induced by metabolic shifts in the cardiomyocyte and not pathological stress responses because these occur as a result of the absence of an increased pressure load. This prior finding suggests that the ANP responds directly to metabolic shifts away from fat oxidation by the pathological heart, with the potential for soluble circulating molecules to serve as metabolic mediators. With such changes in natriuretic peptides, the potential then exists for a secondary metabolic response within adipose tissues to occur at the onset of metabolic remodeling of the pressure-overloaded heart that displays this shift in CPT1 isoforms.

Therefore, we examined the systemic and peripheral tissue responses to cardiac pressure overload in a mouse model designed to minimize acute increases in the pressure gradient across the aortic constriction over the course of several months, leading to eventual pathological hypertrophy and cardiac decompensation. The resulting data are the first to elucidate temporal changes in insulin signaling within peripheral tissues and organs. While systemic metabolic shifts away from fat oxidation by the pathological heart can then be expected to create a shift in the systemic metabolism of LCFA, potentially involving peripheral tissues and organs. While systemic metabolic stress, such as diabetes mellitus, induces disruption of cardiac metabolism and cardiomyopathy, much less is known about the reverse processes associated with the systemic response to altered cardiac metabolism as a consequence of pathological stress on the heart.

Materials and Methods

Animals

Mice were housed in environmentally controlled conditions with a 12-hour light/dark cycle and had free access to standard rodent pellet food and water. The animal protocols were approved by the Institutional Animal Care and Use Committee of University of Illinois at Chicago. Animal care was given in accordance with institutional guidelines. Male C57BL/6 mice were obtained from the Jackson Laboratory (USA). Nine- or 10-week-old C57BL/6 animals were used in our experiments.

Pressure Overload via Transverse Aortic Constriction

Transverse aortic constriction (TAC) was performed on male C57BL/6 mice weighing 20 to 25 g. Each mouse was injected IP with 10 mg/kg etomidate and then placed in an induction chamber filled with 3.5% isoflurane mixed with 100% oxygen. Once sedated, it was intubated with a 20G catheter and placed on a ventilator (MiniVent Model 845; Harvard Apparatus, MA) supplying 2.0% isoflurane in 100% oxygen delivered at 1.0 L/min. Tidal volume was set at 150 μL, and the respiratory rate was 150 beats per minute. The fur in the vicinity of the upper torso was removed using a depilatory cream, the skin scrubbed 3× with alternating swabs of Betadine and 70% alcohol, and a sterile drape placed to maintain aseptic conditions. A midline incision was made in the skin from just above the manubrium to just below the xiphoid process. A partial sternotomy was then performed from the manubrium to just past the third rib, rib spreaders were emplaced to open the chest wall, and the thymus gently moved aside to expose the aortic arch. Adipose tissue was removed from the aortic arch and a titanium microclip placed to reduce the transverse aorta cross section by ~50%. Rib spreaders were removed, the lungs briefly (2–3 seconds) hyperinflated via occlusion of the ventilator’s exhaust line, tidal volume increased to 200 μL, and the chest wall and skin closed in separate layers using 6-0 monofila-

Hemodynamics and Echocardiography

Echocardiography was performed on a VisualSonics’ Vevo 2100 ultrasound machine using an MS550D (40 MHz) transducer. Briefly, mice were anesthetized in an induction chamber filled with 3.5% isoflurane mixed with 100% oxygen and then placed supine on a heated stage containing ECG leads. The heated stage also contained a nosethe one with 1.5% isoflurane in 100% oxygen delivered at 1.0 L/min and an anal temperature probe by which temperature was maintained at 37°C. Transthoracic B-mode, M-mode, pulsed Doppler, and tissue Doppler images were obtained from the parasternal short-axis view (systolic and morphology parameters) and the apical view (diastolic parameters). Results are based on the average of at least 3 cardiac cycles.

Reagents

Dextrose was from Hospira, and insulin humalog used for insulin tolerance test and in vivo insulin stimulation was from Eli Lilly.

Antibodies

Rabbit anti-pAkt (thymoma viral proto-oncogene) and anti–total Akt antibodies were obtained from Cell Signaling. Rabbit anti-uncoupling protein 1 antibody was obtained from Santa Cruz.

RNA Extraction and Real-Time Polymerase Chain Reaction

Total RNA was isolated from tissues with the use of Trizol reagent (Invitrogen) and Direct-zol kit (Zymo Research). cDNA was prepared from 1 μg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Invitrogen) with random hexamer primers, according to the manufacturer’s instructions. The resulting cDNA was diluted 10-fold, and a 1.5 μL aliquot was used in a 6 μL polymerase chain reaction (SYBR Green, Bio-Rad) containing primers at a concentration of 300 nmol/L each. Polymerase chain reactions were run in triplicate and quantitated using the Applied Biosystems ViiA7 Real-Time PCR system. Results were normalized to TBP (TATA box binding protein) expression and expressed as arbitrary units or fold change. Primer sequences are listed in the Data Supplement.

Western Blotting

For total tissue lysate, tissues were lysed in RIPA (Radioimmunoprecipitation assay) buffer supplemented with protease and phosphatase inhibitors cocktail. Total tissue lysates (20 μg) were subjected to SDS-PAGE, and blotting was performed as described. Multiple exposures...
were used to ascertain signal linearity. CPT1a protein expression was measured in mitochondria (3 μg) isolated using a mitochondrial isolation kit (ab110168; Abcam), with VDAC1 (voltage-dependent anion selective channel protein 1) as a mitochondrial loading control.

**Metabolic Parameters**

Plasma insulin was measured with an ELISA kit (Millipore). Non-esterified fatty acids, triglyceride, and cholesterol concentration in serum were measured with non-esterified fatty acids-C and Triglyceride E tests (Wako), respectively. Serum adiponectin and leptin levels were measured with ELISA kits from R&D Systems. Serum ANP and BNP (brain natriuretic peptide) were measured with ELISA kits (RayBiotech and Phoenix Pharmaceutical, Inc, respectively). Plasma catecholamines and cortisol were measured with ELISA kits (Rocky Mountain and Arbor Assays, respectively).

**Physiological Studies, Tissues Collection, and Histological Analyses**

Blood glucose was monitored with an automated glucose monitor (Glucometer Elite, Bayer). GT tests and insulin tolerance tests were performed 16 hours after fasting as described previously. Mice were anesthetized, and tissues, including heart, liver, spleen, skeletal muscle, visceral and subcutaneous adipose tissues, were rapidly dissected after blood collection through cardiac puncture, weighed, and processed for immunohistochemistry as described previously. Adipose tissues around epididymis were collected as representatives for visceral fat, and inguinal fat pads were used as representatives for subcutaneous fat.

**Statistical Analyses**

All data are presented as mean±SEM and analyzed by unpaired 2-tailed Student’s t test or 1-way or 1-way analysis of variance as indicated. When comparing means of unequal variance, a Welch correction was used. A P value <0.05 is considered significant. For repeated measures, a Bonferroni correction is applied, and a P value of 0.00625 is considered significant.

**Results**

**TAC Regulates Systemic Metabolic Homeostasis in Biphasic Manner**

To investigate interaction between the pathologically hypertrophic heart and peripheral organ metabolism, we used a model of cardiac pressure overload, via TAC. The TAC model used produces cardiac hypertrophy but without significant reductions in hemodynamics until 16 weeks post-TAC (Figure 1). Cardiac hypertrophy was detectable as early as 2 weeks post-TAC as shown by increased left ventricle mass (Figure 1A) and heart weight (Figure 1B) and is consistent with elevated cardiac expression of ANP and BNP at 2 and 6 weeks post-TAC (Figure 1C). As previously noted, TAC induced elevated expression of the liver isoform of CPT1, CPT1a, which has been linked to ANP expression in a rodent model of acute CPT1a overexpression (Figure 1D). The increase in CPT1a by 2 weeks TAC is evidence of an early metabolic remodeling in response to this degree of pressure overload that does not result in compromised function until 16 weeks and, thus, coincides with the surprising changes in systemic and adipose metabolism that occur in 2 weeks, as discussed below.

Systolic function was compromised at week 16 (Figure 1E). Despite the delay in systolic dysfunction after TAC, the reduced PGCl-α and CITED4 and elevations of cardiac message levels of MCH-β relative to MHC-α, ANP, and BNP all confirm a pathological, not physiological, hypertrophic response as early as 2 weeks (Figure 1F). No differences in body and organ weights occurred post-TAC versus sham surgery (Figure 1 in the Data Supplement).

Unexpectedly, GT in sham and TAC mice (Figure 2A; Figure IIA and IIB in the Data Supplement) displayed a biphasic response in systemic glucose regulation. TAC mice showed an initial improvement in GT compared with the sham control at 2 weeks, but this effect diminished over time and reversed within 6 weeks, with TAC mice becoming more glucose intolerant. Again, no evidence of physiological hypertrophy occurred during this initial enhancement of GT, as would otherwise be characterized by increased PGCl-α and MHC-α (Figure 1F).

To determine whether these changes in systemic glucose homeostasis are a consequence of altered IS, we performed insulin tolerance tests on sham and TAC mice (Figure 2B; Figure IIC in the Data Supplement). Similar to the GT observations, IS in TAC mice was also regulated in a biphasic manner. Our data suggest that the changes in GT in TAC mice were due in part to changes in systemic IS. To further examine the impact of pathological cardiac stress on systemic metabolic homeostasis, circulating insulin and lipids levels were measured. Consistent with the changes in systemic IS, we found that the fasting plasma insulin levels were initially downregulated and then upregulated in the TAC mice (Figure 2C), whereas the circulating lipid levels remained unchanged at both time points examined (Figure IID in the Data Supplement). Taken together, our data show that in addition to physiological and morphological remodeling of cardiomyocytes, the cardiac response to pathogenic stress plays an important role in systemic metabolic homeostasis during the evolution of cardiac hypertrophy prior to decompensation.

**TAC Modulates Insulin Signaling in Heart, Liver, and White Adipose Tissues**

To identify which tissues contributed to the changes in GT and IS in TAC mice, we performed in vivo insulin stimulation. Western blot results showed that aortic constriction initially improved insulin signaling in liver, epididymal white adipose tissue (eWAT) and inguinal white adipose tissue (iWAT), but not muscle and heart in 2-week TAC mice (Figure 3A). Consistent with the IS data, liver, eWAT, and heart insulin signaling were downregulated in 6-week TAC mice but not iWAT and muscle (Figure 3B). Despite similar pAkt/total Akt ratio, both pAkt and total Akt were found to be downregulated in the heart from 6-week TAC mice. These results suggest inhibition of insulin signaling via regulation of downstream signaling proteins independent of insulin receptor. Because inflammation has been shown to modulate IS, we examined inflammation markers in liver and WAT from sham and TAC mice. Our data show that increased IS in the TAC livers and iWAT at 2 weeks was associated with reduced WAT inflammation in the fed state, while insulin resistance in 6-week TAC mice was associated with systemic inflammation, as determined by elevated hepatic expression of acute-phase proteins (Figure III in the Data Supplement).

To determine the potential functional impact of cardiac hypertrophy on peripheral metabolic organs, we examined the expression levels of glucose and lipid metabolism markers in
the liver. However, despite changes in insulin signaling, neither of these metabolic processes were affected in TAC mice at 2 or 6 weeks (Figure IV in the Data Supplement).

To determine which metabolic processes were affected in WAT in the TAC mice at the different time points, we examined expression markers of oxidative metabolism, lipolysis, and lipogenesis. We observed that lipogenic markers were downregulated in 2-week TAC mice under both fed and fasting conditions (Figure 4A). Also, at this early time point, lipolytic markers are downregulated in the TAC mice under the fed condition while oxidative metabolism markers are downregulated in the fasted TAC mice (Figure 4A). Consistent with the physiological data, we observed that the downregulation observed in the eWAT of TAC mice at the 2-week time point was mostly reversed in 6-week TAC mice (Figure 4B). Fat accumulation and high lipolysis rates in visceral fat are known to contribute significantly to the development of metabolic disorders, and our results suggest that inhibition of lipogenesis and lipolysis in the eWAT could contribute to the improvements in glucose and insulin homeostasis in the TAC mice at the 2-week time point.

Figure 1. Cardiac hypertrophic responses after transverse aortic constriction (TAC). A, Left ventricular (LV) mass in sham and TAC mice measured biweekly after surgery (n=4–6). *P<0.05 (vs sham) unpaired t test. †P<0.00625 Bonferroni correction. B, Heart weight of 2 and 6 weeks sham or TAC mice normalized to respective tibia length (n=5–6). *P<0.05 (vs sham) unpaired t test. C, ANP (brain natriuretic peptide) and BNP (brain natriuretic peptide) mRNA levels in hearts from 2 or 6 weeks sham or TAC mice (n=5–6). *P<0.05 (vs sham) unpaired t test. D, Carnitine palmitoyl transferase I (CPT1) protein expression of liver (CPT1a) isoform was elevated by 2 weeks TAC over that in sham-operated hearts. E, Fractional shortening and ejection fraction of sham and TAC mice measured biweekly after surgery (n=4–6). *P<0.05 (vs sham) unpaired t test. †P<0.00625 Bonferroni correction (vs 2 weeks TAC). F, Hypertrophy markers expression in hearts from 2 and 6 weeks sham and TAC mice (n=5–6). *P<0.05 (vs sham) unpaired t test. F tests were performed to determine variance, and Welch’s correction was used in cases of unequal variance. All quantitative polymerase chain reaction (qPCR) data are normalized with TBP (TATA box binding protein) and presented as mean±SEM.
In contrast to eWAT, we found that markers of thermogenesis, lipolysis, and lipogenesis were upregulated in the iWAT from 2-week TAC mice, but not in 6-week and 17-week iWAT (Figure 4C; Figure V in the Data Supplement). The increased thermogenic markers prompted us to consider possible beige adipocyte formation in the iWAT of TAC mice. To test this idea, we performed hematoxylin and eosin staining of the iWAT sections from both TAC and sham mice. Consistent with the thermogenic marker expression data, a large increase in multilocular adipocytes occurred in iWAT from 2-week TAC, but not 6-week TAC, when compared with the sham mice (Figure 4D).

These results were subsequently confirmed with uncoupling protein 1 immunohistochemical staining (Figure 4E).

Subcutaneous fat is known to play a protective role in whole-body metabolism because of its metabolic flexibility and fat storage capacity, these data suggest that increased browning and adipocyte functions in the iWAT contributes a beneficial metabolic effect during the early phase of cardiac hypertrophy.

In contrast to subcutaneous fat, brown adipose tissue (BAT) is also known to play a key role in maintaining metabolic and energy homeostasis in mice. To investigate the metabolic contribution of interscapular brown fat, we examined BAT from TAC and sham mice at both time points. In contrast to both eWAT and iWAT, BAT lacked significant changes in all functional markers examined, suggesting that BAT might not play a significant role in the beneficial response to cardiac stress observed at the 2-week time point (Figure 5A). Interestingly, both thermogenic and lipogenic markers were downregulated in the BAT from 6-week TAC mice (Figure 5B). Taken together, these data are evidence for impaired metabolic homeostasis at 6-week TAC that is, in part, a consequence of metabolic downregulation in BAT.

Circulating Natriuretic Peptides, Adipokines, and Catecholamine
To identify a possible mediator of the observed metabolic changes in TAC mice, particularly the multiorgan effects, we examined circulating hormones, including ANP, BNP, adiponectin, and leptin. It is well characterized that through the protein kinase A signaling pathway, natriuretic peptides play a role in modulating thermogenesis in brown adipocytes. In addition, leptin is also known to regulate brown fat thermogenesis. On the other hand, circulating adiponectin exerts a beneficial effect on glucose homeostasis. Our analysis showed that plasma ANP, BNP, and adiponectin were upregulated in 2-week TAC mice (Figure 6A and 6B), whereas adiponectin and leptin were downregulated in 6-week TAC mice (Figure 6B). The values obtained with the current assay methods generated plasma BNP in sham-operated mice that seem elevated over previously published values for humans and mice. However, reproducibility in rodent plasma BNP assays, even duplicating prior published methods, has been

Figure 2. Transverse aortic constriction (TAC) regulates systemic metabolic homeostasis in biphasic manner. A, Intraperitoneal glucose tolerance test (IPGTT) after overnight fast at 2, 4, and 6 weeks in sham and TAC mice (n=4–6). B, Intraperitoneal insulin tolerance test (IPITT) after 2 h fast at 2, 4, and 6 weeks in sham and TAC mice (n=4–6). *P<0.05 (vs sham) 2-way analysis of variance and Bonferroni posttest. C, Plasma insulin levels at 2, 4, and 6 weeks in sham and TAC mice (n=4–6). All data are presented as mean±SEM. *P<0.05 (vs sham) unpaired t test.

Browning Effects of TAC on Subcutaneous Adipose Tissue
In contrast to eWAT, we found that markers of thermogenesis, lipolysis, and lipogenesis were upregulated in the iWAT from 2-week TAC mice, but not in 6-week and 17-week iWAT (Figure 4C; Figure V in the Data Supplement). The increased thermogenic markers prompted us to consider possible beige adipocyte formation in the iWAT of TAC mice. To test this idea, we performed hematoxylin and eosin staining of the iWAT sections from both TAC and sham mice. Consistent with the thermogenic marker expression data, a large increase in multilocular adipocytes occurred in iWAT from 2-week TAC, but not 6-week TAC, when compared with the sham mice (Figure 4D). These results were subsequently confirmed with uncoupling protein 1 immunohistochemical staining (Figure 4E). Because subcutaneous fat is known to play a protective role in whole-body metabolism because of its metabolic flexibility and fat storage capacity, these data suggest that increased browning and adipocyte functions in the iWAT contributes a beneficial metabolic effect during the early phase of cardiac hypertrophy.

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elusive, and some cross reactivity, presumably with ANP, may account for these results. Nonetheless, BNP values determined within the constraints of the currently performed assays were also elevated to levels consistent with those of the rat model of ventricular remodeling postmyocardial infarction.

Changes in plasma adiponectin and leptin levels are likely because of changes in their expression in the iWAT. In addition, we observed an increase in the gene expression of the natriuretic peptide receptor type A (NPRA/NPR1; active form) but unchanged natriuretic peptide receptor type C (NPRC/NPR3; clearance receptor) in iWAT from TAC mice (Figure 6D). Increased NPRA expression and circulating ANP and BNP could potentially contribute to the observed browning in iWAT of TAC mice at 2 weeks, while adiponectin could play a direct role in the improvement of GT. In contrast, the downregulation of adiponectin and leptin in 6-week TAC mice may contribute to their glucose intolerance and the inhibition of thermogenesis in BAT of these mice.

To test for the potential for sympathetic contributions to the adipose tissues functional shifts, plasma cortisol and norepinephrine were examined (Figure 6E and 6F). Neither circulating cortisol nor epinephrine was significantly elevated at either 2 or 6 weeks post-TAC in comparison to sham-operated mice.

Figure 3. Transverse aortic constriction (TAC) modulates insulin signaling in heart, liver, and white adipose tissues. Western blot analyses and quantification for p473Akt and total Akt in liver, epididymal white adipose tissue (eWAT), inguinal white adipose tissue (iWAT), heart, and muscle at 2 weeks (A) and 6 weeks (B) from sham and TAC mice stimulated with either saline or insulin after overnight fast (n=5–6). All data are presented as mean±SEM. *P<0.05 (vs sham insulin) unpaired t test.
Figure 4. Transverse aortic constriction (TAC) modulates thermogenesis, lipolysis, and lipogenesis in epididymal white adipose tissue (eWAT). A, Thermogenesis, lipolysis, and lipogenesis markers expression in fed or overnight fasted eWAT from 2 weeks sham and TAC mice. B, Thermogenesis, lipolysis, and lipogenesis markers expression in fed or overnight fasted eWAT from 6 weeks sham and TAC mice (n=5–6). C, Thermogenesis, lipolysis, and lipogenesis markers expression in fed inguinal white adipose tissue (iWAT) at 2 and 6 weeks in sham and TAC mice (n=5–6). D, Representative images from hematoxylin and eosin (H&E) iWAT sections from 2 and 6 weeks sham or TAC mice. E, Representative images from immunohistological staining for uncoupling protein 1 (UCP1) for iWAT section at 2 and 6 weeks in sham and TAC mice. All quantitative polymerase chain reaction (qPCR) data are normalized with TATA box binding protein (TBP) and presented as mean±SEM. *P<0.05 (vs sham) unpaired t test. F tests were performed to determine variance, and Welch’s correction was used in cases of unequal variance.
mice. However, activation of the gene for β3-adrenergic receptor expression in iWAT occurred in response to TAC at 2 weeks, suggesting that a transient change in sensitivity to sympathetic activation in iWAT occurs (Figure 4C).

Discussion

The present study is the first to report a biphasic response of systemic and peripheral organ metabolism to chronic pressure overload of the heart. As shown, pathogenic stress on the heart induces changes in insulin signaling within the heart, liver, and adipose tissue along with changes in circulating adipokines and natriuretic peptides, prior to any hemodynamic deficits. Changes in peripheral organ and systemic metabolism occurred during early evidence of a metabolic remodeling-associated CPT1a isoform distribution, along with elevated message levels for cardiac natriuretic peptide. Gene expression in subcutaneous and visceral adipose tissue was profoundly affected by the induction of pressure overload on the murine heart. Changes in subcutaneous adipose tissue gene expression were consistent with altered thermogenesis and fat browning. These changes in gene expression in the adipose tissues coincided with temporal shifts in the whole body response to glucose and insulin. Indeed, the most surprising aspects of the longitudinal response to the induction of cardiac pressure overload were the initial increases in IS and GT, which coincided with reductions in the expression of genes associated with oxidative metabolism in visceral adipose tissue. While the cardiac response to the pathogenic stress of TAC produced left ventricular hypertrophy in this model, no systolic or diastolic dysfunction was evident until 17 weeks post-TAC, and thus, the metabolic shifts observed in the whole animal, including liver and adipose tissues, were not the consequence of impaired hemodynamics.

The challenge of pressure overload is known to enhance insulin signaling in cardiomyocytes, which is evident within the first minute of challenge via a ligand-independent activation of the cardiac insulin receptor (Insr) in response to cardiomyocyte stretch. However, such temporal observations are highly model-dependent and reflect a specific response to the extent of the induced pressure gradient produced by TAC. In the model used by Shimizu et al for these analyses, left ventricular systolic function was already compromised at 2 weeks, when plasma insulin levels were elevated. Using a TAC model with a less aggressive challenge, our current study produced pressure overload hypertrophy without evidence of impaired systolic function until 17 weeks post-TAC. In this less severe model of pathological cardiac hypertrophy, plasma insulin actually decreased before eventually rising above control levels as hearts progressed toward decompensation. This change in plasma insulin paralleled the biphasic changes in GT observed after TAC. Thus, the acute models of pathological challenge that produce an early systolic dysfunction are too severe to enable elucidation of the biphasic nature of insulin responses to pressure overload, as reported here. Despite the early increases in GT and IS in response to TAC, classic markers of pathological hypertrophy were confirmed in this heart model just 2 weeks post-TAC (Figure 1F).

Impaired IS is a known clinical outcome in patients with congestive heart failure. Impaired insulin signaling in cardiomyocytes of the failing heart has also been reported in patients prior to placement on assist devices. However, the initial response to pathogenic stress that evolves over time, as in the current animal model, is difficult to detect in asymptomatic patients. Thus, the metabolic responses during the pathogenesis of cardiac decompensation, leading to overt heart failure, are poorly documented and understood.

It is now clear that in addition to the endocrine hormones secreted by classical endocrine organs, to achieve intricate regulation of systemic metabolic homeostasis, most metabolically active tissues, including adipose tissues, liver, and skeletal muscle, can also secrete proteins that have paracrine or endocrine actions or both. The data are consistent with elevated CPT1a isoform expression in response to TAC, as well as the increased message levels in response to both TAC and acute CPT1a expression in otherwise healthy rat hearts. It has been long recognized that the heart is capable of secreting regulatory factors known as cardiokines, such as ANP and

Figure 5. Metabolic changes in brown adipose tissue (BAT) after transverse aortic constriction (TAC). Thermogenesis, lipolysis, and lipogenesis markers expression in fed BAT from 2 (A) and 6 (B) wk sham and TAC mice (n=5–6). All quantitative polymerase chain reaction (qPCR) data are normalized with TATA box binding protein (TBP) and presented as mean±SEM. *P<0.05 (vs sham) unpaired t test. F tests were performed to determine variance, and Welch’s correction was used in cases of unequal variance.
BNP, particularly under stress conditions, as well as recently identified new cardiokines, including growth differentiation factor-15, activin A, and the follistatin-like family of proteins (Fstl1, Fstl3). These cardiokines mainly act in an autocrine manner to modulate myocyte death, fibroblast activation, inflammation, and vascular growth and regression. However, it is not known whether the cardiokines play a regulatory feedback role in systemic metabolic homeostasis during the development of heart failure, contributing to a vicious cycle that accelerates disease progression.

Our study examined the molecular and physiological impact of pathogenic cardiac stress, in the absence of additional underlying pathologies, on visceral and subcutaneous adipose tissues. Adverse changes in IS, adipokines secretion, lipolysis, and lipogenesis have been linked to increased risks for cardiovascular diseases and diabetes mellitus. Improvement in IS, lipolysis, and lipogenesis in 2-weeks TAC mice clearly contributed to overall metabolic health of the TAC animals, as demonstrated by the glucose and insulin tolerance tests. In addition, we also observed changes in the expression of thermogenic markers in 2-week TAC mice eWAT. Because the contribution of eWAT thermogenic regulation on systemic metabolic homeostasis has not been carefully investigated and data from limited transplantation studies suggests that the eWAT role is probably minor, the observed downregulation is not likely to significantly alter whole-body metabolism.

The second phase of reverted metabolic responses that were observed after TAC were also considered to be the potential consequence of eventual natriuretic peptide resistance either because of chronic adrenergic drive or peripheral desensitization. Although assays demonstrated no changes in natriuretic peptide receptor expression over the course of the TAC protocol, we cannot exclude the possibility of altered natriuretic peptide receptor signaling. Additionally, an increase in catecholamine release from the failing heart, with eventual depletion from the cardiomyocyte and sustained release of catecholamines from other organs after later stages of heart failure, is well recognized. The model presented here is not expected to be without an eventual and significant contribution to the observed thermogenic markers in 2-week TAC mice eWAT. Because the contribution of eWAT thermogenic regulation on systemic metabolic homeostasis has not been carefully investigated and data from limited transplantation studies suggests that the eWAT role is probably minor, the observed downregulation is not likely to significantly alter whole-body metabolism. The second phase of reverted metabolic responses that were observed after TAC were also considered to be the potential consequence of eventual natriuretic peptide resistance either because of chronic adrenergic drive or peripheral desensitization. Although assays demonstrated no changes in natriuretic peptide receptor expression over the course of the TAC protocol, we cannot exclude the possibility of altered natriuretic peptide receptor signaling. Additionally, an increase in catecholamine release from the failing heart, with eventual depletion from the cardiomyocyte and sustained release of catecholamines from other organs after later stages of heart failure, is well recognized. The model presented here is not expected to be without an eventual and significant contribution to the observed

**Figure 6.** Potential modulators for transverse aortic constriction (TAC)-induced metabolic changes. Plasma ANP (atrial natriuretic peptide), BNP (brain natriuretic peptide; A), adiponectin, and leptin (B) levels at 2 and 6 weeks in sham and TAC mice (n=6). Adiponectin, leptin (C), natriuretic peptide receptor type A (NPR1) and natriuretic peptide receptor type C (NPR3; D) gene expression in inguinal white adipose tissue (iWAT) at 2 and 6 weeks in sham and TAC mice (n=6). Plasma cortisol (E) and noradrenaline (F) levels at 2 and 6 weeks in sham and TAC mice (n=6 for both). All quantitative polymerase chain reaction (qPCR) data are normalized with TATA box binding protein (TBP) and presented as mean±SEM. \(*P<0.05\) (vs sham) unpaired t test. F tests were performed to determine variance, and Welch’s correction was used in cases of unequal variance.
Changes in adipose tissue, but the changes observed in this study occur well before overt heart failure and even preceding systolic dysfunction. Data showing the lack of significant elevation in circulating cortisol and norepinephrine after 2 and 6 weeks of TAC demonstrate that the biphasic, peripheral metabolic responses that this protocol elicits are not likely a consequence of sympathetic release, but rather linked to natriuretic peptides and adipokines. Nevertheless, with the increase in β3-adrenergic receptor gene activation at 2-week TAC in the iWAT, it remains possible that catecholamines play some role in regulating the early responses observed in adipose tissue. Thus, while the contributions of heightened sympathetic drive cannot be separated out from the other mediators of whole body and adipose tissue metabolism, the changes in natriuretic peptides and adipokines are directionally consistent with roles in the early responses to chronic pressure overload of the heart before hemodynamic function is noticeably compromised beyond LV wall hypertrophy.

Subcutaneous fat has been shown to be more insulin sensitive and more resistant to metabolic changes during obesity. We know that accumulation of subcutaneous fat, especially in the gluteofemoral region, confers little metabolic risk or may even be protective. Our 6-week post-TAC data showed that unlike eWAT, iWAT are clearly more resistant to changes in IS during metabolic stress. In addition to its role on lipolysis, lipogenesis, and adipokines secretion, subcutaneous fat is known to form beige cells under appropriate stimuli, which has been proposed to contribute to systemic metabolic homeostasis. A recent study demonstrated an increase in thermogenic markers expression in adipose tissue in a mouse model of heart failure with preserved ejection fraction induced by multiple insults of TAC, uninephrectomy, and infusion of either saline or angiotensin. In contrast, our current study demonstrated for the first time subcutaneous adipose tissue beiging in direct response to direct pathophysiological stress on the heart in the absence of impaired cardiac hemodynamics and a reversal of the gene activation and uncoupling protein 1 expression after 6 weeks of TAC. The appearance of multilocular beige cells at 2 weeks and the disappearance at 6 weeks coincided with a never before recognized, multiphasic changes in glucose and insulin tolerance. These changes strongly suggest a role for subcutaneous beige cells in systemic metabolic regulation after TAC. Several mechanisms are implicated in iWAT beiging; in addition to prolonged cold exposure and chronic adrenergic activation, cardiac natriuretic peptide has more recently demonstrated a role in iWAT beiging. Therefore, the increased circulating cardiac natriuretic peptide and iWAT NPRA expression likely contributed to the observed iWAT beiging in 2-week TAC mice. However, it is unclear at the moment the mechanism behind the disappearance of beige cells in 6-week TAC mice, despite similarly elevated plasma BNP and iWAT NPRA. These observations warrant further study.

Similarly, the impact of pathological cardiac stress on BATs has not been investigated. Brown fat is the key site for thermogenesis. As both triglycerides and glucose are fuels for thermogenesis, BAT has also been recently shown to play an important role in the regulation of systemic lipoprotein and glucose homeostasis. Downregulation of both thermogenesis and lipogenesis observed in the BAT from 6-week TAC mice potentially contributed to the glucose and insulin intolerance observed in the TAC mice.

In conclusion, our study revealed an intriguing biphasic modulation of systemic and peripheral organ metabolism in response to a moderate, chronic pressure overload of the heart prior to evidence of impaired hemodynamics or changes in circulating markers of sympathetic drive. Understanding the molecular pathways leading to metabolic changes in the affected metabolic organs and identification of the circulating modulators could reveal new strategies for treating metabolic and cardiovascular diseases.

Acknowledgments

Dr Liew participated in generating the hypothesis, experimental, aims, and study design, and directly collected data and oversaw data collection, performed data analysis, interpretation and writing of the article. S. Xu, H. Whang Kong, M. McCann participated in data collection, data analysis, and interpretation, as well as contributed to the writing of the article. Dr Wang adopted methods for analytic assays, collected and interpreted data, and contributed to the writing of the article. Dr Carley participated in data collection, data analysis and interpretation, and protocol design for experimental animal models. Dr Pang performed tissue and blood collections and assays, analyzed data, and interpreted results. Dr Fantuzzi participated in study design, performed assays on plasma peptides, performed data analysis, and interpreted results. Dr O’Donnell designed protocols for experimental animal models and oversaw collection of functional hemodynamic data and directly performed analysis on cardiac function data, as well as contributed to writing the article. Dr Lewandowski participated in generating the hypothesis, experimental, aims, and study design, as well as overseeing experimental activities and data collection and directly performed data analysis, interpretation, and writing of the article.

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Disclosures

None.

References


This study elucidates an initial, transient response of increased glucose tolerance and insulin sensitivity with functional changes in adipose tissue in an animal model of cardiac pressure overload. These responses were induced by pathological stress on the heart without any additional confounding pathogenesis. Insulin resistance has long been reported in heart failure patients. Patients on left ventricular assist device support are reported to have impaired insulin signaling in failing myocardium in the absence of type 2 diabetes mellitus. The current study demonstrates a multiphasic response in whole-body, metabolic remodeling that initially improves, before the eventual onset of impaired insulin sensitivity. The ability to resolve this biphasic metabolic response may be a consequence of the unique protocol that initiates a less acute stress than other animal studies in which heart failure occurs within weeks. Our model developed compensatory hypertrophy, but hemodynamics did not decline until 4 months. While sympathetic drive does activate adipose tissue, in this model, there were no early elevations in circulating norepinephrine and cortisol. However, changes in plasma atrial and brain natriuretic peptides, as well as adipokines, occurred in phase with the metabolic shifts, as did fat oxidation enzymes in the stress heart. Thus, interorgan, metabolic signaling initiated at the stressed myocardium induces a favorable metabolic remodeling that is not sustained. These surprising results suggest the potential for early metabolic therapy for hypertensive patients and the potential to sustain the favorable metabolic response that occurs during early pathological stress in advance of early-stage heart failure.
Multiphasic Regulation of Systemic and Peripheral Organ Metabolic Responses to Cardiac Hypertrophy


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Supplemental Material

Multiphasic metabolic regulation in the systemic and peripheral organ responses to cardiac hypertrophy.

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Supplemental Figure Legends

Supplemental Figure 1:
Body (A) and organ (B) weight of sham or TAC mice measured biweekly after surgery (n=4-6). All data presented as mean ± SEM.

Supplemental Figure 2:
(A) Intraperitoneal glucose tolerance test (IPGTT) after overnight fast in 0 wk sham or TAC mice (n=4-6). (B) Area under the curve for IPGTT in 2, 4, and 6 wk sham and TAC mice. (C) Area under the curve for IPITT in 2, 4, and 6 wk sham and TAC mice. (D) Plasma cholesterol, free fatty acid, triglyceride levels in 2, 4 or 6 wk sham or TAC mice (n=4-6). All data presented as mean ± SEM. **,p<0.01 (vs Sham).

Supplemental Figure 3:
Inflammation markers and cytokines expression in fed or overnight fasted liver, gWAT and iWAT from 2 or 6 wk sham or TAC mice (n=4-6). All qPCR data are normalized with TBP and presented as mean ± SEM. *,p<0.05 (vs Sham).

Supplemental Figure 4:
Gluconeogenesis and lipogenesis markers expression in fed or fasted liver in 2 and 6 wk sham or TAC mice (n=5-6). All qPCR data are normalized with TBP and presented as mean ± SEM. *,p<0.05; **,p<0.01 (vs Sham).
Supplemental Figure 5:

Thermogenesis, lipolysis, and lipogenesis markers expression in fed iWAT from 17 wk sham and TAC mice (n=3-5). All qPCR data are normalized with TBP and presented as mean ± SEM. *,p<0.05 (vs Sham).
Supplemental Figure 1

A

Body weight

Week after surgery

B

2 wks Organ Mass/TL

6 wks Organ Mass/TL

gram

gram/mm

iWAT  gWAT  BAT  Liver  Spleen

iWAT  gWAT  BAT  Liver  Spleen
Supplemental Figure 2

A

Week 0 IPGTT

Blood Glucose (mg/dl)

Minutes after glucose injection

Sham

TAC

B

2 wk IPGTT

4 wk IPGTT

6 wk IPGTT

Sham

TAC

AUC

C

2 wk IPITT

4 wk IPITT

6 wk IPITT

AUC

D

2 wk NEFA (fed) 6 wk NEFA (fed)

2 wk TG (fed)

6wk TG (fed)

2 wk Cholesterol (fed) 6wk Cholesterol (fed)

mEq/l

mg/dl

mg/dl

mg/dl
Supplemental Figure 3

2 wks liver (fast)

6 wks liver (fast)

2 wk eWAT (fed)

6 wk eWAT (fed)

2 wk iWAT (fed)

6 wk iWAT (fed)

2 wk gWAT (fast)

6 wk gWAT (fast)
Supplemental Figure 5

17wk SAT TAC fed

Relative expression/ΔCT (A.U.)

- Sham
- TAC

Gene Expression:
- UCP1
- PCG-1a
- Cidea
- Elovl3
- Cox6b
- Db2
- Aorb3
- ATGL
- FASN
- SCD1

* Indicates significant difference compared to Sham.