Load-Induced Changes In Vivo Alter Substrate Fluxes and Insulin Responsiveness of Rat Heart In Vitro

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It has been observed that opposite changes in cardiac workload result in similar changes in cardiac gene expression. In the current study, the hypothesis that altered gene expression in vivo results in altered substrate fluxes in vitro was tested. Hearts were perfused for 60 minutes with Krebs-Henseleit buffer containing glucose (5 mmol/L) and oleate (0.4 mmol/L). At 30 minutes, either insulin (1 mU/mL) or epinephrine (1 μmol/L) was added. Hearts weighed 35% less after unloading and 25% more after aortic banding. Contractile function in vitro was decreased in transplanted and unchanged in banded hearts. Epinephrine, but not insulin, increased cardiac power. Basal glucose oxidation was initially decreased and then increased by aortic banding. The stimulatory effects of insulin or epinephrine on glucose oxidation were reduced or abolished by unloading, and transiently reduced by banding. Oleate oxidation correlated with cardiac power both before and after stimulation with epinephrine, whereas glucose oxidation correlated only after stimulation. Malonyl-coenzyme A levels did not correlate with rates of fatty acid oxidation. Pyruvate dehydrogenase was not affected by banding or unloading. It was concluded (1) that atrophy and hypertrophy both decrease insulin responsiveness and shift myocardial substrate preference to glucose, consistent with a shift to a fetal pattern of energy consumption; and (2) that the isoform-specific changes that develop in vivo do not change the regulation of key metabolic enzymes when assayed in vitro.

WE RECENTLY observed that the heterotopically transplanted (unloaded) rat heart down-regulates the adult isoforms of metabolic enzymes and contractile proteins.[1] Because this pattern of gene expression resembles the one in hypertrophied hearts, we concluded that opposite forms of mechanical stress elicit similar transcriptional responses in the heart.

The aim of the present study was to determine the functional and metabolic consequences of cardiac atrophy (unloaded heart) and of cardiac hypertrophy (pressure-overloaded heart) in an in vitro isolated working heart preparation. Because the transcriptional analyses showed down-regulation of the insulin-stimulated glucose transporter isoform GLUT4 and of the muscle isoform of carnitine palmitoyltransferase I (CPTI) in both states, we tested the hypothesis that the 2 states (atrophy and hypertrophy) also result in similar switches in substrate oxidation from glucose and fatty acids. Furthermore, we were interested in whether the transcriptional changes we observed previously in this model[1] and changes in substrate oxidation would be associated with changes in the activity of pyruvate dehydrogenase complex (PDC). PDC is a key regulatory enzyme for carbohydrate oxidation. We were also interested in levels of malonyl-coenzyme A (CoA), a key regulator of fatty acid oxidation (inhibitor of CPTI) to discriminate effects related to changes in enzyme expression as opposed to short-term allosteric regulation (CPTI) or short-term regulation by phosphorylation (PDC).

We set out to determine whether specific adaptations in substrate oxidation are triggered by mechanical workload in vivo. To test the hypothesis, hearts subjected to unloading or pressure overload in vivo were perfused in a working mode in vitro for determination of rates of glucose and fatty acid oxidation under working conditions. We speculate that the changes observed in this study are the consequence of a physiologic process of adaptation, which ranges from altered gene expression to altered flux through metabolic pathways.

MATERIALS AND METHODS

Animals

Male Wistar-Furth rats (Harlan, Indianapolis, IN) had free access to food and water. The use of animals and the surgical and experimental protocols were approved by the Animal Welfare Committee of the University of Texas-Houston Health Science Center.

Materials

Chemicals, enzymes, and cofactors were obtained from Fisher Scientific (Lexington, MA), Sigma (St Louis, MO), or Boehringer Mannheim (Indianapolis, IN). Regular human insulin (Humulin R) was from Eli Lilly and Co (Indianapolis, IN). [9,10-^{3}H]oleate and [U-^{14}C]glucose were obtained from Amersham (Arlington Heights, IL).

Surgical Procedures

Infrarenal transplantation of the hearts was performed by a modification of the technique by Ono and Lindsey[2] as previously described.[1] Sham operations were not required for the transplantations because the

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recipients' hearts served as controls. The mortality of this procedure was 20%. Banding of the aortic arch was performed by a modification of the technique of Anderson et al[3] as previously described.[1] The surgical mortality of the procedure was less than 5%.

Simultaneous Heart Perfusions

The preparation has been described earlier,[4] including a modification for the collection of $^{14}CO_2$.[5] Hearts were perfused as working hearts at 37°C with recirculating Krebs-Henseleit buffer (200 mL) containing glucose (5 mmol/L) and Na-oleate (0.4 mmol/L) bound to 1% bovine serum albumin (Intergen Company, Purchase, NY) and equilibrated with 95% O_2, 5% CO_2. Perfusate [Ca^{2+}] was 2.5 mmol/L. The transplanted hearts were perfused simultaneously with the hearts of the recipients. The preload was 15 cm H_2O, and the afterload was 100 cm H_2O. Aortic flow, coronary flow, heart rate, and pressures (Millar Instruments, Houston, TX) were measured online and recorded every 5 minutes as described earlier.[6]

Animal Protocol

Hearts were perfused 2 or 7 days after transplantation and 7 or 14 days after aortic banding. The hearts of sham-operated animals served as controls for the animals undergoing aortic banding. [U-^{14}C]Glucose (10 μCi) and [9,10-^{3}H]oleate (10 μCi) were added to the perfusate (200 mL) at the beginning of the experiment (beginning of working mode, t = 0 minutes). At t = 30 minutes, either insulin (1 mU/mL) or epinephrine (1 μmol/L) was added to the perfusate. At 60 minutes, hearts were freeze-clamped.

Measurement of Glucose and Oleate Oxidation

Oleate and glucose oxidation were determined by the rate of $^{3}H_2O$ production from [9,10-^{3}H]oleate or the cumulative production of $^{14}CO_2$ from [U-^{14}C]glucose.[5] The $^{3}H_2O$ in the perfusate or the sum of $^{14}CO_2$ in the perfusate and the exhaust were plotted against time. The slopes of the intervals before (5 to 30 minutes) and after (35 to 60 minutes) the addition of insulin or epinephrine were used to calculate oleate or glucose oxidation rates (μmol/min/g dry wt).

Tissue Analysis

Tissue metabolite content and enzyme activities were determined according to standard biochemical methods.[5,7,8]

Statistical Analysis

All data are presented as means ± SD. Statistical comparison was by 1-way repeated-measures analysis or 1-way analysis of variance (ANOVA) with post hoc comparison by Newman-Keuls test as appropriate.[9] Differences were considered statistically significant when $P < .05$.

RESULTS

Heart and Body Weights

Table 1 shows heart and body weights and their ratios for animals in the control group and animals subjected to aortic banding or transplantation. For reasons of clarity, results from the perfusion of hearts from sham-operated animals and of transplant recipient hearts are presented as a single control group. This is justified by the absence of any differences between these groups in any of the measured parameters. Animals were matched for body weight. We chose 2 and 7 days as time points of investigation for unloading for 2 reasons. First, all changes in gene expression that occurred during a 4-week follow-up were already present after 1 day.[1] We reasoned that although 2 days may still represent a transition period, 7 days represents a new steady state. Second, explantation of the hearts for the purpose of perfusion becomes increasingly difficult because of surrounding tissue adhesions and fibrosis with increasing time. Unloading of the heart by heterotopic transplantation for 7 days caused a significant (35%) reduction in heart weight, whereas the reduction in heart weight at 2 days was not significant. We have previously shown that atrophy stabilizes at 7 days.

Table 1. Heart Weights, Body Weights, and Heart-to-Body Weight Ratios

Groups	Body Weight (g)	Heart Weight (g dry)	Heart-to-Body Weight Ratio (g dry/kg)
Control (n = 22)	274 ± 13	0.20 ± 0.03	0.73 ± 0.12
2d Tr (n = 7)	288 ± 15	0.19 ± 0.04	0.66 ± 0.10
7d Tr (n = 8)	286 ± 10	0.13 ± 0.02*	0.46 ± 0.08*
7d AoB (n = 10)	284 ± 19	0.25 ± 0.02*	0.89 ± 0.07*
14d AoB (n = 9)	301 ± 10	0.25 ± 0.03*	0.84 ± 0.09†

Values are means ± SD. Body weights were obtained before organ harvesting.

*$P < .001$ compared with controls.

†$P < .01$ compared with controls.

We also examined 2 time points during the development of hypertrophy after aortic banding (7 days and 14 days; Table 1). We chose these time points for 2 reasons. At 1 week, echocardiographic studies on banded animals suggested that the trophic changes were complete, and at 2 weeks hypertrophy had stabilized (data not shown).

Cardiac Performance

Cardiac power was 7.47 ± 2.59 mW and 8.02 ± 2.04 mW in the control groups before stimulation with insulin and epinephrine, respectively. Contractile function did not change with insulin administration in any of the groups. Figure 1 shows cardiac power before and after the addition of epinephrine in 4 of the 5 groups. Cardiac power was normalized to dry weight to account for differences in heart weight. Before stimulation with epinephrine, hypertrophic hearts showed decreased contractile function compared with controls (26% at 7 days after aortic banding, 20% at 14 days). Hearts unloaded for 1 week manifested an even greater decrease in contractile function (62%).

As expected, addition of epinephrine produced an immediate increase in heart rate and cardiac power in all groups (Fig 1). In relative terms, the immediate response (70% to 100% increase in power) was similar for all groups. With continued stimulation, only the atrophic hearts maintained increased contractile function. The other groups showed a time-dependent decline in performance. Heart rate remained stable in all groups.

Glucose and Oleate Oxidation

Figure 2 shows rates of glucose and oleate oxidation at baseline (before stimulation with insulin or epinephrine). Perfusion conditions in the first 30 minutes of the experiments were identical, and the baseline values showed no differences within groups in the epinephrine and insulin perfusions (values for the individual groups are given in Tables 2 and 3). There-

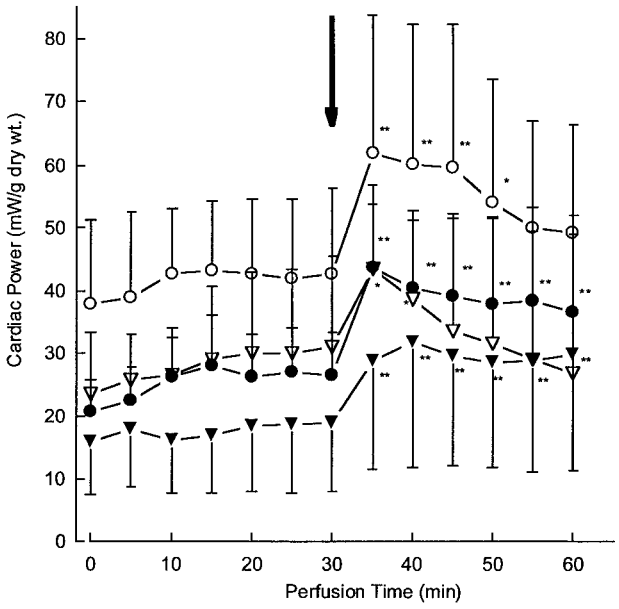

Fig 1. Cardiac power (mean ± STD) of hearts in the 4 groups stimulated with epinephrine (1 μmol/L), ie, of the control hearts (○), hearts perfused 7 (●) or 14 (▽) days after aortic banding, and hearts perfused after 7 days of unloading (▼). The arrow indicates the time point of epinephrine addition.

fore, baseline rates in Fig 2 are pooled values from insulin (n = 13) and epinephrine (n = 9) perfusions (total n = 22). In control hearts, glucose oxidation was 0.21 ± 0.12 and oleate oxidation was 1.09 ± 0.30 μmol/min/g dry wt. Thus, under baseline conditions the hearts oxidized approximately 5 times more oleate than glucose, which is similar to the substrate preference of the heart in vivo. Baseline glucose oxidation in transplanted hearts increased progressively after 2 days and after 1 week. In contrast, baseline glucose oxidation first decreased at 1 week after aortic banding, and then increased above control at 2 weeks. Figure 3 summarizes these results and shows that the ratio of glucose to oleate oxidation increases with atrophy (unloading) and in the stable phase of hypertrophy (aortic banding), ie, the reliance of these hearts on glucose as an oxidative substrate increases.

Table 2 shows rates of glucose oxidation before and after stimulation with either insulin or epinephrine. Left ventricular unloading resulted in a progressive loss of insulin responsiveness. At 7 days, the transplanted hearts were no longer responsive to insulin. Insulin responsiveness was also blunted in hearts after 7 days of aortic banding but returned to baseline by 14 days. In the control group, epinephrine increased glucose oxidation more than 8-fold. Unloading decreased the response of glucose oxidation to epinephrine, as did aortic banding for 7 days (although only in absolute terms).

Table 3 shows rates of oleate oxidation before and after insulin or epinephrine administration. Insulin addition tended to decrease and epinephrine tended to increase oleate oxidation. However, there was no change in oleate oxidation 7 days after transplantation in response to insulin, and there was a decrease 14 days after banding in response to epinephrine. Corresponding to the changes in glucose oxidation, the decrease in oleate oxidation was blunted in hearts with diminished insulin responsiveness (unloaded hearts).

Figure 4 shows glucose and oleate oxidation as a function of

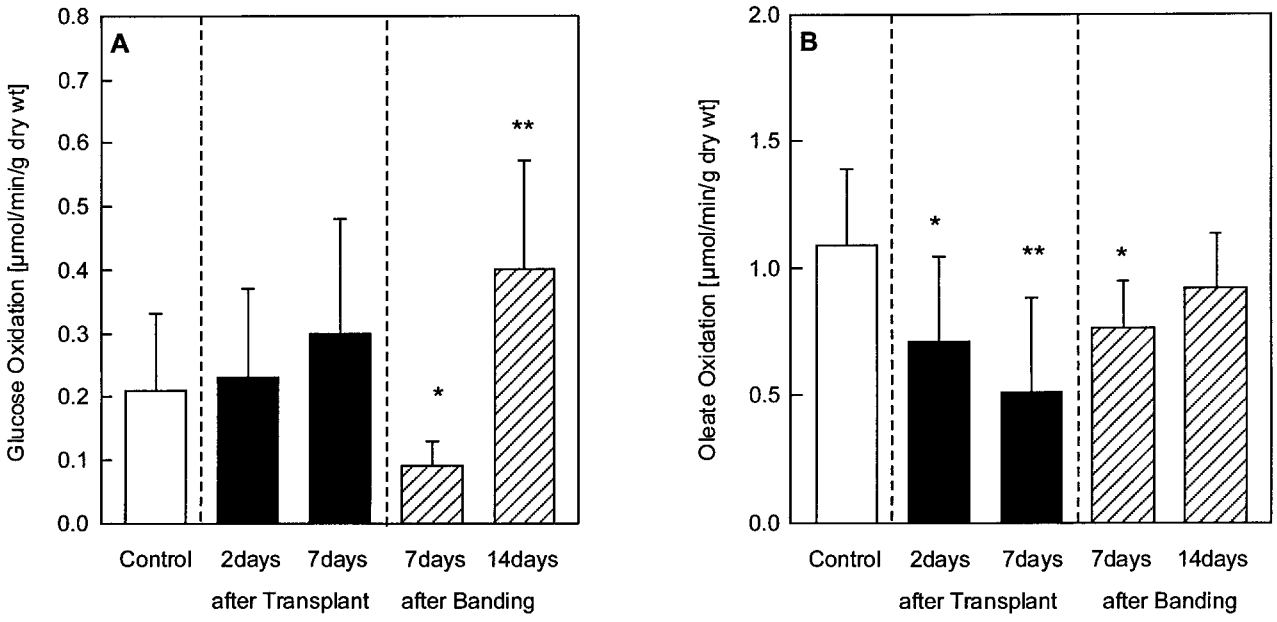

Fig 2. Basal (A) glucose and (B) oleate oxidation of isolated working rat hearts from control animals and animals that underwent heterotopic cardiac transplantation or aortic banding. Hearts were perfused at physiologic workload with Krebs-Henseleit buffer containing glucose (5 mmol/L) and oleate (0.4 mmol/L, 1% albumin). Unloaded hearts were perfused 2 or 7 days after transplantation. Hypertrophied hearts were perfused 7 or 14 days after banding of the aortic arch. Values are means ± SD; *$P < .05$, **$P < .01$ compared with control.

Table 2. Glucose Oxidation of Isolated Working Rat Hearts From Control, Transplanted, or Banded Animals Before and After Addition of Insulin or Epinephrine

Treatment Group	Glucose Oxidation (μmol/min/g dry)			
	Before Insulin	After Insulin (1 mU/mL)	Before Epinephrine	After Epinephrine (1 μmol/L)
Control	0.18 ± 0.13 (n = 13)	0.73 ± 0.24*	0.25 ± 0.09 (n = 9)	2.01 ± 0.78*
2d Tr	0.23 ± 0.14 (n = 7)	0.39 ± 0.25†	ND	ND
7d Tr	0.34 ± 0.17 (n = 4)	0.38 ± 0.21†	0.26 ± 0.20 (n = 4)	1.13 ± 0.58*,‡
7d AoB	0.09 ± 0.03* (n = 5)	0.32 ± 0.05*	0.08 ± 0.06§ (n = 5)	1.15 ± 0.27*,†
14d AoB	0.36 ± 0.19 (n = 5)	0.96 ± 0.20*	0.46 ± 0.16§ (n = 4)	2.08 ± 0.96*

NOTE. Values are means ± SD. ND, not determined; n, number of experiments in each group.
*$P < .05$ compared with before.
†$P < .001$ compared with control.
‡$P < .01$ compared with control.
§$P < .05$ compared with control.

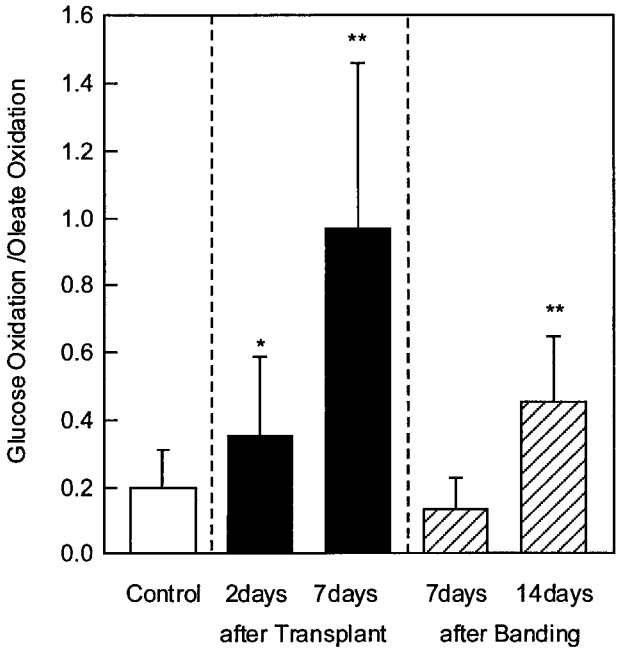

Fig 3. Ratio of basal glucose and oleate oxidation of isolated working rat hearts from control animals and animals that underwent heterotopic cardiac transplantation or aortic banding. Hearts were perfused at physiologic workload with Krebs-Henseleit buffer containing glucose (5 mmol/L) and oleate (0.4 mmol/L, 1% albumin). Unloaded hearts were perfused 2 or 7 days after transplantation. Hypertrophied hearts were perfused 7 or 14 days after banding of the aortic arch. Values are means ± SD; *$P < .05$; **$P < .01$ compared with control. The increased ratios in unloaded and hypertrophied hearts indicate a shift to glucose as energy substrate.

cardiac power before and after addition of insulin or epinephrine. There was no correlation between glucose oxidation and contractile function under baseline conditions (Fig 4A). A significant correlation existed between glucose oxidation and cardiac power after addition of either insulin or epinephrine (Fig 4B). In contrast to glucose, there was a close correlation between oleate oxidation and cardiac power both under baseline conditions and after stimulation with either insulin or epinephrine.

Malonyl-CoA and Glycogen

We measured levels of malonyl-CoA, a regulator of mitochondrial β oxidation (inhibitor of CPTI), to examine whether the changes in oleate oxidation were explainable on this basis.

Table 3. Oleate Oxidation of Isolated Working Rat Hearts From Control, Transplanted, or Banded Animals Before and After Addition of Insulin or Epinephrine

Treatment Group	Oleate Oxidation (μmol/min/g dry)			
	Before Insulin	After Insulin (1 mU/mL)	Before Epinephrine	After Epinephrine (1 μmol/L)
Control	1.06 ± 0.31 (n = 13)	0.72 ± 0.32*	1.12 ± 0.30 (n = 9)	1.21 ± 0.21
2d Tr	0.71 ± 0.33 (n = 7)	0.58 ± 0.26	ND	ND
7d Tr	0.52 ± 0.45 (n = 4)	0.59 ± 0.39	0.51 ± 0.36 (n = 4)	0.75 ± 0.50
7d AoB	0.83 ± 0.14 (n = 5)	0.69 ± 0.17	0.69 ± 0.22 (n = 5)	0.90 ± 0.24
14d AoB	0.94 ± 0.25 (n = 5)	0.59 ± 0.16*	0.90 ± 0.17 (n = 4)	0.60 ± 0.13*,†

NOTE. Values are means ± SD.
*$P < .05$ compared with before.
†$P < .05$ compared with control.

Malonyl-CoA levels in all groups ranged from 4.31 ± 2.69 to 10.25 ± 3.36 nmol/g dry wt. There was no correlation between malonyl-CoA and oleate oxidation in the range of values of β oxidation depicted in Fig 4D.

We determined the tissue content of glycogen in hearts of all groups freeze-clamped at the end of the experiments. We also determined glycogen content in unloaded and control hearts 7 days after transplantation in vivo (additional hearts, not perfused). Glycogen content in vivo did not differ between the groups (85.5 ± 11.5 μmol/g dry wt in control and 82.5 ± 10.5 μmol/g dry wt). The glycogen content in control hearts perfused in the presence of insulin was 97.7 ± 20.2 μmol/g dry wt. In all 3 groups, glycogen content increased in vitro when glucose uptake was stimulated by insulin (data not shown).

Enzyme Activities

We determined the activities of PDC, glycogen synthase, and phosphorylase before perfusion and PDC activities at the end of the perfusions. There was no difference in the total activity of PDC before perfusion (in vivo, 16.4 ± 6.00 μmol/g dry wt) or at the end of the perfusions (in vitro, 15.3 ± 4.77 μmol/min/g dry wt). Before perfusion, 9.92% ± 4.05% and 12.05% ± 8.36% of the enzyme was in the active form in the control and transplanted hearts, respectively. Table 4 shows the activity states of PDC of perfused hearts after stimulation of the iso-

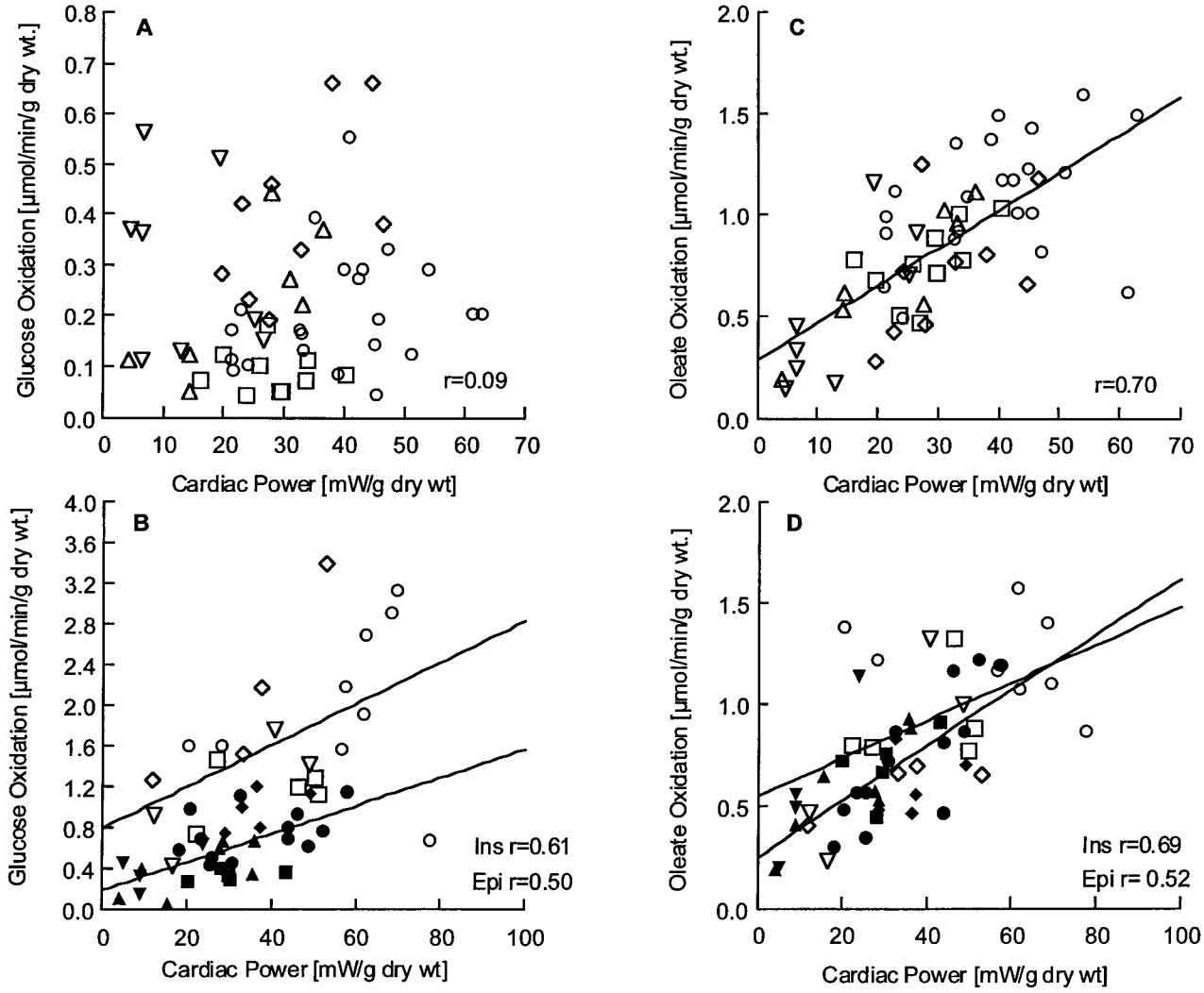

Fig 4. Glucose oxidation (A) before and (B) after insulin (filled symbols) or epinephrine (hollow symbols) administration and oleate oxidation (C) before and (D) after insulin (filled symbols) or epinephrine (hollow symbols) administration as a function of cardiac power in all hearts, ie, unloaded (2 days, △; 7 days, ▽), hypertrophied (7 days, □; 14 days, ◇), and controls (○).

lated hearts with insulin or epinephrine. There was no difference in total PDC activity among groups. After stimulation with insulin, 20% of PDC was in the active, dephosphorylated form (compared with 9.92% before perfusion). Stimulation with epinephrine resulted in a significantly greater increase in activity (46% active). Neither aortic banding nor unloading by transplantation significantly changed the insulin- or epinephrine-stimulated activity state of PDC. There was no difference in total PDC activity among groups at the end of the perfusion.

The enzymes of glycogen turnover (synthase and phosphorylase) were measured in control and transplanted hearts 7 days after transplantation. Compared with the native hearts, glycogen synthase activity was down-regulated in the transplanted heart (5.31 ± 0.49 *v* 3.43 ± 1.05 μmol/min/g dry wt; n = 8; $P < .01$) and inactivated by phosphorylation (40% decrease in the I/D ratio). Consequently, the active form of glycogen synthase was markedly decreased (0.50 ± 0.19 *v* 0.20 ± 0.19 μmol/min/g dry wt; $P < .01$.). Similarly, total phosphorylase was down-regulated by 26% (160 ± 19.4 *v* 118 ± 52.4 μmol/min/g dry wt; $P < .05$) and inactivated by dephosphorylation (41% decrease in the ratio of phosphorylase α to total phosphorylase). Because the activities of glycogen synthase and phosphorylase changed in parallel, glycogen content was not affected (see above).

DISCUSSION

We have previously shown that mechanical unloading of the heart in vivo results in profound transcriptional changes of metabolic genes.[1] We now show that these transcriptional changes in vivo result in dynamic changes of substrate metabolism in vitro. Parallel to the down-regulation of GLUT4 expression in vivo, insulin responsiveness is abolished in the unloaded heart. In the hypertrophied hearts, insulin responsiveness is only temporarily blunted. Despite the profound changes in substrate oxidation in both the unloaded and the hypertro-

Table 4. Activity State of PDC of Isolated Working Rat Hearts From Control, Transplanted, or Banded Animals 30 Minutes After Addition of Insulin or Epinephrine

Group	PDC After Insulin (% active)	PDC After Epinephrine (% active)
Control	19.7 ± 9.4*	46.3 ± 16.8
2d Tr	15.6 ± 15.4	ND
7d Tr	25.6 ± 15.7†	52.9 ± 9.4
7d AoB	9.7 ± 6.8*	41.2 ± 5.5
14d AoB	28.9 ± 18.7†	60.4 ± 6.7

NOTE. Values are means ± SD; n = 4–13 per group. Total PDC activity was 15.3 ± 4.8 μmol/min/g dry wt and was the same in all groups.

*$P < .01$ *v* PDC after epinephrine.

†$P < .05$ *v* PDC after epinephrine.

phied hearts, the hearts retained sensitivity to stimulation of contractile function with epinephrine. We suggest that the described changes are part of a physiologic program of adaptation to changes in cardiac workload. Because there are several differences between this experimental study and the in vivo situation, a critique of the experimental models and their clinical relevance is in order.

Experimental Models

The induction of hypertrophy by aortic banding and the induction of atrophy by heterotopic transplantation are accepted animal models for the investigation of myocardial hypertrophy and unloading, respectively.[1,3]

The isolated working rat heart model permits simultaneous, quantitative measurements of cardiac work and glucose as well as oleate oxidation. Although workload in vivo varies consistently between unloaded and overloaded hearts, the workload in our experiments was fixed at 100 cm H_2O. Thus, under standard conditions in the isolated working heart model, rates of substrate oxidation and contractile function are unlikely to be accurate reflections of the conditions in vivo. However, the measurement of myocardial substrate fluxes in vivo is hampered by the absence of reliable quantitative techniques.[10,11] This technical limitation may also explain the lower cardiac efficiency (ie, power generated per amount of substrate oxidized) in the unloaded hearts than in controls. However, decreased efficiency may also be a feature of the unloaded state. In vivo studies are warranted to investigate this possibility.

Atrophy Versus Hypertrophy

A significant decrease in cardiac mass occurs with unloading, and a significant increase in cardiac mass occurs with aortic banding. The degrees of atrophy and hypertrophy are consistent with those found by others.[3,12,13] The present model of hypertrophy differs from other models with respect to the time interval between intervention and investigation of its effects. Although other investigators performed aortic banding of the ascending or abdominal aorta in young rats 4 to 16 weeks before the assessment of metabolic parameters,[3,12,13] we induced hypertrophy in the adult rat, producing changes within 1 week of the banding procedure. Despite the much shorter time frame in our experiments compared with other studies,[3,12,13] the degree of hypertrophy was similar. The increase in cardiac mass after aortic banding was complete at 1 week, evidenced by the lack of a further increase in mass 2 weeks after banding. However, we found considerable differences in energy substrate metabolism between these 2 time points. One week after aortic banding, glucose oxidation was blunted. After 2 weeks, however, glucose oxidation was increased beyond control values. There is support in the literature for both of these observations. Some investigators observed increased glucose oxidation with hypertrophy,[14,15] and others observed a decrease.[12,16] Based on the assumption that the present model of aortic banding leads to a state of compensated hypertrophy, we suggest that the observed decrease in glucose oxidation 1 week after aortic banding reflects the transition to a newly compensated, hypertrophied state and may serve as an indicator of myocardial stress or states of noncompensation. The apparently conflicting findings of other investigators may then be explained by the relative stages of the hearts at the time of the investigation. In patients, hypertrophy is associated with insulin resistance.[17] The present results suggest that impaired glucose metabolism may be either a part of, or a consequence of, the hypertrophic process. This conclusion is supported by the recent observation of a decrease in uptake of 2-deoxyglucose in rabbit hearts in vivo during the period of transition from compensated hypertrophy to heart failure.[18] In contrast to the hypertrophic heart, increased basal glucose oxidation and insulin resistance are features of the unloaded rat heart.

It is important to identify the reasons for the discrepancy between the effects of unloading in the healthy and the diseased heart. The role of insulin sensitivity in this setting seems to be important but is not clear. McNulty et al[19] concluded from their findings of a decreased stimulation of glycogen synthase by insulin in unloaded hearts that "the performance of repetitive contractile work is necessary to maintain the myocardium maximally responsive to insulin." Here we have come to the same conclusion, based on the decrease in insulin stimulated glucose oxidation in unloaded hearts.

There is already evidence to suggest that the process of atrophy in the unloaded heart is specific and reversible.[1,20] Because the changes investigated in this study were induced in an initially normal heart, it is unlikely that the insulin resistance observed in this setting is a consequence of a disease process outside that of overload-induced hypertrophy. The same may be true for cardiac insulin resistance in patients, ie, insulin resistance may be a consequence of the change in workload. It is reasonable to expand on the hypothesis of McNulty et al[19] and suggest that normal insulin sensitivity is a feature of the normal myocardium.

Regulation of Myocardial Glucose and Oleate Oxidation

Although there is still discussion whether pyruvate dehydrogenase is a target of insulin action, the stimulatory effects of insulin on glucose oxidation in this study are clear. Our observation of decreased insulin-stimulated glucose oxidation in the unloaded hearts cannot be explained by a change in the acute regulation of activity of PDC. Both total PDC activity and the activity state of the enzyme were unchanged after 1 week of unloading. In addition, the effects of unloading on glycogen

synthase were accompanied by similar changes in glycogen phosphorylase activity, rendering glycogen content unchanged. A possible explanation for the lack of an insulin-induced increase in glucose oxidation may lie at the level of glucose transport. We did not measure glucose transport in this study, but the lack of differences in the regulation of other critical steps in glucose metabolism points in this direction. This conclusion is further supported by the observed down-regulation of GLUT4 messenger RNA (mRNA) after 1 day of unloading and thereafter.[21] The remaining insulin sensitivity after 2 days of unloading may be explained by residual GLUT4, and by GLUTl, which is partially insulin sensitive.[22] Thus far, the down-regulation of GLUT4 mRNA is the only identified change in response to unloading that can explain the observed insulin resistance. This conclusion is also consistent with the present but blunted increase in glucose oxidation by epinephrine because epinephrine seems to recruit a different pool of glucose transporters.[23,24] This issue warrants further investigation.

The insulin-induced changes in glucose oxidation in hypertrophied hearts can be interpreted 2 ways. First, there is insulin resistance after 7 days of banding because the absolute increase in glucose oxidation is smaller than in control hearts (Table 2). Second, there is no insulin resistance after 7 days of banding because insulin increases glucose oxidation 3.5-fold both in the control group and after 7 days of banding. We consider the former interpretation more likely. Total PDC activity is the same in all groups (Table 4), ie, the amount of available enzyme is the same. Thus, the potential ability to oxidize glucose in response to insulin should be the same in these groups. Because this is not the case after 7 days of banding (but it is after 14 days), we conclude that hearts after 7 days of banding are less responsive to insulin. This conclusion is further supported by the finding that total epinephrine-stimulated glucose oxidation is also decreased after 7 days of aortic banding. Because PDC activity did not differ among the groups, these results also support the conclusion that the reason for the decreased responsiveness of these hearts to insulin or epinephrine lies at a level upstream from glucose oxidation (ie, possibly glucose uptake, see above), and not in the signaling cascades of the 2 hormones.

Finally, we also showed that fatty acid oxidation, but not glucose oxidation, correlated with contractile activity. This observation suggests a correlation between up-regulation of the oxidative machinery and improved contractile performance.

CONCLUSIONS

Insulin responsiveness is abolished after transplantation, but it is only temporarily blunted after aortic banding. Despite the profound changes in substrate oxidation caused by unloading or hypertrophy, hearts retained the ability to respond to adrenergic stimulation. The blunted response of glucose oxidation is not caused by changes in the activity of PDC but may be localized at the level of glucose transport into the cell.

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