Acute effects of triiodothyronine on glucose and fatty acid metabolism during reperfusion of ischemic rat hearts

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Liu, Que, Alexander S. Clanachan, and Gary D. Lopaschuk. Acute effects of triiodothyronine on glucose and fatty acid metabolism during reperfusion of ischemic rat hearts. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E392–E399, 1998.—Clinical studies have demonstrated improved myocardial recovery after severe ischemia in response to acute triiodothyronine (T_3) treatment. We determined whether T_3 improves the recovery of ischemic hearts by improving energy substrate metabolism. Isolated working rat hearts were perfused with 5.5 mM glucose and 1.2 mM palmitate and were subjected to 30 min of no-flow ischemia. Glycolysis, glucose oxidation, and palmitate oxidation were measured during aerobic reperfusion by adding [5-^{3}H]glucose, [U-^{14}C]glucose, or [9,10-^{3}H]palmitate to the perfusate, respectively. During reperfusion, cardiac work in untreated hearts recovered to a lesser extent than myocardial O_2 consumption ($M\dot{V}O_2$), resulting in a decreased recovery of cardiac efficiency, which recovered to only 25% of preischemic values. Treatment of hearts with T_3 (10 nM) before ischemia increased glucose oxidation during reperfusion, which was associated with a significant increase in pyruvate dehydrogenase (PDH) activity, the rate-limiting enzyme for glucose oxidation. In contrast, T_3 had no effect on $M\dot{V}O_2$, glycolysis, or palmitate oxidation. This resulted in a significant decrease in H^+ production from glycolysis uncoupled from glucose oxidation (2.7 ± 0.3 and 1.9 ± 0.3 μmol·g dry wt^{-1} · min^{-1} in control and T_3-treated hearts, respectively, $P < 0.05$), as well as a 3.2-fold improvement in cardiac work and a 2.3-fold increase in cardiac efficiency compared with untreated postischemic hearts ($P < 0.05$). These data suggest that T_3 can exert acute effects that improve the coupling of glycolysis to glucose oxidation, thereby decreasing H^+ production and increasing cardiac efficiency as well as contractile function during reperfusion of the postischemic heart.

glycolysis; glucose oxidation; fatty acid oxidation; hydrogen production

IT IS WELL-KNOWN that thyroid hormone regulates metabolic and physiological functions in cardiac tissue. The direct effects of 3,5,3′-triiodo-L-thyronine (T_3), the biologically active form of the hormone, on cardiac cells can be nuclear or extranuclear in nature (see Ref. 8 for review). Nuclear effects are delayed in onset and are brought about by binding of thyroid hormone to nuclear thyroid hormone receptors. In vivo, hyperthyroidism can cause increases in heart rate, contractility, and cardiac output, thus raising the inotropic state of the heart, which is accompanied by a high level of energy substrate metabolism, especially glucose utilization (25, 29). Although these effects of thyroid hormone are thought to be the result of changes in myocardial gene expression, attention has recently focused on acute, nonnuclear-mediated actions of T_3 (see Ref. 16 for review).

Extranuclear effects of thyroid hormone are rapid in onset, are not altered by inhibition of protein synthesis, and are mediated by thyroid hormone binding to plasma membrane receptors (see Refs. 16 and 20 for review). Various lines of evidence have documented that T_3 can act as a vasodilator and positive inotrope in vitro (15, 16). The recognition of these effects has resulted in treatment strategies using T_3 that target specific clinical conditions associated with impaired cardiovascular performance and low serum T_3 concentration, including heart failure, cardiac surgery, and acute myocardial infarction (8, 11, 16, 35). In clinical as well as experimental trials, improved myocardial recovery in response to acute T_3 supplementation has been demonstrated after myocardial ischemia and cardiopulmonary bypass (13, 28, 35). The mechanisms responsible for the cardioprotective effects of T_3 have yet to be defined.

In most clinical situations of reperfusion after ischemia, heart muscle is exposed to high levels of fatty acids (see Ref. 21 for review). When hearts are aerobically reperfused after ischemia, glucose oxidation is suppressed because of high rates of fatty acid β-oxidation. This results in a marked imbalance between rates of glycolysis and glucose oxidation (19, 22). In severely ischemic myocardium, production of protons from the hydrolysis of glycolytically derived ATP is a major contributor to acidosis (9, 29). Clearance of H^+ via the Na^+/H^+ exchanger in aerobically perfused hearts subjected to an intracellular acid load leads to a significant decrease in cardiac efficiency (14, 20). This is due to the exchange of H^+ with extracellular Na^+ via the Na^+/H^+ exchanger. The intracellular Na^+ can then decrease the efflux of Ca^{2+} via the Na^+/Ca^{2+} exchanger during reperfusion, resulting in Ca^{2+} overload and cell death. Our previous studies have shown that reducing the source of protons by stimulation of glucose oxidation or inhibition of excessive rates of glycolysis improves cardiac efficiency (2, 10, 20, 26). As a result, modifying glucose metabolism is one potential mechanism by which T_3 could potentially exert its cardioprotective effects. Previous studies have suggested that T_3 can modify both glycolysis and glucose oxidation in the heart (3, 32). Whether the cardioprotective effects of T_3 are attributable to changes in glucose metabolism or a switch in energy substrate preference has not been determined.

In this study we determined whether acute T_3 treatment could improve mechanical function and cardiac efficiency during reperfusion of ischemic hearts by modulation of glucose metabolism. Isolated working rat hearts perfused with high levels of fatty acids were subjected to a 30-min period of global no-flow ischemia, followed by 40 min of aerobic reperfusion. The effects of

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T_3 on the recovery of cardiac work, O_2 consumption, glycolysis, and oxidative metabolism of glucose and fatty acid were measured. Our results demonstrate that by reducing the production of H^+ from glucose metabolism, T_3 significantly improves the recovery of mechanical function and cardiac efficiency in the postischemic heart.

MATERIALS AND METHODS

Heart perfusions. Rat hearts were cannulated for isolated working heart perfusions as described previously (22). Briefly, male Sprague-Dawley rats (250–300 g) were anesthetized with pentobarbital sodium (60 mg/kg ip), and hearts were quickly excised, the aorta was cannulated, and a retrograde perfusion at 37°C was initiated at a hydrostatic pressure of 60 mmHg. Hearts were trimmed of excess tissue, and the pulmonary artery and the opening to the left atrium were then cannulated. After 15 min of Langendorff perfusion, hearts were switched to the working mode by clamping the aortic inflow line from the Langendorff reservoir and opening the left atrial inflow line. The perfusate was delivered from an oxygenator into the left atrium at a constant preload pressure of 11.5 mmHg. Perfusate was ejected from spontaneously beating hearts into a compliance chamber (containing 1 ml of air) and into the aortic outflow line. The afterload was set at a hydrostatic pressure of 80 mmHg. All working hearts were perfused with Krebs-Henseleit solution containing 2.5 mM free Ca^{2+}, 5.5 mM glucose, and 1.2 mM palmitate prebound to 3% bovine serum albumin (fraction V, Boehringer Mannheim).

Spontaneously beating hearts were used in all studies. Heart rate and aortic pressure were measured with a Gould P21 pressure transducer connected to the aortic outflow line. Cardiac output and aortic flow were measured with Transonic T206 ultrasonic flow probes in the preload and afterload lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. The O_2 contents of the perfusate entering and leaving the heart were measured using Yellow Springs Instrument micro oxygen electrodes placed in the preload and pulmonary arterial lines, respectively. Myocardial O_2 consumption ($M\dot{V}O_2$) was calculated according to the Fick principle by use of coronary flow rates and the arteriovenous difference in perfusate O_2 concentration. Cardiac work was calculated as the product of systolic pressure and cardiac output. Cardiac efficiency was defined as a ratio of cardiac work to $M\dot{V}O_2$.

Experimental protocol. Working hearts were initially perfused for a 30-min period under aerobic conditions. Global no-flow ischemia was then introduced by clamping both the left atrial inflow and aortic outflow lines. After 30 min of no-flow ischemia, the left atrial and aortic flows were restored and the hearts were reperfused for a further 40-min period under aerobic conditions. T_3 (Sigma) was added at the onset of the 30-min aerobic working heart perfusion at a final concentration of 10 nM. T_3 was diluted in 1 N NaOH immediately before use, and the same amount of NaOH was added to the control group.

At the end of reperfusion, hearts were quickly frozen with Wollenberger clamps cooled to the temperature of liquid N_2. The atrial tissue was dried in an oven for 12 h at 100°C and weighed. The frozen ventricular tissue was weighed and powdered in a mortar and pestle cooled to the temperature of liquid N_2. A portion of the powdered tissue was used to determine the dry weight-to-wet weight ratio. The dried atrial weight, frozen ventricular weight, and ventricular dry weight-to-wet weight ratio were then used to determine the total dry weight of the heart.

Measurement of glycolysis, glucose oxidation, and palmitate oxidation. Glycolysis and glucose oxidation were measured simultaneously by perfusing hearts with [5-^{3}H/U-^{14}C]glucose (22). Fatty acid oxidation rates were measured with perfusate containing [1-^{14}C]palmitate (19). Total myocardial ^3H_2O production and $^{14}CO_2$ production were determined at 10-min intervals during both the initial aerobic perfusion period and the 40-min period of reperfusion. To measure the rates of glycolysis, ^3H_2O in perfusate samples was separated from [^{3}H]glucose and [^{14}C]glucose with Dowex columns (22). Fatty acid and glucose oxidation rates were determined by quantitative measurement of $^{14}CO_2$ production, including $^{14}CO_2$ released as a gas in the oxygenation chamber and $^{14}CO_2$ dissolved as HCO_3^- in perfusate. The gaseous $^{14}CO_2$ was trapped in hyamine hydroxide solution through an exhaust line in the perfusion system. The $^{14}CO_2$ dissolved as HCO_3^- was released and trapped on filter paper saturated with hyamine hydroxide in the central well of 25-ml stoppered flasks after perfusate samples had been acidified by the addition of 9 N H_2SO_4.

Measurement of pyruvate dehydrogenase activity. Frozen powdered ventricular tissue was divided into two samples (of ~30 mg/sample). One sample was used to determine the active state of pyruvate dehydrogenase (PDH_a); the other sample was used for determination of the total activity of PDH (PDH_t). Tissues were homogenized and used to measure the rate of acetyl-CoA formation from pyruvate, as described by Constantin-Teodosiu et al. (6). The acetyl-CoA was determined as [^{14}C]citrate after condensation with [^{14}C]oxalacetate by citrate synthase (6). PDH_a was measured in homogenates containing NaF and dichloroacetate, and PDH_t was measured after preincubation of homogenates with Ca^{2+}, Mg^{2+}, dichloroacetate, glucose, and hexokinase (to completely dephosphorylate PDH) (6, 7).

Calculation of H^+ production from glucose utilization. If glucose passes through glycolysis to lactate and the ATP so formed is hydrolyzed, a net production of 2 H^+ per molecule of glucose occurs (20, 22). In contrast, if glycolysis is coupled to glucose oxidation, the net production of H^+ is zero. Therefore, the overall rate of H^+ production derived from glucose utilization was determined by subtracting the rate of glucose oxidation from the rate of glycolysis and multiplying by two.

Calculation of tricarboxylic acid cycle rates. The rate of acetyl-CoA production for the tricarboxylic acid (TCA) cycle was calculated on the basis of 2 and 8 mol of acetyl-CoA being produced from glucose and palmitate oxidation, respectively (19, 20).

Statistical analysis. All data are presented as the means ± SE. Data were intially analyzed with the statistical program Instat 2.01 with the Student's *t*-test. When data sets were unevenly distributed, the Mann-Whitney and Wilcoxon nonparametric tests were used to determine the difference between preischemic and postischemic values (when used, this is indicated in the individual tables). Two-way ANOVA was used to compare the preischemic and postischemic values among groups.

RESULTS

Effects of T_3 on cardiac mechanical function of isolated working hearts subjected to 30 min of global no-flow ischemia. Figure 1*A* shows the effects of treatment with 10 nM T_3 on the recovery of cardiac work in hearts subjected to 30 min of global ischemia. After severe ischemia, the recovery of cardiac work was

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depressed in control hearts, returning to only 11% of preischemic values after 40 min of reperfusion. During reperfusion, heart rate, systolic pressure, developed pressure, cardiac output, and coronary flow were all significantly depressed compared with preischemic values. MVO_2 in control hearts recovered to a greater extent during reperfusion than did cardiac work (Fig. 1*B*), resulting in a significant decrease in cardiac efficiency throughout the entire 40-min reperfusion period (Fig. 1*C*).

When 10 nM T_3 was added at the onset of the aerobic perfusion, there was no significant effect on the mechanical function of perfused hearts under aerobic conditions (Table 1, Fig. 1). However, at the end of 40 min of reperfusion, cardiac work recovered to 35% of preischemic values in T_3-treated hearts compared with only 11% in control hearts ($P < 0.05$). Systolic pressure, developed pressure, and cardiac output also recovered to significantly greater values than in control hearts (Table 1). T_3 had no effect on MVO_2 (Fig. 1*B*), but because of the enhanced recovery of function, cardiac efficiency was increased during reperfusion compared with control (Fig. 1*C*).

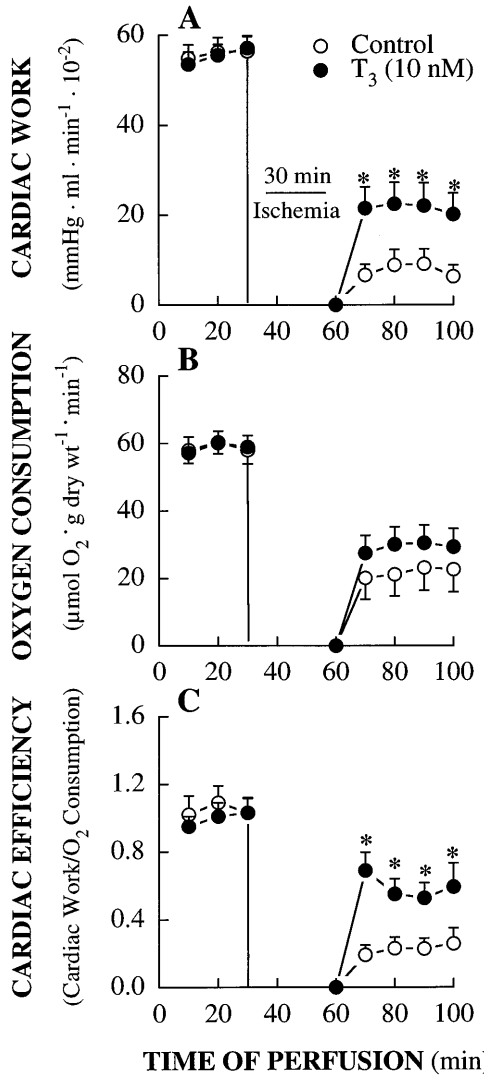

Fig. 1. Effects of 3,5,3′-triiodo-L-thyronine (T_3) on recovery of cardiac work (*A*), O_2 consumption (*B*), and cardiac efficiency (*C*) of isolated working hearts subjected to 30 min of global no-flow ischemia. Values are means ± SE of 17 control hearts (○) and 22 hearts in which T_3 (10 nM) was added at onset of aerobic perfusion (●). *Significantly different from control hearts at corresponding reperfusion time.

Table 1. *Effects of T_3 on recovery of mechanical function of postischemic working rat hearts*

	Control ($n = 17$)	T_3 Treated ($n = 22$)
Preischemic		
Heart rate, beats/min	246 ± 8	226 ± 5
Peak systolic pressure, mmHg	107 ± 3	110 ± 2
Developed pressure, mmHg	26 ± 8	29 ± 8
Cardiac output, ml/min	49 ± 3	51 ± 2
Coronary flow, ml/min	27 ± 2	26 ± 2
Postischemic		
Heart rate, beats/min	96 ± 17†	126 ± 15†
Peak systolic pressure, mmHg	30 ± 7†	59 ± 9*†
Developed pressure, mmHg	4 ± 7†	17 ± 15*†
Cardiac output, ml/min	10 ± 3†	20 ± 4*†
Coronary flow, ml/min	8 ± 3†	11 ± 2†

Values are means ± SE; *n*, no. of hearts. Hearts were subjected to 30 min of aerobic perfusion, 30 min of global no-flow ischemia, and 40 min of aerobic reperfusion. Preischemic values were taken at 30 min of aerobic perfusion. Postischemic values were taken at 40 min of reperfusion. L-3,5,3′-Triiodothyronine (T_3, 10 nM) was added at onset of aerobic perfusion. Statistical analysis of heart reperfused after ischemia involved the Mann-Whitney test. Significantly different ($P < 0.005$) from †preischemic values; *postischemic values in control hearts.

Because T_3 has vasodilatory effects in vitro, it is possible that the beneficial effects of T_3 could partly be explained by alterations in coronary flow. However, as shown in Table 1, T_3 did not increase coronary flow during either the pre- or postischemic period. Furthermore, the increase in MVO_2 was less than that in contractile function postischemia (Fig. 1), suggesting that the beneficial effect of T_3 seen in these hearts is not due to its vasorelaxant properties.

Effects of T_3 on glycolysis, glucose oxidation, and palmitate oxidation during reperfusion of hearts after ischemia. Figure 2 shows cumulative glycolysis (*A*), glucose oxidation (*B*), and palmitate oxidation (*C*). T_3 did not have any significant effects on glycolysis (Fig. 2*A*) and palmitate oxidation (Fig. 2*C*) but did result in a significant increase in glucose oxidation during the reperfusion period (Fig. 2*B*). PDH activity (the rate-limiting enzyme for glucose oxidation) was also measured in hearts frozen at the end of the reperfusion period. T_3 treatment significantly stimulated PDH_a activity without affecting PDH_t in postischemic hearts (Table 2). Although T_3 treatment stimulated glucose oxidation in the reperfusion period, no effect of T_3 on glucose oxidation was observed during the initial aerobic perfusion.

The effects of T_3 on steady-state rates of glycolysis, glucose oxidation, and palmitate oxidation are shown in Table 3. Steady-state rates were calculated for values between 10 and 30 min of the aerobic period and between 10 and 40 min of the reperfusion period (Fig. 2). In control hearts, glycolysis recovered to preischemic rates. T_3 had no significant effect on the rates of

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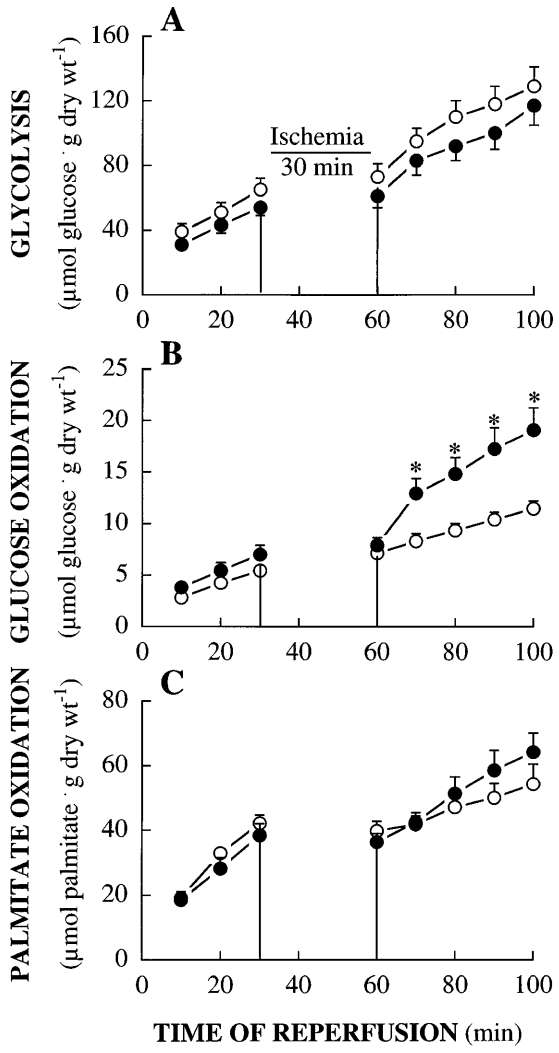

Fig. 2. Effects of T_3 on time course of glycolysis (*A*), glucose oxidation (*B*), and palmitate oxidation (*C*) in hearts reperfused after 30 min of global no-flow ischemia. Values are means ± SE. In *A*, control (○), $n = 12$; T_3 (●), $n = 16$. In *B*, control (○), $n = 13$; T_3 (●), $n = 18$. In *C*, control (○), $n = 5$; T_3 (●), $n = 7$. *Significantly different from control hearts at corresponding reperfusion time.

glycolysis during either the aerobic or reperfusion period. The steady-state rate of glucose oxidation in control hearts was substantially lower than the rate of glycolysis (Table 3). This parallels previous observations in isolated working rat hearts perfused with high levels of fatty acid (5, 13, 20, 22). During reperfusion of control hearts, glucose oxidation did not recover to preischemic rates ($P < 0.05$). Treatment with T_3 resulted in a marked increase in the rate of glucose oxidation during reperfusion compared with control hearts ($P < 0.05$).

The steady-state rates of palmitate oxidation during reperfusion of control hearts did not return to preischemic levels ($P < 0.05$). T_3 had no significant effect on palmitate oxidation rates during reperfusion compared with control hearts.

Effects of T_3 on H^+ production from glucose metabolism. Figure 3 shows the cumulative H^+ production from glucose metabolism during reperfusion of ischemic hearts, calculated from rates of glycolysis and glucose oxidation presented in Fig. 2. Over the course of the 40 min of reperfusion, more than 200 μmol/g dry wt of H^+ was produced from glucose metabolism in control hearts. A significant decrease in H^+ production was seen in T_3-treated hearts compared with control hearts.

Table 2. *Effect of T_3 on active and total pyruvate dehydrogenase activity in hearts reperfused after ischemia*

	PDH_a, nmol·g dry wt^{-1}·min^{-1}	PDH_t, nmol·g dry wt^{-1}·min^{-1}	PDH_a/PDH_t, %
Control	2,300 ± 508	9,578 ± 664	23 ± 4
T_3	3,245 ± 439*	9,585 ± 507	33 ± 4*

Values are means ± SE of 11 hearts in each group. PDH_a and PDH_t, active and total pyruvate dehydrogenase activity, respectively. Hearts were subjected to 30 min of aerobic perfusion, 30 min of global no-flow ischemia, and 40 min of aerobic reperfusion. T_3 (10 nM) was added at onset of aerobic perfusion. Statistical analysis of PDH involved the Mann-Whitney test. *Significantly different ($P < 0.05$) from control hearts.

Table 3. *Effects of T_3 on steady-state rates of glycolysis, glucose oxidation, and H^+ production from glucose utilization before and after ischemia*

	Control	T_3
Preischemic		
Glycolysis	1,270 ± 190 (9)	1,185 ± 183 (13)
Glucose oxidation	160 ± 18 (9)	142 ± 17 (13)
H^+ production	2,220 ± 100 (9)	2,086 ± 365 (13)
Palmitate oxidation	1,350 ± 220 (5)	1,140 ± 106 (7)
Postischemic		
Glycolysis	1,465 ± 153 (9)	1,163 ± 158 (13)
Glucose oxidation	95 ± 8† (9)	230 ± 29* (13)
H^+ production	2,740 ± 310 (9)	1,867 ± 311* (13)
Palmitate oxidation	680 ± 70† (5)	823 ± 100 (7)

Values are means ± SE of nos. shown in parentheses, expressed in nmol·g dry wt^{-1}·min^{-1}. T_3 (10 nM) was added at onset of aerobic perfusion. Statistical analysis of H^+ production rates involved the Mann-Whitney test. Significantly different ($P < 0.05$) from *postischemic values in control hearts; †preischemic values.

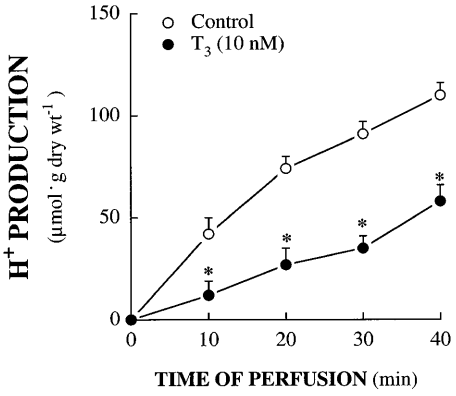

Fig. 3. Effect of T_3 on time course of H^+ production from glucose metabolism in hearts reperfused after 30 min of no-flow ischemia. Hearts were perfused with [5-^{3}H]- or [U-^{14}C]glucose for simultaneous measurement of glycolysis and glucose oxidation. H^+ production from glucose metabolism was calculated as indicated in MATERIALS AND METHODS. Values are means ± SE. Control (○), $n = 9$; T_3 (●), $n = 13$. *Significantly different from control hearts at corresponding reperfusion time.

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Steady-state H^+ production in aerobic and reperfused ischemic hearts is shown in Table 3. By selectively increasing glucose oxidation rates, T_3 improved the coupling between glycolysis and glucose oxidation, resulting in a significant decrease in H^+ production during reperfusion.

Effects of T_3 on rates of TCA cycle activity. To investigate TCA cycle activity during reperfusion, the rate of acetyl-CoA production from glucose oxidation and palmitate oxidation was calculated. As shown in Table 4, the total rate of TCA acetyl-CoA production in control hearts was significantly decreased during reperfusion compared with the preischemic value. This was consistent with the poor recovery of cardiac work. Treatment with T_3 did not alter overall acetyl-CoA production from glucose and palmitate oxidation. However, during reperfusion, T_3 increased acetyl-CoA production from glucose.

DISCUSSION

Acute treatment with physiological or supraphysiological concentrations of T_3 has been shown to have cardioprotective actions in experimental models of ischemia and reperfusion, as well as in the rescue of myocardial function after human cardiopulmonary bypass operations (13, 17, 28, 35). Our data also show that acute T_3 can significantly improve the recovery of contractile function of isolated rat hearts subjected to a severe episode of no-flow ischemia. These effects of T_3 were associated with an improvement in the coupling of glycolysis to glucose oxidation, thereby decreasing H^+ production and increasing cardiac efficiency during reperfusion of the postischemic heart.

T_3 effects on cardiac energy metabolism. Few studies have determined directly the effects of acute T_3 treatment on energy metabolism in the heart. A study by Segal (32) showed that physiological concentrations of T_3 (1 pM to 10 nM) significantly stimulate 2-deoxyglucose uptake in rat heart slices after as little as 10 min posttreatment. However, in both aerobic and postischemic hearts, we observed that T_3 treatment was not associated with any significant effects on glycolysis. Therefore, we suggest that the cardioprotective effects of T_3 are not associated with an increase in glucose uptake and metabolism by glycolysis. Possible reasons for the differences between our study and that performed in rat heart slices are that *1*) relevant levels of fatty acids were present in our perfusate and not in the rat heart slice studies, and *2*) hearts in our study were subjected to physiological workloads. Because circulating fatty acid levels are elevated both during and after clinically relevant conditions of ischemia, and because fatty acids have dramatic effects on glucose metabolism, we felt it necessary to perform our studies in the presence of high levels of fatty acids. In addition, because rates of glucose metabolism are related to workload, all experiments were performed in hearts perfused in the working mode.

Table 4. *Effects of T_3 on source of tricarboxylic acid cycle acetyl-CoA production from glucose and fatty oxidation in aerobic and postischemic hearts*

Source of Acetyl-CoA	Control	T_3 Treated
Preischemic		
Glucose oxidation	0.32 ± 0.04 (9)	0.28 ± 0.03 (13)
Palmitate oxidation	10.8 ± 1.7 (5)	9.1 ± 0.8 (7)
Total TCA cycle activity	11.1 ± 1.7	9.4 ± 0.8
Postischemic		
Glucose oxidation	0.19 ± 0.02† (9)	0.46 ± 0.06*† (13)
Palmitate oxidation	5.4 ± 0.5† (5)	6.6 ± 0.8 (7)
Total TCA cycle activity	5.6 ± 0.5	7.1 ± 0.8

Values are means ± SE of nos. shown in parentheses, expressed in μmol·g dry $wt^{-1}\cdot min^{-1}$. TCA, tricarboxylic acid. Preischemic values were determined between 10 and 30 min; postischemic values were determined between 10 and 40 min of reperfusion. T_3 (10 nM) was added at onset of aerobic perfusion. Statistical analysis of palmitate oxidation rates involved Mann-Whitney test. Significantly different ($P < 0.05$) from *postischemic values in control group; †preischemic values.

Our data show that the primary effect of T_3 on myocardial glucose metabolism is a stimulation of glucose oxidation during reperfusion. A previous study using rat cardiac myocytes also suggested that acute treatment of T_3 directly stimulates glucose oxidation (3). The effects of T_3 on glucose oxidation are unlikely to be due to a generalized increase in oxidative metabolism, because the increase in glucose oxidation seen in T_3-treated hearts was not accompanied by an increase in fatty acid oxidation. Of interest is that the effects of T_3 on glucose oxidation were observed only during reperfusion of ischemic hearts and not under aerobic preischemic conditions.

T_3 treatment significantly increased nonphosphorylated PDH_a activity in postischemic hearts. Because PDH_a plays an important role in regulation of glucose oxidation (4), our data strongly suggest that T_3 stimulates glucose oxidation and improves coupling of glycolysis to glucose oxidation secondary to a stimulation of PDH_a activity. In rat hearts, ischemia or reperfusion has previously been shown to lead to an inactivation of PDH_a under conditions similar to those used in the present study (18, 31). Ischemia is likely to increase intramitochondrial $NADH/NAD^+$ and acetyl-CoA/CoA ratios, which would lead to inactivation of PDH; however, the reduced ATP/ADP would balance this to some extent by favoring activation (18). It has been reported that acute T_3 treatment can reduce intramitochondrial ATP/ADP (33, 34), which may contribute to the observed activation of PDH_a. However, the detailed mechanism of how T_3 regulates PDH activity is still unclear. Future studies are needed to clarify whether acute T_3 treatment has any effect on PDH kinase or phosphatase, both of which also play an important role in regulating PDH activity (6, 7). PDH activity has also been shown to be stimulated by hyperthyroidism (30), although this is probably due to transcriptional regulation. Because acute treatment of T_3 is unlikely to upregulate protein synthesis, and PDH_t was not altered, the observed effects of T_3 on PDH_a in our study were likely the result of changes in the phosphorylated state of PDH.

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Recovery of contractile function, energy metabolism, and cardiac efficiency in the postischemic heart. During reperfusion of the severely ischemic control hearts, a significant decrease in the recovery of cardiac function occurred that was associated with a decrease in cardiac efficiency (Fig. 1). This decrease in cardiac efficiency has also been observed in previous studies (19, 20). However, unlike these previous studies, we did not observe a complete recovery of fatty acid oxidation in control hearts during reperfusion. This difference in the recovery of fatty acid oxidation may be related to the severity of ischemic injury observed in the present study. As shown in Fig. 1, cardiac work recovered to 11% of preischemic rates in control hearts compared with 30–40% in our previous studies (19, 20). However, it should be recognized that, despite this poor recovery of cardiac work, fatty acid oxidation in control hearts recovered to >50% of preischemic levels, resulting in a marked increase in fatty acid oxidation per unit work, a finding consistent with our previous studies. Regardless of the degree of recovery of fatty acid oxidation in this study, our data suggest that the beneficial effects of T_3 are unlikely to be due to any direct effects on fatty acid oxidation.

It is well-known that long-term hyperthyroidism is associated with high levels of $M\dot{V}O_2$. However, with acute treatment of T_3 this is not the case. A recent study by Klemperer et al. (17) showed that acute T_3 treatment improves left ventricular function in isolated rat hearts after ischemia without oxygen-wasting effects. In the present study, we also found that T_3 significantly improves the recovery of cardiac work without a concomitant increase in $M\dot{V}O_2$. Therefore, an improved cardiac efficiency (ratio of cardiac work to $M\dot{V}O_2$) was observed in the postischemic heart.

Previous studies (13) suggest that the combination of global ischemia and depletion of T_3 results in reduced mitochondrial function, inhibition of the TCA cycle, and increased anaerobic metabolism. T_3 replacement therapy leads to improved mitochondrial function and increased anaerobic metabolism. In the present study, we observed that acetyl-CoA production from glucose and fatty acid oxidation was significantly inhibited after 30 min of severe ischemia, whereas glycolysis was unaffected. Treatment with T_3 dramatically increased acetyl-CoA production from glucose oxidation, with minor effects on fatty acid oxidation (Table 4). This suggests that T_3 may directly affect mitochondrial function. Overall acetyl-CoA production was not significantly increased, which suggests that T_3 has no effects on the efficiency of energy production. Rather, T_3 improves the efficiency of energy utilization. Thyroid hormone exerts two types of effects on mitochondria (see Ref. 27 for review). Within a few minutes of administration, T_3 causes a rapid activation of respiration, an effect that is preserved in isolated mitochondria. A direct control of oxidative phosphorylation through binding of T_3 to mitochondria is thought to occur (27, 36, 37). This control of oxidative phosphorylation is observed after administration of physiological amounts of T_3 and is not altered by inhibition of protein synthesis (27, 33). This binding site has been proposed to be the adenine nucleotide translocase (36, 37), an inner mitochondrial membrane carrier that catalyzes exchange between the extra- and intramitochondrial ADP and ATP (12, 33, 38). Of interest is that shifting the mitochondrial ATP/ADP ratio is also involved in the regulation of PDH activity, thereby controlling pyruvate oxidation and consequently glucose oxidation (33, 34). However, studies identifying the adenine nucleotide translocase as a T_3 receptor (as well as the role of adenine nucleotide translocase in short-term activation of respiration) have been questioned (see Ref. 27 for review), and further studies are necessary to clarify this possibility.

In our study, the actual measured TCA cycle rates were lower than predicted TCA cycle rates calculated from measured $M\dot{V}O_2$ values shown in Fig. 1. The reasons for this discrepancy are not absolutely clear. However, in this study we measured both parameters and found that T_3 effects on TCA cycle activity (Table 4) and $M\dot{V}O_2$ during reperfusion of ischemic hearts (Fig. 1) were comparable. That is, T_3 increased TCA cycle activity during reperfusion by 27% (from 5.6 ± 0.5 to 7.1 ± 0.8 μmol·g dry $wt^{-1} \cdot min^{-1}$) and $M\dot{V}O_2$ by 30% (from 22.6 ± 6.7 to 29.4 ± 5.5 μmol·g dry $wt^{-1} \cdot min^{-1}$). This suggests that T_3 is not acting by altering mitochondrial proton leak, and it supports our hypothesis of an increased cardiac efficiency secondary to decreasing proton production from glycolysis uncoupled from glucose oxidation.

Coupling of glycolysis to glucose oxidation in the postischemic heart. The production of H^+ from glucose metabolism is an important contributor to the impaired recovery of mechanism function and to the decrease in cardiac efficiency seen after a severe ischemic episode (19, 20, 22). During reperfusion, treatment with T_3 dramatically stimulated glucose oxidation, with no effect on glycolysis. Each molecule of glucose that passes through glycolysis that is not subsequently oxidized results in the production of 2 H^+ from the hydrolysis of glycolytically derived ATP (19, 20). In the presence of high levels of fatty acids, glucose oxidation rates are 5-fold to 10-fold lower than glycolytic rates (19, 22, 23). Selective stimulation of glucose oxidation improves the coupling of glycolysis to glucose oxidation, leading to a reduction in H^+ production. We have suggested that an increase in H^+ accumulation during the critical period of reperfusion may contribute to cardiac inefficiency (21) and the well-documented Ca^{2+} overload in the postischemic heart that results from an increase in Na^+/H^+ exchange activity coupled with Na^+/Ca^{2+} exchange (14). Because T_3 reduced the H^+ production from glucose utilization during reperfusion, the driving force for the Na^+/H^+ exchange is decreased, and Na^+/Ca^{2+} exchange activity would thus be expected to be reduced during reperfusion. Decreased activity of this exchanger may be responsible for the significant improvement in cardiac efficiency observed during reperfusion.

A number of other pharmacological agents also stimulate glucose oxidation and have a beneficial effect on the

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recovery of mechanical function during reperfusion of the postischemic heart (2, 23). One of these agents is dichloroacetate, a potent PDH activator that also improves the coupling between glycolysis and glucose oxidation. This also results in a significant decrease in H^+ production from glucose metabolism during reperfusion, resulting in a significant increase in cardiac efficiency.

Summary. We demonstrate a significant improvement of recovery in postischemic cardiac function and efficiency in isolated rat hearts by use of moderately supraphysiological amounts of T_3. The cardioprotective effect of T_3 may be due to its stimulation of glucose oxidation secondary to an increase in PDH_a activity, and therefore a reduction in the production of H^+ by coupling glycolysis with glucose oxidation.

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