

# The preferential mobilisation of C<sub>20</sub> and C<sub>22</sub> polyunsaturated fatty acids from the adipose tissue of the chick embryo: potential implications regarding the provision of essential fatty acids for neural development

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## Abstract

The aim of this study was to determine the relative mobilisation of the different fatty acyl components of the triacylglycerol (TAG) of the chick embryo's adipose tissue in the light of the specific requirements of the developing neural tissues of the embryo for C<sub>20-22</sub> polyunsaturated fatty acids. Pieces of adipose tissue, obtained from embryos at various developmental stages, were incubated *in vitro* in Dulbecco's Medium containing serum albumen. The fatty acid compositions of the initial tissue TAG and of the free fatty acid (FFA) mobilised from the tissue during 1 h of incubation were determined and compared. The composition of the FFA released into the medium under conditions of basal (i.e., unstimulated) lipolysis was markedly different in several respects from that of the TAG from which it originated. The polyunsaturated fatty acids, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3, were consistently found to be preferentially released into the medium, whereas the major fatty acyl constituents of the tissue, 16:0 and 18:1n-9, were selectively retained in the TAG. For example, at day 18 of development, the proportions (% w/w of fatty acids) of 20:5n-3 and 22:6n-3 released into the incubation medium were respectively 6.5 and 7.5 times higher than in the original tissue TAG. Glucagon stimulated the overall rate of mobilisation by approx. 2-fold and also partially suppressed the preferential mobilisation of C<sub>20-22</sub> polyunsaturates. These results may be relevant to the elucidation of the means by which essential polyunsaturates are delivered from the yolk to the neural tissues of the embryo, with the implication of a mediatory role for the embryonic adipose tissue in this transfer. © 1997 Elsevier Science B.V. All rights reserved.

**Keywords:** Adipose tissue; Triacylglycerol; Fatty acid; Docosahexaenoic acid; Arachidonic acid; (Chick embryo)

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Abbreviations: TAG, triacylglycerol; FFA, free fatty acid; HSL, hormone sensitive lipase; 16:0, palmitic acid; 16:1n-7, palmitoleic acid; 18:0, stearic acid; 18:1n-9, oleic acid; 18:2n-6, linoleic acid; 18:3n-3,  $\alpha$ -linolenic acid; 20:4n-6, arachidonic acid; 20:5n-3, eicosapentaenoic acid; 22:4n-6, docosatetraenoic acid; 22:5n-3, docosapentaenoic acid; 22:6n-3, docosahexaenoic acid.

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## 1. Introduction

The characteristic incorporation of very high levels of C<sub>20-22</sub> polyunsaturated fatty acids into neuronal and retinal phospholipids during fetal/neonatal life, coupled with evidence that deficiencies in the provision of such fatty acids during defined developmental periods are associated with a range of visual and

behavioural abnormalities [1–10], have raised the question of the route and mechanism by which essential fatty acyl moieties are delivered to the differentiating neural tissues. A series of elegant studies using mammalian systems have generated a series of impressive but somewhat conflicting hypotheses [11–21]. For instance, the considerable evidence that the liver plays a key role in mediating the delivery of polyunsaturates to the brain and retina via the secretion of such fatty acids as components of lipoprotein phospholipids [11–14] does not easily reconcile with the extensive data suggesting that the plasma free fatty acid (FFA) fraction (i.e., non-esterified fatty acid bound to serum albumen or  $\alpha$ -fetoprotein [22]) is the major precursor pool for neural uptake [15–18]. One potential solution to such apparent discrepancies may lie in the proposal, combining aspects of both the above hypotheses, that polyunsaturates may be transported in the circulation from the liver to the brain as components of lyso-phosphatidylcholine bound to serum albumen [19–21]. Another approach is to consider the possibility that, in addition to the role of the liver, other tissues may also be involved in mediating the transport of essential fatty acids to the neural tissues. An obvious candidate is adipose tissue which not only has the capacity to store large amounts of the relevant fatty acyl moieties but can also release these components into the circulation in the FFA form; the preferred form for acyl uptake by both the placenta [23,24] and the brain [15–18].

Recent studies from this laboratory, concerned with the means by which lipid components are transferred from the yolk to the tissues of the chick embryo [25–28], have identified a series of mechanisms which serve to enhance the provision of arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acids to the developing brain [25,26,29,30]. With regard to the latter fatty acid, a series of unique features have been identified in which 22:6n-3 is treated preferentially at successive stages of the lipid transfer process. Firstly, molecular species of phosphatidylethanolamine which contain 22:6n-3 are selectively taken up from the yolk by the surrounding yolk sac membrane prior to the main phase of yolk lipid uptake [25,29]. Secondly, within the yolk sac membrane, 22:6n-3 is uniquely transferred from the yolk-derived phospholipid and incorporated into triacylglycerol (TAG). The assembly of this TAG into lipoproteins which

are secreted from the yolk sac membrane into the embryonic circulation results in the establishment of a highly unusual plasma lipid profile characterised by plasma TAG with a 22:6n-3 content of up to 14% (w/w of fatty acids) [29]. We have proposed that the major consequence of the partitioning of yolk-derived 22:6n-3 into plasma TAG (as opposed to phospholipid) is to render this fatty acid susceptible to hydrolytic release from the lipoprotein by the action of lipoprotein lipase [29]. This enzyme is present at very high activities in the capillaries permeating the embryonic adipose tissue but is not expressed in the developing brain [26]. As a result of the tissue-specific expression of lipoprotein lipase, relatively large amounts of 22:6n-3 are initially diverted to the adipose tissue for temporary storage in the TAG depots [26,29,30] as opposed to the direct transfer of this fatty acid to the brain. Thus from day 14 of development (i.e., at the beginning of the lipid transfer process) to hatching at day 21, the amount of 22:6n-3 stored in adipose TAG is several-fold greater than that present in brain phospholipid [29,30].

These considerations suggest that the adipose tissue of the embryo may act as a temporary store of 22:6n-3 and may therefore function as a mediator in the transfer of this fatty acid from the yolk to the brain. The third example of a unique feature pertaining to the transfer of 22:6n-3 lends considerable weight to this view. Thus it was observed that, over the hatching period, the amount of 22:6n-3 in the adipose depot decreased precipitously to 15–30% of the pre-hatch levels, whereas the amounts of the C<sub>16-18</sub> fatty acids in the tissue TAG remained approximately constant [29,30]. This result suggests that 22:6n-3 may exhibit an unprecedented degree of preferential mobilisation from the tissue TAG with the consequent release of this fatty acid into the circulation as the FFA which may be the preferred plasma source for uptake by the brain [15–18]. The striking observation that the fatty acid profile of the plasma FFA fraction displays levels of 22:6n-3 as high as 17% (w/w of fatty acids) [29] is also consistent with the possibility of preferential mobilisation of this fatty acid from adipose tissue.

The aim of the present study was to test the theory that 22:6n-3 may be selectively released from adipose tissue TAG by investigating the mobilisation of fatty acids from tissue pieces incubated *in vitro*. The re-

sults indicate that 22:6n-3 plus a range of other C<sub>20-22</sub> polyunsaturated fatty acids are preferentially mobilised from adipose tissue under these conditions. Some preliminary aspects of this work have been published as a conference abstract [31].

## 2. Materials and methods

### 2.1. *In vitro* incubation of adipose tissue

The incubation of fertile eggs (Ross 1 broiler-breeder strain) and the dissection of adipose tissue from the subcutaneous depot of the embryos were performed as described in previous publications [26,29,30]. The fresh tissue was chopped into small pieces of approximately 2 mm<sup>3</sup> and washed extensively in incubation medium (see below) in order to remove any fat droplets which may have been released by the chopping process. Aliquots (140–160 mg) of the tissue pieces (which consisted of 40–50% w/w TAG) were incubated in stoppered tubes containing 2 ml of Dulbecco's Modified Eagle's Medium (Hepes buffered) supplemented with 2% (w/v) bovine serum albumen (fatty acid adsorbed). In some cases, glucagon (porcine) was included in the incubation medium at a concentration of 1.0 µg/ml. Previous work has established that glucagon is a major physiological agonist of lipolysis in avian adipose tissue [45]. Incubations were performed at 37°C for 1 h with gentle shaking. Dulbecco's Medium and glucagon were obtained from Sigma Chemicals (Poole, UK) and bovine serum albumen was obtained from First Link Ltd. (Brierley Hill, UK).

### 2.2. Lipid extraction and fatty acid analysis

Portions of freshly dissected unincubated tissue and of tissue and medium after incubation were homogenised in a suitable excess of chloroform/methanol/HCl (100:50:1, v/v) and extracts of total lipid were prepared. TAG and FFA fractions were isolated by thin-layer chromatography on silica gel G using a solvent system of hexane/diethyl ether/formic acid (80:20:1, v/v). Following transmethylation [32], the fatty acid compositions of the identified lipid fractions were determined by gas-liquid chromatography using a capillary column sys-

tem (Carbowax, 30 m × 0.25 mm, film thickness 0.25 µm; Alltech, Carnforth, UK) in a CP9001 instrument (Chrompack, Middleburg, The Netherlands). The identities of the peaks were verified by comparison with the retention times of standard fatty acid methyl esters (Sigma Chemical Co., Poole, UK). Data analysis, using an EZ Chrom Data System (Scientific Software Inc., San Jose, CA, USA), enabled the calculation of the fatty acid compositions and also of the total amounts of TAG and FFA in the extracts.

### 2.3. Expression of results

The extent of lipid mobilisation during incubation is described in terms of % (w/w) of tissue TAG acyl groups mobilised to form FFA in the medium. This was calculated as:  $(f \times 100)/(f + T)$ , where  $f$  is µg FFA released into the medium and  $T$  represents µg TAG acyl groups in the tissue at the end of the incubation period.

The differential release of the various fatty acids is expressed in terms of the 'Relative Mobilisation', defined as the ratio: [% (w/w) in Medium FFA after incubation]/[% (w/w) in tissue TAG before incubation], for each fatty acid [33]. A value greater than 1 indicates preferential mobilisation, whereas a value less than 1 indicates selective retention.

The amounts of FFA released into the medium during the 1 h incubations were quite low, both in absolute terms and as a proportion of the TAG of the tissue pieces (see Section 3, Table 1). Thus it was of crucial importance in these studies to quantify any FFA present in the constituted medium prior to incubation with tissue and to make appropriate corrections. For each incubation, 4 replicate 2 ml portions of the constituted medium (which had not been in contact with tissue pieces) were subjected to lipid analysis as described above (Section 2.2). Although the bovine serum albumen component had been fatty acid adsorbed, these samples were found to contain 15–22 µg FFA per 2-ml portion of medium. This compares with values of 120–430 µg FFA present in the 2 ml of medium after 1 h of incubation with tissue, dependent on the developmental stage and the presence or absence of glucagon. In each case, the initial medium FFA values were subtracted from the values obtained after incubation with tissue in order

to determine the extent of net mobilisation. The composition of the FFA associated with the bovine serum albumen prior to incubation was approx. (w/w) 31% 16:0, 4% 16:1n-7, 34% 18:0, 21% 18:1n-9, 8% 18:2n-6. The fatty acid compositions of the medium after incubation with tissue were subjected to an appropriate correction to take account of this relatively minor contribution from the initial medium. Only those fatty acids which comprised more than 0.1% (w/w) of the total were considered.

All data are expressed as the mean  $\pm$  S.E. of measurements from 4 replicate incubations or fresh tissue samples. Each replicate represents tissue from a different embryo. Statistical comparisons were performed by *t*-test.

### 3. Results

#### 3.1. Differential mobilisation of fatty acids during basal rates of lipolysis

The values for the extent of net lipid mobilisation (% w/w of tissue TAG acyl groups mobilised to form FFA in the medium during a 1-h incubation) are shown in Table 1. Basal mobilisation was significantly greater at day 16 than at day 18 with the highest rate of net release observed at day 22 (day 22 represents 1 day after hatching).

The fatty acid composition of the FFA released into the medium was compared with that of the tissue TAG from which it was mobilised during incubation of adipose pieces under conditions of basal (i.e., unstimulated) lipolysis. Tables 2–4 present the results obtained using adipose tissue from embryos at successive developmental stages. The results clearly demonstrate that the different fatty acyl components of the tissue TAG were not mobilised to the same extent. In particular, the proportions of the C<sub>20-22</sub>

Table 2

Comparison of the fatty acid compositions of tissue TAG and medium FFA under conditions of basal lipolysis using tissue at day 16 of development

Fatty acid	Tissue TAG	Medium FFA	Relative mobilisation
16:0	28.0 $\pm$ 0.2	21.4 $\pm$ 0.7 <sup>a</sup>	0.76
16:1n-7	2.0 $\pm$ 0.1	1.6 $\pm$ 0.1 <sup>a</sup>	0.80
18:0	8.4 $\pm$ 0.1	9.4 $\pm$ 0.2 <sup>c</sup>	1.12
18:1n-9	34.5 $\pm$ 0.1	28.3 $\pm$ 2.4 <sup>e</sup>	0.82
18:2n-6	14.0 $\pm$ 0.3	9.8 $\pm$ 0.4 <sup>a</sup>	0.70
18:3n-3	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1	0.60
20:4n-6	1.6 $\pm$ 0.1	3.5 $\pm$ 0.2 <sup>a</sup>	2.19
20:5n-3	0.3 $\pm$ 0.0	1.0 $\pm$ 0.1 <sup>a</sup>	3.33
22:4n-6	0.4 $\pm$ 0.0	0.6 $\pm$ 0.1	1.50
22:5n-3	0.7 $\pm$ 0.1	1.4 $\pm$ 0.2 <sup>d</sup>	2.00
22:6n-3	5.8 $\pm$ 0.1	20.0 $\pm$ 2.5 <sup>b</sup>	3.45

The fatty acid compositions (% w/w) of the tissue TAG prior to incubation and of the FFA released into the medium during 1 h of incubation are shown. Results are the means  $\pm$  S.E. of 4 replicate incubations. Comparison between medium and tissue composition: <sup>a</sup> *P* < 0.001; <sup>b</sup> *P* < 0.002; <sup>c</sup> *P* < 0.01; <sup>d</sup> *P* < 0.02; <sup>e</sup> *P* < 0.05.

Relative Mobilisation = (% in medium FFA)/(% in tissue TAG) for each fatty acid.

polyunsaturates of both the n-3 and n-6 families were significantly and consistently greater in the medium FFA in comparison with the initial tissue TAG. For example, the proportion of 22:6n-3 in the medium FFA was found to be 3.4–7.5 times greater than in the tissue TAG. As a consequence, very high proportions (> 20% w/w of total fatty acids) of 22:6n-3 were found to be present in the FFA of the medium at days 16 and 18. Also, 20:4n-6, 20:5n-3 and 22:5n-3 were released into the medium at proportions considerably in excess of those found in the initial tissue. For the C<sub>20-22</sub> polyunsaturates, there was a positive relationship between the number of double bonds and

Table 1

Extent of net lipid mobilisation during incubation of adipose tissue pieces for 1 h

Developmental stage (days)	16	18	22
%(w/w) mobilisation			
Basal lipolysis	0.22 $\pm$ 0.05	0.09 $\pm$ 0.01 <sup>z</sup>	0.40 $\pm$ 0.06 <sup>x</sup>
Glucagon-stimulated lipolysis	–	0.24 $\pm$ 0.03 <sup>c</sup>	0.71 $\pm$ 0.10 <sup>ey</sup>

The extent of lipid mobilisation refers to the % (w/w) of tissue TAG acyl groups mobilised to form FFA in the medium. Results are the mean  $\pm$  S.E. of 4 replicate incubations. Comparison between glucagon-stimulated and basal lipolysis: <sup>c</sup> *P* < 0.01; <sup>e</sup> *P* < 0.05. Comparison between stages for the same treatment; difference from preceding stage: <sup>x</sup> *P* < 0.002; <sup>y</sup> *P* < 0.01; <sup>z</sup> *P* < 0.05.

Table 3

Comparison of the fatty acid compositions of tissue TAG and medium FFA under conditions of basal and glucagon-stimulated lipolysis using tissue at day 18 of development

Fatty acid	Tissue TAG	Medium FFA		Relative mobilisation	
		– glucagon	+ glucagon	– glucagon	+ glucagon
16:0	30.2 ± 0.1	20.1 ± 1.2 <sup>a</sup>	25.1 ± 0.6 <sup>ax</sup>	0.67	0.83
16:1n-7	2.3 ± 2.1	2.1 ± 0.3	2.2 ± 0.1	0.91	0.96
18:0	7.8 ± 0.1	9.5 ± 0.5 <sup>d</sup>	6.7 ± 0.5 <sup>x</sup>	1.22	0.86
18:1n-9	35.7 ± 0.1	23.7 ± 1.5 <sup>a</sup>	32.5 ± 0.4 <sup>aw</sup>	0.66	0.91
18:2n-6	14.1 ± 0.1	10.1 ± 0.8 <sup>c</sup>	14.3 ± 0.6 <sup>x</sup>	0.72	1.01
18:3n-3	0.5 ± 0.1	0.3 ± 0.2	0.7 ± 0.1	0.60	1.40
20:4n-6	1.2 ± 0.1	4.5 ± 0.2 <sup>a</sup>	2.6 ± 0.1 <sup>av</sup>	3.75	2.17
20:5n-3	0.2 ± 0.0	1.3 ± 0.2 <sup>b</sup>	0.7 ± 0.1 <sup>cz</sup>	6.50	3.50
22:4n-6	0.3 ± 0.1	0.6 ± 0.1	0.2 ± 0.1 <sup>z</sup>	2.00	0.67
22:5n-3	0.6 ± 0.1	2.4 ± 0.2 <sup>a</sup>	0.9 ± 0.1 <sup>v</sup>	4.00	1.50
22:6n-3	3.0 ± 0.1	22.5 ± 3.3 <sup>b</sup>	10.2 ± 0.2 <sup>ax</sup>	7.50	3.40

Details as for Table 2. Comparison between medium and tissue composition: <sup>a</sup>  $P < 0.001$ ; <sup>b</sup>  $P < 0.002$ ; <sup>c</sup>  $P < 0.01$ ; <sup>d</sup>  $P < 0.02$ ; <sup>e</sup>  $P < 0.05$ . Comparison between media + or – GLUCAGON: <sup>v</sup>  $P < 0.001$ ; <sup>w</sup>  $P < 0.002$ ; <sup>x</sup>  $P < 0.01$ ; <sup>y</sup>  $P < 0.02$ ; <sup>z</sup>  $P < 0.05$ .

the Relative Mobilisation when considering fatty acids of the same chain length. In contrast, the proportions of 16:0 and 18:1n-9 were consistently found to be lower in the medium FFA than in the original tissue TAG. The results for 18:2n-6 and 18:3n-3 were less consistent since both of these fatty acids exhibited selective retention in tissue TAG at days 16 and 18 but not at day 22; in fact 18:3n-3 demonstrated a high degree of preferential mobilisation at the latter developmental stage. There were no significant differences

in TAG fatty acid profiles between the fresh tissue and the tissue after incubation (data not shown); this is consistent with the finding that only a very small proportion (0.4% or less) of the total tissue TAG was mobilised during the 1-h incubation.

The differential release of the various fatty acids is illustrated in Fig. 1 where the Relative Mobilisation values at the different developmental stages are plotted on a logarithmic scale in order to give equivalent but opposite weightings to ratios less than and greater

Table 4

Comparison of the fatty acid compositions of tissue TAG and medium FFA under conditions of basal and glucagon-stimulated lipolysis using tissue at day 22 of development

Fatty acid	Tissue TAG	Medium FFA		Relative mobilisation	
		– glucagon	+ glucagon	– glucagon	+ glucagon
16:0	29.4 ± 0.3	22.7 ± 1.2 <sup>b</sup>	20.6 ± 0.6 <sup>a</sup>	0.77	0.70
16:1n-7	2.3 ± 0.1	2.6 ± 0.1	2.4 ± 0.2	1.13	1.04
18:0	8.2 ± 0.2	8.6 ± 0.7	5.9 ± 0.2 <sup>ay</sup>	1.05	0.72
18:1n-9	40.0 ± 0.2	37.7 ± 0.8 <sup>e</sup>	40.2 ± 1.5	0.94	1.01
18:2n-6	13.7 ± 0.4	14.1 ± 1.4	18.9 ± 0.8 <sup>bz</sup>	1.03	1.38
18:3n-3	0.5 ± 0.0	1.9 ± 0.4 <sup>d</sup>	2.2 ± 0.7 <sup>e</sup>	3.80	4.40
20:4n-6	0.9 ± 0.1	2.4 ± 0.1 <sup>a</sup>	1.7 ± 0.1 <sup>bx</sup>	2.67	1.89
22:4n-6	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	2.00	1.00
22:5n-3	0.3 ± 0.1	0.9 ± 0.1 <sup>c</sup>	0.5 ± 0.1 <sup>z</sup>	3.00	1.67
22:6n-3	1.2 ± 0.1	4.1 ± 0.4 <sup>a</sup>	2.9 ± 0.6 <sup>e</sup>	3.42	2.42

Details as for Table 3. 20:5n-3 was not detected in the samples at this stage.

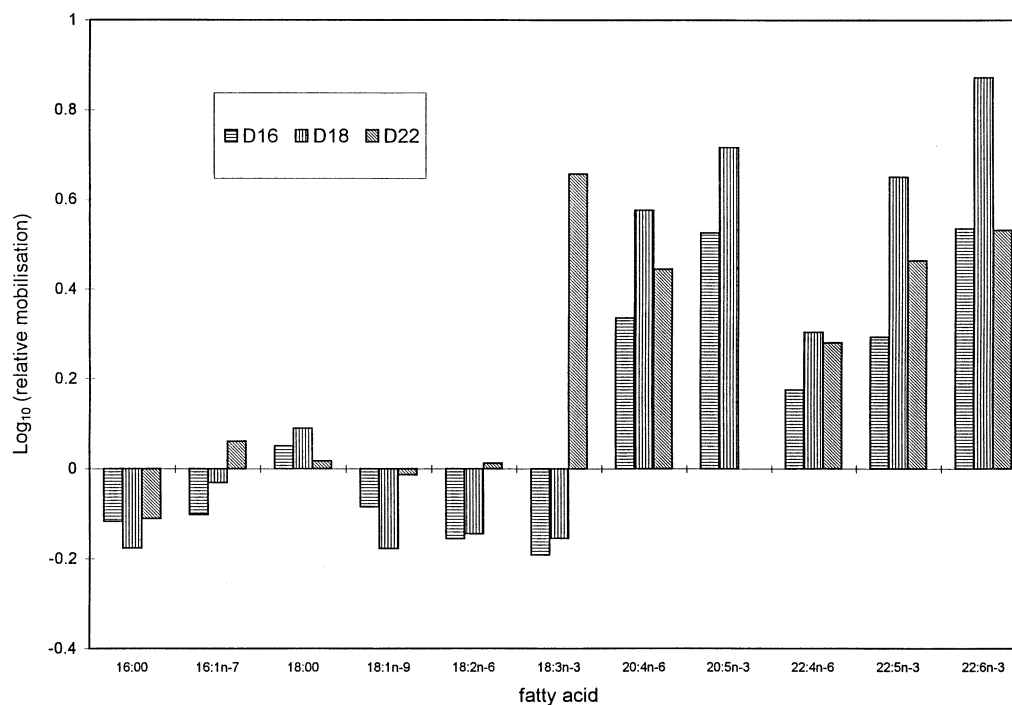


Fig. 1. Differential mobilisation of fatty acids under conditions of basal lipolysis. The values for  $\log_{10}$  (Relative Mobilisation) are shown for the different fatty acids using tissue at days 16, 18 and 22 of development. (20:5n-3 was not detected in the day 22 samples).

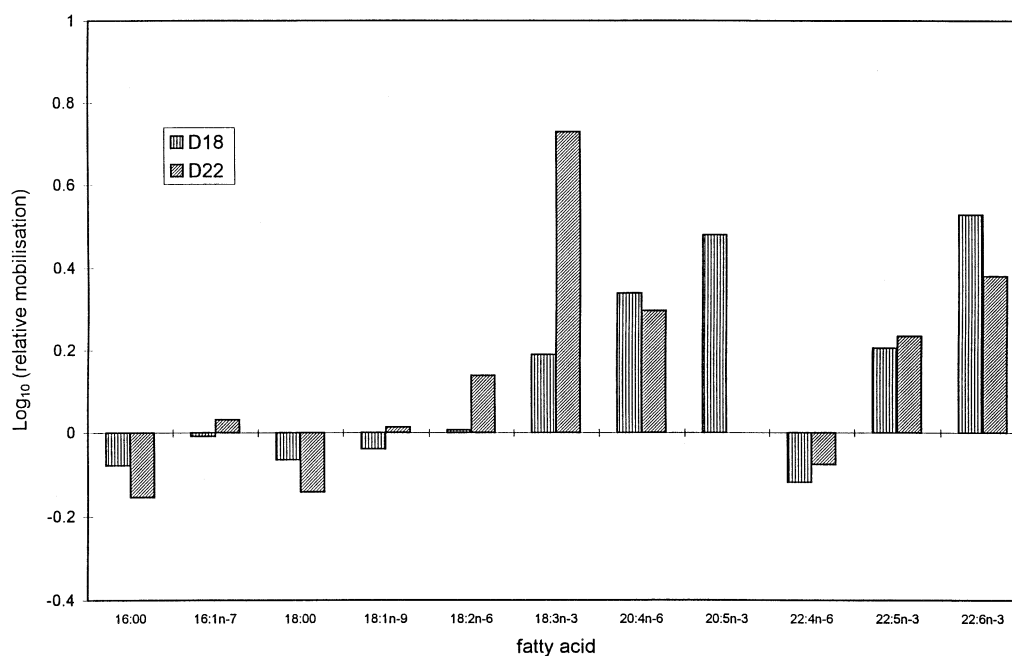


Fig. 2. Differential mobilisation of fatty acids under conditions of glucagon-stimulated lipolysis. The values for  $\log_{10}$  (Relative Mobilisation) are shown for the different fatty acids using tissue at days 18 and 22 of development. (20:5n-3 was not detected in the day 22 samples).

than unity. The results indicate that the extent of preferential mobilisation of the C<sub>20-22</sub> polyunsaturates tended to be greater at day 18 than at days 16 or 22.

### 3.2. Differential mobilisation of fatty acids during glucagon-stimulated lipolysis

The effect of glucagon on the extent of lipid mobilisation and on the pattern of the fatty acids released was investigated using adipose tissue at days 18 and 22 of development. The extent of net lipid mobilisation was significantly stimulated by the presence of glucagon at both developmental stages (by 2.7- and 1.8-fold at days 18 and 22 respectively; Table 1).

In general, many of the features relating to the pattern of differential release of fatty acids which were observed under conditions of basal lipolysis were also found to prevail when lipolysis was stimulated by glucagon (Tables 3 and 4). For example, the C<sub>20-22</sub> polyunsaturates (with the exception of 22:4n-6) were again found to display preferential mobilisation into the medium, whereas 16:0 and also 18:0 were selectively retained in the tissue TAG. However, there were also significant differences in detail between the results obtained in the presence and absence of glucagon. Of greatest relevance was the observation that the preferential mobilisation of the C<sub>20-22</sub> polyunsaturates, whilst still apparent, was considerably attenuated by the presence of glucagon. For example, at day 18, the Relative Mobilisation of 22:6n-3 was reduced from 7.5 to 3.4 when lipolysis was stimulated by glucagon. Also of interest was the emergence under conditions of glucagon-stimulated lipolysis of a positive relationship between the degree of unsaturation and the Relative Mobilisation for fatty acids of the same chain length. Such a relationship was clearly evident for the 4 fatty acids containing 18 carbon atoms and the 3 fatty acids of 22 carbon atoms where, in both cases, the Relative Mobilisation was found to increase progressively with the presence of each successive double bond. The limited information available for fatty acids of 16 and 20 carbon atoms was also consistent with this theme. The present data did not permit the elucidation of any clear relationship between Relative Mobilisation and chain length for fatty acids with the same number of double bonds due to the limited number of such fatty

acids which were present for comparison at a level (> 0.1% w/w of the total) consistent with accurate measurement. It was, however, noticeable that the Relative Mobilisation decreased with increasing chain length when comparing 20:4n-6 with 22:4n-6 and also 20:5n-3 with 22:5n-3. The differential release of the various fatty acids under conditions of glucagon-stimulated lipolysis is illustrated in Fig. 2.

## 4. Discussion

Although the regulation of fatty acid mobilisation from adipose tissue TAG has been an area of intensive research for 3 decades, relatively few studies have focussed on the composition of the mobilised fatty acids. However, over the past 4 years, a number of investigations have specifically addressed the question as to whether the various fatty acyl components of the tissue's TAG may be differentially released [33–36]. A detailed investigation by Raclot and Groscolas [33] not only supplied convincing evidence that certain fatty acids were preferentially mobilised from adipocyte TAG, whilst others were selectively retained but also provided a rational basis for such findings. Their observations on the differential release of 52 fatty acids into the medium during the *in vitro* incubation of rat adipocytes were found to be summarised by the general rule that the Relative Mobilisation of a particular fatty acid is directly proportional to its degree of unsaturation and inversely proportional to its chain length. Thus the proportion of highly polyunsaturated fatty acids such as 20:4n-6 and 20:5n-3 was significantly greater in the medium FFA than in the original cellular TAG. However, it is most pertinent that, in that study, 22:6n-3 was not preferentially released from the rat adipocytes, the explanation being that the promobilisation effect of the high degree of polyunsaturation was cancelled out by the 22-carbon chain length. A mechanistic explanation for selective mobilisation was proposed [33], based on the differential aqueous solubilities of the various fatty acids. It was suggested that TAG species containing the more polar short and unsaturated fatty acids would be preferentially located at the periphery of the cytoplasmic lipid droplet of the adipocyte and would therefore be more accessible to the hormone sensitive lipase (HSL)

acting at the interface of the non-polar and aqueous phases. Further studies demonstrated that the pattern of differential mobilisation observed with the isolated adipocytes was highly consistent with the changes which occurred in the fatty acid compositions of adipose TAG in rats during prolonged fasting [35] and of plasma FFA in rabbits following injection of adrenocorticotrophic hormone [36]. The general conclusion from both of these *in vivo* studies was identical to that previously obtained [33] from the experiments with isolated adipocytes *in vitro*; namely, that preferential mobilisation is favoured by short chain length and a high degree of polyunsaturation. A consistent finding of all these investigations is that the balance between the chain length and unsaturation effects results in a high degree of preferential mobilisation of 18:3n-3, 20:4n-6 and 20:5n-3, but crucially not of 22:6n-3.

In many respects, the present results obtained using adipose tissue from the chick embryo are in concordance with the aforementioned work. For example, 20:4n-6 and 20:5n-3 were found to be preferentially mobilised from the TAG of the tissue pieces, whereas 16:0 and 18:1n-9 were selectively retained. However, a novel feature displayed by the embryonic adipose tissue of the chicken was the unprecedented degree of preferential mobilisation of 22:6n-3, particularly under conditions of basal lipolysis. Moreover, this result is consistent with the *in vivo* changes which occur in the tissue's 22:6n-3 content; in particular, the dramatic and specific decrease in the level of this fatty acid in adipose TAG over the hatching period [29,30]. Why the difference? It is of note that the mammalian studies quoted above [33,35,36] utilised post-weaning animals in which the phase of 22:6n-3 accretion by the neural tissues would have been essentially completed. In contrast, the present study utilised adipose tissue pieces obtained from chick embryos at a stage of development characterised by the dramatic accumulation of 22:6n-3 in the phospholipids of the brain [29,30,38]. It is tempting to speculate that, in this embryonic system, the specific enhancement of 22:6n-3 mobilisation from adipose TAG, to levels which are far higher than would be predicted from the previously established chain length/unsaturation considerations, may represent a developmental adaptation designed to facilitate the transfer of this fatty acid to the brain in a form

suitable for uptake. This proposal is consistent with the evidence that the plasma FFA fraction, bound to serum albumen or  $\alpha$ -fetoprotein, may be the main plasma source of essential fatty acids for neural uptake [15–18].

A second question relates to the mechanism of the enhancement of 22:6n-3 mobilisation. Selective release could possibly be achieved if 22:6n-3 were present in the same TAG molecule as fatty acids with a high natural propensity for preferential release. For instance, a TAG species consisting of 22:6n-3 and, say, two molecules of 20:4n-6 esterified to the glycerol backbone would likely form an effective substrate for HSL at the lipid/water interface, based on polarity considerations. However, analysis by high performance liquid chromatography of the profile of molecular species of TAG present in the adipose tissue of the chick embryo [37] has shown that 22:6n-3 tends to be present in the same TAG molecule as 16:0 and 18:1n-9; two fatty acids with very low Relative Mobilisation values. Since the pattern of differential mobilisation observed in the mammalian examples [33,35,36] appears to be based on sound physico-chemical principles [33], it would seem that some form of perturbation of the natural tendency of TAG species such as 16:0/18:1/22:6 (the major 22:6n-3-containing species in the chick embryo's adipose tissue [37]) to partition within the lipid droplet must be invoked. One possible speculation could involve the induction, at the appropriate stage of development, of a regulatory protein which could bind 22:6n-3-containing TAG species promoting their relocation to the surface of the lipid droplet, thus enhancing accessibility to HSL. Other potential mechanisms for promoting the preferential release of 22:6n-3 could conceivably include a low capacity for re-esterification or a high capacity for transport across the plasma membrane for this polyunsaturate in comparison with other fatty acids.

The present results also indicated that the degree of preferential mobilisation of 22:6n-3 and other C<sub>20-22</sub> polyunsaturates from the adipose tissue of the chick embryo was greater under conditions of basal, as opposed to glucagon-stimulated, lipolysis. This observation could possibly reflect the existence of two distinct functions of adipose tissue during chick development: firstly, the postulated role of the tissue in the specific release of essential polyunsaturates to



support neural development would be most evident under the conditions of basal lipolysis which appear to predominate for most of the embryonic period [39–41]; and secondly, the general mobilisation of fatty acids from the tissue for energy purposes would be more likely to feature mainly during the subsequent hatching and early neonatal periods which are characterised by enhanced plasma levels of glucagon and also by increases in the sensitivity of the chick's adipose tissue to the lipolytic effects of this hormone [39–42]. Possible mechanisms for the attenuation of the preferential mobilisation of C<sub>20-22</sub> polyunsaturates by glucagon could include the phosphorylation of putative regulatory proteins or alterations in the rates of re-esterification of specific fatty acids.

Traditionally, adipose tissue has been regarded as a general repository of TAG, with the bulk mobilisation of the stored lipid providing FFA for oxidation to satisfy the body's energy needs during, for example, starvation or exercise. This view has recently been modified as a result of the studies of Pond and co-workers [43,44], suggesting that some parts of certain adipose depots may display more specialised functions; for example by supplying adjacent tissues such as lymph nodes with polyunsaturated fatty acids for membrane synthesis and regulatory functions. The current work appears to provide a further example of the functional versatility of adipose tissue, whereby, in the chick embryo, C<sub>20-22</sub> polyunsaturated fatty acids are preferentially released from adipose tissue concomitant with the accretion of these fatty acids in the phospholipids of the developing brain [29,30,38]. Further work is needed to confirm whether the brain of the chick embryo is in fact able to utilise directly the polyunsaturated fatty acids which are mobilised from adipose tissue and also to investigate whether any further examples of unique treatment of 22:6n-3, such as preferential uptake by the brain of this fatty acid from the plasma FFA pool, are expressed in this system.

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