Metabolic control analysis of insulin-stimulated glucose disposal in rat skeletal muscle

BEAT M. JUCKER,1 NICOLE BARUCCI,1 AND GERALD I. SHULMAN2
1Department of Internal Medicine and the 2Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06520-8020

Metabolic control analysis of insulin-stimulated glucose disposal in rat skeletal muscle. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E505–E512, 1999.—Metabolic control analysis was used to calculate the distributed control of insulin-stimulated skeletal muscle glucose disposal in awake rats. Three separate hyperinsulinemic infusion protocols were performed: 1) protocol I was a euglycemic (–6 mM)-hyperinsulinemic (10 μU·kg–1·min–1) clamp, 2) protocol II was a hyperglycemic (–11 mM)-hyperinsulinemic (10 μU·kg–1·min–1) clamp, and 3) protocol III was a euglycemic (–6 mM)-hyperinsulinemic (10 μU·kg–1·min–1)-lipid/hepatic (increased plasma free fatty acid) clamp. [1-13C]glucose was administered in all three protocols for a 3-h period, during which time [1-13C]glucose label incorporation into [1-13C]glycogen, [3-13C]lactate, and [3-13C]alanine was detected in the hindlimb of awake rats via 13C-NMR. Combined steady-state and kinetic data were used to calculate rates of glycogen synthesis and glycocalysis. Additionally, glucose 6-phosphate (G-6-P) was measured in the hindlimb muscles with the use of in vivo 31P-NMR during the three infusion protocols. The clamped glucose infusion rates were 31.6 ± 2.9, 49.7 ± 1.0, and 24.0 ± 1.5 mg·kg–1·min–1 at 120 min in protocols I–III, respectively. Rates of glycocalysis were 62.1 ± 10.3, 71.6 ± 11.8, and 19.5 ± 3.6 nmol·g–1·min–1 and rates of glycogen synthesis were 125 ± 15, 224 ± 23, and 104 ± 17 nmol·g–1·min–1 in protocols I–III, respectively. Insulin-stimulated G-6-P concentrations were 217 ± 8, 265 ± 12, and 251 ± 9 nmol/g in protocols I–III, respectively. A top-down approach to metabolic control analysis was used to calculate the distributed control among glucose transport/phosphorylation [GLUT-4/hexokinase (HK)], glycogen synthesis, and glycocalysis from the metabolic flux and G-6-P data. The calculated values for the control coefficients (C) of these three metabolic steps (CGLUT4/HK = 0.55 ± 0.10, CGlycogen Syn = 0.30 ± 0.06, and CGlycogen Syn = 0.15 ± 0.02; where J is glucose disposal flux, and glycogen syn is glycogen synthesis) indicate that there is shared control of glucose disposal and that glucose transport/phosphorylation is responsible for the majority of control of insulin-stimulated glucose disposal in skeletal muscle.

EXPERIMENTAL PROCEDURES

Animals. Sprague-Dawley rats (Charles River, Raleigh, NC) were housed in an environmentally controlled room with a 12:12-h light-dark cycle. At a weight of 300–350 g, rats were chronically catheterized as described elsewhere (33) and allowed to recuperate for another 5–10 days. In vivo experiments. All rats were fasted 24 h before the infusion experiment. Rats were placed in a customized restraining tube that allowed their left hindlimb to be secured...
to the outside of the tube in a manner to limit free movement of the leg for NMR measurements. The rats were transiently anesthetized (<30 s) with a low dose (2.5–5.0 mg) of thiopental (Sigma) to place them in the restraining tube. One of three infusion protocols was started: protocol I was a euglycemic (~6 mM)-hyperinsulinemic (10 mU·kg⁻¹·min⁻¹; Humulin Regular, Eli Lilly) clamp, with 20% dextrose administered at 2.5 min after the commencement of the primed-continuous insulin infusion (n = 10 rats for ¹³C-NMR and n = 6 for ³¹P-NMR); protocol II was a hyperglycemic (~11 mM)-hyperinsulinemic (10 mU·kg⁻¹·min⁻¹) clamp with somatostatin (1 µg·kg⁻¹·min⁻¹; n = 10 for ¹³C-NMR and n = 6 for ³¹P-NMR); and protocol III was a euglycemic (~6 mM)-hyperinsulinemic (10 mU·kg⁻¹·min⁻¹)-lipid/heparin (Liposyn II, Abbott; 1:3 vol/vol saline at 39 µl/min with heparin at 0.0975 IU/min) clamp (n = 9 for ¹³C-NMR and n = 5 for ³¹P-NMR). [¹-¹³C]glucose (99% enriched, 20% wt/vol; Cambridge Isotope Laboratories, Cambridge, MA) was used during the ¹³C-NMR experiments. The glycolytic and glycogen synthesis flux measurements in protocols I–III originated from our previously published work (12–14). All clamps lasted for 180–240 min. Blood samples were drawn during the baseline NMR measurement, at 7.5 min, at 15 min, and every 15 min thereafter for immediate assessment of plasma glucose and lactate concentrations. At the end of the in vivo NMR experiment, rats were euthanized with thiopental (50 mg/kg). Superficial skin was rapidly removed from the left side of the leg for NMR measurements. The rats were transiently anesthetized with excess perchlorate ions. The sample was centrifuged once for saturation and nuclear Overhauser effect contributions. The concentration of muscle [¹³C]glycogen fractional enrichments were determined with the use of the precipitated glycogen from the initial muscle perchloric acid extraction (99% recovery) (13), and absolute glycogen concentrations were measured on a separate portion of muscle (17).

Analytic procedures. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma immunoreactive free insulin was measured with a double-antibody RIA technique (Linco Research, St. Charles, MO). Plasma inactive free insulin was measured with a double-antibody RIA (²²Na assay; Sigma Chemical, St. Louis, MO) modified for tissue analysis. Total creatine concentrations in muscle were measured by ¹³C-NMR analysis performed on a Bruker AM 500 NMR spectrometer system, for which the total [¹³C]creatinine peak intensity [54.4 parts per million (ppm)] was referenced to the [¹³C]acetate peak (24.2 ppm), which was added as an internal concentration standard. Spectra were acquired with a repetition time of 1.4 s, number of scans of 10,000, 16 kilobytes of data, and WALTZ-16 broadband proton decoupling. Peak intensities were corrected for saturation and nuclear Overhauser effect contributions.

Plasma free fatty acids (FFAs) were determined with the use of an acyl-CoA oxidase-based colorimetric kit (Wako NEFA-C; Wako Pure Chemical Industries, Osaka, Japan). Plasma lactate concentrations were measured with the use of a 2300 Stat Plus lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Glycogen synthesis flux calculation. The incremental change in C-1 glycogen peak intensity from [¹-¹³C]glucose incorporation...
tion was measured at 100.5 ppm. Incremental plasma [13C]glucose fractional enrichment as well as final [13C]glucose enrichment and concentrations were used to back extrapolate the glycogen concentration (in μmol/g, which represents μmol glucose units/g muscle wet wt) at each measured time point to baseline, as described by Bloch et al. (3). Glycogen synthesis rates were determined with the use of a linear regression analysis over the individual-time-point glycogen concentrations.

Glycolytic flux calculations. Metabolic steady-state conditions were assumed for the calculation of carbon flux through the glycolytic pathway into the intermediate triose pool of lactate, pyruvate, and alanine. We have previously shown (12, 13) that these intermediates are at steady-state concentrations before and after an euglycemic- or hyperglycemic-hyperinsulinemic clamp. The 13C label incorporation from [1-13C]glucose into [3-13C]lactate and [3-13C]alanine in the hindlimb muscles was observed by 13C-NMR as an indirect marker of pyruvate labeling. Label incorporation into lactate and alanine is a qualitative indicator of glycolytic flux. Differential equations were developed from steady-state rate equations and solved for glycolytic flux (12).

MCA. A brief introduction to MCA theory is described in the appendix. A top-down approach to MCA was used. In doing so, numerous enzymes associated with a particular metabolic step or pathway can be lumped together so that control coefficients for those groups of enzymes can be determined. In this manner, we determined the control of glucose disposal distributed between glucose transport through insulin-stimulated GLUT-4 and phosphorylation (GLUT-4/HK); glycogen synthesis, and glycolysis) in skeletal muscle. J, glucose disposal flux; Vglycogen syn, glycogen synthesis rate; Vglycolysis, glycolytic flux; Glcex, extracellular glucose; G-6-P, glucose 6-phosphate; Ala, alanine; Pyr, pyruvate; Lac, lactate.

We measured the elasticities eglycogen syn and eglycolysis by varying G-6-P concentration ([G-6-P]) indirectly via a hyperinsulinemic clamp at different glycemia levels (i.e., euglycemic and hyperglycemic). We thereby maintained control of insulin, which is a potent regulator of HK-II expression and a covalent modulator of glycogen synthase in all three protocols. Concentrations of known allosteric effectors such as ATP, ADP, Mg2+, pH, and P, were measured with the use of in vivo 31P-NMR. To measure the elasticity of GLUT-4/HK to G-6-P, we maintained external glucose homeostasis as substrate for GLUT-4/HK. It is possible to measure eG-6-P if we can modulate [G-6-P] indirectly by inhibition of glycolysis downstream, for example by increasing lipid oxidation while maintaining external glucose concentrations. This was accomplished with the use of the lipid/heparin infusion. Therefore, we maintained euglycemic-hyperinsulinemic conditions and indirectly modulated [G-6-P] simultaneously. A similar scheme with the use of ketones was used to obtain control coefficients for glucose disposal in heart (16). The control coefficients were calculated by solving the above equations simultaneously.

Statistical analysis. All data are reported as means ± SE. Single-factor ANOVA was performed on data to determine significance at a minimum P < 0.05 threshold among the three protocols. A multiple-comparison Fisher’s protected least significant difference post hoc test was used when necessary to determine significance among protocols. Error analysis of control coefficients was calculated with the use of the SE in the flux and [G-6-P] measurements.

RESULTS

Basal measurements. Basal plasma concentrations of glucose, insulin, and FFA were similar in all three protocols (Table 1).

Protocol I. During the euglycemic-hyperinsulinemic clamp experiment, the plasma glucose concentration was maintained at ~6 mM throughout the study, and the plasma insulin concentration increased to 206 ± 43 μU/ml, whereas plasma FFA decreased to 0.4 ± 0.1 mM (Table 1). The glucose infusion rate was stable throughout the clamp period (31.6 ± 2.9 mg·kg⁻¹·min⁻¹ at 120 min; Table 2). The ATP and total creatine concentrations were 5.1 ± 0.3 and 31.1 ± 7.1 μmol/g, respectively.

Protocol II. During the hyperglycemic-hyperinsulinemic clamp experiment, the plasma glucose concentration increased and was maintained at ~11 mM through-

Table 1. Plasma glucose, insulin, and FFA concentrations in 3 protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Glucose, mM</th>
<th>Insulin, μU/ml</th>
<th>FFA, mM</th>
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<tbody>
<tr>
<td>Protocol I</td>
<td>0 min</td>
<td>6.4 ± 0.2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>6.3 ± 0.3</td>
<td>206 ± 43</td>
</tr>
<tr>
<td>Protocol II</td>
<td>0 min</td>
<td>6.0 ± 0.2</td>
<td>14 ± 3</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>11.3 ± 0.7*</td>
<td>201 ± 35</td>
</tr>
<tr>
<td>Protocol III</td>
<td>0 min</td>
<td>5.8 ± 0.4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>5.9 ± 0.2</td>
<td>229 ± 12</td>
</tr>
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</table>

Data are means ± SE and were obtained from both 13C- and 31P-NMR experiments. Protocol I, euglycemic-hyperinsulinemic clamp; protocol II, hyperglycemic-hyperinsulinemic clamp; protocol III, euglycemic-hyperinsulinemic-lipid/heparin clamp. FFA, free fatty acids. *P < 0.001 vs. protocols I and III at 120 min. †P < 0.0005 vs. protocols I and II at 120 min.
out the study, and the plasma insulin concentration increased to 279 ± 68 µU/ml, whereas plasma FFA decreased to 0.2 ± 0.1 mM (Table 1). The glucose infusion rate was stable throughout the clamp period (49.7 ± 1.0 mg·kg⁻¹·min⁻¹ at 120 min; Table 2). The ATP and total creatine concentrations were 4.9 ± 0.1 and 27.6 ± 2.2 µmol/g, respectively.

Protocol III. During the euglycemic-hyperinsulinemic-lipid/heparin clamp experiment, the plasma glucose concentration was maintained at ~6 mM throughout the study, and the plasma insulin concentration increased to 229 ± 12 µU/ml, whereas the plasma FFA increased to 2.3 ± 0.4 mM as a result of the lipid/heparin infusion (Table 1). The glucose infusion rate decreased throughout the duration of the clamp period and was 24.0 ± 1.5 mg·kg⁻¹·min⁻¹ at 120 min (Table 2). The ATP and total creatine concentrations were 5.2 ± 0.1 and 27.8 ± 2.2 µmol/g, respectively.

In vivo 13C-NMR. Figure 2A illustrates a 15-min acquired baseline spectrum (bottom) and a spectrum taken at 120 min (top), when significant 13C label incorporation into metabolite intermediates was achieved. The β- and α-anomer peaks of [1-13C]glucose appear at 96.8 and 93.0 ppm, respectively, and the large peak slightly downfield at 100.5 ppm corresponds to the C-1 glucosyl unit of the glycogen polymer. [3-13C]lactate and [3-13C]alanine can also be observed at 21.0 and 16.9 ppm, respectively.

The glycolytic flux and glycogen synthesis rate measurements shown in Table 2 originated from our previ-
ously published work (12–14). From these results, it is evident that the decreased glucose disposal observed during the euglycemic-hyperinsulinemic-lipid/heparin clamp was primarily the result of an ~69% inhibition of glycolysis vs. that of the euglycemic-hyperinsulinemic clamp. The increased glucose disposal during the hyperglycemic-hyperinsulinemic clamp, however, was primarily due to a large increase in glycogen synthesis (> ADP, Mg2+ respectively (see APPENDIX). From these elasticity measurements, we were able to calculate the control coefficients by solving the three equations described above (i.e., a change in substrate and/or external effector amount or change in enzyme expression). In this manner, a system of intermediary steps can be analyzed as a whole to determine fractional contribution of any one step to the overall control of flux through a metabolic pathway. Because we cannot obtain relative information with regard to the way in which each individual enzyme in a metabolic pathway reacts to variables affecting it (bottom-up approach) from NMR metabolic flux measurements, we used a top-down approach to MCA, in which we can define control over only a few metabolic components, each consisting of a number of enzymes (e.g., glucose transport/phosphorylation, glycogen synthesis, and glycolysis). In our studies, three separate infusion protocols were necessary to indirectly modulate [G-6-P] to measure the elasticities of these three metabolic components to G-6-P (i.e., \( \epsilon_{G-6-P}^{GLUT-4/HK} \), \( \epsilon_{G-6-P}^{glycogen-syn} \), and \( \epsilon_{G-6-P}^{glycolysis} \)).

It was necessary to maintain concentrations of known allosteric effectors and cofinal modulation of enzymes that make up these pathways. Because the \(^{13}\)C-NMR measurement of glycolytic flux at euglycemia might not be possible (12), we maintained hyperinsulinemic conditions in all three groups, and no significant differences among groups with respect to concentrations of ADP, Mg2+, \( P_i \), or pH were measured (Table 3). Although it has been shown that an acute elevation of plasma FFA can directly regulate insulin signaling or glucose transporter function, these events generally occur only after 3–5 h (9, 23). Additionally, we (13) and others (9) have shown that [G-6-P] values remained elevated during the initial 3–4 h of clamp before elevated plasma FFA could affect insulin signaling or glucose transporter function and result in a decrease in [G-6-P]. Therefore, with proper control of the physiological effectors of these metabolic pathways, one can couple MCA to in vivo metabolic studies in skeletal muscle.

MCA is a logical extension of traditional studies that attempted to define a rate-limiting enzyme in the pathway but were unsuccessful. For example, in heart, when intracellular glucose was not detected as extracellular concentrations varied from 2 to 16 mM, this led to

**DISCUSSION**

MCA theory was developed as an amalgamation of independent work in the early 1970s by Kacer and Burns (15) and Heinrich and Rapoport (10). This theory was formulated to determine how the intermediate steps of a pathway react to a variable that might affect it (i.e., a change in substrate and/or external effector amount or change in enzyme expression). In this manner, a system of intermediary steps can be analyzed as a whole to determine fractional contribution of any one step to the overall control of flux through a metabolic pathway. Because we cannot obtain relative information with regard to the way in which each individual enzyme in a metabolic pathway reacts to variables affecting it (bottom-up approach) from NMR metabolic flux measurements, we used a top-down approach to MCA, in which we can define control over only a few metabolic components, each consisting of a number of enzymes (e.g., glucose transport/phosphorylation, glycogen synthesis, and glycolysis). In our studies, three separate infusion protocols were necessary to indirectly modulate [G-6-P] to measure the elasticities of these three metabolic components to G-6-P (i.e., \( \epsilon_{G-6-P}^{GLUT-4/HK} \), \( \epsilon_{G-6-P}^{glycogen-syn} \), and \( \epsilon_{G-6-P}^{glycolysis} \)).

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<table>
<thead>
<tr>
<th>Table 3. (^{31})P-NMR data</th>
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<tbody>
<tr>
<td>Protocol I</td>
</tr>
<tr>
<td>0 min: 150±11 ( \mu\text{mol/g} )</td>
</tr>
<tr>
<td>120 min: 217±8* ( \mu\text{mol/g} )</td>
</tr>
<tr>
<td>Protocol II</td>
</tr>
<tr>
<td>0 min: 148±2 ( \mu\text{mol/g} )</td>
</tr>
<tr>
<td>120 min: 265±12 ( \mu\text{mol/g} )</td>
</tr>
<tr>
<td>Protocol III</td>
</tr>
<tr>
<td>0 min: 140±17 ( \mu\text{mol/g} )</td>
</tr>
<tr>
<td>120 min: 251±9 ( \mu\text{mol/g} )</td>
</tr>
</tbody>
</table>

Data are means ± SE and were obtained from \(^{31}\)P-NMR experiments only. G-6-P, glucose 6-phosphate; PCR, phosphocreatine. *P < 0.005 vs. protocol II and P < 0.05 vs. protocol III.
the belief that glucose transport was rate limiting for glucose disposal (24). In recent heart work, MCA was used to illustrate that the control of glucose uptake is distributed among glucose transport and phosphorylation, glycogen synthesis, and glycolysis (16). Additionally, the distribution of control was shown to change, depending on substrate availability or hormonal environment.

In skeletal muscle, it has been suggested that the glycogen synthase enzyme controls the rate of glycogen synthesis (4, 21). On the other hand, insulin stimulation has been shown to increase glucose transport via an increase in GLUT-4 translocation to the cell membrane (19). The glycogen synthase enzyme is additionally regulated by insulin by reduction of its degree of phosphorylation. With the unique capability to measure net glycogen synthesis flux and intermediate substrate (G-6-P) by NMR and with the use of a top-down approach, it was shown that glucose transport/phosphorylation could indeed account for almost the entire control of insulin-stimulated glycogen synthesis in normal or diabetic humans (27, 31). In these analyses, control of glycogen synthesis was distributed over glucose transport/phosphorylation and glycogen synthesis only while glycolysis was neglected. This was thought reasonable in light of whole body extrapolation of skeletal muscle glycogen synthesis rates determined by NMR that suggested that glycogen synthesis in skeletal muscle accounts for ~90% of the whole body glucose metabolism under hyperglycemic-hyperinsulinemic conditions (32).

However, through MCA, it has been theoretically shown that glycolytic enzymes do indeed contribute to the control of [G-6-P] (30). Additionally, in rat, glycolysis accounts for a significant portion of the glucose disposal in muscle under euglycemic-hyperinsulinemic conditions (18, 28). Therefore, the potential for glycogen synthesis and glycolysis to account for a significant fraction of control in glucose disposal is evident.

The control of glucose transport/phosphorylation when partitioned into individual flux components of glucose transport and HK activity can be determined if elasticities of these flux components to intracellular glucose are known. We have previously determined that intramuscular glucose is negligible in rat during a hyperglycemic-hyperinsulinemic clamp by using an NMR spectroscopic assay (5), and, therefore, it would most likely be negligible during a euglycemic-hyperinsulinemic clamp. Consequently, we would not have the NMR sensitivity to detect the required modulation of intracellular glucose necessary for calculating elasticities in our experiments. Hypothetically, if intracellular glucose were negligible during both euglycemic- and hyperglycemic-hyperinsulinemic clamps, then, with the use of a bottom-up approach to MCA in which known enzyme kinetic parameters are used, the control of HK would be negligible as well (29).

Insulin sensitivity varies with skeletal muscle fiber composition (11); thus there is the potential for differences in the distribution of control in slow- vs. fast-twitch muscle fibers. For example, it has been shown that the regulation of glycogen synthase is different in fast- vs. slow-twitch muscle after a glucose load (34). Because of the intrinsic sensitivity of the in vivo NMR measurement, it is not possible to make glycolytic and glycogen synthesis flux measurements in small muscles such as soleus and extensor digitorum longus. Therefore, the hindlimb placement over the NMR-sensitive volume of the radio frequency (RF) coil was such that the NMR signal obtained was predominantly from the larger mixed fiber tissue composing the gastrocnemius and biceps femoris muscles. Additionally, it must be noted that the distribution of control might vary depending on the physiological conditions (16, 30).

In conclusion, we have presented a study that takes unique advantage of the in vivo NMR measurements of glycogen synthesis and glycolytic flux and [G-6-P] to apply a top-down analysis of MCA to determine the control of insulin-stimulated glucose disposal in skeletal muscle distributed among glucose transport/ phosphorylation, glycogen synthesis, and glycolysis. It was determined that during insulin stimulation, the majority of control resides at glucose transport/phosphorylation, although glycogen synthesis and glycolysis do share in the control of glucose disposal as well. This approach might be useful in characterizing the control of these pathways under conditions of insulin resistance and/or diabetes.

**APPENDIX**

**MCA Theory**

The following is a brief description of MCA theory (15). The flux control coefficient is defined by the ratio of fractional change of the flux through an enzyme in the pathway to fractional change in enzyme concentration (E)

$$C_a = \frac{\delta J}{J} / \frac{\delta E_a}{E_a}$$

where J is the flux through enzyme a, and $C_a$ is the control coefficient for that enzyme. All of the control coefficients of a pathway are expressible in terms of elasticities. Elasticities are properties of individual enzymes that relate velocities of

<table>
<thead>
<tr>
<th>Protocol</th>
<th>$\delta V_{GLUT-4HK}$</th>
<th>$\delta V_{glycolysis}$</th>
<th>$\delta V_{glycogen-syn}$</th>
<th>$\delta G-6-P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I vs. II</td>
<td>0.15</td>
<td>0.79</td>
<td>0.22</td>
<td>0.69</td>
</tr>
<tr>
<td>I vs. III</td>
<td>-0.34</td>
<td>-2.18</td>
<td>-0.16</td>
<td>3.56</td>
</tr>
</tbody>
</table>

GLUT-4, glucose transporter isoform-4; HK, hexokinase; $V_{glycolysis}$, glycolytic flux; $V_{glycogen-syn}$, glycogen synthesis; $\epsilon$, elasticity.
enzymes with their substrate concentrations in a relationship similar to control coefficients

$$e_{S}^a = \frac{\partial v_{a}}{\partial S} \bigg/ \frac{v_{a}}{S}$$

where $v_{a}$ is the velocity of enzyme $a$, which is equal to the flux $J$ through the enzyme in a steady-state system, $S$ is the substrate for enzyme $a$, and $e_{S}^a$ is the elasticity coefficient for that enzyme.

All of the enzymes or metabolic pathways that can affect the flux through a metabolic system share control of that flux; thus, via the summation theorem, the sum of all of the coefficients equals one, i.e., $\sum_{j} e_{S}^{j} = 1$. If a metabolite is both a substrate and product to separate enzymes in a two-enzyme system, then the use of the connectivity theorem to relate the flux control coefficients to the kinetic properties of the enzyme gives us

$$C_1 e_{S}^1 + C_2 e_{S}^2 = 0$$

For this relationship to hold, all other allosteric effectors of the enzymes must remain constant. Additionally, the branch point theorem (7) states that the ratio of the sum of the flux control coefficients of enzymes in the branches is equal to the ratio of flux through the branches

$$\frac{C_1}{C_2} = \frac{v_1}{v_2}$$

Therefore, because the control coefficients of a pathway are expressible in terms of elasticities, relative fluxes, and substrate concentrations, we can use all of the above theorems together to indirectly calculate control coefficients. This can be accomplished by calculating simultaneous equations for the entire system of enzymes representing a metabolic pathway. Because we cannot define the entire system of enzymes contributing to the flux of a pathway measured with our NMR flux measurements, we must use a top-down approach whereby we assign control coefficients to blocks of enzymes representing a metabolic pathway. General properties, control and\r
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REFERENCES

1. Altura, B. M., A. Gembewold, A. Zhang, B. T. Altura, and R. K. Gupta. Short-term reduction in dietary intake of magne-

sium causes deficits in brain intracellular free Mg\textsuperscript{2+} and [H\textsuperscript{+}], but no high-energy phosphates as observed by in vivo \textsuperscript{31}P-NMR. Biochim. Biophys. Acta 1358: 1-5, 1997.


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We are indebted to Veronika Walton, Laura Burdon, and Kevin Cadman for expert technical assistance. We are grateful to electrical engineers Terry Nixon and Scott Mcintyre for NMR technical improvements and Peter Brown for radio frequency antenna design and construction. We also thank Dr. Douglas L. Rothman for helpful discussions on MCA.

This study was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (RO1-DK-40936 and P30-DK-45735), the American Diabetes Association (Mentor-Based Diabetes and Digestive and Kidney Diseases (RO1-DK-40936 and P30-DK-45735), the American Diabetes Association (Mentor-Based Postdoctoral Fellowship to B. M. Jucker), and an unrestricted grant from Bristol-Myers Squibb.

G. I. Shulman is an investigator for the Howard Hughes Medical Institute.

Address for reprint requests and other correspondence: B. M. Jucker, Dept. of Internal Medicine, Yale Univ. School of Medicine, PO Box 208020, Fitzkin 1, 333 Cedar St., New Haven, CT 06520-8020 (E-mail: Jucker@mrclin1.med.yale.edu).

Received 9 November 1998; accepted in final form 21 April 1999.

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