Intrinsic Diurnal Variations in Cardiac Metabolism and Contractile Function

Martin E. Young, Peter Razeghi, Ari M. Cedars, Patrick H. Guthrie, Heinrich Taegtmeyer

Abstract—Diurnal variation of cardiac function in vivo has been attributed primarily to changes in factors such as sympathetic activity. No study has investigated previously the intrinsic properties of the heart throughout the day. We therefore investigated diurnal variations in metabolic flux and contractile function of the isolated working rat heart and how this related to circadian expression of metabolic genes. Contractile performance, carbohydrate oxidation, and oxygen consumption were greatest in the middle of the night, with little variation in fatty acid oxidation. The expression of all metabolic genes investigated (including regulators of carbohydrate utilization, fatty acid oxidation, and mitochondrial function) showed diurnal variation, with a general peak in the night. In contrast, pressure overload–induced cardiac hypertrophy completely abolished this diurnal variation of metabolic gene expression. Thus, over the course of the day, the normal heart anticipates, responds, and adapts to physiological alterations within its environment, a trait that is lost by the hypertrophied heart. We speculate that loss of plasticity of the hypertrophied heart may play a role in the subsequent development of contractile dysfunction. (Circ Res. 2001;89:1199-1208.)

Key Words: function • gene expression • metabolism • perfusions • rat

Cells are able to anticipate, respond, and adapt to fluctuations in their environment. Anticipation is achieved through self-sustained intracellular clocks, providing advantageous priming of the cell in preparation to a given stimulus.¹ The response of any cell is dictated by the level of the stimulus, as well as the sensitivity to that stimulus. The latter is affected by both intracellular (genotype, circadian clocks) and extracellular (eg, neuronal and humoral factors) influences. The resultant adaptation can be either immediate (alterations in preexisting proteins) or prolonged (changes in gene and protein expression) depending on the length of exposure to the stimulus.

The heart, not unlike other organs, possesses both internal clocks and the ability to respond to external stimuli, both of which could potentially influence gene expression, metabolism, and function.²⁻⁴ It is well known that the onset of heart failure, myocardial infarction, and sudden death is greatest in the early hours of the morning.⁵⁻⁷ For this reason, several studies have investigated diurnal variation in cardiac function in vivo, in both rodents and humans, and have correlated findings with fluctuations in neurohumoral influences.⁵⁻¹¹ However, to date, no study has either postulated or investigated whether the intrinsic properties of the heart fluctuate during the day, or whether loss of synchronization between the presence of a stimulus (eg, sympathetic activity) and responsiveness of the heart plays a role in the development of contractile dysfunction.

We set out to characterize the diurnal variation in contractile function and metabolic flux of the heart in the absence of confounding extracardiac influences by using the isolated working rat heart preparation. Contractile performance, carbohydrate oxidation, and oxygen consumption were greatest in the middle of the night, with little variation in fatty acid oxidation. These changes in the intrinsic properties of the heart were related to the diurnal variations in metabolic gene expression. All metabolic genes investigated showed rhythmic patterns of gene expression. In contrast, pressure overload–induced cardiac hypertrophy completely abolished this diurnal variation of metabolic gene expression. Thus, over the course of the day, the normal heart anticipates, responds, and adapts to physiological alterations within its environment, a trait that is lost in the hypertrophied heart. We speculate that loss of plasticity of the hypertrophied heart plays a role in the subsequent development of contractile dysfunction.

Isolated Working Heart Preparation

Male Wistar rats (300 to 325 g) were placed in a separate environment-controlled room, with a strict 12-hour light/12-hour dark cycle regime (lights on at 7 AM [zeitgeber time; ZT0] and lights off at 7 PM [ZT12]), at least 1 week before experimentation, and received standard laboratory chow and water ad libitum. Hearts were isolated either in the middle of the light phase (ZT6) or the middle...
of the dark phase (ZT18), followed by perfusion using the working heart apparatus as described previously.3 Hearts were initially perfused in the Langendorff mode with Krebs-Henseleit buffer containing 5 mM/L d-glucose, followed by a 30 minute non-recirculating perfusion in the working mode with Krebs-Henseleit buffer containing 5 mM/L d-glucose (plus 20 μCi/[U-14C]-glucose), 0.5 mM/L sodium L-lactate (plus 2 μCi/[U-14C]-lactate), 0.05 mM/L sodium l-pyruvate, 0.4 mM/L sodium oleate (plus 30 μCi/[9,10-3H]-oleate) bound to 1% bovine serum albumin (fraction V, fatty acid free, Intergen, Purchase, NY), and 40 μU/mL insulin (Lilly). After 30 minutes of perfusion (15 cm H2O preload/100 cm H2O afterload), hearts were freeze-clamped and stored in liquid nitrogen prior to dry weight determination. Rates of oleate oxidation, carbohydrate oxidation (combination of exogenous glucose and lactate oxidation), oxygen consumption, as well as cardiac power, were determined as described previously.3

Aortic Constriction
Cardiac hypertrophy was induced in male Wistar rats (160 to 210 g initial weight) by constriction of the ascending aorta, as described previously.2,12 Seven weeks after surgery, hearts were isolated from control and banded rats every 3 hours from 7 AM (ZT0) to 7 AM (ZT24) the following day, as described previously.2 A total of 108 animals were studied, of which 55 were controls and 53 were banded.

RNA Extraction and Quantitative Reverse Transcriptase–Polymerase Chain Reaction
RNA extraction and quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) of samples was performed using previously described methods.13–15 Specific quantitative assays were designed from rat sequences available in GenBank (Table), except in the cases of mgs and cs, for whom the rat sequences were not available. In contrast, these genes have been cloned in the mouse and human, respectively. Multiple assays were then designed using the mouse and human sequences for these genes and subsequently tested for compatibility in the rat. Standard RNA was made for all assays by the T7 polymerase method (Ambion), using total RNA isolated from the rat heart. The correlation between the Ct (the number of PCR cycles required for the fluorescent signal to reach a detection threshold), and the amount of standard was linear over at least a 5-log range of RNA for all assays (data not shown). The level of transcripts for the constitutive housekeeping gene product β-actin was quantitatively measured in each sample, to control for sample-to-sample differences in RNA concentration. The expression of this housekeeping was not significantly different between the groups (data not shown). PCR data are reported as the number of transcripts per number of β-actin molecules.

Plasma Nonesterified Fatty Acid Level Measurement
Plasma nonesterified fatty acid (NEFA) levels were measured as described previously.16

Western Blot Analysis
Total GLUT4 content was determined in heart homogenates as described previously.17 GLUT4 antibodies were purchased from Biogenesis. Band density analysis was made using Image Pro Plus. Total GLUT4 protein is shown in arbitrary units (AU).

Statistical Analysis
Data are presented as the mean±SEM for between 6 and 8 hearts in each group. Statistically significant differences between groups were calculated by the Student’s t test between hearts isolated at ZT6 and ZT18 (perfusion and Western data). A value of P<0.05 was considered significant. For the gene expression studies, data were analyzed by 2-way analysis of variance (ANOVA) using the general linear models procedure in SAS software, version 6.12 (SAS Institute, Inc). Type III sums of squares were computed to account for time points where sample sizes were unequal. Time and banding were handled as class variables and saturated models (main+interaction effects) were evaluated for each gene. Because samples at each time point were from different animals, repeated-measures analysis was not necessary. The null hypothesis for the model effects was rejected at P<0.05.

Results
Increased Cardiac Function and Metabolism During the Dark Phase
We investigated whether intrinsic cardiac function and metabolism showed diurnal variation. For these experiments, working hearts were perfused (under otherwise identical conditions by the same investigators: A.M. Cedars and M.E. Young) at one of 2 time points, ZT6 and ZT18, which are in the middle of the light and dark phases, respectively. The data presented for each parameter is that obtained at time 20 minutes after the initiation of the working heart perfusion (approximately 27 minutes after the isolation of the heart), a time at which steady state cardiac performance and metabolism was established and any residual neurohumoral influences (such as receptor bound hormones) will be lost. Hearts perfused at ZT18 showed significant increases in steady state cardiac power, carbohydrate (glucose and lactate) oxidation, and oxygen consumption, compared with hearts perfused at ZT6 (Figures 1A, 1B, and 1D). No significant difference was observed in the rate of oleate oxidation in hearts perfused at these 2 time points (Figure 1C).

Aortic Constriction Induces Trophic and Gene Expression Markers of Cardiac Hypertrophy
Ascending aortic constriction resulted in increased heart weight-to-body weight (HW/BW) ratios compared with sham-operated controls at all time points investigated (Figure 2A). The average increase in the HW/BW ratio was 30%. This was due to increased heart weight as opposed to any variation in body weight (data not shown). The HW/BW ratio did not undergo any obvious rhythmic fluctuations during the day (Figure 2A).

Pressure overload is known to induce the expression of a number of fetal genes in the heart, such as myosin heavy chain β (mhcβ). Seven weeks after the initial aortic constriction, cardiac mhcβ expression was increased at all time points investigated (Figure 2B). The average increase in mhcβ expression was 53%. In addition, there was a diurnal variation in the expression of mhcβ, in both control and hypertrophied hearts, consisting of a 1.9-fold induction from trough (ZT6) to peak (ZT18) (Figure 2B).

Cardiac Hypertrophy Impairs Diurnal Variation of Carbohydrate Utilization Genes
We investigated diurnal variation in the expression of genes encoding for key regulators of carbohydrate utilization. These included regulators of glucose transport into the cell (glucose transporters 1 and 4; GLUT1 and GLUT4, respectively), glucose incorporation into glycogen
muscle-specific glycogen synthase; mGS), and pyruvate oxidation (pyruvate dehydrogenase kinase-4; PDK4). All genes showed diurnal variation of gene expression (Figure 3). These 4 genes all possessed peak levels of expression at approximately ZT15 (Figure 3). The induction of these genes were greatly attenuated in the hypertrophied heart, with no significant difference in trough expression levels (Figure 3).

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Attenuation of Diurnal Variation of Fatty Acid Metabolism Genes in the Hypertrophied Heart

The diurnal expression of the mRNAs encoding malonyl-CoA decarboxylase (MCD), muscle-specific carnitine palmitoyltransferase I (mCPTI), medium chain acyl-CoA dehydrogenase (MCAD), and long chain acyl-CoA dehydrogenase (LCAD) were investigated in control and hypertrophied hearts. The respective proteins affect fatty acid oxidation by regulating the level of the fatty acid oxidation inhibitor malonyl-CoA, by regulating fatty acyl-CoA entry into the mitochondrion, and by playing a direct role in mitochondrial oxidation of medium and long chain fatty acid oxidation. These genes all possessed rhythmic variations in expression, with a peak at ZT18 and a trough at ZT6 (Figure 4). This diurnal variation of fatty acid metabolism genes was severely attenuated in the hypertrophied heart, with little difference in the basal (trough) level of expression (Figure 4).

Severe Impairment in Rhythmic Expression of Mitochondrial Function Genes in the Hypertrophied Heart

Citrate synthase (CS), oxoglutarate dehydrogenase (OGDH), and uncoupling proteins 2 and 3 (UCP2 and UCP3) are key proteins involved in mitochondrial metabolism and function. Expression of the genes encoding for these four proteins all show diurnal variation, with peak expression at ZT15/18.
Figure 3. Cardiac hypertrophy impairs circadian rhythms of the carbohydrate utilization genes glut1 (A), glut4 (B), mgs (C), and pdk4 (D). Values are shown as the mean±SEM for between 6 and 8 observations in control (□) and banded (■) animals. All expression values are normalized against the housekeeping gene, β-actin. Banding significantly affected the glut4, mgs, and pdk4 expression profiles (P<0.01).

Figure 4. Attenuation of circadian rhythms of the fatty acid metabolism genes mcd (A), mcpt1 (B), mcad (C), and icad (D) in the hypertrophied heart. Values are shown as the mean±SEM for between 6 and 8 observations in control (□) and banded (■) animals. All expression values are normalized against the housekeeping gene, β-actin. Banding significantly affected mcd, mcpt1, mcad, and icad expression profiles (P<0.01).
(Figure 5). Banding severely impaired the rhythmic expression of these mitochondrial function genes (Figure 5).

Dissociation Between Plasma NEFA Levels and Cardiac ppara Expression

To investigate the potential mechanism by which several peroxisome proliferator activated receptor α (PPARα)–regulated genes undergo circadian rhythms (pdk4, med, mcp1, mcad, lcad, and ucp3), we measured the diurnal variation in plasma NEFA levels (the ligand for PPARα) and cardiac ppara expression. During the light phase, plasma NEFA levels rose steadily (Figure 6A). On the onset of the dark phase, plasma NEFA levels dropped significantly (Figure 6A). No difference in plasma NEFA levels were seen between control and aortic constriction animals (Figure 6A).

Cardiac ppara expression is initially low during the light phase, but steadily rises between ZT6 (trough) and ZT15 (peak) (Figure 6B). After ZT15, cardiac ppara expression decreased steadily (Figure 6B). Cardiac ppara expression is decreased in the hypertrophied heart, compared with control hearts (Figure 6B). In addition, the peaks of the ligand (NEFA) and receptor (PPARα) were out of phase by 3 hours (Figure 6).

Increased Total GLUT4 Protein in the Heart During the Dark Phase

Total GLUT4 protein content was determined in hearts isolated either in the middle of the light phase (ZT6) or the middle of the dark phase (ZT18). Consistent with the expression of glut4, total GLUT4 protein content was greater (1.4-fold) in hearts isolated at ZT18 compared with hearts isolated at ZT6 (Figure 7).

Discussion

We investigated the diurnal variation in the intrinsic properties of the heart, at the levels of cardiac function, metabolism, and gene expression. Perhaps the most important finding is that in the working heart preparation, which is devoid of neuronal and humoral influences, diurnal variations in cardiac performance and metabolic flux persist. Intrinsic cardiac power, oxygen consumption, and carbohydrate oxidation peaked in the middle of the dark phase (ZT18), with little variation in fatty acid oxidation. Equally important is the finding that all the metabolic genes investigated showed diurnal variation in expression, with a general induction of metabolic genes during the dark phase (peak at ZT15/18). Diurnal variation of metabolic gene expression was completely abolished in the pressure overload–induced hypertrophied heart. These results show that the intrinsic properties of the heart show diurnal variation to anticipate, respond, and adapt to physiological alterations within the environment; a trait that is lost by the hypertrophied heart. We speculate that loss of plasticity of the hypertrophied heart may play a role in the subsequent development of contractile dysfunction.

Diurnal Variation in Cardiac Performance and Metabolism

We investigated diurnal variation in cardiac performance and metabolism. In order to avoid external influences affecting

Figure 5. Severe impairment in rhythmic expression of the mitochondrial function genes cs (A), ogdh (B), ucp2 (C), and ucp3 (D) in the hypertrophied heart. Values are shown as the mean±SEM for between 6 and 8 observations in control (○) and banded (●) animals. All expression values are normalized against the housekeeping gene, β-actin. Banding significantly affected cs, ogdh, and ucp3 expression profiles (P<0.01).
these parameters, the isolated working heart preparation was used, allowing investigation into the intrinsic properties of the heart. Hearts were isolated at either ZT6 or ZT18, in the middle of the light and dark phases, respectively. All hearts were perfused under the same physiological conditions, with both carbohydrates (glucose, lactate, and pyruvate) and fatty acid (oleate) as fuels. The results were dramatic. Hearts perfused at ZT18 possessed significantly higher cardiac output, oxygen consumption, and carbohydrate oxidation, compared with hearts perfused at ZT6 (Figure 1). No difference was observed in the rates of oleate oxidation at these two time points (Figure 1).

Several of the alterations in cardiac metabolism observed in the perfused heart can be explained by diurnal variation in metabolic genes. In doing so, one assumes that the protein turnover rates for these individual metabolic proteins are sufficient for the changes in gene expression to translate into similar changes in protein content. Measurement of protein turnover rates was beyond the scope of the present study. Hearts perfused at ZT18 possessed significantly higher cardiac output, oxygen consumption, and carbohydrate oxidation, compared with hearts perfused at ZT6 (Figure 1). No difference was observed in the rates of oleate oxidation at these two time points (Figure 1).

In contrast to carbohydrate oxidation, oleate oxidation was not different at the 2 time points, despite increased expression of key regulators of fatty acid metabolism (Figures 1C and 4). This appears to be due to the preferential increase in carbohydrate oxidation, as opposed to fatty acid oxidation, under our perfusion conditions. A possible explanation is that due to the increased glucose influx into the cardiomyocyte, malonyl-CoA levels are maintained at a steady state level even in the presence of increased MCD expression, thereby maintaining a constant rate of fatty acyl-CoA entry into the mitochondrion. However, the increased fatty acid oxidation capacity may be used by the heart during increased workload or prolongation of the fasting period as the rat continues to forage for food (see Potential Mechanisms of Cardiac Circadian Rhythms section).

Hearts perfused at ZT18 also showed increased contractile performance (Figure 1A). This is in agreement with previous in vivo studies that have shown that cardiac output is greater in the dark phase for rats. However, such studies did not investigate the intrinsic properties of the heart alone, where contractile performance in vivo was undoubtedly affected by addition, increased expression of the key Krebs cycle enzymes citrate synthase and oxoglutarate dehydrogenase will promote increased oxidative metabolism (as seen by increased carbohydrate oxidation and total oxygen consumption; Figures 1 and 5). Interestingly, a recent report has shown that lactate dehydrogenase A (ldha) is a clock output gene. Induction of ldha during the onset of the dark phase would promote lactate utilization by the heart.

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Figure 6. Dissociation between plasma NEFA levels (A) and cardiac pparα (B) expression. Values are shown as the mean ± SEM for between 6 and 8 observations in control (○) and banded (●) animals. All expression values are normalized against the housekeeping gene, β-actin. Banding significantly affected the pparα expression profile.

Figure 7. Increased total GLUT4 protein content in hearts isolated in the middle of the dark phase. Representative Western blot for GLUT4 protein in hearts isolated either at ZT6 or ZT18 (A). B, Mean ± SEM for 4 different observations at each time point. Densitometry units are arbitrary units (AU). *P < 0.05 vs ZT6.
external factors, such as sympathetic tone, in addition to the intrinsic sensitivity of the heart to its environment.

Metabolism and function are closely linked in the heart. Increased function will require increased metabolic flux, whereas diminished metabolic activity will decrease the capacity of the heart to work. From the present study, it is not certain which increased first, metabolism or function. Indeed, both metabolic genes (Figures 3 through 5) and contractile protein genes (mhc; Figure 2B) show diurnal variation.

Hearts perfused during the dark phase (ZT18) showed both increased cardiac power and oxygen consumption. However, the increase in contractile function was greater than the increase in oxygen consumption, suggesting increased efficiency of the heart. At first this observation might seem counterintuitive in light of increased UCP2 and UCP3 expression at ZT18 (Figure 5), which are believed to uncouple oxidative metabolism from ATP generation. However, uncoupling proteins may have functions other than simply decreasing ATP synthesis. Uncoupling proteins prevent reactive oxygen intermediate generation during periods of increased oxidative metabolism, as seen at ZT18 for the heart. The apparent increase in efficiency could arise from increased glucose utilization (more ATP generated per O2 consumed compared with fatty acid oxidation) or from increased MHCβ expression (a more efficient MHC isoform).

Potential Mechanisms of Cardiac Circadian Rhythms

The diurnal variation in metabolic gene expression described in the present study could have arisen through (1) circadian rhythms in external factors such as neurohumoral influences (ie, sympathetic activity, circulating hormones, fuel availability); (2) internal clocks that affect the expression of various transcription factors (clock output genes); or (3) a combination of (1) and (2). Distinguishing between these possibilities for all the genes investigated is beyond the scope of the present study; however, we can speculate on the potential mechanisms in broad terms.

Various neurohumoral factors have been shown to undergo circadian rhythms. These include sympathetic activity, circulating insulin, thyroid hormone, and corticosteroids levels, as well as circulating fuels such as glucose, fatty acids, and ketone bodies. All of these factors have the potential to affect cardiac function, metabolism, and gene expression to varying degrees. For example, thyroid hormone can affect the expression of various metabolic genes, allowing the heart to adapt to fatty acid utilization while the animal continues its search for food. As described above, the dissociation between ligand and receptor availability prevents the induction of fatty acid metabolizing genes during the light phase. If we now consider the scenario in which the active animal is unsuccessfully foraging for food, plasma fatty acid levels will continue to rise (in the absence of insulin secretion). At this point, both ligand (fatty acids) and receptor (PPARα) will be high, resulting in a marked induction of fatty acid metabolizing genes, allowing the heart to adapt to fatty acid utilization. Glucocorticoids also affect the rhythmicity of intracellular circadian clocks.

Circadian clocks are believed to provide the selective advantage of anticipation, thereby allowing the cell to respond maximally to a stimulus at the appropriate moment in time. Our results suggest that this role of clocks for anticipation is also true for the heart. We hypothesize that intracellular clocks may allow anticipation of not only increased activity on the onset of the dark period but also the anticipation of prolongation of fasting (as opposed to feeding), when the animal in the wild is unable to successfully forage for food. As described above, the dissociation between ligand and receptor availability prevents the induction of fatty acid metabolizing genes during the light phase. If we now consider the scenario in which the active animal is unsuccessfully foraging for food, plasma fatty acid levels will continue to rise (in the absence of insulin secretion). At this point, both ligand (fatty acids) and receptor (PPARα) will be high, resulting in a marked induction of fatty acid metabolizing genes, allowing the heart to adapt to fatty acid utilization while the animal continues its search for food. The advantage of metabolic circadian rhythms is, therefore, somewhat lost in the laboratory rat, which generally has free access to food. If this hypothesis is true, it would suggest that PPARα, its heterodimerization partner RXR, and/or its coactivators might be under the control of the intracellular circadian clock, which has recently been characterized fully in the heart.

Impaired Metabolic Diurnal Variations in the Hypertrophied Heart

The present study has found that diurnal variations in metabolic gene expression in the hypertrophied heart are severely attenuated, and in most cases, completely abolished (Figure 3 through 5). Seven weeks of mild ascending aortic constriction (as used for the development of hypertrophy in the present study) results in adaptation of the heart, allowing maintenance of cardiac output. Because there were no signs of heart failure in the present study, we speculate that the present intervention will have only affected the heart, with minimal effects on extracardiac tissues. This is exemplified by plasma NEFA levels, which are identical in both control and banded animals (Figure 6A). We therefore assume that the loss of metabolic circadian rhythms in the pressure overloaded heart is primarily due to alterations in the intrinsic properties of the heart, as opposed to altered humoral influences. This is consistent with a recent study, in which we have shown that there is attenuation of the circadian rhythms of clock output genes in the hypertrophied heart. It is tempting to speculate that cardiac metabolic genes are under either direct (eg, ldha) or indirect (eg, ucp3) control by the clock. The attenuation of
PPARα expression in the hypertrophied heart, as well as decreased DNA binding activity and downregulation of coactivators (eg, PGC-1), would then account for diminished diurnal variation in the PPARα regulated genes (eg, ucp3).\textsuperscript{35}

**Clinical Implications**

Several observations indisputably link biological clocks with the development of heart disease. Individuals that regularly work night shifts (and therefore regularly impede the synchrony of environment fluctuations with internal clocks) have been shown to have increased incidence of heart disease compared with their dayshift coworkers.\textsuperscript{36–38} In addition, it has been known for some time that the onset of heart failure, myocardial infarction, and sudden death is greatest in the early hours of the morning.\textsuperscript{5–7} These observations have been explained purely on the basis of alterations in external factors, mainly sympathetic tone, and have ignored the second half of the stimulus-response coupling system, namely the sensitivity of the system for response to the stimulus.\textsuperscript{5,39}

We have found that the intrinsic properties of the heart differ at different times in the day, at the levels of gene expression, metabolism, and function. Furthermore, such diurnal variations, at least at the level of gene expression, are abolished in the hypertrophied heart. We speculate that this rigidity of the hypertrophied heart may lead to heart failure, and we offer the following hypothesis. In terms of gene expression, the normal and hypertrophied hearts are similar when the animal is at rest (the light phase for the rat, which is equivalent to the dark phase for the human). At the onset of the active phase for the animal, metabolic genes increase in the control but not in the hypertrophied heart. During this time sympathetic tone increases, resulting in increased cardiac output, which requires increased ATP turnover. However, in the case of the hypertrophied heart, the impairment in metabolic gene induction during this period of increased sympathetic tone and energy demand may result in energy starvation and failure. It is therefore easy to understand how loss of stimulus-response synchronization (by attenuation of internal clocks by pressure overload or through alterations in external factors due to lifestyle changes) might play a role in the development of organ failure.

**Conclusions**

We have shown that the intrinsic properties of the heart undergo diurnal variation. Levels of gene expression, metabolic fluxes and contractile function of the heart vary dramatically during the day. In contrast, the hypertrophied heart shows little variation, at least at the level of gene expression. Thus, over the course of the day, the normal heart anticipates, responds, and adapts to physiological alterations within its environment, a trait that is lost by the hypertrophied heart. We speculate that loss of plasticity of the hypertrophied heart plays a role in the subsequent development of contractile dysfunction.

**Acknowledgments**

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**References**


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