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An Experimental Study on Transcutaneous Absorption of Ethylesters of Eicosapentaenoic Acid (EPA; 20:5*n*-3) and Docosahexaenoic Acid (DHA; 22:6*n*-3) in Pigs

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Taugbøl, O., T. Framstad and J. R. Rest: An experimental study on transcutaneous absorption of ethylesters of eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) in pigs. Acta vet. scand. 1996, 37, 273-277. – Twenty-two pigs with an average weight of 24.2 kg were divided into two groups. Five grams of an ointment containing 40% of the n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) as ethylesters, was administered cutaneously to the experimental group and orally to the control group twice daily over a 3 day period. Surgical biopsies of subcutaneous fat of all 22 pigs were taken before and after the ethylester administration period. Blood samples were taken from the jugular vein. Skin biopsies were taken after the application period, and the presence of pathological features and inter-animal variation were noted. No increase of EPA and DHA in subcutaneous fat was found in either group. In the oral group the content of EPA had increased in the total plasma lipids on the first day after the end of the experiment. It is concluded that a rapid transcutaneous absorption of EPA and DHA as ethylesters does not seem to occur in pigs.

n-3 polyunsaturated fatty acids; transcutaneous absorption; pig.

Introduction

A study of *Marangoni et al.* (1993) indicated that eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) as ethylesters after only one application penetrated the skin of rats, and were found in subcutaneous fluid, plasma and liver. It is well established that the stratum corneum is the major barrier to most permeants, whereas appendages, such as hair follicles, sweat and sebaceous glands, can provide shunt routes for skin absorption after topical application (*Einstein et al.* 1994). Pigs have a relatively higher ratio of stratum corneum to skin appendages than the rat, and the stratum corneum of pig skin is thicker and containes more lipid bilayers than the skin of rats. In a previous study we found that EPA was incorporated in subcutaneous fat of pigs after 3 days of dietary supplementation with fish oil (*Taugbøl* 1993). The aim of this study was to see if the highly lipophilic ethylesters of EPA and DHA penetrated the skin of young pigs after a 3-day application period.

Materials and methods

Ointment administration and animals

An ointment, containing 64.1% (w/w) EPAX 6000 EE (Pronova Biocare a.s., Sandefjord, Norway), 26.9% water, 7.5% egg-yolk, 0.9% ascorbic acid, 0.5% stabilizer (E410, E412, E415) and 0.1% benzoic acid was used. The EPAX 6000 EE contained 38.3% EPA and 25.2% DHA as ethylesters. Twenty-two pigs of Norwegian Landrace, barrows and gilts, were divided into 2 groups of 11 pigs each. Average initial weight was 24.2 kg. The ointment was administered cutaneously to the experimental group and orally to the control group. In the experimental group the hair of the right side of the pigs' caudal back was removed with electric hair clippers one day before application. Five grams of ointment were applied to the skin in the clipped area (about 450 cm^2) twice daily, whereas 5 grams of ointment were given as a food supplement twice daily to the animals subjected to the oral treatment. The pigs were kept separated until 2 hours after feeding and ointment application. Four pens designed for individual feeding were used in this experiment, and there was an equal number of pigs from both groups per pen. The ethylesters were administered over a 3-day period. The pigs were fed a standard pig mash, twice daily, according to a standard norm. They were housed in an environmentally controlled barn with partially slotted concrete floors.

Sampling

Blood from the jugular vein was collected into heparin vacuum tubes, and the plasma was stored in small plastic tubes at -80°C until processed.

The medication consisted of 4 mg/kg Azaperone i.m. and 2 mg/kg Pethidine i.m. for sedation and Lidocaine 2% with adrenaline for local anaesthesia. Surgical biopsies of subcutaneous fat were taken from the caudal back of all 22 pigs on the day before start, and one and 4 days after the ethylester administration period. From the experimental group subcutaneous fat was taken from both the treated and the untreated sides of the caudal back. The samples, weighing 200-400 mg, were stored in small plastic tubes containing 0.9% saline solution at –80°C until analysed.

Punch biopsies (diameter 8 mm) from the skin of the caudal back were taken one day after the end of the application-period, from 10 pigs, 5 pigs from each group. Skin biopsies from the experimental group were taken from treated areas. The biopsies were embedded in paraffin, cut and stained with hematoxylin and eosin (H&E).

The experiment was approved by the local responsible Laboratory animal science specialist under the surveillance of the Norwegian Experimental Animal Board and registered by the Board.

Analytical methods

The lipids from 150 mg subcutaneous fat and 0.4 ml plasma were extracted by a modification of the procedures of *Folch et al.* (1957), and methylated as described by *Taugbøl* (1993). The methylated fatty acids were extracted with 1 ml hexane and separated on a fused silica wall coated capillary column (Chrompack, WCOT Fused Silica (CP-Sil-5 CB); 50 m x 0.25 mm, film thickness 0.12 μm) in a Dani 86.10 gas chromatograph fitted with a flame-ionization detector and a programmable temperature vaporizer (PTV) injector. Carrier gas used was helium. The injector and detector temperature was 260°C. The column temperature was kept at 60°C for 1.2 min, then increased to 200°C at a rate of 25°C/min, then up to 250°C at a rate of 0.8°C/min (total time 70 min). Identification of major peaks was made by comparing the retention time with those of standard fatty acid-methylesters. The results are reported as rela-

Table 1. Fatty acid composition (area%) in subcutaneous fat from the experimental (cutaneous) and control (oral) groups one day before and one day after the ethylester administration period. The biopsies from the experimental group were taken from the applied area.

	Before	After	
Fatty acid	(both groups) mean (SD)	oral mean (SD)	cutaneous mean (SD)
20:5n-3 (EPA)	0.07 (0.02)	0.05 (0.01)	0.07 (0.03)
22:6n-3 (DHA)	0.47 (0.06)	0.46 (0.06)	0.41 (0.10)

tive distribution of fatty acids from the percentage of the total area under the peaks.

Two H&E sections from each animal were examined without any knowledge of the treatment given. The presence of pathological features and inter-animal variation were noted.

Statistical methods

The results are described as the mean and standard deviation. Missing values were below the detection limit for the variable. Thus, all missing values were set to the minimum value found for each fatty acid in subcutaneous fat or plasma lipids, respectively. (The number of missing values of EPA in subcutaneous fat was 3 and in plasma lipids 8.) The statistical analysis applied was a Repeated Measures ANOVA, with 3 different factors to control for: the time trend, the group difference and the individual pig. The time trend and the group difference were fixed factors and the individual pig was random. The level of significance was set to 0.05.

Results

Subcutaneous fat

In both groups no increase of EPA and DHA in subcutaneous fat was found, Table 1. Neither was there an increase in EPA and DHA in samples taken from the untreated side of the back or in samples collected 4 days after the end of the ethylester application.

Total plasma lipids

In the oral group an increase of EPA was found in the total plasma lipids on the first day after the end of the experiment, Table 2. Furthermore, there was a significant difference ($p<0.001$) between the 2 groups with regard to EPA, with the oral group higher than the cutaneous group. Four days after treatment the differences between groups had disappeared. There was no increase of DHA in any of the groups.

Skin histology

All sections had similar orthokeratosis without

Table 2. Fatty acid composition (area%) of plasma lipids from the experimental (cutaneous) and control (oral) groups one day before and one day after the ethylester administration period.

	Before	After	
Fatty acid	(both groups) mean (SD)	oral mean (SD)	cutaneous mean (SD)
20:5n-3 (EPA)[a]	1.30 (0.14)	4.60 (1.51)	1.73 (0.87)
22:6n-3 (DHA)	3.14 (0.47)	3.12 (0.77)	2.93 (0.39)

[a] There was an increase of plasma EPA in the oral group after feeding the oinment for three days ($p<0.001$). Furthermore, plasma EPA of the oral group was higher than plasma EPA of the cutaneous group ($p<0.001$).

significant variation in thickness. In 2 animals, one from each group, there was focal minimal parakeratosis, and one had an intercorneal pustule. Mitotic figures were rare, and the non-keratinised epithelium (basal, spinous and granular layers) was similar in thickness in all animals. The collagenous dermis was similar in all animals. No variation in ground substance or fibre content was noted. A few perivascular cells (lympho-histiocytic and a few eosinophils) were seen in all animals. Most were in the upper dermis and no eosinophils were noted in the lower dermis. The subcutis (defined as adipose tissue) was present in sections from all but one, and where there was any significant quantity, it was septate and without inflammation.

Discussion

In our experiment we did not find any increase of the n-3 polyunsaturated fatty acids EPA or DHA in the subcutaneous fat or in blood plasma after application to the skin. The data from *Marangoni et al.* (1993) indicated strongly that, when applied as ethylesters on the skin of rats, n-3 polyunsaturated fatty acids entered into the body and were found subcutaneously and in plasma and liver only hours after application. The anatomical differences of the skin of pigs compared to that of rats might be the most important reason for this. The cream used in the study of *Marangoni et al.* (1993) contained partial and complete glycerides of saturated fatty acids and esters of polypropylene glycol, where as the ointment used in our study was an emulsion of ethylesters, egg yolk and water. In our study the ointment was massaged into the skin in a relatively thin layer, 10 mg/cm^2. In the study of *Marangoni et al.* (1993) 200 mg of the cream was applied within a plastic ring, which enclosed a surface area of 1 cm^2, and was glued to the skin. Applied to the skin in a thin layer, the emulsion will change character. Water will evaporate and the polyunsaturated ethylesters become vulnerable for oxidation. In the study of *Marangoni et al.* (1993) the ethylesters might have been protected by the fatty composition of the cream and the thicker application. The rapid absorption seen in rats might also have been a result of rats licking the cream of the skin. In our experiment, the pigs were kept separated for 2 h after application to prevent them from licking the ointment from the back of each other. In addition the pigs were grouped so that all pigs had the same opportunity to lick the ointment in the time they were not individually separated.

We failed to increase the EPA in the subcutaneous fat of the pigs subjected to the oral treatment. *Taugbøl* (1993) reported an increase of the EPA content of subcutaneous fat after only 3 days of oral administration of fish oil to young pigs, and we therefore expected an increase of EPA also after oral supplementation of this ointment. The n-3 polyunsaturated fatty acids of fish oil were triacylglycerides, where as in this experiment EPA and DHA were ethylesters. The intestinal absorption of fatty acids from ethylesters might be lower than from triacylglycerides (*Boustani et al.* 1987, *Reicks et al.* 1990).

DHA did not increase in subcutaneous fat and total plasma lipids in any of the groups. The same was observed in a study in which fish oil was given to hyperlipidemic patients. The EPA content of plasma phospholipids reflected the dietary intake, whereas DHA levels were less discriminating (*Harris* 1989).

The histology of the skin was similar in both groups.

The question whether EPA and DHA as ethylesters penetrate the skin is still controversial. However, a rapid transcutaneous absorption as indicated in rats does not seem to occur in pigs.

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Sammendrag

En eksperimentell studie av perkutan absorbsjon av eikosapentaensyre (EPA; 20:5n-3) og dokosaheksaensyre (DHA; 22:6n-3), begge i form av etylester, hos gris.

Smågris med en gjennomsnittsvekt lik 24,2 kg ble delt i 2 grupper med 11 gris i hver gruppe. Fem g av en salve anriket med langkjedete n-3 fettsyrer, eikosapentaensyre (EPA; 20:5*n*-3) og dokosaheksaensyre (DHA; 22:6*n*-3), i form av etylestere ble påsmurt huden i den eksperimentelle gruppen og gitt som et fôrtilskudd til kontrollgruppen 2 ganger daglig i 3 påfølgende dager. Hårene på huden der salven ble påført var klippet. Fettsyresammensetningen i blodplasma og i underhudsfett fra alle griser ble analysert fra prøver tatt en dag før og en dag etter behandling. Forsøket konkluderer med at etylestere av langkjedete n-3 fettsyrer vanskelig penetrerer huden på gris.

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