Response of lipogenic enzymes and plasma lipids to starvation and refeeding in the adult Japanese quail (*Coturnix coturnix japonica*)

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1. Adult male Japanese quail (*Coturnix coturnix japonica*) were starved for 48 or 60 h and then refed for either 24 or 48 h. Weight and lipid content of carcass and livers were determined, as was the lipid content of plasma. In addition, 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 assayed in liver and in abdominal and neck adipose tissues (AAT and NAT, respectively).

2. Body-weight and carcass lipids failed to return to normal in quail that had been starved for 48 h and refed during 24 h. When starving lasted 60 h, carcass lipids almost resumed the normal level only after 48 h of refeeding. All refeeding treatments caused a 2–3-fold increase in liver weight, with a parallel rise in fat content.

3. In the livers of the refed quail the specific activities of all enzymes, except CBX, reached or slightly exceeded pre-starvation levels. Because of liver enlargement, the total activities of the lipogenic enzymes in the starved–refed quail exceeded pre-starvation levels. In the normally-fed quail the contribution of AAT and NAT to total lipogenesis was insignificant, and in the starved–refed birds it still remained very small compared to that of liver, despite the pronounced relative increases of lipogenic enzyme activities in these adipose tissues.

Hepatic fatty acid synthesis is depressed by starvation and surpasses the *ad lib.*-fed control level on refeeding in both rats (Kornacker & Lowenstein, 1965) and chicks (Goodridge, 1968). The assay of hepatic lipogenic enzymes reveals a difference between these species on refeeding: the rat enzymes show an 'overshoot' (Romsos & Leveille, 1974), whereas in the chick and the young pigeon the enzyme activities merely return to the *ad lib.*-fed control levels (Kornacker & Lowenstein, 1965; Goodridge & Ball, 1966; Leveille, 1969).

In earlier work carried out on overfed male Japanese quail (*Coturnix coturnix japonica*) (Shapira *et al.* 1978*b*) and chicks (Shapira *et al.* 1978*a*), it was shown that hypertrophy of liver and adipose tissue (AT) contributed substantially to the marked increase in total lipogenic enzyme activities. The objectives of this investigation were to study the induction of lipogenic enzyme activities in liver and adipose tissue in refed adult Japanese quails after different periods of starvation and to ascertain the contribution of liver hypertrophy to total enzymic activities. The lipid content of carcass, liver and plasma was also investigated in order to provide additional information on the adaptive changes in the transport system of lipids from the liver to the periphery.

MATERIALS AND METHODS

Animals and diets

Thirty adult male Japanese quail from the flock maintained at the Volcani Center (P.O.B. 6, Bet Dagan 6, Israel) were used in this work. The quail were given a commercial chick starter. The metabolizable energy content of the feed, determined according to Hill & Anderson (1958), was 12·5 J/g. Its composition (Association of Official Analytical Chemists,

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1970) was (g/kg): moisture 126, crude protein (nitrogen × 6·25) 206, diethyl ether extract 28, crude fibre 39, ash 72. Groups of quail were autopsied on two different occasions at 10 d intervals. The quail killed on the first occasion comprised four *ad lib.*-fed birds and eight starved 48 h. Four of the latter were killed after 24 h refeeding and the other four without refeeding. The quail were killed serially by cervical dislocation in blocks of three (four blocks for twelve chicks), starting at 09.00 hours. Each block included one *ad lib.*-fed, one starved and one refed bird. The autopsy and dissection within each block lasted approximately 30 min. On the second occasion there were four *ad lib.*-fed birds and fourteen starved 60 h. Four of the latter were killed without refeeding, four after 24 h refeeding and six after 48 h refeeding. The quail were killed in blocks of four described previously; two extra birds refed 48 h were added, one at the start and one at the end of the autopsy, because previous work with chicks had suggested a great variability was to be expected in this treatment. The values obtained for the *ad lib.*-fed chicks on the two occasions were combined, since no differences were found in body composition and enzyme activities. The different numbers of birds in the six treatments are shown in Table 1. Blood was drawn by heart puncture, using EDTA as the anticoagulant. Plasma was separated from the blood by centrifugation at 2500 *g* for 15 min at 3 °. Carcasses (after removal of blood, liver, AT and contents of the gastrointestinal tract) were kept frozen at −20 ° for lipid analyses.

Enzyme assays

Enzyme activities were assayed in the supernatant fraction from the freshly prepared tissue homogenate: acetyl-CoA carboxylase (*EC* 6.4.1.2; CBX) 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 Hsu *et al.* (1969); (NADP) malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40; ME) by the method of Ochoa (1955). Activity units were: 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. The procedures were described previously (Shapira *et al.* 1978*a*). Since the amount of adipose tissue dissected from the individual quail was minimal, it was pooled for enzymic and soluble protein determinations.

Chemical determinations

Soluble protein was determined by the method of Lowry *et al.* (1951); total lipids in liver and AT were extracted from tissue homogenates according to Folch *et al.* (1957); and determined by the method of Zöllner & Kirsch (1962). Total plasma lipids were similarly extracted and determined by the sensitive colorimetric procedure described by Amenta (1964). Lipid phosphorus was determined in the chloroform extract by the method of Ames & Dubin (1960), assuming an average molecular weight of 800 for the phospholipids. Triglycerides were determined in the same chloroform extract, after removal of the phospholipids with activated zeolite, according to Van Handel (1961) and cholesterol was assayed by the colorimetric ferrous sulphate method of Searcy & Bergquist (1960).

RESULTS

Weight and composition

Body-weight and amount of carcass lipids were directly affected by the length of the starvation period (Table 1). After 48 and 60 h starvation respectively, the body-weight dropped by 14 and 30%, while the reduction in carcass lipids amounted to 53 and 72%, respectively.

The body-weight of the group starved for 60 h was restored after 48 h of refeeding. No

Table 1. *Effect of starvation and refeeding on carcass and tissue weight and on lipid content of adult Japanese quail* (Coturnix coturnix japonica)

(Mean values with their standard errors)

			Starved 48 h, refed for:				Starved 60 h, refed for:					
	Ad lib.-fed		0 h		24 h		0 h		24 h		48 h	
No. of quail	8		4		4		4		4		6	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial wt (g)	107	5	100	4	102	2	104	4	103	3	103	4
Final wt (g)	106^{a}	5	86^{b}	2	88^{b}	3	72^{c}	4	85^{b}	3	106^{a}	4
Carcass lipids* (g/kg)	120^{a}	10	56^{b}	6	56^{b}	1	33^{c}	2	52^{b}	4	92^{a}	8
Liver:												
Wt (g)	$2{\cdot}67^{c}$	0·18	$1{\cdot}54^{d}$	0·06	$4{\cdot}56^{a}$	0·30	$1{\cdot}75^{d}$	0·12	4·50	0·27	$3{\cdot}71^{b}$	0·31
Lipids (g/kg)	43^{b}	2	38^{bc}	4	88^{a}	17	35^{c}	1	106^{a}	2	108^{a}	8
Apipose tissue:												
Abdominal (g)	0·55†		‡		‡		‡		‡		0·26	
Neck (g)	1·53†		‡		§		‡		0·52		0·72	
Plasma lipids (mg/ml):												
Total	$6{\cdot}79^{b}$	0·30	$6{\cdot}34^{b}$	0·16	$7{\cdot}01^{b}$	0·23	$3{\cdot}22^{c}$	0·46	$6{\cdot}36^{b}$	0·59	$9{\cdot}37^{a}$	0·58
Phospholipids	$2{\cdot}76^{b}$	0·21	$2{\cdot}61^{b}$	0·16	$2{\cdot}96^{ab}$	0·10	$0{\cdot}90^{c}$	0·12	$2{\cdot}24^{b}$	0·16	$3{\cdot}44^{a}$	0·24
Triglycerides	$1{\cdot}97^{c}$	0·10	$1{\cdot}44^{d}$	0·09	$2{\cdot}10^{b}$	0·11	$0{\cdot}99^{e}$	0·05	$1{\cdot}74^{cd}$	0·07	$2{\cdot}97^{a}$	0·14
Total cholesterol	$2{\cdot}30^{b}$	0·11	$2{\cdot}76^{ab}$	0·14	$2{\cdot}22^{b}$	0·23	$1{\cdot}79^{c}$	0·09	$2{\cdot}66^{b}$	0·34	$2{\cdot}92^{a}$	0·22

a,b,c,d Values in horizontal rows with different superscript letters differed significantly ($P < 0{\cdot}05$).
* Without gastrointestinal contents, liver and adipose tissue.
† Means of pooled samples.
‡ No adipose tissue detectable
§ Adipose tissue detectable but not weighed.

such return to normal weight was observed in the groups refed 24 h. Carcass lipids of the birds starved for 60 h were essentially restored after 48 h of refeeding but not after 24 h.

Liver weight reached a minimum after 48 h starving (approximately 40% reduction in weight), and hepatic lipid concentration decreased by 16 and 20% after 48 and 60 h starving, respectively. On 24 h refeeding liver weight of the starved birds increased 2·5–3-fold. Further refeeding caused a decrease in the liver weight, but the livers were still larger than those of the control quail. The changes in total liver fat concentration paralleled the weight changes.

AT which was readily detected in the control birds practically disappeared after starvation. Neck adipose tissue (NAT) could be detected again after 24 h refeeding and abdominal adipose tissue (AAT) after 48 h refeeding. Even then AT weight was still much less than before starvation.

Plasma lipids were reduced consistently after 60 h starving. This reduction was due mainly to phospholipids and triglycerides and partly to total cholesterol. Starving for 48 h caused a slight reduction in triglycerides only. Refeeding during 24 h restored all plasma lipids to approximately the control levels. Continued refeeding up to 48 h caused a significant increase in all plasma lipids.

Lipogenic enzymes

The effect of starving and refeeding on hepatic lipogenic enzyme activities is shown in Table 2. All activities (specific and total) declined markedly after 48 h starvation. After an additional 12 h starvation only CBX and ME activities decreased further. Refeeding during 24 h after the 48 or 60 h starvation brought the activity of CCE back to the control (*ad lib.*-fed) level while the activities of FAS and ME exceeded the control values. At this point CBX was the only enzyme which did not return to the control level. Refeeding during an additional 24 h period caused a further increase in all enzymic activities, but this was statistically significant only for ME. The pronounced changes in liver weight brought about by the treatments (Table 1) further accentuated the changes in the total activity of the enzymes. Therefore, while the lowest specific activities after starvation were approximately 30, 40, 60 and 45% of the *ad lib.*-fed group for CBX, FAS, CCE and ME respectively, the corresponding lowest values for total activities were approximately 12, 20, 30 and 25% of the control values. The increases in total enzyme activities after 48 h refeeding were also accentuated by tissue hypertrophy. While the specific activities of CBX, FAS, CCE and ME were 83, 124, 130 and 179% of the *ad lib.*-fed control levels respectively, the corresponding values for total activities were 114, 170, 160 and 226%. The changes in the hepatic soluble protein concentration were not consistent.

The specific activities of the lipogenic enzymes in AT were approximately one-tenth to one-fourth of those found in the liver (Table 3). NAT activities were lowest, approximately one-fourth of the activities found in the AAT. In contrast to the situation in the liver, the highest enzyme activities in NAT were found after 24 h refeeding. Further refeeding for 48 h reduced the activities of the NAT to values below those of the controls. In AAT the activities after 48 h refeeding were slightly higher than the control levels.

DISCUSSION

In the quail the 50% decrease in the specific activities of hepatic lipogenic enzymes due to starvation was quite close to the decrease reported for CCE and ME in the chick; the increase in CCE caused by refeeding was also of the same order as in the chick (Goodridge, 1968), but the ME response was more pronounced. The changes in FAS paralleled those observed for CCE and ME. CBX was the only enzyme which did not return to pre-starvation activity.

Table 2. *Effect of starvation and refeeding on hepatic lipogenic enzyme activities* in adult Japanese quail* (Coturnix coturnix japonica)

(Mean values with their standard errors)

			Starved 48 h, refed for:				Starved 60 h, refed for:					
	Ad lib.-fed		0 h		24 h		0 h		24 h		48 h	
No. of quail	8		4		4		4		4		6	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CBX (*EC* 6.4.1.2):												
Specific activity	$84{\cdot}0^{a}$	4·2	$44{\cdot}5^{b}$	6·9	$55{\cdot}4^{b}$	2·7	$27{\cdot}1^{c}$	1·5	$57{\cdot}3^{b}$	4·1	$70{\cdot}4^{ab}$	7·5
Total activity (10^{-3})	$21{\cdot}1^{a}$	0·6	$7{\cdot}3^{c}$	1·7	$18{\cdot}3^{b}$	0·4	$4{\cdot}2^{c}$	0·3	$18{\cdot}6^{b}$	0·2	$24{\cdot}1^{ab}$	5·0
FAS:												
Specific activity	149^{b}	5	49^{c}	5	164^{ab}	24	61^{c}	5	176^{a}	9	185^{a}	5
Total activity (10^{-3})	$37{\cdot}1^{b}$	0·8	$7{\cdot}6^{c}$	1·3	$54{\cdot}5^{ab}$	9·2	$9{\cdot}4^{c}$	1·2	$57{\cdot}0^{a}$	5·6	$63{\cdot}1^{a}$	9·2
CCE (*EC* 4.1.3.8):												
Specific activity	$33{\cdot}5^{b}$	0·9	$19{\cdot}7^{c}$	2·1	$37{\cdot}1^{b}$	1·8	$21{\cdot}0^{c}$	2·9	$35{\cdot}3^{ab}$	4·0	$43{\cdot}8^{a}$	1·1
Total activity (10^{-3})	$9{\cdot}4^{b}$	0·9	$3{\cdot}0^{c}$	0·5	$12{\cdot}4^{a}$	1·2	$3{\cdot}2^{c}$	0·2	$11{\cdot}4^{a}$	1·2	$15{\cdot}0^{a}$	1·3
ME (*EC* 1.1.1.40):												
Specific activity	164^{c}	5	110^{d}	10	192^{bc}	25	75^{e}	2	211^{b}	8	294^{a}	13
Total activity (10^{-3})	$44{\cdot}5^{c}$	2·9	$17{\cdot}4^{d}$	1·9	$64{\cdot}6^{bc}$	11·5	$11{\cdot}6^{e}$	1·3	$68{\cdot}5^{b}$	6·2	$100{\cdot}6^{a}$	6·8
Soluble protein (mg/g)	93^{b}	2	107^{a}	4	72^{c}	4	88^{b}	5	72^{c}	4	92^{abc}	10

a,b,c,d,e Values in horizontal rows with different superscript letters differed significantly ($P<0{\cdot}05$).

CBX, acetyl-CoA carboxylase; FAS, fatty acid synthetase; CCE, ATP citrate lyase; ME, malate dehydrogenase (decarboxylating) (NADP).

* For details, see p. 440. 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.

Table 3. *Effect of refeeding after 60 h starvation on lipogenic enzyme activities* of abdominal (AAT) and neck (NAT) adipose tissue in adult Japanese quail* (Coturnix coturnix japonica)

(Mean of pooled samples of four quail)

	Ad lib.-fed		Starved 60 h, refed for: 24 h†	48 h	
	AAT	NAT	NAT	AAT	NAT
CBX (*EC* 6.4.1.2):					
Specific activity	10·3	3·9	12·9	12·9	4·5
Total activity	40·8	43·6	71·1	31·9	30·5
FAS:					
Specific activity	21·9	6·5	27·7	39·6	9·6
Total activity	86·7	72·6	152·7	97·8	65·0
CCE (*EC* 4.1.3.8):					
Specific activity	8·0	2·5	9·0	15·5	3·7
Total activity	31·7	27·9	49·7	38·3	25·0
ME (*EC* 1.1.1.40):					
Specific activity	46·0	17·8	53·6	87·6	17·1
Total activity	182·2	198·8	284·3	216·4	115·7
Soluble protein (mg/g)	7·2	7·3	10·6	9·5	9·4

CBX, acetyl-CoA carboxylase; FAS, fatty acid synthetase; CCE, ATP lyase; ME malate dehydrogenase (decarboxylating) (NADP).

* For details see p. 440. 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.

† No AAT was detectable in this treatment.

Our results confirm that avian species differ from the rat, in which a marked 'overshoot' of the lipogenic enzymes is always observed after refeeding (Romsos & Leveille, 1974). The reason for this difference may be found in the fact that the main site of fatty acid synthesis in the quail, as in the instance of other avian species, is the liver (Leveille *et al.* 1975). Therefore the accommodation of the quail to increased lipogenesis would be expected to include adaptive changes in the transport system of lipids from the liver to the periphery. The slow increase in the amount of carcass lipids and plasma triglycerides in the refed quail, together with the marked accumulation of fat in the liver, show that as in the chick (Shapira *et al.* 1979), there is a delay in the transfer of lipids from the liver to AT. This accumulating liver fat, which is essentially of endogenous origin, could exert an inhibitory effect on CBX, usually considered the pacemaker of the lipogenic group (Goodridge, 1975). Exogenous fat has long been recognized as causing inhibition of CBX, and fatty acids mobilized from AT during starvation act similarly (Yeh & Leveille, 1971). In the present work the slower rate of CBX recovery (Tables 2 and 3) compared to other lipogenic enzymes could result from the accumulation of endogenous lipids. One feature common to large fat intakes and fat mobilization from AT is an increase in hepatic levels of fatty acyl-CoA, a known inhibitor of CBX (Yeh, & Leveille, 1971; Goodridge, 1973). Most probably the level of hepatic fatty acyl-CoA is also raised on refeeding, in parallel with lipid accumulation resulting from intensive lipogenesis. The increased lipogenic capacity of the quail liver during refeeding is the result mainly of the increase in lean liver mass. This change might reduce the need for further adaptive changes in the enzyme activities of quail liver.

The picture that emerges from the present results is that the quail, as other avian species, reacts to feeding after starvation by strongly increasing the synthesis of fat in the liver. This

is possible initially because of the high specific enzyme activities of that organ, but a considerable increase in lipogenic capacity further results from liver enlargement. The secretion of lipids from liver into blood does not keep up with the increased rate of lipogenesis and, as a result, lipids accumulate in the liver, and this in turn could eventually inhibit lipogenesis. Additional adaptations occur in AT, which responds by pronounced increases in the normally low lipogenic enzyme activities, but the contribution of AT to total lipogenesis remains very small even then.

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