enzyme activities in muscle (experiment 1). This suggests that the fatty acids deposited in muscle during growth were, at least partly, synthesised in situ. With various dietary fat sources (experiment 2), no relationship was observed, however, between diet-induced changes in enzyme activities and responses in lipid content of muscle, perirenal fat or liver. Rather, the fatty acid composition of tissue lipids mainly reflected that of dietary fat. This suggests that most of the tissue lipids were obtained from circulating triglycerides when rabbits were fed high fat diets. Thus, further investigations are needed to quantify the relative importance of lipogenic enzymes and lipoprotein lipase in the lipid deposition in the rabbit.

Communication no. 17

Activity and regulation of mitochondrial carnitine palmitoyltransferase I are influenced by age, cold exposure and muscle type in piglet skeletal muscle.
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Lipid utilisation increases rapidly during the first postnatal days in pigs, especially during cold-induced muscular shivering thermogenesis. In the liver, carnitine palmitoyltransferase I (CPT I) controls the entry of long-chain fatty acids into the mitochondria and its regulation by malonyl-CoA plays a pivotal role in the regulation of fatty acid oxidation (McGarry et al., Biochem. J. 214 (1983) 21–28). This study investigates the possibility that such a regulatory system is involved in the modulation of fatty acid utilisation in pig skeletal muscle during early postnatal development.

Intermyofibrillar (IM) and subsarcolemmal (SS) mitochondria were isolated from *longissimus thoracis* (LT) and *rhomboideus* (RH) muscles from newborn (n = 10) and 5-d-old piglets. At 5 d, piglets were maintained for a 4-h period in thermoneutral (TN, 30 °C, n = 10) or cold (C, 20 °C, n = 10) conditions before being killed. CPT I activity was assayed as the formation of [^{3}H]-palmitoyl-L-carnitine from [methyl-^{3}H]-carnitine and palmitoyl-CoA according to Herbin (Herbin et al., Eur. J. Biochem. 165 (1987) 201–207). The sensitivity of CPT I to malonyl-CoA inhibition corresponded to the concentration of malonyl-CoA required for 50 % inhibition of the enzyme activity (IC_{50}) in IM mitochondria. Muscle concentration of malonyl-CoA was determined by reversed-phase HPLC on LT and RH muscles.

In SS mitochondria, CPT I activity was lower in LT than in RH muscle at birth (–40 %, $P < 0.001$) and increased by about 600 and 200 % in LT and RH muscles, respectively, within 5 days; there was no effect of cold exposure. In IM mitochondria, CPT I activity was much higher than in SS mitochondria (+620 % at birth, +170 % at 5 d, $P < 0.001$) but was not affected by age, muscle type or cold exposure. Sensitivity of CPT I to malonyl-CoA was similar in both muscles (IC_{50}: 3–6 μM) and 50–100 times lower than in rat muscle (0.035 μM, Mc Garry et al., 1983). It decreased by 47 % ($P < 0.05$) with age. After 4 h of cold exposure, sensitivity of CPT I to malonyl-CoA was unaffected in RH muscle but tended to be greater (+30 %, $P < 0.06$) in LT muscle. Malonyl-CoA concentrations decreased by 27 and 42 % ($P < 0.01$) between birth and 5 d in LT and RH muscles, respectively. Cold stress led to a 27 % ($P < 0.05$) reduction in malonyl-CoA concentration in RH muscle. In conclusion, the postnatal enhancement of CPT I activity in SS mitochondria and weakening of the inhibitory effect of malonyl-CoA upon CPT I activity in IM mitochondria support the suggestion that fatty acid oxidation increased rapidly after birth in piglet skeletal muscle. During cold stress, the muscle-specific decrease in malonyl-CoA observed in RH muscle could partly relieve CPT I inhibition and favour fatty acid oxidation. Finally, the lower sensitivity of CPT I to malonyl-CoA in piglet

than in rat skeletal muscle deserves further molecular studies to determine which isoforms of CPT I are expressed in the piglet skeletal muscle.

Communication no. 18

Isoprostanes (8-epi PGF2a), products of lipid peroxidation, as oxidant stress markers. C. Feillet-Coudray, A. Mazur, E. Rock, Y. Rayssiguier (Laboratoire des maladies métaboliques et micronutriments, Inra, Theix, 63122 Saint-Genès-Champanelle, France)

Free radicals have been implicated in the pathophysiology of a wide variety of diseases including cancer, atherosclerosis, neurodegenerative disorders and even the normal ageing process. Measurement of lipid peroxidation is often employed to evaluate oxidative stress, and more precisely, oxidation products of polyunsaturated fatty acids (TBARS, conjugated diene). These assays, however, suffer from inherent problems related to specificity and sensitivity. Thus, there is a need to provide more reliable markers of oxidative stress in vivo. Recently, isoprostanes have been proposed and seem to be particularly valuable markers. Isoprostanes are produced by the free-radical peroxidation of arachidonic acid. Morrow et al. (Morrow et al., Proc. Natl. Acad. Sci. USA 87 (1990) 9383–9387) were the first to quantify free isoprostanes in plasma and urine by GC/MS, and to demonstrate the increased levels of lipid peroxidation in animal models. Measurement of isoprostanes allowed the detection of oxidative stress in animal models and provided evidence for a role of oxidative stress in human disease. In animals, administration of CCl4 to normal rat and diquat to selenium-deficient rats, combined with vitamin E/selenium and iron overload caused increased levels of isoprostanes. In humans, increased levels of isoprostanes were observed in relation to age, chronic cigarette smoking, diabetes and hypercholesterolemia; administration of vitamin E decreased these levels (Morrow et al., Biochim. Biophys. Acta 1345 (1997) 121–135).

We carried out a large number of nutritional and metabolic disorder studies in animal models, using GC/MS methods (which are difficult and expensive) and recently developed immunoassay methods (which are less time consuming and less expensive). Deficiencies in antioxidant micronutriments (zinc, copper), ageing, alcohol intake and diabetes (STZ) were investigated in rats. Increased urinary levels of isoprostanes were observed in Cu-deficient animals on a prooxidant diet (fructose). Membrane alterations in Cu-deficiency which are related to haemolytic anaemia, may explain this observation. No modification in the levels of isoprostane was observed in other studies. In humans, trials are under way to assess isoprostane levels in degenerative disorders associated with ageing.

The discovery of isoprostanes has opened up new areas of investigation regarding the occurrence of free radical damage in human physiopathology and may provide a valuable approach to evaluate the efficiency of antioxidant micronutriments.

SESSION 3:

TISSUE GROWTH

Communication no. 19

Apoptosis in myogenic cells: effect of oxidative stress and flavonoid antioxidants. A. Orzechowski[a], J. Skierski[b], W. Zimowska[b], B. Balasinska[b], T. Motyl[b], B. Lukomska[c] ([a] Department of Animal Physiology, Warsaw Agricultural University, Nowoursynowska 166, 02-785 Warsaw, Poland; [b] Flow Cytometry Laboratory, Drug Institute, [c] Surgery Research and Transplantation Department, Medical Research Center Institute, Warsaw, Poland)

Muscle differentiation is initiated by a Rb (retinoblastoma) gene product (Okuyama