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COMPUTER SIMULATION OF METABOLISM IN PALMITATE-PERFUSED RAT HEART. III. SENSITIVITY ANALYSIS

Michael C. Kohn

Department of Computer and Information Science
Moore School of Electrical Engineering
University of Pennsylvania
Philadelphia, Pennsylvania

The behavior of a computer model of metabolism in glucose- and palmitate-perfused rat hearts was interpreted by sensitivity analysis to explain why the heart preferentially utilizes fatty acids as fuel even in the presence of substantial exogenous glucose. The sensitivity functions identified those metabolites and enzymes which were most important in regulating the metabolic rate and determined which enzymes set the levels of the critical metabolites. Control of the mitochondrial redox potential and the distribution of coenzyme A thioesters regulated the rate of fatty acid utilization while strong inhibition of citrate synthetase resulted in accumulation of acetyl CoA and suppression of pyruvate oxidation. Glycolysis was limited by the cytosolic ATP/ADP ratio set largely by the creatine shuttle. Metabolic control appears to be widely distributed rather than localized at "key" enzymes. Metabolite levels are usually set by enzymes controlled by modifiers whereas metabolic flux is regulated by the enzymes that produce ligands for the modifier-controlled enzymes.

Keywords *— Sensitivity analysis, Metabolic regulation, Fatty acid utilization, Glucose utilization.*

In the previous two papers in this series (9,10) we described the construction and pseudostationary-state behavior of a computer model of an isolated rat heart perfused with glucose- and palmitate-containing buffer. The experimenters who collected the data on which this model is based (12,15,16) concluded that palmitate utilization was limited by the rate of acetyl CoA oxidation in the citric cycle (12,15). They interpreted the low tissue contents of 2-ketoglutarate and succinyl CoA as due to rate limitation of substrate oxidation at isocitrate dehydrogenase.

Address correspondence to Michael C. Kohn, Department of Physiology, Box 3709, Duke University, Medical Center, Durham, NC 27710.

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Our model suggests (10) that the regulation of fuel utilization in this experiment is much more complex. Control of the metabolism appears to be distributed among a number of enzymes; even the equilibrium enzyme aconitase has an important role. Thus, examination of tissue contents of metabolites may not reveal enough detail to identify the origins of metabolic regulation in this large system. In this work we apply sensitivity analysis to identify which metabolites are most important in regulating the rate of fatty acid utilization, to determine which enzymes are most important in setting the levels of the critical metabolites, and to explain why the heart preferentially utilizes fatty acids as fuel even in the presence of substantial exogenous glucose.

Trajectory sensitivity functions (3) are commonly derived by expanding the equations for the time derivatives of the state variables as functions of certain parameters in a Taylor series about the nominal parameter values. Because only infinitesimal perturbations of the parameter values are considered, the Taylor series can be truncated after the linear terms and rearranged to give a set of first-order differential equations for the sensitivity functions. At steady state the time derivatives of the state variables are zero, and the differential equations for the sensitivity functions reduce to linear algebraic equations. The present sensitivity analysis, a generalization (8) of the metabolic control theory of Kacser and Burns (6) and Heinrich and Rapoport (5), is entirely analogous to the standard approach; the state variables are metabolite concentrations (c_i) and the parameters are enzyme concentrations (e_k). The only mathematical difference is that the sensitivities are calculated as relative sensitivity functions, e.g., $(\partial c_i/\partial e_k) \cdot (e_k/c_i)$, to facilitate comparison of different metabolic states.

The enzyme concentrations in our model are determined by experiments other than the one we are modeling, and while they formally fulfill the role in the sensitivity analysis usually played by parameters, they are constants and *not* parameters in our model. Therefore, the common use of sensitivity analysis to assess the robustness of a model, i.e., to predict the change in state consequent to a finite alteration in the value of some parameter, is not intended here. The fundamental tenet of the present approach is that the sensitivity functions quantitate the instantaneous distribution of metabolic control among the system's constituent enzymes. These functions are specified by the reaction rate laws included in the model and are evaluated for the metabolic state reproduced by the model. Therefore, they identify the origins of metabolic regulation in the experimental preparation.

CONTROLLERS OF FATTY ACID UTILIZATION

Virtually every pathway in the catabolism of fatty acids has at some time been identified as the major regulator of fatty acid utilization. Normann and Flatmark (12) found fatty acid activation to be rate limiting. This is supported by the finding of Oram et al. (16) that cytosolic nonesterified coenzyme A (CoASH) never exceeds its K_m for acyl CoA synthetase in rat heart and that the rate of

activation depends on the availability of CoASH. Pande (18), on the other hand, interpreted his experimental data as indicating that acyl CoA synthetase is not rate limiting. Oram et al. (15) concluded that fatty acid uptake or activation is limiting only at low extracellular fatty acid concentrations.

Rate limitation of fatty acid utilization at "high" rates of oxidative phosphorylation was attributed to translocation of acyl groups across the mitochondrial membrane (14,15). The "inner" isozyme of carnitine acyltransferase was identified as the most likely site of regulation. However, Pande's (18) data apparently exclude this possibility, and Bremer (1) determined that the maximal activity of carnitine acyltransferase in mitochondria exceeds the capacity to oxidize acyl CoA.

Pande (18) concluded that fatty acid utilization was limited by the ability to produce acetyl CoA by β-oxidation. Fong and Schulz (4) identified 3-ketoacyl CoA thiolase as the limiting enzyme, but Pande's conclusion was disputed by Bremer and Davis (2). The data of Whitmer et al. (20) indicate that β-oxidation is limiting in anoxic but not in aerobic hearts.

At low work load, control of the rate of fatty acid utilization was attributed to the oxidation of acetyl CoA in the citric acid cycle (15). The shift of CoA moieties from CoASH and succinyl CoA to acetyl CoA with increasing external palmitate concentration was identified as the source of the rate limitation. In addition, these changes in mitochondrial coenzyme A levels affect the rate of fatty acid activation (16). The mitochondrial CoASH/acetyl CoA ratio affects the carnitine acetyltransferase equilibrium, which Oram et al. (15,16) conclude is important in coupling the citric acid cycle activity to fatty acid uptake and activation.

In their computer simulation of the citric acid cycle in heart mitochondria, McMinn and Ottaway (11) showed by sensitivity analysis that control of cycle flux was distributed among a number of enzymes. The most important controller was citrate synthetase. As the cycle flux increased in their simulations, Ottaway and McMinn (17) found that a major share of the control shifted to the electron transport chain. In all cases regulation of the distribution of coenzymes, including CoASH and its thioesters, among their various forms, was the crucial factor. The results of our simulation (10) are in agreement with these findings.

These disparate observations suggest that control of fatty acid utilization is not localized, is distributed, and depends strongly on the biochemical state of the tissue. As there are degrees of workload, hypoxia, etc., there must be smooth changes in the distribution of enzymatic control as the state of the tissue changes. Sensitivity analysis of a model can quantitate the distribution of control of the metabolism.

SENSITIVITY FUNCTIONS

Because the distribution of coenzymes has been implicated in the control of this metabolism, we need to compute indices of control of metabolite levels as well as indices of control of pathway flux. We also must consider the effects of

the slow nonenzymatic reactions (e.g., molecular diffusion through the interstitial space) and the fast spontaneous equilibria (e.g., nucleotide chelation) included in our model. Regulation of such a system is summarized by the following relative sensitivity functions. Their derivations and properties have been described elsewhere (8).

The *effector index* is the *relative* change in the flux v_k through an *isolated* reaction consequent to a *fractional* change in a metabolite concentration c_j:

$$E^e_{jk} = \frac{\partial v_k / v_k}{\partial c_j / c_j}, \tag{1}$$

where a superscript e denotes an enzymatic reaction, and a superscript s would denote a slow nonenzymatic reaction. Because fast equilibria always have zero net flux, the corresponding semirelative effector index

$$E^f_{jk} = \frac{\partial v_k}{\partial c_j / c_j} \tag{2}$$

is used to avoid division by zero. [As only ratios of such quantities are involved in this sensitivity analysis (see below), the "missing" velocity would cancel anyway.] The above quantities reflect the control exerted by reactants or modifiers on a given chemical reaction.

Suppose metabolites i and j appear in the same spontaneous equilibrium. If the concentration of i (c_i) is perturbed, the concentration of j (c_j) will change in a negligible period of time (compared to the rates of the other chemical reactions) so as to restore the equilibrium. If chemical j participates in a slower reaction, the perturbation in c_i will "instantaneously" induce a change in the flux through the slow reaction because of its effect on c_j. We define the *induced effector index* as the relative change in reaction rate consequent to such a perturbation:

$$I^e_{ik} = \sum_{j=1}^{n} \frac{\partial v_k / v_k}{\partial c_j / c_j} \cdot \frac{\partial c_j / c_j}{\partial c_i / c_i} = -\sum_{j=1}^{n} E^e_{jk} \sum_{q=1}^{N_f} \frac{E^f_{iq}}{E^f_{jq}}, \tag{3}$$

where the superscript e denotes an enzymatic reaction, a superscript s would indicate a slow nonenzymatic reaction, n is the number of metabolites, and N_f is the number of fast equilibria.

The *regulation index* of an enzyme is the relative change in a metabolite concentration consequent to a fractional change in that enzyme's concentration, e_k, in a *multienzyme* system:

$$R_{jk} = \frac{\partial c_j / c_j}{\partial e_k / e_k}. \tag{4}$$

Unlike effector indices which reflect the affinities of the enzymes for their ligands, regulation indices are properties of the total system. These quantities tell which enzymes cooperate in the regulation of a given metabolite level.

Any metabolic system may be considered as being composed of one or more intricately connected pathways (e.g., glycolysis, β-oxidation, and the citric acid cycle in the present simulation). The net flux (v_{net}) through a given pathway is the net rate of production of an end product (e.g., lactate, acetyl CoA, and CO_2 for the above three pathways). The *acceleration index* of a metabolite is the relative change of pathway net flux consequent to a fractional change in that metabolite's concentration:

$$A_j = \frac{\partial v_{net}/v_{net}}{\partial c_j/c_j}. \tag{5}$$

The *control index* of an enzyme is the relative change in pathway net flux consequent to a fractional change in that enzyme's concentration:

$$C_k = \frac{\partial v_{net}/v_{net}}{\partial e_k/e_k}. \tag{6}$$

The control indices are complicated functions of the acceleration indices.

The acceleration and control indices may have different values for each pathway in the system. So different groups of enzymes may regulate metabolism in different pathways. On the other hand, if two pathways use the same cofactors (e.g., CoASH, ATP), the enzymes which regulate these cofactor levels may have high control indices for both pathways.

The various sensitivity functions were calculated for the pseudo-steady state predicted by our model (9,10), using a computer program (8) named MENSA (*ME*tabolic *N*etwork *S*ensitivity *A*nalysis). This program shares several subroutines with our simulator program, whose output serves as the input to MENSA. This sensitivity analysis does not require any mathematical or computer expertise from the modeler, who only needs to supply a list of end products for the pathways to be analyzed. The above relative sensitivity functions are ultimately dependent on the affinities of the enzymes for their ligands. Indeed they are complicated functions of the effector indices. Thus, the results of such a sensitivity analysis reflects the control of a complex multienzyme system by the properties of its individual constituent enzymes.

CONTROL OF METABOLITE LEVELS

Effector Indices

A representative sample of the computed effector indices is given in Table 1. The effector index is positive for a substrate or activator and negative for a

TABLE 1. Effector indices and induced effector indices for selected enzymes.

Enzyme*	Metabolite†	Effector Index	Induced Effector Index
ACS	Fatty acid	0.108	
	$MgATP^{2-}$	0.050	
	CoASH	0.821	
	AMP^{2-}	− 0.074	
	$MgPP_i^{2-}$	− 0.089	
	Acyl CoA	− 0.097	
ECH	Enoyl CoA	60.6	
	3-Hydroxyacyl CoA	−60.6	
ICDH	ADP^{3-}	0.362	
	ATP^{4-}	− 0.250	
	Mg isocitrate	2.23	
	NAD	0.049	
	NADH	− 0.004	
HACDH	ATP^{4-}		−0.111
	$ATPH^{3-}$		−0.132
	ADP^{3-}		−0.111
	$ADPH^{2-}$		0.164
	AMP^{2-}		−0.111
	$AMPH^{-}$		−6.22

*Abbreviations: ACS, acyl CoA synthetase; ECH, enoyl CoA hydratase; ICDH, NAD-linked isocitrate dehydrogenase; HACDH, 3-hydroxyacyl CoA dehydrogenase.
†Metabolites are in the mitochondrial compartment for all enzymes except ACS, for which metabolites are cytosolic.

product (if the enzyme is reversible) or inhibitor. For irreversible enzymes exhibiting hyperbolic kinetics, the effector index approaches unity below the K_m (or K_i) of the ligand and approaches zero as the ligand approaches saturating concentrations. For irreversible enzymes exhibiting sigmoidal kinetics, the effector index exceeds unity at ligand concentrations much less than the K_m (or K_i), is close to unity near the K_m, and approaches zero as the ligand becomes saturating. This latter behavior is shown by Mg-isocitrate for isocitrate dehydrogenase (the substrate is subsaturating here), whereas the other ligands of this enzyme show hyperbolic kinetics.

Reversible enzymes show sigmoidal kinetics with the inflection point of the enzymatic velocity vs. concentration curve (Fig. 1) falling on the concentration axis at the equilibrium substrate concentration. The effector index, proportional to the slope of this curve at the corresponding ligand concentration, is very large near the inflection point and tends to zero as the ligand approaches either zero or saturating concentrations. The greater the tissue capacity of the enzyme, the less deviation from equilibrium is needed to "drive" the enzyme at a given rate. This is illustrated by the effector indices (Table 1) of acyl CoA synthetase and enoyl CoA hydratase, a low and a high tissue capacity enzyme, respectively.

Oxidation of 3-hydroxyacyl CoA by NAD at 3-hydroxyacyl CoA dehydrogenase proceeds with production of a hydrogen ion. Because the mitochondrial

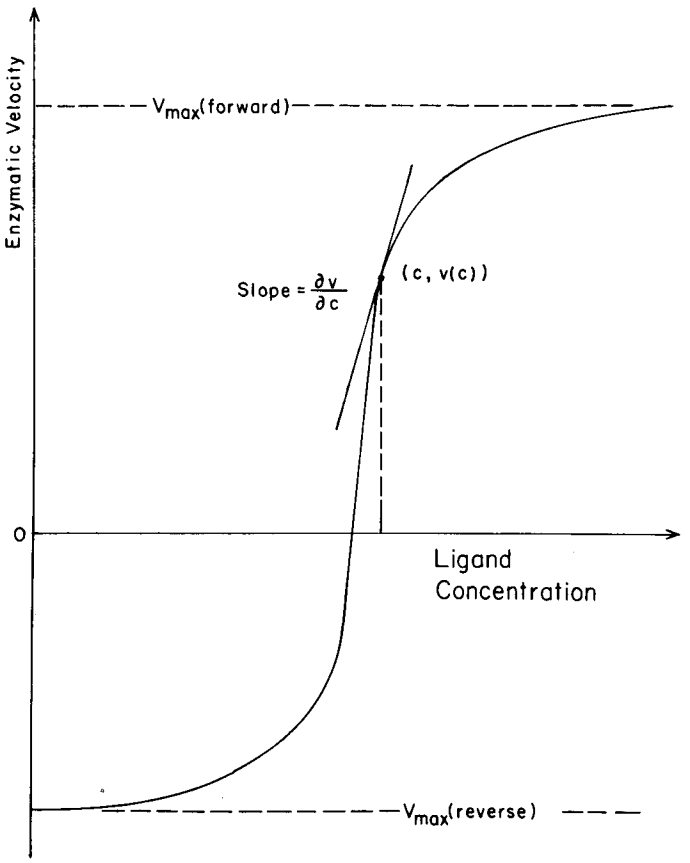

FIGURE 1. Enzymatic velocity vs. concentration of one substrate with the concentrations of all other substrate fixed. The slope of the curve equals the effector index of the varied substrate times the ratio of velocity to concentration.

adenine nucleotides participate in protonation and metal ion chelation equilibria, perturbations in the nucleotide levels would cause a change in pH (all other factors being held constant). This change would induce a change in the flux through the above enzyme. This behavior is reflected by the induced effector strengths listed in Table 1. Note that the magnitude of the effect increases with decreasing pK_a of the protonated nucleotide:

$$\text{AMPH } (pK_a = 6.4) > \text{ADPH } (pK_a = 6.8) > \text{ATPH } (pK_a = 7.0).$$

Regulation Indices

The computed regulation indices of the enzymes for intermediates in fatty acid metabolism, citric acid cycle, and glycolysis are given in Tables 2, 3, and 4, respectively. In order to scale the regulation indices for each pathway identically,

TABLE 2. Regulation of fatty acid metabolic intermediates. Normalized regulation indices.

	ACS	ACDH	CS	STK	SDH
Cytosol					
Fatty acids	−0.005	−0.083	−0.012	−0.022	−0.878
Triglycerides	1.000				
CoASH	−0.005	−0.083	−0.012	−0.022	−0.878
Acyl CoA	0.005		0.013	0.024	0.958
Carnitine	−0.005	−0.083	−0.012	−0.023	−0.878
Acyl carnitine	0.005		0.013	0.023	0.960
Acetyl CoA	0.911		−0.506	−1.405	
Acetyl carnitine	−0.014		0.269	0.745	
Mitochondria					
CoASH	0.002	0.086	0.001	0.001	0.911
Acetyl CoA	0.001	0.085	0.002	0.006	0.905
Acyl CoA	0.004	0.083	0.011	0.019	0.883
Carnitine	−0.005		−0.013	−0.025	−0.957
Acyl carnitine	0.005		0.012	0.022	0.961
Acetyl carnitine	−0.082		0.296	0.787	
Enoyl CoA		0.086	−0.001	−0.002	0.917
3-Hydroxyacyl CoA		0.086	−0.001	−0.002	0.917
3-Ketoacyl CoA		0.086		−0.002	0.915
NADH	0.011	−0.093	0.026	0.044	−0.988
$CoQH_2$		−0.086			−0.914

*Unusual abbreviations: ACS, acyl CoA synthetase; ACDH, acyl CoA dehydrogenase; CS, citrate synthetase; STK, succinate thiokinase; SDH, succinate dehydrogenase.

the indices in each table have been normalized by dividing by the absolute value of the sum of the regulation indices for the particular metabolite in that table (i.e., divided by the magnitude of the row's sum). This facilitates comparing the shares

TABLE 3. Normalized regulation indices of citric acid cycle enzymes.*

	CS	Acon	ICDH	STK	SDH
CoASH	0.001	0.001		0.002	0.997
Acetyl CoA	0.003	0.002	−0.001	0.006	0.990
Citrate	−0.089	0.375	0.017	−0.081	0.777
Mg-citrate	0.586	0.402		−0.001	0.013
Isocitrate	0.478	0.399	0.003	−0.014	0.134
Mg-isocitrate	0.090	−0.377	−0.018	0.082	−0.777
2-Ketoglutarate	0.001	0.001		0.004	0.994
Succinyl CoA	0.002	0.002		0.005	0.992
Succinate	0.003	0.002		0.005	0.991
Fumarate	0.005	0.004	−0.001	0.004	0.988
Malate	0.005	0.004	−0.001	0.004	0.988
Oxaloacetate	0.006	0.005	−0.001	0.005	0.984
NADH	0.028	0.024	−0.006	0.049	−1.10
$CoQH_2$					−1.00

*Unusual abbreviations: CS, citrate synthetase; Acon, aconitase; ICDH, isocitrate dehydrogenase; STK, succinate thiokinase; SDH, succinate dehydrogenase.

TABLE 4. Normalized regulation indices of glycolytic enzymes.*

	HK	PFK	PGK	PK
Glucose	0.517	0.518	-1.02	-1.02
G6P	0.498	0.482	-1.00	-0.979
F6P	0.498	0.482	-1.00	-0.979
FDP	0.486	0.450	-0.980	-0.956
GAP	-0.501	-0.489	1.01	0.985
DHAP	-0.488	-0.467	0.996	0.959
1,3-DPG	0.516	0.516	-1.02	-1.02
3-PGA	0.516	0.516	-1.02	-1.02
PEP	0.516	0.516	-1.02	-1.02
Pyruvate	0.923	1.26	-1.33	-1.85
Lactate	0.516	0.516	-1.02	-1.02
NADH	0.521	0.526	-1.02	-1.03

*Unusual Abbreviations: HK, hexokinase; PFK, phosphofructokinase; PGK, 3-phosphoglycerate kinase; PK, pyruvate kinase.

in the control of their respective pathways by two particular enzymes. Enzymes with negligible regulation indices for the indicated metabolite are not listed.

Most of the regulation of fatty acyl intermediate levels (Table 2) is due to citric acid cycle enzymes. Increases in long-chain acyl intermediates occur at the expense of nonesterified fatty acids, carnitine, and cytosolic CoASH, which have negative regulation indices. Mitochondrial CoASH is regenerated in the citric acid cycle, and its positive regulation index shows that its concentration is not lowered consequent to a small increase in cycle activity. Succinate dehydrogenase, which largely controls the mitochondrial redox potential (Table 2), is the primary regulator of long-chain acyl intermediates. Because the electron transport chain is represented by three simplified net reactions in our model, rather than by a detailed enzymatic mechanism, the magnitude of the regulation indices of succinate dehydrogenase may be too great. If the enzymology of respiration were represented explicitly, those enzymes would share in the control and the regulation indices for succinate dehydrogenase might be lower. Acyl CoA dehydrogenase, which also utilizes coenzyme Q (CoQ), is the only β-oxidation enzyme that has a significant regulation index under the conditions of this simulation. The transport of acyl groups across the mitochondrial membrane also has negligible control under these conditions. Acyl intermediate levels are apparently controlled not by the enzymes that produce acetyl CoA, but by those that set the mitochondrial redox potential.

The mitochondrial acetyl CoA level also is set by succinate dehydrogenase. The other acetyl derivatives are regulated by citrate synthetase and succinate thiokinase (and acyl CoA synthetase for cytosolic acetyl CoA). These CoASH utilizing enzymes evidently cooperate in transferring acetyl groups to carnitine (acetyl carnitine has a positive regulation index) for storage as a reserve fuel supply. Triglyceride synthesis depends almost entirely on acyl CoA synthetase for supply of acyl CoA substrate for glycerophosphate acyltransferase. Carnitine acetyltransferase has a negligible share of the control of these processes; its

equilibrium passively follows the coenzyme A distribution that is directed by the citric acid cycle. Under the conditions simulated here, the shift of coenzyme A moieties among CoASH and its thioesters apparently is more important in directing excess acyl groups to "fuel storage" than in directing acyl groups to oxidation.

The regulation of citric acid cycle intermediates is given in Table 3. Succinate dehydrogenase has the greatest share in the control of the levels of these intermediates. Increasing the activity of this enzyme would result in oxidation of the mitochondrial matrix (the regulation indices of NADH and $CoQH_2$ are negative) because the perturbation would ultimately increase the rate of electron transport. This effect on the redox potential is responsible for most of the control. Isocitrate dehydrogenase has little control of the intermediate concentrations.

The accumulation of citrate and isocitrate in the hearts simulated here is largely due to the properties of citrate synthetase and aconitase. Our simulation (10) indicated that citrate synthetase was largely inhibited. Indeed, the effector indices of the inhibitors are all very small, indicating near saturation of their binding sites. This is likely the reason for so great a share in the control of these intermediate levels by these two enzymes. The negative regulation indices of aconitase for Mg-isocitrate reflects the effect of Mg^{2+} ions on the isomerization equilibrium. The equilibrium citrate/isocitrate ratio is nearly ten times higher at high Mg^{2+} than at low Mg^{2+} concentration (7). As Mg^{2+} increases with increasing cycle flux (7), the shift of the aconitase equilibrium allows oxidation of Mg-isocitrate without depletion of citrate.

The regulation of glycolytic intermediates is shown in Table 4. The three irreversible kinases of glycolysis have important shares of the control, as would be expected, and most of the equilibrium glycolytic enzymes have negligible control. 3-Phosphoglycerate kinase, however, has an unexpectedly high regulation index for a high tissue capacity reversible enzyme. This may reflect the significant amount of inhibition of this enzyme by binding of uncomplexed nucleotides at a regulatory site (10). Hexokinase and phosphofructokinase mostly have positive regulation indices because they tend to deplete inhibitory ATP; phosphoglycerate kinase and pyruvate kinase mostly have negative regullation indices because they produce ATP. The signs are reversed for the trioses, however. The control of NADH follows that of the other glycolytic intermediates, and increased oxidation of the cytosol consequent to increased glycolytic activity (the sum of the regulation indices for NADH is negative) decreases the small triose pools by the glyceraldehyde phosphate dehydrogenase and triose phosphate isomerase equilibria.

CONTROL OF PATHWAY FLUX

The sensitivity functions that most closely parallel the traditional concept of rate limitation are the control indices. The net fluxes of the glycolytic, fatty acid

metabolic, and citric acid cycle pathways are given by the net rates of lactate, acetyl CoA, and CO_2 production, respectively. The control indices (Table 5) for each of these pathways were normalized by dividing by the absolute value of the sum of the control indices to facilitate comparison of control in the three pathways. Enzymes with negligibly small control indices are not listed.

For all pathways considered, cytoplasmic (as opposed to mitochondrial) creatine kinase has a major share in the control of flux, probably reflecting its crucial role in transport of high energy phosphates (19). Because cytoplasmic creatine kinase makes ATP, it contributes to the inhibition of glycolytic enzymes (hence the negative effect on lactate production rate) and assists in fatty acid activation (hence the positive effects on acetyl CoA and CO_2 production rates).

The control of adenine nucleotide levels is distributed over a large number of enzymes, and the corresponding regulation indices of creatine kinase are quite large. Indeed, creatine kinase has a significant regulation index for most of the metabolites in the model. Numerically, this result may be exaggerated because the heart model does not yet include all the major ATPases and kinases. However, the general importance of creatine kinase points to the ATP/ADP ratio (or to the phosphate potential) in both cellular compartments as major controllers of the metabolism simulated here.

Control of Lactate Production Rate

Although the control indices of the sarcolemmal glucose carrier and of hexokinase for lactate production (Table 5) are significant, most of the control of lactate production originates in the mitochondria (see below). Under the conditions of this simulation, ATP^{4-}, ADP^{3-}, and glucose 6-phosphate have small negative effector indices for hexokinase, indicating that the enzyme is substantially inhibited. The negative control indices of the glucose carrier can be understood as indicating that the increased cytosolic glucose level resulting from an increase in carrier activity would raise the glucose 6-phosphate level and, with activities of the other glycolytic kinases unchanged, increase the inhibition of hexokinase.

Citrate synthetase, succinate dehydrogenase, and mitochondrial malate dehydrogenase are major controllers of the lactate production rate, and pyruvate dehydrogenase and its specific, inactivating kinase have less but still significant control. The signs of the control indices can be understood by the effect on the pyruvate level of an increase in the activity of the corresponding enzyme. Pyruvate dehydrogenase activity consumes pyruvate, thus depriving lactate dehydrogenase of substrate, whereas the specific kinase inactivates the dehydrogenase, thus providing more substrate for lactate production. Pyruvate dehydrogenase phosphatase activates the dehydrogenase, but the increased production of acetyl CoA would further inhibit the latter enzyme, cancelling the effect of the phosphatase. Increased citrate synthetase activity ultimately would

TABLE 5. Normalized control indices for several sink chemicals.*

	Lactate	Mitochondrial Acetyl CoA	CO_2
Glycolytic and related enzymes			
Glucose carrier	−0.107	0.044	0.584
HK	0.112	−0.044	−0.602
PFK		−0.010	0.004
Aldolase	0.010		−0.060
GPDH	0.044	−0.001	−0.254
PGK	−0.002	−0.007	−0.013
PGM + Enolase	0.003	−0.004	−0.015
PK	0.002	−0.021	−0.017
CK	−0.544	0.596	2.87
Fatty acid metabolizing enzymes			
Fatty acid carrier	0.024	−0.022	−0.123
ACS	0.074	−0.041	−0.435
TGL	−0.093	0.098	0.495
GPAT	0.029	0.002	−0.096
c-CAT	−0.029	−0.014	0.065
CTL	−0.008	−0.010	−0.084
m-CAT	0.028	−0.051	−0.246
ACDH	0.064	−0.033	−0.707
ECH	0.003	−0.002	−0.014
HACDH	0.032	−0.016	−0.161
KACT	0.137	−0.083	−0.752
Citric acid cycle and related enzymes			
PDH	−0.113	0.226	0.328
PDHK	0.137		
PDHP	−0.007	−0.291	
CS	0.364	−0.055	−1.91
m-Aconitase	−0.001	−0.049	−0.011
m-ICDH	−0.001	0.016	0.012
KGDH	0.002	−0.007	−0.010
STK		0.014	−0.034
SDH	−0.298	0.364	−1.95
Fumarase	−0.003	0.001	0.015
m-MDH	−0.259	0.091	1.31
Malate-aspartate shuttle and related enzymes			
ATL	−0.138		0.761
m-GOT	−0.305	0.160	1.60
c-MDH	−0.001	0.002	0.004
KTL	−0.356	0.164	1.88
c-Aconitase		−0.004	
Malic enzyme	0.201	−0.014	−1.05

*Abbreviations not obvious from the context: CK, creatine kinase; GPDH, glyceraldehyde 3-phosphate dehydrogenase; TGL, triglyceride lipase; GPAT, glycerophosphate acyltransferase; CAT, carnitine acyltransferase; CTL, carnitine translocator; KCAT, 3-ketoacyl CoA thiolase; PDH(K) or (P), pyruvate dehydrogenase (kinase) or (phosphatase); GOT, glutamate: oxaloacetate transaminase; ATL, aspartate/glutamate translocator; KTL, 2-ketoglutarate/malate translocator. The prefixes c- and m- denote cytosolic and mitochondrial, respectively.

increase the citrate level, thus inhibiting this enzyme still further and causing an accumulation of pyruvate. The pyruvate dehydrogenase complex and citrate synthetase apparently function as a "gate," directing pyruvate either toward oxidation or toward lactate production. Raising the malate dehydrogenase activity lowers pyruvate by supplying extra oxaloacetate to citrate synthetase (Table 3), overcoming the competitive inhibition by citrate and consuming the acetyl CoA that is responsible for inactivation of pyruvate dehydrogenase. Succinate dehydrogenase lowers the matrix redox potential, thus increasing the oxidation of pyruvate.

The most surprising result of this sensitivity analysis is the large control indices of most of the malate-aspartate shuttle enzymes. By transferring more malate to the mitochondria, they increase the mitochondrial oxaloacetate by the mitochondrial malate dehydrogenase equilibrium and overcome the inhibition of citrate synthetase. More important, because they increase cytosolic oxaloacetate as well as lower cytosolic malate, increasing the activity of these enzymes tends to consume the NADH necessary for lactate production. Cytosolic malate dehydrogenase itself, however, remains near equilibrium and has little effect on lactate production. Thus, these enzymes, which have negligible regulation indices themselves, cooperate in determining the flux through key regulatory enzymes by affecting the latter's substrate levels.

Why does the heart preferentially utilize fatty acid as fuel even in the presence of large amounts of exogenous glucose? The conventional wisdom is that the elevated citrate levels resulting from fatty acid oxidation inhibit phosphofructokinase. The control index of this enzyme for lactate production is negligible. Although citrate is a moderately important inhibitor of the kinase (its effector index is -0.068), the enzymes which set the cytosolic citrate level, cytosolic isocitrate dehydrogenase and cytosolic aconitase, have negligible effects on the glycolytic flux. This sensitivity analysis suggests that the adjustment of the cytosolic ATP/ADP ratio by energy metabolism and the creatine phosphate shuttle (19) results in rate limitation at hexokinase. None of the hexokinase inhibitors has a sufficiently small effector index (each is approximately -0.1) to explain this rate limitation by itself. It is the synergistic effects of all of them that turn off glycolysis.

Control of Mitochondrial Oxidations

The control of acetyl CoA production is, not surprisingly, to some extent a mirror image of the control of lactate production. Because there are more enzymes contributing to coenzyme A metabolism than pyruvate metabolism, control of the acetyl CoA production rate is more widely distributed (the normalized control indices are smaller), and citrate synthetase does not have so dramatic a share of the control. Of particular interest is the control by the pyruvate dehydrogenase complex. Control shifts from the inactivating kinase (for lactate

production) to the activating phosphatase (for acetyl CoA production). While increasing the phosphatase activity would have little influence on the lactate production rate, the consequent increase in acetyl CoA overcomes the inhibition of citrate synthetase and increases the rate of acetyl CoA consumption. Increasing the activity of the kinase would decrease acetyl CoA production, but this effect would be cancelled by the resultant reduced citrate synthetase rate.

The CO_2 production rate is largely controlled by citrate synthetase, succinate dehydrogenase, and mitochondrial malate dehydrogenase (Table 5). Part of this effect is due to the tendency of the dehydrogenases to oxidize the matrix (Table 3). In addition, citrate synthetase was shown to direct pyruvate toward lactate production under the conditions simulated, and this would reduce the rate of CO_2 production by pyruvate dehydrogenase. Succinate dehydrogenase tends to increase acetyl CoA and succinyl CoA (Table 3), inhibitors of the decarboxylating enzymes pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase, respectively. Furthermore, succinate dehydrogenase also tends to decrease Mg-isocitrate, the substrate for isocitrate dehydrogenase, the other decarboxylating enzyme. Note that the highly inhibited enzymes are controlled by enzymes that regulate inhibitor levels, while the uninhibited but unsaturated isocitrate dehydrogenase is controlled by the enzyme that has the greatest share in the regulation of its substrate level (Table 3).

Malate dehydrogenase is an exception to the above rule. By increasing the production of the small pool of matrix oxaloacetate, it helps overcome the competitive inhibition of citrate synthetase. Similarly, enzymes of the malate aspartate shuttle, which supply malate to the mitochondria, enchance this effect, and cytosolic malic enzyme, which shunts malate from the mitochondria, reduces this effect, as can be seen from their control indices (Table 5).

Oxaloacetate has often been termed a "sparker" of the citric acid cycle. Indeed, its acceleration index for CO_2 production is greater than that for any other cycle intermediate (Table 6). For comparison, the acceleration indices of

TABLE 6. Normalized acceleration indices of citric acid cycle and fatty acyl intermediates for CO_2 production.

Acetyl CoA	−0.001
CoASH	0.577
Citrate	−1.34
Mg-citrate	−0.178
Isocitrate	1.35
Mg-isocitrate	0.195
2-Ketoglutarate	0.000
Succinyl CoA	−0.869
Succinate	−1.50
Fumarate	1.27
Malate	0.000
Oxaloacetate	1.50
Acyl CoA	−5.52
Enoyl CoA	5.34
Pyruvate	0.000

long-chain acyl CoA, enoyl CoA, and pyruvate are included in Table 6. Long-chain acyl CoA is highly inhibitory for 3-ketoacyl CoA thiolase and citrate synthetase and accordingly has a negative acceleration index. The acceleration index of enoyl CoA is more representative of the contribution of β-oxidation intermediates to the overall metabolic rate. The insignificant effect of pyruvate on the flux is a reflection of the strong inhibition of pyruvate dehydrogenase.

DISCUSSION

This sensitivity analysis suggests that fatty acid utilization under the conditions simulated here is not limited by fatty acid uptake or activation, acyl transfer across the mitochondrial membrane, β-oxidation, or inhibition of isocitrate dehydrogenase. Mitochondrial regulation of coenzyme levels controls the rate of fatty acid utilization, largely at citrate synthetase. These results are consistent with those of an earlier sensitivity analysis (11). Regulation of metabolite levels was calculated as originating in the mitochondria. The regulatory effects are transmitted to the cytosol by the creatine phosphate and the malate–aspartate shuttles. The first shuttle sets the cytosolic ATP/ADP ratio and turns off glycolysis. The second shuttle participates in the regulation of lactate production. M. J. Achs, in the course of constructing many of the models which are predecessors to the one described here [e.g., (7)], reached qualitatively similar conclusions to those reported here. As outlined above, some experimenters also recognized the importance of the CoA distribution, but their experimental results did not identify the origin of that distribution.

The β-oxidation intermediates were computed to be regulated mostly by the control of the mitochondrial redox potential by succinate dehydrogenase. Acetyl esters were computed to be largely regulated by citrate synthetase and succinyl thiokinase, which controlled the distribution of coenzyme A moieties among CoASH and its thioesters and directed excess acyl groups to storage as triglycerides and acetyl carnitine. Citric acid cycle intermediates also were computed as set by the redox potential except for citrate and isocitrate, which were computed to be regulated by citrate synthetase and aconitase. Glycolytic intermediates were computed to be regulated by the four kinases of glycolysis; phosphoglycerate kinase is predicted to be as important a regulator as the irreversible kinases. Glycolysis was computed as limited by hexokinase in response to the cytosolic adenine nucleotide levels set by mitochondrial energy metabolism and the creatine phosphate shuttle. The pyruvate dehydrogenase complex and citrate synthetase were predicted to act in concert to distribute pyruvate between oxidation to acetyl CoA and reduction to lactate. The malate–aspartate shuttle enzymes were calculated as cooperating in this by affecting the rate of supply of oxaloacetate to citrate synthetase and of NADH to lactate dehydrogenase. Palmitate utilization was computed as limited by citrate synthetase, succinate

dehydrogenase, and malate dehydrogenase, partly by their regulating the mitochondrial redox potential and partly by their controlling the rate of supply of ligands to regulatory enzymes.

Metabolic regulation in large systems is too complex to permit interpretation of experimental results by inspection. Because different enzymes utilize common substrates and modifiers, the control properties of an enzyme in a particular metabolic state are not readily predictable and are frequently nonintuitive. Although simpler models are easier to understand and interpret, eliminating enzymes or intermediates considered unimportant or condensing several submodels into one overall reaction would destroy the predictive ability of this model. Indeed, the unimportance of certain enzymes and intermediates in the control of flux could not have been identified by such a simplified model. In fact, a sensitivity analysis of a complete model is necessary to determine which processes are relatively unimportant. Furthermore such simplifications, may cause overestimates of the influence of the remaining processes in the model. For example, the exaggerated importance assigned to succinate dehydrogenase by our sensitivity analysis is a result of the present insufficiently detailed model of respiration.

In every metabolic system we have examined, control of flux is distributed among a number of enzymes – which enzymes share in the control may change with the changing metabolic state of the system. Even quite small systems exhibit this behavior (8). The simplistic idea of assigning control of a pathway to an irreversible regulatory enzyme has never been supported by these or other (11,17) sensitivity analyses. Nevertheless, some generalizations suggest themselves. Metabolite levels are usually set by the regulatory (usually allosteric and irreversible) enzymes. The enzymes that produce substrates and modifiers for the regulatory enzymes have more control of the flux through the system than do the regulatory enzymes themselves. Frequently this latter group includes readily reversible (equilibrium) enzymes.

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