Thyroid hormone controls myocardial substrate metabolism through nuclear receptor-mediated and rapid posttranscriptional mechanisms

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Thyroid hormone controls myocardial substrate metabolism through nuclear receptor-mediated and rapid posttranscriptional mechanisms. Am J Physiol Endocrinol Metab 290: E372–E379, 2006. First published October 4, 2005; doi:10.1152/ajpendo.00288.2005.—Thyroid hormone regulates metabolism through transcriptional and posttranscriptional mechanisms. The integration of these mechanisms in heart is poorly understood. Therefore, we investigated control of substrate flux into the citric acid cycle (CAC) by thyroid hormone using retrogradely perfused isolated hearts (n = 20) from control (C) and age-matched thyroidectomized rats (T). We determined substrate flux and fractional contributions (Fc) to the CAC by ¹³C-NMR spectroscopy and isotopomer analyses in hearts perfused with [1,3-¹³C]lactoacetic acid (0.17 mM), [1-¹³C]lactoacetic acid (LAC, 1.2 mM), [U-¹³C]long-chain mixed free fatty acids (FFA, 0.35 mM), and unlabeled glucose. Some T hearts were supplied triiodothyronine (T₃, 10 nM; TT) for 60 min. Prolonged hypothyroid state reduced myocardial oxygen consumption, although T3 produced no significant change. Hypothyroidism reduced overall CAC flux but selectively altered only FFA₉₉ among the individual substrates, though LAC tended upward. T₃ rapidly decreased lactate Fc and flux. ¹³C labeling of glutamine through glutamate was increased in T with further enhancement in TT. The glutamate-to-glutamine ratio was significantly lower in T and TT. Immunoblots detected a decrease in hypertrophic thyroid hormone for muscle carnitine palmitoyltransferase I (CPT I) and a marked increase in pyruvate dehydrogenase kinase (PDK-2) with no changes in liver CPT I, PDK-4, or hexokinase 2. TT, but not T, displayed elevated glutamine synthetase (GS) expression. These studies showed that T₃ regulates cardiac metabolism through integration of several mechanisms, including changes in oxidative enzyme content and rapid modulation of individual substrates fluxes. T₃ alsomoderates forward glutamine flux, possibly by increasing the overall activity of GS.

The current study investigated control of substrate flux into the citric acid cycle (CAC) by thyroid hormone. This included a standard study design invoking nuclear mediated responses through thyroidectomy. First, we postulated that induction of hypothyroidism alters fractional contributions (Fc) of acetyl-CoA to the CAC or substrate flux in an isolated perfused rat heart model. Second, we tested the hypothesis that T₃ regulates substrate oxidation through immediate posttranscriptional pathways in the hypothyroid state. Accordingly, we determined whether acute T₃ supplementation rapidly altered Fc and substrate oxidative flux in this hypothyroid model.

¹³C magnetic resonance spectroscopy and isotopomer analyses provided the principal tools of this study. In addition, we followed results of substrate flux studies with protein analyses for enzymes regulating pathways that were altered by our perturbations. During this study, we also noted T₃-mediated changes in glutamine labeling via the CAC, thereby identifying a route for loss of CAC intermediates. Therefore, we also evaluated components of this pathway, including the primary substrate glutamate and the controlling enzyme glutamine synthetase.

MATERIALS AND METHODS

Animals. All rats for these experiments were obtained from Charles River Laboratories (Wilmington, MA). They supplied two groups: control Sprague-Dawley (SD) males, weighing 407 ± 13 g, and age-matched (3–4 mo) thyroidectomized (T) SD males, which were provided calcium gluconate supplement in water until they were euthanized. Thyroidectomies were performed between 15 and 20 days after a 2-week recovery period.
before our perfused-heart protocol. Effectiveness of thyroidectomy was confirmed by serum total T₃ <30 ng/dl at the time the animals were euthanized.

Heart perfusion procedure. Rats were anesthetized with pentobarbital sodium (45 mg/kg ip) and heparinized (700 U/kg ip). The heart was rapidly excised and submerged in ice-cold physiological salt solution (PSS), pH 7.4, containing (in mM) 118.0 NaCl, 25.0 NaHCO₃, 4.7 KCl, 1.23 MgSO₄, 1.2 NaH₂PO₄, 5.5 d-glucose, and 1.2 CaCl₂.

The aorta was cannulated in the standard Langendorff mode, and the spontaneously beating heart was perfused with PSS containing the following ¹³C-labeled substrates in addition to unlabeled glucose (5.5 mM): [1,3-¹³C]acetacetic acid (AA, 0.17 mM), t-[3-¹³C]lactic acid (LAC, 1.2 mM), [U-¹³C]long-chain mixed free fatty acids (FFA, 0.35 mM) bound to 0.75% (wt/vol) delipidated bovine serum albumin (BSA). The mixture contained pre-dominantly saturated and unsaturated fatty acids ranging from 14 to 22 carbons in length, with palmitic and linoleic as the most prominent. Details regarding isotope preparation and labeling strategy have been previously published (14). This mixture had been equilibrated with 95% O₂-5% CO₂ at 37°C and passed twice through filters with 3.0 µm pore size. Perfusion pressure was maintained at 70 mmHg. The entire perfusion system was jacketed and maintained at 37°C.

An incision was made in the left atrium, and an empty latex balloon was passed through the mitral orifice and placed in the left ventricle. The balloon was filled with saline and connected to a pressure transducer for continuous measurement of left ventricular pressure (LVP), from which its first derivative with respect to time (dP/dt) was calculated. The caudal vena cava, cranial vena cava, and the azygous vein were ligated. The pulmonary artery was cannulated to enable collection of coronary flow, which was measured with a flowmeter (T106; Transonic Systems, Ithaca, NY). The analog signals were continuously recorded on a chart recorder (Gould, Cleveland, OH) and an online computer (Macintosh, Biopac Signal Acquisition System). To characterize cardiac function, left ventricular developed pressure (LVDVDP) was defined as peak systolic pressure minus end-diastolic pressure. Myocardial oxygen consumption (MVₐO₂) was calculated as MVₐO₂ = CF × [(PaO₂ - P(Paco₂))/P(c/760)] × [(c/760)], where CF is coronary flow (ml·min⁻¹·g wet tissue⁻¹), PaO₂ is the difference in the partial pressure of oxygen (mmHg) between perfusate and coronary effluent, and c is the Bunsen solubility coefficient of O₂ in perfusate at 37°C (22.7 µl O₂·atm⁻¹·ml⁻¹). PaO₂ was determined with anABL5 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Experimental protocol. Hearts were divided into three groups: control (C, n = 6), hypothyroid (T, n = 6), and hypothyroid with T₃ infusion (TT, n = 8). All of the hearts were perfused for a 30-min equilibration period after which the baseline measurements were taken. After heart isolation and preparation, a left ventricular balloon volume was defined to provide a developed pressure between 100 and 140 mmHg. This volume remained unchanged throughout the protocol. Hearts with end-diastolic pressures >8 mmHg at baseline were not accepted (20). Also, data from hearts characterized by developed pressures <100 mmHg or >140 mmHg were not used in the analysis. Baseline measurements were followed by the perfusion period of 60 min when the function measurements were taken every 15 min. TT hearts were infused with T₃, supplied as liothyronine, with a final concentration of 10 nM for the entire 60 min. After 90 min of total perfusion, nonventricular tissue was removed, and hearts were immediately freeze-clamped with copper tongs that had been chilled in liquid nitrogen.

Extraction. Briefly, freeze-clamped hearts were ground into fine powder under liquid nitrogen, extracted with 0.6 M perchloric acid, and neutralized with cold KOH to pH 7.4. The final supernatant was lyophilized overnight at −50°C for later NMR analysis.

¹³C-NMR and isotopomer analyses. Lyophilized heart extracts were dissolved in 99.8% ²H₂O for NMR spectral acquisition. De-coupled ¹³C-NMR spectra of the samples were acquired at 187.5 MHz on a Bruker DMX 750 spectrometer with a 45° pulse and a 4-s recycle delay. Free-induction decays were baseline corrected, zero filled, and Fourier transformed. All of the labeled carbon resonances (C₁–C₅) of glutamate were integrated using the Lorentzian peak-fitting subroutine in the acquisition program (NUTS; Acorn NMR, Livermore, CA). The individual integral values were used as starting parameters for the CAC analysis-fitting algorithm tcaCALC, kindly provided by Drs. C. R. Malloy and F. M. Jeffrey. This algorithm provided the Fc for each substrate to the acetyl-CoA pool entering CAC.

Amino acid measurements. Multiple amino acid concentrations (Arg, Asn, Asp, Gln, Glu, Gly, Ser, Tau, Thr) in the lyophilized heart extracts dissolved in ¹H₂O were determined by HPLC. Amino acids were precolumn derivatized with o-phthalialdehyde (Sigma, St. Louis, MO), separated, and measured as previously described (38).

Western blotting. Fifty micrograms of total protein extracts from rat heart tissue were electrophoresed through 4.5% stacking and 7.5 or 10% running SDS-polyacrylamide gels and electroblotted onto PVDF membranes. The blots were blocked for 1 h at room temperature with 5% nonfat milk in Tris-buffered saline plus Tween-20 (TBST; 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween-20), followed by overnight incubation at 4°C with antibodies diluted in blocking buffer directed toward glutamine synthetase (GS), hexokinase II (HKII) (Santa Cruz Biotechnology, CA), liver carnitine palmitoyltransferase I (L-CPT I), muscle (M-CPT I) (8, 39), and pyruvate dehydrogenase kinase (PDK)-2 and -4 (23, 36). After two 5-min washes with TBST and one 5-min wash with TBS, blots were incubated at room temperature for 1 h with the appropriate secondary antibody conjugated to horseradish peroxidase (HRP). The blots were washed twice for 10 min with TBST and visualized with enhanced chemiluminescence after exposure to Kodak biomass light ML-2 film. The blots were stripped by treating them twice for 30 min with 200 mM glycine, 0.1% SDS, and 1% Tween-20, (pH adjusted to 2.2) followed by two 5-min washes with TBST and one 5-min wash with TBS. The blots were again blocked for 1 h as above, followed by overnight incubation at 4°C with β-actin antibody (Santa Cruz Biotechnology) in blocking solution. The next day, the blots were washed (as above), the appropriate secondary HRP antibody was applied, and the remaining procedures as described above were followed. The β-actin was used to quantify the multiple protein signals by densitometry after standardization for loading. The densitometric intensities were determined using the ImageJ 1.32 program (NIH). Western blots were repeated three times to confirm consistency of the findings.

Statistical analysis. Reported values are means ± SE in text, tables, and figures. Data were analyzed with repeated-measures analysis of variance (ANOVA) within groups and single-factor ANOVA tables, and figures. Data were analyzed with repeated-measures analysis of variance (ANOVA) within groups and single-factor ANOVA across groups (StatView 4.5; Abacus Concepts, Berkley, CA), as well as Fisher’s test and unpaired t-tests when appropriate. Criterion for significance was P < 0.05 for all comparisons.

RESULTS

Cardiac function and V₀₂. Functional parameters (Fig. 1) and V₀₂ (Fig. 2) are reported for 15-min intervals. These parameters did not deviate significantly over the time of the protocol, thereby demonstrating the required functional and metabolic steady state for the tcaCALC algorithms. No significant differences in systolic function (developed pressure, dP/dmax) were noted among the three groups (Fig. 1, A and B). However, −dP/dmax, representing ventricular relaxation speed, was significantly depressed in the two thyroidectomy groups, consistent with previous literature (Fig. 1B) (9). Also, spontaneous heart rate was lower in the thyroidectomy groups throughout the protocol (Fig. 1C). T₃ infusion did not yield a significant change in heart rate or −dP/dmax. The prolonged
hypothyroid state reduced MV\(\dot{O}_2\) for the duration of the experiment. T\(_3\) infusion did not significantly alter MV\(\dot{O}_2\) (Fig. 2).

**Substrate selection.** Acetyl-CoA enters the CAC either through the acyl-CoA synthase or pyruvate dehydrogenase. Fc of acetyl-CoA into CAC for each substrate during the 60-min steady-state period were determined from glutamate peak areas using \(^{13}\)C isotopomer analysis (12) and appear in Fig. 3. No significant Fc differences occurred between control and hypothyroid groups, although we detected a trend toward higher ketone (acetoacetate) Fc in the hypothyroid hearts (\(P = 0.053\)). T\(_3\) infusion decreased lactate Fc compared with the hypothyroid group. Although individually neither FFA nor acetoacetate Fc reciprocally increased with the decrease in lactate Fc, their combined contribution was elevated by T\(_3\) infusion.

**Substrate flux.** The absolute flux for the CAC and oxidative flux for individual substrates were calculated from MV\(\dot{O}_2\) and the stochiometric relationships between MV\(\dot{O}_2\) and citrate formation from the various substrates as described by Jeffrey et al. (12). Although calculated, this value accounts for changes in oxidative rates as well as the anaplerotic contribution to the CAC. Briefly, MV\(\dot{O}_2\)/CAC flux = \(FC_{FFA}\)R\(_{FFA}\) + \(FC_{LAC}\)R\(_{LAC}\) + \(FC_{AA}\)R\(_{AA}\) + \(FC_{end}\)R\(_{end}\) + \(yRa\), where Fc are fractional contributions for each substrate determined by isotopomer analysis and R is an assumed respiratory quotient (R\(_{FFA}\) = 2.8, R\(_{LAC}\) = 3, R\(_{AA}\) = 2, R\(_{end}\) = 2.9). \(yRa\) represents the anaplerotic component (12). The endogenous component (\(FC_{end}\)R\(_{end}\)) in these studies is most likely due to unlabeled glucose, glycogen, and a minor contribution from endogenous triglycerides. The calculated CAC flux was normalized for each substrate by dividing the total CAC flux by the number of acetyl-CoA esters yielded per molecule of that substrate (FFA = 8.5, LAC = 1, AA = 2), and multiplying by corresponding Fc. The calculated total CAC flux and flux rates for each substrate are presented in

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**Fig. 1.** Cardiac functional parameters over 60 min. **A:** developed pressure stayed the same in all groups during the experimental protocol, indicating functional steady state. **B:** \(-dP/dt_{max}\) was significantly lower in both hypothyroid and hypothyroid + triiodothyronine (T\(_3\)) infusion groups compared with controls. \(+dP/dt\) was not different between groups. **C:** heart rate was significantly lower in both hypothyroid and hypothyroid + T\(_3\) infusion groups compared with controls. Values are means ± SE. *\(P < 0.05\) vs. control.

**Fig. 2.** Myocardial oxygen consumption (MV\(\dot{O}_2\)) was reduced in hypothyroid hearts with or without T\(_3\) infusion compared with controls. Values are means ± SE. *\(P < 0.05\) vs. control.

**Fig. 3.** Fractional contributions of acetyl-CoA from substrates for the citric acid cycle (CAC). *\(P < 0.05\) vs. hypothyroid.
As expected, hypothyroidism reduced overall CAC flux but selectively altered only FFA flux among the individual substrates, although lactate flux trended upward. However, T3 infusion dramatically decreased lactate flux. Anaplerotic contribution determined by the tcaCALC algorithm was <8% of the total CAC flux in all groups.

Glutamine labeling. 13C labeling of glutamine through glutamate was increased in hypothyroid hearts with further enhancement by T3. The ratio of [13C]glutamate to glutamine (total peak areas determined by 13C-NMR spectroscopy, means ± SE) were 0.00 ± 0.00 for C, 0.16 ± 0.07 for T, and 0.43 ± 0.06 for TT. The ratio of labeled glutamine to glutamate was significantly higher in TT vs. T (P < 0.05). A representative carbon-3 spectrum for each group is presented in Fig. 5.

Amino acid content. Conceivably, alterations in glutamine flux either yield or result from changes in the glutamate or glutamine pools. Therefore, we measured these pools, as well as concentrations of multiple other amino acids including Arg, Asn, Asp, Gly, Ser, Tau, and Thr. Amino acids were measured simultaneously by HPLC and are presented in micromoles per gram of wet weight in Table 1. Taurine, glutamate, and glutamine presented the majority of the amino acid pool. Glutamate and glutamine concentrations were not statistically different between groups. However, the glutamate/glutamine ratio was significantly lower in hypothyroid hearts with or without T3 infusion. Taurine, which represents the majority of the cytosolic amino acid pool, was decreased significantly in hypothyroid hearts compared with controls. Other amino acid concentrations were relatively low compared with glutamine, glutamate, and taurine and were unaffected by thyroid state.

Western blot analyses. The reductions in flux for substrate pathways, as well as the apparent change in glutamine flux in the hypothyroid hearts, prompted protein analyses for enzymes involved in these pathways. We analyzed protein concentration for several enzymes involved in regulation of substrate oxidation. Results as well as representative blots for each analyzed protein appear in Fig. 6. A decrease was noted in hypothyroid hearts for M-CPT I and a marked increase in PDK-2 with no change in t-CPT I, PDK-4, or HKII. Although we detected no difference in GS expression between control and hypothyroid hearts, T3 infusion did elevate detectable GS protein within 1 h (Fig. 7).

Fig. 4. Calculated flux rates (μmol·g⁻¹·min⁻¹) for the total CAC and each substrate. Values are means ± SE. *P < 0.05 vs. control; †P < 0.05 vs. hypothyroid. Lactate flux indicates directional flux from lactate toward acetyl-CoA and CAC entry.

Fig. 5. Representative glutamate carbon-3 spectrum for each group. Differences in the amount of glutamine labeling are evident in this model, as T3 markedly increased it in hypothyroid hearts, already showing elevated values.
DISCUSSION

Our results indicate that thyroid control of substrate oxidation occurs through multiple mechanisms involving nuclear receptor-mediated and posttranscriptional processes. In this study, these mechanisms were defined by onset of action and the assumption that T3 requires more than 1 h to achieve a protein or physiological response through transcriptional activation. Previous studies have seemingly confirmed transcriptional mechanisms by showing thyroid modulation of mRNA for various genes encoding proteins involved in substrate oxidation (4, 11, 19). Some investigators have shown that hyperthyroidism modifies protein content or specific activity for key enzymes regulating glycolysis and glucose oxidation (33). In particular, thyroid hormone supplementation decreases pyruvate dehydrogenase (PDH) complex activity through transcriptional upregulation of PDK, which phosphorlates and inhibits PDH (26, 33). A prolonged hyperthyroid state also attenuates insulin-initiated glucose uptake by freshly isolated cardiomyocytes (22). These results from multiple studies using various experimental preparations are all difficult to reconcile.

### Table 1. Amino acid concentrations in isolated perfused rat hearts

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Control (n = 5)</th>
<th>Hypothyroid (n = 5)</th>
<th>Hypothyroid + T3 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>0.08±0.03</td>
<td>0.07±0.01</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.09±0.02</td>
<td>0.07±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.16±0.02</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.15±0.03</td>
<td>0.15±0.04</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Serine</td>
<td>0.12±0.02</td>
<td>0.08±0.01</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>Taurine</td>
<td>18.49±2.00</td>
<td>11.62±1.20*</td>
<td>12.38±1.44*</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.13±0.02</td>
<td>0.12±0.01</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1.76±0.21</td>
<td>1.33±0.16</td>
<td>1.24±0.15</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.69±0.15</td>
<td>0.86±0.18</td>
<td>1.1±0.25</td>
</tr>
<tr>
<td>Glutamate/glutamine</td>
<td>2.78±0.29</td>
<td>1.68±0.21*</td>
<td>1.39±0.29*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in μmol/g heart wet wt. T3, triiodothyronine. *P < 0.05 vs. control.
with data from isolated perfused rat hearts, which have uniformly demonstrated enhanced rates of glucose oxidation and glycolysis in the hyperthyroid state (28, 31). However, those latter studies were somewhat biased or hampered in scope, as isolated hearts were proffered glucose as the exclusive oxidative substrate, ignoring probable effects of thyroid state on FFA or ketone body oxidation. Additionally, past experiments have been performed almost exclusively using models chronically exposed to excess thyroid hormone.

Study design. Accordingly, we designed a study to determine the influence of thyroid state on fractional contribution and flux through multiple substrate oxidation pathways. Our principal hypothesis, supported by the published PDH activity data (26, 33), assumes that the hypothyroid heart promotes PDH flux over fatty acid flux as a pathway to acetyl-CoA production. Regulation of fatty acid oxidation by the hypothyroid state has not been previously evaluated. To evaluate multiple substrate pathways simultaneously, we used relatively physiological concentrations of four principal substrates previously defined in rat experiments (29). This isotopic labeling strategy permits a robust change in fractional contributions to the CAC during various perturbations such as epinephrine stimulation (14). Glucose was not specifically labeled with isotope, as we (14) have previously shown that glucose contribution to CAC flux under these substrate provision conditions remains relatively low. However, glucose contribution is represented as a portion of the unlabeled fractional contribution (Fig. 3), along with presumably minor contributions from endogenous substrates. PDH flux in these experiments is supplied mainly through lactate oxidation, whereas FFA and ketones supplied the overwhelming majority of acetyl-CoA to the CAC in all hearts.

Selective FFA\textsubscript{flux} inhibition. Using the stated conditions, we found a selective decrease in FFA\textsubscript{flux} in both hypothyroid groups. On the basis of earlier studies by other investigators, we presumed that the decrease in total CAC\textsubscript{flux} and FFA\textsubscript{flux} in the hypothyroid hearts was caused by thyroid-mediated transcriptional or translational control over other pivotal enzymes involved in fatty acid oxidation. To further explore this possibility, we evaluated protein content for a limited number of proteins over this time course, including PDK-2. The prevailing evidence shows that T3 requires at least 30 min to induce a detectable change in mRNA levels (5), whereas the minimal time required for translation is unknown. We (14) have previously shown similar modifications on substrate flux in euthyroid rat hearts in only 20 min. We also induced T3-mediated change in phosphorylation potential in hypothyroid hearts of the intact animal over a similar time period (25). Thus these data together support the contention that these rapid modulations in substrate flux are not transcriptionally mediated. We cannot eliminate posttranscriptional modification as a T3-mediated mechanism, as all prior studies evaluating protein synthesis rates of the relevant enzymes were performed over days, not minutes (13, 27). Liu et al. (17) observed acute T3 enhancement of posts ischemic active PDH activity, although not total activity, thereby supporting a rapid mechanism of allosteric or covalent modification of PDH in heart. Their data are directionally inconsistent with other studies (22, 26, 33) and our own results, possibly reflecting differing sensitivities of enzymes to T3 in the aerobic and posts ischemic states. Recent data in some cell types suggest the hypothesis that T3 exerts immediate effects by activating specific kinases and covalently modifying key enzymes. For instance, in alveolar epithelial cells and L6 myoblasts, T3 stimulates several kinases, such as phosphoinosotide 3-kinase and the src kinase family, resulting in Na-K-ATPase or Na\textsuperscript+/H\textsuperscript+ exchanger activation (6, 15). Future studies are needed to clarify whether a similar mechanism modulates cardiac substrate metabolism.

Glutamine labeling. We have serendipitously discovered thyroid modulation of glutamine labeling via glutamate. The reactions leading from \(\alpha\)-ketoglutarate to glutamate provide a cataplerotic pathway, as well as a mechanism for reducing intracellular ammonia. Cohen et al. (3) noted forward GS flux by using \(^{13}\)C labeling but were unable to demonstrate the reverse deamidase reaction through glutaminase under their experimental conditions. Our \(^{13}\)C isotope experiment identified glutamine labeling, which nearly mirrored the glutamate labeling pattern, suggesting forward flux through GS. Under these experimental conditions, glutamine labeling was virtually undetectable in euthyroid hearts. The hypothyroid state en-
hances labeling through GS, and surprisingly, T₃ supplementation appeared to further accelerate glutamine labeling in hypothyroid hearts during our experiments. Unfortunately, we could not determine through the ¹³C experiments whether the enhanced glutamine labeling reflected more rapid forward flux through GS compared with control hearts or instead represented decreased glutamine metabolism and/or release into the circulation. Our Western blot analysis did not show elevated GS protein in hypothyroid hearts, thereby eliminating one possible cause for the increase in forward flux. The HPLC data showed that, although glutamate and glutamine levels were preserved in hypothyroid hearts, the glutamate-to-glutamine ratio was decreased. This finding seems to support the notion that forward flux is increased to maintain glutamine. Surprisingly, acute T₃ enhancement of glutamine labeling was accompanied by ~20% higher levels of GS than occurring in hypothyroid hearts without T₃ supplementation. Although we are skeptical that the elevation in protein content is due to enhanced synthesis over a 1-h period, conceivably T₃ stabilizes the enzyme and slows degradation, or modifies the protein, making it more accessible to immunologic detection. Ample evidence exists in the literature supporting posttranscriptional T₃ modulation of protein synthesis and degradation (1, 10). This work was funded by National Heart, Lung, and Blood Institute Grant HL-60666 to M. A. Portman.

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