*Biochimica et Biophysica Acta* 891 (1987) 103–114
Elsevier



BBA 42518

# Substrate-dependent functional defects and altered mitochondrial respiratory capacity in hearts from guinea pigs with iron deficiency anemia

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(Received 13 June 1986)

Key words: Cytochrome; Heart function; Iron deficiency; Substrate difference; (Mitochondria)

**Iron deficiency anemia was induced by dietary means in weanling guinea pigs. A 25% higher ventricular wall mass per 100 g body mass was seen after 6 weeks of feeding. Myocardial performance was determined in isolated perfused hearts using an isovolumic Langendorff preparation. All hearts exhibited a 25% decrease in left ventricular developed pressure (LVDP) and decreased $dP/dt$ when substrate was switched from 10 mM pyruvate to 16.6 mM glucose. The glucose reduction in LVDP resulted from decreased systolic pressure, which completely reversed when hearts again metabolized pyruvate. With glucose as substrate, left ventricular developed pressure-end diastolic volume relationships were indistinguishable. However, with pyruvate, iron-deficient hearts appeared to be less responsive to the increased energy demands required by elevated diastolic volumes. Rates of state 3 respiration were 18% below control with glutamate + malate as substrate, and 38% lower with pyruvate + malate in mitochondria isolated from anemic animals. No differences in respiration were noted with succinate. Cytochrome $a + a_3$ content, cytochrome oxidase activity and total mitochondrial protein content appeared to be unchanged. In contrast, cytochromes $b$, $c + c_1$, and the flavoproteins were significantly decreased. The data suggest that iron deficiency anemia induces cardiac hypertrophy with a fixed but defective mitochondrial population, potentially placing the heart in an energetic imbalance. These differences in mitochondrial function were expressed by decreased myocardial performance when the heart metabolizes pyruvate, an exclusively aerobic substrate.**

## Introduction

It is well appreciated that iron deficiency anemia decreases the skeletal muscle activities of iron-containing oxidative enzymes [1–5] and mitochondrial respiratory activity [6–9]. The diminished capacity for cellular oxidative metabolism has been linked to an increased fatigability of skeletal muscle. For instance, severely affected rats have significantly less exercise endurance capacity than normals [7,8]. Also, contracting hindlimb muscles of iron deficient animals exhibit lower rates of oxygen utilization and fatigue more easily, even in the presence of a normal oxygen supply [10]. However, little is known about the metabolic and functional consequences of iron deficiency anemia in the myocardium of such animals.

In rat heart preparations, substrate utilization rates may vary as a function of the perfusion substrate [11]. As a result, phosphocreatine-to-creatine ratios, tissue NADH levels and citric acid cycle intermediate levels are elevated when pyru-

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vate is substituted for glucose [12]. A direct correlation between the rates of oxidative metabolism and tension development in vitro was seen in rabbit papillary muscles using different substrates, with pyruvate being favored over glucose [13,14]. Thus, in myocardial tissue there appears to be a rather fine balance between energy supply and demand. This can be expressed by changes in myocardial function due to differences in the relative flux rates of the energy-producing pathways of oxidative metabolism.

Relative cardiac hypertrophy due to the enlargement of the mitochondrial compartment has been reported in iron deficient rats [15,16], along with a diminished respiratory enzyme activity in isolated heart mitochondria [6]. However, the relationship between these mitochondrial defects and cardiac function were not defined. Presumably, the effectiveness of a substrate to support myocardial performance would be a function of its rate of oxidation, and ability to supply the ATP required for contraction. Even in the normal heart, the rates of ATP turnover are very high relative to the total pool of high-energy phosphates [17,18]. Therefore, if the mitochondrial population were defective, metabolic flux rates could be reduced, placing the heart in a functionally limited state. The experiments reported in this communication were designed to explore this possibility.

## Materials and Methods

*Dietary iron deficiency.* Chronic iron deficiency anemia was produced in female weanling guinea pigs (Hartley strain from Beckberg; Tomkins Cove, NY) by limiting the intake of dietary iron for 6 weeks, as previously described [9]. Test and control groups were each fed different modified Reid-Briggs guinea pig diets (Teklad; Madison, WI), which except for the iron content, were formulated identically. The elemental iron content of the two diets was 19.9 ppm (iron deficient) and 79.9 ppm (iron-fed). All animals were given a daily supplement of 0.5 g/l ascorbic acid in their drinking water.

Hemoglobin levels and hematocrits were measured weekly. 24-h prior to death, a final blood sample was taken and analyzed on a Coulter Model S-Plus electronic blood cell counter.

*Isolated heart preparation.* Guinea pigs, injected with sodium heparin at least 30 min prior to death, were killed by cervical dislocation. Hearts were removed immediately and suspended from a column via an aortic cannula. Coronary flow was quickly established and maintained at a perfusion pressure of 81 mmHg using an overflow system [19]. The perfusate was a modified Krebs-Henseleit bicarbonate buffer (pH 7.40) with the desired concentration of substrate and the following components: 117.4 mM NaCl, 4.7 mM KCl, 3.0 mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM $KH_2PO_4$, 0.55 mM $Na_2EDTA$ and 24.6 mM $NaHCO_3$ [20]. Perfusates were maintained at 37°C in water-jacketed reservoirs and were bubbled continually with 95% $O_2$/5% $CO_2$. In order to ensure that a temperature differential was not created, perfusates were pumped through short lines of equal length converging into a common line attached to the perfusion column. Also, both the perfusion column and the reservoir into which the hearts were immersed were water-jacketed and maintained at 37°C. Perfusates were pumped through an Ultipor blood filter (Pall Biomedical Products Corp.; Glen Cove, NY).

Immediately after cannulation, a ventricular drain was created at the apex of the left ventricle to minimize ventricular filling via Thebesian circulation. An incision was made in the left atrium, and a saline-filled latex balloon attached to P-E tubing was inserted through the mitral valve into the left ventricle and secured. The balloon was attached to a Statham P23 Db pressure transducer via a three-way stopcock. Intraventricular pressure and the differentiated output ($dP/dt$) were recorded on a Gould 2400S chart recorder. A water-jacketed reservoir containing perfusate at 37°C was raised so that the ventricles were fully immersed. The volume of the balloon was adjusted to an end-diastolic pressure of approx. 10 mm Hg. This constituted the initial reference volume used to normalize additional measurements. Generally, the hearts were allowed to beat at an intrinsic rate of 240 beats per min. However, some hearts were arrhythmic and were paced via the right ventricle using a Grass SD9 stimulator.

Hearts were perfused initially with buffer containing 10 mM pyruvate. After a 10 min stabilization period, a left ventricular developed pressure-

end diastolic volume curve was obtained by expanding the balloon volume in 0.02 ml increments. The end diastolic pressure was then returned to its initial value, and a short period of time was allowed for recovery of stable myocardial function before switching to perfusate containing 16.6 mM glucose. A 10 min stabilization period was again allowed before a second assessment of performance was obtained. When heart mitochondria were to be isolated, the hearts were immediately removed from the perfusion column and placed in cold perfusate.

*Mitochondrial isolation.* After excision of the atria, both ventricles were opened, lightly blotted to remove excess perfusate and weighed. The tissue was cut into small pieces and homogenized briefly with a Brinkman PT 10-35 tissue homogenizer in an isotonic salt medium (pH 7.40) consisting of: 120 mM KCl, 50 mM Tris, 3 mM $MgCl_2$, 1 mM EGTA, with 1% fatty-acid free bovine serum albumin and 2 mg per 15 ml (final homogenate volume) of Nagarse. Mitochondria were isolated by differential centrifugation [21], washed and suspended in 0.225 M mannitol plus 0.075 M sucrose. Aliquots of this preparation were used for subsequent assays.

*Assessment of mitochondrial functions.* Oxygen consumption was measured polarographically at 28°C in a water-jacketed Gilson oxygraph chamber, using a suspension medium consisting of 0.225 M mannitol, 0.075 M sucrose plus 20 mM Tris and 10 mM inorganic phosphate (pH 7.40) [22]. Measurements were made with a Clarke type oxygen electrode attached via an oxygen probe amplifier to a Kipp & Zonen BD41 chart recorder. State 3 and state 4 respiration rates were measured, and the ADP/O ratios along with the respiratory control ratio (state 3/state 4) (RCR) were calculated for each substrate [23]. Substrates were 7 mM glutamate + 7 mM malate; 7 mM pyruvate + 7 mM malate; and 7 mM succinate (plus 7 $\mu$M rotenone). Protein was measured using the biuret reaction [24].

Cytochrome oxidase activity was determined by a spectral assay [25,26]. The reaction mixture consisted of 0.1% bovine serum albumin, 0.01 M potassium phosphate (pH 7.4) and 0.033 mM cytochrome *c* (Sigma Type III horse heart) reduced with equimolar ascorbate. Reactions were initiated by addition of aliquots of mitochondria pre-treated with 0.2% lubrol [27] and were measured at 28°C in Gilford 2000 recording spectrophotometer. Oxidation of ferrocytochrome *c* was monitored as decreasing absorbance at 550 nm. Enzyme activity was calculated from linear regression plots and mitochondrial protein content was estimated by calculating the total cytochrome oxidase activity in lubrol-treated muscle homogenates compared to the cytochrome oxidase specific activity in the mitochondrial preparations [28].

Mitochondrial cytochromes and combined flavoproteins were measured spectrally [29,30]. Difference spectra of dithionite-reduced versus air-oxidized suspensions of mitochondria were generated on a Cary 118C spectrophotometer, and the following wavelength pairs and millimolar extinction coefficients were used to calculate the concentrations of individual components: 605–630 nm (cytochrome $a + a_3$), 16.0; 562–575 nm (cytochrome *b*), 20.0; 551–540 nm (cytochrome $c + c_1$), 19.1; 510–465 nm (flavoproteins), 11.0.

*Statistical analyses.* The significance of differences between the means of unpaired and paired data sets was determined by generating *t* statistics which test the null hypothesis $u_1 - u_2 = 0$. Probability values were read from a table of *t* distributions (two-tailed tests) [31].

## Results

*Induction of iron deficiency anemia*

Table I shows that after being on the specially formulated diets for 6 weeks, hematocrits and hemoglobin levels in the iron deficient animals were 58% and 52%, respectively, of values in the iron-fed controls. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC) were also significantly lower in the iron deficient animals compared to controls. For controls, mean body mass was $509 \pm 59$ g ($n = 5$), compared to $453 \pm 34$ g ($n = 7$) for iron deficient animals. Ventricular wall mass, both left and right, was $1.60 \pm 0.25$ g in controls and $1.78 \pm 0.20$ g in iron deficient hearts. Thus, iron deficient animals exhibited relative cardiac hypertrophy, since the ventricular mass per 100 g body mass was $0.313 \pm 0.015$ and $0.393 \pm 0.032$ in control and iron deficient animals, respectively ($P < 0.001$). These data

TABLE I

HEMATOLOGIC FINDINGS IN GUINEA PIGS AT DEATH

Hct, hematocrits; Hb, hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin content.

| | Hct (%) | Hb (g/dl) | MCV (fl) | MCHC (g/dl) |
|---|---|---|---|---|
| Control | 39.5 ± 0.9 (5) | 13.0 ± 0.4 (5) | 69.9 ± 2.8 (5) | 32.9 ± 0.8 (5) |
| Iron deficient | 22.9 ± 3.0 (6) | 6.8 ± 0.9 (7) | 52.7 ± 3.0 (7) | 30.0 ± 0.7 (6) |
| | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

show that the iron deficit in the test group was severe enough to induce measurable changes in both blood and myocardial parameters.

*Effects of substrates on heart performance*

The left ventricular functional data presented in Fig. 1 show the effects of changing perfusate substrate in an isovolumic heart from a normal guinea pig. In the upper traces (A and B), the heart was perfused initially with 10 mM pyruvate, then switched to 16.6 mM glucose. Upon representation of glucose to the heart, there was an initial rise in the left ventricular developed pressure, followed by a gradual decline to a stable level of function well below control (Fig. 1A). Returning the heart to the pyruvate perfusate elicited an initial drop in generated pressure followed by a gradual rise back to the initial pyruvate level of performance (Fig. 1C). Parallel changes in $dP/dt$ were also recorded (Fig. 1B and 1D). End-systolic and end-diastolic pressure curves measured with each substrate showed that with glucose the reduction in developed pressure resulted from a decrease in end-systolic pressure (Fig. 2), which was completely reversed upon returning to pyruvate. End-diastolic pressure appeared to be unaffected by available substrate.

At a fixed end-diastolic pressure of 8–10 mmHg, the developed pressure in iron-deficient hearts was the same as that in control hearts when perfused with glucose. However, in all hearts the elevated ventricular performance with pyruvate was noted, and the magnitudes of these changes were equivalent in iron deficient and control hearts (Table II). Within each group there was a 25% decrease in generated pressure due to a decline in end-systolic pressure upon switching from pyruvate to glucose (Table II). Therefore, under these conditions the hearts from the two groups performed in an apparently equivalent manner.

Myocardial function was evaluated under energetically more demanding conditions where tension development was increased by changing end-diastolic volume. Increasing ventricular volume ultimately results in peak developed pressure, which has been shown to correlate with maximum oxygen consumption in similar Langendorff type preparations [32]. In the present experiments, only

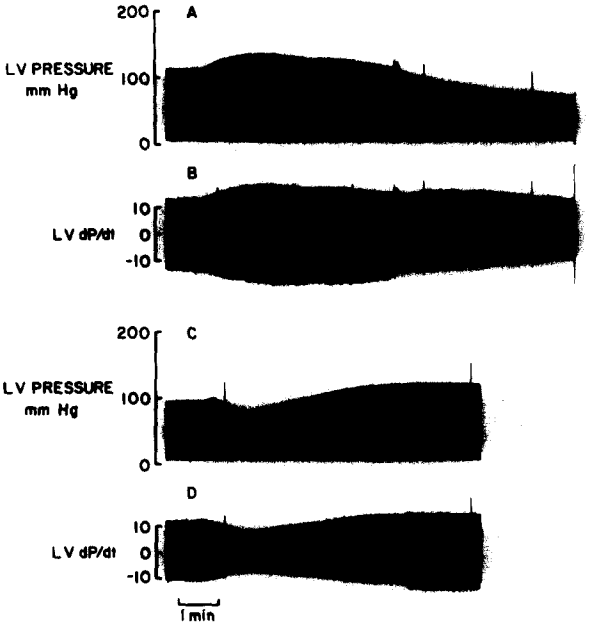



Fig. 1. Effect of changing perfusion substrates on felt ventricular (LV) developed pressure in an isovolumic control heart. The heart was perfused initially with 10 mM pyruvate. At the beginning of panels A and B the perfusate was switched to one containing 16.5 mM glucose. At the beginning of panels C and D the original perfusate containing 10 mM pyruvate was then reintroduced. The $dP/dt$ scale is relative.

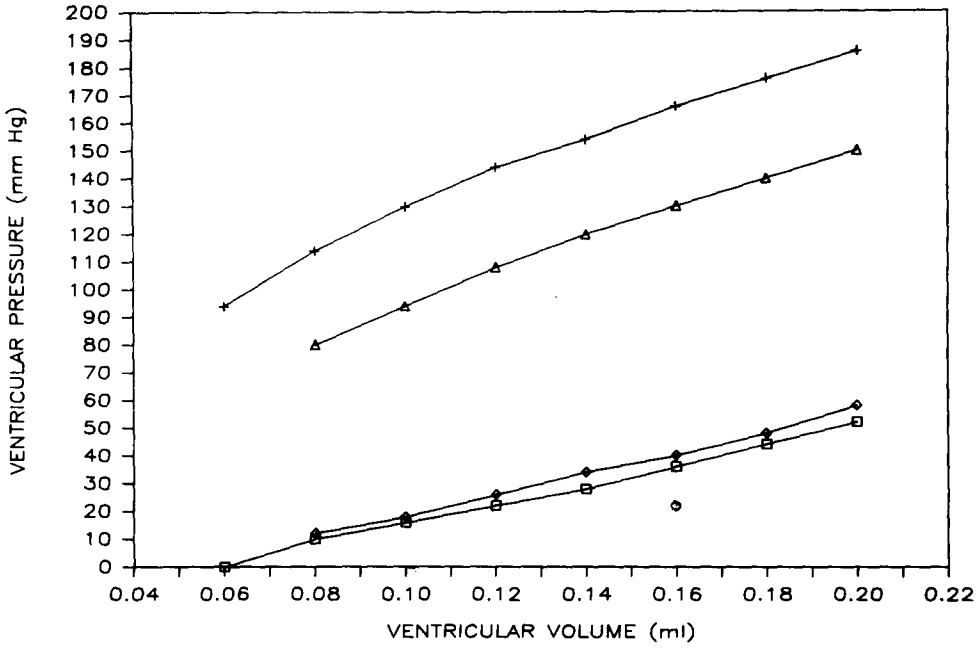



Fig. 2. End-systolic and end-diastolic pressures generated by the control heart in Fig. 1 at different ventricular volumes with perfusate containing pyruvate or glucose: pyruvate end-diastolic pressure (□), pyruvate end-systolic pressure (+) and glucose end-diastolic pressure (◇) and glucose end-systolic pressure (△).

TABLE II

LEFT VENTRICULAR PRESSURE DEVELOPMENT IN GUINEA PIG HEARTS WITH PYRUVATE AND GLUCOSE

| Heart no. | Pyruvate (mmHg) | | | Glucose (mmHg) | | |
|---|---|---|---|---|---|---|
| | Ped | Pes | P | Ped | Pes | P |
| Control group | | | | | | |
| 1 | 8 | 116 | 108 | 8 | 76 | 68 |
| 2 | 6 | 122 | 116 | 8 | 88 | 80 |
| 3 | 9 | 144 | 135 | 12 | 102 | 90 |
| 4 | 5 | 126 | 121 | 4 | 104 | 100 |
| 5 | 6 | 136 | 130 | 6 | 110 | 104 |
| Mean ± S.E. | 6.8 ± 0.7 | 129 ± 5 | 122 ± 5 | 7.6 ± 1.3 | 96 ± 6 | 88 ± 7 |
| Iron deficient group | | | | | | |
| 1 | 10 | 126 | 116 | 12 | 68 | 56 |
| 2 | 9 | 94 | 85 | 12 | 72 | 60 |
| 3 | 9 | 144 | 135 | 7 | 128 | 121 |
| 4 | 12 | 118 | 106 | 12 | 102 | 90 |
| 5 | 8 | 124 | 116 | 8 | 100 | 92 |
| 6 | 7 | 130 | 123 | 14 | 102 | 88 |
| 7 | 6 | 124 | 118 | 10 | 106 | 96 |
| Mean ± S.E. | 8.7 ± 0.7 | 123 ± 6 | 114 ± 6 | 10.7 ± 0.9 | 97 ± 8 | 86 ± 8 |

Ped, end-diastolic pressure; Pes, end-systolic pressure.

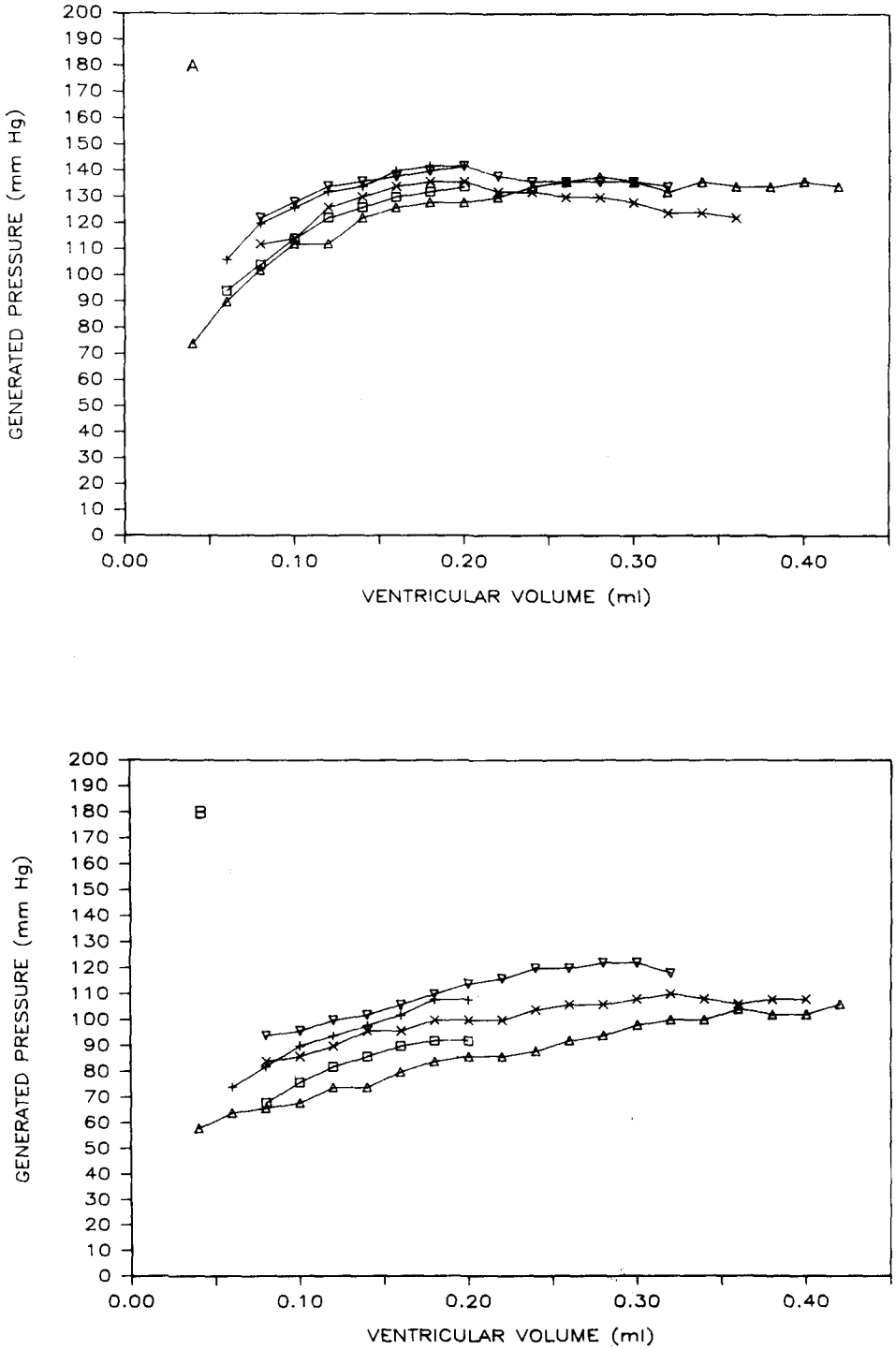



Fig. 3. Pressure-volume curves obtained from the hearts of control guinea pigs by increasing balloon volume in the left ventricle. Hearts were perfused first with pyruvate (panel A), then with glucose (panel B). The left ventricular developed pressure (end-systolic minus end-diastolic pressure) of each heart are represented by the same individual symbol in both panels.

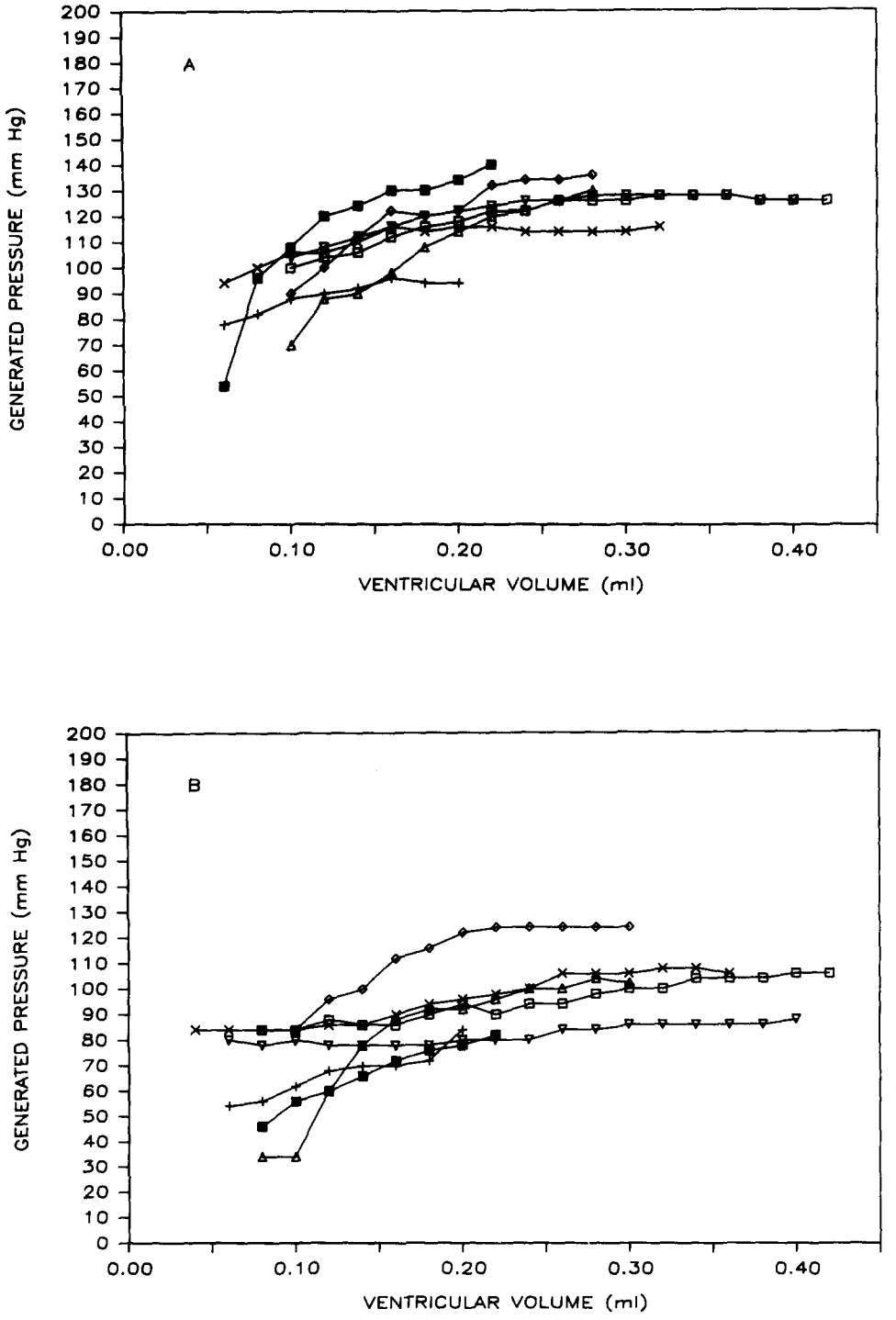
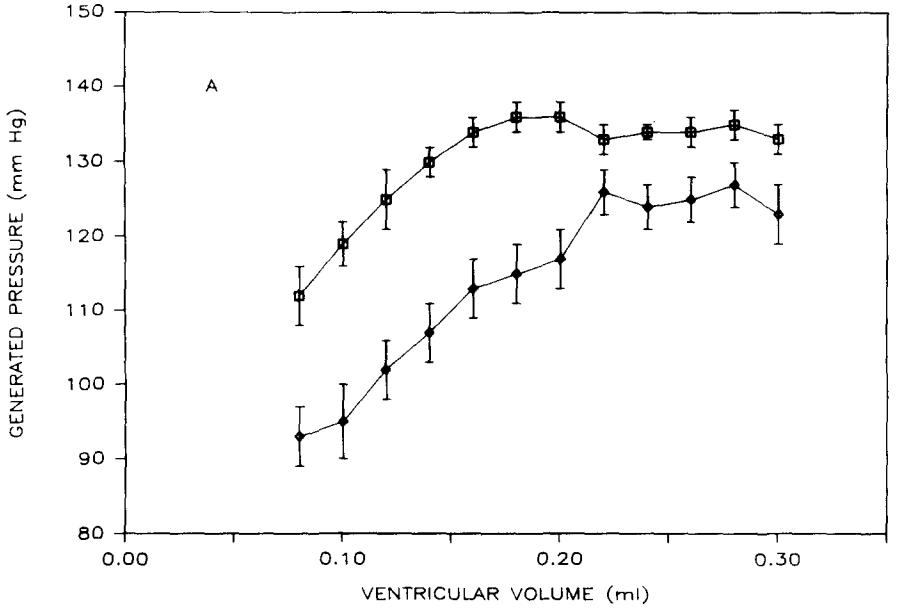



Fig. 4. Pressure-volume curves obtained from the hearts of iron-deficient guinea pigs in the same manner as those in Fig. 3. Hearts were perfused first with pyruvate (panel A), then with glucose (panel B). The left ventricular developed pressure (end-systolic minus end-diastolic pressure) of each heart are represented by the same individual symbol in both panels.



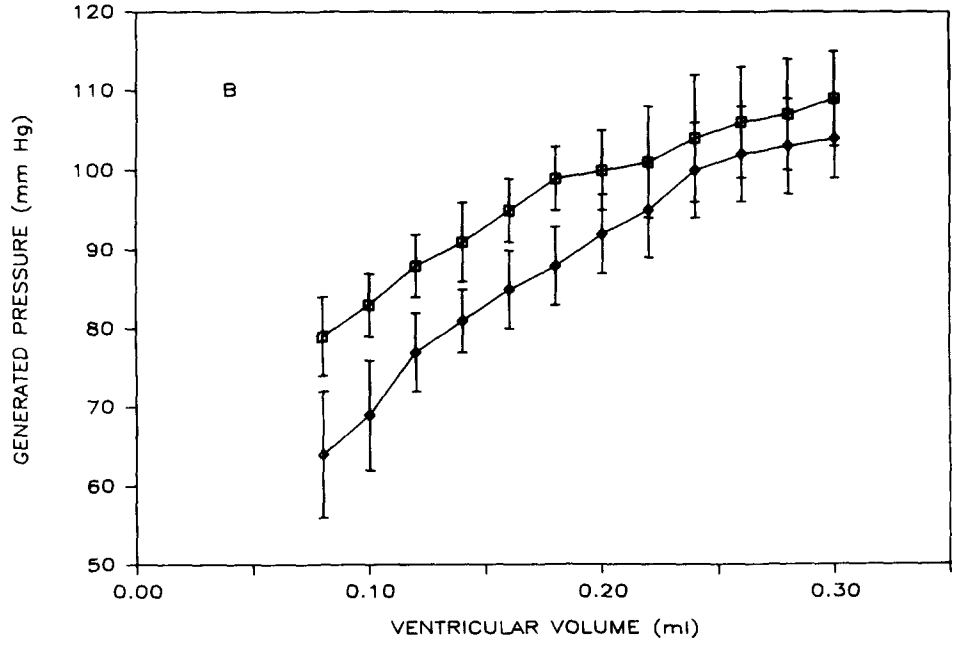




Fig. 5. Mean pressure-volume curves from the hearts of control (□) and iron-deficient (△) animals. Hearts perfused with pyruvate are represented in panel A, while those perfused with glucose are represented in panel B. Vertical bars correspond to the standard error of the mean of left ventricular developed pressure at each volume.

TABLE III

RESPIRATORY FUNCTION IN HEART MUSCLE MITOCHONDRIA

| Substrate | State 3 respiration * | | RCR | | ADP/O | |
|---|---|---|---|---|---|---|
| | control | iron deficient | control | iron deficient | control | iron deficient |
| Glutamate + malate | 144 ± 12 (5) | 118 ± 18 (7) | 8.9 ± 1.1 (5) | 7.3 ± 1.7 (7) | 2.4 ± 0.4 (5) | 2.3 ± 0.1 (7) |
| | $P < 0.025$ | | n.s. | | n.s. | |
| Pyruvate + malate | 120 ± 14 (5) | 74 ± 28 (7) | 6.8 ± 1.3 (5) | 4.3 ± 1.8 (70 | 2.6 ± 0.1 (5) | 2.5 ± 0.4 (7) |
| | $P < 0.01$ | | $P < 0.05$ | | n.s. | |
| Succinate (+ rote) | 108 ± 10 (5) | 98 ± 6 (5) | 2.3 ± 0.7 (5) | 2.4 ± 0.4 (5) | 1.1 ± 0.4 (5) | 1.3 ± 0.1 (5) |
| | n.s. | | n.s. | | n.s. | |

* ng atoms oxygen · $min^{-1}$ · $mg^{-1}$ mitochondrial protein at 28°C.

moderate increases in balloon volume were employed in order to avoid compression of the ventricle wall and limit coronary flow. Therefore, ventricle volume has never increased to the point where developed pressure began to decline. When it was felt that a significant increase in developed pressure had been achieved, the balloon volume was brought back to the initial reference volume. Because of differences among preparations in this end-point, and because of anatomical differences in ventricular cavity size among different hearts, we show the curves for each heart (Figs. 3 and 4). In Fig. 3A we see the functional characteristics of control hearts perfused with pyruvate. In curves obtained from control hearts exhibited a decrease in amplitude as well as a flattened initial response to increased energy demand (i.e., increased balloon volume) when pyruvate was replaced by glucose (Fig. 3B). Similar functional changes between pyruvate (Fig. 4A) and glucose (Fig. 4B) were seen in the hearts from iron deficient animals. To more directly compare the function of iron deficient versus control hearts for each substrate, the data for individual hearts in Figs. 3 and 4 are combined to provide the mean functional data presented in Fig. 5. With this type of analysis, we now can see significant functional differences during pyruvate perfusion (Fig. 5A). At each ventricular volume, control hearts clearly exhibit a higher developed pressure. Such marked functional differences are not observed during glucose perfusion (Fig. 5B), when the hearts are performing under conditions of a generally lower energy demand.

*Respiratory function of isolated mitochondria*

The data in Table III show that the mitochondrial fraction from the hearts of both groups were well coupled. However, mitochondria isolated from iron deficient animals exhibited a marked decrease in state 3 respiratory rates compared to controls when either glutamate plus malate or pyruvate plus malate were used as respiratory substrates. The decrease in respiratory capacity was most prominent with pyruvate. In contrast, no significant differences in respiratory rates were observed when site I was blocked with rotenone, and succinate was the substrate. Other differences were not noted in respiratory or coupling parameters. The data in Table IV show that cytochrome oxidase activity in total tissue and the specific activities in isolated mitochondria were identical in both groups of animals. Therefore, the total mitochondrial protein content appears to be

TABLE IV

CYTOCHROME OXIDASE ACTIVITY IN HEART MUSCLE MITOCHONDRIA

| | Muscle activity [a] | Mitochondrial activity [b] |
|---|---|---|
| Control | 36.8 ± 18.5 (5) | 710 ± 231 (5) |
| Iron deficient | 36.4 ± 18.8 (7) | 713 ± 195 (7) |

[a] mmol cytochrome *c* oxidized · $min^{-1}$ · $g^{-1}$ (wt wt.) muscle.
[b] nmol cytochrome *c* oxidized · $min^{-1}$ · $mg^{-1}$ mitochondrial protein.

TABLE V

RESPIRATORY CHAIN COMPONENTS IN HEART MUSCLE MITOCHONDRIA

Values are nmol·mg$^{-1}$ mitochondrial protein.

| | $a + a_3$ | $b$ | $c + c_1$ | flavoprotein |
|---|---|---|---|---|
| Control | 0.252 ± 0.032 (5) | 0.129 ± 0.024 (5) | 0.327 ± 0.040 (5) | 0.391 ± 0.043 (5) |
| Iron deficient | 0.249 ± 0.033 (7) | 0.077 ± 0.013 (7) | 0.244 ± 0.032 (7) | 0.329 ± 0.32 (7) |
| | n.s. | $P < 0.001$ | $P < 0.005$ | $P < 0.025$ |
| | $b/(a + a_3)$ | $(c + c_1)/(a + a_3)$ | $fp/(a + a_3)$ | |
| Control | 0.51 ± 0.08 (5) | 1.30 ± 0.07 (5) | 1.55 ± 0.07 (5) | |
| Iron deficient | 0.31 ± 0.05 (7) | 0.98 ± 0.10 (7) | 1.33 ± 0.11 (7) | |
| | $P < 0.001$ | $P < 0.001$ | $P < 0.005$ | |

approximately equivalent in both sets of hearts. These data are consistent with data in Table V, where mitochondrial cytochrome $a + a_3$ content was also the same in iron deficient and control mitochondria. However, significant loss of cytochromes $b$ and $c + c_1$ as well as of flavoproteins was seen in mitochodnria from iron deficient animals (Table V). Overall, these data document specific respiratory chain defects in the hearts of iron deficient animals. These contribute to observed reductions in mitochondrial respiratory rates, particularly when pyruvate is the substrate.

## Discussion

The present investigation demonstrates that moderately severe iron deficiency anemia decreases the respiratory capacity of guinea pig heart mitochondria in a manner similar to that previously observed in skeletal muscle mitochondria [9]. Diminished mitochondrial function is associated with preferential loss of electron transport chain components preceding cytochrome oxidase, which remains unchanged both in concentration and activity. The lack of statistically significant differences in respiratory rates of mitochondria from both groups of animals with succinate plus rotenone as substrate might suggest that iron sulfur proteins are affected to a greater extent than other components of the electron transport chain. Experiments with severely iron deficient rats lends support to this view [33]. The large decrease in state 3 respiratory rates in iron deficient mitochondria with pyruvate plus malate as compared to glutamate plus malate suggests additional derangements in the metabolic pathways preceding the electron transport chain. While pyruvate dehydrogenase is an important regulator of carbohydrate metabolism in the heart [34], inhibition of flux through the citric acid cycle might account for the extra decrease in state 3 rates in iron deficient mitochondria when pyruvate is the sole substrate. For example, aconitase, a key enzyme in the citrate to isocitrate, is an iron-dependent enzyme whose activity is decreased in iron deficient rats [2].

The results presented in this communication also show that iron deficiency anemia produces relative cardiac hypertrophy in the guinea pig. Resultant distortions in left ventricular chamber volumes may account for the somewhat greater scatter in performance data seen with hearts from iron deficient animals relative to controls. Our observations do not support the idea [15] that the relative increase in ventricular wall mass is due to a disproportionate increase in mitochondrial mass. Previous conclusions were based largely on morphological estimates of relative mitochondrial mass in electron micrographs of affected myocardial tissue. In contrast, the estimates of mitochondrial content presented in this report were based on direct assay of enzyme activity. It may be that diminished production of high-energy phosphate in affected hearts leads to swelling of the mitochondrial compartment [35]. If so, the relative cardiac hypertrophy in iron-deficient animals may reflect a physiological bias favoring normal cardiac growth as various organ systems compete for an

ever diminishing iron supply.

Our data suggest that moderately severe iron deficiency anemia results in a fixed, but defective mitochondrial population in the heart. Such a loss of mitochondrial respiratory capacity might lead to an energy deficit which could induce changes in myocardial function. This would be especially true under conditions of increased energy demand, as with increasing myocardial work loads or with increasing left ventricular volume. Our data show such functional defects. The potential reasons for such changes are straightforward. Calculations based on measurements of rat heart ATP turnover, mitochondrial content, and mitochondrial respiration rates suggest that during periods of maximum work load, the rate of ATP turnover approaches the theoretical upper limits of ATP production [36]. Therefore, even small changes in mitochondrial capacity to regenerate ATP could alter the balance of energy supply and compromise myocardial function.

The degree to which these changes are expressed could necessarily be substrate specific. With glucose, the flux of substrates into the citric acid cycle is limited, and mitochondrial NADH production is thus diminished [11,37]. Under these conditions, fluxes through the citric acid cycle or the electron transport chain would not be rate limiting, so that only the severest disruption of either pathway would be detected as functional changes. On the other hand, pyruvate oxidation is normally associated with much higher metabolic cycling rates through the citric acid cycle and increased rates of mitochondrial NADH production. Therefore, with pyruvate even modest changes in the enzymatic capacities of either the citric acid cycle or the mitochondrial electron transport chain would more likely be reflected by noticeable changes in myocardial function.

In the present experiments, we have shown that left ventricular developed pressure in perfused isovolumic hearts is affected by the perfusion substrate. It is unlikely that the 25% decrease in left ventricular developed pressure observed during the transition from pyruvate to glucose arises from a limitation of sarcolemmal glucose transport. Using $^{31}$P-NMR spectroscopy, we saw no changes in function or in the high-energy phosphates in isovolumic guinea pig hearts perfused with 16.6 mM glucose when insulin was added to the perfusate (W.J., unpublished observations). It has also been reported that glucose consumption by isolated working rat hearts is unaffected by insulin addition under near-physiological conditions when glucose is the only substrate [38].

We conclude that differences in the flux rates of energy-producing mitochondrial reaction pathways in the heart may be expressed as changes in myocardial function, especially when the heart is forced to metabolize an exclusively aerobic substrate. These findings have possible clinical importance in terms of what might be expected to occur when the heart is severely stressed, such as during extreme exercise or during periods of moderate ischemia. Increased heart rates during exercise attributable to iron loss alone in human subjects have been reported [39], while post-ischemic treatment of swine hearts in situ with pyruvate has been reported to significantly improve recovery [40,41]. Recent experiments utilizing $^{31}$P-NMR have demonstrated that [PCr]/[$P_i$] ratios are increased 3-fold in normal guinea pig hearts when perfusion substrate is switched from glucose to pyruvate [42]. Furthermore, the increase in [PCr]/[$P_i$] with pyruvate is maintained during a 2-fold pace-jump stress and after 30 min of global ischemia. Given that the pool of high-energy phosphates is quite small and quickly depleted in the absence of fully competent metabolic pathways, the physiological evidence presented in this report, as well as other data from the post-ischemic heart [36], suggests that any loss of energy reserve can potentially limit heart function.

## Acknowledgements

This work was supported by the following grants: HL-02799, HL-20658, and HL-33592 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD. The authors wish to express their appreciation for the excellent technical assistance provided by Mr. Koenraad M. Vandegaer and Mrs. Patricia J. Hathaway during the course of this work.

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