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A study of acetyl CoA-carboxylase in adipose tissues

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Introduction

The *de-novo* biosynthesis of fatty acids in tissues depends on the activities of lipogenic enzymes (1). Acetyl-CoA-carboxylase (EC 6.4.1.2.) has frequently been described as the rate-limiting enzyme (2–6), but this claim has been disputed in at least two cases (7, 8). When it was found (preceding paper) that two layers of porcine subcutaneous adipose tissue differ considerably in some biochemical parameters relevant for lipogenesis, we attempted to reinvestigate the rate control of acetyl-CoA-carboxylase in fatty acid biosynthesis. Special attention was paid to enzymologically as well as nutritively competent experimental designs.

In the course of studies of the hereditability of acetyl-CoA-carboxylase in the pig, and by comparison of nutritive effects on different adipose tissues in laboratory rodents, informations on this enzyme were obtained which form the basis of this communication.

Materials and methods

Pigs (Deutsche Landrasse) were kept under standardized conditions in the Growth Performance Station Forchheim, the Experimental Station Unterer Lindenhof, or in the Department of Animal Nutrition, University of Hohenheim. Albino rats SIV 50 were obtained from Ivanovas Animal Farm, Kißlegg; obese NZO mice were a present from Prof. W. Staib, Düsseldorf.

Special nutritive measures are given in the legends to figures and tables; for a comprehensive description see l.c. (9). Chemicals, buffer substances and solvents of analytical grade were obtained from E. Merck, Darmstadt; fine biochemicals and enzymes were supplied by Boehringer Mannheim, Serva, Heidelberg, and Sigma, St. Louis.

Adipose tissue from pigs was taken by biopsy during a short thiobarbiturate narcosis. Adipose tissues from laboratory rodents were excised after cervical dislocation. Rat liver was taken under freeze-clamp conditions. All tissue samples were kept deeply frozen until used; liver was lyophilized and powdered.

Enzyme activities were measured at 334 nm in $30 \times 10^3 \times$ g-30 min supernatants of tissue homogenates after removal of visibly separated lipid material. Acetyl-CoA-carboxylase was extracted with 0.05 M Tris/HCl buffer of pH 7.4, containing 0.1 M KCl and 0.02 M K-citrate and measured via ADP formation (l.c. 9, 10). NADPH-forming dehydrogenases were extracted with 0.15 M KCl and assayed according to

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(l.c. 9, 11, 12). Soluble protein was determined after Lowry *et al.* (13) with bovine serum albumin as reference.

Pig body composition was estimated by standardized inspection methods (animals from Forchheim), or by back fat thickness (ultrasonic echo probe, animals from Unterer Lindenhof). Rodent corpses were lyophilized, ground and extracted in a Soxhlet apparatus with low boiling petrol ether (b.p. 40–60 °C). The dried extract was weighed.

Results

Genetic data

Activity patterns of lipogenic enzymes in the subcutaneous adipose tissue of the pig are given in table 1. Since the hereditability of the four NADPH yielding dehydrogenases was established (14) previously, the correlation coefficients of acetyl-CoA-carboxylase (table 1) prove indirectly the hereditability of this enzyme. Table 2 demonstrates changes in the activity levels of acetyl-CoA-carboxylase in a selection experiment, when pigs are bred for low or high activities of the four dehydrogenases. These data thus demonstrate the genetic constitution of adipose tissue acetyl-CoA-carboxylase in the pig, which renders itself toward breeding experiments.

Table 1. Activity levels of lipogenic enzymes in subcutaneous adipose tissue of the pig (inner layer).

	Acetyl-CoA-carboxylase	Malic enzyme	Glucose-6-phosphate dehydrogenase	6-Phosphogluconate dehydrogenase	Isocitrate dehydrogenase
Activity (mU/mg soluble protein)	135	980	380	215	325
Coefficient of correlation		0.80	0.78	0.77	0.78
Significance		< 0.001	< 0.001	< 0.001	< 0.001

Table 2. Acetyl-CoA-carboxylase in subcutaneous adipose tissue of the pig (outer layer) in breeding selection for low (E^-) and high (E^+) activities of NADPH generating dehydrogenases, respectively.

	E^- (mU/mg soluble protein)	E^+ (percent of E^-)
First daughter generation	165 ± 144 (n = 125)	+ 12 (n = 120)
Second daughter generation	111 ± 95 (n = 112)	+ 90 (n = 122)
Third daughter generation	47 ± 43 (n = 81)	+ 176 (n = 84)

Table 3. Acetyl-CoA-carboxylase and extent of body fat deposition. Upper part: mU/mg soluble protein; enzyme activity as ratio outer/inner layer of subcutaneous adipose tissue of the pig (age 120 days) versus back fat thickness (in cm) (ultrasonic measuring, age 150 days); lower part: morphometric data on slaughtering versus enzyme activities as above).

Breeding line	Enzyme ratio	Back fat
E^-	0.58 ± 0.38	1.46 ± 0.08
E^+	0.79 ± 0.41	1.62 ± 0.12

Correlations	Back fat thickness	Number of high-fat cuttings	Planimetry of fat areas
mU/mg in outer layer	0.09 (n = 74)	−0.13 (n = 76)	0.00 (n = 71)
mU/mg in inner layer	−0.09 (n = 166)	−0.18 (n = 155)	−0.10 (n = 166)
mU/mg in outer/inner layer	0.20 (n = 71) $p < 0.05$	0.32 (n = 76) $p < 0.01$	0.21 (n = 71) $p < 0.05$

Different adipose tissues

If acetyl-CoA-carboxylase activities are measured in the outer or inner layer of subcutaneous adipose tissue of the pig (preceding paper and table 3), the ratio outer: inner layer correlates significantly with the thickness of the back fat (upper part of table 3) in breeding lines for high/low dehydrogenase levels, as well as with morphometric parameters obtained at the time of slaughtering of the pigs. The lower part of table 3 illustrates the fact that not enzyme levels in the layers of adipose tissue as such, but only their ratios lead to positive correlations.

When genetically obese mice are fed a highly fattening ration (fig. 1), again a ratio of acetyl-CoA-carboxylase activities in two different adipose

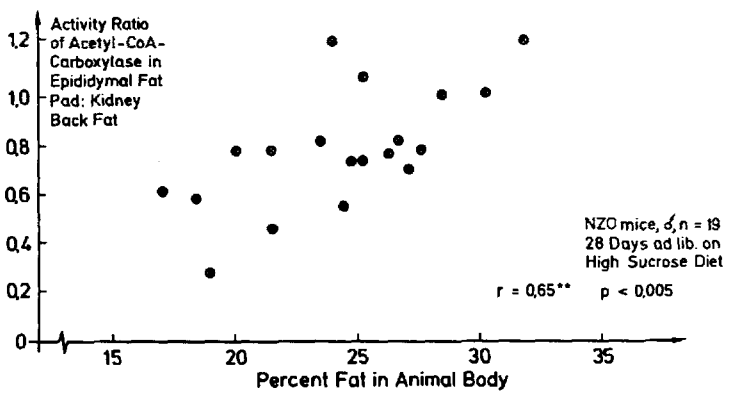

Fig. 1. Correlation of the activity ratio of acetyl-CoA-carboxylase in epididymal/perirenal adipose tissues with triglyceride deposition in obese male mice. Ration: 30 % casein, 64 % sucrose, 1 % sunflower oil, 5 % vitamin-salt mixture.

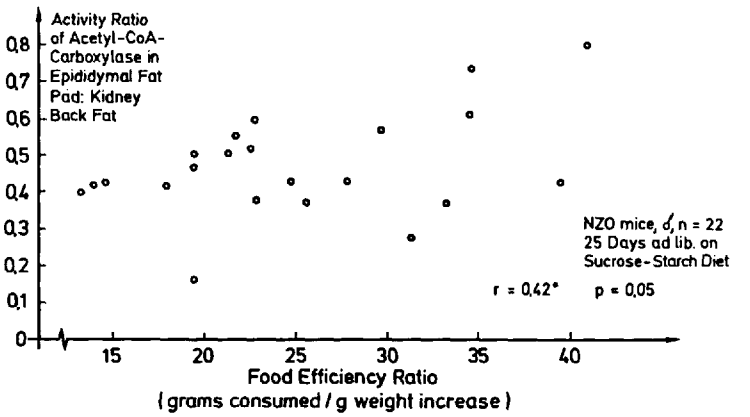

Fig. 2. Correlation of the activity ratio of acetyl-CoA-carboxylase in epididymal/perirenal adipose tissues with food efficiency (grams food consumed per g weight increase) in obese male mice. Ration: 32 % corn starch, 32 % sucrose, otherwise as in figure 1.

tissues permits the calculation of a positive correlation coefficient between this ratio and total body fat. Under a less fattening diet (fig. 2), and with a numerically decreased ratio of acetyl-CoA-carboxylase activities in epididymal fat pads/perirenal adipose tissue, again a positive correlation is obtained between food efficiency ratio and enzyme ratio. These data are taken as first evidence of the validity of the pacemaker role of acetyl-CoA-carboxylase in lipogenesis – in these experiments not confined to fatty acid biosynthesis but extended to triglyceride deposition as body and/or organ fat.

Table 4. Acetyl-CoA-carboxylase in adipose tissues of different anatomical localization (mU/mg soluble protein).

Species (n) weight sex	Adipose tissue		Activity	P	
Pig (n = 93) 100 kg ♀	subcutaneous	layer			
		outer	69 ± 46		< 0.001
		inner	99 ± 65		
Rat (n = 24) 218 ± 17 g ♂	subcutaneous		187 ± 83		
	perirenal		135 ± 41	n.s.	< 0.001
	epididymal fat pad		120 ± 42	< 0.01	
NZO mice (n = 42) 49 ± 4 g ♂	subcutaneous		80 ± 55		
	perirenal		56 ± 35	n.s.	< 0.01
	epididymal fat pad		47 ± 32	0.01 < p < 0.02	

Table 5. Nutritive effects on acetyl-CoA-carboxylase activity in the layers of subcutaneous adipose tissue of the pig (mU/mg soluble protein in male castrates of 30 kg body weight).

Ration		Layers outer	Layers inner	Significance
High-fat	23.0 % protein 35.5 % fat 36.5 % carbohydrate	24 ± 15 (n = 8) A	45 ± 26 C (n = 8) B	A < 0.001 B < 0.05 C < 0.05 D n.s.
Low-fat	18.5 % protein 4.5 % fat 72.0 % carbohydrate	86 ± 31 (n = 7)	77 ± 25 D (n = 7)	

Different locations of adipose tissue in three different mammals are compared in table 4, which once again points out that studies of lipogenesis in a single type of tissue may be quite misleading. These data also show that genetically obese mice contain rather low specific activities of acetyl-CoA-carboxylase. All enzyme activities were obtained under conditions of saturation of the enzyme with acetyl-CoA; in adipose tissues rather high concentrations of acetyl-CoA are required.

Table 6. Effect of dietary carbohydrates on acetyl-CoA-carboxylase activities in different adipose tissues of rats and mice (mU/mg soluble protein).

Dietary carbohydrate	Epididymal fat pad		Perirenal adipose tissue		Subcutaneous adipose tissue	
	NZO-mice	rats	NZO-mice	rats	NZO-mice	rats
32 % sucrose	33 ± 11 (n = 25)	27 ± 7 (n = 10)	38 ± 15 (n = 25)	123 ± 42 (n = 10)	62 ± 17 (n = 25)	107 ± 66 (n = 10)
	④	①	⑤	②	⑥	③
64 % glucose	136 ± 30 (n = 10)	156 ± 38 (n = 10)	62 ± 17 (n = 10)	88 ± 25 (n = 10)	86 ± 16 (n = 10)	134 ± 66 (n = 10)
	⑦		⑧		⑨	
64 % sucrose	54 ± 33 (n = 17)		57 ± 30 (n = 17)		82 ± 26 (n = 17)	

Significances:			
	① < 0.001	④ < 0.001	⑦ < 0.001
	② < 0.05	⑤ < 0.01	⑧ n.s.
	③ n.s.	⑥ < 0.001	⑨ n.s.

Table 7. Data of lipogenesis in biotin deficiency (percent of control left).

		Rats	NZO-mice
Acetyl-CoA-carboxylase (mU/mg soluble protein)	Subcutaneous adipose tissue	51 > 0.1 < 0.2	40 < 0.001
	Perirenal adipose tissue	67 < 0.001	30 < 0.001
	Epididymal fat pad	34 < 0.02	102 n.s.
	Liver	38 < 0.05	80 < 0.05
	Epididymis/subcutaneous	67 < 0.05	64 < 0.001
Malic enzyme (mU/mg soluble protein)	Liver	78 n.s.	
Glucose-6-phosphate dehydrogenase (mU/mg soluble protein)	Liver	30 < 0.001	
Triglyceride content in the body (%)		55 < 0.05	92 n.s.
Weight gain (g/day)		86 < 0.01	76 < 0.05
Food efficiency ratio (grams food per g body weight)		111 = 0.05	125 < 0.01

n.s. = not significant

Nutritive influence

Both in the pig and in laboratory rodents acetyl-CoA-carboxylases in different adipose tissues respond in their activity levels to the amount and type of dietary carbohydrate (tables 5 and 6), cf. also figure 2.

Biotin deficiency

In order to lower the enzyme activity of acetyl-CoA-carboxylase, a partial biotin deficiency was installed by adding avidin to a biotin-less diet of rats and NZO mice. Table 7 demonstrates the different degrees, and the

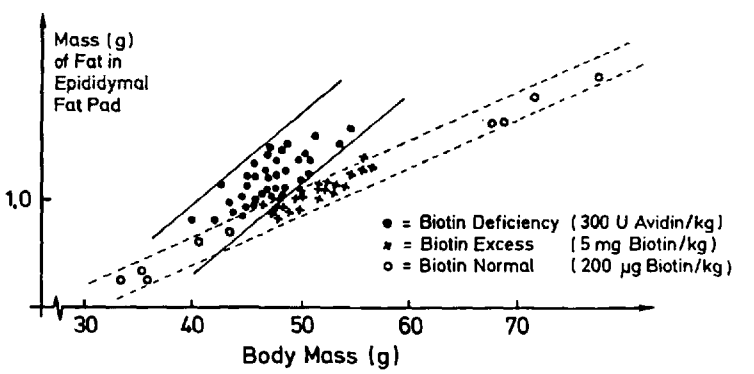

Fig. 3. Correlation of body weights with fat content in the epididymal fat pad (both in grams) in obese male mice at different levels of biotin supply. Biotin and avidin are given per kg ration; it contained 20 % dry egg white, 37 % corn starch, and 37 % sucrose; sunflower oil, biotinless vitamin mixture and inorganic salts as in figure 1.

inter-species differences, of diminished acetyl-CoA-carboxylase activities in different adipose tissues and in liver. Not shown are differences in the extent of reactivation by preincubation of the enzyme source with biotin plus ATP. Glucose-6-phosphate dehydrogenase in rat liver is diminished like acetyl-CoA-carboxylase, but malic enzyme is not. Body fat and food utilization change in the same sense as the enzyme activities in partial biotin deficiency.

However, most prominent is a correlation between the level of biotin supply and the fat content of the epididymal fat pad (fig. 3). There is a definitely steeper slope in biotin deficiency than under either normal or excess dietary biotin supply, which may indicate that epididymal adipocytes are less affected than major adipose tissues by an incomplete biotin deficiency.

Discussion

Acetyl-CoA-carboxylase in adipose tissues obviously constitutes a member in a block of lipogenic enzymes, comprising in addition the four major NADPH-generating dehydrogenases, which manifests itself e.g. in breeding (table 1) and nutritional (table 7) experiments. Both carbon and hydrogen supply for fatty acid biosynthesis seem to underly some uniform regulation. Such interpretations (tables 1 and 2) are confined to adipose tissue where pentose formation, compared with NADPH production, has probably much less relevance than e.g. in liver.

Whereas enzymatic activities as such do not bear much information, activity ratios of anatomically different adipose tissues are of considerable interest. The ratio of acetyl-CoA-carboxylase activity in the outer/inner layer of porcine subcutaneous adipose tissue has predictive value whether a growing pig will finally belong to fat or lean phenotypes (9). Similar ratios, constructed e.g. from epididymal fat pad/perirenal adipose tissue in rats and mice, are significantly correlated with triglyceride deposition in the body (fig. 1) and food efficiency (fig. 2). It is suggestive to propose, from such ratios, a lipogenic potential, like a gradient of biosynthetic pressure on different adipocytes. Such a gradient is certainly dependent on the carbohydrate moiety of the ration (tables 5 and 6) and may constitute an expression of the intensity of substrate (carbohydrate intermediate) flux in the body and thus between different tissues.

When acetyl-CoA-carboxylase activities are correlated with body fat deposition (tables 2 and 3, fig. 1), always significant positive coefficients were obtained. These data do not permit to sustain doubts (7, 8) about the rate-limiting function of the enzyme in fatty acid biosynthesis. Rather, from the fact that acetyl-CoA-carboxylase is correlated with body fat deposition, one ought to conclude that formation and storage of triglycerides in adipose tissues are essentially regulated by this enzyme, not by any other factor; thus, not only the chain of events leading to fatty acid synthesis, but also subsequent steps of esterification and deposition of triglycerides are governed by acetyl-CoA-carboxylase.

Biotin deficiency expresses itself to varying degrees in enzyme activities and fat deposition. Experiments of this type (table 7 and fig. 3) again stress the key role of acetyl-CoA-carboxylase, which apparently

responds to graded biotin depletion in rather individual ways in different species and tissues. The role of physico-chemical events like apoenzyme-coenzyme interactions as well as the biology of the enzyme protein (renewal rate as an example) may deserve more thorough studies in the future.

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Summary

Acetyl-CoA-carboxylase activities were measured in adipose tissues of pigs during a breeding experiment for a low-fat line, and of rats and obese mice under different nutritional conditions. Acetyl-CoA-carboxylase behaves uniformly with the four major NADPH-generating dehydrogenases, like a block of lipogenic enzymes, and is found to be genetically determined in pigs.

Correlation with body fat under a variety of experimental conditions confirms the rate-limiting character of acetyl-CoA-carboxylase, not only for the biosynthesis of fatty acids, but obviously also for their esterification and for triglyceride deposition. Activity ratios of this enzyme in different adipose tissues, e.g. outer *versus* inner layer of subcutaneous adipose tissue in pigs, epididymal *versus* subcutaneous, or epididymal *versus* perirenal adipose tissue in rats and obese mice, correlate well with predicted fattening in pigs and with fat deposition in laboratory rodents.

Moderate biotin deficiency in obese mice leads to a preferred fat deposition in the epididymal fat pad in comparison with normal biotin supply. The concept of a lipogenic potential in the body is derived from the activity ratios of acetyl-CoA-carboxylase.

Zusammenfassung

Die Aktivität der Acetyl-CoA-Carboxylase wurde im Fettgewebe von Schweinen bei einem Züchtungsversuch auf magere Tiere und bei Ratten und Mäusen unter verschiedenen Ernährungsbedingungen gemessen. Das Enzym verhält sich gleichförmig mit den vier hauptsächlichen NADPH liefernden Dehydrogenasen wie ein Block lipogener Enzyme und wird in Schweinen als genetisch determiniert gefunden.

Wird die Enzymaktivität unter einer Reihe verschiedener Versuchsbedingungen mit dem Körperfett korreliert, so wird die geschwindigkeitsbestimmende Funktion der Acetyl-CoA-Carboxylase nicht nur für die Fettsäuren-Biosynthese bestätigt, sondern auf deren Veresterung und Ablagerung im Gewebe ausgedehnt. Quotienten der Enzymaktivität in der äußeren : inneren Schicht des Unterhaut-Fettgewebes vom Schwein, auch in epididymalem : subkutanem oder epididymalem : perirenalem Fettgewebe bei Ratte und Maus stehen in guter Korrelation mit der vorhergesagten Fettablagerung beim Schwein und mit der Verfettung bei Labornagern.

Gestufter Biotinmangel führt bei fettsüchtigen Mäusen zu einer Bevorzugung der Fetteinlagerung in den epididymalen Fettkörper im Vergleich zu normaler Biotinversorgung. Aus den Aktivitätsquotienten der Acetyl-CoA-Carboxylase wird das Konzept eines lipogenen Potentials in den Versuchstieren abgeleitet.

Key words: acetyl-CoA-carboxylase, adipose tissue, biotin, fatty acid biosynthesis, epididymal fat pad, liver

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