

**PLASMA LIPOPROTEIN METABOLISM AND GENETIC VARIATION  
OF FATNESS IN BROILERS**

by

**KUNDE GUO  
MSc (Nanjing)**

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**University of Edinburgh**

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#### DECLARATION

I declare that this thesis describes the results of the research carried out by myself, unless otherwise stated, at the Poultry Department, AFRC Institute for Grassland and Animal Production, Roslin, Midlothian, during the period between October 1985 and September 1988.

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**ABSTRACT**

Investigation of in vivo lipogenesis in commercial broilers at 5 weeks of age using tritiated water showed that total adipose tissue lipogenesis was 80% of the amount of liver lipogenesis. Total lipid deposition in the broilers at 6-7 weeks of age was calculated to be 5.5 g/d/kg body weight of which 55% was estimated to be incorporated from plasma lipoprotein triglyceride using <sup>14</sup>C-labelled very low density lipoproteins (VLDL) and <sup>3</sup>H-labelled portomicrons. 80% of the lipid deposited in the abdominal fat pad was estimated to be derived from plasma lipoprotein triglyceride. These results confirmed that plasma portomicrons and VLDL play an important role in body fat deposition in broilers, but also indicated adipose tissue lipogenesis cannot be neglected.

Possible relationships between fatness and lipoprotein lipase (LPL) activity in adipose tissue and plasma from heparinized birds were examined in 7-week-old male and female broilers. Total LPL activity in abdominal fat was significantly correlated ( $r=0.5$ ) with fat pad weight, but there was no correlation between specific activity of the enzyme and fat pad weight. LPL activity in post-heparin plasma showed no correlation with either abdominal fat or total body fat content. The results indicate that measurements of LPL activity in biopsy samples or in post-heparin plasma are of no value in predicting fat content of live birds.

LPL was purified from post heparin plasma, abdominal fat, heart and leg muscle of broilers by affinity chromatography on heparin-Sepharose. The presence of different forms of LPL was examined by SDS polyacrylamide gel electrophoresis and isoelectric focusing and identified using immunoblotting techniques. Most of the enzyme purified from post-heparin plasma and tissues had a molecular weight of 54 kD, though a minor component with a molecular weight of 29.5 kD was also present. Isoelectric focusing in the 6 M urea gel separated more than 10 species, with isoelectric points ranging from pH 3.5 to 7.0. No difference was found in the isoelectric focusing patterns between the samples from different tissues. These results indicate that the various tissue LPL released into post-heparin plasma cannot be distinguished by molecular weight or isoelectric point.

Birds divergently selected for plasma VLDL concentration for 7 generations showed 2.8 fold difference in abdominal fat weight. The amount of plasma VLDL incorporated into the whole abdominal fat pad in high VLDL birds was estimated to be about 8-fold greater than that in low VLDL birds. However, both specific and total LPL activities in the abdominal fat pads from fat birds were lower than those from lean birds, as were the specific LPL activities in heart and leg muscle. These results suggest that muscle LPL in low VLDL birds may make a greater contribution to plasma VLDL removal than in high VLDL birds.

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## ABBREVIATIONS

AU	absorbance units
dpm	disintegrations per minute
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
FCR	feed conversion ratio
FFA	free fatty acid
LPL	lipoprotein lipase
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
PBS	phosphate buffered saline
pI	isoelectric point
PMSF	phenylmethyl sulphonyl fluoride
SDS	sodium dodecyl sulphate
TEMED	N,N,N',N'-tetramethyl ethylenediamine
Tris	Tris (hydroxymethyl) aminomethane
VLDL	very low density lipoproteins

## Chapter 1

### SELECTION FOR LEANNESS AND PLASMA LIPOPROTEIN METABOLISM IN

#### REGULATION OF FATNESS IN BROILERS

##### - GENERAL INTRODUCTION

### 1 INTRODUCTION

Body weight of broiler chickens at 47 days of age has been increased by about 50g/generation during the last three decades (Chambers et al., 1981). This faster growth rate is associated with excessive fat deposition (Becker et al., 1979; Lin et al., 1980; Chambers et al., 1981; McCarthy and Siegel, 1983; Cahaner and Nitsan, 1985). Excessive fat deposition is becoming an increasing concern to the broiler industry. On average, about 2 to 3% of live body weight of broilers is abdominal fat. Part of it is lost from the carcass during processing, thus reducing meat yield and increasing the amount of offal and the fat content of waste water (Heath et al., 1980). It is estimated that the world broiler meat output will be over 22 million tons in 1988 (see Poultry International Feb. 1988). This means that about 1 million tons of abdominal fat will be produced and a considerable amount of it will be lost during the processing. The production of unused fat represents a loss to the producer.

Although the total body fat content in chickens is relatively low in comparison with those in cattle, sheep and pigs, it is not

unusual for fat to constitute 15-20% of the total body weight in modern broiler strains (Leenstra, 1986). The consumers in many countries have become more and more critical of the fatness of carcasses in relation to the advisability of reducing total fat consumption in the human diet. There is about 30% of fat in the carcasses of ducks at 56 days of age (Campbell et al., 1985) and this high proportion of fat and a poor feed conversion ratio compared with those in other poultry species result in limited duck meat consumption and production.

Poultry body fat is derived from dietary fat as well as from lipogenesis. The amount of fat deposited depends on energy intake, the amount of energy needed for maintenance and activity and for the growth of non-fat tissues. Fat deposition is influenced by genetic, dietary and environmental factors. Environmental factors, such as temperature and house design, have some effect on body fat deposition, but these are too small to be worthwhile influencing by changing management practises (Leenstra, 1986). In general, as the protein to energy (P : E) ratio of a diet increases, so body fat in broilers decreases (Whitehead, 1986). A disadvantage of higher protein diets is the greater costs. Dietary fat per se, within normal nutritional limits, has little obvious effect on body fatness in broilers (Whitehead, 1986). From the long term point of view selection provides the most satisfactory solution for reducing body fat content and several selective improvement methods for reducing fatness have been effectively used in practice (Leclercq et al., 1980; Griffin et al., 1982; Powell, 1984). A complete understanding of the factors regulating excessive fat accumulation

is still far from clear. To develop a complete strategy to reduce fat deposition to match the needs of the market is still a challenge for scientists.

In this chapter, the selection of lean chickens, plasma lipoprotein metabolism and fat deposition are reviewed and the objectives of the research in this thesis are described.

## 2 SELECTION OF LEAN CHICKENS

### 2.1 Variation of Fat Deposition in Chickens

Studies on the precursors of modern broiler strains have shown there are significant differences among breeds in fat deposition. Hunt (1965) showed that the body nitrogen : water ratio, which is an indicator of body composition, was different between two strains, Ottawa Meat Control and New Hampshire x Barred Plymouth Rock cross chicks. Significant differences were found among 12 broiler strains when fat content of the eviscerated carcasses was analyzed (Goodwin et al., 1969). Purebred White Rock carcasses, regardless of sex, contained 2 to 4% more fat than the Cornish or their reciprocal crosses (Moran et al., 1970). Edwards and Denman (1975) observed that there were significant differences in the percentage of total lipid in the carcass among five breeds of chickens at four weeks of age. The Light Brahma contained the largest amounts of total lipid (10.4%), followed by the White Plymouth Rock (10.2%), Black Jersey Giant (9.5%), Single Comb White Leghorn (8.8%), and the Dark Cornish

(8.6%). Washburn et al. (1975) also noticed that there were strain differences in carcass lipid.

Breeds also showed significant differences in the contents of fatty acids (myristic acid, palmitoleic acid and linoleic acid) of adipose tissue. Furthermore, there are differences of adipocytes between different breeds. For example, March and Hansen (1977) found that the adipocytes of White Leghorn were smaller in number and size than those of broilers.

The amount of abdominal fat (g/100g body weight) has been found to vary among broiler strains (Littlefield, 1972; Merkley, 1977; Nordstorm, 1978; Griffiths et al., 1978). Within strain, the coefficient of variation for abdominal fat was much larger than the coefficients of variation for live and carcass weight (24% vs 8.9%) in broilers at 50 days of age (Becker et al., 1984). In another experiment, coefficients of variation of abdominal fat were found above 30%, whereas those of the weights of muscular and skeletal tissue were only about 10% (Cahaner and Nitsan, 1985). It is evident that carcass and abdominal fat contents differ among breeds and strains and individuals.

Although the coefficients of variation of abdominal fat weight are high, it appears that the weight of abdominal fat is influenced by both nutritional and genetic factors (Cherry et al., 1978; Leenstra, 1986). The genetics of fat deposition has usually been assumed to be under polygenic control (Lin et al., 1980; Leclercq et al., 1980). Friars et al. (1983) reported that the

heritabilities of abdominal fat weight, abdominal fat weight as a percentage of carcass weight, and carcass fat percentage in broilers at 44 days of age were  $0.51 \pm 0.11$ ,  $0.62 \pm 0.12$ , and  $0.48 \pm 0.11$  respectively. Leenstra and Pit (1988) estimated that the heritability of abdominal fat as a percentage of carcass weight was  $0.45 \pm 0.15$  in cage housed birds at 42 days of age and  $0.53 \pm 0.09$  in litter housed birds at 43-44 days of age. Cahaner and Nitsan (1985) found that the heritability of abdominal fat weight (g) of broilers at 9 weeks of age was higher ( $0.82 \pm 0.28$ ). These results indicate that about half of the variability of abdominal fat and total body fat is genetic and provide clear evidence that the amount of fat might be decreased by selection.

## 2.2 Selection for Reducing Body Fat Content

Direct selection for reducing body fat content seems impossible, because there are at present no methods for directly measuring body fat content in live birds. Several indirect selection methods have been used in practice in recent years, and these include selection on abdominal fat pad weight, feed conversion ratio and biochemical indicators.

### 2.2.1 Abdominal fat

Becker et al. (1979) reported that abdominal fat weight (g or percentage of body weight) was a good predictor of total fat in broiler carcass ( $r = 0.8$ ) and suggested that selection for abdominal

fat should decrease fat in other tissues without changing fat-free weight. The measurements of abdominal fat have been usually conducted by hand palpation, caliper determination and sib analysis.

#### 2.2.1.1 Hand palpation measurements

In a programme of selection (Lilburn et al., 1982) for differences in abdominal fat pad size in layer type chickens while maintaining constant body weight, the size of the abdominal fat pad was judged by hand palpation of mature females. After 8 generations of selection, the adult females in lean and obese lines showed considerable difference in abdominal fat; body weight, however, had also diverged to some extent. Although the selection was made on mature hens, studies showed that the obesity could develop as early as 6 weeks of age. Sorensen (1984) has also reported the estimation of abdominal fat in live broilers by hand palpation. The correlation between the palpation scores and abdominal fat corrected for body weight in his studies was 0.40. He believed that it should be possible to reduce the amount of fat by 1 g per generation by using this very simple method in his selected lines. Because of relatively low correlation between the palpation scores and the abdominal fat weight, the application of this method is limited.

#### 2.2.1.2 Caliper measurements

Pym and Thompson (1980) found that a cloacal caliper technique was efficient in estimating the amount of abdominal fat in live broilers, reporting phenotypic correlations between caliper

measurement and the proportion of abdominal fat of 0.80. The correlation between the caliper measurement and the total body fat was about 0.5 in 3 out of 4 groups but the correlation was low (0.15) in the fourth (Whitehead and Griffin, 1982). It is possible that this method could be used in a breeding programme although there is so far no report of its use in breeding practice.

The accuracy of caliper measurements is largely dependent on the judgement of individual operators and there are likely to be differences between the scores judged by different persons on the same bird. A similar criticism can be applied to hand palpation.

#### 2.2.1.3 Sib analysis

Another approach was developed by Leclercq et al. (1980) who used the ratio of abdominal fat : body weight of sibs for selecting broilers. Four sons and one daughter per dam were slaughtered, for each line the progeny of the 20 dams whose sons showed the greatest deviation (positive or negative, depending on lines) from the mean were retained. After three generations the differences in abdominal fat content were already perceptible at 28 days of age; no significant change was found for body weight. After seven generations, the fat line had up to four times more abdominal fat than the lean line, whereas body weight remained almost equal in the two lines (Leclercq, 1983). Sib selection has also been practiced in Israel (Cahaner et al., 1985) and in the Netherlands (Leenstra and Pit, 1988) and produced results similar to those of Leclercq (1983).

Sib analysis is an effective method for selection against fatness. Its major disadvantage is that it is laborious and expensive because it involves slaughter of a large number of birds. If there are 200 dams in a line, and four sons and one daughter per dam have to be slaughtered (according to Leclercq et al., 1980), a total of 1,000 birds will be killed for weighing of abdominal fat in each generation. This is very hard work for breeders and the costs are quite high. Costs would be greater in using sib analysis with ducks and turkeys.

#### 2.2.2 Feed conversion ratio

In chickens, there is a significant positive phenotypic correlation between feed conversion ratio (FCR) and carcass lipid (Thomas et al., 1958; Washburn et al., 1975). A successful experiment in which selection for decreased FCR resulted in a substantial decrease in body fat was reported by Pym and Solvyns (1979). Compared with the control line, the line selected for decreased FCR for five generations decreased 21.2% (82.5:104.7 g/kg) in body fat at 9 weeks of age and 17.7% (88.1:107.1 g/kg) in body fat at a given body weight (about 1275g). Leenstra and Pit (1987) also reported that selection for a favourable feed conversion for five generations produced broiler chickens with better feed conversion (1.72 vs 1.77) and less abdominal fat (2.23% vs 2.91%) than selection for body weight. They calculated that the genetic correlation between feed conversion ratio and abdominal fat weight (g/100g of body weight) was 0.44.

In ducks, selection for improved feed conversion ratio over 8 generations in 3 diverse pure-bred lines has resulted in substantial improvements in FCR by 9-13% as well as reducing carcass skin and fat by 9-15% and growth rate by 7-12% (Powell, 1984).

Measuring feed conversion ratio individually is demanding of labour and time. If accurate data is to be collected, specially designed troughs are needed to avoid waste of feed. A potential problem is that the FCR from the bird kept in the individual cage may be different from that from the same bird reared on a deep litter system. From this author's experience, the birds with better FCR records in single cages are relatively inactive, and the inactive birds generally have poorer reproductive performance. It is therefore difficult to be certain of the long-term effects of selection for FCR.

### 2.2.3 Biochemical criterion - VLDL

Various metabolic factors are involved in lipid synthesis and transport, among them, plasma very low density lipoprotein (VLDL) concentrations give a good indication of body fatness (Griffin et al., 1982; Whitehead et al., 1984). A turbidimetric assay for VLDL based on the selective precipitation with heparin and magnesium ions was developed (Griffin and Whitehead, 1982) and correlations between plasma VLDL concentration measured turbidimetrically and body fat content were similar to or higher than those obtained previously using a chemical assay. Phenotypic correlations for broiler grandparent stock fed on a low-fat diet were 0.70 and 0.65 for males

and females respectively. Up to 1987, male and female broilers of a pure line had been selected over 7 generations on the basis of high or low concentration of plasma VLDL at 7 weeks of age. The abdominal fat and body lipid in the lean line decreased about 35% and 20% respectively and the feed conversion efficiency improved by 6.2% compared with those of control line. There was no difference between the lines in 7-week body weight (Whitehead, 1988). This method appears to be the first successful example of indirect selection against fatness using a biochemical criterion in livestock production.

In turkeys, birds with lowest plasma VLDL concentrations had significantly less abdominal and total fat than those with the highest concentrations, indicating that plasma VLDL concentration can also be used as an indirect method of estimating fatness in this species too (Griffin and Whitehead, 1985). The heritability of plasma VLDL concentration in Japanese Quail has been estimated as 0.5 and its genetic correlation with body fat content as 0.5 also (Garwood, 1987). In contrast, plasma VLDL concentrations in ducks are not well correlated with body fat content (H.D. Griffin and J. Powell, personal communication).

The VLDL selection is a simple and cheap method. Taking about 1 ml blood sample per bird from <sup>a</sup> wing vein and measuring the VLDL concentration as described by Griffin and Whitehead (1982) is not laborious and several hundreds of blood samples can be treated in one day. Because no birds need to be killed, this method is much cheaper than sib analysis. Grunder and Chambers (1988) reported the

genetic gains in reducing fatness from selection for low VLDL was at least as effective as the gains from selection either against fatness on the basis of a slaughtered sib test or for FCR. This sufficiently simple method therefore provides an alternative to expensive sib analysis. However, Grunder and Chambers (1988) also pointed out that selection for low VLDL would result in lower body weight and poorer FCR compared with direct selection for body weight or feed efficiency.

Other biochemical parameters related to fatness have been sought in poultry. For example, Leclercq et al. (1987) reported that selection for high and low plasma glucose concentrations in meat-type chickens has been carried out for 5 generations. The realised heritability of plasma glucose concentration was close to 0.25. Phenotypic correlations between plasma glucose at 3 weeks of age and abdominal fat to body weight ratio were estimated to be -0.10 and -0.24 in males and females respectively, and the genetic correlation estimates were -0.61 and -1.03 respectively. The birds from the high glucose line were significantly leaner than those from low glucose line, but the efficiency of selection for high glucose on reducing fatness appears to be poorer than that from sib analysis.

#### 2.2.4 A general comment on these methods

Although several selection methods have been used and the results of selections are favourable in general, less is known about the long term effects of selection against fatness on growth rate,

feed conversion ratio, reproductive traits and economic performance. The challenge is to reduce fatness without affecting growth rate.

There are also some estimates of the association between fat deposition and growth rate. Friars et al. (1983) reported estimates for the genetic and phenotypic correlations between average daily gain and percent abdominal fat were  $-0.31 \pm 0.21$  and  $0.18 \pm 0.04$  respectively, and suggested that simultaneous improvement of growth and lean carcass content should be possible in broilers. However, Becker et al. (1984) found the genetic correlations between abdominal fat weight and live weight or carcass weight were positive and ranged from 0.43 to 0.50 in males and 0.32 to 0.40 in females (for combined components). They believed that these genetic correlations provided strong evidence that if selection was applied for reduced abdominal fat weight, a correlated response of lower live and carcass weight would occur. This view appeared to be confirmed by a selection experiment in ducks, in which the carcass skin and fat decreased 9-15% and the growth rate also decreased 7-12% (Powell, 1984). In some experiments in which broilers were selected for high and low levels of fatness, there was no difference in body weight between the birds from two lines (Leclercq, 1983; Whitehead and Griffin, 1984; Cahaner et al., 1985), but their growth rates have not been compared with the birds directly selected for growth rate. Body weight of the birds directly selected for growth rate tends to be heavier than that selected for reducing body fat content by using feed efficiency, VLDL or sib analysis methods (Pym and Nicholls, 1979; Whitehead and Griffin, 1984; Leenstra and Pit, 1988). This loss creates a

dilemma for the commercial breeder, who wants to provide the fastest growing bird on the market with the best FCR and meat yield.

Generally, there is still a lack of a suitable technique for assessing the abdominal fat weight that is both simple and accurate. Computerized tomography had been investigated to predict the amount of abdominal fat in broilers and the correlation between predicted and observed values was estimated to be about 0.8 (Bentsen and Sehested, 1986). However, the equipment costs more than 1 million pounds and running costs are high. This method is unlikely to be used in breeding practice until its precision is increased further and becomes much higher than those of cheaper methods.

### 3 PLASMA LIPOPROTEIN METABOLISM AND FAT DEPOSITION

Selection on the basis of VLDL concentration depends on the high correlations between plasma VLDL concentrations and body fat deposition in broilers. The function of plasma lipoproteins is to transport lipids derived from the diet or synthesized primatively in the liver to extrahepatic tissues. Plasma triglyceride-rich lipoproteins are hydrolyzed by lipoprotein lipase (LPL) as they pass through the peripheral circulation, and the fatty acids released are either oxidised or re-incorporated into triglyceride for storage in the surrounding tissues. In this section, the characteristics and functions of plasma lipoproteins and LPL will be introduced. Adipose tissue development will be described briefly.

### 3.1 Plasma Lipoprotein metabolism

The composition and function of plasma lipoproteins in poultry has been recently reviewed in detail (Griffin and Hemier, 1988). The plasma lipoproteins are usually classified according to their ultracentrifugal flotation properties at particular solvent densities (Havel et al., 1955). Different plasma lipoproteins have similar structures. In the core of particles, there are triglyceride and cholesterol ester and the particles are surrounded by a layer of phospholipid, cholesterol and apolipoproteins (Shen et al., 1977). In lipoproteins, the lipid and proteins are not covalently joined but held together largely by hydrophobic interactions between the nonpolar lipid and the protein components of apolipoproteins. These apolipoproteins have specific physiological functions, for example, apo-C-II is important for normal hydrolysis in the circulation (Smith et al., 1978).

Triglyceride that accumulates in avian adipose tissue is thought to be synthesized mainly in the liver or derived from the lipid in diet. Newly synthesized lipid secreted from the liver into the plasma and transported to the extra-hepatic tissues is in the form of very low density lipoproteins. Dietary lipid is transported from avian intestine directly into the portal vein as portomicrons (Bensadoun and Rothfeld, 1972) and non-esterified fatty acids (Sklan et al., 1984). This route of entry of dietary lipid into the bloodstream is different from that in mammals, because the intestinal lymphatic system is poorly developed in birds. The majority of triglyceride-rich lipoproteins in the plasma of broilers

fed standard commercial diets are VLDL (Table 1.1), though portomicrons can accumulate in the circulation of birds fed diets with high levels of fat (Griffin et al., 1982).

Table 1.1 Typical lipid composition of lipoproteins from immature chickens

	Portomicrons	VLDL	IDL	LDL	HDL
Density	<1.013	<1.013	1.013 -1.023	1.023 -1.046	1.052
Lipid composition: (weight %)					
triglyceride	88.8	59.3	16.3	7.9	2.5
phospholipid	6.2	14.2	20.2	22.9	28.6
cholesterol	3.6	5.2	7.7	9.7	3.2
cholesterol ester		11.1	30.9	32.5	22.5
Protein:	1.4	11.3	25.4	26.8	43.2

From Griffin and Hermier (1988).

The low density lipoproteins (LDL) are primarily derived from VLDL catabolism. Their major role is probably to transport cholesterol to the peripheral tissues where they may also regulate intracellular synthesis of cholesterol. The high density lipoproteins (HDL) arise from a variety of sources, including the liver. They also promote cholesterol transport from extrahepatic tissues to the liver and may also transfer some cholesterol to other lipoprotein species (Smith et al., 1978).

### 3.2 Lipoprotein Lipase

The activity of LPL is a major factor controlling the rate of fat deposition in adipose tissues (Cryer, 1981). Total blockage of

VLDL and portomicron triglyceride removal from the circulation of chickens by intravenous injection of an anti-LPL antiserum provides direct experimental evidence that LPL is indispensable for the transfer of plasma triglyceride to tissues (Kompaing et al., 1976). LPL is synthesized in the tissue cells, but only the part of activity that has migrated to the capillary endothelial cell wall can be considered to be functionally active. LPL is bound to the luminal surface of the capillary wall via attachment to heparin-like compounds, and it can be displaced from this site into the general circulation by intravenous injection of heparin (Cryer, 1981).

Results accumulated over many years have provided substantial evidence that LPL activity has a major influence upon the rate of uptake of plasma triglyceride into tissues. In rats, for example, fasting decreases LPL activity adipose tissue and increases that in heart, and lactation increases LPL activity in the mammary gland and decreases that in adipose tissue (Cryer, 1981).

LPL appears to be responsible for at least some of the differences in fatness produced by selection or mutation. Selection for high and low backfat thickness in swine over 18 generations led to a three-fold increase in dorsal subcutaneous adipose tissue LPL specific activity (expressed per mg of protein and per g of tissue) in 110 days fetal pigs of the high line compared to the low line. LPL activity in muscle of 110 d fetuses was not significantly different between low and high lines (McNamara and Martin, 1982). At 110 d of gestation, high line fetuses had a greater capacity for triglyceride fatty acid uptake in adipose tissue than in muscle,

while the opposite was the case in the low line. The body composition of the two lines at this age was not different. Selection, therefore, caused a change in activity of LPL before any measurable change in adipose tissue accumulation.

In the rat, tissue specific LPL activities have their own patterns. Muscle LPL activity was found to be lower in neonatal genetically obese Zucker rats than in lean rats (Boulangé et al., 1981), but in adults, muscle LPL activities were similar in obese and lean animals (McNamara et al., 1982). At all stages during the postsucking period, the obese rat had significantly more LPL activity in the epididymal fat pad regardless of whether activity was expressed per whole epididymal adipose depot or per fat cell. Early increases in LPL activity in adipose tissue of the "pre-obese" rat may contribute significantly to the early fat cell hypertrophy seen during the development of this genetic obesity (Gruen et al., 1978). LPL activity ( $\mu$ moles FFA released/g of fresh tissue) in heart and epididymal fat pad were higher in adult genetically obese mice (ob/ob). In heart, the enzyme was unchanged from 8 to 48 weeks of age in lean mice, but in obese mice it increased between 8 and 12 weeks of age and remained elevated (Enser, 1972). De Gasquet and Pequignot (1972) reported that the specific activities (units/g of tissue) of LPL in epididymal adipose tissue, heart, and diaphragm from genetically obese mice (ob/ob) were similar to those from controls (lean mice), although the total LPL activities (units/whole tissue pad) in these tissues were significantly higher in obese mice.

LPL in avian tissues seems much less responsive to nutritional state than in mammalian tissues. Total activity in adipose tissue and skeletal muscle is either unaffected or slightly reduced by fasting, whereas that in cardiac muscle increases only slightly (Evans, 1972; Husbands, 1972; Benson and Bensadoun 1977; Griffin and Butterwith, 1988). An inverse relationship existed between fat pad weight and specific LPL activity of the tissue in White Leghorn pullets (Pfaff et al., 1977). A negative correlation between the abdominal fat weight and lipase activity per gram of the tissue was also found in broilers ( $r=-0.31$ ,  $P<0.01$ ; Grunder and Chambers, 1988). It was also reported that the difference in LPL activity did not appear to be related to differences on body fat deposition observed between broilers and turkeys (Borron et al., 1979). However, the total LPL activity in abdominal fat pad from genetically fat broilers was higher than that in lean birds (Leclercq, 1988). LPL in adipose tissue is likely to increase with number of fat cells and therefore reflect the degree of adipocyte hyperplasia, and perhaps the lipogenesis of fat depots, as well as their capacity to take up and re-esterify lipid from the plasma (Griffin and Hermier, 1988).

### 3.3 Adipose Tissue Development

The increase of adipose tissue in immature birds, as in mammals, depends on increases in adipocyte hyperplasia (cell number) and cell hypertrophy (cell size). Hyperplasia remains active for approximately 14 weeks of life in broilers and the size of

adipocytes also increases with fat deposition in the birds from 1 to 14 weeks of age (Hood, 1982; Simon and Leclercq, 1982), but the relative contribution of fat cell size and number to adiposity in broilers at market age is not clear.

Leaner birds produced by selection for feed efficiency have smaller adipocytes (Hood and Pym, 1982). Divergent selection for abdominal fat ratio showed that the difference in abdominal fat weight of the birds in fat and lean selected <sup>lines</sup> could be largely accounted for by the difference of adipocyte size (Leclercq, 1984). This was similar in both lines and sexes at 1 week of age, but it was higher in fat line males than in lean line males at 2 weeks of age and thereafter remained consistently higher ( $P < 0.01$ ) in both sexes of the fat line. In males at 9 weeks of age, the adipocyte diameter was  $50.0 \mu\text{m}$  in the fat line and 39% greater than in the lean line (Simon and Leclercq, 1982).

Adipocytes in the neck and shoulder dorsal-most subcutaneous tissue from obese pig (selected for backfat thickness over 18 generation) fetuses at 110 days of gestation were larger ( $P < 0.01$ ) than cells from lean fetuses (Hausman et al., 1983). At 14 to 20 days, 56 to 70 days, 180 days obese pigs also had larger ( $P < 0.01$ ) cells than lean pigs (Hausman and Martin, 1981).

During the post-sucking development period, fat cell size in epididymal adipose tissue from genetically obese rat was significantly increased (Gruen et al., 1978). Adipocytes in inguinal adipose tissue were also larger than their lean controls

(Boulange et al., 1979)

Generally, the fat animals which are produced either by selection or gene mutation have larger adipocytes than those from their lean controls. LPL is likely to be a major determinant of cell size (Griffin et al., 1987), particular in birds, which derive a large part of their fat from the plasma.

#### 4. OBJECTIVE OF THE RESEARCH

As described above, excessive fat deposition in poultry for meat production is becoming of increasing concern to the poultry industry. Plasma VLDL concentration in fed broilers gives a good indication of body lipid content of live birds at 7 weeks of age and has been used as a basis for selection for leanness. However, from the 4th generation of selection for low plasma VLDL concentration, plasma VLDL concentration seemed not to decrease further, and neither did the correlated response to body fat content (from the 4th to the 7th generation, plasma VLDL concentrations in the birds were 0.070, 0.068, 0.064, 0.060 absorbance units, total body lipid were 137, 134, 126, 134 g/kg respectively, see Whitehead, 1988). The selection appears to have reached a limit. Additional criteria are therefore needed to improve the accuracy of selection and take the procedure to lower levels of fat. The aim of the present research is to further investigate plasma lipoprotein metabolism and fat deposition in broilers and to examine possible ways of improving the accuracy of prediction of body fat content using biochemical

determinations. The research consisted of the following parts:

i) Determination of the contribution of plasma lipoprotein triglyceride and adipose tissue lipogenesis to adipose tissue lipid deposition (Chapter 2), with the view of defining relative importance of hepatic lipogenesis and adipose tissue lipogenesis and providing clues to new criteria for use in selection.

ii) Investigation of the variation in adipose tissue LPL activity between individual broilers, and measurement of the correlations between plasma VLDL concentrations, adipose tissue and post-heparin plasma LPL activities and body fat content (Chapter 3). This work aims to investigate whether post-heparin plasma LPL activity or tissue LPL activity might be indicators of fat deposition.

iii) Investigation of the possibility of distinguishing post-heparin plasma LPLs released from different tissues by their molecular weights and isoelectric points (Chapter 4). Since plasma VLDL triglyceride hydrolyzed in muscle is largely used for oxidation and that hydrolyzed in adipose tissue is mainly used for storage, selection for high muscle LPL activity and low adipose tissue LPL activity in post-heparin plasma might produce leaner birds.

iv) Comparison of in vivo lipoprotein metabolism in birds divergently selected for plasma VLDL concentration (Chapter 5), to determine the effect of selection on tissue LPL activity and the fate of circulating VLDL.

The conclusions are discussed in Chapter 6.

## Chapter 2

## PLASMA LIPOPROTEIN METABOLISM IN COMMERCIAL BROILERS

## SUMMARY

Investigation of in vivo lipogenesis in commercial broilers at 5 weeks of age using tritiated water showed that tritium incorporation into liver lipid (dpm/g of tissue) was 8-fold greater than that incorporated into the lipids of abdominal fat pad. Total adipose tissue lipogenesis was estimated to be about 80% of the amount of liver lipogenesis.  $^3\text{H}$ -labelled portomicrons and  $^{14}\text{C}$ -labelled very low density lipoprotein (VLDL) showed similar rates of clearance from plasma and 53% of  $^3\text{H}$  and 51% of  $^{14}\text{C}$  injected was estimated to be incorporated into adipose tissue. Only 0.3% of the total radioactivity from intravenously-injected  $^{14}\text{C}$ -palmitic acid was incorporated into the abdominal fat pad. Total lipid deposition in the broilers at 6-7 weeks of age was calculated to be 5.5 g/d/kg body weight of which 55% was estimated to be incorporated from plasma lipoprotein triglyceride. 80% of the lipid deposited in the abdominal fat pad was estimated to be derived from plasma lipoprotein triglyceride. These results confirmed that plasma portomicrons and VLDL play an important role in body fat deposition in broilers, but indicated that adipose tissue lipogenesis cannot be neglected.

## INTRODUCTION

As described in Chapter 1, selection for plasma low VLDL concentration to reducing body fat content is a successful example of using a biochemical criterion in poultry breeding practice. Since body fat deposition is related to many different biochemical processes, further investigation of the mechanism of broiler fat deposition may provide clues to increase the accuracy of selection for low VLDL in reducing body fat content.

Although the correlation between the plasma VLDL concentration and body fat content in broilers is high ( $r=0.7$ , Griffin et al., 1982), many details about plasma lipid metabolism, such as the contribution of VLDL triglyceride to adipose tissue growth and relative importance of adipose tissue lipogenesis are not clear. Lipogenesis in chicken has been investigated in several experiments (O'Hea and Leveille, 1969; Leveille et al., 1975; Brady et al., 1976; Saadoun and Leclercq, 1983, 1986). These results indicated that 70-90% of fatty acid synthesis occurred in liver and about 5-30% in extra-hepatic tissues. However, the birds in these experiments were not modern commercial broilers (O'Hea and Leveille, 1969) and either the birds were very young (only 300-400g body weight, Brady et al., 1976) or the tritiated water was administered intraperitoneally (Saadoun and Leclercq, 1983, 1986).

In the present experiment, lipogenesis was measured in modern commercial broilers at 6 weeks of age using tritiated water and the

results from intravenous injection and intraperitoneal injection were compared. In addition, lipid deposition, lipid intake from diet, plasma VLDL accumulation, portomicron and VLDL catabolism and the utilization of fatty acids by adipose tissue were investigated. The rate of production of plasma VLDL was determined using an anti-LPL serum to block the clearance of VLDL. The metabolism of portomicrons and VLDL were compared in vivo by intravenous injection of  $^3\text{H}$ -labelled portomicrons and  $^{14}\text{C}$ -labelled VLDL into the same birds at the same time. The fate of fatty acids was studied by intravenous administration of  $^{14}\text{C}$ -palmitic acid.

#### MATERIALS AND METHODS

##### Birds

Commercial male broilers from D.B. Marshall Ltd, Newbridge, Scotland were reared from day old and kept in individual cages from 4 to 7 weeks of age on a 23 h light : 1 h dark photoperiod with lights off between midnight and 0100 h. A standard commercial broiler finisher diet containing 190 g/kg of crude protein, 31 g/kg of ether-extractable fat and 3168 kcal ME/kg was fed ad libitum from 4 to 7 weeks of age. Individual food intake was measured on 8 birds from 43 to 50 days of age.

Determination of in vivo lipogenesis by the incorporation of tritiated water

The use of tritiated water to estimate lipogenesis is considered more accurate than  $^{14}\text{C}$  labelled glucose or acetate, since it estimates the rate of fatty acid synthesis from all carbon precursors and minimizes the problem of isotope dilution which exists with glucose and acetate tracers (Brady et al., 1976; Goodbole and York, 1978). Tritiated water was administered either by intravenous injection or intraperitoneal injection.

Because most of the fat in rapidly growing broilers killed at 6-7 weeks of age is deposited in the preceeding 2-3 weeks, birds at 5 weeks of age (over 1,200 g body weight) were used in the present study for the determination of in vivo lipogenesis. Birds were given 0.5 mCi tritiated water in 1 ml of saline by intravenous (n=4) or intraperitoneal injection (n=4). Ten min after the injection, 1 ml of blood was collected from the wing vein. The birds were then killed quickly by intravenous injection of sodium pentobarbitone (Expiral, Abbott Laboratories, Queenborough, Kent) and the liver and abdominal fat were removed, rinsed in ice-cold saline, weighed and immediately frozen in liquid nitrogen.

Tissue lipids were extracted using the method of Folch et al. (1957). Duplicate one gram samples of crushed tissue were homogenized in 9 ml of chloroform/methanol (2:1, v/v) with a Polytron homogenizer (Kinematica, Lucerne, Switzerland). The head of the homogenizer was then washed with another 3 ml of the solvent.

The sediments in the initial extracts and washings were removed by filtration. The extract was then washed with saline three times to remove radioactivity not incorporated into lipid. In each wash the extract was mixed with saline (2.5 ml the first wash and 5 ml in the second and third) vigorously and the upper aqueous phase was removed after being centrifuged at 500 g for 5 min. The chloroform in infranatant was evaporated under nitrogen. The lipid residue was dissolved in 4 ml of Optiphase 'X' (LKB) and radioactivity was determined using a liquid scintillation counter (LKB 1216 RackBeta II). Duplicate 0.2 ml sample of plasma were directly mixed with 4 ml of Optiphase 'X' and then counted.

#### Measurement of lipoprotein secretion rate

Normally, the amount of VLDL secreted into the plasma is difficult to estimate, since the VLDL is rapidly hydrolyzed by the LPL when it circulates to the capillaries. In the present study, plasma VLDL production rate was determined by measuring the rate of VLDL accumulation after inhibition of LPL activities by intravenous injection of an anti-LPL serum (Kamplang et al., 1976). Eight birds at 6 weeks of age were intravenously injected with 2 ml of antiserum for inhibition of LPL activity. The antiserum had been raised in sheep by repeated injection of LPL purified from broiler adipose tissue by heparin-affinity and concanavalin-A-affinity chromatography (S.C. Butterwith and H.D. Griffin, unpublished). Blood samples were taken immediately before injection and <sup>at</sup> 10, 20, 30 and 40 min after injection. Plasma VLDL concentration was measured turbidimetrically (Griffin and Whitehead, 1982).

Metabolism of  $^3\text{H}$ -portomicrons and  $^{14}\text{C}$ -VLDL in vivo

Newly synthesized lipid is secreted from the liver into the plasma and transported to the extra-hepatic tissues in the form of VLDL, whereas dietary lipid is transported from avian intestine directly into the portal vein as portomicron (Bensadoun and Rothfeld, 1972) and non-esterified fatty acids (Sklan et al., 1984). In the present study, metabolism of portomicrons and VLDL was investigated in vivo using different labels.

Labelled portomicrons and VLDL were prepared by H.D. Griffin and F. Acamovic. 100  $\mu\text{Ci}$   $^{14}\text{C}$ -palmitic acid was complexed to 2 ml of chicken plasma and injected intravenously into a 6 week old broiler chicken. 2 ml of anti-LPL antiserum was injected 20 min later. The bird was killed after 60 min by intravenous injection of sodium pentobarbitone and blood was collected from the jugular vein.  $^{14}\text{C}$ -labelled VLDL were isolated by centrifuging plasma overlaid with 0.9% NaCl at 100,000 g for 20 h.  $^3\text{H}$ -labelled portomicrons were prepared using a similar procedure, except that 250  $\mu\text{Ci}$   $^3\text{H}$ -palmitic acid was incubated into the crop. Portomicrons were separated from the plasma by centrifuging at 100,000 g for 1 h. Thin-layer chromatography of lipids extracted from  $^3\text{H}$ -portomicrons and  $^{14}\text{C}$ -VLDL prepared in this way had previously shown that more than 95% of the label was present in lipoprotein triglyceride.

Each of 8 birds at 6 weeks of age was intravenously injected with 1 ml of the mixture of  $^3\text{H}$  labelled portomicrons (1,237,500 dpm) and  $^{14}\text{C}$  labelled VLDL (625,000 dpm). The birds were bled after 24

min and killed immediately for sampling of abdominal fat, neck fat thigh fat and liver. Sample collection, lipid extraction and radioactivity determination were the same as those described above. The lipids were weighed after evaporation of chloroform. Plasma volume in the birds was estimated to be 4.7% of the body weight (H.D. Griffin, unpublished).

#### Metabolic utilisation of $^{14}\text{C}$ -palmitic acid in vivo

In order to study the fate of blood free fatty acids, four birds were intravenously injected with  $^{14}\text{C}$ -palmitic acid (720,000 dpm each) complexed to 1 ml of broiler plasma. Blood samples were taken from two of them at 1, 2, 3, 5, 8 and 9.5 min after the injection. All the four birds were killed at 10 min. The procedures for tissue sampling, lipid extraction and radioactivity counting were the same as described previously.

#### Carcass analysis

Carcasses were stored at  $-20^{\circ}\text{C}$  prior to analysis. Each frozen carcass was cut into small pieces with a Hobart cutting machine (Model VCM 25) and then reduced to a homogeneous paste by being passed three times through a Hobart 2.2 kw mincing machine using a fine screen. Duplicate 200 g samples of paste were weighed, freeze-dried and reweighed to determine dry matter content. The fat content of the dried samples was determined by continuous extraction with petroleum ether (bp  $40-60^{\circ}\text{C}$ ) in a Soxhlet apparatus.

## RESULTS AND DISCUSSION

Food intake and body fat content of the birds

The food intake in birds between 43 and 50 days of age was 158 g/d/bird and gain in body weight was 68 g/d/bird. Body lipid content at 50 days of age was 160g/kg (Table 2.1). If body composition did not change between 43 and 50 days of age, the birds deposited about 11 g lipid per day, or about 5.5 g/kg body weight per day. The abdominal fat pad contained about 8.25% of total body lipid (13.2/160 g of kg body weight, Table 2.1): 0.45 g of lipid was deposited in abdominal fat pad/day/kg body weight. Because the diet contained 31 g/kg of lipid (see Materials and Methods), the maximum lipid intake from diet was 4.9 g/d/bird, or 2.5 g/d/kg body weight.

Table 2.1 Food intake, gain and body fat content of male broilers in the period from 43 to 50 days of age

	mean + S.E	(n=8)
body weight (g)		
at 43 d of age	1649+45	
at 50 d of age	2193±38	
gain (g/d) <sup>1</sup>	68.1+3.1	
food intake (g/d) <sup>1</sup>	158±3.3	
body lipid		
(g/kg body weight) <sup>2</sup>	160±2.4	
abdominal fat pad weight		
(g/kg body weight) <sup>2</sup>	18.9±1.2	
abdominal fat lipid <sup>2,3</sup>		
(g/kg body weight) <sup>2,3</sup>	13.2±0.84	

<sup>1</sup>mean from 43 to 50 days of age.

<sup>2</sup>at 50 days of age.

<sup>3</sup>calculated from fat pad weight and lipid content in the fat pad (700 mg lipid/g of fat pad).

### In vivo incorporation of tritium from injected tritiated water

The specific activity of tritium reached a plateau 5 min after intravenous (i.v.) injection and 7 min after intraperitoneal (i.p.) injection (Fig. 2.1). However, only 1 min after i.v. injection, the radioactivity in the plasma was close to that at final equilibration. After i.p. injection, equilibration occurred more slowly. Similar specific radioactivities (dpm/ml) were obtained in the plasma when equilibration was reached, regardless of the method of administration.

The amount of the tritium incorporated into the tissue lipids is shown in Table 2.2. The birds in the two treated groups (i.v. injection and i.p. injection) had similar body weight, abdominal fat weight and liver weight. The total radioactivity retained in the plasma of i.v. injected birds was higher than that in i.p. injected birds ( $P < 0.05$ ) although the specific activities (dpm/ml) in plasma were not significantly different ( $P > 0.05$ ) between the birds from different treatments. The tritium incorporated in the liver lipid from the birds given i.v. injections was slightly higher than that from the birds given i.p. injections, although the difference was not significant ( $p > 0.05$ ). The tritium incorporated into the lipids of the abdominal fat pad was substantially lower in the birds given i.v. injection ( $P < 0.01$ ) when results were expressed as either total activity or specific activity incorporated. These results suggest that abdominal fat pad in birds given tritiated water intraperitoneally might directly incorporate the tritium from tritiated water present within the abdominal cavity. Therefore, the

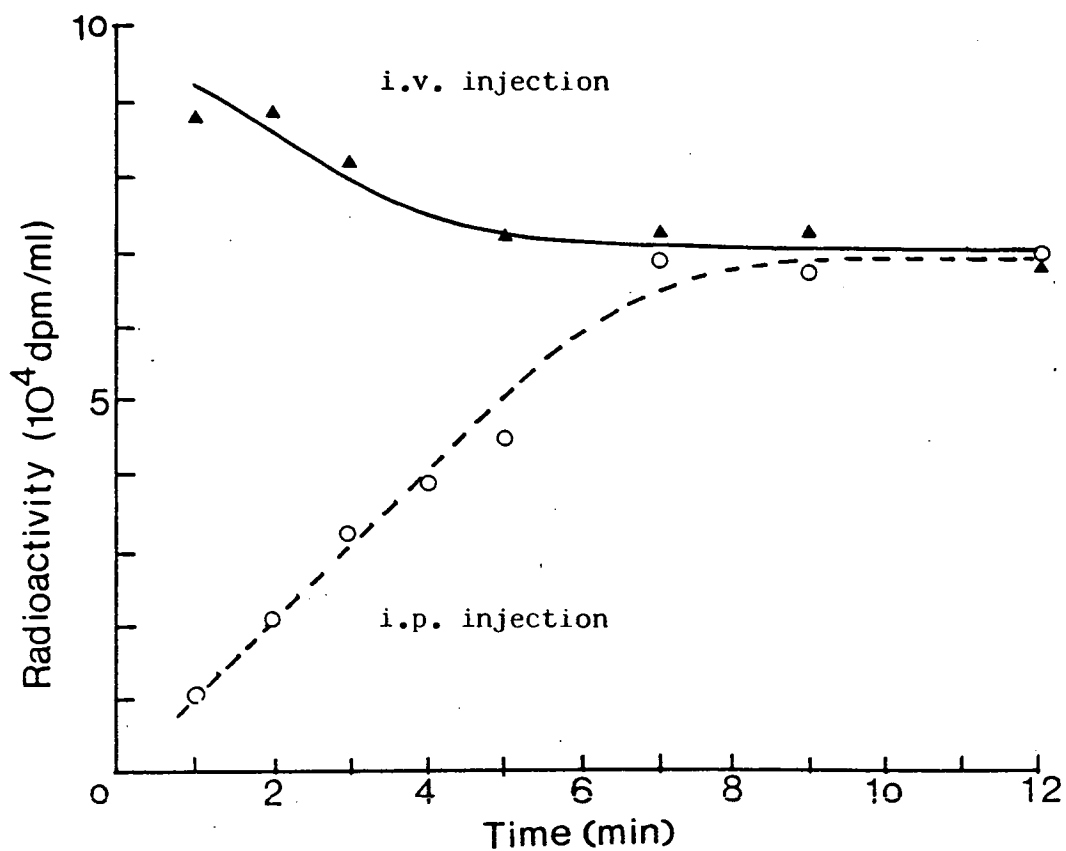


Fig. 2.1 Equilibration of tritiated water in blood after intravenous (i.v.) and intraperitoneal (i.p.) injection. Two birds of 6 weeks of age received the same amount of tritiated water (97,667,000 dpm) by i.v. or i.p. injection respectively.

Table 2.2 In vivo incorporation of tritium into broiler liver, abdominal fat pad lipid and plasma from broilers given the intravenous (i.v.) and intraperitoneal (i.p) injection of 0.5 mCi tritiated water<sup>1</sup>

	i.v.injection	i.p. injection	P
plasma			
dpm/ml x10 <sup>3</sup>	207+17	176+8.5	>0.05
dpm/total plasma x10 <sup>5</sup>	118+2.5	107+2.3	<0.05
liver			
dpm/g of tissue	461+70	385+31	>0.05
dpm/whole tissue (x10 <sup>2</sup> )	188+25	169+20	>0.05
abdominal fat			
dpm/g of tissue	53.3+7.0	188+20	<0.01
dpm/whole tissue (x10 <sup>2</sup> )	7.93+0.83	27.2+2.8	<0.01
body weight (g)	1226+72	1214+36	>0.05
abdominal fat weight (g)	15.6+2.2	13.3+1.0	>0.05
liver weight (g)	41.3+2.0	39.7+2.7	>0.05

<sup>1</sup>Mean+S.E. of four birds at 5 weeks of age.

lipogenesis in the abdominal organs and tissues contaminated with tritiated water by the intraperitoneal injection would be overestimated.

Liver lipids were much more highly labelled than adipose tissue lipids. In the group injected intravenously, the total activity incorporated into liver lipids was 23-fold greater than that in abdominal fat pad lipid and the specific activities were 8 times greater. This indicates that the rate of lipogenesis in the liver is much greater than that in abdominal fat pad in commercial broilers at 5 weeks of age, as also found by Saadoun and Leclercq (1983). Nevertheless, if it is assumed that these birds had the

same body composition as those at 7 weeks of age (160 g lipid/kg body weight) and the specific radioactivities incorporated in all adipose tissue depots were similar to that in abdominal fat pad, the total rate of lipogenesis in adipose tissue might be 80% of that in the liver.

#### VLDL secretion rate

VLDL secretion rate was measured after LPL activity was inhibited using an anti-LPL antiserum. In order to check the inhibition of LPL by antiserum, the disappearance of labelled portomicrons and VLDL from the plasma was examined in four birds, two in the absence and two in the presence of anti-LPL serum. Results are presented in Fig. 2.2. In the absence of anti-LPL serum, 92% of  $^3\text{H}$  radioactivity injected and 90% of  $^{14}\text{C}$  radioactivity injected were removed within 20 min of the injection. During the first 20 min after injection of anti-LPL serum, 38% of radioactivity of  $^3\text{H}$  and 35% of  $^{14}\text{C}$  were removed; but between 20 and 40 min after the injection the radioactivities in the plasma remained constant.

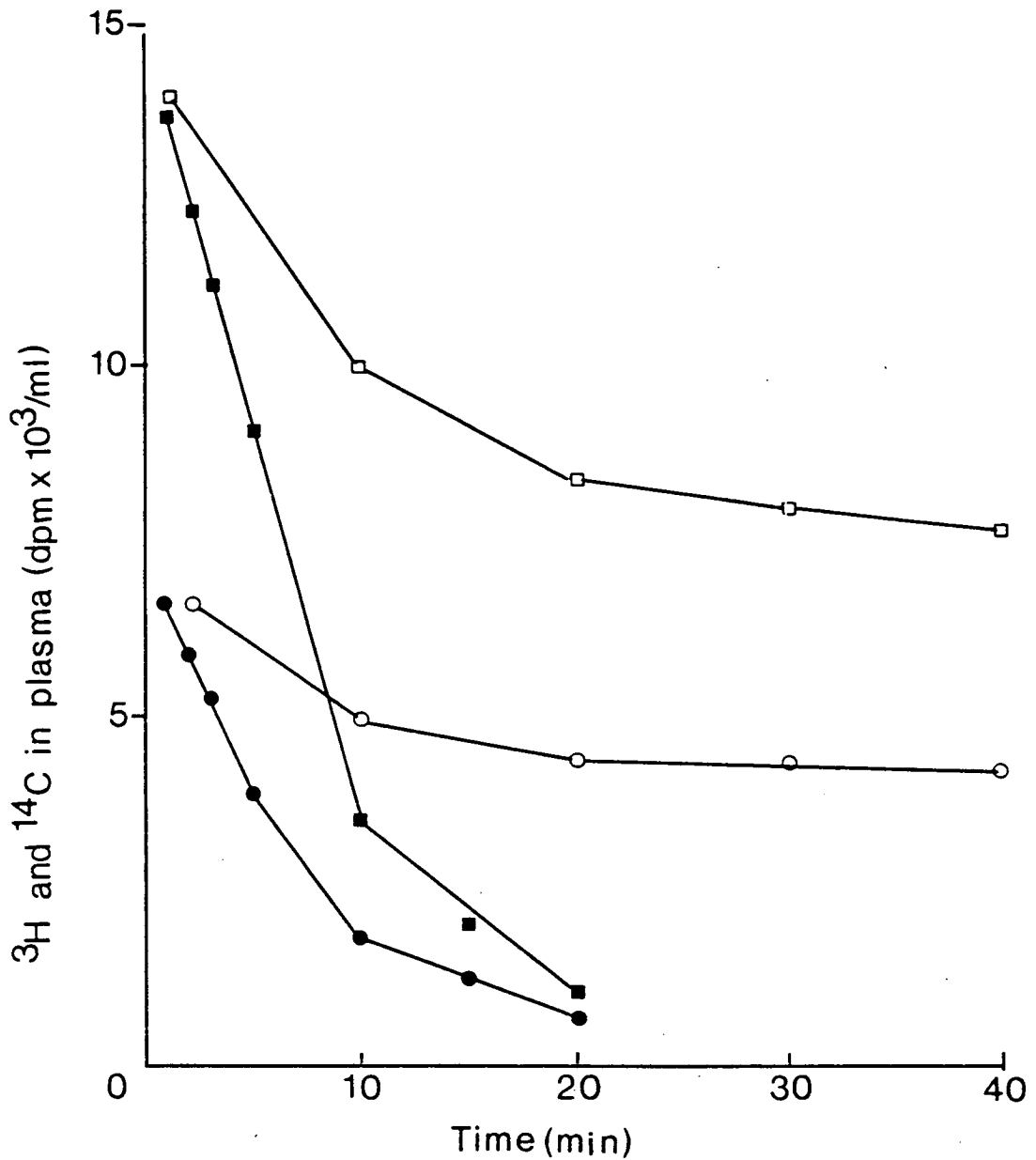


Fig. 2.2 Inhibition of removal of labelled plasma portomicrons and VLDL by anti-LPL serum.  $^3\text{H}$ -labelled portomicrons and  $^{14}\text{C}$ -labelled VLDL were injected 1 min after the administration of 2 ml anti-LPL serum and blood samples taken at the times indicated.  $\circ$ ,  $\bullet$ , portomicrons;  $\square$ ,  $\blacksquare$ , VLDL. Open symbols describe removal of label in the presence of antiserum, closed symbols in its absence.

After injection of anti-LPL serum, plasma VLDL concentration increased linearly (Fig. 2.3). The linear increase of plasma VLDL and triglyceride concentrations after inhibition of LPL by the antiserum confirmed that LPL activity was effectively blocked. But the radioactivities in plasma after intravenous injection of labelled portomicrons and VLDL decreased 35-38% during the first 20 min. This decrease may be caused by removal of portomicrons and VLDL damaged during preparation. A rapid disappearance of freeze-thawed labelled VLDL injected after the administration of anti-LPL antiserum has been observed previously (H.D. Griffin, unpublished).

In an experiment conducted by Kompiang, Bensadoun and Yang (1976), the birds received multiple anti-serum injections at 0, 30, 60 and 90 min and the labelled VLDL was injected at 30 min. This procedure may be more effective than a single dose of antiserum, but 12-24% of the initial radioactivity was still removed during the first 60 min. Alternatively, the time of plasma lipoprotein equilibration might not be as rapid as expected and the lipoproteins injected might equilibrate with non-plasma pools. Sniderman et al. (1975) indicated the total extravascular low density lipoprotein (LDL) pool in pigs was about 20-30% of the size of the plasma LDL pool. The existence of non-LPL dependent mechanism for removing VLDL also could not be eliminated.

Forty min after anti-LPL serum been injected into 8 six week old birds, plasma VLDL concentrations increased 4-fold on average (Fig. 2.3). Plasma triglyceride concentrations were  $1379 \pm 180$

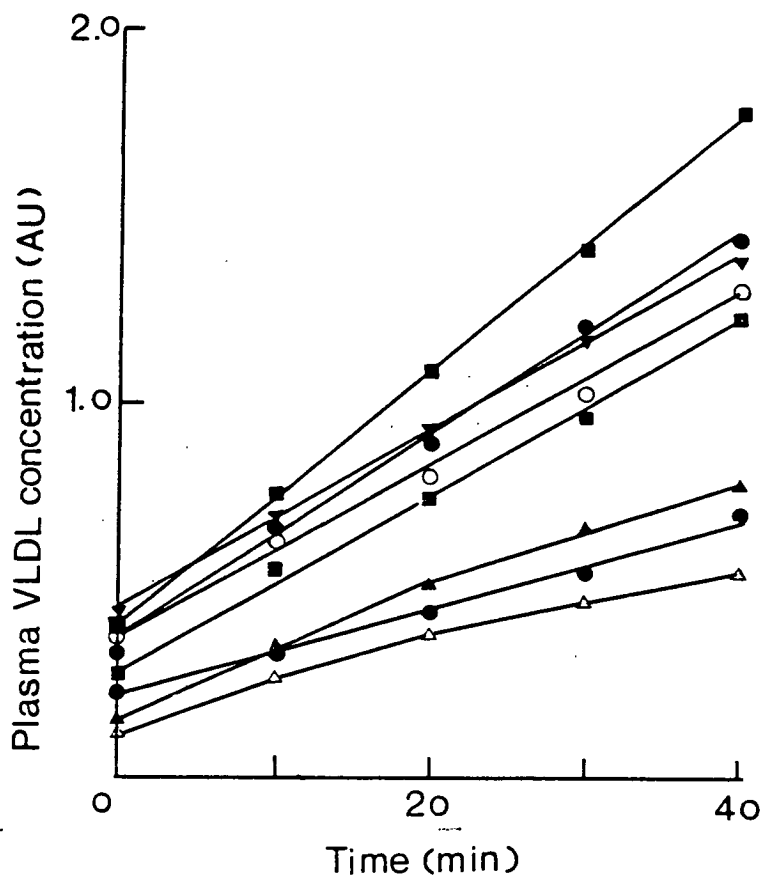


Fig. 2.3 Effect of anti-LPL serum on plasma VLDL accumulation. At time 0, 2 ml of anti-LPL serum was injected in eight birds at 6 weeks of age, with  $1427 \pm 58$  (g) of body weight and  $188 \pm 24$  (g/kg) of abdominal fat. Rates of VLDL production were calculated from initial rate of VLDL accumulation.

( $\mu\text{g/ml}$ , mean+S.E.) at 0 time and  $4894 \pm 554$  ( $\mu\text{g/ml}$ ) at 40min after the injection of antiserum, i.e.  $87.8 \mu\text{g/min/ml}$  accumulated. Mean plasma triglyceride production was estimated as  $5.95 \text{ g/day/kg}$  of body weight. This conclusion assumes that VLDL production rates measured over a short period are typical of rates over the whole day. Birds were fed ad libitum on a 23 h light and 1 h dark photoperiod and would be expected to be feeding continually.

#### Fate of labelled portomicrons and VLDL

Twenty four min after the administration of labelled portomicrons and VLDL, more than 90% of the radioactivity had been removed from the plasma and about 6% (either  $^3\text{H}$  or  $^{14}\text{C}$ ) was incorporated into abdominal fat (Table 2.3). There were no significant differences ( $P > 0.05$ ) between the proportion of administrated  $^3\text{H}$  and  $^{14}\text{C}$  incorporated into the abdominal fat pad and that remaining in the plasma. However, the percentage of  $^3\text{H}$  from portomicrons incorporated into liver lipids was higher than that of  $^{14}\text{C}$  from VLDL ( $P < 0.05$ ).

There were no significant differences ( $P > 0.05$ ) between the specific radioactivities (dpm/g of lipid) of  $^3\text{H}$  and  $^{14}\text{C}$  incorporated in different fat depots, although the activities incorporated in abdominal fat pad tended to be higher (Table 2.4). The ratios of  $^3\text{H}$  and  $^{14}\text{C}$  incorporated into the different fat depots were similar and very close to the ratio of  $^3\text{H}$  and  $^{14}\text{C}$  injected, indicating no selective uptake of portomicrons or VLDL.

(%)

Table 2.3 Incorporation of label from intravenously injected  $^3\text{H}$ -portomicrons and  $^{14}\text{C}$ -VLDL into broiler tissues<sup>1</sup>

	$^3\text{H}$	$^{14}\text{C}$	P
liver	14.4+1.09	10.5+0.775	<0.05
abdominal fat	6.35+0.342	5.69+0.234	>0.05
plasma	7.13+0.925	5.22+0.750	>0.05

<sup>1</sup>Mean+S.E. of 8 birds. The body weight, abdominal fat weight and liver weights were 1817+63.2, 37.8+1.87 and 57.2+1.41 g respectively. Tissue samples were collected 24 min after the injection. The means of  $^3\text{H}$  and  $^{14}\text{C}$  incorporated into the same tissue were compared by t-test.

Table 2.4 Specific radioactivities incorporated into the fat tissues

	$^3\text{H}$ (dpm/g of lipid)	$^{14}\text{C}$	ratio ( $^3\text{H} : ^{14}\text{C}$ ) <sup>1</sup>
abdominal fat	2753+199 <sup>a</sup>	1249+72.7	2.02
neck fat <sup>2</sup>	2125+281	1017+133	2.08
thigh fat	2063+204	1032+94.1	1.99

<sup>1</sup>ratio of  $^3\text{H}$  and  $^{14}\text{C}$  injected was 1.98 (1,237,500/652,000 dpm).

<sup>2</sup>neck fat and thigh fat indicated the subcutaneous fat pads on neck and thigh.

<sup>a</sup>Mean+S.E. of 8 birds. Means within columns do not differ significantly ( $p > 0.05$ , F test).

The means of the specific radioactivities from abdominal, neck and thigh adipose tissues were 2313 and 1099 (dpm/g of lipid) for  $^3\text{H}$  and  $^{14}\text{C}$  respectively. According to carcass analysis on a similar group of birds, body total lipid was 160+2.6 g/kg of the body weight

(Table 2.1). Assuming that the birds receiving isotopes had a similar body composition and that most ~~of the~~ body fat was present in adipose tissue, the  $^3\text{H}$  and  $^{14}\text{C}$  incorporated in total lipid of the body were estimated to be about 53% and 51% of the labels administered respectively.

#### Metabolic utilization of $^{14}\text{C}$ -palmitic acid in broilers

The removal of  $^{14}\text{C}$  radioactivity from the plasma was very rapid. More than 98% of the radioactivity was removed in 5 min after  $^{14}\text{C}$ -palmitic acid injection and the plasma radioactivity attained a steady-state after 5 min (Fig. 2.4).

The specific radioactivities incorporated in abdominal, neck and thigh fat were not significantly different ( $P > 0.05$ , Table 2.5). About 40% of radioactivity injected was incorporated in the liver and only 0.3% into abdominal fat 10 min after the injection (Table 2.5). This indicated that plasma non-esterified fatty acids had little chance to be incorporated into adipose tissue directly and that most of the fatty acids incorporated from the plasma into adipose tissue is derived from plasma lipoprotein triglyceride.

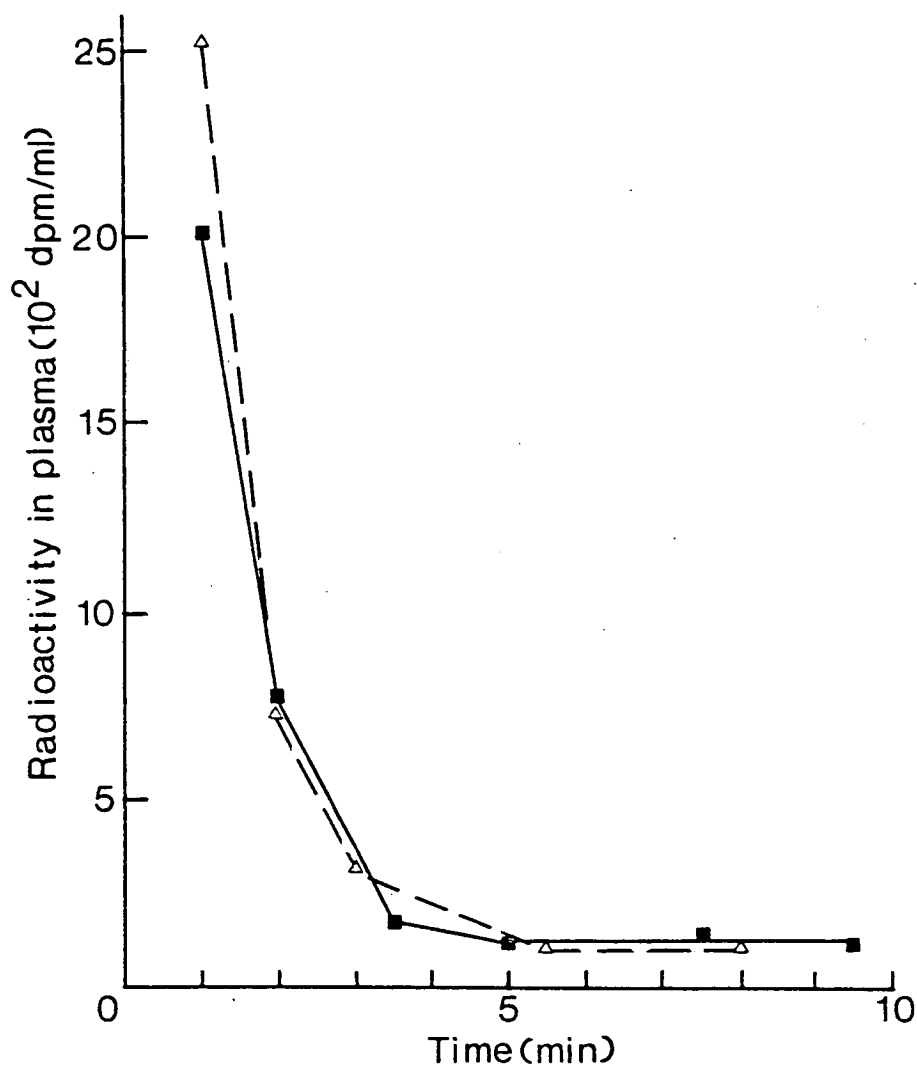


Fig. 2.4 Clearance of  $^{14}\text{C}$ -palmitic acid from plasma. Two birds with 1990 and 1980 g body weight respectively were intravenously injected with *the* same amount of  $^{14}\text{C}$ -palmitic acid (720,000 dpm). Blood samples were taken at the times indicated.

Table 2.5 Metabolic utilization of  $^{14}\text{C}$ -palmitic acid in broilers<sup>1</sup>

	specific activity	total activity in liver or fat pad (% of injected radioactivity)
liver	5299 $\pm$ 284 (dpm/g tissue)	39.2 $\pm$ 2.54
fat		
abdominal	107 $\pm$ 18.9 (dpm/g lipid)	0.299 $\pm$ 0.045
neck	78.8 $\pm$ 26.4 "	
thigh	143 $\pm$ 32.3 "	

<sup>1</sup>Mean $\pm$ S.E. of 4 birds. The total radioactivity injected was 720,000 dpm per bird. Body weight, abdominal fat weight and liver weight (g) were 1943 $\pm$ 3.20, 32.8 $\pm$ 1.29 and 53.5 $\pm$ 2.86 respectively. The definition of neck fat and thigh fat are given in table 2.4. There was no significant difference between the specific radioactivities incorporated in abdominal fat, neck fat and thigh fat tissues ( $P > 0.05$ , F test).

### Conclusions

As mentioned previously, male broilers between 43 and 50 days of age deposited approximately 5.5 g lipid/d/kg body weight of which about 0.45 g/d/kg was deposited in the abdominal fat pad. The rate of plasma triglyceride production was estimated to be 5.95 g/kg/d. Assuming that the calculation that 53% of portomicrons and 51% of VLDL triglyceride were incorporated into adipose tissue is correct, only about 3.0 g/kg/d of lipid deposited in adipose tissue in the whole animal is derived from diet and hepatic lipogenesis. In other words, 55% of total lipid deposited comes from diet lipid and hepatic lipogenesis and is transported as plasma lipoproteins. According to these estimates, the rest (45%) presumably comes from adipose tissue lipogenesis. These estimates therefore confirmed that plasma VLDL triglyceride is a major source of body fat deposition.

Similar calculations can be performed with respect to growth of the abdominal fat pad. Plasma triglyceride secretion rate was estimated to be 5.95 g/kg/d and about 6% of plasma chylomicrons and VLDL triglyceride was incorporated into abdominal fat pad. Therefore about 0.357 g of abdominal fat pad lipid came from plasma lipoproteins. The rate of lipid accumulated in the abdominal fat pad is estimated to be 0.45 g/kg/d. Plasma lipoprotein triglyceride therefore contributed about 80% of lipid deposited in abdominal fat pad: the rest (20%) might be derived from lipogenesis of abdominal fat tissue itself.

The calculation was conducted on the assumption that little lipid deposited in adipose tissue is mobilized and used as energy, since the birds were fed ad libitum with 23 h photoperiod and would be expected to be feeding continually, the utilization of adipose tissue lipid as energy might not occur.

Saadoun and Leclercq (1983) reported that 65-70% of de novo lipogenesis in slower growing broiler strains occurred in the liver. They did not specifically investigate the contribution of adipose tissue lipogenesis to fat deposition, although the present study indicates that the lipogenesis in adipose tissue (also found by Brady et al., 1976) may contribute a significant proportion of total fatty acids to adipose tissue growth. Adipose tissue lipogenesis may be particularly important in depots other than the abdominal fat pad, although this needs further investigation.

### Chapter 3

#### BIOCHEMICAL INDICATORS OF FATNESS IN MEAT-TYPE CHICKENS:

#### LACK OF CORRELATION BETWEEN LIPOPROTEIN LIPASE

#### ACTIVITY IN POST-HEPARIN PLASMA AND BODY FAT

#### SUMMARY

Possible relationships between fatness and lipoprotein lipase activity in adipose tissue and plasma from heparinized birds were examined in 7-week-old male and female broilers. Total lipoprotein lipase activity in abdominal fat was significantly correlated ( $r=0.5$ ) with fat pad weight, but there was no correlation between specific activity of the enzyme and fat pad weight. Lipoprotein lipase activity in post-heparin plasma showed no correlation with either abdominal fat or total body fat content. The results indicate that measurements of lipoprotein lipase activity in biopsy samples or in post-heparin plasma are of no value in predicting fat content of live birds.

## INTRODUCTION

Avian adipocytes have a limited capacity for lipogenesis and much of the fat that is deposited in avian adipose tissue is synthesised in the liver or derived from the diet. Results from the previous Chapter showed that about 55% of body fat deposited in 7 weeks old broilers came from plasma lipoprotein triglyceride. Most of the triglyceride in broiler plasma is present in very low density lipoproteins (VLDL) of hepatic origin and it has been shown that the concentration of VLDL in the plasma of fully fed broiler chickens is sufficiently well correlated ( $r$  up to 0.7) with body fat content to be used as an indirect measure of fatness (Griffin et al., 1982; Whitehead and Griffin, 1982). Divergent selection for plasma VLDL concentration in a commercial broiler grandparent strain has produced lines with markedly different body composition and the leaner, low VLDL line has a considerably improved efficiency of feed and protein conversion (Whitehead 1988).

Uptake of triglyceride from plasma lipoproteins into extra-hepatic tissues is mediated by lipoprotein lipase (LPL). Part of the activity of this enzyme is present at the capillary bed and catalyses the hydrolysis of both circulating portomicrons and VLDL. The fatty acids released then enter the surrounding cells to be re-esterified or oxidised. Lipoprotein lipase is anchored to the endothelial cell wall by attachment to heparin-like compounds and the enzyme can be released from this site into the general circulation by intravenous injection of heparin (Cryer, 1981;

Pedersen et al., 1983).

LPL activity is elevated in obesity. Gruen et al. (1978), for example, reported that the genetically obese rats (fa/fa) had more LPL per epididymal fat pad and per fat cell than lean rats. McNamara and Martin (1982) also reported that the LPL activity (units/mg of protein and units/g of tissue) in dorsal subcutaneous adipose tissue of 110 days fetal pigs was three-fold greater in the high backfat line compared to the low line. Taskinen (1987) found that post-heparin plasma LPL activity in humans was positively correlated with the enzyme activity in both adipose tissue ( $r=0.6$ ,  $P<0.01$ ) and skeletal muscle ( $r=0.64$ ,  $P<0.01$ ). Since taking blood samples is much easier and <sup>more</sup> convenient than taking tissue samples, if there were significant correlation between post-heparin plasma LPL activity and chicken body fat content, post-heparin plasma LPL activity would become a useful indicator of chicken body fat content in breeding practice. Measurements of LPL activity in post-heparin plasma and tissues from broilers might be combined with plasma VLDL concentration to increase the accuracy at prediction of fatness for selecting lean birds.

In the present study, the extent of variation of adipose tissue lipoprotein lipase activity within a commercial broiler population and its relationship with fatness were investigated. The possibility that such variation might be determined in vivo by measurement of lipoprotein lipase activity of biopsy samples or in post-heparin plasma was also examined.

## MATERIALS AND METHODS

Birds

Day old chicks of a commercial broiler strain were obtained from D.B. Marshall Ltd, Newbridge, Scotland. They were reared to 6-7 weeks of age in pens on a 23 h light: 1 h dark photoperiod with lights off between midnight and 0100 h. Standard broiler growing diets appropriate for age were fed ad libitum.

Blood samples

Blood was removed from the wing-vein and EDTA (2 mg/ml of blood) used as anti-coagulant. Plasma was prepared by centrifugation at 1000 g for 10 min at 4°C and stored at -70°C.

Measurement of post-heparin lipase activity

Heparin (1000 U/kg of body weight) was injected into the wing vein in 0.9% NaCl (1000 U/ml) and blood sampled 2 min later. Lipase activity in post-heparin plasma was assayed using Intralipid (Kabi Vitrum, Stockholm, Sweden) as substrate. Intralipid (20%) was activated by incubating for 1 h at 37°C with immature broiler serum (3 ml of serum/ ml of Intralipid). The post-heparin plasma was thawed at room temperature and diluted 8-fold with distilled water. Duplicate 20, 40 and 60  $\mu$ l aliquots of diluted plasma were incubated with 10  $\mu$ moles activated Intralipid triacylglycerol in 250  $\mu$ l of 50 mM Tris/HCl, pH 8.0 containing 2% (w/v) defatted bovine serum

albumin and a final concentration of 10  $\mu\text{g}$  heparin/ml. Reactions were stopped after 1 h at 37°C by addition of 2.4 ml of 0.1 M glycine buffer, pH 2.7. The free fatty acids released were extracted by 3.2 ml of di-n-butyl-ether. Free fatty acids were measured as described by Bowyer et al. (1978) using a Technicon Autoanalyser. Palmitic acid was used as standard.

The contribution of lipoprotein lipase to total lipase activity was determined by immunoprecipitation with an anti-lipoprotein lipase antiserum. This antiserum had been raised in sheep by repeated injection of lipoprotein lipase purified from broiler adipose tissue by heparin-affinity and concanavalin-A affinity chromatography (S.C. Butterwith and H.D. Griffin, unpublished). Aliquots (100  $\mu\text{l}$ ) of post-heparin plasma or extracts of acetone-ether powders were incubated for 30 min at 4°C with an equal volume of sheep antiserum diluted 20-fold with 0.9% NaCl. Precipitation of the enzyme-antibody complex was encouraged by addition of 10  $\mu\text{l}$  of a donkey anti-sheep IgG antiserum (Scottish Antibody Production Unit, Carlisle, Scotland) and incubation for a further 30 min at 4°C. Precipitated enzyme was removed by centrifugation for 20 min at 3000 g and 4°C and lipase activity was determined in the supernatant.

#### Measurement of tissue LPL activities

Birds were killed by rapid intravenous injection of phenobarbitone (Expiral, Abbott Laboratories, Queenborough, Kent). Tissues were rapidly removed, washed in ice-cold saline, frozen in

liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . The whole of the abdominal fat pad and samples of heart, muscle and liver were homogenized in water containing  $20\ \mu\text{g}$  heparin/ml at  $0^{\circ}\text{C}$  (normally 10 ml/g of tissue) using a Polytron homogenizer (Kinematica, Lucerne, Switzerland). After 30 min at  $0^{\circ}\text{C}$  the infranatant was decanted from beneath the floating fat. Aliquots of 2 ml were mixed with 10 ml ice-cold acetone and 10 mg bovine serum albumin (Cohn Fraction V, Sigma Chemical Co.) added as carrier. After 30 min at  $0^{\circ}\text{C}$ , precipitated protein was recovered by centrifuging at 1000 g for 10 min and the protein pellet was washed once with 10 ml acetone and once with 10 ml diethyl ether, both at  $0^{\circ}\text{C}$ . Residual ether was removed by evaporation under  $\text{N}_2$  at room temperature and acetone-ether powders stored at  $-70^{\circ}\text{C}$ . Samples of homogenates were retained for determination of tissue protein and DNA content.

Acetone-ether powders prepared from 2 ml of tissue homogenates were resuspended in 4 ml or 8 ml of 20 mM Tris/HCl, pH 8.0 containing  $20\ \mu\text{g}$  heparin/ml. Extracts of the powders were used to determine the LPL activities using the same procedure as described for post-heparin plasma.

#### Other assays

Protein was determined according to Lowry et al. (1951) using bovine serum albumin as standard. DNA was assayed using a fluorometric microassay method (Legros and Kepes, 1985). Plasma

VLDL concentration was measured turbidimetrically (Griffin and Whitehead, 1982) and expressed as absorbance units (AU). Carcass analysis has been described in Chapter 2.

## RESULTS

### Development of methodology

1) Time course and dose-response of release of tissue LPL into plasma after intravenous injection of heparin.

The first experiment was designed to investigate the time course of tissue LPL release into the general circulation after intravenous injection of heparin. LPL activity was released into the blood stream very rapidly after the injection of heparin (Fig. 3.1) and reached a maximum within only 1 min. There was little further change over the next 4 min.

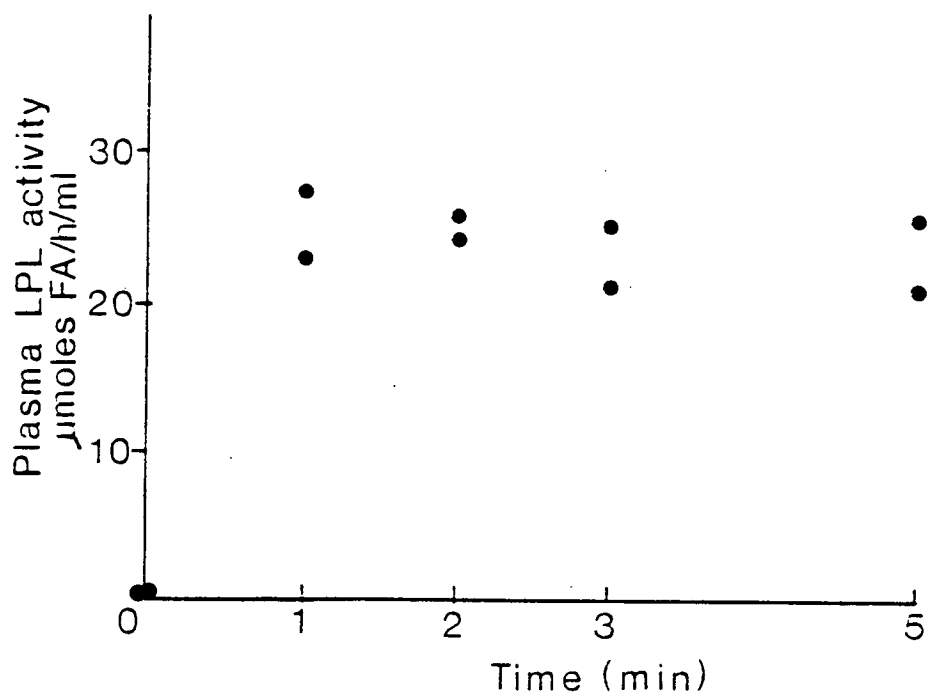
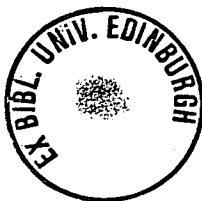


Fig. 3.1. Time course of LPL release. Two birds were bled before heparin injection and 1, 2, 3 and 5 min after the injection of 1000 U of heparin/kg body weight. Each point represents an individual bird.



The specific activity of LPL released into the circulation appeared to increase with increasing amount of heparin injected but there was little change when the concentration of heparin was greater than 700 units per kg body weight (Fig. 3.2). A dosage of 1000 U/kg body weight was chosen for further studies.

## 2) Recovery of LPL from fat tissue

Tissue LPL activity was extracted from acetone-ether powders which were prepared from tissue homogenates. Each abdominal fat pad could have been directly homogenized in acetone, and further delipidated with ether. However, this would have needed a large volume of organic solvents. In the present study, the adipose tissue was homogenized and disrupted in water first. The floating lipid was removed and only aliquots (usually 2 ml) of the infranatant were used to prepare acetone-ether powders.

This procedure assumed effective release of LPL into the aqueous homogenate. To test this, one frozen abdominal fat pad from a bird at 9 weeks of age was crushed and duplicate samples of 2 g each were homogenized in 10, 20, 30, 40 ml ice-cold water containing 20  $\mu$ g heparin/ml. After 30 min, the samples were centrifuged at 2000g for 30 min at 4°C. Total floating lipid and 2 ml of infranatant from each sample were used to prepare acetone-ether powders. Assay of LPL activity showed (Fig. 3.3) that recovery of

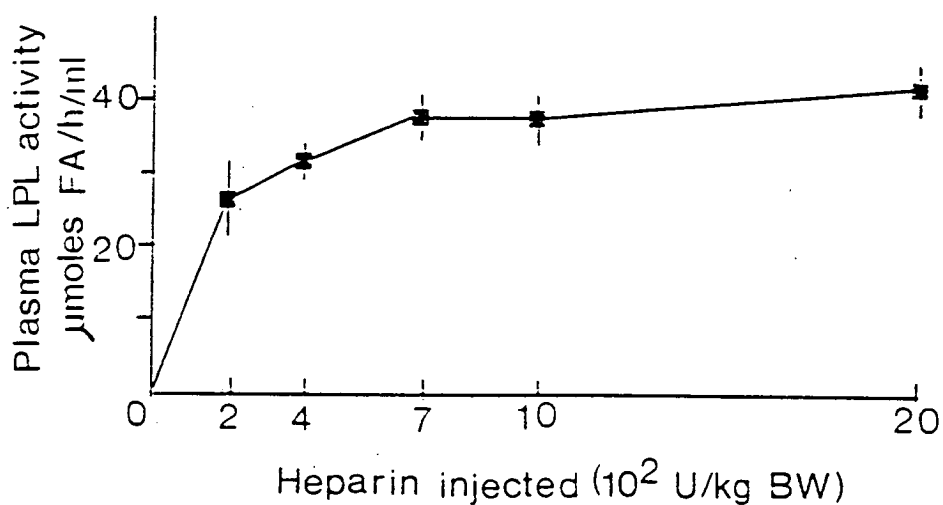


Fig. 3.2 Dose-response curve for the release of lipase activity after intravenous injection of heparin. 34 female birds at 7 weeks of age were divided into 5 groups (4 groups of 7 and one group of 6) and injected with 200, 400, 700, 1000 and 2000 units of heparin per kg body weight respectively. Blood samples were taken 2 min later. Data are expressed as mean±S.E.

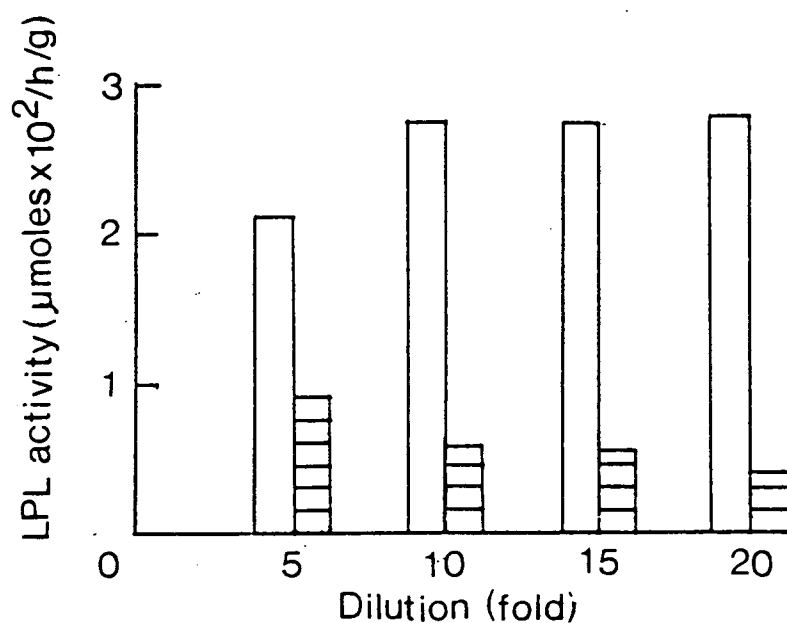


Fig. 3.3 Effect of homogenate volume on the LPL activity extracted. Duplicate 2 g samples of abdominal fat tissue were homogenized in 5, 10, 15, 20 mls of water/g. Open bars represent LPL activity in the infranatant after centrifugation, shaded bars represent LPL activity in floating lipid.

LPL from the aqueous phase was over 80%, when the the sample dilution was at least 10-fold. Assuming that the floating lipid layer contained 50% of trapped aqueous phase, more than 90% of LPL activity in adipose tissue was extracted by this procedure. A 10-fold dilution was chosen for the homogenization of adipose tissue in subsequent experiments.

### 3) Reproducibility of the measurements of LPL activity

The reproducibility of the assay of LPL activity was examined using LPL in post-heparin plasma and a sample of adipose tissue. Fifteen aliquots from one post-heparin plasma sample were assayed for LPL activity at the same time. The coefficient of variation was only 4.1% (Table 3.1). In order to estimate the reproducibility of the measurements of adipose tissue LPL activity, one abdominal fat pad was crushed while frozen and divided into 12 aliquots of 2 g each. Sample homogenization, acetone-ether powder preparation, LPL extraction and lipase activity assay were performed as described above. The coefficient of variation of LPL in the 12 samples from the same fat pad was 6.0% (Table 3.1). These results indicate that the systematic error from the assay of plasma and tissue lipase activity could be controlled and the results of the assay of LPL activity would be reliable.

Table 3.1 Reproducibility of assay of LPL activity

	N <sup>1</sup>	mean	S.E.	C.V.
post-heparin plasma	15	96.49 <sup>a</sup>	1.0	4.1%
abdominal fat pad	12	189.7 <sup>b</sup>	3.3	6.0%

<sup>1</sup> number of replicates.

<sup>a</sup>  $\mu$ moles/h/ml.

<sup>b</sup>  $\mu$ moles/h/g of tissue.

#### 4) Contribution of LPL to total post-heparin lipase activity and total tissue lipase activity

Intravenous injection of heparin into the circulation of mammals releases two lipase activities: LPL from extrahepatic tissues and a triglyceride lipase from the liver. The latter may have a role in the further catabolism of the triglyceride-depleted lipoproteins produced by the action of LPL in the peripheral circulation (Smith et al., 1978) and in the clearance of phospholipid and cholesterol associated with the lipoproteins (Cheng et al., 1985).

Two methods were used here to determine the contribution of LPL to total post-heparin lipase activity in broiler plasma. The first is based on the observations that LPL is almost completely inhibited by high NaCl concentrations whereas hepatic lipase is virtually unaffected (Brockerhoff and Jenson, 1974). The second relies on a difference in their immunological characteristics (Smith et al., 1978) and used a specific antiserum to selectively precipitate LPL.

When the lipase activity in post-heparin plasma sample was assayed in the presence of 0.9 M NaCl, 95.7% of the lipase activity was inhibited. Immunoprecipitation removed 98.6% of the lipase activity. Therefore, greater than 95% lipase activity released into the circulation in post-heparin plasma is LPL activity.

After immunoprecipitation with anti-LPL antiserum, tissue lipase activities remaining were less than 5% of total activity in each case (Table 3.2). This indicates that more than 95% of tissue lipase activity is LPL activity.

Table 3.2 Contribution of LPL to total tissue lipase activities

Tissue	n	lipase activities	
		control ( $\mu$ moles/h/g)	after immunoprecipitation (% of control)
heart muscle	4	64.5 $\pm$ 7.2 <sup>a</sup>	2.2%
wing muscle	4	0.975 $\pm$ 0.063	b
leg muscle	4	7.20 $\pm$ 1.5	b
breast muscle	4	0.625 $\pm$ 0.24	b
gizzard muscle	4	26.5 $\pm$ 4.2	3.5%
liver	4	1.18 $\pm$ 0.55	b
lungs	4	1.98 $\pm$ 0.15	b
abdominal fat	4	82.0 $\pm$ 19	3.8%

<sup>a</sup> mean $\pm$ S.E.

<sup>b</sup> the activity was below the limit of detection.

Experiment 1. Abdominal fat tissue LPL activity and fatness

The variation in specific and total activities of lipoprotein lipase in the abdominal fat of female broilers at 7 weeks of age was high (Table 3.3). Total lipase activity in abdominal fat was significantly correlated with fat pad weight (Fig. 3.1), but correlations between abdominal fat and specific activity of LPL were low and not significant (Table 3.3).

Table 3.3 Correlations between adipose tissue LPL activity, plasma VLDL concentration and abdominal fat pad weight in female broilers at about 7 weeks of age

	Mean (n=36)	C.V. (%)	Correlations with	
			Abdominal fat (g)	Mean VLDL concentration
Body weight (g)	2076	10		
Abdominal fat (g)	43	27		
Lipoprotein lipase:				
$\mu$ moles/h/fat pad	3308	42	0.52**	-0.08
$\mu$ moles/h/g of tissue	78	34	-0.18	-0.20
$\mu$ moles/h/mg of protein	9.7	26	-0.01	-0.17
$\mu$ moles/h/ $\mu$ g of DNA	0.39	39	-0.13	-0.10
Protein (mg/g of tissue)	8.3	16	-0.48**	-0.12
DNA ( $\mu$ g/g of tissue)	209	17	-0.18	-0.15

Blood samples were taken for measurement of plasma VLDL concentration at 47 and 49 days of age. Birds were killed at 51 days of age. \*\*  $p < 0.01$

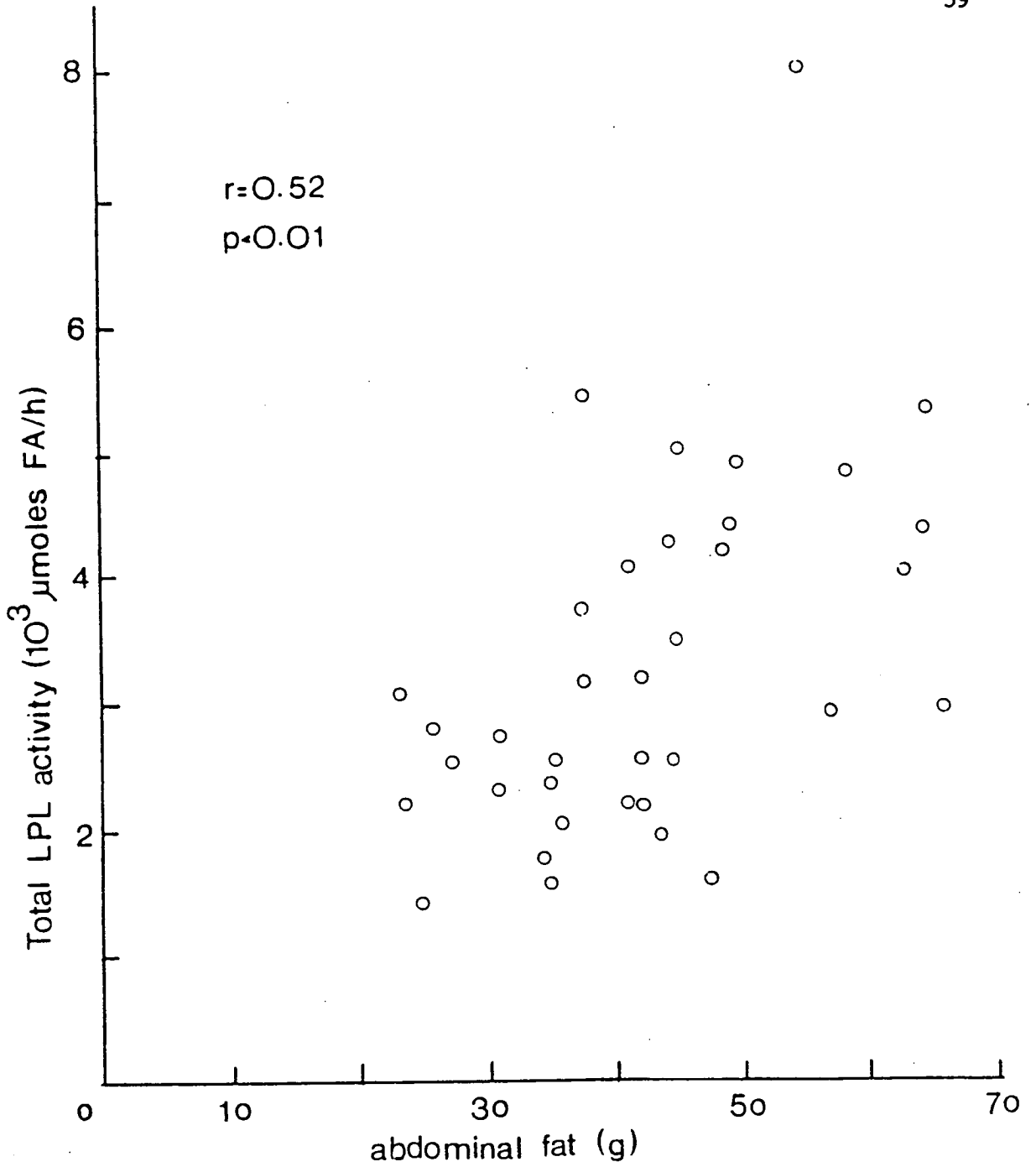


Fig. 3.4 Relationship between total fat pad LPL activity and abdominal fat weight in female broilers at 51 days of age.

Experiment 2. Post-heparin lipase activity, plasma VLDL concentration and fatness

Post-heparin lipase activities, plasma VLDL concentrations and fatness were similar in male and female broilers at about 7 weeks of age (Table 3.4). Individual variation in post-heparin lipase activity was also high, but showed no correlation with abdominal fat pad weight or total body fat content or with plasma VLDL concentration in both sexes.

Plasma VLDL concentration was well correlated with body fat content and abdominal fat pad weight, confirming previous observations (Griffin and Whitehead, 1982). However, correlations between plasma VLDL concentration and body weight were also significant (0.55 for females and 0.53 for males,  $P < 0.01$ , Table 3.4).

The correlation between abdominal fat pad weight and total body fat content was high and significant (0.83 in males and 0.80 in females,  $p < 0.01$ ). This result is consistent with many previous reports that the weight of abdominal fat pad is a good indicator of total body fat content. For example, Becker et al. (1979) reported that the correlation between abdominal fat pad weight and body fat content was 0.82.

Table 3.4. Correlations between body weight, body fatness, plasma VLDL concentration and LPL activity in post-heparin plasma of broilers at about 7 weeks of age

	Mean	CV (%)	correlations with			
			Body wt(g)	Abdominal fat(g)	Body fat (g/kg)	Plasma VLDL(A)
<b>Females (n=39)</b>						
Post-heparin LPL ( $\mu$ moles/ml/h)	65.7	26	-0.03	0.04	0.00	-0.03
Body weight (g)	1944	11	-	0.50**	0.32*	0.55**
Abdominal fat (g)	39.6	31	-	-	0.80**	0.69**
Body fat (g/kg)	143	14	-	-	-	0.58**
Plasma VLDL (A)	0.195	28	-	-	-	-
<b>Males (n=38)</b>						
Post-heparin LPL ( $\mu$ moles/ml/h)	74.4	24	-0.18	-0.04	-0.10	0.10
Body weight (g)	2119	10	-	0.59**	0.48**	0.53**
Abdominal fat (g)	42.9	34	-	-	0.83**	0.70**
Body fat (g/kg)	131	14	-	-	-	0.68**
Plasma VLDL (A)	0.193	23	-	-	-	-

Values for plasma VLDL concentration are the means from blood samples taken at 46 and 48 days of age. Post-heparin plasma LPL activity was measured at 50 days of age and birds were killed immediately afterwards. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

Experiment 3. Effect of intravenous injection of heparin on tissue LPL activity

An estimate of the relative contribution of different tissues to LPL activity in post-heparin plasma was attempted by comparing the specific activity of LPL in tissues of birds injected with heparin with those of control birds (Table 2.5). Of the tissues examined, adipose tissue contained the highest LPL activity/g of tissue. Leg and gizzard muscle also contained substantial quantities of enzyme, but activity in breast muscle was very low. Studies in mammals have shown that red muscle fibres contain much more LPL than white muscle (Linder et al., 1976) and this difference in fibre type may explain the variation in activity between different muscles found in the present study.

Since broiler plasma contributes 4.7% of the body weight (H.D. Griffin, unpublished), injection of heparin released about 4200 U ( $\mu$ moles of fatty acid released/h) of LPL into the circulation of the birds used in the experiment described in Table 3.5. Comparison with the specific activity of LPL in adipose tissue and muscle suggests that this represents a substantial proportion of the total activity in the bird. Nevertheless, heparin injection caused only a small decrease in LPL activity in adipose tissue, had little or no effect on activity in leg and gizzard muscle and increased activity in heart.

Table 3.5. Effect of intravenous injection of heparin on tissue lipoprotein lipase activities

	Control	Heparin-treated
Body weight (g)	1391 $\pm$ 51	1401 $\pm$ 36
Lipoprotein Lipase		
Plasma ( $\mu$ moles/h/ml)	0	64.1 $\pm$ 4.4
Liver ( $\mu$ moles/h/g)	0	25.6 $\pm$ 3.5
Heart ..	77.5 $\pm$ 4.2	97.5 $\pm$ 6.8*
Gizzard ..	17.3 $\pm$ 2.1	18.8 $\pm$ 1.5
Leg ..	16.7 $\pm$ 3.7	11.8 $\pm$ 3.1
Breast ..	0.983 $\pm$ 0.28	1.10 $\pm$ 0.19
Abdominal fat		
( $\mu$ moles/h/g)	192 $\pm$ 20	157 $\pm$ 17*
( $\mu$ moles/h/mg of protein)	22.9 $\pm$ 1.6	18.6 $\pm$ 1.9*

Treated birds were injected with 1000 U heparin/kg of body weight via the wing vein. Control birds received the same volume of saline. Blood was sampled 2 min later and birds rapidly killed by intravenous injection of phenobarbitone. Values are means  $\pm$  S.E. of the results from 9 birds at 6 weeks of age/group. Those for treated birds that are significantly different (Student's test) from those for controls are indicated by: \*,  $p < 0.05$ , \*\*,  $p < 0.01$ .

## DISCUSSION

Release of LPL after heparin injection was very rapid, the LPL activity in the plasma reached a maximum within only 1 min and there was little further change over the next 4 min after heparin intravenous injection. A very similar result was obtained in 6-12 month old White Leghorn male chickens by Benson et al. (1975), in which the post-heparin lipase activities were little changed from 1 to 10 min after injection of heparin. This rapid LPL release after injection of heparin may be a result of the very rapid circulation of blood in birds. Benson et al. (1975) noted that increasing the intravenous dose of heparin resulted in release of more lipase into plasma, but the amount of heparin for maximum release was not determined.

Very good reproducibility of the assay of LPL from post-heparin plasma and tissue indicates that the technique for assay of LPL is reliable. The coefficient of variation in the assays of post-heparin lipase was only 4.1% and that in the assays of adipose tissue lipase was 6.0% (Table 3.3). The error caused by the assay itself can not account for the variation between the birds, because the coefficient of variation for post-heparin lipase between individuals was 25% (Table 3.4) and for lipase in abdominal fat pad was 34% (Table 3.3).

Since total LPL activity equals to specific activity  $\times$  weight of fat pad, it must correlate with the weight of fat pad. Therefore, the correlation (Fig. 3.4) between total abdominal fat LPL activity and the weight of fat pad does not suggest that total

LPL is an important factor in determining the rate of fat deposition in broilers, <sup>although</sup> similar observations of the relationships between total fat pad LPL activity and the weight of pad weight have been reported for mouse perirenal adipose tissue (De Grasquet and Pequignot, 1972), rat inguinal fat pad (Boulangue et al., 1979) and rat epididymal fat pad (Gruen et al., 1978). Negative correlations between abdominal fat weight and tissue protein content and DNA content (Table 3.3) suggest that some of the variation in abdominal fat weight is due to the variation of cell size. The variation of total LPL in the adipose tissue of birds may be due to the difference in both adipocyte number and LPL/cell. For example, Griffin et al. (1987) reported a 24-fold difference of fat pad LPL activity between the broilers and layers which they attributed to differences in both cell number and LPL/cell. The low and non-significant correlations between specific LPL activities with fat pad weight (Table 3.3) indicate that the measurement of tissue LPL activity on biopsy samples would not be a suitable method of predicting the fatness in live birds. However, Grunder and Chambers (1988) reported that the specific lipase activity (activity/g of tissue) was significantly correlated with abdominal fat weight ( $r=-0.31$ ,  $P<0.01$ ). This different result may be due to the different broiler strains investigated.

The absence of correlation between plasma VLDL concentration and post-heparin lipase activity is surprising, since the total functional lipoprotein lipase activity in the bird would be expected to be a major determinant of the rate of VLDL removal from the circulation. In humans, the concentration of VLDL triglycerides

correlates inversely with post-heparin plasma LPL activity ( $r=-0.56$ ,  $P<0.01$ ; Taskinen, 1987). Studies in rats have shown that intravenous injection of heparin releases only a small proportion of total tissue lipoprotein lipase into the circulation, most of which is present within the adipocytes or muscle cells (Cryer 1981). A similar small proportion of total LPL activity appears to be heparin-releasable in avian tissues (Table 3.5), but the variation in activity within treated and control groups prevented any assessment of the contribution of different tissues to post-heparin plasma LPL activity. LPL that is released into the circulation is believed to be taken up and degraded by the liver (Peterson et al., 1985; Chajek-Shaul et al., 1985) and this process presumably accounts for the appearance of LPL activity in the liver of heparin-treated birds. The activity in the liver 2 min after heparin injection was more than 30% of total activity present in the plasma (estimated from Table 3.5), but much of the enzyme released from tissues after longer time periods may be taken up from the plasma and degraded.

The lack of correlation between body fat content and LPL activity in post-heparin plasma may mean that the amount of LPL at the capillary bed is not an important influence on the rate of fatty acid uptake by adipose tissue. It seems more probable, although difficult to prove, that the contribution of other tissues to post-heparin plasma lipase activity is substantial and that this obscures any relationship between fatness and post-heparin lipase activity.

## Chapter 4

### ARE THERE TISSUE-SPECIFIC LIPOPROTEIN LIPASES?

#### SUMMARY

Lipoprotein lipase (LPL) was purified from post-heparin plasma, abdominal fat, heart and leg muscle of broilers by affinity chromatography on heparin-Sepharose. The presence of different forms of LPL was examined by SDS polyacrylamide gel electrophoresis and isoelectric focusing. Separated proteins were transferred to nitrocellulose paper by electroblotting and LPL was identified using an anti-LPL antiserum. Most of the enzyme purified from post-heparin plasma and tissues had a molecular weight of 54 kD, though a minor component with a molecular weight of 29.5 kD was also present. Isoelectric focusing in the presence of 6 M urea separated more than 10 species of LPL, with isoelectric points ranging from pH 3.5 to 7.0. No difference was found in the isoelectric focusing patterns between the samples from different tissues. These results indicate that the various tissue lipoprotein lipases released into post-heparin plasma cannot be distinguished by molecular weight or isoelectric point.

## INTRODUCTION

Functionally active LPL is bound to the luminal surface of the capillary wall via attachment to heparin-like compounds and can be displaced from this site into the general circulation by intravenous injection of heparin. Post-heparin plasma contains LPL displaced from all tissues. The lack of any correlation between lipoprotein lipase activity in post heparin plasma and body fat content (see previous chapter) suggests that the contribution of other tissues to post-heparin plasma lipase activity may obscure the relationship between fatness and the activity released from adipose tissue.

Most of plasma VLDL triglyceride hydrolyzed in capillaries in adipose tissue will be re-esterified for storage. That hydrolyzed in muscle is largely used for oxidation (Cryer, 1981). Although LPL activity per gram of tissue in muscle is lower than that in adipose tissue, muscle forms a large portion of the body mass and the contribution of the LPL in muscle to the removal of triglyceride-rich lipoproteins from circulation can be equal to or greater than that of adipose tissue (Borensztajn, 1987). If adipose tissue and muscle LPLs could be distinguished in post-heparin plasma, selection for low adipose tissue LPL activity and high muscle LPL activity might create lean animals which use less plasma VLDL triglyceride for storage.

There is some evidence for the existence of tissue specific LPLs. Fielding (1978) reported that functional LPL from rat heart

had a molecular weight of 34-37 kD and that the enzyme from adipose tissue had a molecular weight of 69-71 kD. These two LPL species could be recovered from plasma after injection of heparin and appeared to represent the metabolically active species released from muscle and adipose tissues. Fielding (1978) also reported a significant difference in the Michaelis constant and substrate affinity of rat adipose tissue and heart tissue LPL. Ben-Zeev et al. (1983) reported that the expression of heart and adipose LPL activity in mice was under independent genetic control. A single major gene seems to control the adipose tissue lipoprotein lipase activity, whereas the inheritance segregation of heart activity could imply involvement of more than one gene.

On the other hand, Bensadoun and Kompiang (1979) reported that LPL from chicken heart, adipose tissue, liver, and granulosa cells activities were all completely inhibited in vitro by an antiserum raised against the LPL purified from chicken adipose tissue. This indicates that LPL from different tissues share a common immunological determinant. Several authors suggested that the minor components with lower molecular weight present in many LPL preparations are degradation products of LPL (Socorro and Jackson, 1985; Friedman et al., 1986; Vydelingum et al., 1986). It is possible that the lower molecular LPL form in post-heparin plasma reported by Fielding et al. (1974) represents a specific cleavage product of the high molecular form of the enzyme.

In the present study, the possible presence of multiple forms or tissue-specific LPL in chicken tissues and post-heparin plasma

was examined by comparing their molecular weights and isoelectric points. LPL was identified by using a monospecific antiserum. The development of the techniques used are described as well as the results obtained.

## MATERIALS AND METHODS

### Birds

Day old chicks of a commercial broiler strain were obtained from D.B.Marshall Ltd, Newbridge, Scotland. They were reared to 6-7 weeks of age in cages on a 14 h light:10 h dark photoperiod. Standard broiler growing diets appropriate for age were fed ad libitum.

### Purification of post-heparin plasma LPL and tissue LPL

In order to investigate the characteristics of LPL in post-heparin plasma and different tissues, it proved essential to separate the enzyme from other plasma and tissue proteins. All procedures for the purification of post-heparin plasma and tissue LPLs are based on the affinity of the enzyme for heparin (Egelrud and Olivecrona, 1972). Heparin-Sepharose 4B was used for affinity chromatography of LPL and separate columns were used each time to prevent the cross-contamination of samples from different sources.

### 1) Purification of post-heparin plasma LPL

Blood samples were collected 2 minutes after intravenous injection of heparin (1000 U/kg body weight) and immediately added to sufficient phenylmethyl sulphonyl fluoride (PMSF) to give a final concentration of 100  $\mu\text{g/ml}$  of blood. Plasma was separated by centrifugation at 1000 g for 10 minutes at 4°C. Each 100 ml of plasma was diluted with 67 ml of 0.65 M NaCl, 30% glycerol 5 mM phosphate and pH 7.0 buffer. 45 ml of diluted plasma sample was loaded at a rate of 12 ml/h onto a heparin-Sepharose 4B (Sigma) column (8 cm length x 1.1 cm diameter) which had been previously equilibrated with 0.3 M NaCl in 5 mM phosphate, 30% glycerol, pH 7.0. The column was washed with 50 ml of 0.3 M NaCl buffer and then eluted with a linear gradient from 0.3 and 2 M NaCl in 30% glycerol, 5mM phosphate, pH 7.0. Total volume of the gradient was 40 ml (i.e. 5 column volumes). Fractions of 2 ml were collected and those containing the peak of enzyme activity were combined and dialysed against 20mM Tris/HCl, 30% glycerol, pH 7.0 overnight at 4°C and then stored at -70°C. LPL activity was assayed as described in Chapter 3.

### 2) Purification of tissue LPL

Tissue LPL was extracted from acetone-ether powders. Full details of the methods used to prepare acetone powders have been described in Chapter 3. Powders were homogenized in 1.2 M NaCl, 10 mM phosphate, 30% glycerol, pH 7.0 (50 mg/ml of buffer), in the presence of 50  $\mu\text{g}$  PMSF/ml. After 30 minutes at 0°C, the samples

were centrifuged at 1000 g at 4°C for 20 min. The supernatants (about 40 ml, without floating lipid) were dialysed against phosphate buffer to bring the the final salt concentration to 0.3-0.4M and loaded onto the heparin-Sepharose column without further dilution. The procedures of heparin-affinity chromatography for these extracts were usually the same as those for post-heparin plasma samples, as described previously.

In some experiments, the dialysed extracts or diluted plasma were directly mixed with 8 ml of packed heparin-Sepharose and incubated at 4°C with gentle stirring for 2.5 h. The heparin-Sepharose together with bound enzyme was recovered by centrifugating at 4°C in 1000 g for 10 minutes and the supernatant decanted. The gel was washed with 0.3 M NaCl buffer containing 20 mM phosphate, 30% glycerol, pH 7.0 and packed into a column for gradient or stepwise elution.

Multiple aliquots of purified enzyme were stored at -70°C. individual aliquot was taken for use each time so that enzyme degradation caused by re-freezing and thawing was avoided.

### SDS gel electrophoresis

Initially tissue LPLs were compared by SDS gel electrophoresis. In this technique, proteins are reacted with the anionic detergent, sodium dodecyl sulphate (SDS). The SDS binds to proteins and the overwhelming negative charge provided by the SDS coating makes any

charge contributed by the protein negligible. Separation of such protein-SDS complexes by polyacrylamide gel electrophoresis is therefore almost entirely dependent on the molecular size of the proteins.

Electrophoresis was performed in a vertical slab system (Biorad Protean II system) using the buffer system described by Laemmli (1970). Acrylamide/bis was prepared from a stock solution of 29.2 per cent by weight of acrylamide and 0.8 per cent by weight of N,N'-bis-methylene acrylamide. Fifteen ml of stacking gel solution contained 5% (w/v) acrylamide/bis and 30 ml of separating gel solution contained 10% (w/v) of acrylamide/bis were prepared for a gel with dimensions of 142x160x1.5 mm. The final buffer concentrations were as follows: 0.125 M Tris/HCl (pH 6.5), 0.1 per cent SDS in the stacking gel and 0.375 M Tris/HCl (pH 8.6), 0.1 per cent SDS in the separating gel.

The gels were polymerized with tetramethyl-ethylenediamine (TEMED) and ammonium persulphate. The separating gel solution was mixed with 10  $\mu$ l of TEMED and 150  $\mu$ l of freshly prepared 10% (w/v) persulphate and poured immediately into the gel mould to a level about 5 cm below the top of mould. Propan-2-ol was carefully overlaid on the top of the gel solution to eliminate oxygen and generate a flat top to the gel. After polymerization, propan-2-ol was poured from the gel and the gel top was washed with water. The stacking gel solution (15 ml) was mixed with 10  $\mu$ l of TEMED and 150  $\mu$ l of persulphate. The top of separating gel was rinsed with about 2 ml of stacking gel mixture and the rest of the mixture was then

poured on the top of the separating gel at once. Wells were formed in stacking gel by inserting a comb into the gel solution before it polymerized.

The electrode buffer contained 0.025 M Tris and 0.19 M glycine and 0.1% SDS (w/v). The solution was about pH 8.3 without adjustment. The sample buffer consisted of 0.17 M Tris/HCl, pH 8.6, 3.3% SDS, 33% glycerol, 4% 2-mercaptoethanol and 0.0001% bromophenol blue as marker dye. Samples and molecular weight markers were incubated at 100°C for 5 minutes with an equal volume of sample buffer and then transferred to the wells. A prestained marker mixture was obtained from Sigma (SDS-7B) containing alpha-2-macroglobulin (human plasma, MW 180,000), beta-galactosidase (*E. coli*, MW 116,000), fructose-6-phosphate kinase (rabbit muscle, MW 84,000), pyruvate kinase (chicken muscle, MW 58,000), fumarase (porcine heart, MW 48,500), lactic dehydrogenase (rabbit muscle, MW 36,500) and triosephosphate isomerase (rabbit, MW 26,600). Electrophoresis was carried out with a current of 30 mA until the marker dye had entered the separating gel and 60 mA thereafter, and it was stopped when the marker dye reached the bottom of the gel.

### Isoelectric focusing

Isoelectric focusing is an electrophoretic method for the separation of protein molecules according to their isoelectric point (pI): this is the pH at which they possess no net electric charge. A pH gradient is formed by the migration of Ampholines in a gel

between cathode and anode during electrophoresis and proteins migrate until they reach their pI on this pH gradient. As the protein is not charged in this position, it remains stationary and becomes 'focused'. Isoelectric focusing is a sensitive and reproducible technique, particularly valuable in the separation of closely related proteins that may not be easily separated by other techniques (Divall, 1984).

In later experiments, gels were run in the presence of 6 M urea to insure samples remained soluble and inhibit complex formation. Ultrapurified urea (ARISTAR grade, BDH) was used and urea solutions were freshly prepared and deionized with resin MB-1 (BDH) immediately before use. Gels were prepared with 5.2% (w/v) acrylamide/bis (36.5 : 1), 2% (w/v) Ampholines (pH 3-10, Sigma) and 2% (w/v) Nonidet P-40 in 6M urea. Oxygen in the solution was removed under vacuum for 30 min before the addition of detergent. Gels were polymerized with TEMED (2  $\mu$ l/ml of gel solution) and ammonium persulphate (10% solution, 3  $\mu$ l/ml of gel solution) in a vertical slab system (142x160x1.5 mm). Normally, polymerization was complete in 1 h. Samples were diluted with 2 volumes of sample buffer containing 3% (w/v) Nonidet P-40, 3% (w/v) Ampholines (pH 3-10) and 100 mM dithiothreitol in 9 M deionized urea and loaded into the wells of the gel. An isoelectric point marker mixture (pH 3.5-9.3, Sigma) was treated similarly and loaded at the same time. The mixture contained amyloglucosidase from Aspegillus oryzae (pI 3.50), trypsin inhibitor from soybean (pI 4.55), beta-lactoglobulin A from bovine milk (pI 5.20), carbonic anhydrase B from bovine erythrocytes (pI 5.85), carbonic anhydrase B from human erythrocytes

(pI 6.55), myoglobin from horse heart (pI 6.85 and 7.35), L-lactic dehydrogenase from rabbit muscle (pI 8.15, 8.45 and 8.65) and trypsinogen from bovine pancreas (pI 9.30). The upper reservoir (anode) was usually filled with 0.02M glacial acetic acid and the lower tank (cathode) contained 0.02M NaOH. The gels were run at the fixed power of 15 watts for 4-5 hours.

### Immunoblotting

Although polyacrylamide gel electrophoresis is an extremely powerful tool for the analysis of complex protein mixtures, it is restricted in that the separated proteins remain buried within the dense gel matrix and not readily available for further investigation. Protein blotting promises to overcome many of these problems, and the use of antibodies to identify the proteins transferred on the nitrocellulose sheets provides a very sensitive and specific method for identifying individual proteins.

The transfer of proteins from SDS gels to nitrocellulose paper was conducted in a LKB 2117-005 Multiphor II Novablot Electrophoretic Transfer Unit using a continuous buffer system containing 39 mM glycine, 48 mM Tris, 0.0375% SDS and 20% methanol. The nitrocellulose paper (0.2  $\mu$ m thick, Schleicher and Schuell, W. Germany) was placed facing the anode. With gels containing urea, transfer was found to be most effective in a continuous acid buffer system containing 0.7% acetic acid, 20% methanol, with the paper facing the cathode, as recommended by Beisiegel (1986). The

transfers were run at a current of 0.8 mA per square centimetre for 1 hour.

LPL on the blot was identified using the anti-LPL antiserum described in Chapter 3. The nitrocellulose paper was incubated with 10% (v/v) horse serum and 0.05% (v/v) Tween 20 in phosphate-buffered saline (PBS) at room temperature for 40 minutes. The paper was then washed 5 times with PBS for 10 minutes each time and incubated with sheep anti-chicken LPL antiserum diluted 1:200 in PBS for 1 hour. The paper was washed another 5 times with PBS and incubated for 1 h at room temperature with donkey anti-sheep IgG (whole molecule) alkaline phosphatase conjugate (Sigma) diluted 1:500 in PBS. The paper was washed with PBS 5 times again and then alkaline phosphatase substrate (Vector Laboratories, SK 5200) diluted in 100mM Tris, pH 9.5 was added. The colour reaction was developed for 20-30 minutes in the dark at room temperature. The paper was then washed with water for 5 minutes and dried at room temperature.

The proteins on the blots were stained with Pelikan drawing inks (W. Germany). First, the blots were washed with 0.5 M NaOH for 5 min and with PBS contained 0.05% (v/v) Tween-20 4 times for 10 min each time. The blots were stained with 0.1% solution of drawing ink in PBS-Tween for 2 h or overnight and then washed 3-4 times with water and dried at room temperature. The Pelikan Fount India drawing ink recommended by Sutherland and Skerritt (1986) failed to stain proteins in this study. In contrast, the Pelikan Tinta China drawing ink worked very well. The reason for this difference between inks is not clear. It is possible that Indian ink would not

like to work for a Chinese student.

## RESULTS

### Purification of LPL

A typical separation of post-heparin plasma LPL on an heparin affinity column is shown in Fig. 4.1. Most protein was not bound and passed through the column during the loading. Most LPL activity was bound to the column and bound activity was eluted in a single peak at a salt concentration of 1.0–1.2 M NaCl. Similar results were obtained during purification of LPL from pig adipose tissue (Bensadoun et al., 1974), bovine milk (Iverius and Ostlund-Lindqvist, 1976), rat post-heparin plasma (Fielding et al., 1977) and guinea pig adipose tissue (Semb and Olivecrona, 1986).

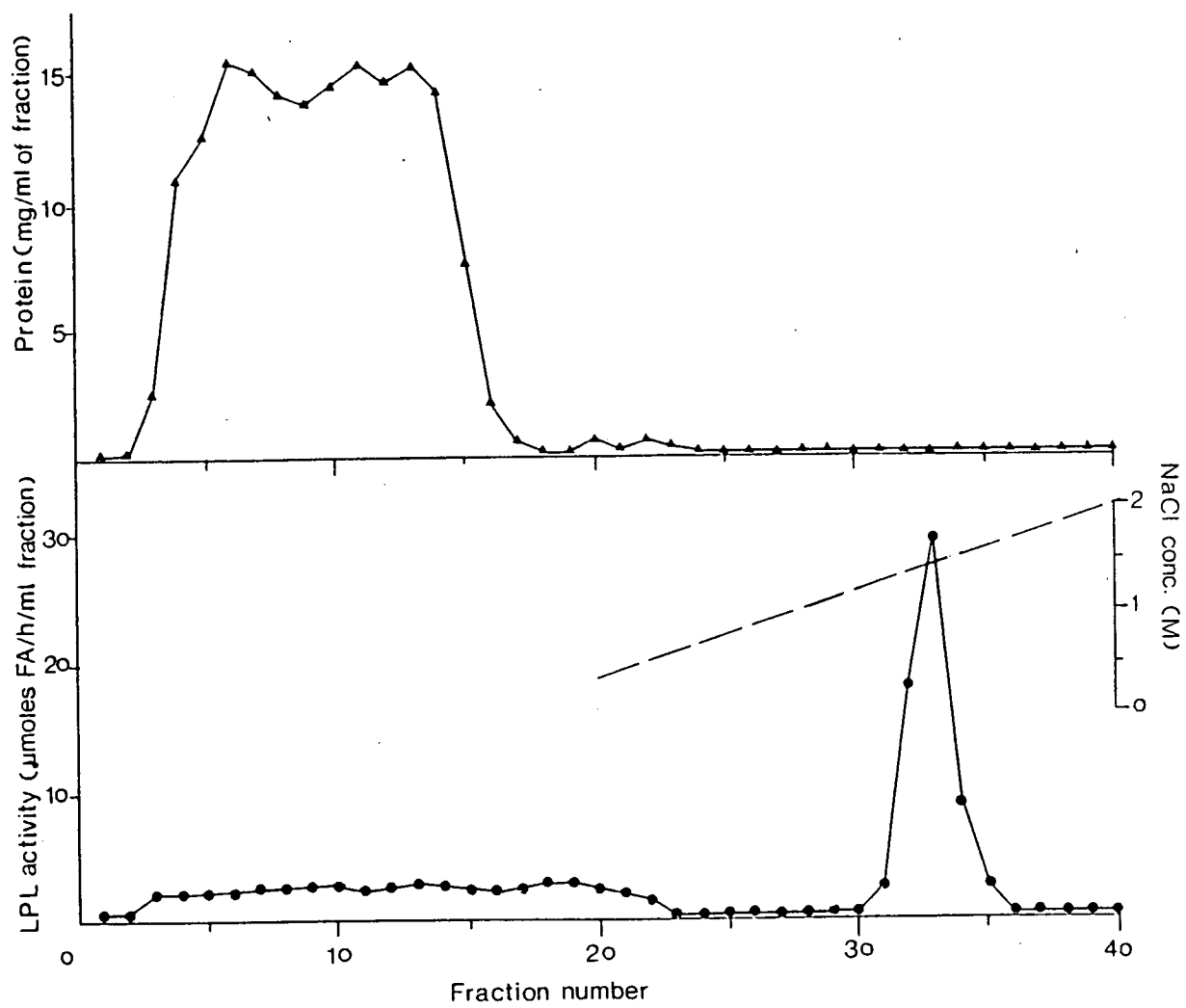


Fig. 4.1 Chromatography of post-heparin plasma on heparin-Sepharose

The salt concentration used to elute the column is very important for effective separation of LPL from contaminating proteins. When the column was eluted sequentially with 0.3, 0.5 and 1.2 M NaCl buffer and the LPL from the peak of activity was examined by SDS electrophoresis, the purified LPL contained a lot of contaminating proteins (Fig. 4.2), especially with protein-rich tissue extracts such as those from heart and leg muscle. When the column was loaded with the same samples and washed with 0.75 M NaCl buffer before the elution of LPL with 1.2 M NaCl buffer, most of the contaminating proteins were removed (Fig. 4.2b). A concentration of 0.75 M NaCl was used routinely in subsequent work.

The recovery of LPL applied on heparin affinity chromatography depended upon the methods used to load the samples (Table 4.1). The recovery of LPL activity from post-heparin plasma applied directly to the heparin-Sepharose column was only 14%, but when the sample was bound by incubation with the gel on a magnetic stirrer the recovery was 52%. The proportions of the enzyme bound to the total activities applied were all quite high. The recoveries from tissue extracts loaded with the latter method were also high (40-74%, Table 4.2) and the sample from heart muscle had the highest recovery (74%).

The reason for the differences of recoveries is not clear. Part of the sample loaded directly to the column may aggregate at the top of column and may not be eluted from the column even with high salt solutions.

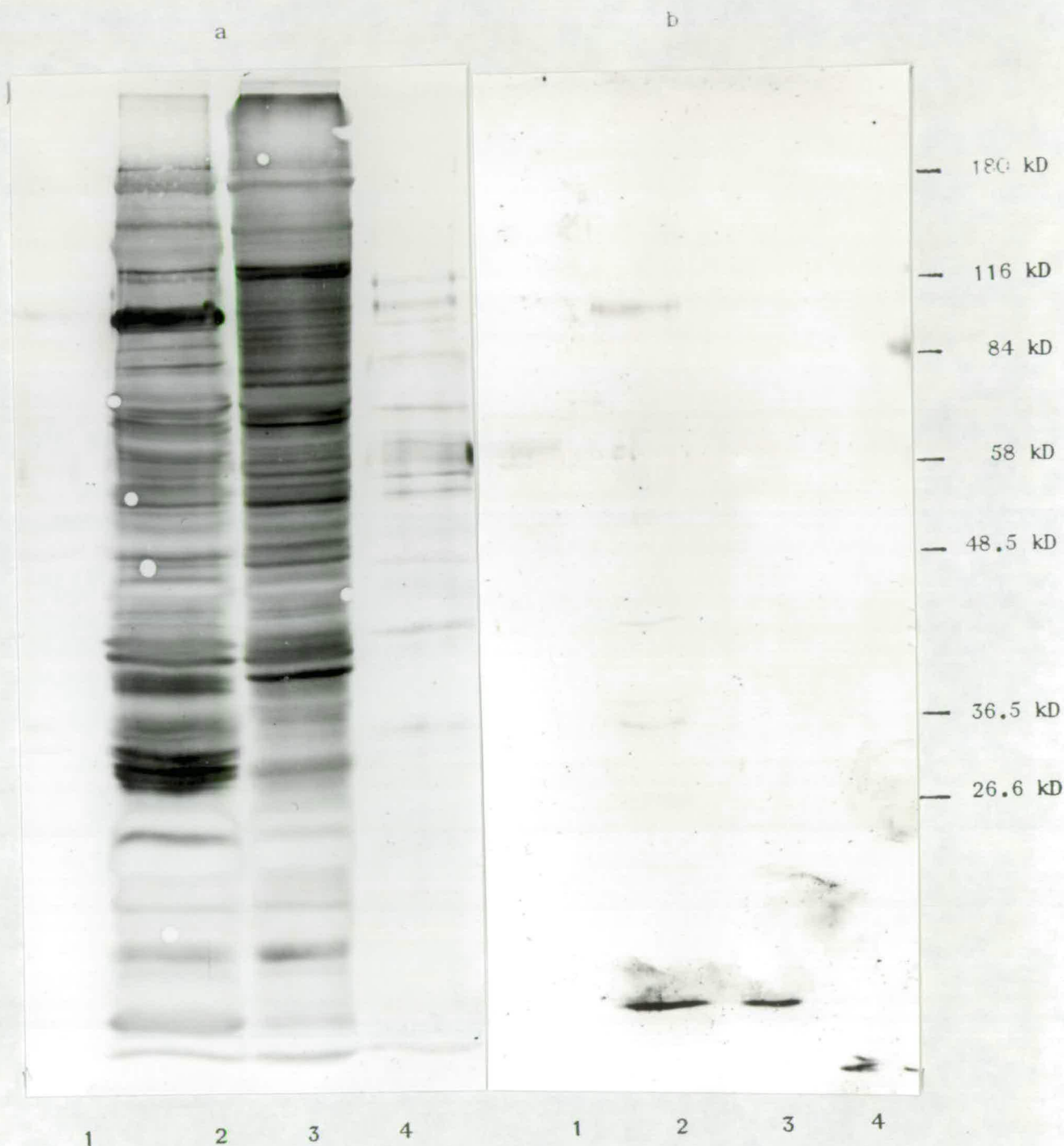


Fig. 4.2 SDS electrophoresis with LPL samples purified from heparin affinity chromatography a) after 0.3, 0.5 and 1.2 M NaCl washes, b) after 0.3, 0.5, 0.7 and 1.2 M NaCl washes. The blots were stained with Pelikan Chinese ink. Sample from 1) post-heparin plasma obtained by gradient salt elution as control, 2) leg muscle, 3) heart muscle, and 4) abdominal fat. Molecular weight markers ran as indicated.

Table 4.1 Effect of methods of binding on the recoveries of LPL activity from heparin affinity chromatography column<sup>1</sup>

LPL activity	A <sup>2</sup>	B <sup>3</sup>
activity applied ( $\mu$ moles of FFA/h)	1836	1344
activity bound (% of applied)	94	81
recovery (% of applied)	14	52

<sup>1</sup> post-heparin plasma samples were prepared from the broilers at 4 weeks of age.

<sup>2</sup> plasma loaded directly onto the column.

<sup>3</sup> plasma incubated with heparin-Sepharose by gentle stirring before packing into the column.

Table 4.2 Recoveries of LPL activity from different tissue extracts on heparin affinity chromatography

	sample from		
	abdominal fat	heart muscle	leg muscle
LPL activity applied ( $\mu$ moles of FFA/h)	527	597	636
LPL activity bound (% of applied)	87	96	99
recovery (% of applied)	40	74	40

Samples were collected from the broilers at 9 weeks of age. The extracts from acetone-ether powders were incubated with 8 ml of packed volume of heparin-Sepharose 4B for 2.5 h at 4°C prior to packing into a column. All the samples were run under the same conditions.

### Purification of antiserum

In preliminary studies, LPL purified from post-heparin plasma was run on SDS gel and two major bands were identified using crude anti-LPL antiserum (Fig. 4.3a). Several authors have reported that there is only one major molecular weight component of LPL from chicken adipose tissue (Gershenwald et al., 1985), from bovine milk (Socorro and Jackson, 1985; Voyta et al., 1985) and from perfused rat heart (Friedman et al., 1986) using similar Westernblotting analysis. Therefore, one of these two major bands found in the present study might be contaminant.

Since the post-heparin plasma LPL had been purified from post-heparin plasma by heparin affinity chromatography, the contaminant must be a plasma protein which had high affinity for heparin. Normal plasma was collected and run through the heparin affinity column under the same conditions as used with post-heparin plasma. Samples from the fractions which would have normally contained LPL were run on SDS gel. Immunoblotting analysis showed proteins in normal plasma could be recognized by non-purified antiserum (Fig. 4.3b) and the major band was located in the same position as one of two major bands on Fig. 4.3a. Antithrombin, a major plasma protein, has a high affinity for heparin-Sepharose columns and consequently is a common contaminant of LPL fractions (Ostlund-Lindqvist and Boberg, 1977; Cheng et al., 1985). It is also a glycoprotein with a similar molecular weight to that of the LPL (Ostlund-Lindqvist, 1979).

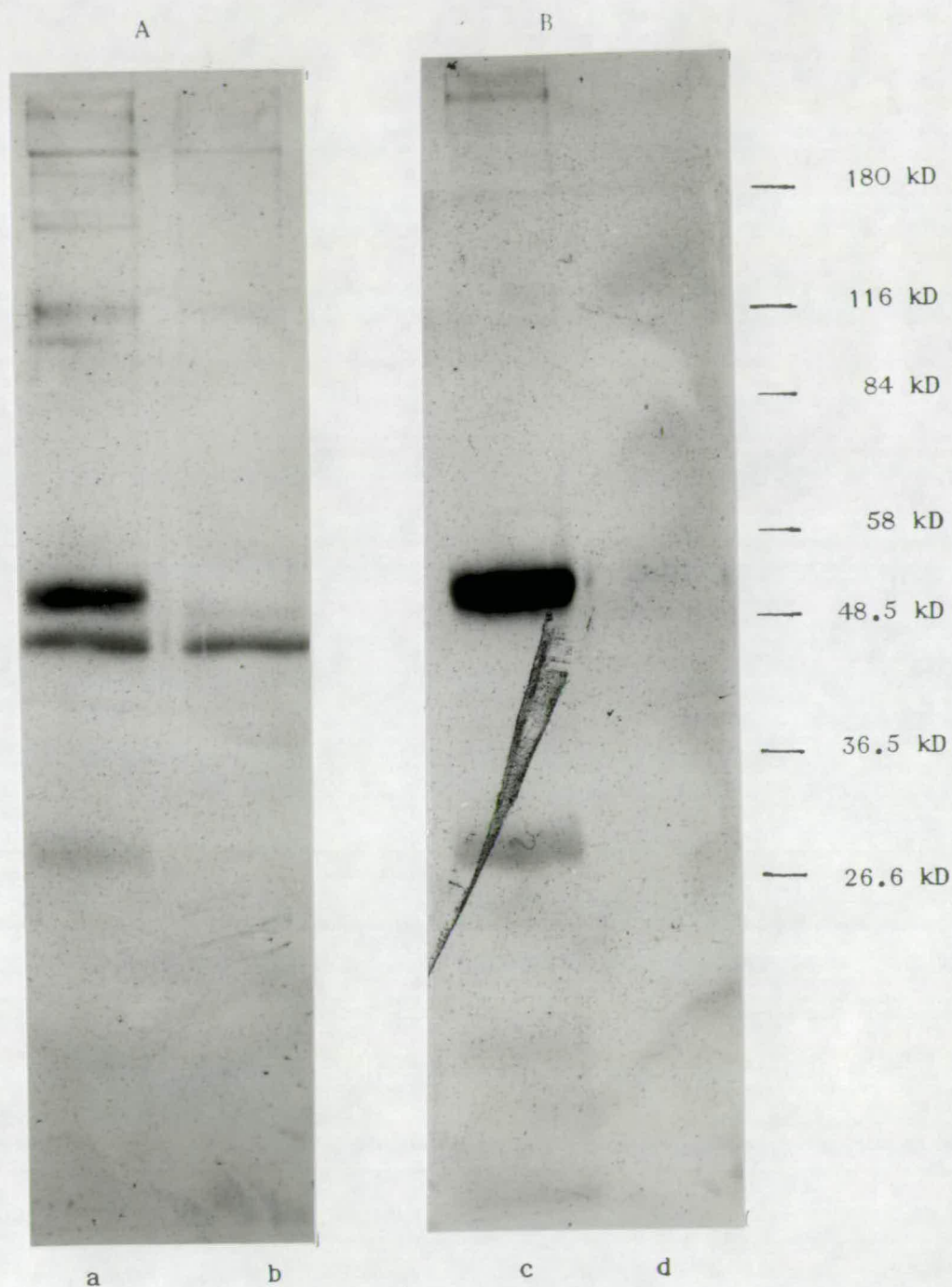


Fig. 4.3 Westernblotting analysis of partially purified post-heparin plasma LPL with non-purified (A) or purified antiserum (B). a) Two major bands and several minor bands from post-heparin plasma LPL sample are identified with non-purified antiserum. b) One major band from normal plasma is identified with non-purified antiserum. c) One major band from post-heparin plasma LPL is identified with purified antiserum. d) No band is identified in normal plasma with purified antiserum.

Why should this contaminant be recognized by the anti-LPL antibodies? The polyclonal antiserum had been raised by injections of purified LPL in sheep. The LPL was purified by successive affinity chromatography steps on heparin-Sepharose 4B and Concanavalin A-Sepharose 4B (S.C. Butterwith and H.D. Griffin, unpublished). Antithrombin has been found in the LPL purified from milk (Olivecrona and Bengtsson-Olivecrona, 1987) and adipose tissue (D.S. Robinson, personal communication). The LPL purified from chicken adipose tissue used in raising the antiserum may also have contained antithrombin and generated antibodies to that protein as well as LPL. Because there is no LPL in normal plasma, the non-LPL specific antibody can be separated from the antiserum by immunoadsorption on a plasma protein affinity column and the details of the methods used are described below.

2.4 g of cyanogen-Bromide activated Sepharose 4B (Sigma) was suspended in 100 ml of 1 mM HCl and centrifuged at 1000g for 10 minutes at 4°C. The gel was washed in 1 mM HCl a further three times. The washed gel was resuspended in 15 ml of 0.1 M NaHCO<sub>3</sub> at pH 8.0 and stirred with 2 ml of normal plasma from a six week old broiler at 4°C overnight. Any remaining active groups were deaminated by adding 0.75 ml of ethanolamine and stirring for 4 hours at 4°C. The gel was washed with 2x50 ml of distilled water, 2x50 ml of 1.2 M NaCl and 2x50 ml of distilled water and then packed in a column (0.5 cm diameter x 4 cm length) This plasma protein-Sepharose 4B column was washed with PBS buffer until no protein could be detected in the elution. 1 ml of LPL antiserum was loaded onto the column which was then eluted with PBS. The

fractions with highest protein contents were collected and the aliquots were stored at  $-20^{\circ}\text{C}$ .

The results of Western blot analysis using the antiserum purified by this method were quite different from that using non-purified antiserum. Identification with the purified antiserum revealed only one major band on the immunoblot from a SDS gel loaded with post-heparin plasma LPL (Fig. 4.3c) and no band on the blot from a heparin-affinity purified plasma protein sample (Fig. 4.3d). These results indicated that the purification of the antiserum by this immunoadsorption method had been very effective.

#### Separation of tissue and post-heparin plasma LPL on the basis of molecular weight

A typical separation of partially purified LPL samples from tissues and post-heparin plasma on 10% polyacrylamide gel is shown in Fig. 4.4. LPL from different sources produced major bands at the same position on the gel indicating a molecular weight of 54 kD and faint bands with a molecular weight of 29.5 kD.

The result of running a gradient SDS gel (10-16%) with the same samples was similar to that with normal SDS gel, although the bands on the former look sharper (data not shown). The scanning densitometry of the blot from the gradient gel was performed using a LKB UltroScan XL and showed more clearly that LPL purified from different tissues and from post-heparin plasma had the same or very

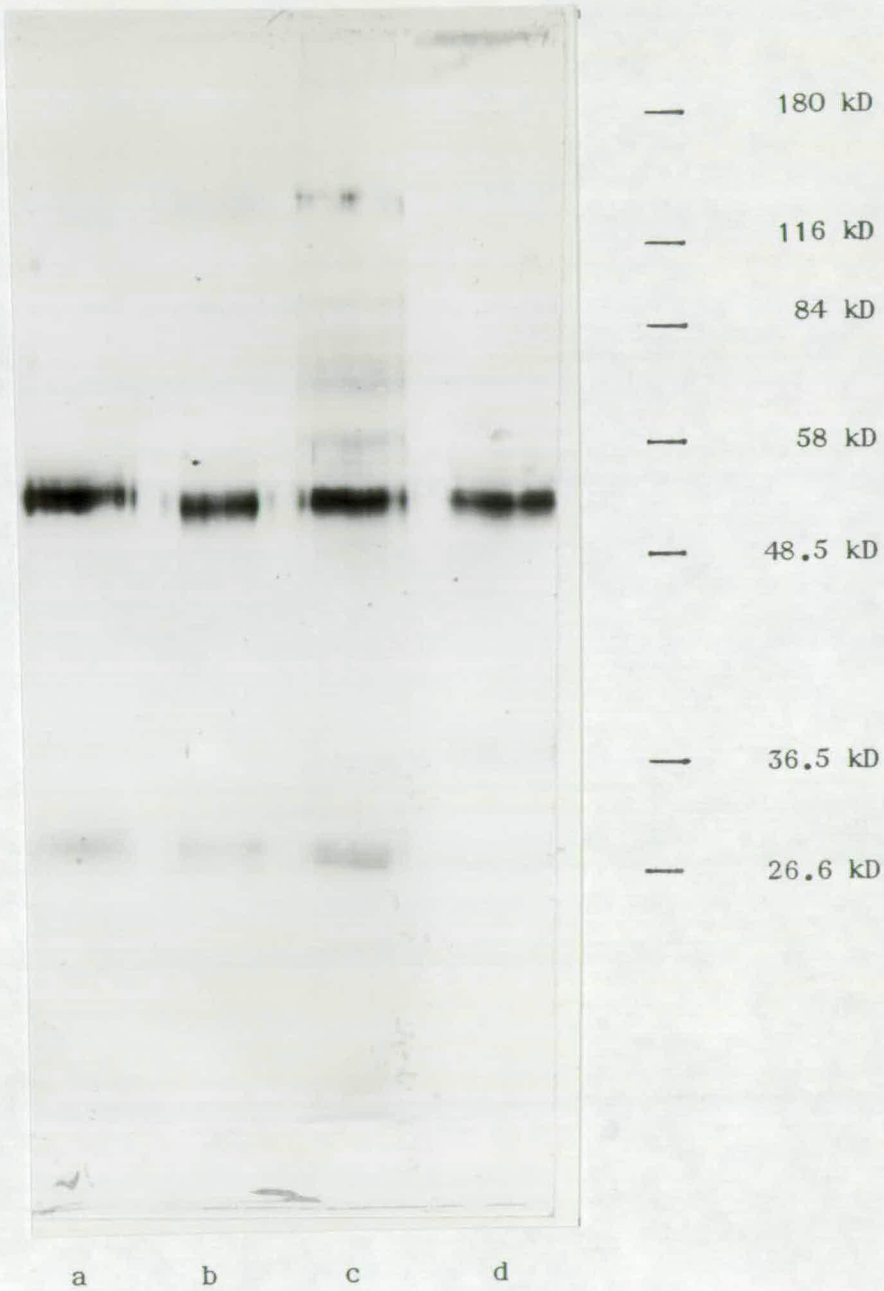


Fig. 4.4 Immunoblot from SDS electrophoresis of LPL samples purified from different tissues and from post-heparin plasma. LPL from a) abdominal fat, b) heart muscle, c) leg muscle and d) post-heparin plasma.

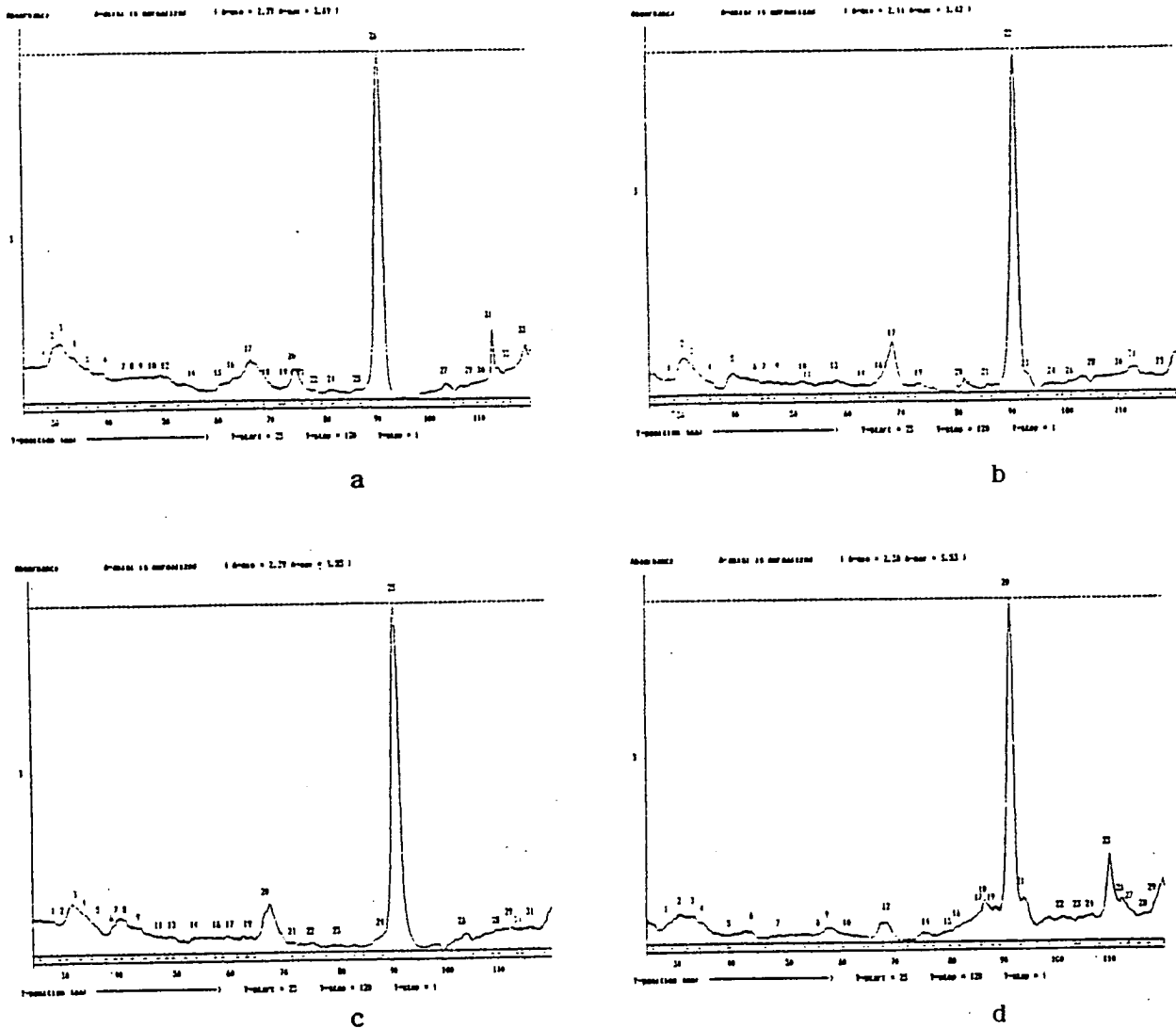


Fig. 4.5 Scanning densitometry of the immunoblot after separation of LPL on a gradient SDS gel (10-16%). Samples purified from a) post-heparin plasma, b) abdominal fat, c) heart muscle, and d) leg muscle.

similar molecular weight (Fig. 4.5).

#### Evidence for proteolytic degradation of the native enzyme

Minor components with lower molecular weight have been identified by immunoblotting analysis in LPL purified from milk (Socorro and Jackson, 1985), perfused rat heart (Friedman et al., 1986) and rat adipose tissue (Vydelingum et al., 1986) and have been suggested to be degradation products. Similar minor components also existed in chicken LPL samples (Fig. 4.4, Fig. 4.5) and these might be the result of proteolysis.

Protease inhibitors were used to try to prevent the production of these lower molecular weight fragments in vitro. Post-heparin blood was directly collected from the wing vein into syringes containing a mixture of leupeptin (2  $\mu\text{g/ml}$ ), aprotinin (10  $\mu\text{g/ml}$ ), 2 mM DTT, 2 mM EDTA and 2 mM benzaminidine. Washing and elution buffers used in chromatography contained 1 mM benzaminidine. Alternatively, blood samples were collected in presence of 1 mM PMSF and PMSF was included at a concentration of 0.5 mM in the buffers used for heparin-affinity chromatography.

The lipoprotein lipase purified from post-heparin plasma in presence of the protease inhibitors leupeptin and benzaminidine still contained minor components of lower molecular weight as shown in Fig. 4.6. When the sample was incubated at 37°C for 24 h, an

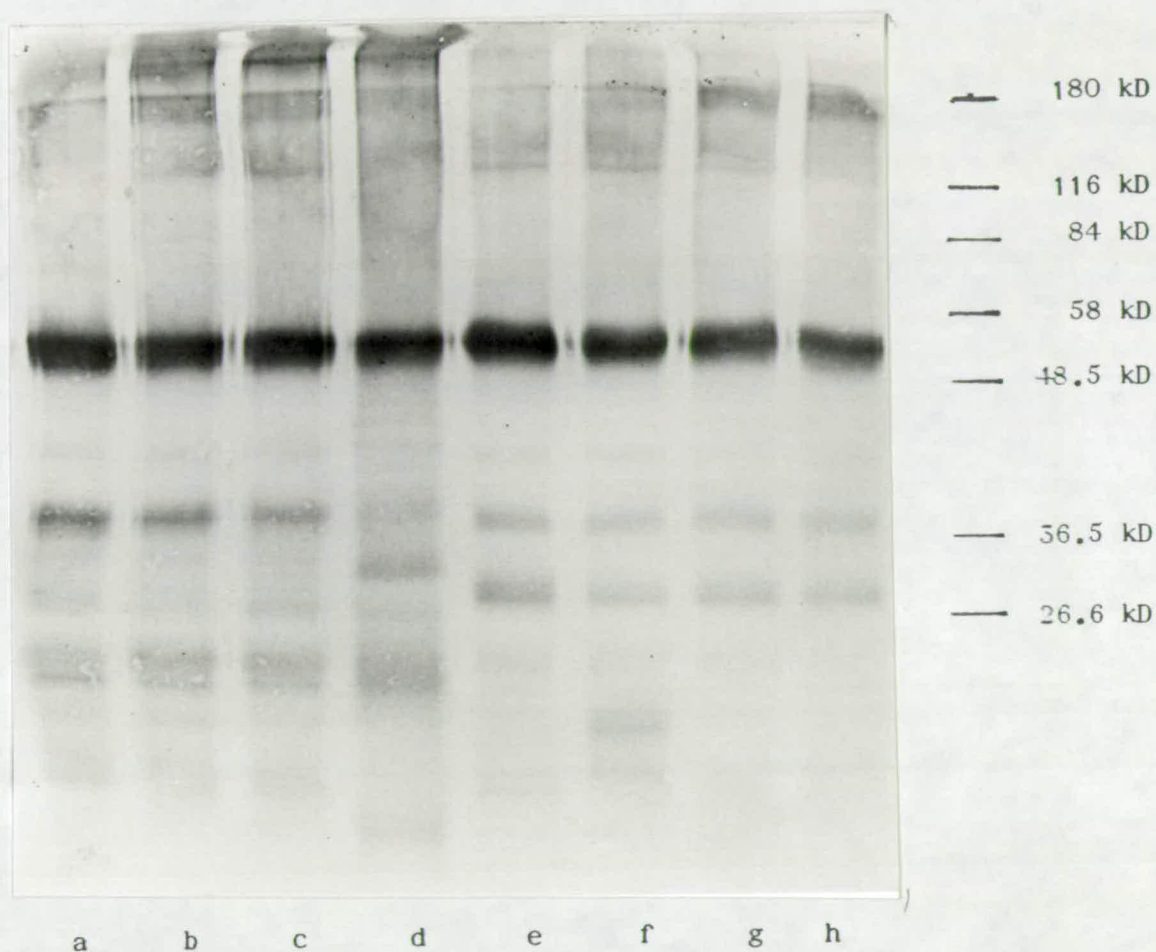


Fig.4.6 Effect of protease inhibitors on the degradation of LPL. Lanes on the left (a-d) contained LPL purified from post-heparin plasma in presence of the protease inhibitors, leupeptin, aprotinin and benzaminidine and incubated at 37°C for 0, 1, 4 and 24 h respectively. The arrow indicates the band appeared after 24 h incubation. Lanes on the right (e-h) shows LPL purified from post-heparin plasma in presence of PMSF only and incubated at 37°C for 0, 1, 4 and 24 h respectively. LPL was identified using purified antiserum.

apparently new minor component with a molecular weight of 31 kD and several faint bands with molecular weight between 34 kD and 53 kD appeared (Fig. 4.6d).

PMSF appeared to be more effective as a protease inhibitor since it prevented the appearance of additional minor components either before or after the incubations at 37°C (Fig. 4.6 e-h). Nevertheless, minor bands were still presented, suggesting that proteolysis might be a normal fate of LPL in vivo. Socorro and Jackson (1985) observed the same phenomenon with LPL from milk.

#### Characterisation of LPL by isoelectric focusing

In preliminary studies, isoelectric focusing was performed on a pH gradient from 3-10 in the absence of urea. Under these conditions, the LPL either precipitated at the application point or produced a trailing pattern. This occurred even when non-ionic detergents Triton X-100 and Nonidet P-40 were added to both sample and gel to solubilize the proteins and all particulate material was carefully removed from samples by centrifugation.

When isoelectric focusing was performed on the gels containing 6 M urea (see Material and Methods), precipitation of LPL still occurred when samples were applied at the cathode (Fig. 4.7). This suggested that the pH at the cathode favoured aggregation of LPL even though urea and other detergents were present in both the sample buffer and gel. However, when the samples were applied at

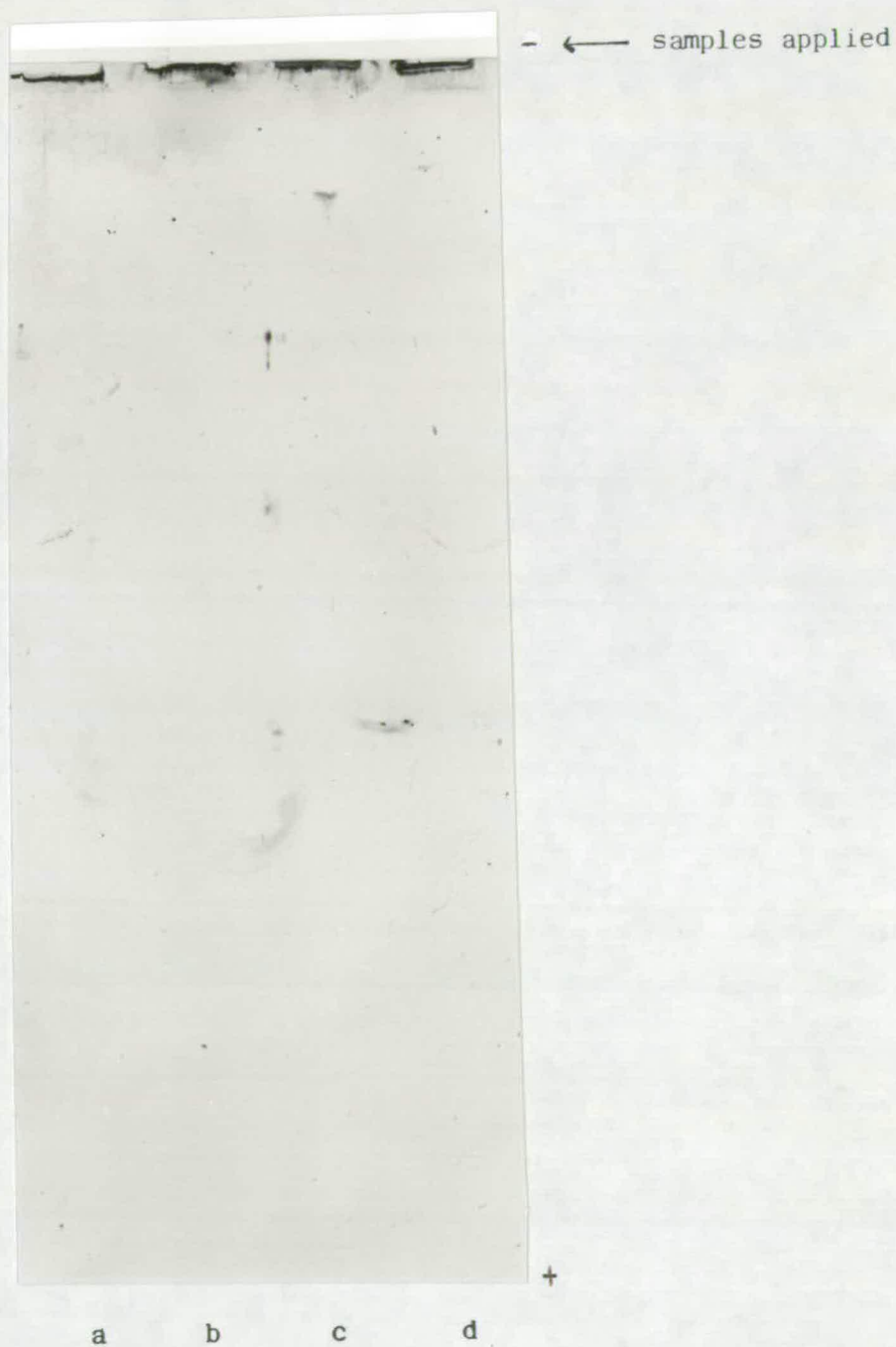


Fig. 4.7 Sample precipitations at the cathode of isoelectric focusing gel. LPL purified from a) leg muscle, b) heart muscle, c) adipose tissue, and d) post-heparin plasma. Samples were applied at the ~~anode~~ cathode.

anode, no aggregation occurred and reproducible isoelectric patterns similar to those in Fig 4.8 were obtained. More than 10 individual variants ranging in isoelectric point from pH 3.5 to 7.0 were separated from each sample loaded. Adipose tissue, heart and muscle LPL showed similar heterogeneity and no tissue-specific LPL patterns could be identified.

Neuraminidase (Sigma) and endoglycosidase H (Boehringer Mannheim Biochemica, W. Germany) were used to examine the effects of removal of sialic acid and high mannose components from LPL. LPL preparations were incubated with neuraminidase (0.17 U/ml) or endoglycosidase H (0.03 U/ml) at 37°C for 18 h. The reaction mixture also contained 5 mM PMSF and 150 mM phosphate adjusted to pH 5.0. After digestion, the samples were mixed with sample buffer and loaded into the wells of the gel for isoelectric focusing.

There was no apparent change in the isoelectric focusing pattern of LPL digested with neuraminidase or endoglycosidase H (Fig. 4.9). It seems that the sialic acid and high-mannose are not the major components of carbohydrate in LPL which would cause the formation of multiple bands seen in the present study.

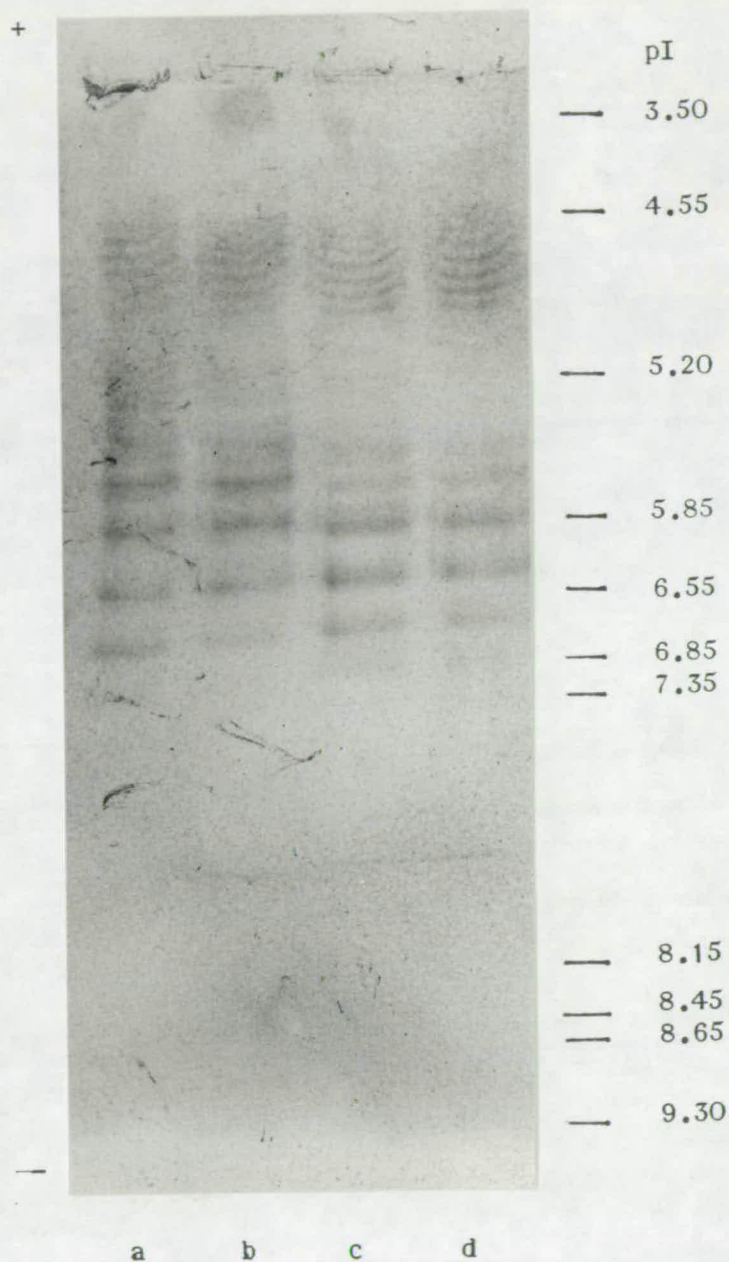


Fig. 4.8 Isoelectric focusing pattern from immunoblotting of LPL. LPL sample purified from a) leg muscle, b) heart muscle, c) abdominal fat and d) post-heparin plasma. Samples were applied at the anode. Position of isoelectric point markers is as shown.

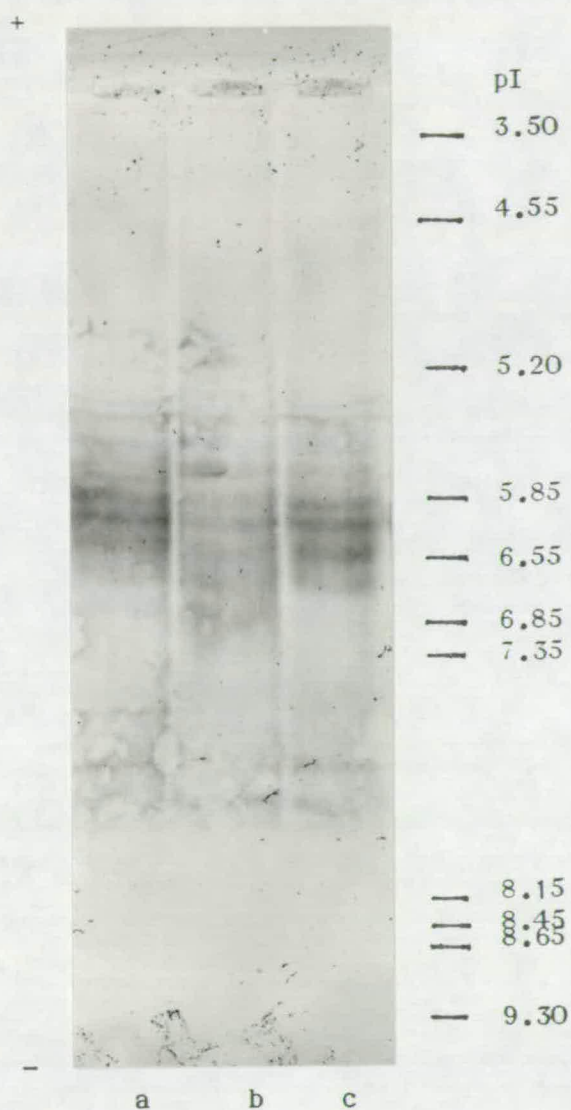


Fig. 4.9 Isoelectric focusing patterns of neuraminidase and endoglycosidase H treated LPL samples. LPL was purified from adipose tissue. LPL samples were incubated at 37°C for 18 h a) with neither neuraminidase nor endoglycosidase H, b) with neuraminidase, c) with endoglycosidase H. Position of pI markers is as shown.

## DISCUSSION

In this study, most of the LPL from post-heparin plasma and different tissues from broiler chickens was present in a component with a molecular weight of 54 kD. Each sample also contained a minor component with a molecular weight of 29.5 kD. Gershenwald et al. (1985) reported that chicken adipose tissue LPL contained one component with a molecular weight of 60 kD and one minor species corresponding to a molecular weight of 32.8 kD. Socorro and Jackson (1985) found that most of the LPL purified from bovine milk had an apparent molecular weight of 55 kD, although minor components with molecular weight of 36 kD and 18-22 kD were also present.

Socorro and Jackson (1985) suggested the presence of the minor components with smaller molecular weight in Westernblotting analysis of LPL from milk indicated that the proteolysis of LPL occurs in vivo or is an extremely rapid process in vitro since they also found some breakdown of LPL from milk even in the presence of PMSF. In the present study, the inclusion of PMSF during sample collection and purification could not prevent the appearance of at least one lower molecular weight component. PMSF was capable of inhibiting further proteolysis of the enzyme incubated at 37°C for 24 h. Nevertheless, minor components were still present, suggesting that proteolysis might be a normal fate of chicken LPL in vivo. If the proteolysis of LPL occurs in vivo, are the proteolytic products artefacts?

The present study is the first in which the pI point of LPL has been analyzed using immunoblotting techniques. No single isoelectric point of the enzyme could be determined because of the appearance of multiple bands on the isoelectric focusing spectrum in the study. Greten and Walter (1973) reported that the pI point of LPL from rat adipose tissue was 4.2 and Bensadoun et al. (1974) reported that LPL from pig adipose tissue was 4.0. In these two experiments, density gradient columns were used in isoelectric focusing and the pI point was determined on the pH of the peak of the enzyme activity in the fractions collected. The activity of the enzyme was spread over a wide pH range. For example, in the experiment of Bensadoun et al. (1974), LPL activity was present in the fractions ranging from pH 3 to pH 7, although its peak activity was at pH 4.0. It was possible that the LPL was focused on the multiple pH points along the density gradient column, and if the volume of fractions collected had been smaller, multiple peaks of the enzyme would have been discovered. Using the same kind of density gradient system, Bengtsson and Olivecrona (1977) found that LPL purified from bovine milk formed several peaks on isoelectric focusing. Bengtsson and Olivecrona (1977) also reported that LPL focused in 5 M urea polyacrylamide gel slabs with a pI point around pH 9. But that LPL isoelectric focusing band had not been identified by anti-LPL antiserum and it might be an impurity existed in the LPL preparations rather than LPL itself.

The presence of many more isoelectric focusing bands than expected perhaps means that they are the result of artefact formation. The multiple forms may be caused by the combination with

heparin. Nader et al. (1974) and McDuffie et al. (1975) reported that heparin could be fractionated by isoelectric focusing into 21 components, with pI between pH 3.2 and 4.2. This result has been shown to be a consequence of a strong interaction between the polysaccharide and different amphoteric species in the ampholine mixture (Righetti and Gianazza, 1978). The ability to bind to heparin appears to be a general property of LPL (Egelrud, 1973; Olivecrona et al., 1977) and all the LPL samples in <sup>the</sup> present study had been partially purified by heparin-affinity chromatography. It was possible therefore that a LPL-heparin complex existed in sample preparations and the interaction between heparin and carrier ampholytes would elicit artefacts. However, Righetti and Gianazza (1978) reported that samples run in high concentration of urea appeared to fully dissociate any complexes of heparin and carrier species. In the present study, the enzyme samples were focused in 6M urea which would be expected to completely disrupt any complex of sample and ampholines.

A second possible cause of artefact formation might be carbamylation. Urea in aqueous solution tends to be hydrolyzed into  $\text{NH}_4^+$  and cyanate, which eventually form an equilibrium pair. The cyanate can react with  $\text{NH}_2$  and SH groups of proteins, particularly at high temperature ( $>37^\circ\text{C}$ ) and high pH ( $>$  or  $= 8$ ), and to form neutral derivatives having altered isoelectric points (Isoelectric Focusing, pp 92-93, Pharmacia Fine Chemicals). In the present study the risk of carbamylation was minimized by always using fresh solution of high quality urea which had been deionized with Ambertite MB-1.

Other methods suggested by Gianazza and Righetti (1980) have also been tried to demonstrate whether the sample heterogeneity is artefactually generated. Alteration of the enzyme/Ampholine ratio was conducted. If complexes are formed, increasing sample loads will give increased polydispersity. When the enzyme was diluted upto 10 fold against Ampholine background, there was no apparent difference between their isoelectric focusing patterns (not shown).

The reason for formation of multiple bands of LPL on isoelectric focusing is not clear. They may be the products from distinct structural genes or the result of different post-translational modifications of the same gene product. LPL is a glycoprotein containing 8.3% carbohydrate (Iverius and Ostlund-Lindqvist, 1976) and multiple bands could be a result of non-uniform distribution of saccharide units between different molecules (Gianazza and Righetti, 1980). Multiple forms observed in the isoelectric focusing of the glycoprotein ApoH completely disappeared when ApoH was treated with neuraminidase to remove sialic acid (Sprecher et al., 1984). In the present study, samples digested with neuraminidase still showed multiple form and this suggests that the multiple form of LPL are not caused by the variation in its sialic acid component.

The similar molecular weights and isoelectric focusing patterns for the LPL purified from different tissue indicated that tissue specific LPLs can not be distinguished from the post-heparin plasma by their molecular weight and pI point.

## Chapter 5

VERY LOW DENSITY LIPOPROTEIN METABOLISM IN BROILERS SELECTED  
FOR HIGH AND LOW PLASMA VERY LOW DENSITY  
LIPOPROTEIN CONCENTRATIONS

## SUMMARY

The birds in this study were from two lines divergently selected for plasma very low density lipoprotein (VLDL) concentration for 7 generations. The difference in plasma VLDL concentrations between the birds from the lines was about 7-fold and the difference of abdominal fat weights was 2.8-fold. The amount of plasma VLDL incorporated into the whole abdominal fat pad in high-VLDL birds was estimated to be about 8-fold greater than that in low-VLDL birds. However, both specific and total lipoprotein lipase (LPL) activities in the abdominal fat pads from fat birds were lower than those from lean birds. The specific LPL activities in heart and leg muscle were greater in the lean birds. These results suggest that muscle LPL in low-VLDL birds may make a greater contribution to plasma VLDL removal than in high-VLDL birds.

## INTRODUCTION

Divergent selection for plasma very low density lipoprotein (VLDL) concentrations in birds from a commercial broiler strain has produced birds with 6-fold difference in plasma VLDL concentration and 2.45-fold difference in abdominal fat pad weight (Whitehead, 1988). The difference in fat deposition between lean and fat birds is presumably caused by the difference in VLDL metabolism. Previous studies using the same approach as described in Chapter 2 have shown that the VLDL secretion rate in the birds from high-VLDL line was 3 times greater than that in the birds from low-VLDL line (H.D. Griffin and F. Acamovic, unpublished), but the fates of plasma VLDL in the birds from these two lines have not been compared. In this chapter, the effect of the selection on the uptake of VLDL incorporated into adipose tissue was investigated using labelled VLDL.

## MATERIALS AND METHODS

Birds

Sixteen female birds from each of the two lines divergently selected for plasma VLDL concentration for 7 generations were reared from day old and then kept in individual cages from 4 to 7 weeks of age. Standard broiler finisher diet was fed ad libitum during this period. The photoperiod was 23 h light : 1 h dark, with lights off between midnight and 0100 h.

### Labelled VLDL administration and sample collection

At 7 weeks of age, blood samples were taken for the assay of plasma VLDL concentration and the birds were then intravenously injected with 1 ml of  $^{14}\text{C}$  labelled VLDL (radioactivity: 688,780 dpm, prepared by H.D. Griffin and F. Acamovic as described in Chapter 2). Blood samples were collected 20 minutes after the labelled VLDL was injected into the birds from the low-VLDL line and 40 minutes after for the birds from the high-VLDL line. Different time intervals were chosen, since preliminary observations showed different rates of clearance of VLDL from the circulation of the low- and high-VLDL lines. EDTA was added as an anticoagulant when blood samples were taken and the plasma was separated by centrifugation.

As soon as blood samples were taken, birds were killed by rapid intravenous injection of phenobarbitone (Expiral, Abbott Laboratories, Queenborough, Kent) and the abdominal fat, heart, liver and leg muscle samples were collected and stored as described in Chapter 2.

### The assays

Measurement of the radioactivities in tissue and plasma samples was the same as described in Chapter 2. Plasma VLDL concentrations and tissue LPL activities were determined as described in Chapter 3. Total plasma volume in both lines was assumed to be similar to that

in normal birds (4.7% of body weight, H.D. Griffin, unpublished).

Results from the two lines were compared by Student's t-test.

## RESULTS AND DISCUSSION

### The growth of the birds

Body weight, abdominal fat weight, liver weight and plasma VLDL concentrations in the birds from the two lines used in the present study are shown in Table 5.1.

Table 5.1 Mean+S.E. of body weight, abdominal fat weight, liver weight and plasma VLDL concentration of the birds from high-VLDL and low-VLDL lines at 7 weeks of age

	high-VLDL line	low-VLDL line	P
number of birds	8	8	-
body weight (g)	2086 $\pm$ 63	1910 $\pm$ 36	<0.05
VLDL conc. (abs. unit $\times 10^{-3}$ )	589 $\pm$ 92	81.9 $\pm$ 5.3	<0.01
abdominal fat:			
(g)	74.3 $\pm$ 9.6	26.9 $\pm$ 1.7	<0.01
(% of B.W.)	3.51 $\pm$ 0.38	1.40 $\pm$ 0.090	<0.01
liver weight:			
(g)	72.5 $\pm$ 6.5	51.2 $\pm$ 2.4	<0.05
(% of B.W.)	3.46 $\pm$ 0.25	2.67 $\pm$ 0.11	<0.01

The birds from the high-VLDL line were significantly heavier ( $P < 0.05$ ) than those from the low-VLDL line, in contrast to the previously published results which indicated that there was no between-line difference in body weight (Whitehead and Griffin, 1984). The plasma VLDL concentrations in the birds from these two

lines were very different, as expected, the concentration being over 7-fold higher in the high-VLDL line. There were significant differences ( $P < 0.05$ ) in liver weight between the high-VLDL and low-VLDL birds, whether expressed in absolute values or as a percentage of body weight. The weight of abdominal fat pad in the birds from the high-VLDL line was 2.8 times greater than that from low-VLDL line and 2.5 times when expressed as a percentage of body weight. This amount of difference in abdominal fat weight between the female birds from these two lines was similar to that between the female birds from the lines divergently selected for percentage abdominal fat weight (Leclercq, 1988). Grunder and Chambers (1988) have indicated that the genetic gain in reducing body fat content from selection for low VLDL in reducing body fat content was at least as great as that from direct selection for low abdominal fat weight.

#### Uptake of labelled VLDL

The radioactivities in tissues and plasma after the injection of labelled VLDL are shown in Table 5.2. There were no differences between the high-VLDL and low-VLDL lines in specific activities in the abdominal fat and leg muscle of the birds between the high-VLDL and low-VLDL lines. The specific activities in the heart muscle and liver from the low-VLDL birds were higher than those from the high-VLDL birds and the activity remaining in plasma was higher in high-VLDL birds ( $P < 0.05$ ).

Table 5.2 The radioactivities (mean+S.E.) in the tissues and plasma of the birds from high-VLDL and low-VLDL lines<sup>1</sup>

	High-VLDL line	Low-VLDL line	P
number of birds	8	8	-
<u>specific activity:</u>			
abdominal fat (dpm/g)	1150+79	1234+38	>0.05
liver (dpm/g)	1197+104	2724+218	<0.01
heart (dpm/g)	224+19	308+12	<0.05
leg muscle (dpm/g)	27+3.3	28+3.7	>0.05
plasma (dpm/ml)	1240+206	602+45	<0.05
<u>percentage activity<sup>2</sup>:</u>			
abdominal fat (%)	11.9+1.4	4.84+0.35	<0.01
liver (%)	12.1+0.82	19.9+1.3	<0.01
plasma (%)	17.7+3.2	7.87+0.69	<0.05

<sup>1</sup>Samples were collected from the birds from the high-VLDL line at 40 min and from low-VLDL line at 20 min after <sup>14</sup>C labelled VLDL administration (688,780 dpm/bird).

<sup>2</sup>the percentage of the radioactivity incorporated into the whole tissue to the total radioactivity injected.

In this experiment, birds were killed at 20 and 40 min to compensate for the differences in rates of clearance. This was only partly successful since the total activity remaining in the plasma from the fat birds was higher in fat birds (17.7% vs 7.87%,  $P < 0.05$ ). However, more than 80% of the radioactivity administered was removed from the blood of the birds of both high- and low-VLDL lines during the time periods given. The specific radioactivity in abdominal fat pads of the birds from the two lines was not different ( $P > 0.05$ , Table 5.2), but the total activity in the abdominal fat pads of the birds from the high-VLDL line was 2.5-fold greater than that in low-VLDL birds (11.9% vs 4.8%, Table 5.2).

Little of the lipid taken up in the adipose tissue of fully fed broilers is likely to be mobilised over the short period of the experiment. 40 min after the injection of labelled VLDL into the birds from high-VLDL line, 17.7% of label injected remained in the plasma (Table 5.2), i.e. 82.3% of the label was removed from the plasma. Since 11.9% of total radioactivity injected was incorporated into the abdominal fat pad, this represented 14.5% of the label removed from the circulation. This proportion is time independent. A similar calculation with data from the birds from the low-VLDL line showed that only 5.3%. Therefore, the difference in the proportions of the labelled VLDL incorporated into the whole abdominal fat pads was about 2.8-fold (14.5% vs 5.26%). Assuming that labelled VLDL and native VLDL are metabolized in a similar way, and the VLDL secretion rate in the birds from high-VLDL line is 3 times higher than that in the birds from low-VLDL line (H.D. Griffin and F. Acamovic, unpublished), the amount of plasma VLDL incorporated into the whole abdominal fat pad in high-VLDL birds might be about 8 times that in low-VLDL birds.

#### Tissue LPL activity

The lipoprotein lipase activities in abdominal fat, heart and leg muscle of the low-VLDL birds were higher than those of high-VLDL birds. Because the specific lipase activity in abdominal fat pads of the low-VLDL birds was 3.3 fold greater than that in high-VLDL birds ( $P < 0.01$ , Table 5.3), the total lipase activity from the fat pads in lean birds was still higher than that in fat birds ( $P < 0.05$ , Table 5.3).

Table 5.3 Tissue lipoprotein lipase activities (mean+S.E.) in the birds from high-VLDL and low-VLDL lines

	High-VLDL line	Low-VLDL line	P
number of birds	8	8	-
abdominal fat ( $\mu$ moles/g/h)	82+11	267+18	<0.01
( $\mu$ moles/whole pad/h)	5515+468	6997+218	<0.05
heart ( $\mu$ moles/g/h)	80+9.4	110+3.0	<0.05
leg muscle ( $\mu$ moles/g/h)	7.9+0.47	13+1.6	<0.05

The higher incorporation rate of VLDL into the abdominal fat pad is not therefore correlated with the adipose tissue LPL activities. Studies on humans have shown a more consistent, but still variable, inverse relationship between LPL per gram of adipose tissue and serum triglyceride content (Eckel, 1987). In normal broilers, lipase activity per gram of abdominal fat tissue is also negatively correlated with the fat pad weight ( $r=-0.31$ ,  $P<0.01$ ; Grunder and Chambers, 1988). The reason for the low VLDL birds having higher abdominal fat tissue LPL activity is not clear.

The specific LPL activities in heart and leg muscles from the lean birds were all higher than those from the fat birds. Because a large portion of the body mass is made up of muscle, the contribution of muscle LPL to the removal of plasma VLDL may be at least the same as that in adipose tissue, as reported for the rat (Borensztajn, 1987). The higher LPL activity in muscles from low-VLDL birds suggests that more VLDL triglyceride may be

hydrolyzed and used for oxidation in the muscles in low-VLDL birds. Part of the reason for accumulation of VLDL in the plasma in the high-VLDL line may be its lower muscle LPL activity.

Changes in the concentration of triglyceride-rich lipoproteins in the plasma may influence the pattern of distribution of circulating triglyceride to individual tissues. Fielding (1976) reported that the affinity of rat muscle LPL for lipoprotein substrates was considerably higher than that of adipose tissue LPL. This result suggests that when VLDL concentrations are low, VLDL triglyceride would be preferentially taken up by muscle; when plasma VLDL concentrations are high, the LPL in muscle would be saturated and a greater proportion taken up by adipose tissue. The results of the present study are at least consistent with this hypothesis.

## Chapter 6

### GENERAL DISCUSSION

Whether an indirect criterion can be used in breeding practice depends on it : 1) being effective - it must have a high heritability and a high genetic correlation with the trait to be improved, 2) being easy to measure, 3) having a low cost. Selection on the basis of plasma VLDL concentration was designed to be simple and rapid. Collection of blood samples is relatively easy and a development of the assay for plasma VLDL concentration gives a rapid analysis of 40 samples/h (Griffin and Whitehead, 1982). Since the samples are taken from live birds, the cost is obviously lower than that based on killing animals. The heritability of plasma VLDL concentration was estimated to be 0.5 in the first generation of the selection and the original phenotypic correlation between plasma VLDL concentration and body fat content was estimated to be 0.7 in the broiler strain at Roslin (Whitehead and Griffin, 1984). Similar phenotypic correlations between plasma VLDL concentration and fatness were found in the present study in broilers from a different genetic background.

The genetic correlation between plasma VLDL concentration and body fat content has not been estimated in the strains developed at Roslin. However, studies elsewhere appear to have substantiated the approach. For example, Garwood (1987) reported that both the heritability of plasma VLDL concentration and the genetic

correlation between plasma VLDL concentration and body fat content in Japanese Quail were over 0.5. Grunder and Chambers (1988) found that the heritability of plasma VLDL concentration was 0.25 and the genetic correlation between plasma VLDL and percentage abdominal fat weight was  $0.97 \pm 0.04$  in broilers. They had compared expected genetic gains from various types of selection at the same intensity of 1.4 (80% culled) to change abdominal fat percentage in broilers. The selection criteria were abdominal fat percentage, abdominal fat weight, body weight at 42 days, feed consumption, feed efficiency, plasma VLDL concentration, lipase activity expressed per mg of protein or per g of fat, and protein content of the enzyme preparation expressed as  $\mu\text{g}$  of protein/ml of enzyme preparation. The results showed that selection for low VLDL should be at least as effective as direct selection based on slaughtered sibs in reducing abdominal fat percentage.

However, because the selection at Roslin did not have a true nonselected control line for proper assessment of progress, the efficiency of the selection for reducing body fat content is difficult to evaluate. From the 4th generation to the 7th generation, the VLDL concentrations in the birds of the low VLDL line were 0.070, 0.068, 0.064 and 0.060 absorbance units; the total body fat contents were 137, 134, 126 and 134 g/kg (Whitehead, 1988). The selection seems to have reached a plateau. In contrast selection for high VLDL continued to make further progress.

The limit of the selection may be caused by changes in the heritability of plasma VLDL concentration and/or the genetic

correlation between VLDL and body fat content. The heritability of plasma VLDL concentration was estimated to be 0.50 from the birds in the first generation of the selection (Whitehead and Griffin, 1984), but it has not been re-estimated since then. The phenotypic correlation between plasma VLDL concentration and body fat content has dropped from 0.70 to 0.15 after selection for low VLDL for 7 generations (Whitehead, 1988) and this large change suggests that the genetic correlation of these two traits may also have decreased.

Since the function of plasma VLDL is to transport the lipid mainly synthesized in liver to extrahepatic organs to meet their physiological demands or for storage, it may be necessary to keep a certain secretion rate of VLDL for the growth of the birds. Continual selection for reduced plasma VLDL concentration may be alternatively opposed by natural selection, i.e. the selection may have reached a physiological limit.

Leclercq (1988) reported that direct selection against abdominal fat weight using sib analysis had not reached a plateau after 7 generations of selection. In this divergent selection experiment, the birds from the lean and fat lines showed differences in lipid metabolism. For example, the birds from the lean line had lower hepatic lipogenesis rates, less plasma VLDL, lower LPL activity per abdominal fat pad, a smaller volume of adipocytes and less adipocyte number in abdominal fat pad. In contrast after 7 generations of selection for plasma VLDL concentration, the low-VLDL birds (lean) had higher abdominal fat tissue LPL activity (expressed in both per gram tissue or per whole abdominal fat depot) than that

in high-VLDL birds (fat; Chapter 5). The limit of selection for low-VLDL in reducing body fat content may be partly a result of the lack of correlated response in LPL activity in adipose tissue.

As described previously, LPL-catalysed hydrolysis of circulating lipoproteins is an obligatory step in uptake of plasma triglyceride into tissues. However, the assay of tissue LPL activity in live birds can only be conducted using post-heparin plasma samples or tissue biopsy samples. In Chapter 3, a very low and non-significant correlation between post-heparin plasma LPL activity and body fat content was found. The correlation between LPL activity per gram of abdominal fat weight and fat pad weight was non-significant. Grunder and Chambers (1988) however reported that there was a significantly negative correlation between the lipase activity per gram of tissue and abdominal fat weight in the broilers developed in the Animal Research Centre, Canada. The difference in these correlations may be due to the different broiler strains used or a difference in the methods used for measuring lipase activity.

The major problem in assessing the functional LPL activity in vivo is in its measurement. It is generally considered that LPL activity in acetone-ether preparations represents the total tissue activity, i.e. both the intracellular and the extracellular pools, including the physiologically active fraction at the endothelial surface (Nilsson-Ehle et al., 1980). Upon incubation of tissue samples in a heparin-containing buffer, LPL is released into the medium, providing a convenient source for assay of enzyme activity. LPL activity in this preparation depends on the incubation

conditions. For example, it is virtually impossible to exclude losses of enzyme activity during incubation, or to conclusively demonstrate which pool(s) of enzyme is recovered in heparin eluates under various conditions (Nilsson-Ehle, 1987). In addition, there is limited knowledge of the extent of the heparin-releasable pool of enzyme and its relative importance to the in vivo function of the enzyme (Eckel, 1987).

Tissue LPL activity can also be expressed in different ways, i.e. per g wet weight, per mg protein, per  $\mu$ g DNA, per adipocyte, or per organ. The problem is to determine which method of expression most closely reflects the activity in vivo. Apparent discrepancies in the literature about the relationship between the tissue LPL activity and fat deposition might result from the methods of expression of enzyme activity. For example, Eckel (1987) found that, with one exception, all reports of heparin-releasable adipose tissue LPL in obese humans had demonstrated greater enzyme activity per cell compared to lean controls. When the data were expressed per g of adipose tissue, greater, lower, or similar enzyme activities had been found when compared to the lean controls. As a possible indicator to be used to predict fatness, the expression of LPL activity must have a consistent and significant correlation with body fat deposition. The results of this study show that such a correlation can not be achieved. New methods for the assay of LPL from which the results more closely represent the functional active LPL activity need to be developed.

Recently, Enerback et al. (1988) reported that the patterns of

guinea pig mRNA species differ between heart and adipose tissue. In heart, a 3.8 kb mRNA dominates but in adipose tissue, the LPL mRNAs of 3.3 and 2.1 kb occur in similar abundance in addition to the 3.8 kb species. This study suggested that LPL expression can be regulated at the level of mRNA content and this regulation is tissue specific. Their observations provide further evidence that LPLs from muscle and adipose tissue may have different isoenzymes. Chicken LPL from different tissues however could not be distinguished by their molecular weight and pI point in the present study (see Chapter 4). Isoelectric focusing is a sensitive and reproducible technique, particularly valuable in the separation of closely related proteins that may not be easily separated by other techniques (Divall, 1984). In Chapter 4, it was shown that LPLs could be separated into more than 10 species when the enzymes were focused and the LPL purified from different tissue were also separated into more than 10 species in a similar pattern. It is possible that the abundance of corresponding species of LPLs from different tissues is different. This possibility can not be confirmed or excluded at the moment, because little is known whether the species separated from the samples purified from different tissues have <sup>the</sup> same efficiency in electroblotting and immunostaining.

Selection for VLDL concentration is based on the importance of plasma VLDL as a precursor of fat deposition in broilers. In the present study, it was estimated that 55% of body fat deposition came from plasma lipoprotein triglyceride and 45% from adipose tissue lipogenesis. This indicated that adipose tissue lipogenesis is an important source for fat deposition in broilers. O'Hea and Leveille

(1969) reported that 90-95% of de novo fatty acid synthesis in the chick appeared to take place in the liver. However, they did not investigate what proportion of the fatty acid synthesized in liver could be incorporated into adipose tissue for storage. Saadoun and Leclercq (1983) investigated the lipogenesis of the birds at 5 weeks of age from the 6th generation of divergent selection for abdominal fat/body weight ratio. They found that abdominal fat lipogenesis in fat birds was greater than that in lean birds, but the importance of adipose tissue lipogenesis appeared to be underestimated. They observed that the total incorporation of  $^3\text{H}_2\text{O}$  into the liver of the lean and fat birds was 54,300 dpm and 73,300 dpm respectively. Corresponding radioactivity in the abdominal fat pad of the two lines was 700 and 1,760 dpm respectively. Although adipose tissue lipogenesis in the abdominal fat was only 1.3% and 2.4% of that in the liver, a small proportion of plasma VLDL triglyceride secreted by the liver is taken up by the abdominal fat pad (see Chapter 2). Assuming that all the fatty acid synthesized in the liver is secreted as plasma lipoprotein triglyceride and the proportion of plasma lipoprotein triglyceride incorporated into the abdominal fat pad in these two lines is similar to that in normal broilers (about 6%, see Chapter 2), then about 1/4 of the lipid deposited into abdominal fat pad is a consequence of lipogenesis in abdominal fat pad of these selected birds. This is a significant proportion.

In Chapter 2, it was estimated that abdominal fat tissue lipogenesis contributed 20% of the lipid deposited in this depot. It may be greater in other depots. However, the estimation of the

contribution of adipose tissue lipogenesis to the fat deposition was based on some important assumptions, i.e. that  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  incorporated into lipid was incorporated into fatty acids, and that all ether-extractable fat was in adipose tissue. The estimates need to be confirmed: for example, by determining the proportion of  $^3\text{H}$  from  $^3\text{H}_2\text{O}$  incorporated into fatty acid in adipose tissue rather than into total lipid.

If adipose tissue lipogenesis is important, it might be difficult to find any biochemical parameter which can be used in poultry breeding practice, although the sum of the activity of NADPH-generating enzymes has been used in pig breeding as a selection criterion to indirectly reduce the backfat thickness (Muller, 1986). It may prove easier to further improve on selection for VLDL, by measuring VLDL secretion rates, perhaps combined with beta-hydroxybutyrate, LPL activity or glucose (Leclercq et al., 1987). The relationship between LPL activity and body fat deposition in broilers is uncertain and new methods to measure functional LPL activity need to be developed, although it is difficult to imagine how this might be done. If progress in these areas were made, the limit in reducing body fat content in broilers by selection for low VLDL might be overcome.

Of all poultry species, fast growing ducks deposit the greatest amount of body fat. For example, the body fat content of Pekin ducks at 7 weeks of age is about 30% (males 35%, females 27%) of the body weight (Leeson, et al., 1982), whereas the body fat content in modern broilers usually contribute 15-20% of the total body weight

(Leenstra, 1986). High body fat content in ducks has seriously restricted the duck meat consumption and production. Fast growing ducks may be a particularly interesting system for research on the mechanisms of hepatic or adipose tissue lipogenesis and plasma lipoprotein metabolism. A large amount of body fat in ducks is deposited subcutaneously: skin and subcutaneous fat contributed 38% of body <sup>over ready</sup> weight in Pekin ducks at 48 days of age (Stadelman and Meinert, 1977). The relative importance of hepatic lipogenesis to adipose tissue lipogenesis in ducks may be different from that in broilers. This seems to be supported by the experimental observations that there was no correlation between VLDL concentration and body fat deposition in ducks (H.D. Griffin and J. Powell, personal communication). Selection for food conversion ratio has been used in duck breeding to reduce body fat content (Powell, 1984) but a biochemical criterion cannot be used in ducks until we have a better understanding of lipid metabolism. Research on duck lipid metabolism and the reduction of body fat content in ducks particularly interest the author of this thesis, the results of research on broilers at Roslin should be a useful guide to tackling the problem with ducks.

## REFERENCES

- Becker, W.A., Spencer, J.V., Mirosh, L.W. and Verstrate, J.A. (1979) Prediction of fat and fat free live weight in broiler chickens using backskin fat, abdominal fat and live body weight. *Poultry Science* 58: 835-842
- Becker, W.A., Spencer, J.V., Mirosh, L.W. and Verstrate, J.A. (1984) Genetic variation of abdominal fat, body weight and carcass weight in a femal broiler line. *Poultry Science* 63: 607-611
- Beisiegel, U. (1986) Protein blotting. *Electrophoresis* 7: 1-18
- Bengtsson, G. and Olivecrona, T. (1977) Does lipoprotein lipase bind Ampholytes? In 'Electrofocusing and Isotachopheresis' (eds. Radola, B.J. and Graesslin, D.), pp. 189-195, Walter de Gruyter, Berlin
- Bensadoun, A. and Rothfeld, A. (1972) The form of absorption of lipids in the chicken, Gallus domesticus. *Proc. Soc. Exp. Biol. Med.* 141: 814-817
- Bensadoun, A., Ehrholm, C., Steinberg, D. and Brown, W.V. (1974) Purification and characterization of lipoprotein lipase from pig adipose tissue. *J. Biol. Chem.* 249: 2220-2227
- Bensadoun, A. and Kompiang, I.P. (1979) Role of lipoprotein lipase in plasma triglyceride removal. *Federation Proceedings* 383: 2622-2626
- Benson, J.D. and Bensadoun, A. (1977) Response of adipose tissue lipoprotein lipase to fasting in the chicken and rat - a species difference. *Journal of Nutrition* 107: 990-997
- Benson, J.D., Hearn, V., Boyd, T. and Bensadoun, A. (1975) Triglyceride hydrolase of chicken and rat pre-and post-heparin plasma. Effect of fasting and comparison with adipose tissue lipoprotein lipase. *Int. J. Biochem.* 6: 727-734
- Bentsen, H.B. and Sehested, E. (1986) Computerized tomograph of chichens. *Proceedings of 28th British Poultry Breeders Roundtable conference.*
- Ben-Zeev, O., Lusic, A.J., LeBoeuf, R.C., Nikazy, J. and Schotz, M.C. (1983) Evidence for independent genetic regulation of heart and adipose lipoprotein lipase activity. *J. Biol. Chem.* 258: 13632-13636

- Borensztajn, J. (1987) Heart and skeletal muscle lipoprotein lipase. In 'Lipoprotein Lipase' (ed. Borensztajn, J.), pp. 133-148, Everner, Chicago
- Borron, D.C., Jensen, L.S., McCartney, J.C. and Britton, W.M. (1979) Comparison of lipoprotein lipase activities in chickens and turkeys. *Poultry Science* 58: 659-662
- Boulangé, A., Planche, E. and de Gasquet, P. (1979) Onset of genetic obesity in the absence of hyperphagia during the first week of life in Zucker rat (fa/fa). *Journal of Lipid Research* 20: 857-846
- Boulangé, A., Planche, E. and de Gasquet, P. (1981) Onset and development of hypertriglyceridemia in the Zucker rat (fa/fa). *Metabolism* 30: 1045-1052
- Bowyer, D.E., Cridland, J.S. and King, J.P. (1978) A novel, semiautomated method for the estimation of free fatty acid in serum or plasma. *J. of Lipid Research* 10: 274-280
- Brady, L., Romsos, D.R. and Leveille, G.A. (1976) In vivo estimation of fatty<sub>3</sub> acid synthesis in chicken (*Gallus domesticus*) utilizing H<sub>2</sub>O. *Comp. Biochem. Physiol.* 54 B: 403-407
- Brockerhoff, H. and Jensen, R.G. (1974) *Lipolytic Enzymes*. Academic Press, New York
- Cahaner, A. and Nitsan, Z. (1985) Evaluation of simultaneous selection for live body weight and against abdominal fat in broilers. *Poultry Science* 64: 1257-1263
- Cahaner, A., Krinsky, M. and Nitsan, Z. (1985) The response to one cycle of divergent selection for abdominal fat in broilers raised under different conditions. *Poultry Science* 64: 1813-1820
- Campbell, R.G., Karunajeewa, H. and Bagot, I. (1985) Influence of food intake and sex on the growth and carcass composition of Pekin ducks. *British Poultry Science* 26: 43-50
- Chajek-Shaul, T., Friedman, G., Stein, O., Etienne, J. and Stein, Y. (1985) Endogenous plasma lipoprotein lipase activity in fed and fasting rats may reflect the functional pool of endothelial lipoprotein lipase. *Biochim. Biophys. Acta* 837: 271-278
- Chambers, J.R., Gavora, J.S. and Fortin, A. (1981) Genetic changes in meat-type chickens in the last twenty years. *Can. J. Anim. Sci.* 21: 555-563
- Cheng, C.F., Bensadoun, A., Bersot, T., Hsu, J.S.T. and Melford, K.H. (1985) Purification and characterization of human lipoprotein lipase and hepatic triglyceride lipase. *J. Biol.*

Chem. 260: 10720-10727

- Cherry, J.A., Siegel, P.B. and Beane, W.J. (1978) Genetic-nutritional relationships in growth and carcass characteristics of broiler chickens. *Poultry Science* 57: 1482-1487
- Cryer, A. (1981) Tissues lipoprotein lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* 13: 525-541
- De Gasquet, P. and Pequignot (1972) Lipoprotein lipase activities in adipose tissues, heart, and diaphragm of the genetically obese mouse (ob/ob). *Biochem. J.* 127: 445-447
- Divall, G.B. (1984) Isoelectric focusing in ultrathin polyacrylamide gels. in 'Methods in Molecular Biology', Volume I, Proteins. (ed. Walker, J.M.), pp. 105-111, Humana Press Inc, Clifton, New Jersey
- Eckel, R.H. (1987) Adipose tissue lipoprotein lipase. In 'Lipoprotein Lipase' (ed. Borensztajn, J.), pp. 79-132, Everner, Chicago
- Edwards, H.M., Jr. and Denman, F. (1975) Carcass composition studies 2. influences of breed, sex and diet on gross composition of the carcass and fatty acid composition of the adipose tissue. *Poultry Science* 54: 1230-1238
- Egelrud, T. (1973) Reversible binding of lipoprotein lipase from hen adipose tissue to insolubilized heparin. *Biochim. Biophys. Acta* 296: 124-129
- Egelrud, T. and Olivecrona, T. (1972) The purification of a lipoprotein lipase from bovine skim milk. *J. Biol. Chem.* 252: 995-962
- Enerback, S., Semb, H., Tavernier, J., Bjursell, G. and Olivecrona, T. (1988) Tissue-specific regulation of guinea pig lipoprotein lipase: effects of nutritional state and of tumor necrosis factor on mRNA levels in adipose tissue, heart and liver. *Gene* 64: 97-106
- Enser, M. (1972) Clearing-factor lipase in obese hyperglycaemic mice (ob/ob). *Biochem. J.* 129: 447-453
- Evans, A.J. (1972) Lipoprotein lipase activity in adipose tissue of the domestic duck: the effect of age, sex and nutritional state. *International Journal of Biochemistry* 4: 199-206
- Fielding, C.J. (1976) Lipoprotein lipase: evidence for high- and low-affinity enzyme site. *Biochemistry* 15: 879-884
- Fielding, C.J. (1978) Origin and Properties of remnant lipoproteins. In 'Disturbances in Lipid and Lipoprotein

- Metabolism' (eds. Dietschy, J.M., Gotto, Jr., A.M. and Ontko, J.A.), pp. 83-97, American Physiological Society, Bethesda, Maryland
- Fielding, P.E., Shore, V.G. and Fielding, C.J. (1974) Lipoprotein lipase: properties of the Enzyme isolated from post-heparin plasma. *Biochemistry* 13: 4318-4323
- Fielding, P.E., Shore, V.G. and Fielding, C.J. (1977) Lipoprotein lipase: isolation and characterization of a second enzyme species from postheparin plasma. *Biochemistry* 16: 1896-1900
- Folch, H., Lees, M. and Sloane-Stanley, G.H. (1975) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 497-506
- Friars, G.W., Lin, C.Y., Patterson, D.L. and Irwin, L.N. (1983) Genetic and phenotypic parameters of fat deposition and associated traits in broilers. *Poultry Science* 62: 1425 (abstr.)
- Friedman, G., Chajek-Shaul, T., Etienne, J., Stein, O. and Stein, Y. (1986) Characterization of the lipoprotein lipase in the functional pool of rat heart by immunoblotting. *Biochim. Biophys. Acta* 875: 397-399
- Garwood, V.A. (1987) Estimates of heritability for plasma very low density lipoprotein concentration and genetic correlations with lipid content, body volume, and density in Coturnix coturnix japonica. *Poultry Science* 66: 1272-1275
- Gershenwald, J.E., Bensadoun, A. and Saluja, A. (1985) Monoclonal antibodies to avian lipoprotein lipase. Purification of the enzyme by immunoaffinity chromatography. *Biochim. Biophys. Acta* 836: 286-295
- Gianazza, E. and Righetti, P.G. (1980) Facts and artefacts in isoelectric focusing. In "Electrophoresis" 79 (Radola, B.J. ed.), pp. 129-140, Walter de Gruyter, Berlin, New York
- Goodbole, V. and York, D.A. (1978) Lipogenesis in situ in the genetically obese Zucker fatty rat (fa/fa): role of hyperphagia and hyperinsulinaemia. *Diabetologia* 14: 191-197
- Goodwin, T.L., Andrews, L.D. and Webb, J.E. (1969) The influence of age, sex, and energy level on the tenderness of broilers. *Poultry Science* 48: 548-552
- Greten, H. and Walter, B. (1973) Purification of adipose tissue lipoprotein lipase. *FEBS LETTERS* 35: 36-40
- Griffin, H.D. and Whitehead, C.C. (1982) Plasma lipoprotein concentration is an indicator of fatness in broilers: development and use of a simple assay for plasma very low

- density lipoproteins. *British Poultry Science* 23: 307-313
- Griffin, H.D. and Whitehead, C.C. (1985) Identification of lean or fat turkeys by measurement of plasma very low density lipoprotein concentration. *British Poultry Science* 26: 51-56
- Griffin, H.D. and Butterwith, S.C. (1988) Effects of *Escherichia Coli* endotoxin on tissue lipoprotein lipase activities in chickens. *British Poultry Science* 29: 379-386
- Griffin, H.D. and Hermier, D. (1988) Plasma lipoprotein metabolism and fattening in poultry. In 'Leanness in Domestic Birds' (eds. Leclercq, B. and Whitehead, C.C.), pp. 175-201, Butterworths, London
- Griffin, H.D., Whitehead, C.C. and Brodbent, L.A. (1982) The relationship between plasma triglyceride concentrations and body fat content in male and female groilers - a basis for selection? *British Poultry Science* 23: 15-23
- Griffin, H.D., Butterwith, S.C. and Goddard, C. (1987) Contribution of lipoprotein lipase to differences in fatness between broiler and layer-strain chicks. *British Poultry Science* 28: 197-206
- Griffiths, L., Leeson, S. and Summers, J.D. (1978) Studies on abdominal fat with four commercial strains of male broiler chicken. *Poultry Science* 57: 1198-1203
- Gruen, R., Hietanen, E. and Greenwood, M.R.C. (1978) Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa). *Metabolism* 27(Suppl. 2): 1955-1966
- Grunder, A.A. and Chambers, J.R. (1988) Genetic parameters of plasma very low density lipoproteins, abdominal fat lipase, and protein, fatness, and growth traits of broiler chickens. *Poultry Science* 67: 183-190
- Hausman, G.J. and Martin, R.J. (1981) Subcutaneous adipose tissue development in Yorkshire (lean) and Ossaban (obese) pigs. *J. Animal Science* 52: 1442-1449
- Hausman, G.J., Campion, D.R. and Thomas, G.B. (1983) Adipose tissue cellularity and histochemistry in fetal swine as affected by genetic selection for high or low backfat. *Journal of Lipid Research* 24: 223-228
- Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34: 1345-1353
- Heath, J.R., Covey, R.C. and Owens, S.L. (1980) Abdominal leaf fat separation as a result of evisceration of broiler carcasses.

Poultry Science 59: 2456-2461

- Hood, R.L. (1982) The cellular Basis for growth of the abdominal fat pad in broiler type-chickens. Poultry Science 61: 117-121
- Hood, R.L. and Pym, R.A.E. (1982) Correlated responses for lipogenesis and adipose tissue cellularity in chickens selected for body weight gain, food consumption, and food conversion efficiency. Poultry Science 61: 122-127
- Hunt, J.R. (1965) Factors influencing body N : H<sub>2</sub>O ratio of growing chicks. Poultry Science 44: 236-240
- Husbands, D.R. (1972) The distribution of lipoprotein lipase in the tissues of the domestic fowl and the effects of feeding and starving. British Poultry Science 13: 85-90
- Iverius, P-H. and Ostlund-Lindqvist, A-M. (1976) Lipoprotein lipase from bovine milk. J. Biol. Chem. 251: 7791-7795
- Kompiang, I.P., Bensadoun, A. and Yang, M.W.W. (1976) Effect of an anti-lipoprotein lipase serum on plasma triglyceride removal. J. Lipid Research 17: 498-505
- Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227: 680-685
- Leclercq, B. (1983) The influence of dietary protein content on the performance of genetically lean or fat growing chickens. British Poultry Science 24: 581-587
- Leclercq, B. (1984) Adipose tissue metabolism and its control in birds. Poultry Science 63: 2044-2054
- Leclercq, B. (1988) Genetic selection of meat type chicken for high or low abdominal fat content. In 'Leanness in Domestic Birds' (ed. Leclercq, B Whitehead, C.C.), pp25-40, Butterworths, London.
- Leclercq, B., Blum, J.C. and Boyer, J.P. (1980) Selecting broilers for low or high abdominal fat: initial observations. British Poultry Science 21: 107-113
- Leclercq, B., Simon, J. and Ricard, F.H. (1987) Effects of selection for high and low plasma glucose concentration in chickens. British Poultry Science 28: 557-565
- Leenstra, F.R. (1986) Effect of age, sex, genotype and environment on fat deposition in broiler chickens. World's Poultry Science Journal 42: 12-22
- Leenstra, F.R. and Pit, R. (1987) Fat deposition in a broiler sire strain. 2. Comparisons among lines selected for less abdominal fat, lower feed conversion ratio, and higher body weight after

- restricted and ad libitum feeding. *Poultry Science* 66: 193-202
- Leenstra, F.R. and Pit, R. (1988) Fat deposition in a broiler sire strain. 3. Heritability of and genetic correlations among body weight, abdominal fat, and feed conversion. *Poultry Science* 67: 1-9
- Leeson, S., Summers, J.D. and Proulx, J. (1982) Production and carcass characteristics of the duck. *Poultry Science* 61: 2456-2462
- Legros, M. and Kepes, A. (1985) One-step fluorometric microassay of DNA in procaryotes. *Analytical Biochemistry* 147: 497-502
- Leveille, G.A., Romsos, D.R., Yeh, Y.-Y. and O'Hea, E.K. (1975) Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. *Poultry Science* 54: 1075-1093
- Lilburn, M.S., Leach, R.M., Jr., Buss, E.G. and Martin, R.J. (1982) The developmental characteristics of two strains of chickens selected for differences in mature abdominal fat pad size. *Growth* 46: 171-181
- Lin, C.Y., Friars, R.M. and Moran, E.T. (1980) Genetics and environmental aspects of obesity in broilers. *World's Poultry Science Journal* 36: 103-111
- Linder, C., Chernik, S.S., Fleck, T.R. and Scow, R.O. (1976) Lipoprotein lipase and uptake of chylomicron triglyceride by skeletal muscle of rats. *American Journal of Physiology* 231: 860-864
- Littlefield, L.H. (1972) Strain difference in quantity of abdominal fat in broilers. *Poultry Science* 51: 1829 (abstr.)
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin reagent. *Journal of Biological Chemistry* 193: 265-275
- March, B.E. and Hansen, G. (1977) Lipid accumulation and cell multiplication in adipose bodies in White Leghorn and broiler-type chicks. *Poultry Science* 56: 886-894
- McCarthy, J.C. and Siegel, P.B. (1983) A review of genetical and physiological effects of selection in meat-type poultry. *Animal Breeding Abstracts* 51: 87-94
- McDuffie, N.M., Dietrich, C.P. and Nader, H.B. (1975) Electrofocusing of heparin: fractionation of heparin into 21 components distinguishable from other acidic mucopolysaccharides. *Biopolymers* 14: 1473-1486
- McNamara, J.P., and Martin, R.J. (1982) Muscle and adipose tissue

lipoprotein lipase in fetal and neonatal swine as affected by genetic selection for high or low backfat. *J. Animal Science* 55: 1057-1061

- McNamara, J.P., Azain, M., Kasser, T.R. and Martin, R.J. (1982) Lipoprotein lipase and lipid metabolism in muscle and adipose tissues of Zucker rats. *Amer. J. Physiol.* 243 12R: 258-264
- Merkley, J.W., Littlefield, L.H., Malone, G.W. and Chaloupka, G.W. (1977) Fresh eviscerated yield of five commercial broiler strains. *Poultry Science* 56: 1738 (abstr.)
- Moran, E.T., Orr, H.L. and Larmond, E. (1970) Dressing, grading and meat yielding with broiler chicken breed. *Fd. Technol. Champaign.* 24-73
- Muller, E. (1986) Physiological and biochemical indicators of growth and composition. In 'Exploiting New Techniques in Animal Breeding' (eds. Smith, C., King, I.W.B. and Mekay, J.C.), pp. 132-139, Oxford Science Publications, Oxford
- Nader, H.B., McDuffie, N.M. and Dietrich, C.P. (1974) Heparin fractionation by electrofocusing: presence of 21 components of different molecular weight. *Biochemical and Biophysical Research Communications* 57: 488-493
- Nilsson-Ehle, P. (1987) Measurement of lipoprotein lipase activity. In 'Lipoprotein Lipase' (ed, Borensztajn, J.), pp. 59-78, Evener, Chicago
- Nilsson-Ehle, P., Garfinkel, A.S. and Schotz, M.C. (1980) Lipolytic enzymes and plasma lipoprotein metabolism. *Ann. Rev. Biochem.* 49: 667-693
- Nordstorm, J.O. (1978) Influence of genetic strain, sex, and dietary energy level on abdominal fat deposition in broilers. *Poultry Science* 57: 1176 (abstr.)
- O'Hea, E.K. and Leveille, G.A. (1969) Lipid biosynthesis and transport in the domestic chick (Gallus Domesticus). *Comp. Biochem. Physiol.* 30: 149-159
- Olivecrona, T. and Bengtsson-Olivecrona (1987) Lipoprotein lipase from milk—the model enzyme in lipoprotein lipase research. in *Lipoprotein lipase* (ed. Borensztajn, J.), pp. 15-58, Evener, Chicago
- Olivecrona, T., Bengtsson, G., Marklund, S-E., Lindahl, U. and Hook, M. (1977) Heparin-lipoprotein lipase interactions. *Fedn. Prod. Fedn. Am. Soc. Exp. Biol.* 31: 60-65
- Ostlund-Lindqvist, A-M. (1979) Properties of salt-resistant lipase and lipoprotein lipase purified from human post-heparin plasma. *Biochem. J.* 179: 555-559

- Ostlund-Lindqvist, A-M. and Boberg, J. (1977) Purification of salt resistant lipase and lipoprotein lipase from human post-heparin plasma. FEBS LETTERS 83: 321-236
- Pedersen, M.E., Cohen, M. and Schotz, M.C. (1983) Immunocytochemical localization of the functional fraction of lipoprotein lipase in the perfused heart. Journal of Lipid Research 24: 512-518
- Peterson, J., Olivecrona, T. and Bengtsson-Olivecrona, G. (1985) Distribution of lipoprotein lipase and hepatic lipase between plasma and tissues: effect of hypertriglyceridemia. Biochimica et Biophysica Acta 837: 262-270
- Pfaff, F.E., Benson, J.D. and Austic, R.E. (1977) Influence of diet on adiposal lipoprotein lipase and hepatic triglyceride synthetase activities in the developing pullet (Gallus Domesticus). Comp. Biochem. Physiol. 58B: 345-348
- Powell, J.C. (1984) Selection for improved feed conversion ratio in the domestic ducks. Proceeding and Abstracts of World's XVII Poultry Congress, pp. 108-109, Helsinki
- Pym, R.A.E. and Nicholls, P.J. (1979) Selection for food conversion in broilers: direct and correlated responses to selection for body weight gain, food consumption and food conversion ratio. British Poultry Science 20: 73-86
- Pym, R.A.E. and Solvyns, A.J. (1979) Selection for food conversion in broilers: body composition of birds selected for increased body-weight gain, food consumption and food conversion ratio. British Poultry Science 20: 87-97
- Pym, R.A.E. and Thompson, J.M. (1980) A simple caliper technique for the estimation of abdominal fat in live broilers. British Poultry Science 21: 281-286
- Righetti, P.G. and Gianazza, E. (1978) Isoelectric focusing of heparin: evidence for complexing with carrier ampholytes. Biochimica et Biophysica Acta 532: 137-146
- Saadoun, A. and Leclercq, B. (1983) Comparison of in vivo fatty acid synthesis of the genetically lean and fat chickens. Comp. Biochem. Physiol. 75B: 641-644
- Saadoun, A. and Leclercq, B. (1986) In vivo lipogenesis of genetically lean and fat chickens of various ages. Comp. Biochem. Physiol. 83B: 607-611
- Semb, H. and Olivecrona, T. (1986) Lipoprotein lipase in guinea pig tissues: molecular size and rate of synthesis. Biochim. Biophys. Acta 878: 330-337
- Shen, B.N., Scanu, A.M. and Kezdy, F.J. (1977) Structure of human

- serum lipoproteins inferred from composition analysis. Proc. Natl. Acad. Sci. USA 74: 838-841
- Simon, J. and Leclercq, B. (1982) Longitudinal study of adiposity in chickens selected for high or low abdominal fat content: further evidence of a glucose-insulin imbalance in the fat line. J. Nutr. 112: 1961-1973
- Sklan, D., Geva, A., Budowsky, P. and Hurwitz, S. (1984) Intestinal absorption and plasma transport of lipids in chicks and rats. Comparative Biochemistry and Physiology 78A: 507-510
- Smith, L.C., Powmall, H.J., and Gotto, Jr., A.M. (1978) The plasma lipoproteins: structure and Metabolism. Ann. Rev. Biochem. 47:751-777
- Sniderman, A.D., Carew, T.E., and Steinberg, D. (1975) Turnover and tissue distribution of  $^{125}\text{I}$ -labeled low density lipoprotein in swine and dogs. J. Lipid Research 16: 293-298
- Socorro, L. and Jackson, R.L. (1985) Monoclonal antibodies to bovine milk lipoprotein lipase. J. Biol. Chem. 260: 6324-6328
- Sorenson, P. (1984) Estimation of abdominal fat in live chickens by palpation. Proceeding and abstracts of XVII World's Poultry Congress, pp. 185-186, Helsinki
- Sprecher, D.L., Taam, L. and Brewer, Jr., A.B. (1984) Two-dimensional electrophoresis of human plasma apolipoproteins. Clinical Chemistry 30/12: 2084-2092
- Stadelman, W.J. and Meinert, C.F. (1977) Some factors affecting meat yield from young ducks. Poultry Science 56: 1145-1147
- Sutherland, M.M. and Skerritt, J.H. (1986) Alkali enhancement of protein staining on nitrocellulose. Electrophoresis 7: 401-406
- Taskinen, M-R, (1987) Lipoprotein lipase in hypertriglyceridemias. in Lipoprotein lipase (ed. Borensztajn, J.), pp. 201-228, Everner, Chicago
- Thomas, C.H., Glazener, E.W., and Blow, W.L. (1958) The relationship between feed conversion and ether extract of broilers. Poultry Science 37: 1177-1179
- Voyta, J.C., Via, D.P., Kinnunen, P.K.J., Sparrow, J.T., Gotto, Jr., A.M. and Smith, L.C. (1985) Monoclonal antibodies against bovine milk lipoprotein lipase. J. Biol. Chem. 260: 893-898
- Vydelingum, N.A., AlQuadan, F., Kissebah, A.H. and Etienne, J. (1986) Characterization of rat adipose tissue lipoprotein lipase using a monospecific antibody. Biochim. Biophys. Acta 876: 339-412

- Washburn, K.W., Guil, R.A. and Edwards, Jr., H.M. (1975) Influence of genetic difference in feed efficiency on carcass composition of young chickens. *J. Nutr.* 105: 1311-1317
- Whitehead, C.C. (1986) Nutrition effects of fat in broilers. *Poultry-Misset* February 24-25
- Whitehead, C.C. (1988) Selection for leanness in broilers using plasma lipoprotein concentration as selection criterion. In 'Leanness in Domestic Birds' (eds. Leclercq, B. and Whitehead, C.C.), pp 47-51, Butterworths, London
- Whitehead, C.C. and Griffin, H.D. (1982) Plasma lipoprotein concentration as an indicator of fatness in broilers: effect of age and diet. *British Poultry Science* 23: 299-305
- Whitehead, C.C. and Griffin, H.D. (1984) Development of divergent lines of lean and fat broilers using plasma very low density lipoprotein concentration as selection criterion: the first three generations. *British Poultry Science* 25: 573-582
- Whitehead, C.C., Hood, R.L., Heard, G.S. and Pym, R.A.E. (1984) Comparison of plasma very low density lipoproteins and lipogenic enzymes as predictors of body fat content and food conversion efficiency in selected lines of broiler chickens. *British Poultry Science* 25: 277-286

## PUBLISHED WORK

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## BIOCHEMICAL INDICATORS OF FATNESS IN MEAT-TYPE CHICKENS: LACK OF CORRELATION BETWEEN LIPOPROTEIN LIPASE ACTIVITY IN POST-HEPARIN PLASMA AND BODY FAT

K. GUO, H. D. GRIFFIN AND S. C. BUTTERWITH

*AFRC Institute for Grassland and Animal Production, Poultry Department, Roslin, Midlothian EH25 9PS, Scotland*

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- Abstract**
1. Possible relationships between fatness and lipoprotein lipase activity in adipose tissue and plasma from heparinised birds were examined in 7-week-old male and female broilers.
  2. Total lipoprotein lipase activity in abdominal fat was significantly correlated ( $r=0.5$ ) with fat pad weight, but there was no correlation between specific activity of the enzyme and fat pad weight.
  3. Lipoprotein lipase activity in post-heparin plasma showed no correlation with either abdominal fat or total body fat content.
  4. The results indicate that measurements of lipoprotein lipase activity in biopsy samples or in post-heparin plasma are of no value in predicting the fat content of live birds.

### INTRODUCTION

Excessive fatness is a problem in modern strains of meat-type (broiler) chickens, as in other species of livestock. Fatness in chickens is variable and highly heritable ( $h^2 \sim 0.5$ ) and a relatively rapid reduction in body fat content could be achieved by genetic selection if body composition could be reliably estimated in live birds. Unfortunately the physical methods used to estimate body fat content in other livestock species (for example, ultrasonics) have so far not proved applicable to broilers. Selection on the basis of biochemical variables correlated with body fat content represent an alternative approach.

Avian adipocytes have a limited capacity for lipogenesis and much of the fat that is deposited in the adipose tissue of broilers is synthesised in the liver (Saadoun and Leclercq, 1983) or derived from the diet. Most of the triglyceride in broiler plasma is present in very-low-density lipoproteins (VLDL) of hepatic origin and we have shown that the concentration of VLDL in the plasma of fully fed broiler chickens is sufficiently well correlated ( $r$  up to 0.7) with body fat content to be used as an indirect measure of fatness (Griffin *et al.*

Requests for reprints should be addressed to Dr Griffin.

1982; Whitehead and Griffin, 1982). Divergent selection for plasma VLDL concentration in a commercial broiler grandparent strain has produced lines with markedly different body composition and the leaner, low VLDL line has a considerably improved efficiency of food and protein conversion (Whitehead and Griffin, 1984).

Uptake of plasma triglyceride into extra-hepatic tissues is mediated by lipoprotein lipase. Part of the activity of this enzyme is present at the capillary bed and catalyses the hydrolysis of circulating portomicrons and VLDL. The fatty acids released then enter the surrounding cells to be re-esterified or oxidised. Lipoprotein lipase is anchored to the endothelial cell wall by attachment to heparin or heparin-like compounds and the enzyme can be released from this site into the general circulation by intravenous injection of heparin (Cryer, 1981; Pedersen *et al.*, 1983).

The activity of lipoprotein lipase in the abdominal fat pad of broilers is up to 24-fold greater than in chicks of a layer strain and this high activity appears to be an important contributor to the very rapid growth of the abdominal fat pad in broilers (Griffin *et al.*, 1987). In this paper we have examined the extent of variation of adipose tissue lipoprotein lipase activity within a commercial broiler population and its relationship with fatness and investigated whether such variation might be determined *in vivo* by measurement of lipoprotein lipase activity of biopsy samples or from post-heparin plasma.

#### MATERIALS AND METHODS

This study consisted of 3 parts. In experiment 1 we examined the relationships between lipoprotein lipase activity in the abdominal fat pad of female broilers at 7 weeks of age and the weight of their abdominal fat. In experiment 2 we investigated potential relationships between lipoprotein lipase activity in post-heparin plasma and fatness in both male and female broilers at about 7 weeks of age. In experiment 3 we attempted to determine the relative contribution of tissues to post-heparin plasma lipoprotein lipase activity in 6-week-old female broilers.

#### *Birds*

The birds used in all experiments were from a commercial broiler strain obtained from D. B. Marshall, Newbridge, Scotland, UK. Chicks were reared from 1-d-old to 6 or 7 weeks of age in cages with a 14 h light : 10 h dark cycle with lights on at 07.00 h. The birds were fed on standard broiler growing diets appropriate for their age and containing a calculated 230 g crude protein and 12.55 MJ metabolisable energy/kg. Food and water were available *ad libitum*.

#### *Blood samples*

Blood was removed from the wing-vein and the sodium salt of EDTA

(2 mg/ml of blood) was used as anti-coagulant. Plasma was prepared by centrifugation at 1000 g for 10 min and stored at  $-70^{\circ}\text{C}$ .

#### *Measurement of post-heparin lipase activity*

Heparin (1000 units (U)/kg of body weight) was injected into the wing vein in NaCl solution (0.154 mol/l) and blood was sampled 2 min later. This protocol was used because preliminary studies showed that intravenous injection of heparin produced a very rapid appearance of lipase activity in the plasma: activity reached a maximum after only 1 min and there was little further change over the next 10 min. The activity in the plasma tended to increase with the amount of heparin injected but maximum release was obtained by injection of 500 to 1000 U/kg of body weight. Very similar results were obtained by Benson *et al.* (1975) in 6 to 12 month old White Leghorn male chickens.

Lipase activity in post-heparin plasma was assessed by measuring the rate of release of fatty acid from activated Intralipid (Kabi Vitrum, Stockholm, Sweden) as described by Griffin *et al.* (1987). The contribution of lipoprotein lipase to total lipase activity was determined by immunoprecipitation with an anti-lipoprotein lipase antiserum. This was raised in sheep by repeated injection of lipoprotein lipase purified from broiler adipose tissue by heparin-affinity and concanavalin-A-affinity chromatography (Butterwith and Griffin, unpublished). Samples (100  $\mu\text{l}$ ) of post-heparin plasma or extracts of acetone-ether powders were incubated for 30 min at  $4^{\circ}\text{C}$  with an equal volume of sheep antiserum diluted 20-fold with NaCl solution (0.154 mol/l). Precipitation of the enzyme-antibody complex was encouraged by addition of 10  $\mu\text{l}$  of a donkey anti-sheep IgG antiserum (Scottish Antibody Production Unit, Carlisle, Scotland) and incubation for a further 30 min at  $4^{\circ}\text{C}$ . Precipitated enzyme was removed by centrifugation for 20 min at 3000 g and  $4^{\circ}\text{C}$  and lipase activity was determined in the supernatant.

#### *Measurement of tissue lipoprotein lipase activities*

Birds were killed by rapid intravenous injection of sodium phenobarbitone (Exprial, Abbott Laboratories, Queenborough, Kent, U.K.). Tissues were rapidly removed, washed in ice-cold saline, frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . The whole abdominal fat pad and samples of heart, thigh muscle and liver were homogenised in water containing 20  $\mu\text{g}$  heparin/ml at  $0^{\circ}\text{C}$  (10 ml/g of tissue) using a Polytron homogeniser (Kinematica, Lucerne, Switzerland). Acetone-ether powders were prepared from duplicate 2 ml aliquots of homogenates and samples of homogenates were retained for determination of tissue protein and DNA content. Full details of methods used to prepare acetone powders and to measure their lipoprotein lipase activity are described by Griffin *et al.* (1987).

Briefly, acetone powders were prepared by homogenising the abdominal fat pad in water and precipitation of lipoprotein lipase by acetone in the

presence of bovine serum albumin as a protein carrier. Extracts of the acetone powder were assayed using an activated Intralipid substrate.

### *Carcase analysis*

Total body fat content of carcasses were determined by extraction with petroleum ether (bp 40 to 60°C) as described by Griffin *et al.* (1982).

### *Other assays*

Protein in extracts of acetone-ether powders was determined according to the method described by Lowry *et al.* (1951) using bovine serum albumin as standard. DNA was assayed using a fluorometric microassay method (Legros and Kepes, 1985). Plasma VLDL concentration was measured turbidimetrically (Griffin and Whitehead, 1982) and expressed as absorbance units (A).

## RESULTS AND DISCUSSION

### *Experiment 1*

The variation in specific and total activities of lipoprotein lipase in the abdominal fat of female broilers at 7 weeks of age was high (Table 1). Total lipase activity in abdominal fat was significantly correlated with fat pad weight, but correlations between abdominal fat and specific activity of lipoprotein lipase were low and not significant.

TABLE 1

*Correlations between adipose tissue lipoprotein lipase activity, plasma VLDL concentration and abdominal fat pad weight in female broilers at about 7 weeks of age*

	Mean	Coefficient of variation (%)	Correlations ( $r$ ) with	
			Abdominal fat (g)	Mean plasma VLDL concentration
Body weight (g)	2076	10		
Abdominal fat (g)	42.5	27		
Lipoprotein lipase:				
$\mu$ moles/h/fat pad	3308	42	0.52**	-0.08
$\mu$ moles/h/g of tissue	78	34	-0.18	-0.20
$\mu$ moles/h/mg of protein	9.7	26	-0.01	-0.17
$\mu$ moles/h/ $\mu$ g of DNA	0.39	39	-0.13	-0.10

Blood samples were taken for measurement of plasma VLDL concentration from 36 female broilers at 47 and 49 d of age. Birds were killed at 51 d of age.

\*\*  $P < 0.01$ .

### *Experiment 2*

Individual variation in post-heparin lipase activity was also high, but showed no correlation with abdominal fat pad weight or total body fat content

in either males or female broilers (Table 2). More than 0.95 of the lipase in post-heparin plasma was precipitated with the anti-lipoprotein lipase antiserum, so the post-heparin plasma of broilers therefore contained little hepatic lipase. Similarly low concentrations of hepatic lipase were found in chicken plasma by Benson *et al.* (1975).

TABLE 2

*Correlations between body weight, body fatness, plasma VLDL concentrations and lipoprotein lipase activity in post-heparin plasma in male and female broilers at about 7 weeks of age*

	Mean	Coefficient of variation (%)	Body weight (g)	Correlations (r) with		
				Abdominal fat (g)	Body fat (g/kg)	Plasma VLDL(A)
<b>Females</b>						
Body weight (g)	1944	11	...	0.50**	0.32*	0.55**
Abdominal fat (g)	39.6	31	...	...	0.80**	0.69**
Body fat (g/kg)	143	14	...	...	...	0.58**
Plasma VLDL (A)	0.195	28	...	...	0	...
Post-heparin lipase ( $\mu$ moles/ml/h)	65.7	26	-0.03	0.04	0	-0.03
<b>Males</b>						
Body weight (g)	2119	10	...	0.59**	0.48**	0.53**
Abdominal fat (g)	42.9	34	...	...	0.83**	0.70**
Body fat (g/kg)	13.1	14	...	...	...	0.68**
Plasma VLDL (A)	0.193	23	...	...	...	...
Post-heparin lipase ( $\mu$ moles/ml/h)	74.7	24	-0.18	-0.04	-0.10	0.10

Values for plasma VLDL concentration are the means from blood samples taken from 38 male and 39 female broilers at 47 and 49 d of age. Birds were killed at 51 d of age.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

Plasma VLDL concentration was strongly correlated with body fat content and abdominal fat pad weight, confirming previous observations (Griffin and Whitehead, 1982) but correlations between plasma VLDL concentration and body weight were higher than reported in earlier studies (Griffin *et al.* 1982; Whitehead and Griffin, 1982). Divergent selection for plasma VLDL has produced lines with no difference in body weight and this indicates that plasma VLDL and body weight are not genetically correlated. The absence of any correlation between plasma VLDL concentration and post-heparin lipase activity is surprising, because the total functional lipoprotein lipase activity in the bird would be expected to be a major determinant of the rate of VLDL removal from the circulation.

### Experiment 3

An estimate of the relative contribution of different tissues to lipoprotein lipase activity in post-heparin plasma was attempted by comparing the specific activity of lipoprotein lipase in tissues of birds injected with heparin with those of control birds (Table 3). Of the tissues examined, adipose tissue contained the highest lipoprotein lipase activity/g of tissue. Heart, thigh and gizzard muscle also contained substantial quantities of enzyme, but activity in breast

muscle was very low. Studies in mammals have shown that red muscle fibres contain much more lipoprotein lipase than white muscle (Linder *et al.*, 1976) and this difference in fibre type may explain the variation in activity between different muscles found in the present study.

TABLE 3

*Effect of intravenous injection of heparin on tissue lipoprotein lipase activities in female broilers at 6 weeks of age*

	Control	Heparin-treated
Body weight (g)	1391 ± 51	1401 ± 36
Lipoprotein lipase:		
Plasma (μmoles/h/ml)	0	64.1 ± 4.4**
Liver (μmoles/h/g)	0	25.6 ± 3.5**
Heart (μmoles/h/g)	77.5 ± 4.2	97.5 ± 6.8*
Gizzard (μmoles/h/g)	17.3 ± 2.1	18.8 ± 1.5
Thigh (μmoles/h/g)	16.7 ± 3.7	11.8 ± 3.1
Breast (μmoles/h/g)	1.0 ± 0.3	1.1 ± 0.2
Abdominal fat (μmoles/h/g)	192 ± 20.0	157 ± 17*
Abdominal fat (μmoles/h/mg of protein)	22.9 ± 1.6	18.6 ± 1.6*

Treated birds were injected with 1000 U heparin/kg of body weight via the wing vein. Control birds received saline. Blood was sampled 2 min later and birds rapidly killed by intravenous injection of sodium phenobarbitone. More than 0.95 of lipase activity in tissues and post-heparin plasma was precipitated by an anti-lipoprotein lipase antiserum. Values are the means ± SE of 9 birds/group; those for treated birds that are significantly different (*t* test) from those for controls are indicated by:

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

If blood constitutes about 0.07 of total body weight (Kotula and Helback, 1966) and plasma about 0.70 of blood (Medway and Kare, 1959) injection of heparin released about 4000 U (μmoles fatty acid released/h) of lipoprotein lipase into the circulation of the birds used in the experiment described in Table 3. Comparison with the specific activity of lipoprotein lipase in adipose tissue and muscle suggests that this represents a substantial proportion of the total activity in the bird. Nevertheless, heparin injection caused only a small decrease in lipoprotein lipase activity in adipose tissue, had little or no effect on activity in leg and gizzard muscle and increased activity in heart muscle.

Studies in rats have shown that intravenous injection of heparin releases only a small proportion of total tissue lipoprotein lipase into the circulation, most of which is present within the adipocytes or muscle cells (Cryer, 1981). A similarly small proportion of total lipoprotein lipase activity appears to be functionally active in avian tissues (Table 3), but the variation in activity within treated and control groups prevented any assessment of their contribution to plasma lipoprotein lipase activity. Heparin has been shown to inhibit degradation of lipoprotein lipase and stimulate its release from within cells (Cupp *et al.* 1987). It seems unlikely that these effects would contribute to release of lipase activity into the plasma within the 2 min period between intravenous injection and blood sampling used in the present study, but they may have caused some increase in activity measured within tissues. Lipoprotein lipase that is released into the circulation is believed to be taken up and degraded by the liver and

this process presumably accounts for the appearance of lipoprotein lipase activity in the liver of heparin-treated birds. The activity in the liver 2 min after heparin injection was less than 0.10 of total activity present in the plasma, but much of the enzyme released from tissues after longer time periods may be taken up from the plasma and degraded.

The results of this study confirm previous observations that plasma VLDL concentrations are strongly correlated with body fat content in broilers. Total lipoprotein lipase activity in the abdominal fat pad was significantly correlated with fat pad weight but the absence of any correlation with specific activity of the enzyme indicates that the variation in adipose tissue lipoprotein lipase activity cannot be detected from measurements on biopsy samples.

The lack of any correlation between body fat content and lipoprotein lipase activity in post-heparin plasma may mean that the amount of lipoprotein lipase at the capillary bed is not an important influence on the rate of fatty acid uptake by adipose tissue. It seems more probable, although difficult to prove, that the contribution of other tissues to post-heparin plasma lipase activity is substantial and that this obscures any relationship between fatness and the activity released from adipose tissue. The identification of tissue-specific lipoprotein lipases in post-heparin plasma is a possible solution to this problem and their existence is being investigated at present.

#### REFERENCES

- BENSON, J.D., HERN, V., BOYD, T. & BENSADOUN, A. (1975) Triglyceride hydrolase of chicken and rat pre- and post-heparin plasma. Effects of fasting and comparison with adipose tissue lipoprotein lipase. *International Journal of Biochemistry*, **6**: 727-734.
- CRYER, A. (1981) Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *International Journal of Biochemistry*, **13**: 525-541.
- CUPP, M., BENSADOUN, A. & MELFORD, K. (1987) Heparin decreases the degradation rate of lipoprotein lipase in adipocytes. *Journal of Biological Chemistry*, **262**: 6383-6388.
- GRIFFIN, H.D. & WHITEHEAD, C.C. (1982) Plasma lipoprotein concentration as an indicator of fatness in broilers: development and use of a simple assay for plasma very low density lipoproteins. *British Poultry Science*, **23**: 307-313.
- GRIFFIN, H.D., WHITEHEAD, C.C. & BROADBENT, L.A. (1982) The relationship between plasma triglyceride concentrations and fatness in male and female broilers—A basis for selection? *British Poultry Science*, **23**: 15-23.
- GRIFFIN, H.D., BUTTERWORTH, S.C. & GODDARD, C. (1987) Contribution of lipoprotein lipase to differences in fatness between broiler and layer-strain chicks. *British Poultry Science*, **28**: 197-206.
- KOTULA, A.W. & HELBACK, N.V. (1966) Blood volume of live chickens and influence of slaughter technique on blood loss. *Poultry Science*, **45**: 684-688.
- LEGROS, M. & KEPES, A. (1985) One-step fluorometric microassay of DNA in prokaryotes. *Analytical Biochemistry*, **147**: 497-502.
- LINDER, C., CHERNIK, S.S., FLECK, T.R. & SCOW, R.O. (1976) Lipoprotein lipase and uptake of chylomicron triglyceride by skeletal muscle of rats. *American Journal of Physiology*, **231**: 860-864.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin reagent. *Journal of Biological Chemistry*, **193**: 265-275.
- MEDWAY, W. & KARE, M.R. (1959) Blood and plasma volume, haematocrit, blood specific gravity and serum protein electrophoresis of the chicken. *Poultry Science*, **38**: 624-631.
- PEDERSEN, M.E., COHEN, M. & SCHOTZ, M.C. (1983) Immunocytochemical localisation of the

- functional fraction of lipoprotein lipase in the perfused heart. *Journal of Lipid Research*, **24**: 512-518.
- SAADOUN, A. & LECLERCQ, B. (1983) Comparison of *in vivo* fatty acid synthesis of the genetically lean and fat chickens. *Comparative Biochemistry and Physiology*, **75B**: 641-644.
- WHITEHEAD, C.C. & GRIFFIN, H.D. (1982) Plasma lipoprotein concentration as an indicator of fatness in broilers: effect of age and diet. *British Poultry Science*, **23**: 299-305.
- WHITEHEAD, C.C. & GRIFFIN, H.D. (1984) Development of divergent lines of lean and fat broilers using plasma very low density lipoprotein concentration as selection criterion: the first three generations. *British Poultry Science*, **25**: 573-582.