Interactions between gluconeogenesis and non-esterified fatty acid oxidation in hepatocytes from 1-day-old rats

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Studies carried out previously in this laboratory have shown that starved newborn rats become rapidly hypoglycaemic owing to a low rate of gluconeogenesis when compared with suckling rats (Girard *et al.*, 1975). The low rate of gluconeogenesis in the starved newborn rats is strongly stimulated *in vivo* by triacylglycerol feeding, which increases plasma non-esterified fatty acids as well as their hepatic oxidation (Ferré *et al.*, 1978). The interactions between hepatic gluconeogenesis and non-esterified fatty acid oxidation have thus been qualitatively established in the newborn rat (Ferré *et al.*, 1979). However, this phenomenon is difficult to quantify *in vivo*. This is why we have performed studies *in vitro* using hepatocytes from 1-day-old rats.

Hepatocytes were prepared by the method described by Berry & Friend (1969), with the following modifications: livers from six 1-day-old rats (unfed or suckling) are perfused simultaneously in order to allow a sufficient yield of cells; after anaesthesia of the animal (sodium pentobarbital, 1.5 mg/kg body wt.), a needle is inserted in the inferior vena cava and the perfusion medium enters the liver by hepatic veins and leaves it by the portal vein, which is simply cut; resistance at heart valves ensures a satisfactory flow through the portal vein. After isolation, hepatocytes are still contaminated with haematopoietic cells, abundant in the liver 1 day after birth; however, these latter cells are easily distinguishable from hepatocytes, owing to their small diameter as seen by light microscopy. The number and viability of hepatocytes is determined with a haemocytometer by using a phase-contrast microscope. Endogenous glucose production has been substracted in the results, which are expressed as μmol of glucose produced/h per 10^9 viable hepatocytes and as means $\pm$ S.E.M. of six to ten observations. In the 1-day-old suckling rat, hepatocytes produce glucose from 10mM-lactate at a rate of 270 ± 22. These values are higher than those obtained with hepatocytes from unfed adult rats, 160 ± 6 (Demaugre *et al.*, 1978). In contrast, hepatocytes from 1-day-old starved rats produce glucose from 10mM-lactate at a rate of 55 ± 10; after addition of 1 mM-oleate bound to fatty acid-free albumin, glucose production from 10mM-lactate in hepatocytes of starved rats rises to 183 ± 36 ($P < 0.01$). If hepatocytes of suckling rats are incubated in presence of an inhibitor of long-chain fatty acid oxidation, 2-tetradecylglycidic acid (Tutwiler & Dellevigne, 1979), at a concentration of 20μM, the gluconeogenic rate decreases from 271 ± 22 to 149 ± 20 ($P < 0.01$); 2mM-octanoate added together with the inhibitor restores a normal rate of gluconeogenesis, 262 ± 19. This shows that the inhibition of fatty acid oxidation was responsible for the decreased gluconeogenesis and not a toxic effect of the inhibitor itself.

In conclusion: (1) the procedure described here allows the isolation of viable newborn-rat hepatocytes in amounts sufficient to perform metabolic studies; (2) these hepatocytes from suckling newborns have a rate of gluconeogenesis similar to that of 48h starved adult rats; (3) an active hepatic non-esterified fatty acid oxidation is required to sustain a high rate of gluconeogenesis in the hepatocytes of newborn rats.

Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520

Demaugre, F., Leroux, J.-P. & Cartier, P. (1978) *Biochem. J.* **172**, 91–96

Ferré, P., Pégorier, J.-P., Assan, R., Marliss, E. B. & Girard, J. R. (1978) *Am. J. Physiol.* **234**, E129–E136

Ferré, P., Pégorier, J.-P., Williamson, D. H. & Girard, J. R. (1979) *Biochem. J.* **182**, 593–598

Girard, J. R., Guillet, I., Marty, J. & Marliss, E. B. (1975) *Am. J. Physiol.* **229**, 466–473

Tutwiler, G. F. & Dellevigne, P. (1979) *J. Biol. Chem.* **254**, 2935–2941

Lipids of marine teleost fish (Teleostei)

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Lipid composition of marine fish has been reported by different authors interested in the nutritional aspects of fish (Lambertsen, 1973; Ackman, 1974, and references therein). Therefore, most of the data have been limited to cod (*Gadus morrhua*), plaice (*Pleuronectes platessa*), mackerel (*Scomber scombrus*) and the like. Moreover, those reports often compare lipid compositions of fish captured in very distant areas, or in different seasons. We have attempted a systematic study of the lipid composition of Eastern Atlantic fish, concerning the comparative and phylogenetic aspects of the subject but without neglecting its potential commercial interest.

All the fish used in the present study were captured in August 1979 by fishing boats operating from Vigo (north-western Spain). The fish was kept on ice until arrival at port (less than 12h) where one or several portions of muscle were dissected and frozen until analysed.

Lipids were extracted (Santiago *et al.*, 1964) and determined microgravimetrically. Lipid analysis were performed as described previously (Goñi & Macarulla, 1977). The degree of unsaturation (D.U.) refers to the number of double bonds per 100 fatty acid molecules.

The main results corresponding to 21 species are summarized in Table 1. Most of these fish species are edible, although only a few of them are of real commercial value. There are considerable differences in total lipid contents of the various species, from 1.4 mg/g muscle in *Trisopterus luscus*, and 2.7 in *Psetta maxima* (turbot), to 23.4 in *Scorpaena scropha*. Differences in cholesterol contents are smaller; extreme values are found in two members of the family Serranidae, *Serranus cabrilla* and *Dicentrarcus labrax*. Variations in phospholipid contents are in turn important; *S. scombrus* or *Belone belone* contain 10 times more phospholipid than *T. luscus*. The amount of depot triacylglycerols may be approximately estimated by subtracting from the total lipid contents the combined amounts of cholesterol and phospholipid. In this way we obtain again the extreme values in *T. luscus* or *P. maxima* (lower extreme) and *S. scropha* (higher extreme). Thus the lipid class that determines chiefly the total lipid contents are the triacylglycerols. An increased lipid proportion does not mean an increased cholesterol content; this is noteworthy in view of the pathogenic role of cholesterol in atherosclerosis.

Important variations are also observed in the D.U. of the fish lipids. In general, fish lipids are much more unsaturated than

Table 1. *Lipid composition of muscle in various species of Eastern Atlantic Teleostei*

[a] $C_{14:0} > 5\%$; [b] contains fatty aldehydes (~4%); [c] $C_{16:0} > 37\%$; [d] $C_{16:0} < 25\%$; [e] $C_{16:1} > 10\%$; [f] $C_{18:0} > 10\%$; [g] $C_{18:1} > 30\%$; [h] $C_{18:1} < 10\%$; [i] $C_{20:5} > 20\%$; [j] $C_{20:5} < 5\%$; [k] $C_{22:6} > 25\%$; [l] $C_{22:6} < 5\%$. Abbreviation used: D.U., degree of unsaturation.

Order/family	Species	Content (mg/g of muscle)			D.U.
		Total lipid	Sterol	Phospholipid	
Pleuronectiformes					
Scophthalmidae	*Psetta maxima*	2.7	0.28	1.9	259
	Lepidorhombus whyff[d]	4.2	0.32	2.6	251
Pleuronectidae	*Plathicthys flessus*[d,e,l]	6.9	0.41	3.0	222
Soleidae	*Solea vulgaris*	10.6	0.38	3.0	187
Anguilliformes					
Anguillidae	*Anguilla anguilla*[g]	17.0	0.96	4.9	215
Congridae	*Conger conger*[e]	12.6	0.59	3.0	170
Beloniformes					
Belonidae	*Belone belone*[e,f]	8.2	0.37	5.0	208
Lophiiformes					
Lophiidae	*Lophius budegassa*[d,l]	4.2	0.44	2.5	286
Gadiformes					
Gadidae	*Trisopterus luscus*	1.4	0.25	0.49	234
	Micromessistios poutassou	3.2	0.26	1.0	157
Perciformes					
Serranidae	*Serranus cabrilla*[d]	4.8	0.23	2.6	193
	Dicentrarchus labrax[d,i,k]	15.7	0.94	3.9	323
Sparidae	*Pagellus bograves*[c]	5.1	0.40	3.9	212
	Boops boops[c,j]	6.1	0.37	3.5	200
Sciaenidae	*Sciaena umbra*[c,f,j,l]	10.6	0.65	1.9	85
Carangidae	*Trachurus trachurus*[k]	4.8	0.56	3.9	277
Mugilidae	*Lisa sp.*[b,d,h,l]	13.8	0.70	4.3	258
Scorpaenidae	*Scorpaena scropha*[k]	23.4	0.70	3.5	353
Thunnydae	*Thunnus albacares*	12.0	0.51	3.4	161
Scomberomoridae	*Sarda sarda*[a]	13.4	0.70	4.6	161
Scombridae	*Scomber scombrus*[a,l,j]	15.0	0.32	5.0	115

those of mammals, especially because of the presence of fatty acids containing five and six double bonds. In virtually all species, the main fatty acids were $C_{16:0}$, $C_{18:1}$, $C_{20:5}$ and $C_{22:6}$. This is in accord with the data collected by Ackman (1974). Nevertheless, there are important interspecific variations, some of which are also indicated in Table 1.

It is not easy at the present moment to establish a relationship between similarity of lipid composition and phylogenetic proximity. Similarities exist indeed among members of the same family in Scophthalmidae or Sparidae, or among members of related families, like Thunnydae, Scomberomoridae and Scombridae, all three belonging to the sub-order Scombridei.

However, it is difficult to observe any similarities at all in higher taxonomic levels, e.g. order, and even at the level of family, similar lipid composition is not always the rule (cf. Gadidae). More data are required in order to ascertain whether or not a correlation can be established between phylogeny and lipid composition of fishes. At present, we are extending our studies to a variety of fish species from the Bay of Biscay.

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Ackman, R. G. (1973) *Proc. FAO Conf. Fishery Products, Tokyo*, pp. 112–131

Goñi, F. M. & Macarulla, J. M. (1977) *Laboratorio (Granada, Spain)* **384**, 501–519

Lambertsen, G. (1973) *Wiss. Veroeff. Dtsch. Ges. Ernaehr.* **24**, 25–31

Santiago, E., Mule, S., Redman, M., Hokin, M. R. & Hokin, L. E. (1964) *Biochim. Biophys. Acta* **84**, 550–562

Translation *in vitro* of rabbit reticulocyte carbonic anhydrase

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The synthesis *in vitro* of carbonic anhydrase (EC 4.2.1.1) by both human and animal reticulocytes has been described in several reports (Meyers *et al.*, 1969; Edwards, 1970; Beuzard *et al.*, 1973; Desimone *et al.*, 1973; Anyaibe & Headings, 1975). Recently rabbit reticulocyte lysates have been employed to show incorporation of a radioactive amino acid into specific immunoprecipitable material (Taniguchi *et al.*, 1978) and using this system, carbonic anhydrase isoenzymes (CAI the 'low-activity' isoenzyme and CAII the 'high-activity' isoenzyme) were apparently labelled at similar rates. In contrast, however, Cramer *et al.* (1978) have shown predominant synthesis of CAI by reticulocyte lysates.

To investigate these previous results further, the synthesis *in vitro* of rabbit CAI and CAII, by using both total reticulocyte RNA preparations in cell-free systems and intact erythrocyte precursors, was monitored by affinity chromatography on a sulphonamide resin (Osborne & Tashian, 1975). This technique has the advantage that the sulphonamide ligand, which specifically inhibits CAI and CAII activity, will only bind isoenzymes that have the active-site configuration of the native enzyme.

Linear incorporation of radioactively labelled amino acids