Nutritional Regulation of the Activities of Lipogenic Enzymes of Rat Liver and Brown Adipose Tissue

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Nutrition-induced effects on the activity of enzymes of lipogenesis, fatty acid synthase (FAS; EC 2.3.1.85), ATP citrate lyase (ACL; EC 4.1.3.8), malic enzyme (ME; EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44) were investigated in liver and interscapular brown adipose tissue (BAT) of rats. The lipogenic enzymes could be grouped into two categories according to their response to dietary manipulations: FAS and ACL, both key enzymes of lipogenesis, responded fast and strongly to dietary manipulations. ME, G6PDH and PGDH, enzymes which also contribute to metabolic pathways other than lipogenesis, responded in a more sustained and less pronounced fashion.

Feed deprivation caused the specific activities of lipogenic enzymes to decline several-fold. Refeeding of previously fasted (up to 3 days) animals increased the activities dramatically (10-to 25-fold) to far above pre-fasting levels ("overshoot"). Repetition of the fasting/refeeding regimen increasingly impaired the ability of both tissues to synthesize overshooting enzyme activities in the subsequent refeeding period. The fasting-induced decline of the activities was prevented when sugars were provided to the animals via drinking water. The sugars displayed different effectivities: sucrose= glucose> fructose> maltose>> lactose. Sugars as the sole nutrient after fasting were also able to induce overshooting enzyme activities. Again, activities of FAS and ACL responded in a more pronounced fashion than the other three enzymes.

Transition from feeding one diet to feeding a new diet of different composition led to adaptation of the lipogenic enzyme activities to levels characteristic for the new diet. Replacing a low-carbohydrate with a high-carbohydrate diet proceeded with major alterations of enzyme activities. This process of attaining a new level took up to 20 days and involved pronounced oscillations of the specific activities. In contrast, when a high-carbohydrate diet was replaced with another diet, particular one high in fat, transition to new enzyme activities was completed within 2–3 days and proceeded without oscillations. All dietary manipulations caused more pronounced responses in young (35d-old) than in adult (180d-old) animals.

Introduction

In mammals, the major sites of synthesis of fatty acids are liver, white and brown adipose tissue, and during lactation, mammary gland. The rates of lipid synthesis in these tissues vary profoundly with the nutritional status of the animal; the transformation of mostly carbohydrate into triglycerides to store the chemical energy occurs in response to excess caloric intake of the organism. The lipogenic process must, therefore, be regulated precisely in response to the ever changing energy needs and supplies (Wakil *et al.*,1983). The rate of lipid synthesis and the activities of the lipogenic enzymes are reduced with starvation, consumption of a fat-rich diet, or insulin deficiency, while refeeding, high insulin levels, or a low-fat, high-carbohydrate diet will markedly increase the activities of the lipogenic enzymes and restore the capability of the tissues to form triglycerides (Geelen *et al.*, 1980).

Although lipogenesis comprises all metabolic steps from glucose to triglycerides, attention has mostly focused on the enzymes which catalyze the

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formation of fatty acids from two-carbon precursors and on the auxiliary systems which generate NADPH. In this study five enzyme activities were investigated to cover a variety of steps of lipogenesis, viz. supply of small precursor units (ACL), chain elongation (FAS), supply of reducing equivalents (ME) as well as NADPH generation (G6PDH, PGDH). For a recent review on the regulation of fatty acid synthesis see (Hillgartner *et al.*, 1995).

The present paper gives a comprehensive description of various factors affecting lipogenic activity: dietary composition, i.e. fat vs. carbohydrate; various carbohydrates, i.e. starch vs. sucrose or sucrose vs. other dietary sugars; the response to feed deprivation; the influence of age on the ability to respond to nutritional stimuli. Particulary the influence on lipogenesis of various dietary sugars other than sucrose and fructose have so far not been described. The results should be very helpful in the assessment of the physiological responses following fasting regimens or changes of diet.

Materials and Methods

Animals and diets

Male Wistar rats from our own husbandry were used. They were kept on a standard stock diet (diet tpf 1324, Altromin, D-49828 Lage, Germany) in a 12 h light/dark cycle (lights on: 6 a.m. to 6 p.m.) in a temperature- and humidity-controlled room at 25 ± 2 °C. The animals received the above diet for at least 14 days before they were weighed and used for the experiments. At that time they were approximately 35, 75 and 180 days old. Under standard feeding conditions daily feed consumption was 11–13 g per animal for 35d old- and 16–18 g for 180d old individuals. Experimental diets were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio, U. S. A. Their composition is summarized in Table I. Daily feed consumption varied up to 10 percent between the different diets offered. Under conditions of refeeding previously starved animals (see text and Fig. 3) feed intake was 12–15 and 18–20 g /day for 35 and 180d old animals, respectively. This slightly increased feed intake persisted for the entire refeeding period investigated (up to 10 days) (see Boll *et al.*, 1994). Except under conditions of feed deprivation (1–3 days) animals had free access to feed. The animals had also free access to tap water. Sugar-containing tap water was supplied in liquid feeder tubes. For details concerning the individual feeding regimens see the figure and table captions.

Tissue sampling and preparation of crude extract

Livers and interscapular brown adipose tissue (BAT) were removed between 8 and 9 a.m. from ether-anesthetized animals and rapidly chilled in ice-cold buffer (0.1M sucrose, 0.05M KCl, 0.04M KH_2P0_4, 0.03M EDTA, pH 7.2). Contaminating tissue was carefully removed from BAT. Liver aliquots were homogenized in the above buffer (1 g tissue/3 ml buffer) in an ice-cooled Potter-Elvehjem homogenizer with 20 strokes. The homogenates were centrifuged at 20,000x*g* for 30 min, the resulting supernatant centrifuged at 105,000x*g* for 60 min and the resulting cytosols used as source of the enzymes. Interscapular BAT was homogenized as described for liver and the homogenates were centrifuged at 105,000x*g* for 45 min. The clear infranatant was then decanted from the fat layer and used as source of enzymes. All procedures were carried out at 4 °C.

Enzyme assays

Lipogenic enzymes were assayed with established spectrophotometric procedures: FAS (Hsu *et al.*, 1963), ACL (Inoue *et al.*, 1966), ME (Hsu and Lardy 1967), G6PDH (Löhr and Waller 1974) and PGDH (King 1974). Specific activities are expressed as μmol product formed per min x mg protein at 25 °C. Protein was determined with the Lowry method (Lowry *et al.*, 1951), using bovine serum albumin as standard. Biochemicals were obtained from Sigma Chemical Company, D-82041 Deisenhofen, Germany.

Results

Basic activities of lipogenic enzymes in liver and brown adipose tissue

The basic activities of five lipogenic enzymes as expressed in liver and BAT upon feeding of different diets are displayed in Table I together with the composition of the respective diets. The values shown are those found after feeding the diets for

Table I. Activities of lipogenic enzymes in liver and brown adipose tissue of rats fed diets of different composition.

	Diet* (ingredients in percent)		Specific activity [μmol/min x mg protein]									
			FAS		ACL		ME		G6PDH		PGDH	
			Liver	BAT	Liver	BAT	Liver	BAT	Liver	BAT	Liver	BAT
1	Carbohydrate	61	12	8.2	25	4.1	45	30	3.8	4.1	8.5	4.2
	Protein	19										
	Fat	7										
2	Sucrose	69	31	21	55	8.8	125	82	17	17	22	5.7
	Protein	22										
	Fat	0										
3	Sucrose	68	10	7.1	15	2.3	41	27	5.2	5.6	8.5	2.5
	Protein	18										
	Fat	8										
4	Sucrose	22	6.7	4.6	11	1.7	32	21	3.5	3.3	4.9	1.3
	Protein	64										
	Fat	8										
5	Starch	58	6.9	2.9	9.5	1.1	28	15	2.8	3.0	3.2	0.9
	Protein	27										
	Fat	10										
6	Starch	78	5.1	nd	7.5	nd	34	nd	35	nd	4.9	nd
	Protein	8										
	Fat	10										

* The standard stock diet (diet 1) contained a mixture of cereal-derived carbohydrates, protein (extracted from soy bean grist), fat (soy bean), 5% fibrous material, 3% minerals and vitamins and 5% ashes. Experimental ICN diets (diet 2–6) contained casein as source of protein, cotton seed oil as fat and corn starch (diet 5 and 6). They also had 1.5% brewers yeast and 4% salt- and vitamin mixture. Diet 2, in addition, contained 5% fibrous material. Animals received the indicated diet for 28 days (see Methods) before enzyme activities were determined; (nd= not determined). Values are the mean of 6 animals. Standard errors (5–14%) omitted for clarity. BAT, brown adipose tissue; FAS, fatty acid synthase; ACL, ATP citrate lyase; G6PDH, glucose-6-phosphate dehydrogenase; PGDH, 6-phosphogluconate dehydrogenase.

approximately 4 weeks and they represent stable values which on continuation of feeding were no longer altered. Generally the activities of the lipogenic enzymes in BAT were lower than in liver. The most pronounced diet-induced differences in the enzyme activities of liver and BAT, respectively, were observed with ACL and PGDH. The activities of G6PDH in liver and BAT were virtually identical (Table I). Diet-induced changes of enzyme activities always occurred at similar ratios in the two tissues, and experimental manipulations of the activities will further be reported only for one tissue and generally for selected enzymes in an exemplary fashion.

Both the amount and the nature of the carbohydrate portion of a diet influenced the activities of the lipogenic enzymes in either tissue (Table I). The stock diet (diet 1), containing a mixture of cereal-derived carbohydrates, resulted in twice as high enzyme activities as the similarly composed experimental diet 5, which contained only corn starch as carbohydrate. If sucrose was substituted for starch the enzyme activities increased strongly (compare diets 5 and 6 to diets 2 and 3).

The highest activities, as expected, ensued from the fat-free diet 2. However, the amount of sucrose played a similarly important role: switching the portions of sucrose and protein in the presence of normal amounts of fat (diet 3 vs. diet 4) resulted in relatively low enzyme activities. High dietary fat caused the lowest activities (compare diet 6 to diets 1 and 2). Enzyme activities shown in Table I are for young male animals. In addition there are significant differences in the enzyme levels between male and female as well as between young and old individuals (Boll *et al.*, 1994).

Change of diet

When during standard feeding conditions a diet was replaced by another one of different composi-

tion, the activities of the lipogenic enzymes adapted to a level characteristic for the new diet. With higher carbohydrate or lower fat content of the new diet adaptation would result in an increase of the activities, and in a decrease with high fat and low sugar, respectively. Fig. 1 summarizes the changes of the activities on transition from a low-sucrose, fat containing diet (diet 4) to diets of different composition. The adaptation of enzyme activities was not a fast and straight-forward process: depending on the diet it would take several weeks during which the enzyme activity would oscillate with a roughly three-day cycle, increasing as much as 15-fold over starting levels (Fig. 1). The kinetics of the adaptation exhibited a maximum of the specific activities around day 16 (Fig. 1). Different enzymes were affected in different ways: FAS and ACL (Fig. 1 A, B) responded very strongly to the fat-free diet 2, and less to diet 3, whereas diets 5 and 6 caused only minor changes. The response of G6PDH activity was basically the same but less pronounced (Fig. 1 C). In contrast, ME activity responded very strongly to the low-protein, fat-rich diet 6, somewhat less to the high-starch diet 5, and even less pronounced to the high-sucrose diets 2 and 3 (Fig. 1 D). In any case, upon continued feeding of a given diet the oscillations and overshoot would eventually stop and enzyme activity would gradually adjust to the level characteristic for the new diet (see Table I).

Feeding rats for several weeks with a high-sucrose fat-free diet (diet 2) resulted in very high activities of the lipogenic enzymes in liver and BAT (Table I). In this case a transition to diets of different composition always caused the enzyme activities to decline. This is summarized in Fig. 2. The velocities of decline varied with the different

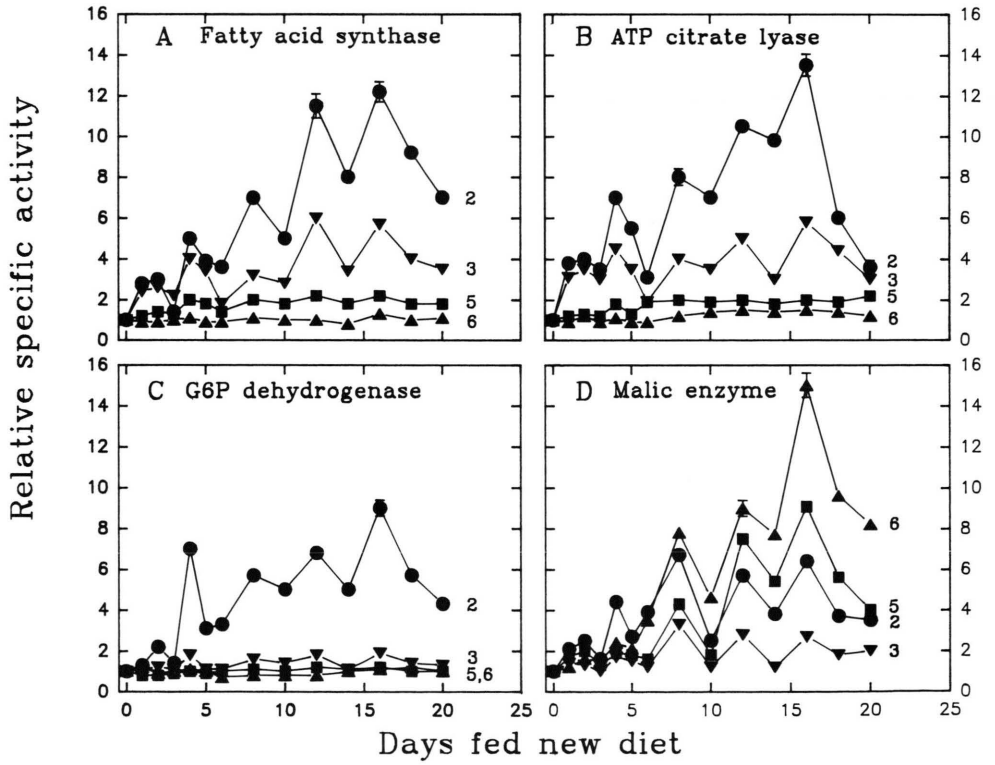

Fig. 1. The effect of a change in the diet composition (initial diet: low-sucrose, fat-containing diet 4) on the activities of liver lipogenic enzymes.
Animals were fed with diet 4 of Table I for 28 days before being transfered to the different diets of the figure at time zero. Numbers are diets of Table I. Specific activity of the enzymes in animals fed diet 4 was set at 1. For absolute values of initial specific activity see diet 4 in Table I. Means of 6 animals. Standard errors shown for selected points only; errors in the other points are in corresponding range.

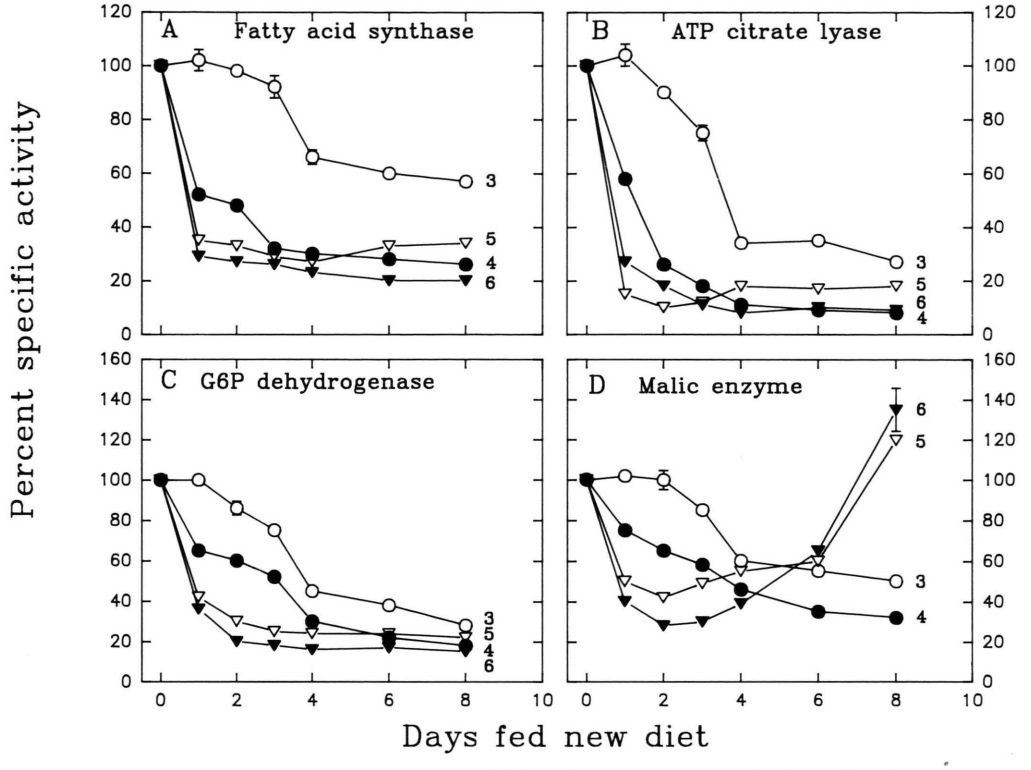

Fig. 2. The effect of a change in the diet composition (initial diet: high-sucrose, fat-free diet 2) on the activities of liver lipogenic enzymes.
Animals were fed with diet 2 of Table I for 28 days before being transfered to the different diets of the figure at time zero. Numbers are diets of Table I. Specific activity of the enzymes in diet 2 was set at 100 percent. Values are percent activity of the initial values. For absolute values see diet 2 in Table I. Means of 6 animals ± SEM. For further details see legend to Fig. 1.

diets. It was slowest with diet 3 which, like diet 2, contained high amounts of sucrose but also fat which supresses the activities (Geelen *et al.*, 1980). Enzyme activities decreased substantially faster with diet 4 having less sucrose than diet 3, while with diets 5 and 6, containing high amounts the less easily digestible carbohydrate starch, the decline was still faster in response to the dietary change. Thus, decline of the lipogenic activities obviously reflects quality and quantity of the carbohydrate portion of the new diet as well as its fat content. Again the pattern of change of ME activity was different (Fig. 2 D): The response of this enzyme's activity to switching from diet 2 (initial diet) to diets 3 and 4 was essentially the same as with the other enzymes. Diets 5 and 6, however, caused a rapid decrease of ME activity to a minimum within 2 days, which was followed by a gradual increase until day 6, and then a rapid increase exceeding initial levels by day 8 (Fig. 2 D).

Levels of the serum lipids (triglycerides, cholesterol and phospholipids) in rats were also different among the offered diets (Boll *et al.*, 1996). A change of the diet composition here also resulted in an adaptation to the levels of the new diet with variations, including oscillations (Boll *et al.*, 1996). It is speculated that these oscillating responses are a reflection of the activity changes of the synthesizing enzymes.

Fasting and refeeding responses

The common response of lipogenic enzyme activities in liver and BAT to feed deprivation is decline (Gibson *et al.*,1972; Waerie and Kanagabasai 1982; Boll *et al.*, 1994). These well-known facts

will not be repeated here. Suffice to say that upon fasting the activities of FAS and ACL in the present study generally decreased more rapidly than those of ME, G6PDH and PGDH. When the animals were deprived for 3 days of the previously fed high-sucrose diets 2 or 3, activities of FAS and ACL both declined 80%, ME decreased 65%, G6PDH 55% and PGDH 50%, respectively (percent decreases as compared to values in Table I). Most of these declines occurred within the first day of fasting. If the diet before the starvation period was high in starch (diet 5 or 6) the decreases were not so pronounced (viz., 55–37% maximum). However, the decline occurring here on the first day of fasting was small and became more pronouced on the following two days.

Refeeding of starved animals results in a transient increase of the activities of lipogenic enzymes to levels far higher than those observed before feed deprivation. This phenomenon has been observed repeatedly (Muto and Gibson 1970; Gibson *et al.*, 1972; Yagil *et al.*, 1974; Boll *et al.*, 1994; Boll *et al.*, 1995), and has been termed "overshoot" (Berdanier and Shubeck 1979). As shown in Fig. 3 the increase of the activities of lipogenic enzymes upon refeeding animals deprived of feed for 3 days, will also reflect the composition of the refeeding diet. The activities of FAS, ACL, G6PDH and also PGDH (not shown) increased mostly with diet 2, followed by diet 3. Diets 4, 5 and 6 were less effective in producing an overshoot of enzyme activity. As with previously experiments the response of ME activity to refeeding various diets differed appreciably from the responses of the other enzymes. The high-sucrose diets 2 and 3 elicited comparatively moderate responses,

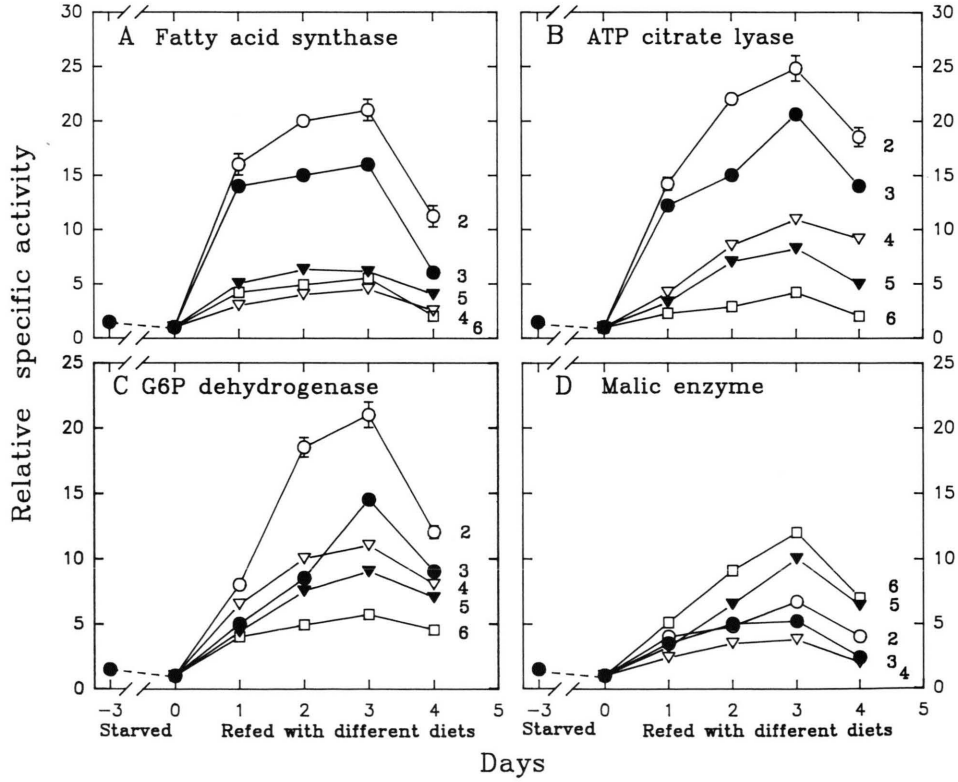

Fig. 3. The effect of fasting and subsequent refeeding with diets of different composition on the activities of liver lipogenic enzymes.
Animals previously fed with the standard stock diet (diet 1 of Table I) were deprived of feed for 3 days and subsequently refed with the different diets of the figure. Numbers are diets of Table I. Specific activity of the enzymes in animals fed the standard diet (basal activty) was set at 1. For absolute values of base specific activities see diet 1 in Table I. Means of 4 animals ± SEM. For other details see legend to Fig. 1.

whereas the starch-containing diets 5 and 6 resulted in a 10-fold overshoot by 3 days of refeeding (Fig. 3 D).

With all enzymes the maximum overshoot occurred on the 3rd day of refeeding (Fig. 3). It could, however, depending on the experiment, also occur already on the second day of refeeding (Boll *et al.*, 1994). Thereafter, oscillations of enzyme activities were observed (Boll *et al.*, 1994). The amplitude of the oscillations reflected the composition of the refeeding diets and the highest amplitudes ensued from the high-sucrose, fat-free diet 2 (Boll *et al.*, 1994). The magnitude of the overshoot increased with the duration of the previous feed deprivation. For example FAS activity in BAT of 35d old animals was induced 8.5-, 11-, and 15-fold over fasting activity within 1 day of refeeding after feed had been withheld for 1,2, and 3 days, respectively. Likewise the amplitude of the oscillations of enzyme activity which were observed here reflected the duration of the foregoing fasting period, being greater after longer fasting periods. When the 3 day fasting / 3 day refeeding regimen was repeated up to 3 times in a row, the extent of the response of enzyme activities diminished with each repetition. At the third repetition the response was only one-third to one-fifth of that after the first fasting/refeeding transition (Table II).

Table II. The effect of a repeated starved- to refed transition on the capacity of induction of the activity of the lipogenic enzymes from brown adipose tissue.

	Fold increase of specific activity		
Enzymes	A	B	C
FAS	36.1 ± 3.26	20.3 ± 2.24	6.9 ± 0.63
ACL	22.2 ± 1.91	10.3 ± 1.21	7.2 ± 0.75
ME	13.4 ± 1.44	4.5 ± 0.48	2.5 ± 0.18
G6PDH	8.5 ± 0.79	4.8 ± 0.49	2.5 ± 0.27
PGDH	6.5 ± 0.68	4.0 ± 0.39	2.3 ± 0.23

Animals (35 days-old), previously held on the standard stock diet (diet 1), were divided into groups A-C. Each group was then deprived of feed for 3 days and subsequently refed with the high-sucrose, fat-free diet (diet 2) for 3 days. The procedure of fasting and subsequent refeeding was repeated once in group B and twice in group C. Each time enzyme activities were determined at the end of feed deprivation and after the 3 day refeeding period. Values are fold increase of enzyme activity over the value of the respective period of feed deprivation. For absolute values see Table I, diet 1. Means of 4 animals ± SEM.

Effect of different sugars as dietary source of carbohydrate

Among a variety of possible dietary manipulations the carbohydrate portion of a diet exerted the strongest effect on the activities of enzymes of lipogenesis. The 3 day starvation-induced decline of these activities could be completely prevented if a sugar was provided as the sole nutrient via drinking water. This is shown in Table III for liver FAS. At an average intake of 15 g/d/100 g body weight sucrose, glucose, fructose and maltose all completely prevented a decline of the lipogenic activities induced by feed deprivation (Table III), while lactose could only partly prevent this decline. Lowering the sugar intake revealed that the sugars have different capabilities to prevent the decrease of enzyme activities. Sucrose and glucose were still fully effective at an intake of 7.5 g/d/100 g body weight. Fructose was still completely effective at 10 g/d/100 g body weight, maltose only at 15 g/d/100 g and lactose was only partially effective (Table III).

The activities of the lipogenic enzymes responded likewise with recovery and overshoot when feed depriviation was followed by refeeding with sugars as the sole nutrient. The minimum amount of sucrose to affect enzyme activities varied with the enzyme under consideration. This is summarized in Table IV. The activities of FAS and ACL responded to as little sucrose intake as 1.87 g/d/100 g body weight, ME activity began to increase at 3.75 g/d/100 g. The activities of G6PDH and PGDH started to respond only above 7.5 g of sucrose. The activities of FAS, ACL and ME, but not those of G6PDH and PGDH, increased significantly beyond prestarvation levels (A) during 2 days with the highest daily intake of sucrose (22.5 g/d/100 g body weight) (C). However, even this highest daily intake of sucrose achieved only part of the overshoot in enzyme activity observed after 2 days refeeding the complete high-sucrose diet 2 (D). The amount of sucrose from diet 2 which was consumed by the animal (20–24 g/d/100 g body weight) matched the amount of the sucrose intake via drinking water (22.5 g/d/100 g body weight). Refeeding for two days with the

Table III. The effect of different sugars on the starvation-induced decrease of liver fatty acid synthase activity.

Treatment of animals			Fatty acid synthase (specific activity)		
A control fed			12.2 ± 1.1		
B starvation, 3 days			3.1 ± 0.32		
C starvation, 3 days + sugars (g/d/100 g body weight)	Sucrose	Glucose	Fructose	Maltose	Lactose
15.0	13.1 ± 1.35	12.5 ± 1.31	11.6 ± 1.12	13.4 ± 1.35	6.5 ± 0.68
10.0	12.4 ± 1.22	14.1 ± 1.48	10.9 ± 1.05	8.8 ± 0.91	3.8 ± 0.42
7.5	12.1 ± 1.24	11.0 ± 1.08	7.9 ± 0.82	5.9 ± 0.63	3.1 ± 0.33
3.75	7.3 ± 0.74	6.9 ± 0.71	4.5 ± 0.46	3.8 ± 0.39	– –
1.85	4.1 ± 0.42	3.8 ± 0.40	3.0 ± 0.31	3.3 ± 0.31	3.2 ± 0.33

Rats (35 days-old) were fed the standard stock diet (A) before feed was withdrawn. Animals were then kept for 3 days on tap water only (starvation) (B) or on tap water supplemented with the indicated concentrations of the different sugars (C). Sugar intake was measured from the daily volume of fluid intake. Control daily intake of drinking water containing different amounts of the sugars was measured in a total of 8 animals. Values are means of 4 animals ± SEM.

Table IV. The ability of sucrose to induce the activity of liver lipogenic enzymes after feed deprivation.

Treatment of animals	Specific activity FAS	ACL	ME	G6PDH	PGDH
A control fed	12.2 ± 1.14	25.3 ± 2.48	42.1 ± 4.22	3.6 ± 0.36	8.4 ± 0.85
B fasting	2.9 ± 0.26	4.3 ± 0.44	17.4 ± 1.75	1.8 ± 0.18	4.4 ± 0.46
C refed with sucrose					
1.87	4.7 ± 0.49	6.1 ± 0.62	18.3 ± 1.85	1.3 ± 0.12	2.3 ± 0.24
3.75	5.9 ± 0.63	10.0 ± 1.14	23.4 ± 2.38	1.4 ± 0.14	2.6 ± 0.25
7.5	8.7 ± 0.84	13.2 ± 1.32	36.7 ± 3.66	1.8 ± 0.17	3.3 ± 0.33
15.0	16.1 ± 0.17	18.2 ± 1.81	49.8 ± 4.85	2.3 ± 0.23	3.8 ± 0.39
22.5	25.5 ± 0.26	46.1 ± 4.66	55.3 ± 5.71	4.5 ± 0.36	9.3 ± 0.83
D refed with diet 2	39.4 ± 3.95	85.2 ± 8.60	138.7 ± 13.5	16.0 ± 1.65	24.5 ± 2.50
E refed with diet 2 + sucrose	37.5 ± 3.81	78.4 ± 7.95	141.0 ± 14.2	16.5 ± 1.72	23.2 ± 3.32

Animals (35 days old) had been kept on the standard stock diet (diet 1) before feed deprivation was initiated (A). The rats, after starvation for 3 days (B), received for 2 days either different amounts of sucrose via drinking water (g/d/100 g body weight) (C), high-sucrose, fat-free diet (diet 2) (D) or this diet plus 15 g of sucrose/d/100 g body weight (E). Values are means of 4 animals ± SEM.

high-sucrose, fat-free diet 2 plus additional sucrose in the drinking water had no further stimulatory effect (E). Activities of FAS, ACL and ME began to oscillate after the third day of refeeding 22.5 g sucrose/d/100 g body weight (data not shown).

Influence of age

Between 35 and 180 days of age the base activities of the lipogenic enzymes of liver and BAT decreased considerably (Table V). FAS, ACL, and ME activities decreased more during this time span than those of G6PDH and PGDH. The major portion of this decline of the specific activities occurred between 35 and 75 days of age (Table V). The enzyme activities will continue to decline beyond an age of 180 days, albeit slowly (Boll *et al.*, 1994).

The ability of the lipogenic enzymes to respond to changes in the dietary intake decreased in magnitude with increasing age. The decrease of the activities upon feed deprivation (see above) was 10–25 percent smaller in 180d old animals than in 35d old animals. Likewise, the increase of the lipogenic activities occurring in response to refeeding starved 180d old was only 50–60 percent of that of 35d old individuals. As shown in Table VI this also was the case when sucrose was the only nutrient during refeeding. Thus, the animals displayed considerably higher degrees of en-

Table V. Decrease of base activities of lipogenic enzymes in liver and adipose tissue between the age of 35 and 180 days.

Enzymes	Specific activity					
	Liver			BAT		
	35d	75d	180d	35d	75d	180d
FAS	12.0 ± 1.33	6.5 ± 0.68	3.7 ± 0.38	8.2 ± 0.85	5.3 ± 0.52	3.6 ± 0.37
ACL	25.3 ± 2.58	13.4 ± 1.44	10.0 ± 1.11	4.1 ± 0.40	2.2 ± 0.21	1.8 ± 0.19
ME	45.1 ± 4.65	26.2 ± 2.70	18.4 ± 1.91	31.4 ± 3.35	20.3 ± 2.14	17.6 ± 1.78
G6PDH	3.8 ± 0.37	2.9 ± 0.31	2.4 ± 0.23	4.2 ± 0.43	3.2 ± 0.34	2.7 ± 0.26
PGDH	8.5 ± 0.86	7.1 ± 0.72	5.3 ± 0.58	2.5 ± 0.26	1.9 ± 0.20	1.6 ± 0.17

Experimental animals were fed the standard stock diet (diet 1). Values are specific activities at the indicated days. Means of 4 animals ± SEM.

Table VI. The effect of age on the increase of liver lipogenic activities after refeeding starved animals with sucrose.

Enzymes	Fold increase of specific activity	
	35 days old	180 days old
FAS	8.1 ± 0.76	5.2 ± 0.53
ACL	7.2 ± 0.81	4.3 ± 0.43
ME	3.0 ± 0.29	1.9 ± 0.18
G6PDH	1.1 ± 0.13	0.7 ± 0.06
PGDH	0.7 ± 0.07	0.4 ± 0.04

Animals previously kept on the standard stock diet (diet 1) were deprived of feed for 2 days and subsequently received sucrose for 1 day. Sucrose, applied via drinking water, was 15 g/d/ 100 g body weight. Values are fold increase after 1 d in the presence of sucrose over the starvation level. Means of 4 animals ± SEM.
For absolute values *cf.* Table V.

zyme induction, and adult individuals responded in a more protracted fashion to the fasting/refeeding challenge. Keeping in mind that the basic enzyme activities in adult animals were already lower than in young ones (Table V), the maximum effect on lipogenesis that could be elicited by dietary manipulations was comparatively small in mature animals.

Discussion

The components in dietary fat which inhibit lipogenesis are most likely polyunsaturated fatty acids (Wilson *et al.*, 1990) as mono-unsaturated fatty acids, e.g., oleate do not inhibit enzymes of lipogenesis (Clarke *et al.*, 1976, 1977; Baltzell and Berdanier, 1985). The effect of polyunsaturated fatty acids in a diet is so strong that it can overwhelm the inducing effect of a high-carbohydrate portion (Baltzell and Berdanier 1985). The experimental ICN diets 3 through 6 used in this study contained cotton seed oil, which consists to 42% of the unsaturated linoleic acid, explaining their high efficiency to limit lipogenesis (Table I).

A high carbohydrate portion in the diet can induce lipogenesis only if the carbohydrate is readily available, e.g., in the form of sucrose rather than of starch (compare diets 2 and 3 to diets 1,5 and 6; Table I). Diet 4, with highly available, but low-level carbohydrate, did not induce lipogenesis above base levels. This suggests that not only the amount of carbohydrate in a given diet regulates lipogenesis, but also the rate of absorption through the alimentary tract, i.e., the change per unit time and peak levels of blood glucose. There must be, however, additional factors beyond dietary sugar which regulate lipogenesis. The data of Table IV indicate that, although sucrose alone can induce lipogenesis far beyond base levels (Table IV C), a fat-free diet with high sucrose contents was at least twice as effective (Table IV D). The additional factor(s) may be protein, or vitamins and minerals which supply cofactors for converting enzymes.

The carbohydrate component which ultimatively affects the extent of lipogenesis is glucose (Hillgartner *et al.*, 1995). Most diets, however, contain composite carbohydrates, i.e., starch, fructose, sucrose, lactose, etc. They are differently effective in inducing lipogenesis. It has been shown that a single dose of fructose is already sufficient for synthsis and increase of FAS activity and that fructose is more effective than glucose itself (Volpe and Vagelos, 1974). Somewhat different from these results the present study indicates that glu-

cose and sucrose were similar effective to induce lipogenesis, and fructose was slightly less effective (Table III). These are very common dietary sugars, whereas maltose and lactose are not as common, and accordingly the latter sugars were far less effective to induce lipogenesis. The differences most likely reflect low effectiveness or, particularly in the case of lactose, low capacity in adult animals of enzyme systems which convert composite sugars ultimately into the standard currency of carbohydrate metabolism, viz., glucose. These findings have importance for the assessment of metabolic regulation: not only hormones, but also nutritional components in blood (above all glucose and polyunsaturated fatty acids) directly influence lipogenesis (*cf.* Hillgartner *et al.*,1995).

Of the enzyme activities investigated here only FAS and ACL are involved exclusively in lipogenesis, the other enzymes contribute more or less to other metabolic pathways. Major dietary manipulations which change the substrate flux through the pathway of lipogenesis several-fold must ultimately affect also the activities of collateral enzymes. The data reported here (see e.g. Figs. 2 and 3) indicate that the activities of the collateral enzymes reponded slower and leveled off at less extreme values than the immediately involved enzymes FAS and ACL, corroborating both their participation in other metabolic pathways and a more remote nature of their regulation via dietary manipulations.

In the experiments shown in Fig. 1 (switch from low-sucrose, fat-containing diet to other diets), Fig. 2 (switch from high-sucrose, fat-free diet to other diets) and Fig. 3 (fasting/ refeeding challenge) the activity of ME responded to the transitions in a different fashion than the other enzyme activities. We have no evident explanation for this phenomenon; however, it could be related to rate of change and/or peak levels of blood glucose as mentioned above. A slow increase of blood glucose with ultimately high levels may signal to the regulator of ME activity a higher need for shuttling reducing equivalents between metabolic compartments than does a rapid increase of blood glucose.

Oscillations of the activities of lipogenic enzymes as a result of a dietary manipulation have been observed repeatedly and reproducibly (Gibson *et al.*, 1972; Yagil *et al.*, 1974; Boll *et al.*, 1994). Their origin is yet unknown. One could suspect that after fasting they are caused by cycles of initial overeating followed by voluntary fasting but in the present study feed intake was found to be constant on all days where oscillations of enzyme activities were seen. Additional proof for an endogenous origin of the oscillating enzyme activities comes from the experiments where fasting was followed by feeding sugars via drinking water: again the intake was steady over several days, yet the oscillations were seen as before.

Maturation and aging diminished the capability of the organism to induce lipogenesis. Lower activities of acetyl CoA carboxylase (Czech *et al.*,1977), FAS (Czech *et al.*,1977) and ACL (Hoffmann *et al.*,1979) have been reported to occur in the adipose tissue with aging of animals. This might be a consequence of the obesity which develops with age particularly in males and to a much lesser extent in females. The age-related decrease of lipogenic activity in the liver of rats was more pronounced in males than in females (Boll *et al.*,1994) . The two enzymes which supply NADPH for lipogenesis from the pentose-phosphate pathway, viz. G6PDH and PGDH, were reduced only in mature males but not in females (Boll *et al.*,1994). In the case of ACL it has been shown that the age-related decrease is a function of obesity rather than of age (Hoffmann *et al.*,1980). Thus, the general conclusion might be drawn that the reduction of lipogenic enzyme activities occurring with age is a consequence of the obesity which develops concomitantly.

The mechanisms controlling the response of lipogenic enzymes to nutritional stimuli are allosteric control via humoral factors (short-term), phosphorylation/ dephosphorylation and/ or synthesis/ degradation of enzymes (long-term) (Hillgartner *et al.*,1995). Considering the time frame of most changes demonstrated in this study, viz., one day to a few days to attain maximum changes, clearly speak in favor of long-term regulation at the mRNA level. It has been shown that weaning of suckling rats to a high-fat diet prevents increases of FAS and acetyl CoA carboxylase mRNA abundances and of enzyme activities, whereas weaning to high-carbohydrate diet increased mRNA abundances and enzyme activities (Perdereau *et al.*,1990). However, the immediate point of regulation differs with enzyme. As compiled by Hillg-

artner *et al.*, (1995) in rat liver the mRNA of FAS is regulated at the transcriptional level, that of G6PDH transcriptionally with some effect on mRNA stability, that of ME at the post-transcriptional level, and that of PGDH pre-translationally. The receptors and effectors active at the gene level have not yet been identified.

Baltzell J. K. and Berdanier C. D. (1985), Effect of the interaction of dietary carbohydrate and fat on the responses of rats to starvation-refeeding. J. Nutr. **115**, 104–110.

Berdanier C. D. and Shubeck D. (1979), Interaction of glucocorticoid and insulin in the responses of rats to starvation-refeeding. J. Nutr. **109**, 1766–1771.

Boll M., Weber L. W. D., Stampfl A. and Messner, B. (1994), Lipogenic enzymes of rat liver and adipose tissue. Dietary variations and effect of polychlorinated biphenyls. Z. Naturforsch. **49c**, 665–678.

Boll M., Weber L. W. D. and Stampfl A. (1995), The effect of γ-hexachlorocyclohexane (Lindane) on the activities of liver lipogenic enzymes and on serum lipids in rats. Z. Naturforsch. **50c**, 135–142.

Boll M., Weber L. W. D. and Stampfl A. (1996), The response of rat serum lipids to diets of varying composition or contaminated with organochlorine pesticides. Z. Naturforsch. **51c,** 91–100.

Clarke S. D., Romsos D. R. and Leveille G. A. (1976), Specific inhibition of hepatic fatty acid synthesis exerted by dietary linoleate and linolenate in essential fatty fatty acid adequate rats. Lipids **11**, 485–490.

Clarke S. D., Romsos D. R. and Leveille G. A. (1977), Differential effects of dietary methyl esters of long-chain saturated and polyunsaturated fatty acids on rat liver and and adipose tissue lipogenesis. J. Nutr. **107**, 1170–1181.

Czech M. P., Richardson D. K., and Smith C. J. (1977), Biochemical basis of fat cell insulin resistance in obese rodents and man. Metabolism **26**, 1057–1078.

Geelen M. J. H., Harris R. H., Beynen A. C. and Mc Cune S. A. (1980), Hormonal control of hepatic lipogenesis. Diabetes **29**, 1006–1022.

Gibson D. M., Lyons R. T., Scott D. F. and Muto Y. (1972), Synthesis and degradation of the lipogenic enzymes of rat liver. Adv. Enzyme Regul. **10**, 187–204.

Hillgartner F. B., Salati L. M. and Goodridge A. G. (1995), Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. Physiol. Rev. **75**, 47–76.

Hoffmann G. E., Kreisel C., Wieland O. H. and Weiss L. (1979), Pyruvate dehydrogenase and ATP citrate (*pro*-3S)-lyase activities in adipose tissue and liver of the young lean and the older obese rat. Hoppe-Seyler's Z. Physiol. Chem. **360**, 45–50.

Hoffmann G. E., Kraupe P., Andres H. and Weiss L. (1980), Influence of age and obesity on ATP citrate (*pro* 3 S)-lyase activities in the rat. Hoppe-Seyler's Z. Physiol. Chem. **361**, 1121–1123.

Hsu R. Y. and Lardy H. A. (1967), Pigeon liver malic enzyme. II. Isolation, crystallization and some properties. J. Biol. Chem. **242**, 520–526.

Hsu R. Y., Wasson G. and Porter J. W. (1963), The purification and properties of fatty acid synthetase of pigeon liver. J. Biol. Chem. **240**, 3736–3746.

Inoue H., Suzuki F., Fukunishi K., Adashi K. and Takeda J. (1966), Studies on ATP citrate lyase of rat liver. I. Purification and some properties. J. Biochem. (Tokyo) **60**, 543–553.

King J.(1974), 6-Phosphogluconat-Dehydrogenase. In: Methoden der enzymatischen Analyse, (H. U. Bergmeyer, ed.).Verlag Chemie, Weinheim, pp. 668–672,

Löhr G. W. and Waller H. D. (1974), Glucose-6-phosphat dehydrogenase. In: Methoden der enzymatischen Analyse (H. U. Bergmeyer, ed.). Verlag Chemie, Weinheim, pp. 673–681.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall J. (1951), Protein measurement with the Folin phenol reagent. J Biol. Chem. **193**, 265–275.

Muto Y. and Gibson D. M. (1970), Selective dampening of lipogenic enzymes of liver by exogenous polyunsaturated fatty acids. Biochem. Biophys. Res. Commun. **38**, 9–15.

Perdereau J. D., Narkewisz M., Coupe C., Ferre P. and Girard J. (1990), Hormonal control of specific gene expression in the rat liver during the suckling-weaning transition. Adv. Enzyme Regul. **30**, 91–108.

Volpe J. J. and Vagelos P. R. (1974), Regulation of mammalian fatty acid synthetase. The roles of carbohydrate and insulin. Proc. Natl. Acad. Sci. USA **71**, 889–893.

Wakil J. S., Stoops J. K. and Joshi V. C. (1983), Fatty acid synthesis and its regulation. Annu. Rev. Biochem. **52**, 532–579.

Wearie P. J. and Kanagabasai T. F. (1982), Effects of high-carbohydrate diets on lipogenesis in rat interscapular brown adipose tissue. Biochem J. **206**, 667–669.

Wilson M. D., Blake W. L., Salati L. M. and Clarke S. D. (1990), Potency of polyunsaturated and saturated fats as short-term inhibitors of hepatic lipogenesis in rats. J. Nutr. **120**, 544–552.

Yagil G., Shimron F. and Hizi A. (1974), On the mechanism of glucose-6-phosphate dehydrogenase regulation in mouse liver. 1. Characterization of the system. Eur. J. Biochem. **45**, 189–200.