Response of lipogenic enzymes to intensity and duration of overfeeding in the adult Japanese quail (*Coturnix coturnix japonica*)

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1. Adult male Japanese quail (*Coturnix Coturnix Japonica*) were overfed at different intensities during varying periods: (1) by gradually increasing the daily amount of food up to twice the control level in a period of 11 d and keeping this level constant during the subsequent 12 d period (prolonged overfeeding); (2) by gradually increasing the daily amount of food up to approximately twice the control level in a period of 6 d (short, low-intensity overfeeding; SL); (3) by gradually increasing the daily amount of food up to approximately 2·5-fold the control level in a period of 7 d (short, high-intensity overfeeding; SH).

2. 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) and malate dehydrogenase (decarboxylating) (NADP) (*EC* 1.1.1.40; ME) were determined in adipose tissue (AT) and liver of the overfed and *ad lib.*-fed chicks. Size and fat content of carcass, AT and liver were also determined in order to evaluate the extent of obesity.

3. The weight of carcass, lean body mass, liver and AT and the extent of fat deposition increased with the total amount of food consumed. Obesity was particularly pronounced in quail after prolonged overfeeding.

4. Lipogenic enzymes in liver were much more active than those of AT. The specific activities of CBX, FAS and CCE in liver and AT in overfed Japanese quail increased consistently in the SL and SH groups only and the increase was related to the level of food intake in these groups. ME was the only enzyme whose specific activity increased in all overfed groups. Total AT enzyme activities were increased most strongly after prolonged overfeeding because of the pronounced tissue enlargement in that treatment. But in liver, where tissue enlargement was less and differences in size due to the three overfeeding treatments were less marked, total activities depended mainly on the enhancement of specific activities.

5. The results lead to the conclusion that the increase in hepatic lipogenic enzymes roughly reflects the current food intake and may thus be considered to represent the primary adaptation in the overfed Japanese quail. Secondary adaptation, involving tissue enlargement, occurs principally in AT of Japanese quail after prolonged overfeeding, in which situation AT contributes significantly to total lipogenic activity.

The response of lipogenic enzyme activities to excess food intake is weaker in chicks than in rats. This was shown by Leveille, Romsos, Yeh & O'Hea (1975) who measured enzyme activities and incorporation of ^{14}C precursors into lipids in starved–refed chicks and rats. Recently we investigated the effects of overfeeding on lipogenic enzymes in chicks and reported only a moderate increase in specific activities, 2–3-fold in a light breed and even less in a heavy breed (Shapira, Nir & Budowski, 1977). We suggested that hypertrophy of the liver and a substantial increase in the mass and enzymic activities of adipose tissue (AT) in chicks might account for the observed differences in enzymic response to an increased substrate flux between the two species.

The rapid growth of the young chick causes a situation in which the food intake level is not constant. Moreover in light-breed chicks a substantial part of the excess food is converted to lean body mass (Nir, Shapira, Nitsan & Dror, 1974). It was therefore considered that adult birds might be a more suitable model than growing chicks for studying lipogenic enzyme responses to overfeeding. The Japanese quail (*Coturnix coturnix japonica*) was selected because of its small size and rapid growth and since it was shown in a previous study that it could be readily overfed and made obese (Nir, 1977).

The objective of this investigation was to study the induction of lipogenic enzyme

activities in liver and AT in overfed adult Japanese quail in situations in which the daily food intake was increased to different levels over short periods and excess food was administered at a constant level during a longer period.

MATERIALS AND METHODS

Experimental procedures

Adult male Japanese quail weighing approximately 100 g were divided into four groups. One group served as an *ad lib.*-fed control. The second group was force-fed increasing amounts of food twice daily (08.00 and 17.00 hours) during 11 d and thereafter received a constant amount (30 g, approximately twice the control level) during twelve consecutive days (prolonged overfeeding; PO). The third group was force-fed increasing amounts of food three times daily during 6 d. At this time the amount of food consumed reached approximately twice the control level (short, low-intensity overfeeding; SL). The fourth group was force-fed in the same way as the SL group during 7 d, but by the end of this period, the amount of food force-fed to this group exceeded that of the SL group by approximately 20% (short, high-intensity overfeeding; SH). The trial with the SH group and one with half the control birds was done later than the experiment with PO and SL groups, but since the values for food intake, body and liver composition and enzymic activities of the control groups, determined on both occasions, were identical, the experimental results are presented together.

The food was a crumbled commercial chick starter diet containing (/kg) 12·5 MJ metabolic energy and 210 g protein. Overfeeding was achieved by intubation of the moistened food into the crop as described previously (Nir *et al.* 1974). The birds were killed by cervical dislocation 3 h after force-feeding the morning meal and the livers and AT were removed. The contents of the gastrointestinal tract were removed and the carcasses were then frozen at $-18°$.

Chemical determinations

The carcasses without livers, AT and digesta were autoclaved at 22 lb/sq. in. (1·5 atm) for 3 h, cooled to room temperature and homogenized in a Waring Blendor. Samples were extracted with chloroform–methanol (2:1, v/v), the extract was filtered and the fat was determined gravimetrically after evaporation of the solvent mixture.

Fat content of liver and AT was determined by the colorimetric method of Zöllner & Kirsch (1962) in the portions of tissue remaining after sampling for enzyme assays. Soluble protein in the enzyme extracts was determined according to Lowry, Rosebrough, Farr & Randall (1951).

Enzyme assays

The activities of all enzymes were assayed in the 105000 *g* supernatant fractions of the freshly-prepared homogenates. Acetyl-CoA carboxylase (*EC* 6.4.1.2; CBX) was determined by the method of Dakshinamurti & Desjardins (1969); ATP citrate lyase (*EC* 4.1.3.8; CCE) according to Srere (1962); fatty acid synthetase (FAS) according to Kumar, Dorsey, Mursing & Porter (1970) and malate dehydrogenase (decarboxylating) (NADP) (*EC* 1.1.1.40; ME) by the method of Ochoa (1955). Details of the assay procedures were given previously (Shapira *et al.* 1977).

Activity units were calculated as follows: CBX, nmol $^{14}CO_2$ fixed/min per mg soluble protein; CCE, nmol NADH oxidized/min per mg soluble protein; FAS, nmol NADPH oxidized/min per mg soluble protein; ME, nmol $NADP^+$ reduced/min per mg soluble protein.

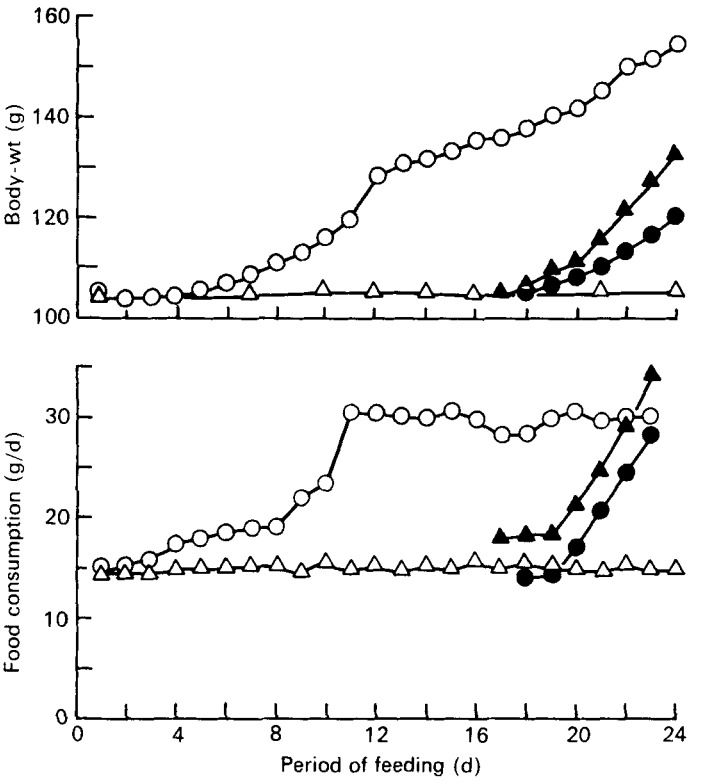

Fig. 1. Food consumption and body-weight of Japanese quail (*Coturnix coturnix japonica*) fed *ad lib.* (*n* 10) (△); overfed for 6 d (short, low-intensity overfeeding; SL group; *n* 6) (●); overfed for 7 d (short, high-intensity overfeeding, SH group, *n* 6) (▲) and overfed for 23 d (prolonged overfeeding; PO group; *n* 8) (○). For details of feeding regimens, see p. 290.

RESULTS

Weight gain and food consumption

Food consumption and body-weight remained unchanged in the *ad lib.*-fed birds in the experimental period (Fig. 1). In the force-fed groups, body-weight increased steadily with the increase in the amount of food delivered into the crop.

Body composition

The surplus of food directly affected the final weight and composition of body, liver and AT. In the PO group, body-weight was increased by 50%, liver weight by 2·6-fold and abdominal AT weight by 10-fold (Table 1). The corresponding increases in the SL and SH groups were approximately 20% and 30% for body-weight, 80% and 2-fold for liver weight and 3-fold for abdominal AT weight respectively. Liver lipid concentration was tripled in the PO group and doubled in the other groups. Overfeeding also increased the lean body mass of the chicks, the increase being greatest in the PO group.

Lipogenic enzymes

When the Japanese quail were exposed to increasing daily amounts of food (SL and SH groups), the specific activities of the lipogenic enzymes in livers and AT increased in

Table 1. *Effect of intensity and duration of overfeeding* on weight and lipid content of body, liver and adipose tissue of adult Japanese quail* (Coturnix coturnix japonica)

(Mean values with their standard errors)

			Overfed for:					
	Ad lib.-fed		6 d		7 d		23 d	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No. of quail	10	—	6	—	6	—	8	—
Food consumption (g/d)	15·5	—	20·5	—	24·1	—	24·8	—
Initial wt (g)	103	2	103	3	102	4	103	3
Final wt (g)	104	2^{d}	120	2^{c}	131	3^{b}	155	3^{a}
Lean body-wt (g)	92	2^{c}	105	3^{b}	106	4^{b}	114	3^{a}
Carcass lipids (g/kg)	119	5^{d}	151	12^{c}	187	6^{b}	266	8^{a}
Liver:								
Wt (g)	2·35	$0·04^{d}$	4·26	$0·35^{b,c}$	5·10	$0·23^{b}$	6·20	$0·5^{a}$
Lipids (g/kg)	52	4^{c}	94	6^{b}	119	11^{b}	174	18^{a}
Adipose tissue:								
Abdominal (g)	0·64	$0·15^{c}$	1·80	$0·41^{b,c}$	2·03	$0·16^{b}$	6·16	$0·48^{a}$
Neck (g)	1·42	$0·22^{b}$	ND	—	3·29	$0·24^{a}$	ND	—

a, b, c, d, values in horizontal rows with different superscript letters differed significantly ($P < 0·05$).
ND, not determined.
* For details, see p. 290.

proportion to the rate of increase of food intake (Table 2). In the livers of the SH group the activities of CBX, CCE and ME were doubled and the activity of FAS was tripled. In the SL group the response was slight and non-significant for CBX and FAS, but for CCE and ME there was a substantial increase of 64%. In the PO group, which received a high but constant level of food, the situation was similar to that seen in the SL treatment, with increases of approximately 50% being found for CCE and ME.

In all groups the liver enzymes were much more active than the abdominal AT enzymes by factors of approximately 6–11 for CBX and 3–5 for the other enzymes. Enzyme activities in neck AT, determined in the *ad lib.*-fed control and SH groups only, were even lower than in abdominal AT.

Unlike hepatic enzymes, AT enzymes responded consistently to all force-feeding treatments. Activity increases were approximately 1·5-, 2·0- and 2·5-fold for the PO, SL and SH groups respectively. These responses were statistically significant ($P < 0·05$) for all enzymes in the SH and SL groups but only for ME in the PO group. The enzymes in neck AT responded more markedly to overfeeding than the abdominal AT enzymes.

The increase in the total activities (which reflects both specific activities and tissue growth) was especially pronounced in AT (Fig. 2). While the greatest response in total hepatic enzyme activities was obtained in the SH group, the response in total abdominal AT activity was highest in the PO group.

DISCUSSION

Our results on Japanese quail are in agreement with previous studies on other avian species showing that the main site of fatty acid synthesis in birds is the liver (Goodridge, 1968; O'Hea & Leveille, 1968). However the specific activities of lipogenic enzymes in the livers of adult male Japanese quail were 2–5-fold those found in light- and heavy-breed chicks, using the same procedures (Shapira *et al.* 1977). The present work emphasizes the direct relationship existing between the flux of substrate, the specific activities of lipogenic

Table 2. *Effect of intensity and duration of overfeeding* on the specific activities of lipogenic enzymes† in the liver and adipose tissues (AT) of adult Japanese quail* (Coturnix coturnix japonica)

(Mean values with their standard errors)

	Ad lib.-fed		Overfed for: 6 d		7 d		23 d	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No. of quail	10	—	6	—	6	—	8	—
CBX (*EC* 6.4.1.2):								
Liver	77	$4^{b,c}$	86	2^{b}	161	4^{a}	74	4^{c}
Abdominal AT	6·6	$1·1^{b}$	13·5	$0·5^{a}$	16·4	$3·8^{a}$	9·5	$1·4^{a,b}$
Neck AT‡	2·4	—	ND	—	16·3	4·3	ND	—
FAS:								
Liver	90	7^{b}	106	11^{b}	265	11^{a}	108	7^{b}
Abdominal AT	18·6	$1·4^{c}$	30·2	$2·0^{b}$	39·1	$2·8^{a}$	23·7	$1·6^{b,c}$
Neck AT	6·9	—	ND	—	31·1	3·5	ND	—
CCE (*EC* 4.1.3.8):								
Liver	33·0	$2·6^{c}$	54·3	$4·2^{a}$	61·6	$3·6^{a}$	45·7	$2·6^{b}$
Abdominal AT	6·9	$0·5^{c}$	17·1	$2·0^{a}$	18·2	$2·5^{a}$	10·7	$0·6^{b}$
Neck AT	2·4	—	ND	—	11·8	8·6	ND	—
ME (*EC* 1.1.1.40):								
Liver	169	14^{c}	277	10^{b}	351	28^{a}	259	20^{b}
Abdominal AT	44	2^{c}	90	7^{a}	109	9^{a}	67	6^{b}
Neck AT‡	17	—	ND	—	57	9	ND	—
Soluble protein (mg/g fresh tissue):								
Liver	98·4	5·4	90·6	8·0	85·3	6	88·2	3·5
Abdominal AT	9·0	0·15	9·9	0·8	6·9	0·6	7·1	0·4
Neck AT	8·2	—	ND	—	6·9	0·9	ND	—

a, b, c, d, values in horizontal rows with different superscript letters differed significantly ($P < 0·05$).

ND, not determined; CBX, acetyl-CoA carboxylase; FAS, fatty acid synthetase; CCE, ATP citrate lyase; ME, malate dehydrogenase (decarboxylating) (NADP).

* For details, see p. 290.

† For details, see p. 290. Enzyme values are expressed in activity units/mg soluble protein as follows: CBX, nmol $^{14}CO_2$ fixed/min; CCE, nmol NADH oxidized/min; FAS, nmol NADPH oxidized/min; ME, nmol $NADP^+$ reduced/min.

‡ Neck AT from *ad lib.*-fed Japanese quail were pooled.

enzymes and the hypertrophy of liver and adipose tissues. When the Japanese quail were exposed to short periods of increasing daily food consumption (SL and SH groups), the specific activities of lipogenic enzymes were substantially increased in both liver and AT and the responses were related to the level of overfeeding in these groups. When the level of overfeeding was kept constant over a long period, as in the PO treatment, the enzyme activities (ME excepted) returned to approximately control levels. The reasons for this regression may be various and are complicated by the adiposity induced by this treatment and the resulting physiological and endocrine changes. It was reported that in cocks and Japanese quail, obesity induced by overfeeding had an effect very similar to that of castration and caused a decrease in blood testosterone, an increase in blood luteinizing hormone and a marked shrinking of the testes; combs in cocks, and cloacal foam glands in the Japanese quail, also decrease in size (Nir *et al.* 1974; Nir, 1977). It was reported earlier that testosterone exerts a major control over the processes governing accumulation of fat in cockerels' adipose tissue (Snapir, Nir, Furuta & Lepkovsky, 1969, 1974).

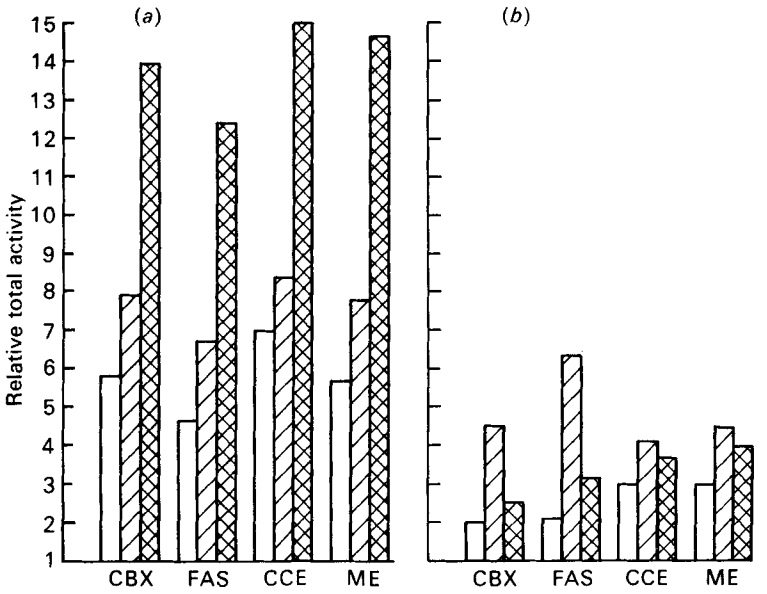

Fig. 2. Total lipogenic enzyme activities in abdominal adipose tissue (*a*) and livers (*b*) of overfed Japanese quail (*Coturnix coturnix japonica*) relative to the value for *ad lib.*-fed controls (*n* 10). Japanese quail were overfed for 6 d (short, low-intensity overfeeding; SL group; *n* 6) (□), 7 d (short, high-intensity overfeeding; SH group; *n* 6) (▨), and 23 d (prolonged overfeeding; PO group; *n* 8) (▩). For details of feeding regimens, see p. 290. CBX, acetyl-CoA carboxylase (*EC* 6.4.1.2); FAS, fatty acid synthetase; CCE, ATP citrate lyase (*EC* 4.1.3.8); ME, malate dehydrogenase (decarboxylating) (NADP) (*EC* 1.1.1.40), for details, see p. 290.

The hypertrophy of the liver, and particularly that of the abdominal AT in the obese Japanese quail, could result in total enzyme activities sufficient to deal with the increased substrate flux. The weaker enhancement of total activities in the livers of the PO group would be compensated by the substantial increase in the total activities of the AT (Fig. 2). An estimate of the contribution of total AT to lipogenesis according to the approach of Goodridge & Ball (1966) shows that in the PO group it reached about 15% of the lipogenic capacity of the liver. Thus, although the contribution of AT to lipogenesis is normally negligible, in conditions of increased adiposity it becomes quantitatively important.

The lipogenic enzyme activities from different anatomical locations and their respective responses to overfeeding varied in the Japanese quail. In the *ad lib.*-fed birds the specific activities in the neck AT were lower than in the abdominal AT, but they responded much more strongly to overfeeding. Differences in the activities of AT from different anatomical locations were also reported for the pig (Anderson, Dauffman & Kastenschmidt, 1972).

The present results strengthen our previous suggestion that an increase in lipogenic enzymes occurs when hypertrophy of the lipogenic sites does not satisfy the lipogenic needs of the overfed bird (Shapira *et al.* 1977).

It has been reported that while extrahepatic tissues may account for more than 50% of the total fatty-acid-synthesizing activity in the chick (Yeh & Leveille, 1972, 1973), only 12% are actually contributed by the skin and intestine. In birds the existence of other as yet unidentified sites could be of physiological importance. Elucidation of this question would provide additional information on the lipogenic response of birds to the feeding status.

The present work emphasizes the importance of the quantitative aspects of food consumption on lipogenesis and enzyme activities. Short-term overfeeding with increasing

amounts of food causes only mild adiposity but evokes an adaptive response expressed primarily by increased hepatic lipogenic activity. The latter reflects current food intake even when overfeeding is continued over longer periods at a high but constant level of feeding; but in this situation marked obesity develops and the enlargement of AT constitutes an additional adaptive change enabling this tissue to contribute to the conversion of excess energy into fat, while serving as a depository for the fat formed.

REFERENCES

Anderson, D. B., Dauffman, R. G. & Kastenschmidt, L. L. (1972). *J. Lipid Res.* **13**, 593.
Dakshinamurti, K. & Desjardins, P. R. (1969). *Biochim. biophys. Acta* **176**, 221.
Goodridge, A. G. (1968). *Am. J. Physiol.* **214**, 897.
Goodridge, A. G. & Ball, E. G. (1966). *Am. J. Physiol.* **221**, 803.
Kumar, S., Dorsey, J. A., Mursing, R. A. & Porter, J. W. (1970). *J. biol. Chem.* **245**, 4732.
Leveille, G. A., Romsos, D. R., Yeh, Y. Y. & O'Hea, E. K. (1975). *Poult. Sci.* **54**, 1075.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Nir, I. (1977). *Br. Poult. Sci.* **18**, 245.
Nir, I., Dror, Y., Nitsan, Z. & Shapira, N. (1977). *Br. J. Nutr.* (In the Press.)
Nir, I., Shapira, N., Nitsan, Z. & Dror, Y. (1974). *Br. J. Nutr.* **32**, 229.
Ochoa, S. (1955). *Meth. Enzym.* **1**, 739.
O'Hea, E. K. & Leveille, G. A. (1968). *Comp. Biochem. Physiol.* **26**, 111.
Shapira, N., Nir, I. & Budowski, P. (1977). *Br. J. Nutr.* (In the Press.)
Snapir, N., Nir, I., Furuta, F. & Lepkovsky, S. (1969). *Endocrinology* **84**, 611.
Snapir, N., Nir, I., Furuta, F. & Lepkovsky, S. (1974). *Gen. Comp. Endocr.* **24**, 53.
Srere, P. A. (1962). *Meth. Enzym.* **5**, 641.
Yeh, S. J. C. & Leveille, G. A. (1972). *J. Nutr.* **102**, 349.
Yeh, S. J. C. & Leveille, G. A. (1973). *Proc. Soc. exp. Biol. Med.* **142**, 115.
Zöllner, N. & Kirsch, K. (1962). *Z. ges. Exp. Med.* **135**, 545.

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