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Adaptation of liver enzymes associated with gluconeogenesis

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INTRODUCTION

The effects of diets and hormones on enzyme activities have been examined for the past 50 years. These enzyme activities have been associated with the metabolism of amino acids, carbohydrates, lipids, purines and other processes (1, 2). The emphasis of this manuscript will be related to enzymes associated with gluconeogenesis, three in a positive association and one in a negative association.

The three enzymes that are positively associated with gluconeogenesis and adaptable are: glucose-6-phosphatase (G-6-Pase), fructose-1, 6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK). The enzyme negatively associated with gluconeogenesis is pyruvate kinase (PyrK). These enzymes are changed in amount and activity in response to dietary and hormonal regimens which affect the flux of gluconeogenesis. These responses are probably protective in nature and not flux determining as the potential flux through many of these steps are well in excess of the actual flux. Furthermore, changes in enzyme amount is too slow to meet immediate demands. These are most probably met by allosteric and covalent controls.

Diets and hormones

In early studies Maley and Lardy (3) showed that thyroxine administration increased hepatic G-6-Pase in the rat. G-6-Pase was also increased by other treatments causing increased gluconeogenesis (Table 1). These include glucocorticoids (4), high fructose diets (5,6), high protein (5) and high fat (5) diets devoid of carbohydrate. This was interpreted as a response of an increased flux through G-6-Pase to maintain glucose supply.

A further study of the response of FBPase to these and other treatments showed some interesting relationships (Table 1). The treatments that caused a general increase in gluconeogenesis requiring flow through both G-6-Pase and FBPase increased the activities of both enzymes (7), whereas the exclusion of glucose and glucose polymers and substitutions of galactose or mannose which only require flux through G-6-Pase, but not FBPase, increased G-6-Pase but not FBPase activity (7). Thus the enzymic responses appear to be related to flux and are not general gluconeogic responses.

The response of PEPCK to treatments causing increased gluconeogenesis also appear to be pathway dependent. High protein diets and cortisol treatments which cause an increased gluconeogenesis from amino acids causes an increase flux through PEPCK and an increase in PEPCK activity (8), whereas high fructose feeding, where increased flux only occurs at G-6-Pase and FBPase, does not cause any increase in PEPCK activity (9). In addition, the activity of pyruvate kinase, which is negative in regard to gluconeogenesis causing substrate cycling, is also affected by diets and hormones (10, 11). Those treatments causing a general increase in gluconeogenesis as cortisol, thyroxine and high protein diets cause a decrease in PyrK activity (10,12). This would be commensurate with the negative role of PyrK in gluconeogenesis. However, high fructose diets, which only require increased gluconeogenic flux at FBPase and G-6-Pase, have no diminishing effect on PyrK activity (13), but in fact markedly increase PyrK activity. This increase in PyrK with high fructose diets may be related to lipogenic effects of high fructose diets (13).

After prolonged periods (22 days) of receiving a high protein or high fat diet, the G-6-Pase activity returned to control values. This was attributed to adaptation of using fats or amino acids more efficiently in the periphery and reducing the need for gluconeogenesis (14). This was tested by switching high protein fed rats to high fat diets and vice versa. This caused a marked increase in the animals switched to high protein diets and a more modest increase in the animals switched to the high fat diet (Table 2). The lesser increase in the animals switched to the high fat diet was probably due to previous adjustment to fatty acid catabolism in the periphery. When high sucrose diets were fed there was no decrease in G-6-Pase, even after 31 days. This was probably due to the fact that the peripheral tissues could not utilize the fructose portion on sucrose and required its conversion to glucose throughout the feeding period.

Endocrine role in dietary responses

The question arises as to whether the nutritionally mediated changes in enzyme activity is hormonally mediated or require normal hormone levels. This can be answered by using hormonally altered animals. If the response is similar in nature and degree in adrenalectomized and hypothesectomized animals as it is in intact animals, this would indicate the response was independent of hormonal influence. If the increases fail to occur in these surgically altered animals, this would indicate hormone involvement. In the latter case, replacement therapy could differentiate between a response of greater hormone release and a permissive effect of the hormone. In regard to G-6-Pase, it can be seen in Table 3 that endocrinectomy decreased the response to thyroxine, but had a lesser or no effect on the response to high protein or high fructose diets. Thus it appears that the dietary induction is independent of hormonal influences, with the exception perhaps of insulin. The absence of hormonal influence has been seen with the response of a large number of enzymes to high protein and high fructose diets (15, 16).

The interaction of insulin and cortisol on several of these enzymes had led to the proposal of a gluconeogenic operon (12). Cortisol, as previously indicated, causes increases in G-6-Pase and FBPase and a decrease in PyrK. Insulin itself in normal animals has little if any effect on these enzymes (Table 4), however it blocks, either fully or partially, the response to cortisol which would be consistent with an

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operon where insulin was the turn-off signal. If indeed there was an operon with insulin as a turn-off signal, then this hormone should prevent the dietary induction and repression of these enzymes. However, insulin had no appreciable effect on the responses of these enzymes to a high protein or high fructose diet (13), thus it is more likely that there is an insulin-cortisol antagonism than an operon controlled by insulin in a negative fashion.

TABLE 1
Effects of diets and hormones on enzymes associated with gluconeogenesis

Enzyme Effect on Gluconeogenesis	G-6-Pase +	FBPase +	PEPCK +	PyrK -
Treatment				
Cortisol	2.1	1.5	4.0	0.6
Thyroxine	2.1	0.6		0.7
High fat	1.4	1.2		
High protein	1.3	1.6	2.5	0.3
Fructose	1.8	1.2	1.0	1.8
Galactose	1.4	0.9		
Mannose	1.2	0.9		

Control: Dextrin + Normal protein and Fat = 1.0

TABLE 2
Diet switching and G-6-Pase

Treatment	Days	G-6-Pase	Switch	G-6-Pase
High protein	4	1.73		
High protein	22	1.06	High fat	1.68
High fat	4	1.85		
High fat	22	1.03	High protein	1.15
Sucrose	2	1.77		
Sucrose	31	1.76		

Control activity = 1.0

TABLE 3
Effect of endocrinectomy on increases of G-6-Pase in response to diets and thyroxine

Treatment	Intact	Adrenalectomized	Hypothesectomized
Thyroxine	2.1	1.6	1.3
Fructose	1.6	1.6	1.6
High protein	1.7	1.3	1.4

TABLE 4
Effect of insulin on the response of three gluconeogenic enzymes to diets and hormones

	G-6-Pase	FBPase	Pyr Kinase
Insulin	0.8	1.1	1.2
Cortisol	1.9	2.1	0.6
Cortisol + Insulin	1.15	1.3	1.05
Fructose	1.6	1.3	1.8
Fructose + Insulin	1.4	1.5	2.8
High Protein	1.7	1.4	0.3
High protein + Insulin	1.2	1.5	0.6

Normal control values = 1.0

Possible controls at DNA transcription

The question arose during studies on these enzyme increases whether the changes in activity were due to more enzyme synthesis or an activation of existing enzyme[s]. Furthermore, if there were an increased synthesis, was this due to increased translation (protein synthesis/unit m-RNA) or increased transcription (m-RNA synthesis). These considerations were examined 20 years before one could measure specific m-RNA in an efficient manner. The early approaches here were to use protein synthesis inhibitors, such as cyclohexamide and puromycin, and m-RNA synthesis inhibitors such as actinomycin D and azaguanine. If the increase were blocked by protein synthesis inhibitors, this would indicate that the increases were due to new protein synthesis and not activation. If the increases were blocked by m-RNA synthesis, this would indicate that increased m-RNA was needed and increased translation of existing m-RNA was not the cause of the increases.

The fructose (or sucrose) induced increases in G-6-Pase and PyrK were prevented by both inhibitors of protein synthesis and inhibitors of m-RNA synthesis (Table 5) indicating that both increases in m-RNA synthesis is required for the increased synthesis of these enzymes in response to dietary fructose (17,18). Actinomycin D, an inhibitor of m-RNA synthesis prevented the cortisol induced increases in G-6-Pase, FBPase and PEPCK (Table 5) once more indicating the need of increased m-RNA for these enzymic increases (18). This is consistent with the known mechanism of glucocorticoids on the process of transcription (19).

The question of presence of m-RNA versus the translation of m-RNA in regard to PyrK activity was examined using nutritional manipulations. Diets high in glucose but devoid of protein should produce increased m-RNA, but fail in the translational process due to absence of sufficient amino acids. Diets high in protein but devoid of glucose should cause a decreased amount of m-RNA (20). Both of these produced low levels of PyrK (Table 6). Switching the rats for one day to the opposite diets caused a marked increase in PyrK, supposedly resulting from increased m-RNA after high carbohydrate feeding and increased translation of existing m-RNA after high protein feeding. This hypothesis was tested by treating the animals with 8-azaguanine, an inhibitor of m-RNA synthesis, prior to and during the dietary changes. The change to the high protein diet resulted in the expected increase in PyrK, indicating m-RNA for this enzyme was present, but not being translated when no protein was present in the diet. However, when protein was fed there was increased translation and PyrK activity. The switch to the high glucose diet in the presence of 8-azaguanine failed to cause any increase in PyrK, this indicates that despite a residual supply of amino acids that the lack of m-RNA prevented the increase in PyrK observed when 8-azaguanine was not administered.

Twenty years later, when it was possible to measure specific m-RNA levels, it was shown that many treatments increasing enzymes also increased the corresponding m-RNAs. In the case of PEPCK it was shown that c-AMP, starvation and diabetes caused an increase in both PEPCK activity and PEPCK m-RNA (21). The increases due to diabetes could be reversed by insulin. Thus the assumptions of nearly 40 years ago in relation to nutrition and/or hormones on m-RNA and thus the observed changes in enzyme activities are now being proven using molecular biological techniques.

TABLE 5
Effects of inhibitors of protein synthesis and m-RNA synthesis on enzyme increases in response to diets and hormones

Treatment	Enzyme	Normal Response	Inhibitor	Activity
Sucrose	G-6-Pase	1.68	Ethionine	1.03
Fructose	G-6-Pase	1.43	Cyclohexamide	.93
Fructose	Pyr Kinase	1.49	Cyclohexamide	.77
Cortisol	G-6-Pase	2.1	Actinomycin D	1.0
Cortisol	FBPase	1.5	Actinomycin D	1.0
Cortisol	PEPCK	4.0	Actinomycin D	0.6
Fructose	PyrKinase	1.8	Actinomycin D	0.9
Fructose	G-6-Pase	1.43	Actinomycin D	1.04
Fructose	Pyr Kinase	1.49	Actinomycin D	0.99

TABLE 6
Dietary and 8-Azaguanine effects on liver pyruvate kinase

High glucose-Protein free	Carbohydrate free 1 day high protein	1 day high protein + AG
23.2	55.1	67.9
High protein-Carbohydrate free	**1 day high glucose**	**Protein free 1 day high glucose + AG**
26.2	47.9	27.5

Flux and increased activity

The question arises whether the increase in enzyme activity is needed for increased flux or is an excess protective process. The maximum rate of gluconeogenesis from fructose is about 2.6 μ moles/gm liver and about 2 μ moles/gm liver for lactate (22). In contrast the activity in glucose fed rats of G-6-Pase is 13.5 μ moles/gm and for FBPase is over 6 μ moles/gm liver (5). Thus, even without the increases in activity, there is an excess of enzymic activity compared to flux. Concentrations of hepatic G-6-P are close to Km of G-6-Pase (23), thus even at one-half Vm there is an excess of enzyme activity. There are also studies showing that increases in flux occur either before increases in enzyme activity is observed (24) or without changes in enzyme amount (25).

Table 7 shows that the potential for gluconeogenesis with fructose or dihydroxyacetone is much greater than that from lactate and also amino acids or glycerol (22). Thus, for amino acids, there is an excess of G-6-Pase and FBPase, however a high protein diet causes an increase in both of these enzymes despite their excess. Thus the increases appear to be an overprotective process.

TABLE 7
Perfused liver gluconeogenesis

	No additions	+ Glucagon	+ Butyrate	B + G
Endogenous	0.14	0.22		
Lactate	1.06	1.86	1.41	2.20
Dihydroxyacetone	2.07			
Fructose	2.68	2.94		

Rates are in μmoles/min per gram of liver.

It appears that changes in rates of gluconeogenesis can occur without any change in amount of enzymes in the perfused liver (Table 7; (25)). The addition of glucagon or butyrate causes a marked increase in gluconeogenesis from lactate, although at this time there is no change in enzyme amount. Thus the importance of allosteric effectors, as butyrate increases mitochondrial acetyl CoA stimulating pyruvate carboxylase, is apparent. In the case of glucagon covalent modifications of enzymes play a major role, such as phosphorylation of PyrK and phosphofructokinase 2, which can increase gluconeogenesis by causing decreases in activity of both of these enzymes. Studies in isolated hepatocytes have indicated that major effect of glucagon on increasing gluconeogenesis is the phosphorylation of PyrK, decreasing the recycling of PEP back to pyruvate (26). Thus it appears that changes in enzyme amount are not controlling rates of gluconeogenesis but responding as an overcompensation. In gluconeogenesis allosteric and covalent modifications are more critical. However, there are some cases where enzyme activity or amounts are critical in controlling metabolism, such as tryptophan pyrrolase for tryptophan catabolism (27) and serine dehydratase for serine catabolism (28).

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