CONSTANT AND SPECIFIC PROPORTION GROUPS OF ENZYMES IN RAT MAMMARY GLAND AND ADIPOSE TISSUE IN RELATION TO LIPOGENESIS

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1. Introduction

The observation by Pette et al. [1–3] on the existence of constant and specific proportion groups of enzymes and the value of these groups in differentiating enzymes of especial importance in the individual metabolism of different tissues has prompted a number of studies in a variety of tissues which attempt to specify enzymes of particular significance to those tissues [4–7]. This principle has been extended to investigate a single tissue under different functional conditions and it has been applied here to the changes of enzyme content found in the mammary gland as the tissue progresses from the relatively quiescent state found in late pregnancy to the highly active state characteristic of the peak of lactation. For comparison, the relative enzyme changes have also been recorded for adipose tissue in conditions of normal, depressed and hyperlipogenic states. This comparison is a relevant one in that both tissues have metabolic patterns largely directed to fatty acid synthesis although it may be noted that, while the mammary gland is an open-ended secretory system and is virtually a unidirectional biosynthetic tissue, adipose tissue may, at different times, act either as a lipogenic tissue or as a fatty acid exporter.

In the mammary gland there is a marked increase in enzymic activity from pregnancy to the height of lactation. When these activities are expressed relative to that of the rate-limiting enzyme of the glycolytic sequence (namely PFK) a distinct pattern emerges, the enzymes falling into three groups the members of each of which appear to be functionally related. A more limited range of data is available for adipose tissue but this, nevertheless, shows that similar groupings exist in this tissue with two outstanding exceptions, glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme. Apart from these two enzymes, there was a remarkable consistency in the enzyme profile between lactating mammary gland and adipose tissue. The differences which exist are discussed in relation to special features and control in the two tissues.

The constant and specific proportionality appears to extend, in large measure, to the metabolite profiles in these tissues and, when phosphorylated intermediates of the glycolytic and pentose phosphate pathways are expressed relative to the tissue content of glucose-6-phosphate, a striking consistency is again apparent. Such a proportionality is, presumably, a necessary corollary of the enzyme pattern.

The initiation of lactation and the increasing yield of milk as lactation advances are accompanied by considerable changes of activity of the mammary gland enzymes. All enzymes measured so far respond to these physiological demands by increasing in activity, but they appear to do so in a differential manner. In order to distinguish any pattern in this adaptive behaviour, all activities have been expressed relative to the activity of the rate-limiting enzyme of the glycolytic sequence, PFK. The results of this calculation are shown in table 1. It may be seen from this table that the enzymes of the mammary gland fall into 3 distinct groups. In the first of these are the

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Table 1
Tissue enzyme profiles – Activities relative to phosphofructokinase.

	Mammary gland enzymes				Adipose tissue enzymes			
	Pregnancy 20 days	Lactation 1st day	Lactation 14th day	Relative change during lactation	Alloxan-diabetic	Control	Starved refed high carbo-hydrate	Relative change during hypo- to hyperlipogenesis
Glycolytic pathway								
Hexokinase	1.3	1.0	0.9	→	0.6	0.6	0.9	→
PGI	39	43	42	→	30	34	33	→
PFK	1	1	1	→	1	1	1	→
GADPH/3PGK	7.0	9.0	9.4	→	7.7	10.6	8.0	→
PK	13	13	14	→	12	14	19	→
LDH	43	62	60	→	46	49	50	→
αGPDH	10	8	11	→	53	66	62	→
Pentose phosphate pathway								
G6PDH	2.2	4.4	13.0	↑	2.0	2.1	2.6	→
6PGDH	1.5	2.2	2.1	→	2.6	3.2	3.2	→
R5P isomerase	3.8	3.0	3.3	→	2.3	4.4	4.6	→
Ru5P epimerase	18.2	13.9	9.4	→	4.6	9.5	9.6	→
Transketolase	0.7	0.5	1.0	→	0.8	0.8	1.5	→
Transaldolase	1.6	1.2	0.9	→	1.5	1.5	1.8	→
Tricarboxylic acid cycle								
Pyruvate dehydrogenase	0.36	0.34	0.23	→				
Pyruvate carboxylase	0.61	0.63	0.48	→				
Citrate synthase	1.41	2.94	4.81	↑				
ICDH	0.75	0.59	0.25	↓				
Succinoxidase	0.77	0.57	0.15	↓				
MDH (NAD^+)	13.2	9.3	3.4	↓				
GLUDH	0.43	0.69	0.17	↓				
Fatty acid synthesis and related enzymes								
Citrate synthase	1.4	2.9	4.8	↑	–	2.6	–	
Citrate cleavage enzyme	1.3	2.1	3.8	↑	0.3	2.0	8.3	↑
Ac CoA carboxylase	0.04	0.16	0.12	↑	0.09	0.2	0.5	↑
Fatty acid synthetase	0.5	1.7	3.4	↑	0.2	1.0	1.9	↑
G6PDH	2.2	4.4	13.0	↑	2.0	2.1	2.6	→
Malic enzyme ($NADP^+$)	5.2	5.8	5.2	→	0.9	5.2	17.4	↑
ICDH ($NADP^+$) cytosol	3.8	2.3	0.9	↓	1.2	1.7	1.7	→
MDH (NAD^+) cytosol	264	147	74	↓	–	–	–	

Table 1

The activities of enzymes of pathways of carbohydrate and lipid metabolism, in mammary gland at different stages of the lactation cycle, and in adipose tissue, in various lipogenic states, expressed relative to the activity of PFK in each condition.

The enzyme profile is shown on the basis of a PFK value of unity. The PFK activity of mammary gland at 20 days pregnancy, 1 and 14 days lactation, were respectively 0.56 ± 0.12; 0.85 ± 0.14 and 3.09 ± 0.52 μmoles substrate converted/min at 25°/g tissue corrected for milk content (present communication). The PFK activity of adipose tissue from alloxan-diabetic, control and starved rats refed high carbohydrate diet for 3 days were, respectively, 3.83 ± 0.74; 4.56 ± 0.21 and 4.03 ± 0.17 μmoles substrate used/hr at 25°/mg tissue N [16].

The mammary gland profile was compiled from figures of Gumaa et al. [17] and from unpublished data of these authors with the exception of acetyl CoA carboxylase which was from Howanitz and Levy [18]. Adipose tissue data was drawn from the following sources: glycolytic pathway, Saggerson and Greenbaum [16]; acetyl CoA carboxylase, fatty acid synthetase and ICDH, Saggerson and Greenbaum [19]; pentose phosphate pathway, Gumaa et al. [20]; citrate lyase and malic enzyme, Ball [11]; citrate synthase, Coore et al. [9].

The arrows in columns 5 and 9 indicate whether the particular enzyme changes faster than, slower than, or at the same rate as, PFK in the adaption to the stress of lactation from late pregnancy to the height of lactation in mammary gland, and in the transition from hypo- to hyperlipogenic states in adipose tissue.

enzymes of the glycolytic and the majority of those of the pentose phosphate pathways. The enzymes of the glycolytic sequence behave as a constant proportion group and, relative to PFK, do not change their correspondence during the lactation cycle. Certain enzymes concerned with pyruvate metabolism, namely pyruvate dehydrogenase, pyruvate carboxylase and 'malic' enzyme, also fall into this category as do the enzymes of the pentose phosphate pathway with the notable exception of G6PDH. The second group contains a number of enzymes of the tricarboxylic acid cycle and these increase more slowly than the first group. Thus, relative to PFK, they decline with the initiation and progression of lactation. The enzymes in this group are NADP-linked mitochondrial isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and glutamate dehydrogenase. The third group contains enzymes related to fatty acid synthesis, and these show a pattern opposite to that exhibited by the second group in that they increase more rapidly than PFK. The enzymes of this group are citrate synthase, citrate cleavage enzyme, acetyl CoA carboxylase, fatty acid synthetase and G6PDH.

The present results are in agreement with those of Baldwin and Milligan [8] with respect to the more rapid increase of G6PDH and fatty acid synthetase relative to a glycolytic marker enzyme during lactation. However, substantially different results were obtained for the mitochondrial tricarboxylic acid cycle enzymes. The two studies are complementary in that a different spectrum of enzymes was examined in each case and it is apparent from the work of these authors that the lactose synthesizing enzymes fall into the group which increases faster than those of the glycolytic sequence.

Examination of another lipogenic tissue, normal rat epididymal adipose tissue, reveals that the enzyme profile, relative to PFK, is, with certain notable exceptions, remarkably similar to that of the lactating mammary gland. The outstanding differences are α-glycerophosphate dehydrogenase (αGPDH), which is some 6-fold higher, and G6PDH, which is lower by a factor of 6 in adipose tissue. The relative activities of pyruvate carboxylase:pyruvate dehydrogenase differ in the two tissues, the ratio in lactating mammary gland being 2 (table 1) and in adipose tissue being 10 [9]. Considering next the changes in

Table 2
Metabolite profiles relative to the glucose-6-phosphate content.

	Mammary gland				Adipose tissue			
	20 days pregnancy	1 day lactation	14 days lactation	Relative change	Anti-insulin serum	Control	Insulin	Relative change
G6P	100	100	100	→	100	100	100	→
F6P	34	31	33	→	42	37	35	→
FDP	21	20	23	→	15	19	22	→
DHAP	33	28	43	→	27	28	35	→
3PG	40	47	37	→	–	69	68	→
PEP	7	5	7	→	–	–	–	
PYR	47	48	63	→	–	–	–	
αGP	120	261	423	↑	440	420	480	→
MAL	137	271	324	↑	132	112	147	→
6PG	16	32	53	↑	11	35	62	↑
CIT	415	243	203	↓	292	219	161	↓

The metabolite profile of mammary gland and adipose tissue in different functional states expressed relative to the tissue content of G6P in each condition.

The table is compiled from mammary gland data of Gumaa et al. [17] and adipose tissue data of Saggerson and Greenbaum [21]. The tissue content of G6P is given a value of 100 and all other metabolites are expressed as a percentage of the G6P content.

The values for G6P as mμmoles/ml intracellular water are: for mammary gland at 20 days pregnancy, 1 and 14 days lactation, 188 ± 25; 209 ± 19 and 188 ± 23, respectively. The corresponding figures for adipose tissue are: 190 ± 18 in presence of anti-insulin serum; 250 ± 54, control; 450 ± 44 in the presence of insulin.

adipose tissue in hypo- and hyperlipogenic states (i.e. in alloxan diabetes and in starved rats refed a high carbohydrate diet), it may be seen that the glycolytic enzymes and the entire sequence of the pentose phosphate pathway, including G6PDH, remain constant relative to PFK (group 1). Of the enzymes concerned with fatty acid synthesis (group 3), it may be seen that these all increase faster than PFK and that this group contains malic enzyme but not G6PDH, two marked points of contrast to the mammary gland at different stages of the lactation cycle. There is, at present, insufficient data to make a comparison of the remaining group, which contains the enzymes of the tricarboxylic acid cycle.

A further finding was that, when a metabolite profile of mammary gland was established (by expressing the tissue steady-state content of the intermediates relative to the content of G6P, arbitrarily assigned a value of 100, the intermediates of glycolysis again fell into a fixed pattern relative to one another and that the same pattern held, to an appreciable extent, for adipose tissue (table 2).

With the initiation and progress of lactation, 3 metabolites linked to fatty acid synthesis increase (αGP, 6PG and malate), while citrate falls, relative to the glycolytic intermediates, a profile not inconsistent with the known enzymic changes and expected changes in allosteric modifiers. The different pattern of behaviour of αGP and malate with increased lipogenesis in adipose tissue and mammary gland is perhaps a reflection of the control at αGPDH and of the relative importance of the malate cycle in the two tissues.

2. Discussion

The present study makes use of the concept, first proposed by Pette [1–3], that the existence of constant and specific proportion enzymes can be used to identify enzymes or pathways of especial significance in the metabolic activities of different tissues. Examination of the enzyme profile (table 1) of 2 tissues, each with a metabolism largely directed towards lipogenesis, shows the existence of major groups of enzymes, the component enzymes of each of which act essentially as a constant proportion group, the relative proportions being the same in both tissues and in all states studied. However, although

each group retains this remarkable internal consistency, no such parallelism exists between groups. For example, in both mammary gland and adipose tissue the enzymes of lipogenesis increase much faster than those of glycolysis. The results also reveal the existence of enzymes which do not conform to this general pattern, enzymes of the specific proportion type, e.g. G6PDH in mammary gland and αGPDH and malic enzyme in adipose tissue. Where major differences in enzyme profile emerge between the two tissues, these may be clearly related to differences in requirement for αGP, to the source of hydrogen for reductive synthesis and, integrated with this latter, to the relative importance of the malate cycle.

In mammary gland, G6PDH increases in parallel with the enzymes of fatty acid synthesis whereas malic enzyme does not and is, in fact, a number of the glycolytic enzyme constant proportion group, which would imply that high rates of lipogenesis are met by adaptive change of the dehydrogenase. The reverse is true for adipose tissue, implying that hyperlipogenesis in this tissue requires high levels of malic enzyme. The data thus support the views of Flatt and Ball [10, 11] that, in adipose tissue, while the pentose phosphate pathway can supply most of the hydrogens required for lipogenesis at low rates of fatty acid synthesis, at high rates this pathway cannot respond adequately and up to half the total hydrogens required are generated via the malic enzyme. The table also supplies some supplementary data on this theme. A corollary to a high functional activity of malic enzyme in the generation of NADPH in the cytosol will be that the anaplerotic reaction, the conversion of pyruvate to oxaloacetate by pyruvate carboxylase, will also need to be correspondingly high [12–14]. Thus, some measure of the significance of the malate cycle in a particular tissue may be adduced from a consideration of the relative activities of an enzyme intimately linked to lipogenesis (e.g. citrate cleavage enzyme) and of pyruvate carboxylase, which may be considered a key enzyme in the replenishment of mitochondrial oxaloacetate. In normal adipose tissue the ratio of pyruvate carboxylase:citrate cleavage enzyme is 1:1.7, while in lactating mammary gland this ratio is 1:7.6, showing the greater significance of the carboxylation reaction and, by implication, of the malate cycle, in adipose tissue.

The return of malate, as opposed to pyruvate, to the mitochondrial compartment could be of considerable significance in the energy balance of the cell; this has been discussed by Flatt [14] with respect to adipose tissue. The transhydrogenation of NADH to NADPH by malic enzyme is, at once, energy consuming and a diversion of glycolytic NADH from an oxidative pathway. When the reductive requirement of lipogenesis is met from NADPH generated in the pentose phosphate pathway, not only is there no energy requirement for the anaplerotic reaction, but the glycolytic NADH is now available for energy generation. The net energy yield from the two processes is vastly different.

Atkinson [15] has recently re-emphasized the value of a teleological approach in considering the functional significance of interrelated pathways. Viewed in such a light it might be considered that the mammary gland, with an open-ended secretory system manufacturing in quantity a range of milk components (fats, proteins, lactose), has a much higher energy requirement than adipose tissue. The use of the highly efficient pentose phosphate pathway by mammary gland would confer real evolutionary advantage compared with the relatively expensive, in terms of ATP, malate cycle.

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