Effect of Exercise and Dietary Carbohydrate on Glucose Oxidation in Rats (35902)

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The effect of high-fat vs high-carbohydrate diets on the pentose cycle (PC) in rats has been partially clarified. Rats show a high activity of glucose-6-phosphate dehydrogenase, the initial enzyme in the cycle, when their diet is high in carbohydrate and adequate in protein. Substitution of fat for carbohydrate in the diet lowers the level of this enzyme (1–3) and also reduces the amount of glucose available for catabolism by any pathway. It is not clear how the dietary ratio of fat to carbohydrate affects the fraction of glucose catabolism occurring by way of the PC.

In the present studies, the relative role of the pentose cycle in glucose catabolism was studied by measuring expired $^{14}CO_2$ after the injection of glucose labeled at various sites. Observations were made during the feeding of high-fat vs high-carbohydrate diets under conditions in which caloric intake, physical activity, and kind of dietary carbohydrate were varied. Since rat strains are known to differ in tissue levels of glucose-6-phosphate dehydrogenase (4) and appear to vary in their use of the pentose cycle, two strains were used in the present study.

Methods. Treatment of Animals. The present studies were carried out in two stages with approximately 150-day-old male rats which had been eating a commercial stock diet.[3] Animals were then given, for 6 or 8 weeks, either a high-fat or high-carbohydrate diet. These diets (5, 6) contained the same adequate quantities of protein, vitamins, and minerals per calorie and provided either 12 or 54% of metabolizable calories as carbohydrate and, respectively, 62 or 20% as a 3:1 mixture of beef tallow and corn oil. At intervals during the experimental diet period, a total of five animals per treatment group were injected intraperitoneally with 1-^{14}C-glucose, 6-^{14}C-glucose, or uniformly labeled U-^{14}C-glucose. Each rat received about 1 μCi of radioactivity in not more than 1 μmole of glucose. Expired CO_2 was collected over two successive 6 hr intervals and analyzed for $^{14}CO_2$, as previously described (7).

In the first study, the animals used were from a Wistar-derived strain. The carbohydrate provided in the high- and low-fat diets was cornstarch. All animals were fed once daily in the early morning. Half the animals were offered more than they would eat, while the other half were restricted to 65% of the *ad libitum* intake. Half the animals subjected to each of the four dietary regimens were forced to swim twice daily for 30 min in 37° water for a total of 1 hr/day; while the remaining half were left undisturbed in their separate cages.

The second study dealt with *ad libitum* fed Sprague-Dawley rats. The carbohydrate, consisting either of sucrose, lactose, raw cornstarch, or of a mixture (40% cornstarch, 20% lactose, 10% glucose, 10% sucrose, 10% fructose, and 10% dextrin), was fed at the two

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previously described levels in otherwise identical diets. Half the animals receiving each of the eight diets were forced to run daily on a treadmill, while the remaining rats received only the voluntary activity which they exhibited in rotating treadmill activity cages (6).

Procedures for the isotopic studies started at 7 a.m. when animals were placed with their daily rations in individual respiration chambers. The labeled glucose was injected 1 hr later. In the first study, each animal received only one of the three labeled forms of glucose used. In the second study, each rat received 1-^{14}C-, 6-^{14}C-, and U-^{14}C-glucose, in random order at 4-day intervals.

Model for interpreting data. Variously labeled forms of glucose were injected intraperitoneally. Most of the injected tracer was thought to enter the blood and to label the circulating glucose pool. The basic parameter measured was the fraction (F_1, F_6, and F_U) of injected label, respectively, from 1-^{14}C-, 6-^{14}C-, or U-^{14}C-glucose, which was converted to expired $^{14}CO_2$ within 6 or 12 hr. These values were considered to represent the portion of the C-1, C-6, or of all the carbon atoms of circulating glucose which was converted by the various tissues to CO_2 over a period of less than 6 or 12 hr.

An experimental model was set up to use average values of F_1, F_6, and F_U in estimating the relative role of the pentose cycle in glucose catabolism. At points of uncertainty, the model tended consistently to underestimate the actual role of the PC. Values derived by use of the model represented minimal estimates of the PC role, which applied to a main portion, but not to the whole, of glucose catabolism in the body. Specifically, they applied to the metabolic fate of glucose which moves from blood into relatively labile tissue pools.

($F_1 - F_6$) was taken as a measure of the fraction of injected glucose which was selectively decarboxylated at the C-1 position by the pentose cycle. Specifically, a 0.08 value for ($F_1 - F_6$) was interpreted as indicating that, under the specified condition, 8% of injected glucose underwent that metabolic fate.

Further interpretation of ($F_1 - F_6$) required an estimate of the fraction of injected glucose which, after undergoing glycolysis, was irreversibly oxidized, *i.e.*, yielded up CO_2 from the oxidation of pyruvate. Several assumptions and approximations were made in estimating this second fraction of the injected glucose. First, if one of the two 3-carbon products of glycolysis escaped oxidative decarboxylation, *i.e.*, became the glycerol moiety of a lipid molecule or entered the gluconeogenic pathway, only half a glucose molecule was assumed to have undergone oxidation. Secondly, the fates of the two halves of the glucose molecule were considered identical, except when the C-1 was selectively oxidized by the PC. Thus either F_3 or F_4 was thought to be equal to the fraction of injected glucose which, following glycolysis, underwent irreversible oxidation.

Neither F_3 nor F_4 were directly measured, for the appropriately labeled glucose was not available. However, F_3 was estimated from measurements of F_1, F_6, and F_U. By definition, $6F_U$ was equal to the sum of F_1, F_2, . . . F_6. It followed that ($F_3 + F_4$) equaled ($6F_U - F_1 - F_2 - F_5 - F_6$). As noted above, F_3 was assumed to equal F_4. It was further assumed that F_2 and F_5 were each equal to F_6. The following formula for F_3 was developed: $0.5(6F_U - F_1 - 3F_6)$. ($F_1 - F_6$) was then added to the above, yielding the quantity ($3F_U + 0.5F_1 - 2.5F_6$), which was used as the formula for estimating the total fraction of injected glucose which underwent irreversible oxidation.

The relative role of the PC was expressed as the ratio, ($F_1 - F_6$):($3F_U + 0.5F_1 - 2.5F_6$). These were completely different terms for expressing PC participation than were used in the *in vitro* model systems of Landau and Katz (8).

Results and Discussion. Average values of F_1, F_6, and F_U for the various treatment groups in the first portion of these studies are presented in Table I. Similar values for the second portion of the studies are not shown. However, in all cases, F_1 was found to exceed F_6 and to indicate PC activity. In Table II,

TABLE I. Effect of Diet, Food Intake, and Exercise on the Fraction (F) of Injected 1-^{14}C-, 6-^{14}C-, or U-^{14}C-Glucose Recovered in Expired CO_2 of Wistar Rats.

Condition of animal								
Diet high in	Dietary intake (% of *ad libitum*)	Exercise (swimming)	During 6 hr			During 12 hr		
			F_1	F_6	F_U	F_1	F_6	F_U[a]
Fat	65	2×/day	0.23	0.19	0.26	0.29	0.24	0.31
Starch	65	2×/day	0.22	0.14	0.28	0.28	0.18	0.32
Fat	100	2×/day	0.25	0.11	0.24	0.31	0.16	0.30
Starch	100	2×/day	0.28	0.14	0.23	0.34	0.18	0.30
Fat	65	Never	0.29	0.08	0.22	0.37	0.22	0.29
Starch	65	Never	0.34	0.21	0.29	0.40	0.30	0.35
Fat	100	Never	0.30	0.12	0.30	0.36	0.23	0.36
Starch	100	Never	0.33	0.18	0.32	0.39	0.34	0.38
Main effects (from analysis of variance)								
Exercise			Decrease[b]	NS[c]	NS	Decrease[b]	Decrease[b]	NS
Restricted dietary intake			NS	NS	NS	NS	NS	NS
Composition of diets			NS	NS	NS	NS	NS	NS

[a] Subscript indicates position of ^{14}C on injected glucose.

[b] Decrease is significant at $p < .05$.

[c] NS means not significant at $p < .05$.

the relative role of the PC under the various test conditions is expressed in terms of the previously described model.

The first portion of the study provided data about caloric restriction. Restricted animals developed the practice of consuming their daily quota of food within several hours in the morning and were meal-eaters. Among the sedentary animals, caloric balance permitted some growth; and there was no reduction in the extent of glucose catabolism by the pentose cycle. However, animals which were both forced to exercise and restricted in food intake showed a persistent weight loss and a reduced utilization of the PC. A similarly reduced utilization of the PC has been reported in rats which were not forced to exercise but were severely underfed (9).

Estimates of the PC participation, as based on 6 and 12 hr collections of $^{14}CO_2$, may be compared. In the first portion of the work, PC participation among *ad libitum* fed rats appeared greater when based on 6 hr than 12 hr data. This observation was expected, for the pentose phosphate resulting from the C-1 oxidation of glucose is known to undergo further oxidation and to yield up expired CO_2 derived from what had been the C-6 of the injected glucose. This implied that ($F_1 - F_6$) underestimates PC activity, especially as the time of observation is increased. In the second phase of the study, estimates of the relative role of the PC, as based on 12 hr data, exceeded those based on 6 hr data. This was not expected. Apparently, whole-body utilization of the pentose cycle was, in fact, greater between 1 and 7 p.m. than during the late morning. These data suggest that the Sprague-Dawley rats had developed a diurnal rhythm in the degree of utilization of the PC, perhaps in response to a diurnal rhythm in their diet intake.

The second half of the study provides data regarding effects of kind of dietary carbohydrate on the relative role of the PC in glucose catabolism. Data are consistent with previous reports that dietary sucrose (2, 10) and lactose (6) enhance PC activity. However, the present data suggest that these effects of kind of dietary carbohydrate are relatively small.

It is further observed that the relative participation of the PC in glucose catabolism was similar in animals fed high-fat vs high-carbohydrate diets. This trend was observed under a variety of conditions in which kind

TABLE II. Effect of Diet, Food Intake, and Exercise on the Relative Role of the Pentose Cycle (PC) in the Catabolism of Injected Glucose.

Dietary carbohydrate	Dietary intake (% of *ad libitum*)	Form of exercise	Diet:	Portion of oxidized ^{14}C-glucose initially decarboxylated at C-1[a]			
				Based on 6 hr data		Based on 12 hr data	
				High fat	High carbohydrate	High fat	High carbohydrate
				First stage[b]			
Starch	65	Swimming		0.09	0.14	0.11	0.16
	65	None		0.34	0.25	0.30	0.20
	100	Swimming		0.26	0.29	0.24	0.27
	100	None		0.23	0.22	0.19	0.10
		Av		0.23	0.225	0.205	0.18
				Second stage[b]			
Starch	100	Forced		0.26	0.20	0.28	0.28
	100	Voluntary		0.25	0.16	0.30	0.23
Sucrose	100	Forced		0.33	0.17	0.30	0.24
	100	Voluntary		0.21	0.21	0.30	0.37
Lactose	100	Forced		0.20	0.30	0.28	0.37
	100	Voluntary		0.25	0.16	0.37	0.23
Mixed	100	Forced		0.19	0.20	0.28	0.30
	100	Voluntary		0.17	0.21	0.22	0.26
		Av		0.23	0.20	0.29	0.29

[a] Portion of ^{14}C-glucose initially oxidized at C-1 is estimated to be $(F_1—F_6)$; while total ^{14}C-glucose oxidized, *i.e.*, yielding some expired CO_2, is calculated as $(3F_U+0.5F_1—2.5F_6)$. See text for derivation.

[b] Wistar and Sprague-Dawley rats were used, respectively, in the first and second stages of the study.

of carbohydrate fed and form of exercise provided were varied. Apparently, carbohydrate restriction reduced to a similar degree the oxidation of glucose by the PC and via the oxidation of products of glycolysis. The fact that traffic on the PC pathway was not disproportionately inhibited by carbohydrate restriction reflected the major role of dietary protein in stimulating glucose-6-phosphate dehydrogenase (1, 2, 11). The high-fat and high-carbohydrate diets of the present studies provided identical amounts of the same proteins.

In two strains of rats under a variety of conditions, at least 25% of the injected glucose which was being irreversibly oxidized was first decarboxylated at the C-1 position by the PC over extended periods of the 24 hr day. This value appears conservative because of three points of inaccuracy in the experimental model. $(F_1 — F_6)$ underestimates the portion of the injected glucose selectively decarboxylated at C-1 by the PC in two ways. First, as previously indicated, a glucose molecule may be first acted upon by the PC and later yield up its C-6 as expired CO_2. Secondly, some glucose is selectively oxidized at the C-6 by the hexuronic acid cycle (12). Both of these factors are omitted from consideration in the model. On the other hand, $(3F_U+0.5F_1—2.5F_6)$ overestimates the fraction of tracer glucose which was partially or completely converted to CO_2 and water. This comes about through the fact that F_2 and F_5 were presumably intermediate between F_6 and F_3 and were underestimated in the model (as being equal to F_6).

These considerations indicate that, under a variety of conditions, at least 25% of the glucose which left the blood for relatively labile pools was selectively decarboxylated at the C-1 position, and that the PC participation may actually have far exceeded this value. The PC role becomes particularly impres-

sive in view of the low level of the pathway known to exist in heart and skeletal muscle (13). These facts indicate a particularly high level of utilization of the PC in adipose tissue and liver, even when dietary carbohydrate was in short supply.

Summary. The effect of high-fat vs high-carbohydrate diets on the relative role of the pentose cycle (PC) in glucose catabolism was studied in exercised and sedentary rats fed different carbohydrate sources. Expired $^{14}CO_2$ was measured after injection of ^{14}C-glucose labeled at C-1 or C-6 or at all carbons, and data were fitted to an experimental model. *Ad libitum* fed Sprague-Dawley rats appeared to show a diurnal rhythm in the degree of utilization of the PC in glucose catabolism. Exercise of calorically restricted animals reduced the relative role of the PC; but altering the fat:carbohydrate ratio of the diet of *ad libitum* fed animals had no apparent effect. Under a variety of conditions, at least 25% of the glucose which entered relatively labile pools was selectively decarboxylated at the C-1 position.

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