Response of lipogenic enzymes to overfeeding in liver and adipose tissue of light and heavy breeds of chicks

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1. Chicks, 3-d-old, of a heavy breed (HB) and a light breed (LB) were overfed for 18 d. The activities of acetyl-CoA carboxylase (*EC* 6.4.1.2; CBX), fatty acid synthetase (FAS), ATP citrate lyase (*EC* 4.1.3.8; CCE), NADP-malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40; ME), 6-glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PDH) and phosphogluconate dehydrogenase (*EC* 1.1.1.44; 6PGDH) were determined in abdominal adipose tissue (AT) and liver samples of overfed and *ad lib.*-fed chicks. Size and fat content of liver and adipose tissue were also determined in order to evaluate the extent of obesity.

2. On *ad lib.*-feeding HB chicks consumed more food, gained more weight and deposited more fat than the corresponding LB chicks. Their lipogenic enzymes were more active than in the LB chicks in both adipose tissue and liver. The increase in food consumption (%) that could be achieved by overfeeding was three times greater in the LB chicks than in the HB chicks.

3. Overfeeding increased the weight and fat content of liver and AT in both breeds. The specific activities of CBX, FAS, CCE and ME in liver and AT increased in the LB chicks only and the total activities of liver and AT enzymes increased much more in the LB chicks than in the HB chicks in which the increase was derived mainly from tissue enlargement.

4. The activity of the pentose cycle dehydrogenases was very low in liver, but in AT about one third of the NADPH generating capacity could be accounted for by these dehydrogenases.

5. The results show that lipogenic enzymes of chicks respond to an increased substrate flux. It is suggested that the enlarged liver, the higher participation of AT in lipogenesis and the uninterrupted supply of crop-stored excess food enable the chick to accommodate the increased amounts of substrate with only moderate enzymic adaptation.

Chicks differ from rats in the relative contribution of adipose tissue (AT) and liver to lipogenesis. While in the rat AT constitutes the main site of fatty acid synthesis, in the chick the contribution of this tissue is negligible and the liver is the main site of this process. Another difference between these species is in the relation between the rate of fatty acid synthesis (as measured by substrate incorporation in vitro) and the activity of lipogenic enzymes. While in the rat starvation and refeeding influence lipogenesis and enzymatic activity in the same way, in the chick under similar conditions, fatty acid synthesis 'overshoots' the control rate on refeeding, whereas lipogenic enzyme activities merely return to normal (Goodridge, 1968*a*; Leveille, Romsos, Yeh & O'Hea, 1975).

It was recently found that light-breed (LB) chicks could be easily overfed (Nir, Shapira, Nitsan & Dror, 1974) but heavy-breed (HB) chicks could not (Nir, Nitsan, Dror & Shapira, 1978). An attempt was therefore made in the present study to measure the activities of some enzymes involved in lipogenesis in the liver and AT of overfed, obese chicks. The following questions were investigated: (1) effect of breed; (2) the response of the enzymes to increased food supply; (3) the relation between the enzymic response and the resistance of HB chicks to overfeeding.

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METHODS

LB chicks (New Hampshire × Leghorn) and HB chicks (White Rock) were used in this study. At the age of 3 d they were divided into four groups: five chicks of each breed were force-fed during 18 d by the technique described by Nir *et al.* (1974) and five chicks of each breed were fed *ad lib.* The food used was a crumbled commercial starter supplying (US) National Research Council (1971) requirements.

On the 19th day of the treatment, 3 h after force-feeding the morning meal, the chicks were killed by cervical dislocation. Livers and abdominal AT were immediately removed and weighed, and samples were homogenized during 10 s in 3 or 2 vol. extraction solution, respectively, using an Ultra Turrax homogenizer (Janke & Kunkel KG, Stauffen, West Germany). The extraction solution contained 150 mM-KCl, 5 mM-$MgCl_2$, 5 mM-EDTA and 1 mM-dithiothreitol (DTT) and was brought to pH 7·4. Homogenates were centrifuged for 60 min at 10000 *g* in an ultracentrifuge (Model L_3-50, type Ti-50; Beckman Instruments Corp., Stanford, Palo Alto, Calif., USA). Enzymic activities were assayed in the supernatant fraction after suitable dilution with the extraction solution. All operations preceding the enzyme assays were carried out at 0–5° except for homogenization of AT which was carried out at room (22°) temperature. Assays for each tissue were run consecutively immediately after centrifugation, following the order of decreasing enzyme lability as described for pig AT (Anderson, Kauffman, & Kastenschmidt, 1972).

Enzyme assays: Acetyl-CoA carboxylase (*EC* 6.4.1.2; CBX) was assayed by the method of Dakshinamurti & Desjardins (1969). Activation was as follows: 200 μl liver extract (12·5 mg/ml) or 200 μl AT extract (400 mg/ml) were mixed with 100 μl of a solution containing 100 mM-Tris brought to pH 7·5 using hydrochloric acid, albumin (50 g/l), 25 mM-potassium citrate, 10 mM-ATP, 75 mM-$KH^{14}CO_4$ (specific activity 66 Ci/mmol; The Radiochemical Centre, Amersham, Bucks) and 1·25 mM-acetyl-CoA. Periods of incubation for liver and AT assays were 3 and 6 min respectively. The reactions were stopped by addition of 0·5 ml trichloroacetic acid solution (50 g/l). Traces of $^{14}CO_2$ were removed by solid carbon dioxide, according to Anderson, Kauffman & Kastenschmidt (1972). Scintillation mixture (5 ml; (g/l) 2,5-diphenyloxazole (PPO) 5, 1,4-bis{2(4-methyl-5-phenyloxazyl)} benzene (POPOP) 0·3, Triton X-100 (Packard Instrument International, Zurich, Switzerland) in toluene 330) was added and the samples were counted in a Tricarb Scintillator, Model 3003; Packard Instrument Co., Inc., Downers Grove, Ill., USA. The activity unit was nmol $^{14}CO_2$ fixed/min per mg soluble protein. 'Blanks' without acetyl-CoA were run in parallel.

The activities of the following enzymes were measured spectrophotometrically by monitoring the extinction at 340 nm using a spectrophotometer (Cary-15 DB Varian, Palo Alto, Calif., USA), the reaction temperature being adjusted by circulation of water around the cuvette compartment.

ATP citrate lyase (CCE, *EC* 4.1.3.8) was assayed according to Srere (1962). The complete assay mixture was as described by Anderson *et al.* (1972) and contained: 10 mM-$MgCl_2$, 4 mM-DTT, 1 mM-KCN, 5 IU malate dehydrogenase (*EC* 1.1.1.37), 20 mM-potassium citrate, 0·4 mM-CoA, 0·2 mM-NADH, 5 mM-ATP, 1 mM-Tris at pH 7·5 and 200 μl liver extract (25 mg/ml) or 300 μl AT extract (400 mg/ml). Final reaction volume was 1 ml. CoA was omitted during the 10 min pre-incubation period at 37° and was only added to start the reaction. Incubation was continued until linearity was achieved. The unit of activity was nmol NADH oxidized/min per mg soluble protein.

Fatty acid synthetase (FAS)was determined according to Hsu, Butterworth & Porter (1969) and Kumar, Dorsey, Mursing & Porter (1970). Liver extract (25 mg/ml; 100 μl) or 200 μl AT extract (400 mg/ml) were pre-incubated during 80 min with 500 μl of a solution containing

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Table 1. *Effect of overfeeding on weight and lipid content of body, liver and abdominal adipose tissue (AT) of light and heavy breeds of chicks, overfed from 3 to 21 d of age*

(Mean values with their standard errors for five chicks/group)

	Light breed				Heavy breed			
	Ad lib.-fed		Overfed		*Ad lib.*-fed		Overfed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food consumption (g)	467*		671*	12	691*		781	14
Growth								
Initial wt (g)	47	1	49	1	54	1	50	1
Final wt (g)	264^{c}	5	344^{b}	7	432^{a}	14	455^{a}	9
Liver								
Wt (g)	7·05^{c}	0·13	21·7^{a}	0·6	12·1^{b}	0·3	23·9^{a}	1·5
Lipids (mg/g)	47·8^{c}	1·1	161^{a}	11	49·3^{c}	1·5	90·7^{b}	10·3
AT								
Wt (g)	0·86^{d}	0·07	7·80^{a}	0·59	2·83^{c}	0·08	4·95^{b}	0·30
Lipids (mg/g)	357†		541^{b}	9	538^{b}	7	643^{a}	6

a, b, c, values in horizontal rows with different superscript letters were significantly different: $P < 0·05$.
* Group intake.
† Pooled tissues.

200 mM-phosphate buffer (pH 6·5), 5 mM-EDTA and 5 mM-DDT, in order to ensure re-association of the enzyme complex. The complete reaction mixture (1 ml) contained: 0·1 mM-acetyl-CoA, 0·15 mM-NADPH, bovine albumin (2 g/l) and 0·175 mM-malonyl-CoA. The reaction was started by adding malonyl-CoA and incubating at 30° until linearity was achieved. The unit of activity was nmol NADPH oxidized/min per mg soluble protein.

NADP-malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40; ME) was assayed by the method of Ochoa (1955). The reaction mixture (1 ml) contained: 25 mM-glycylglycine (pH 7·5), 1·5 mM-$MnCl_2$, 0·15 mM-$NADP^+$, 1 mM-L-sodium malate, 25 μl liver extract (25 mg/ml) or 50 μl AT extract (400 mg/ml). By adding the malate to the ice-cold reaction mixture before bringing the temperature to 30°, the initial reaction velocity could be maintained constant over 15 min. The unit of activity was nmol $NADP^+$ reduced/min per mg soluble protein.

Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PDH) and phosphogluconate dehydrogenase (*EC* 1.1.1.44; 6PGDH) were assayed by the methods of Kornberg & Horecker (1955) and Horecker & Smyrnotis (1955) respectively. The reaction mixture (1 ml) contained: 20 mM-glycylglycine (pH 7·5), 20 mM-$MgCl_2$, 0·15 mM-$NADP^+$, and 1·25 mM-glucose-6-phosphate or 6-phosphogluconate, 50 μl liver extract (250 mg/ml) or 100 μl AT extract (400 mg/ml). The assays were started by adding the substrates and were carried out at 30°. The unit of activity was nmol $NADP^+$ reduced/min per mg soluble protein.

Reagents were buffered to the pH stated for each assay. The linearity and the proportionality of reaction rates to concentration of tissues extracts were tested before each assay.

Chemical determinations. Total lipids in liver and AT homogenates were determined by the method of Zöllner & Kirsch (1962) and soluble protein by the method of Lowry, Rosebrough, Farr & Randall (1951). AT samples of *ad lib.*-fed LB chicks were pooled since they were too small for individual determinations.

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Table 2. *Effect of overfeeding on the specific activities* of lipogenic enzymes in the livers and abdominal adipose tissue (AT) of light and heavy breeds of chicks*

(Mean values with their standard errors for five chicks/group)

	Light breed				Heavy breed			
	Ad lib.-fed		Overfed		*Ad-lib.*-fed		Overfed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Acetyl-CoA carboxylase CBX (*EC* 6.4.1.2)								
Liver	$38{\cdot}5^{c}$	2·2	$86{\cdot}6^{a}$	5·1	$63{\cdot}2^{b}$	8·7	$78{\cdot}7^{ab}$	9·8
AT	2·48†		$10{\cdot}4^{a}$	0·8	$3{\cdot}05^{b}$	0·30	$7{\cdot}04^{ab}$	2·58
FAS Fatty acid synthetase								
Liver	$15{\cdot}5^{c}$	1·5	$47{\cdot}6^{a}$	2·7	$30{\cdot}8^{b}$	3·3	$35{\cdot}1^{b}$	1·3
AT	2·38†		6·10	0·79	4·23	0·64	4·80	0·44
ATP citrate lyase CCE (*EC* 4.1.3.8)								
Liver	$15{\cdot}6^{c}$	1·6	$49{\cdot}7^{a}$	3·2	$34{\cdot}8^{b}$	3·6	$45{\cdot}3^{ab}$	4·1
AT	1·82†		6·25	0·73	5·35	0·60	5·72	0·40
NADP-malate dehydrogenase (decarboxylating) ME (*EC* 1.1.1.40)								
Liver	123^{c}	14	435^{a}	33	282^{b}	17	232^{b}	39
AT	32·1†		48·0	5·6	48·7	5·4	39·6	4·2
Phosphogluconate dehydrogenase 6PGDH (*EC* 1.1.1.44)								
Liver	$6{\cdot}22^{b}$	0·94	$10{\cdot}1^{a}$	1·2	$8{\cdot}94^{ab}$	1·75	$10{\cdot}2^{ab}$	1·2
AT	10·5†		$18{\cdot}7^{a}$	0·9	$13{\cdot}8^{b}$	0·8	$12{\cdot}9^{b}$	1·6
6-Glucose-6-phosphate dehydrogenase G6PDH (*EC* 1.1.1.49)								
Liver	5·62	1·03	4·52	0·73	7·28	0·93	5·60	0·90
AT	7·87†		8·62	1·21	6·70	1·32	6·82	1·22
Soluble protein (mg/g)								
Liver	80·8	4·3	69·7	5·2	79·2	6·1	77·5	7·6
AT	15·9†		$7{\cdot}50^{b}$	0·24	$12{\cdot}3^{a}$	0·7	$11{\cdot}5^{a}$	1·1

a, b, c, values in horizontal rows with different superscript letters were significantly different: $P < 0{\cdot}05$.
* For details of units, see pp. 152–153.
† Pooled tissues.

RESULTS

Body, liver and AT weights and lipid deposition

When fed *ad lib.*, the HB chicks consumed more food, gained more weight and had larger livers and more abdominal AT than the LB chicks (Table 1). However, liver weight in relation to body-weight was similar in both breeds (2·7%) and so was the hepatic lipid concentration. On the other hand the relative weight of the abdominal AT and its lipid content were much higher in the *ad lib.*-fed HB chicks than in the LB chicks.

The excess food which the chicks could be made to ingest on force-feeding during the experimental period differed markedly between the breeds. The HB chicks were resistant to overfeeding and the excess of food which could be introduced into the gastrointestinal tract exceeded the *ad lib.* level by only 13%. But in the force-fed LB chicks the food intake exceeded the *ad lib.* level by 44%. The amount of surplus food directly affected the weight and composition of body, liver and abdominal AT. In the HB chicks body-weight was increased by only 4% (non-significant), liver weight by 98% and abdominal AT weight by 75%.

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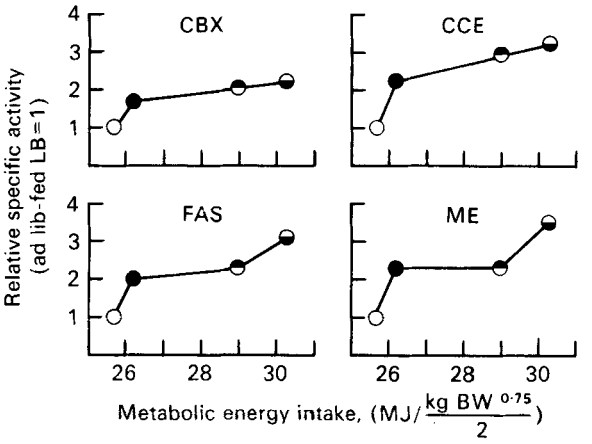

Fig. 1. Relationship between metabolic energy intake and specific activities of lipogenic enzymes in light (LB) and heavy (HB) breeds of chicks overfed from 3 to 21 d of age. Energy intake is expressed per one-half final metabolic weight ($BW^{0.75}$), as the latter approximates the average metabolic weight during the entire experimental period. Enzyme activities, taken from Table 2, are plotted on a relative scale, with a value of 1 assigned to *ad lib.*-fed LB chicks. (○), *ad lib.*-fed LB; (●), *ad lib.*-fed HB; (◓), overfed HB; (◒), overfed LB. CBX, acetyl-CoA carboxylase (*EC* 6.4.1.2); CCE, ATP citrate lyase (*EC* 4.1.3.8); FAS, fatty acid synthetase; ME, NADP-malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40).

The corresponding increases in the LB chicks were 30%, 3-fold and 9-fold respectively. Liver lipid concentration was doubled in the HB chicks and tripled in the LB chicks while the AT lipid concentration increased by 20 and 50% in the HB and LB chicks respectively.

Lipogenic enzymes

In *ad lib.*-fed chicks the specific activities of CBX, FAS, CCE and ME showed a pronounced breed difference, being higher in the HB chicks than in the LB chicks (Table 2). In both breeds the liver enzymes were much more active than the AT enzymes by factors of approximately twenty for CBX and six to ten for the other enzymes. ME was the only enzyme not to follow this trend.

The response in the enzyme specific activities caused by overfeeding was related to the amount of excess food consumed. In the HB chicks the enzyme response was slight and inconsistent. But in the LB chicks a marked increase was obtained in all the lipogenic enzymes, the highest response (a 4-fold increase) being that of CBX in AT.

The pentose phosphate dehydrogenases differed from the other enzymes by their relatively low specific activities in the liver. A clear increase due to overfeeding was seen only for 6PGDH in the tissues of the LB.

The increase in the total activities (which reflects both the increase in specific activities and the greater organ sizes) was especially pronounced in the LB chicks in which 6–9-fold increments were obtained in the liver and up to 18-fold increases in the AT. The corresponding increments for the HB chicks were approximately 2- and 4-fold respectively.

DISCUSSION

Our results are in agreement with previous evidence showing that in contrast to the rat, the chick synthesizes fatty acids mainly in the liver, the contribution of AT being much less than that of the liver in this respect (Goodridge, 1968*a*; O'Hea & Leveille, 1968).

The increase in the hepatic lipogenic enzyme activities during prolonged overfeeding

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roughly paralleled the metabolic energy intake expressed on a per unit metabolic weight basis, regardless of breed (Fig. 1).

The only enzyme that failed to respond was ME in HB chicks. ME could therefore be a limiting factor in the capacity of the HB chicks to divert excess substrate resulting from overfeeding to fat synthesis. ME is considered a lipogenic enzyme because it is involved in the generation of NADPH which is required for fatty acid synthesis (Young, Shrago & Lardy, 1964).

The importance of ME in the livers of overfed chicks is emphasized by the fact that the generation of NADPH by the dehydrogenases of the pentose cycle was very low, in agreement with Goodridge (1968*b*) and Madappaly, Paquet, Mehlman & Tobin (1971). Moreover the specific activities of G6PDH and 6PGDH in the livers of HB chicks did not respond to overfeeding while in the LB chicks, a slight response was obtained for 6PGDH only. A similar resistance of these enzymes to overfeeding was reported earlier for the chick (Nitsan, Dror, Nir & Shapira, 1974) and also for the goose (Nitsan, Nir, Dror & Bruckental, 1973). Thus hepatic ME plays an important role in the generation of NADPH for fatty acid synthesis in chicks.

In the chick the magnitude of enzymic response to an increased substrate flux resulting from refeeding after starvation is much lower than in the rat (Romsos & Leveille, 1974). In the present overfeeding experiment the magnitude of the response in the liver was only 2–3-fold in the LB chicks and even less in the HB chicks. It seems that in the HB chick the liver accommodates the increased flux of substrate at first by increasing its size. This was also seen in chicks fed intermittently each second day, when the liver mass was doubled after each feeding day (Nir & Nitsan, unpublished results). An increase in the specific activity of lipogenic enzymes occurs only when hypertrophy alone does not satisfy the lipogenic need of the liver. This was probably the situation in the LB chicks in which a further increase in the lipogenic capacity was achieved by an increase in the specific activities of the lipogenic enzymes.

Enzymic adaptation to increased substrate by organ hypertrophy is not restricted to the liver only. It has been reported that in the chick, the increase in total activity of the digestive enzymes obtained by overfeeding was due essentially to hypertropy of the pancreas and intestine (Nitsan *et al.* 1974; Nir *et al.* 1978).

The activity of CBX in AT was particularly low compared with its activity in the liver, suggesting that this enzyme could be rate-controlling in the conversion of acetyl-CoA to fatty acids (Goodridge, 1973). In contrast to CBX, the activities of the dehydrogenases of the pentose cycle were much higher in AT where their NADPH-generating activity attained 50% of that of the ME. Thus the contribution of these enzymes to NADPH generation in AT is considerable, whereas in the liver, it is very small, as also reported by Goodridge (1968*b*).

The relative contribution of abdominal AT to total lipogenesis in LB chicks (approximately 0·5%) was tripled by overfeeding. However, the abdominal AT is only a very small proportion of the total chick AT. If we assume a proportional response for the total amount of AT, the latter's contribution to lipogenesis in the overfed chick could be considerable. This, together with the alternative mechanisms of adaptation to excess substrate suggested previously, could partly explain the difference in the enzymic response to increased substrate flux between chicks and rats (Leveille *et al.* 1975).

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