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Triidothyronine and epinephrine rapidly modify myocardial substrate selection: a ^{13}C isotopomer analysis

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Krueger, Julia J., Xue-Han Ning, Barisa M. Argo, Outi Hyyti, and Michael A. Portman. Triidothyronine and epinephrine rapidly modify myocardial substrate selection: a ^{13}C isotopomer analysis. *Am J Physiol Endocrinol Metab* 281: E983–E990, 2001.—Triiodothyronine (T_3) exerts direct action on myocardial oxygen consumption ($M\dot{V}O_2$), although its immediate effects on substrate metabolism have not been elucidated. The hypothesis, that T_3 regulates substrate selection and flux, was tested in isovolumic rat hearts under four conditions: control, T_3 (10 nM), epinephrine (Epi), and T_3 and Epi (TE). Hearts were perfused with [1,3-^{13}C]acetoacetic acid (AA, 0.17 mM), L-[3-^{13}C]lactic acid (LAC, 1.2 mM), U-^{13}C-labeled long-chain free fatty acids (FFA, 0.35 mM), and unlabeled D-glucose (5.5 mM) for 30 min. Fractional acetyl-CoA contribution to the tricarboxylic acid cycle (Fc) per substrate was determined using ^{13}C NMR and isotopomer analysis. Oxidative fluxes were calculated using Fc, the respiratory quotient, and $M\dot{V}O_2$. T_3 increased ($P < 0.05$) Fc_{FFA}, decreased Fc_{LAC}, and increased absolute FFA oxidation from 0.58 ± 0.03 to 0.68 ± 0.03 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1} ($P < 0.05$). Epi decreased Fc_{FFA} and Fc_{AA}, although FFA flux increased from 0.58 ± 0.03 to 0.75 ± 0.09 $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1}. T_3 moderated the change in Fc_{FFA} induced by Epi. In summary, T_3 exerts direct action on substrate pathways and enhances FFA selection and oxidation, although the Epi effect dominates at a high work state.

metabolism; mitochondria; free fatty acids; nuclear magnetic resonance

SEVERAL HORMONES MEDIATE CHANGES in substrate supply or metabolic pathways, which control reductions in equivalent delivery to the tricarboxylic acid cycle (TCA) during variations in energy utilization and demand (7, 11). This regulatory process influences cytosolic and mitochondrial NAD redox state, as well as cytosolic phosphorylation potential (19, 35). Thus hormones participate in the integration of systems that either directly or indirectly determine the free energy of ATP hydrolysis and thus the efficiency of oxygen utilization.

Modulation of myocardial substrate pathways by hormones such as insulin, glucagon, and catecholamines has been investigated in detail (7). Although thyroid hormone is often implicated as a regulator of substrate pathways, relatively few studies involving its rapid action on cardiac metabolism have been published. This is surprising, because triiodothyronine has recently received attention as a therapeutic agent for myocardial dysfunction during several clinical conditions (17, 26, 31). Thyroid hormone, 3,3′,5-triiodo-L-thyronine (T_3), modulates myocardial energy metabolism, principally through nuclear-mediated regulation of specific enzyme systems (30). These include enzymes that facilitate substrate entry into the TCA, as well as those that regulate nucleoside transport into the mitochondria (2, 8, 30). T_3 also elicits immediate and probably direct effects on cardiac metabolism through actions at the plasma or mitochondrial membrane (18, 20).

Immediate thyroid action on myocardial metabolism might be caused by T_3-promoted changes in substrate utilization or preference. Some investigators have shown that T_3 binds directly to the mitochondrial membrane and alters function of specific enzyme systems (38, 39). In particular, Sterling (38) and Sterling and Brenner (39) demonstrated immediate action by T_3 at the adenine nucleotide translocator in liver mitochondria (38, 39). Others have shown rapid T_3 action on enzymes that regulate long-chain fatty acid synthesis in liver (25, 37). These findings imply that T_3 exerts direct and immediate actions at the mitochondrial membrane that could alter and possibly enhance fatty acid metabolism. Some thyroid action has been attributed to changes in myocardial sensitivity to catecholamines, such as epinephrine (1, 4, 40). However, other studies indicate that catecholamines inhibit acute stimulatory effects of T_3 on cytosolic Ca^{2+} entry, which may play a role in activation of some substrate pathways (16).

Accordingly, this study's primary objective was to test the hypothesis that T_3 alters myocardial substrate selection rapidly and through other than nuclear-mediated mechanisms. A secondary objective was to de-

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termine whether metabolic synergism exists between thyroid hormone and epinephrine. Additionally, thyroid hormone action could change at higher rates of oxygen consumption induced by catecholamine stimulation. These experiments, performed in isolated rat hearts, used ^{13}C magnetic resonance spectroscopy with isotopomer analysis (15, 22, 23) to determine relative substrate contribution to the TCA cycle.

MATERIALS AND METHODS

Materials. Standard reagents were obtained from Sigma Chemical (St. Louis, MO). [1,3-^{13}C]acetoacetic acid (99.0 atom percent), L-[3-^{13}C]lactic acid (sodium salt), and D-[U-$^{13}C_6$]glucose were obtained from Isotec (Miamisburg, OH). Long-chain fatty acid preparations uniformly enriched with ^{13}C to 99% were obtained from Martek (Columbia, MD). The fatty acid preparation consisted of the sodium salts of the following acids: palmitic (23.2%), palmitoleic (4.4%), hexadecadienoic (10.7%), hexadecatrienoic (7.6%), oleic (12.0%), linoleic (26.2%), and linolenic (10.0%). Fatty acid-free bovine serum albumin (fraction V), 3,3′,5′-triiodo-L-thyronine (free acid), and deuterium oxide (^2H_2O) were obtained from Sigma Chemical. Animal procedures were in accordance with guidelines of the University of Washington Animal Care Committee and the National Institutes of Health.

Acetoacetate-[1, 3-^{13}C]ethyl ester (22.2 μl) was hydrolyzed in 40 ml of deionized water to acetoacetate at pH 12.0 (1 M NaOH) (13). After 2.5 h, the pH was adjusted to ~7.2 with 1 M HCl before addition to 1 liter of physiological salt solution (PSS) for perfusion.

Heart perfusion. Male Sprague-Dawley rats weighing 350–450 g were anesthetized with pentobarbital sodium (45 mg/kg ip) and heparin (700 U/kg ip). The heart was rapidly excised and immersed momentarily in ice-cold PSS (pH 7.4) containing (in mmol) 118.0 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.23 $MgSO_4$, 1.2 NaH_2PO_4, 5.5 D-glucose, and 1.2 $CaCl_2$. The aorta was cannulated in the standard Langendorff mode. The heart was perfused with PSS, which had been equilibrated with 95% O_2-5% CO_2 at 37°C and passed twice through filters with 3.0-μm pore size. Perfusion pressure was maintained at 70 mmHg. An incision was made in the left atrium, and a fluid-filled latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was connected to a pressure transducer for continuous measurement of left ventricular pressure (LVP) and its first derivative with respect to time (LV dP/dt). The caudal vena cava, the left and right cranial venae cavae, and the azygous vein were ligated. The pulmonary artery was incised and cannulated to enable collection of coronary flow, which was measured with a flowmeter (T 106, Transonic Systems, Ithaca, NY). Myocardial O_2 consumption ($M\dot{V}O_2$) was calculated from the differences in O_2 content of perfusion in the supply line and coronary effluent from the pulmonary artery, as described previously (28).

The analog signals were continuously recorded on a chart recorder (Gould, Cleveland, OH) and an on-line computer (Macintosh, Biopac Analog Signal Acquisition System). To characterize cardiac function, left ventricular developed pressure (LVDP) is defined as peak systolic pressure (PSP) minus end-diastolic pressure (EDP). $M\dot{V}O_2$ was calculated as $CF \times [(Pa_{O_2} - Pv_{O_2}) \times (c_{O_2}/760)]$, where CF is coronary flow ($ml \cdot min^{-1} \cdot g$ wet tissue^{-1}), $(Pa_{O_2} - Pv_{O_2})$ is the difference in the partial pressure of O_2 (mmHg) between perfusate and coronary effluent (determined with a Radiometer ABL5 blood gas analyzer), and c_{O_2} is the Bunsen solubility coefficient of O_2 in perfusate at 37°C (22.7 μl O_2/atm − ml perfusate). O_2 extraction was calculated as $M\dot{V}O_2$ divided by the O_2 content in the perfusate.

Perfusate protocol. Spontaneously beating hearts were used throughout the experiment. After heart isolation and preparation, a left ventricular balloon volume was defined that would provide an LVDP between 100 and 140 mmHg. This volume was maintained throughout the protocol. Hearts were excluded from statistical analyses if this volume produced EDPs >8 mmHg. All hearts were initially stabilized via perfusion for 30 min with the indicated standard PSS buffer. The perfusate was switched to PSS containing ^{13}C-labeled substrates: 0.17 mM acetoacetate-[1,3-$^{13}C_2$]ethyl ester, 1.2 mM L-[3-^{13}C]lactic acid, and 0.35 mM of U-^{13}C-labeled long-chain free fatty acids (FFA) bound to 0.75% (wt/vol) delipidated bovine serum albumin reconstituted with deionized water. The substrate concentration of this mixture conforms to physiological concentrations in rat artery, as reported by Remesy and Demigne (32). However, acetoacetate supplied the entire ketone body composition for labeling purposes. The entire perfusion system was jacketed and maintained at 37 ± 1°C.

The labeling patterns of the ^{13}C-enriched substrates were selected to quantify unambiguously the contribution of exogenous substrates to acetyl-CoA. The hearts were divided into four groups. The control group (C) was perfused for 30 min with the perfusate containing the ^{13}C-labeled substrates. The T_3 group was supplied with triiodothyronine diluted in 1 N NaOH (34) at the onset of the 30-min period of perfusion at a perfusate concentration of 10 nM. The epinephrine (Epi) group received epinephrine in the ^{13}C-labeled perfusate at a final concentration of 1 μM, also at the onset of the 30-min perfusion period. The fourth group, TE, was subject to treatment with both T_3 and Epi in the perfusate at the same concentrations as with the individual reagents. Functional and metabolic parameters were recorded every 5–10 min. After 60 min of total perfusion, nonventricular tissue was removed, and hearts were freeze-clamped with copper tongs that had been chilled in liquid nitrogen.

Extraction. One gram of the frozen tissue was ground into fine powder by use of a mortar and pestle under liquid nitrogen and transferred into four Eppendorf tubes, each containing 1 ml of 3.5% cold perchloric acid (4°C). The acid extraction was centrifuged for 25 min at 20,800 *g* to remove insoluble tissue. The supernatant was neutralized with 5 M KOH to pH 2, with 1 M KOH to pH 5, and finally with 0.1 M KOH to pH 7.4. The neutralized samples were spun for 15 min at 20,800 *g* to remove insoluble $KClO_4$ salts. The final supernatant was lyophilized at −50°C overnight and stored at −80°C for later NMR analysis.

^{13}C NMR and isotopomer analysis. Lyophilized heart extracts were dissolved in 0.5 ml ^2H_2O (99.8%) for NMR spectral acquisition. ^{13}C NMR spectra of the samples were acquired at 125.7 MHz on a Bruker AM 500 spectrometer with a 45° pulse and a 3-s recycle delay by use of 16-K data points to digitize 13 kHz. Protons were decoupled with a Waltz-16 decoupling scheme. Before Fourier transformation, the free-induction decays were baseline corrected and zero-filled. Generally, spectra with adequate signal-to-noise ratio were obtained in 4–5 h.

All of the labeled carbon resonances (C_1 to C_5) of glutamate were integrated using the Lorentzian peak-fitting subroutine in the acquisition program (Tecmag MacFID 1D 5.2). The raw integral values were entered into a spreadsheet to calculate ratios of components in each carbon's multiplet pattern. These ratios were used as starting parameters for the TCA analysis-fitting algorithm. The TCA analysis software, tcaSIM and tcaCALC, were provided by Dr. C. R. Malloy and

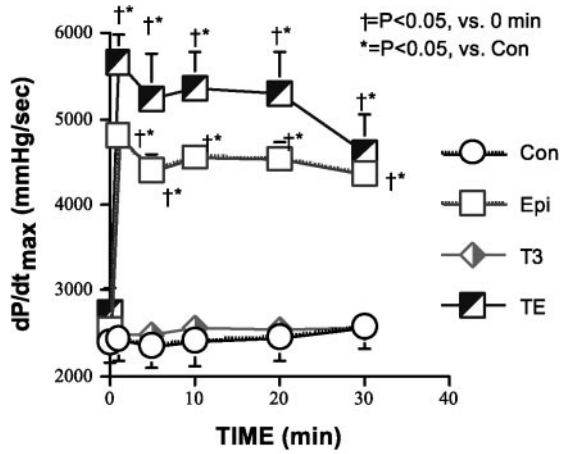

Fig. 1. First derivative relative to time (dp/dt_{max}) for left ventricular pressure expressed for 4 protocols: control (C or Con), epinephrine (Epi), triiodothyronine (T_3), and T_3 and Epi combined (TE). Con and T_3 dp/dt_{max} values do not change through the 30-min protocol, indicating that a hemodynamic steady state has occurred. A marked increase in dp/dt_{max} occurs with Epi and TE, followed by stabilization.

Dr. F. M. Jeffrey through the website www2.swmed.edu/rogersmr2/.

Statistical analyses. Reported values are means ± SE. The Statview 4.5 (FPV) program (1995, Abacus Concepts, Berkeley, CA) was used for statistical analysis. Data were evaluated with repeated-measures analysis of variance (ANOVA) within groups and single-factor ANOVA across groups. When significant F values were obtained, individual group means were tested for differences using the unpaired t-test. The criterion for significance was $P < 0.05$ for all comparisons.

RESULTS

Cardiac function parameters. No significant changes in heart rate occurred with either Epi or T_3 infusion. LVDP and maximum DP/dt increased with Epi alone and in combination with T_3 but did not change with T_3 infusion alone. Figures 1 and 2 illustrate these parameters through the protocols and demonstrate achievement of steady-state levels of function.

$M\dot{V}O_2$. Elevation in $M\dot{V}O_2$ occurred with Epi and with TE treatments. Statistical comparisons with baseline and control groups are shown in Fig. 3. T_3 alone did not elicit a change in $M\dot{V}O_2$. After the initial $M\dot{V}O_2$ increase (1 min) in the two groups receiving Epi, a steady rate was achieved. $M\dot{V}O_2$ did not change significantly when compared with the 1-min value. Changes in $M\dot{V}O_2$ resulted from combined alterations in both coronary flow and oxygen extraction. However, these indexes did not change significantly when analyzed individually. Coronary flow in the Epi groups exhibited the greatest range, extending from 42.8 ± 5.1 at baseline to 52.7 ± 2.5 $ml \cdot min^{-1} \cdot g$ dry wt^{-1} at peak. Similar baseline values occurred in the C and T_3 groups, with minimal detectable change through the protocol.

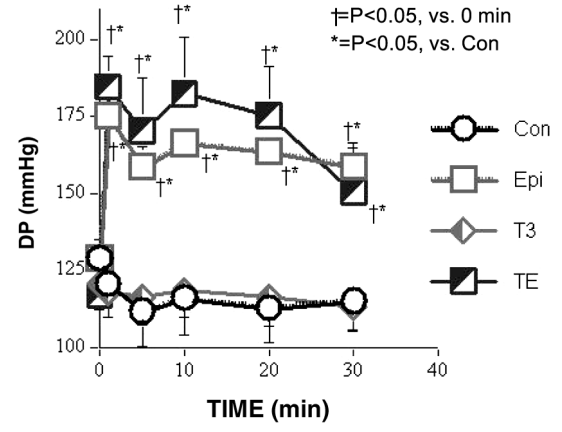

Fig. 2. Developed pressure (DP) expressed for the 4 protocols described in Fig. 1.

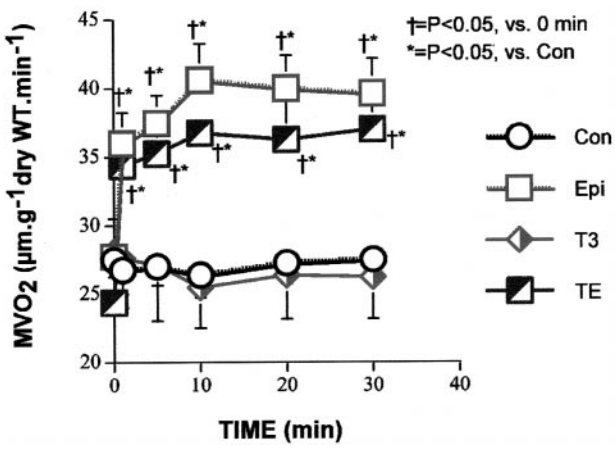

Fig. 3. Myocardial oxygen consumption ($M\dot{V}O_2$) vs. time for the 4 protocols. $M\dot{V}O_2$ changes parallel functional changes noted in Fig. 1. All 4 protocols achieve a steady-state period during which labeled substrates are provided.

Isotopomer analysis. Several previous publications have described ^{13}C isotopomer modeling and analyses (13). An example of raw spectra and synthetic corresponding spectra generated by the Lorentzian fit routine is demonstrated in Fig. 4. The control heart spec-

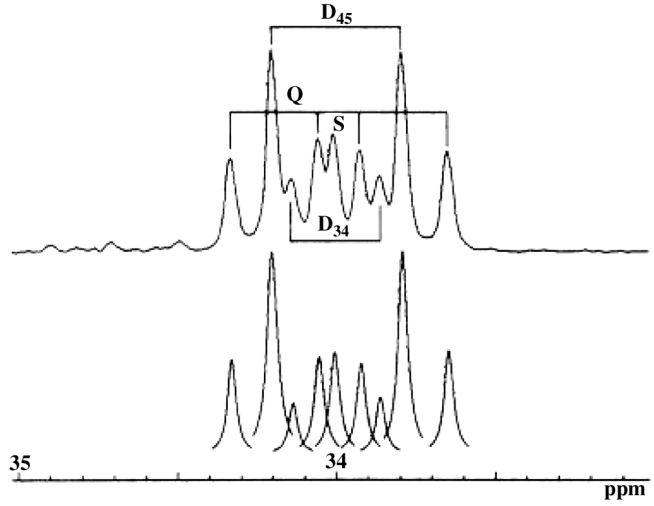

Fig. 4. A spectrum from control heart extract shows the glutamate C_4 from a proton-decoupled ^{13}C NMR spectrum at 34 ppm. Each multiplet is labeled as a singlet (S), doublets (D) with relevant coupling noted as a subscript, and a quartet (Q). *Top*: raw spectrum; *bottom*: Lorentzian fit.

trum at C_4 (Fig. 4) yielded a quartet from coupling between labels at C_4. At the two adjacent sites, C_3 and C_5, a doublet, D_{45}, arises from coupling between labels at C_4 and C_5. A doublet, D_{34}, arises from similar coupling between labels at C_4 and C_3, and a singlet results from label at C_4, but with no adjacent labeling.

Substrate selection. Acetyl-CoA enters the TCA cycle either through the acyl-CoA synthase or pyruvate dehydrogenase pathways. Fractional acetyl-CoA contributions of individual substrates were determined in these experiments from ^{13}C isotopomer analyses. Representative spectra from each experimental condition are shown in Fig. 5. Differences in specific resonance patterns among the various conditions can be noted. For instance, the singlet in the C4 region predominates relative to other spectra from the epinephrine-infused heart. Anaplerosis contribution to the TCA was also calculated. However, the anaplerotic contribution never exceeded 4% in these protocols.

The fractional oxidative contribution (Fc) during each condition by individual substrate is summarized in Table 1. The Fc reported represents results of calculations obtained with a steady-state model (Dr. Malloy). However, all data were also subjected to analyses with a non-steady-state model by use of tcaCALC. Results from steady-state and non-steady state analyses were virtually identical, indicating that steady state had been achieved by completion of all our protocols.

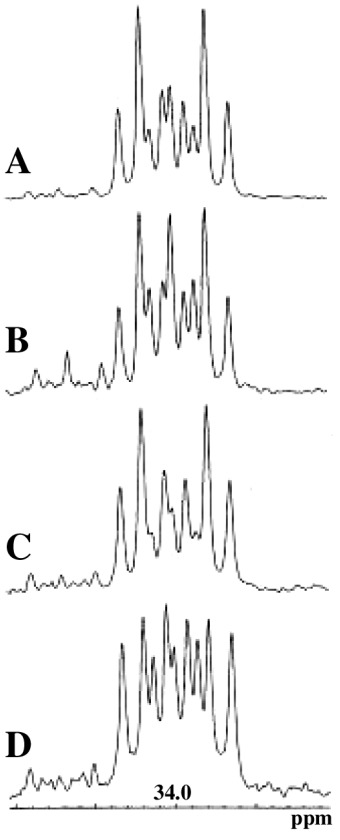

Fig. 5. Spectra from the glutamate C_4 region are shown from heart extracts after the various protocols. Splitting patterns and assignments are similar to those in Fig. 4. Compared with Con (*A*), Epi (*B*) induces increases in the singlet S and doublet D_{34}. T_3 (*C*) yields depression in those peaks. The TE pattern (with T_3 and Epi) shifts (*D*) back toward Con compared with Epi alone.

Table 1. *Fractional contributions from substrates to tricarboxylic acid cycle*

	Control (n = 10)	T_3 (n = 10)	Epi (n = 8)	TE (n = 8)
Free fatty acids	0.48 ± 0.04	0.57 ± 0.01*	0.42 ± 0.02*	0.45 ± 0.03
Acetoacetate	0.29 ± 0.04	0.28 ± 0.04	0.20 ± 0.01*	0.19 ± 0.01*
Lactate	0.14 ± 0.01	0.07 ± 0.02*	0.18 ± 0.01	0.21 ± 0.02*
Unlabeled substrates	0.10 ± 0.01	0.08 ± 0.02	0.18 ± 0.02*	0.14 ± 0.03

Values are means ± SE. Control, triiodothyronine (T_3), epinephrine (Epi), and a combination of T_3 and Epi (TE) treatment groups are compared. *$P < 0.05$ vs. Control. There are no significant differences between Epi and TE.

Thyroid hormone promotes a significant shift toward selection of FFA. This occurs in association with a relatively reduced Fc through lactate, although no significant change in acetoacetate oxidation occurs. Thyroid does not cause a change in the Fc of unlabeled substrate.

Epi significantly decreased the Fc by FFA (Fc_{FFA}) and acetoacetate (Fc_{AA}) while increasing contribution of endogenous unlabeled substrate. A trend toward an increase in lactate (Fc_{LAC}) occurred during Epi infusion, which did not reach significance ($P = 0.053$).

There were no significant differences between Epi and TE groups. However, T_3 slightly modified shifts in substrate selection caused by Epi. Thus reductions noted in FFA Fc during Epi, which reached statistical significance, were less pronounced and were not significant with thyroid hormone present (C vs. TE). The FC_{LAC} response to Epi was also slightly modified by T_3.

Substrate flux. The ^{13}C isotopomer analyses do not directly provide absolute measures of substrate flux. However, Jeffrey et al. (14) derived equations for estimating citric acid cycle and substrate flux when TCA fractional enrichment and total $M\dot{V}O_2$ rates are known (see Ref. 14 for details). In general, this method utilizes an assumed respiratory quotient (R) value ($M\dot{V}O_2/TCA_{flux}$) for each substrate, where R is 2 for AA, 3 for LAC, and 2.8 for FFA. The composition of the endogenous (end) substrate contribution was not determined but was greatest during Epi stimulation. Because previous work indicates that this contribution is provided through glycogen and endogenous triglycerides, an R value of 2.9 is assumed. Thus $M\dot{V}O_2/TCA_{flux} = Fc_{FFA}R_{FFA} + Fc_{LAC}R_{LAC} + Fc_{AA}R_{AA} + Fc_{end}R_{end} + yRa$, where Fc is the fractional contribution to TCA from each substrate, and yRa represents the anaplerotic component. Because anaplerosis contributes <4% to total oxygen consumption even at high workloads (14), this component is considered negligible for these experimental calculations. Flux through the TCA, which is synonymous with oxidation rate for each substrate, can be calculated by normalizing individual TCA_{flux} values to the number of acetyl-CoA esters

yielded per molecule of that substrate, e.g., LAC 1, AA 2, and FFA 8.5. Results of these calculations are summarized in Table 2. Epi substantially increases TCA flux, defined as the overall acetyl-CoA oxidation rate. T_3 increases FFA flux through the TCA and diminishes lactate flux. Although the FFA Fc is reduced by Epi, the absolute oxidation rate is increased substantially. Acetoacetate flux is reduced by Epi, whereas lactate oxidation is increased. Statistical comparisons between the Epi and the TE groups reveal no significant differences between these groups with regard to Fc values or calculated flux rates. Thus these comparisons between groups indicate that responses to Epi or high work state are not directly modified by T_3. However, although Epi lowers Fc_{FFA} relative to the C group, this effect is ameliorated by addition of T_3. Furthermore, the TE group demonstrates a significantly higher Fc_{LAC} than the C group, which is not demonstrated in the Epi group.

The described labeling patterns were not directed toward analyses of direct glucose contribution to the TCA cycle, because such contribution has been shown to be minimal under the substrate provisions provided in these experiments at control conditions. However, a glucose contribution might have occurred during epinephrine stimulation provided in these experiments. To determine whether an important glucose contribution did exist, D-[U-$^{13}C_6$]glucose was added to the perfusate, with labeled acetoacetate and lactate but unlabeled fatty acids. Spectra obtained during C ($n = 2$) and Epi conditions ($n = 3$) and subjected to isotopomer analysis detected minimal evidence of glucose (Fc_{Glc} <3%) contribution, even at the higher work state. This is demonstrated in Fig. 6, where the uniformly labeled substrates are expected to contribute to the glutamate C_4 D_{45} and C_4 quartet. The marked diminution of these peaks, when the ^{13}C enrichment scheme is changed from uniformly labeled FFA to uniformly labeled glucose, demonstrates the low contribution of the glucose to the C_4 glutamate spectrum.

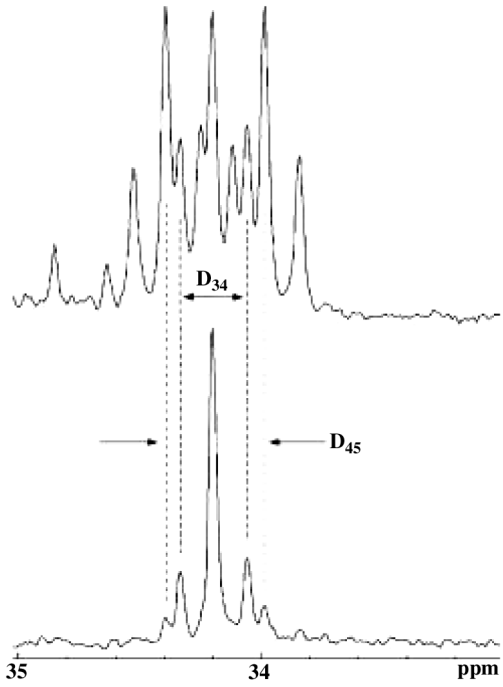

Fig. 6. Spectra (C_4 glutamate) at *top* were obtained using [1,3-^{13}C]acetoacetate, [3-^{13}C]lactate, U-^{13}C-labeled free fatty acids (FFA), and D-[^{12}C]glucose. *Bottom*: D-[U-$^{13}C_6$]glucose replaced unlabeled glucose, and unlabeled FFA replaced the uniformly ^{13}C-labeled FFA. Peak assignments for the D_{34} and D_{45} are shown (see assignments in Fig. 4). The marked diminution of the D_{45} and the near disappearance of the C_4 quartet demonstrate the negligible contribution of labeled glucose to the spectrum.

DISCUSSION

Evidence for thyroid promotion of myocardial fatty acid utilization represents the principal and novel finding in this study. The ^{13}C isotopomer analyses, combined with oxygen consumption measurement, provide the Fc to the TCA cycle for multiple substrates, as well as absolute substrate flux calculations. Using this format, we detected T_3 alterations in FFA acetyl-CoA Fc and calculated absolute FFA flux rate, which occurred independently of changes in oxygen consumption or cardiac functional parameters. Thus these data indicate that changes in substrate utilization result from hormonal action and not solely through elevations in cardiac work state.

Table 2. *Calculated flux rates through the TCA*

	Control	T_3	Epi	TE
TCA	10.3 ± 0.8	10.1 ± 0.7	15.1 ± 1.1*	13.8 ± 0.9*
Free fatty acids	0.58 ± 0.03	0.68 ± 0.03*	0.75 ± 0.09*	0.73 ± 0.06*
Acetoacetate	2.99 ± 0.16	2.83 ± 0.15	1.51 ± 0.17*	1.31 ± 0.16*
Lactate	1.45 ± 0.15	0.71 ± 0.13*	2.73 ± 0.31*	2.89 ± 0.27*

Values are means ± SE for flux rates expressed in $\mu mol \cdot min^{-1} \cdot g$ dry wt^{-1} through the tricarboxylic acid (TCA) cycle. Calculations are described in MATERIALS AND METHODS. TCA represents the total rate of acetyl-CoA oxidation. *$P < 0.05$ vs. control.

Previously, Liu et al. (20) reported that T_3 at a similar dose caused no significant change in palmitate oxidation in the isolated working heart. Their results seem to conflict with our findings, indicating that T_3 promotes FFA selection and absolute oxidation rate. However, those investigators examined substrate oxidation under conditions that are substantially different from those applied in our study. The principal objective of those experiments was to determine whether T_3 could improve mechanical function and cardiac function by modulation of glucose metabolism. Accordingly, the investigators proffered substrates at concentrations that would maximize palmitate oxidation rates during control and reperfusion conditions (36). Specifically, those investigators used perfusate with a relatively high palmitate concentration (1.2 mM) and containing no lactate or ketone bodies. This substrate provision differs substantially from the physiological composition used in the current study and from that used in detailed experiments with rats (32). The physiological perfusate substrate concentrations in our studies were appropriate for addressing the

principal hypothesis that thyroid hormone directly promotes fatty acid oxidation and/or selection. The elevation of palmitate oxidation, possibly to near-maximal flux rates, limited the capability of detecting further increases in FFA oxidation during Liu's experiments. Thus apparent discrepancies between these studies can be explained by differences in substrate provision.

Direct thyroid hormone action presumably occurs along two principal pathways that regulate substrate selection, those leading to the pyruvate dehydrogenase complex and those leading to acyl-CoA synthase (10). In the current experiments, T_3 increased FFA flux while coordinately decreasing oxidation of lactate, which represented the principal substrate source for the acetyl-CoA contribution through pyruvate dehydrogenase. This pattern supports the postulated reciprocal mode of regulation of these pathways (33). Activation of enzymes that regulate myocardial fatty acid utilization and/or transport provides a plausible explanation for the T_3-induced elevation in FFA acetyl-CoA Fc and absolute oxidation rate. For example, carnitine palmitoyl transferase I catalyzes the initial reaction of mitochondrial import of long-chain fatty acids. This tightly regulated transfer presumably participates in the control of fatty acid oxidative flux rate. T_3 directly diminishes utilization of exogenous [^{14}C]palmitate for synthesis of longer chain fatty acids in isolated rat hepatocytes (25). This effect can be reversed by blocking fatty acid oxidation through octanoylcarnitine, a direct carnitine palmitoyl-transferase I inhibitor, although evaluation of this phenomenon has not been reported in heart (25). Goodwin and colleagues (9, 10) showed that β-oxidation rates in rat heart correlate inversely with malonyl-CoA levels. Thus acetyl-CoA carboxylase, the enzyme responsible for synthesis of malonyl-CoA, provides another candidate site for direct T_3 regulation of long-chain FFA oxidation. Further studies will be required to elucidate the mechanisms involving activation of the FFA pathways by T_3 in heart.

A secondary objective of this study was to determine whether thyroid hormone effects on substrate preference dominated during abrupt increases in cardiac workload caused by Epi. Although several investigators have used Epi stimulation as a mode to examine substrate oxidation patterns during acute elevations in work state (3, 6, 9, 10), rapid thyroid hormone action under comparable conditions has not been well investigated. Thyroid hormone promotes increases in cardiac β-adrenoreceptor density and sensitivity (12, 29). Although these actions are nuclear mediated, they provide the general impression that thyroid and epinephrine work synergistically. This contention stems in part from studies suggesting that thyroid hormone enhances the cardiac inotropic response to epinephrine (1, 4, 40).

Accordingly, the effects of Epi in this study should be considered in context to understand possible metabolic interactions between these two hormones. First, one must evaluate the consequences of supplying Epi alone (without T_3) in the current model. Considerable inconsistencies exist in the scientific literature with respect to Epi influence on substrate metabolism in isolated perfused rat hearts. Early studies by Crass et al. (5) and Neely et al. (27) indicate that palmitate oxidation increases in response to elevation in work state and/or Epi. More recently, Collins-Nakai et al. (3) found that Epi increased ATP production through selective increases in glucose oxidation and glycolysis. Goodwin et al. (10) found that Epi immediately stimulated glycogen contribution to oxidation, induced glucose oxidation later, and caused a trend toward increased oleate oxidation (although it was not statistically significant). Virtually all these studies employed different protocols using a wide variety of substrate mixtures, perfusion conditions, and methodology. Whereas Collins-Nakai et al. perfused hearts with palmitate and glucose, but no lactate or ketone bodies, Goodwin et al. used a complex protocol involving glycogen depletion by substrate deprivation followed by repletion of glycogen during perfusion with multiple substrates including lactate, glucose, first β-hydroxybutyrate, and then oleate. Thus those attempting to make comparisons among these studies, as with the current data, must consider the specific conditions of each experiment.

Epi in these experiments elevated the acetyl-CoA Fc through lactate and unlabeled sources. These changes in relative Fc occurred in conjunction with absolute flux increases for FFA and lactate, as well as decreases in acetoacetate Fc and absolute flux. The composition of unlabeled substrate sources cannot be strictly defined in this analysis but should include exogenous substrate, i.e., glucose, and endogenous substrates such as triglycerides and glycogen. Under baseline conditions, both with and without T_3, the unlabeled substrates contribute no more than 10% of the acetyl-CoA entering the TCA. This value corresponds well to data from other studies employing ^{13}C isotopomer techniques (13, 21), supporting the tenet that glucose contributes minimally without Epi stimulation, even if this substrate represents the entire unlabeled source. Further evidence that glucose is not a major contributor in the unstimulated state is provided by several recent studies employing $^{14}CO_2$ production from labeled substrates and indicating that glucose contributes 3–20% TCA acetyl-CoA, depending on perfusate substrate composition as well as the presence or absence of insulin (3, 9, 20). The increase in Fc from unlabeled sources to 18% during Epi stimulation in our experiments could be secondary to increased unlabeled glucose oxidation. This hypothesis was considered and tested by providing a separate group of hearts with D-[U-$^{13}C_6$]glucose in the place of U-^{13}C-labeled long-chain FFAs. The minimal glucose contribution (<3%) during Epi stimulation is dramatically demonstrated in the spectra illustrated in Fig. 6. Thus these experiments, employing both labeled and unlabeled glucose schemes, indicate that endogenous stores, and not glucose, are responsible for the increase in unlabeled

Fc during Epi infusion. These data are consistent with experimental results reported by Goodwin et al. (9, 10), who demonstrated a burst of glycogen breakdown and oxidation, as well as elevated triglyceride turnover, early during Epi infusion. Those investigators detected subsequent increases in glucose oxidation in their working heart model, which might be delayed (5, 27) and therefore go undetected in these Langendorff heart experiments. One might question the validity of the steady-state assumptions in the current experiments, and whether errors in isotopomer analyses contributed to results indicating a minimal oxidative contribution from glucose. However, as previously noted, data were also subjected to non-steady-state analyses (24). Rigorous statistical comparisons of the data analyzed using these different models (steady state vs. nonsteady state) validated the assumptions and indicated that metabolic steady state had indeed been achieved for all protocols in these experiments.

Generally, the direct comparison data (Epi vs. TE) indicate that T_3 does not substantially alter substrate selection or flux during high work state elicited by Epi. Thus no obvious synergism or cooperation existed between these two hormones with regard to substrate metabolism. The Epi influence dominated, which was manifested by the increase in calculated TCA flux and lactate oxidation rate. T_3 appears to slightly modify Fc values for FFA, lactate, and unlabeled substrates when statistical comparisons of control vs. Epi are considered. The direction for Fc_{FFA} modification could be expected when one considers shifts toward FFA selection by T_3 at the lower work states. The Fc_{LAC}, which demonstrated no change with Epi (vs. control), also showed a significant increase when T_3 was included (C vs. TE). This finding was surprising when we consider the reduction in Fc_{LAC} by T_3 at low work state. However, these data could be explained by a T_3 enhancement of exogenous over endogenous substrate selection at high work state. As stated previously, because the composition of the unlabeled source is not strictly defined in these experiments, flux rates for this portion cannot be calculated. Alternative models, which employ endogenous source labeling, would be required to further evaluate T_3 effects on this substrate pool at high work states.

In summary, these studies demonstrate that thyroid hormone directly and rapidly shifts myocardial substrate preference. The time frame of these experiments indicates that these responses are not nuclear mediated. Although T_3 might enhance the contractile response to Epi in some models, no synergism exists with regard to FFA contribution to the TCA. These studies have particular significance because of the recently established clinical importance of T_3 supplementation after cardiopulmonary bypass (26), which causes drops in circulating thyroid hormone levels. T_3 supplementation improves postoperative cardiac function, although the mechanisms and their relationship to myocardial metabolism have not been established.

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