Proceedings of the Nutrition Society

Abstracts of Original Communications

The 3rd French–British Meeting on Nutrition, a joint meeting of the Nutrition Society, Association Française de Nutrition and Société de Nutrition et de Diététique de Langue Française was held at Nancy, France on 30 September–2 October 1998, when the following papers were presented.

All abstracts are prepared as camera-ready material by the authors.

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Restoration of full reproductive capacity in male and female *ob/ob* mice by chronic leptin administration. By S.M. McBENNETT, E. SMYTH and J.F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

The *ob/ob* mouse has a defective *ob* gene and is thus unable to produce an active form of the *ob* gene product leptin. In addition to the striking obesity which develops shortly after weaning in the *ob/ob* mouse, one of the other manifold effects of their lack of active leptin is the failure to mature sexually. Thus the *ob/ob* mouse is sterile, with immature gonads in both males (undescended testes) and females (immature ovaries with no occurrence of oestrus cycle). Chronic injection of leptin has been shown to reverse some of the reproductive deficits in female mice (Chehab *et al.* 1996) and male mice (Mounzih *et al.* 1997). The aim of the present study was a more extended investigation over the full reproductive cycle to see if it were possible to fully restore reproductive capacity, both physiological and behavioural. Could chronically leptin-injected *ob/ob* mice of both sexes produce offspring? Could males develop fully mature testes and develop mature sperm and in addition have restoration of mating behaviour to copulate with and successfully fertilize proven normal females? Could female mice develop fully mature ovaries, have restoration of oestrus cycle, be receptive to proven normal males and thus become pregnant, carry their fetus to term and successful delivery and, most importantly, then feed and 'mother' their offspring to successful weaning? This study proved that chronic leptin administration was able to restore reproductive capacity in this most complete sense, in both male and female *ob/ob* mice.

Young, 7-week-old male (n 7) and female (n 7) Aston strain *ob/ob* mice were identified. They were treated twice daily with injections (i.p.) of 25 μg leptin dissolved in sterile saline. Control animals (two male, two female) were saline treated only. Animals were allowed food and water *ad libitum* and were housed at 20°. As expected (Pelleymounter *et al.*. 1995) leptin treatment reduced food intake, increased metabolic rate and normalized body-weight gain to that of non-obese animals.

Males were killed after 30 d treatment with leptin. Testicular function was unequivocally stimulated, with full descent and increased weight of testes (8.25(SE 0.66) *v* 4.02(SE 0.41) in controls, mg/g body weight) and histological evidence of spermatogenesis, with many sperm heads, though few sperm tails had yet formed, changes absent in the untreated controls. After 50 d, allowing sufficient time for full sperm maturation, one leptin *ob/ob* mouse successfully sired a litter when paired with a proven normal female.

All seven treated female animals had passed through their first oestrus cycle by 27 d of treatment. Cycling was established by cytological examination of vaginal smears. Two of these cycling animals, maintained on leptin, were paired with proven male normal mice and became pregnant, successfully carrying their fetus to term and normal delivery. One of the pair lactated and successfully raised her litter of eight pups to weaning.

Chehab FF, Lim ME & Lu R (1996) *Nature Genetics* **12**, 318-320.

Mounzih K, Lu R & Chehab FF (1997) *Endocrinology* **138,** 1190-1193.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F (1997) *Science* **269,** 540-543.

Comparison of dietary restriction alone with leptin treatment on spermatogenesis in male *ob/ob* mice. By S.M.McBENNETT, E.SMYTH and J.F.ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

The male *ob/ob* mouse, lacking functional leptin, is sterile with immature undescended testes. Leptin treatment of young male *ob/ob* mice for 30 d restores testicular function with descent of the testes, normal histology and spermatogenesis. After 50 d, fully functional sperm are present with total restoration of reproductive capacity as demonstrated by a treated *ob/ob* malemaking a normal female pregnant with subsequent delivery of healthy offspring (McBennett *et al.* 1999). Leptin treatment brings about a dramatic reduction in the *ad libitum* food intake of treated animals compared with that of vehicle-treated control *ob/ob* male mice and is accompanied by reduction in metabolic rate with, consequently, a normalization of body weight (Pelleymounter *et al.* 1995). As dietary restriction alone, with reduction in body weight, has been shown to cause some restoration of the testicular function of *ob/ob* mice (Mounzih *et al.* 1997) we argued that the effect of leptin, in normalizing the energetics and fat content of the body which allow restoration of reproductive capacity, may not be a direct, but rather a permissive effect of leptin on reproductive function.

Therefore, four male mice were studied, pair fed to the *ad libitum* food intake of four leptin-treated *ob/ob* mice. *ob/ob* saline-injected mice had an average *ad libitum* food intake of 9 g/d (two control animals were retained on this regimen). Leptin treatment (25 μg leptin in saline, twice daily, i.p.) reduced this by one-third to 6 g/d, which amount was offered to the pair fed *ob/ob* saline injected cohort. After 30 d treatment animals were killed, testes were dissected and weighed and studied histologically. *Ad libitum* -fed *ob/ob* mice, as expected, had highly abnormal undescended testes, weighing 4.02(SE 0.41) mg/g body weight with no sperm present; leptin-treated animals had fully descended testes weighing double those of untreated animals (8.25(SE 0.66) mg/g body weight), demonstrating normal histology with immature sperm present, without tails yet developed (full sperm maturation requires 50 d). Food restricted animals had partial, not full testicular descent with a modest 25% increase in weight, 5.0(SE 0.53) mg/g body weight, with an intermediate appearance showing some evidence of spermatogenesis commencing. This study demonstrates that leptin is required for full restoration of testicular capacity. Indeed, recently it has been shown that there are leptin receptors on both male and female gonads (Chehab *et al.* 1996). It is an intriguing observation, however, that reduction in food intake alone also has some beneficial effect on testicular performance, in animals which totally lack functional leptin. Perhaps the central regulatory mechanisms of reproduction have been normalized in these animals, but leptin is required in a permissive role at gonadal level for full restoration of function? This requires further investigation.

Chehab FF, Lim M. & Lu R. (1996) *Nature Genetics* **12**, 318-320.

McBennett SM, Smyth E & Andrews JF (1999) *Proceedings of the Nutrition Society* (In the Press).

Mounzih K, Lu R & Chehab FF (1997) *Endocrinology* **138,** 1190-1193.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F (1997) *Science* **269,** 540-543.

Circulating form of human leptin. By V. OGIER[1], D. LAMBERT[1], L. MEYER[2], O. ZIEGLER[2], J.P. NICOLAS[1] and L. MEJEAN[1], *[1] INSERM U308, Nancy, France. [2] Service de Diabétologie, Maladies métaboliques et nutrition, CHU de Nancy, France*

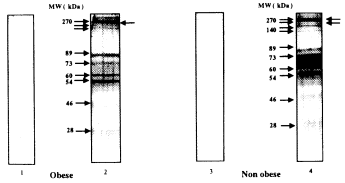
Leptin, the product of the *ob* gene, is secreted by adipose tissue and has been implicated in the regulation of food intake and energy expenditure. In man, circulating leptin concentrations are about four times higher in obese than in normal-weight subjects. This suggests that obesity is associated with leptin resistance. We investigated the physiology of circulating leptin by trying to ascertain whether leptin interacts with other circulatory components.

The distribution of leptin in plasma was analysed using Sephadex G-100 gel filtration and radioimmunoassay (RIA Kit Linco Inc., St Charles, MO, USA) of plasma from twenty five subjects: twenty one subjects were obese (BMI: 39.6 (SD 8.0) kg/m^2; leptin: 37.6 (SD 19.5) ng/ml; age: 46 (SD 11) years) and four subjects were normal weight (BMI: 20.8 (SD 0.7) kg/m^2; leptin: 10.1 (SD 11.0) ng/ml; age: 34 (SD 11) years).We demonstrated that leptin circulated in two forms: free leptin and leptin bound to macromolecules.

We used affinity chromatography and SDS PAGE to characterize these macromolecules. Two different protocols of affinity chromatography are described and compared. In the first, human recombinant leptin (R&D Systems) was coupled with Affi-Gel 15 (Bio-Rad)(0.5 mg/ml beads): 1 ml human plasma was incubated with 200 µl beads overnight at 4 °, washed with 20 ml PBS; 1% Tween 20; 200 mM-NaCl and 60 ml PBS; 1% Tween 20; 500 mM-NaCl and eluted with PBS; 1% SDS (Gavrilova *et al.* 1997). In a second protocol we used the Aminolink Plus Immobilization Kit (Pierce): 0.4 mg human recombinant leptin was coupled with 2 ml beads and incubated with 1 ml human plasma overnight at 4 °. The proteins were washed with 80 ml 50 mM-phosphate buffer; 0.2% Tween 20; 500 mM-NaCl; pH 7.5 and eluted with 0.1 M-glycine buffer pH 2.8 (Sinha *et al.* 1996).

In the two protocols eluted and washed proteins were concentrated using ultrafiltration (Centricon 3 Amicon) and electrophoresed in a 10% SDS PAGE gel. Preliminary analyses of proteins bound to leptin for an obese and a non-obese patient were performed.

The washing step was more efficient when leptin was bound to Affi-Gel 15. With the Aminolink Plus Immobilization Kit, proteins were still present before elution even when using a large volume of washing buffer. Affinity chromatography on Affi-Gel 15 followed by SDS PAGE showed many proteins: ten bands were obtained for the obese subject, and twelve bands were obtained for the non-obese subject. A major band was not detected at molecular mass of 140 kDa for the obese subject. The amounts of protein of the same molecular mass in the obese and non-obese subjects were different. There was more peptide of molecular mass 73 kDa in the non-obese than in the obese subject.



SDS PAGE of proteins eluted from leptin affinity column (Affi-Gel 15) of an obese subject (BMI: 35.4 kg/m^2; leptin: 27.5 ng/ml; age 24 years) (lanes 1 and 2) and of a non-obese subject (BMI: 20.9 kg/m^2; leptin: 12.7 ng/ml; age 28 years) (lanes 3 and 4). Lanes 1 and 3: last wash before elution (PBS, 1% Tween 20, 500 mM-NaCl) Lanes 2 and 4: elution (PBS, 1% SDS)

Work is in progress to confirm the difference between obese and non-obese subjects. We will check the specificity of the binding of these proteins to leptin and the binding of anti-Ob-Re to one of these proteins to determine the relation between the Ob-Re form of leptin receptor and the proteins.

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Effects of adrenaline, noradrenaline and isoprenaline on circulating leptin levels in mice. By ALISON MOSTYN, D. VERNON RAYNER and PAUL TRAYHURN, *Molecular Physiology Group, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Noradrenaline and ß-adrenoceptor agonists are potent inhibitors of the expression of the *ob* gene, this gene encoding the 16 000 M*r* hormone, leptin (Trayhurn *et al.* 1995; 1998). Leptin production by adipocytes in cell culture is inhibited by catecholamines, but whether circulating levels of the hormone in animals are affected by these agents has not been established. In the present study we have investigated the acute effects of adrenaline, noradrenaline and isoprenaline (a synthetic ß-adrenoceptor agonist) on plasma leptin in mice.

Male mice (Aston strain), aged 8-10 weeks, from a colony maintained at the Rowett were caged individually at 21°. The mice (eight in each group) received subcutaneous injections of either saline (controls), noradrenaline (750 µg/kg body weight), adrenaline (1000 µg/kg body weight) or isoprenaline (700 µg/kg body weight). The mice treated with adrenaline and noradrenaline were injected at 0, 2 and 4 h, while isoprenaline was administered at 0 and 2 h. All animals were killed 6 h after the first injection; truncal blood was collected and plasma obtained by centrifugation. The epididymal fat pads were removed. Plasma leptin levels were measured by ELISA with a mouse recombinant leptin standard (Hardie *et al.* 1996). *ob* mRNA was measured by Northern blotting using digoxigenin-labelled antisense oligonucleotide probes (Trayhurn *et al.* 1995).

The administration of noradrenaline led to a 65 % reduction in the level of circulating leptin by 6 h after the first injection ($P<0.05$; all changes relative to controls). Leptin levels were reduced by 60 % ($P<0.01$) following the injection of isoprenaline. A 76 % decrease in leptin was observed after treatment with adrenaline ($P<0.05$). The level of *ob* mRNA in the epididymal white adipose tissue was reduced by 80 % ($P<0.01$) in the mice injected with adrenaline.

Previous studies in mice have shown that both noradrenaline and isoprenaline inhibit *ob* gene expression in white adipose tissue (Trayhurn *et al.* 1995), and the present work indicates that this leads to a rapid fall in circulating leptin. Our results also show that adrenaline can inhibit leptin production, this occurring through the inhibition of *ob* gene expression. Adrenaline could play a role physiologically in the regulation of leptin production in situations in which there is a marked increase in the secretion of this catecholamine.

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Hardie LJ, Rayner DV, Holmes S & Trayhurn P (1996) *Biochemical and Biophysical Research Communications* **223**, 660-665.

Trayhurn P, Duncan JS, Hoggard N & Rayner DV (1998) *Proceedings of the Nutrition Society* **57**, 413-419.

Trayhurn P, Duncan JS & Rayner DV (1995) *Biochemical Journal* **311**, 729-733.

Effect of cholera toxin on glutamine metabolism and transport in rabbit ileum. By M. ABELY, P. DALLET and J.F. DESJEUX, *INSERM U.290 et Laboratoire de Biologie, Conservatoire National des Arts et Metiers, 75003 Paris, France*

Cholera is a disease often associated with malnutrition. Cholera toxin triggers a sudden increase in intestinal water and electrolyte secretion that may lead to rapid dehydration. Glucose-containing oral rehydration solution (ORS) has a dramatic effect on rehydration. However, glucose is not the main energetic substrate in the intestine. Thus, glutamine has been proposed as a substrate for ORS, in order to stimulate Na^+ absorption and provide additional energy to the intestine.

The aim of the present study was to evaluate the effect of cholera toxin on intestinal glutamine metabolism and glutamine-dependent Na absorption. NaCl and L-glutamine (gln) transport and metabolism across isolated ileum previously stimulated with cholera toxin of fasted weaning rabbits were studied in Ussing chambers. Ion fluxes were estimated by electrical variables (Isc) and isotopic tracers. For each animal, measurements were assessed in eight control and eight cholera tissues. Gln transport and metabolism were studied in seven other rabbits, simultaneously using ^{14}C fluxes and HPLC. Unlabelled 10 mM-gln and 74 kBq [^{14}C]gln were added in the mucosal compartment. The ^{14}C flux (J) across the epithelium representing the overall flux of glutamine and ^{14}C metabolites was expressed as Jgln-equivalent. In the same tissues, the flux of each [^{14}C]amino acid (Jgln, Jcit...) was studied by HPLC from the determination of amino-acid specific activity, and glutamine oxidation by measurement of $^{14}CO_2$ and alanine flux (glutamate and pyruvate pathway).

Results: (1) In control tissues, gln stimulated Na^+ absorption (Isc=2.2 (SE 0.22) µEq/h per cm^2) that was further stimulated by glucose. Gln was mainly oxidized ($J^{14}CO_2$ = 1.3 (SE 0.37) and Jala = 0.146 (SE 0.03) µmol/h per cm^2). In addition, the flux of intact gln (Jgln = 0.6 (SE 0.18) µmol/h per cm^2) represented 66 % of Jgln-equivalent (0.9 (SE 0.156) µmol/h per cm^2); the other metabolites were glutamate (glu) and to a lesser degree citrulline, ornithine and proline (P5C pathway). (2) Cholera toxin did not alter gln-stimulated Na^+ absorption (Isc 2.2 (SE 0.36) µmol/h per cm^2), gln oxidation (Jala = 146 (SE 43) nmol/h per cm^2), transport (Jgln-equivalent = 0.9 (SE 0.19) µmol/h per cm^2) and metabolism (Jgln = 0.7 (SE 0.2) and Jglu = 0.115 (SE 0.04) µmol/h per cm^2).

In conclusion, the present results confirm the presence in the small intestine of gln-dependent Na absorption and gln oxidation, absorption and metabolism; they further indicate the relative contribution of gln to the different functional and metabolic pathways. In addition, they strongly suggest that cholera toxin does not alter gln intestinal function and metabolism. Thus, gln may be beneficial to intestinal epithelial function of dehydrated patients with cholera, especially if they are malnourished.

Effects of ruminal or postruminal fish oil supply on conjugated linoleic acid (CLA) content of bovine milk fat. By Y. CHILLIARD[1], J.M. CHARDIGNY[2], J. CHABROT[1], A. OLLIER[1], J.L. SEBEDIO[2] and M. DOREAU[1], *[1]Laboratoire Sous-Nutrition des Ruminants, INRA, Theix, 63122 St-Genès-Champanelle and [2]Unité de Nutrition Lipidique, INRA, 21034 Dijon cedex, France*

CLA is naturally occurring in ruminants' milk fat, and could be a potential anticarcinogenic agent (Parodi, 1997). Supplementation of dairy rations with vegetable oils has been shown to increase CLA concentration in cow's milk fat (Kelly *et al.* 1997). Addition of fish oil (FO) to dairy cow diet decreases the milk fat content (Chilliard & Doreau, 1997), and this could facilitate the management of milk fat quotas by farmers. It is, however, necessary to evaluate the consequences of FO supply in terms of the quality of milk fat. The aim of the present study was to compare the respective effects of a control diet (C), and either a ruminal (R) or a duodenal (D) infusion of FO (menhaden type, 300 ml/d for 4 weeks), on the concentration of CLA and other C18 fatty acids in milk. A 3 x 3 Latin square was designed with six mid-lactation cows fitted with rumen and duodenum cannulas. Milk fat CLA content was determined by a combination of reverse phase HPLC and GLC analysis.

Treatment	Milk fat (g/d)	18:0*	18:1*n*-9	18:1*n*-7	18:2*n*-6	CLA
C	783[a]	7.2[a]	16.5[a]	1.1[a]	2.0[a]	0.5[a]
R	567[b]	2.3[b]	5.9[b]	12.5[b]	1.7[b]	2.6[b]
D	737[a]	8.1[a]	16.1[a]	1.0[a]	2.2[a]	0.4[a]

[a], [b]Values within a column bearing unlike superscripts were significantly different, $P < 0.01$.

* g/100g butyl esters.

D infusion of FO did not change milk fat yield or the proportions of C18 fatty acids (Table). R infusion sharply decreased milk fat yield and the proportions of stearic acid and 18:1*n*-9 (mainly oleic acid), and to a lower extent linoleic acid. It increased sharply (11- and 6-fold respectively) the proportions of 18:1*n*-7 (mainly *trans*-vaccenic acid) and CLA. The increase in CLA was due mainly (96 %) to the 9 *cis* – 11 *trans* isomer.

This study shows that milk fat secretion and the proportions of C18 fatty acids can be changed to a large extent by addition of FO to the diet of the dairy cow. This effect is mainly due to changes in ruminal metabolism of fatty acids, since D infusion was without effect. The sharp increase in CLA content of milk fat could be of interest for human consumption, although potential effects of the simultaneous increase in *trans*-18:1 and possibly *trans*-18:2 fatty acids requires careful evaluation.

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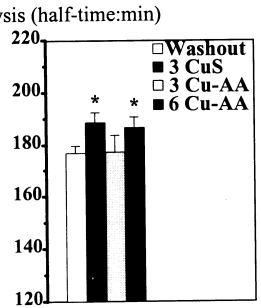
Parodi PW (1997) *Journal of Nutrition* **127**, 1055-1060.

Copper supplementation in middle aged people decreased the susceptibility to peroxidation of erythrocytes. By E. ROCK[1], A. MAZUR[1], A-M. ROCK[1], C. COUDRAY[1], Y. RAYSSIGUIER[1], C. KEHOE[2], J.M. O'CONNOR[2], M.P. BONHAM[2] and J.J. STRAIN[2],[1]*Unité Maladies Métaboliques et Micronutriments, INRA, 63122 Saint Genès Champanelle, France.* [2]*University of Ulster, Cromore Road, Coleraine, BT52 1SA*

Cu is an essential trace element and its deficiency may be involved in a number of degenerative and inflammatory diseases. No recommended dietary allowance has been set for Cu so that the estimated intake of about 1-2 mg/d may not be optimal. Cu is considered to be an antioxidant but it may also have pro-oxidant activity by itself and large dietary Cu intakes may also lead to oxidative stress. Therefore, the objective of the present study was to provide data on the significance of increased dietary Cu as a pro- or antioxidant *in vivo* in free living middle aged men and women. Free radical-induced haemolysis of erythrocytes *in vitro* has been used for that purpose.

Twelve males and thirteen females, with mean ages of 59 and 56 years, were included in the study after medical screening. They were supplemented daily for 6-week periods with capsules containing either placebo or $CuSO_4$ (3 mg) or Cu amino acid chelate (Cu-aa ; 3 mg or 6 mg). Analyses were performed on blood collected at the end of each 6-week periods. Oxidizability of erythrocytes was assessed by following haemolysis induced by an azo initiator, 2,2'-azo-bis(2-amidinopropane) hydrocloride (AAPH).



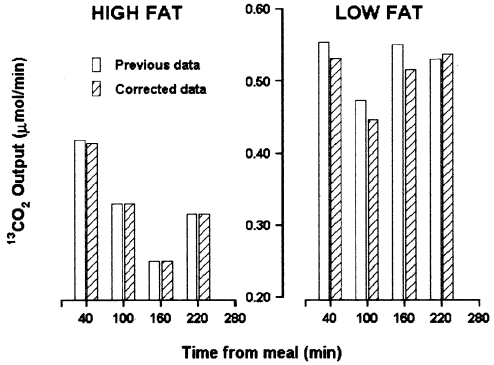
In the whole population (*n* 25), the mean time necessary for 50 % haemolysis induced by AAPH was significantly higher after supplementation with 3 mg $CuSO_4$/d and 6 mg Cu-aa as compared with the preceding placebo period (Fig.). No differences were found with people supplemented with 3 mg Cu-aa/d. Decreased susceptibility of erythrocytes to peroxidation occurred without changes in several indices of Cu status including erythrocyte (Cu-Zn) superoxide dismutase activity. However, significant increases were found in diamine oxidase activity. Together, these findings suggest that intake of Cu as high as 7 mg/d has no pro-oxidant activity erythrocytes.

This work was conducted under the FoodCue contract - EU-FAIR CT 95 0813.

Impact of endogenous ^{13}C-labelling of a foodstuff on $^{13}CO_2$ output during stable-isotope studies of postprandial fat metabolism. By C. J. SEAL and J. C. MATHERS, *Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU*

We have previously reported the results of a study on postprandial fat metabolism (Moore *et al.* 1997) in which the oxidation of free fatty acid after a meal was estimated by measurement of $^{13}CO_2$ production during a continuous intravenous infusion of [1-^{13}C]palmitate. These results indicated that the proportion of the palmitate flux which was oxidized was higher after consumption of a low-fat meal (LF; 20 % of dietary energy from fat) compared with a high-fat meal (HF; 60% of dietary energy from fat) throughout the postprandial phase. The LF meal included a glucose drink prepared from hydrolysed maize starch (providing approximately 45 g glucose), and the plasma glucose response after consumption of this meal was significantly higher than after the HF meal. It was suggested that the increased $^{13}CO_2$ output may be due to oxidation of glucose from the glucose drink which is naturally enriched with ^{13}C arising from C4-metabolism in the maize plant.

Four (out of five) healthy, weight-stable non-obese volunteers (two males) who had taken part in the previous experiment returned to the Wellcome Laboratory Metabolic Unit to repeat the study without stable-isotope infusions. Each consumed the LF and HF meals as before and samples of expired air were collected at regular intervals for a total of 6 h after the meal. Indirect calorimetry was used to measure CO_2 output over the same time period. $^{13}CO_2$ output from the second study was compared with results obtained during isotope infusions to evaluate the impact of dietary ^{13}C on apparent oxidation of ^{13}C-labelled palmitate.



Breath $^{13}CO_2$ enrichment rose after consumption of the LF meal to approximately 0.0008 atoms percent excess above background levels but did not change after the HF meal. The Fig. Shows that there was no significant difference (P = 0.59 - 1.00) between $^{13}CO_2$ output corrected for the small contribution made by ^{13}C in the glucose drink compared with the previous data at all time points. The results confirm that palmitate oxidation is higher during the postprandial phase after consumption of the LF meal and that the measurement of this variable in this study is not affected by contributions from dietary ^{13}C sources.

The authors are grateful to Anneli Bähr for her help with the repeat study.

Moore R, Seal C J & Mathers J C (1997) *Proceedings of the Nutrition Society* **56**, 193A.

Effects of changes in polyamine metabolism on DNA methylation during growth inhibition of human colonic cancer cells. By BENOIT DURANTON[1], GERARD KEITH[2], RENE SCHLEIFFER[1], CHRISTIAN BERGMANN[1], FRANCINE GOSSE[1] and FRANCIS RAUL[1], *[1]CJF INSERM 9509, Institut de Recherche contre les Cancers de l'Appareil Digestif, and [2] UPR 9002 CNRS, IBMC, Strasbourg, France*

S-adenosylmethionine (AdoMet) is a common substrate for polyamine biosynthesis and DNA methylation. Putrescine and spermidine are ubiquitous biogenic amines that are essential for cell growth and differentiation (Pegg & McCann, 1982). Changes in cellular polyamine metabolism may affect the degree of DNA methylation and consequently modify gene expression (Heby, 1995).

The effects of CGP 48664 (10 μM) and 2-difluoromethylornithine (DFMO) (100 μM), selective inhibitors of S-adenosylmethionine decarboxylase (AdoMetDC), and ornithine decarboxylase (ODC), the key enzymes in polyamine biosynthesis, were investigated on growth, polyamine metabolism and DNA methylation in the Caco-2 cell line. Both inhibitors caused growth inhibition and induced to a comparable degree the initial expression of the differentiation marker sucrase.

	Polyamine content (pmol/mg protein)				Enzymes of polyamine metabolism (pmol/mg protein per h)				DNA methylation	
	Putrescine		Spermidine		ODC		AdoMetDC		% dm^{5}Cytidine	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	133^a	16	3520^a	202	48^a	8	24^a	0.3	2.70^a	0.17
CGP48664	7487^b	903	2503^b	240	360^b	19	26^a	1	3.37^b	0.12
DFMO	0		163^c	7	35^a	4	733^c	43	3.51^b	0.17

Results are means ± SEM of three independent experiments performed in triplicate.
^{abc} Mean values within a column with unlike superscript letters were significantly different, p<0.05

The Table shows that in the presence of CGP 48664 (the AdoMetDC inhibitor), ODC activity was enhanced after 5 d of culture more than 13-fold, and the intracellular pool of putrescine increased 100-fold, whereas the spermidine pool was decreased by 30 %. In the presence of DFMO (the ODC inhibitor), AdoMetDC activity was significantly enhanced and putrescine became undetectably low in the cells. A 20-fold drop in the intracellular pool of spermidine was observed. The degree of global DNA methylation increased by 20 % during growth inhibition by DFMO and CGP48664. In addition, we observed that spermidine (but not putrescine) inhibited endogenous cytosine-DNA methyltransferase activity by 50 %.

In conclusion, the only common features produced in Caco-2 cells by both inhibitors were the extensive depletion of spermidine and the enhancement of DNA methylation. This suggests that the depletion of spermidine may cause the enhancement of DNA methylation, and represent a crucial step in the regulation of Caco-2 cell growth and differentiation.

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Guar gum increases but dietary cholesterol suppresses intestinal tumourigenesis in the *Min* mouse. By C.E. VALLANCE, J. COAKER and J.C. MATHERS, *Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Colorectal cancer incidence rates vary 20-fold throughout the world with much of this variation attributable to dietary factors. Tumours result from clonal selection driven by mutations in proto-oncogenes and in tumour suppressor genes such as *APC* (Reale & Fearon, 1996). *Min* mice have a STOP mutation at codon 850 in the *Apc* gene which results in spontaneous development of multiple intestinal adenomas and carcinomas within 3 months (Su *et al.* 1992). Mutations in this gene are also found in the majority of human large bowel tumours (Powell *et al.* 1992) making the *Min* mouse a valuable model for studies of intestinal tumourigenesis.

At weaning, *Min* mice were allocated at random to one of four experimental diets (twenty mice/diet). The basal diet contained (g/kg) maize starch 305, sucrose 350, casein 200, maize oil 50, cellulose 50, mineral premix 35, vitamins 10 and was modified by the addition of 100 g guar gum/kg (+GG), 10 g cholesterol/kg (+Chol) or both (+GG+Chol) at the expense of maize starch. After 12 weeks of feeding, the number, size and intestinal location of tumours were recorded.

Diet	No of tumours				SEM	Significance of effects		
	Basal	+GG	+Chol	+GG+Chol		GG	Chol	GGxChol
Small intestine	10.4	17.6	6.1	14.4	1.25	***	*	NS
Colon	0.2	0.4	0.2	0.2	0.08	NS	NS	NS
Total	10.7	18.0	6.3	14.6	1.25	***	*	NS

* P<0.05, *** P<0.001.

Most of the tumours occurred in the small intestine where tumour multiplicity was increased significantly (P<0.001) by guar gum and reduced by cholesterol feeding (P<0.05). There was no interaction between guar gum and cholesterol and no significant (P>0.05) effects of treatment on colonic tumourigenesis. To our knowledge, this is the first report that guar gum feeding can increase, or cholesterol reduce, tumour development in the small bowel. The underlying mechanisms for these responses are unknown. This study demonstrates that tumour development in the intestine of *Min* mice is readily modified by dietary factors and establishes the value of this model in studies of chemoprevention by diet.

C.V. holds a MAFF Research Studentship and we acknowledge additional support from MAFF contract ANO 317.

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Thermogenic effect of slight hyperglycaemia during a lipid infusion. By V. RIGALLEAU[1], M. BEYLOT[2], M. LAVILLE[3], C. PACHIAUDI[3], S. NORMAND[3], E. NLEND[1] and H. GIN[1], *[1]Nutrition et Diabétologie, Hôpital Haut-Lévêque, 33600 Pessac, France, [2]Laboratoire de Physiopathologie Métabolique et Rénale, Lyon, [3]Centre de Recherche en Nutrition Humaine de Lyon, France*

Resistance to the glucoregulatory action of insulin is a common finding in obesity and may affect thermogenesis (Ravussin *et al.* 1985). In twelve healthy subjects, we studied the effect of an acute state of insulin resistance produced by a lipid infusion ('Ivélip' 20 %; 0.015 ml/kg per min for 6 h) on substrate-induced thermogenesis (measured by indirect calorimetry) in the post-absorptive state (n 4), during a euglycaemic hyperinsulinaemic clamp (n 4×2) and an oral glucose tolerance test (OGTT) (n 8×2). Clamps and OGTT were performed twice in the same subjects, with and without the lipid infusion. The energy cost of storage was lower ($P<0.05$) for lipids (3.9 (SD 0.9) %) than for glucose (clamp: 12.0 (SD 0.6) %, OGTT: 14.9 (SD 4.0) %). During the clamp, the lipid infusion decreased glucose storage (controls: 3.98 (SD 0.54) mg/kg per min, lipid: 0.90 (SD 0.53) mg/kg per min; $P<0.05$) and thermogenesis increased less (controls 7.53 (SD 0.84) kJ/kg per min, lipid 4.6 (SD 1.26) kJ/kg per min; $P<0.05$). During the OGTT, the lipid infusion increased glucose storage (controls: 1.76 (SD 0.22) mg/kg per min, lipid: 2.94 (SD 0.27) mg/kg per min; $P<0.05$) and thermogenesis increased more (controls: 3.77 (SD 0.84 kJ/kg per min, lipid: 8.37 (SD 1.26) kJ/kg per min; $P<0.05$). The opposite effect was attributed to the lipid-induced impairment of glucose tolerance. A slight elevation of plasma glucose level in response to insulin resistance can affect thermogenesis, by redirecting the storage of substrates from lipids to glucose, which costs much more energy.

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Ravussin E, Acheson K, Vernet O, Danforth E, Jéquier E. (1985) *Journal of Clinical Investigation* 7 6, 1268-1273.

Effects of the nature and the quantity of dietary lipids on the expression of retinoic acid (RAR) and triiodothyronine (TR) nuclear receptors in the rat liver. By CATHERINE NOEL-SUBERVILLE, STEPHANE BONILLA, ANABELLE REDONNET and PAUL HIGUERET, *Laboratoire de Nutrition, Université Bordeaux I, 33405 Talence cedex, France*

Recent studies have shown that the expression of triiodothyronine (T3) and retinoic acid (RA) nuclear receptors (TR and RAR) is modulated by hormones and nutrients (Glass, 1997). In a previous study, we have shown that a diet rich in saturated fat induces a decrease in the expression of these receptors in rat liver (Noel-Suberville *et al.* 1998). In the present experiment, the effects of the nature and the quantity of dietary faty acids on signalling pathways of RA and T3 were studied.

Male Wistar rats weighing 120 g were fed for 4 weeks on diets containing 50 or 250 g/kg lipids: coconut oil (containing 76 g saturated fatty acids (SFA)/100 g) or olive oil (containing 76 g monounsaturated fatty acid (MUFA)/100 g) or safflower oil (containing 77 g polyunsaturated fatty acid (PUFA)/100 g). The maximal binding capacity (Cmax) of the binding of T3 and RA to their nuclear receptors was determined using radiolabelled T3 and a synthetic analogue of RA (CD367) followed by a Scatchard analysis. Quantification of RAR and TR mRNA was performed by semi-quantitative reverse transcriptase polymerase chain reaction.

The main results obtained were: (1) a decreased expression of RAR in rats fed on diets containing 250 g lipids/kg compared with rats fed on diets containing 50 g of lipids/kg, whatever the nature of lipids in the diet (in the range 23 - 30 % reduction for Cmax and between 25 and 43 % for mRNA); (2) a decrease in the expression of TR in rats fed on diets containing 250 g coconut oil/kg (27 % reduction for Cmax and 28.2 % for mRNA) or safflower oil (25 % reduction for Cmax and 25 % for mRNA) compared with rats fed on diets containing 50 g of these same oils/kg.

In conclusion, our results showed that diets rich in lipids induce decreased expression of RAR and TR in rat liver. Now, there are several arguments leading us to consider that the cell signalling pathway of fatty acids is mediated by activation of nuclear receptors called peroxysome proliferators activated receptor (PPAR) and that these PPAR form heterodimers with RXR (the TR and RAR common partner for heterodimerization) in order to regulate gene expression (Desvergne *et al.* 1998).

We hypothesize that the modifications observed in this experiment could be due to crosstalk between retinoids and fatty acid signalling pathways.

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Noel-Suberville C, Pallet V, Audouin-Chevallier I, Higueret P, Bonilla S, Martinez AJ, Zulet MA, Portillo PM & Garcin H (1998) *Metabolism* **47**, 301-307.

Characterization of the regulatory regions of the glucose-6 phosphatase gene promoter in HepG2 cells. By FABIENNE RAJAS, STEPHANE DUVERNAY, SANDRINE TARPIN and GILLES MITHIEUX, *INSERM U. 449, Faculté Laennec, Rue G. Paradin, 69372 Lyon Cédex 08, France*

Glucose-6 phosphatase (*EC* 3.1.3.9; G6Pase) is an essential enzyme in glucose metabolism. It allows the gluconeogenic tissues to release glucose into blood. Its expression is increased in the course of diabetes and fasting and normalized upon insulin treatment and refeeding respectively. To understand better the transcriptional control of G6Pase gene expression, we have characterized G6Pase promoter activity by transient transfections in HepG2 hepatoma cells and in control HeLa cells. The –1640/+60 base pair (bp) G6Pase gene region was progressively 5'-deleted and cloned upstream from a luciferase reporter gene.

The –80/+60 bp fragment was active in HepG2 cells but inactive in HeLa cells; this region therefore appears to be essential for liver-specific expression. When hepatocyte nuclear factor such as HNF1 and/or HNF4 were cotransfected in HeLa cells, all fragments were active. In HepG2 cells, the fragments shorter than the –500/+60 bp fragment showed the same minimal activity. In contrast, the –500/+60 bp fragment was 7-fold more active. This suggests the presence of a positive regulatory element between –320 and –500 bp. A lower activity of the –730/+60 bp fragment was observed, suggesting the presence of a negative regulatory element between –500 and –730 bp. Another positive element might be localized between –1320 and 1480 bp. Forskolin (10 μM) strongly stimulated the promoter activity (3- to 10-fold) of the –500/+60 bp to –1640/+60 bp fragments and this effect was suppressed by insulin (100 nM). These results suggest that the positive element localized between –320 and –500 bp could be dependent on cAMP. Dexametheasone (1μM) and glucose (25 mM) had no effect on the promoter activity of any fragment.

In conclusion, we have delineated the main regulatory regions involved in the specific expression of the G6Pase gene in HepG2 cells.

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Clinical features of food allergy in France based on 703 patients (1116 cases of food allergy). By G. KANNY, P. LEMERDY, F. RANCE, G. DUTAU and D.A. MONERET-VAUTRIN, *CICBAA, Internal Medicine D, Clinical Immunology and Allergology, Hôpital Central, 29 av. de Lattre de Tassigny 54035 NANCY Cedex France.*

This study concerns the clinical features of food allergy (FA) in France and was carried out on the basis of 703 patients (1116 observations of FA) collected by CICBAA, Circle of Clinical and Biological Investigations in Food Allergy, in an Allergy Centre which handles patients of all ages and a Paediatric department from July 1995 to January 1998. All these cases of FA were selected according to the proposals of the European Food Allergies Study Group and confirmed by the following criteria: either a positive provocation test, or clinical improvement by avoidance for at least 6 months, or the occurrence of an anaphylactic shock to the sensitizing food, or the existence of an oral syndrome to fruit and vegetables in a patient allergic to pollen. After the validation of the observations, the data were computerized using Impromptu and Powerplay software (Cognos, Canada).

Atopic dermatitis was the earliest symptom of FA, representing 80 % of clinical manifestations of FA between the ages of 0 and 1 year, 64 % between 1 and 3 years, 34 % between 3 and 6 years, 16 % between 6 and 15 years and 4 % in adults after 15 years. Asthma was particularly common in adolescents and young adults. The frequency of anaphylactic shock (AS) increased with age, whether concerning a single or recurring AS. AS represented 30 % of symptoms after the age of 30 years, while AS was rare during early infancy. The food allergens involved changed according to age of patient. Their number increased with diversification of diet. The prevalence of egg and milk FA decreased according to age when peanut FA persisted.

These data demonstrate that clinical manifestations of FA change according to age. If FA seems to be at the moment slightly more common in infancy, symptoms become more severe with age. These findings underline the necessity to pay close attention from the onset of FA manifestations, in order to prevent worsening of FA symptoms.

Epidemiological study of food allergy in France. By D.A. MONERET-VAUTRIN, G. KANNY and F. THEVENIN, *Internal medicine D, Clinical Immunologie and Allergology, Hôpital Central, 54035 Nancy Cedex France*

An epidemiological survey was carried out in order to determine the frequency and clinical aspects of food allergy (FA) in France.

A questionnaire was sent out to a representative sample of the French population on a scale of 1/1000 and under the age of 61 years (permanent sample survey basis METASCOPE of the French institute SOFRES). 4In all, 44 000 people (20 000 homes) received this questionnaire. 33 110 people answered questions. A second questionnaire was sent out to food allergic subjects.

Initially, 1,253 subjects responded positively to the questions about FA. This group was largely composed of adult women. Atopic history occurred in 57 % of the cases of FA (*v.* 17% for the subjects without FA), 16 % had an occupational risk of food sensitisation (*v.* 7 %) (health profession, catering, agroalimentary industry). The most recent allergic reactions were urticaria (71 %), digestive symptoms (37 %), Quincke oedema (20 %), atopic dermatitis (10 %), asthma (5 %) and anaphylactic shock (2 %). Food allergens were fruits and vegetables (44 %), fish, crustaceans and shellfish (27 %), dairy products (9 %), eggs (7 %) and nuts (6 %). Of these subjects 37 % underwent an allergologic check-up which confirmed FA in 56 % of cases. Of food allergic subjects 5 % presented latex allergy, (*v.* 2 %) with frequent fruit and vegetable allergy.

A total of 947 subjects answered the second questionnaire. An evolving FA was confirmed in 3 % of the total population and 0.5 % were cured or asymptomatic with an eviction diet. Alcohol or drugs (non-steroidal anti-inflammatory drugs, ß-blockers, angiotensin converting enzyme inhibitors) were associating factors in 28 % of FA; 2.4 % had exercise-induced FA.

In conclusion, the prevalence of FA in France can be estimated at 3.5 %. FA concerns predominantly atopic subjects with a high rate of adult women, and a relative predominance of occupations at risk of food sensitization. The low rate in the the paediatric population and the predominance of immediate symptoms leads us to suppose that FA in children is probably still underestimated.

Energy balance during post-partum amenorrhea among chronically malnourished women in rural Bangladesh. By SOPHIE VINOY[1], LYLIANE ROSETTA[1,2] and C.G. NICHOLAS MASCIE-TAYLOR[3], *[1]Laboratoire de Physiologie des Adaptations, Faculté Cochin Port-Royal, Paris, France, [2]CNRS EP 1781, Paris, France, [3]Department of Biological Anthropology, University of Cambridge, Cambridge CB2 3DZ.*

In order to test the possible effect of energy balance on the duration of post-partum amenorrhea, a longitudinal study was carried out in Bangladesh among chronically malnourished women. They were all of rural origin, living in the same villages. Approximately half the sample (*n* 23) were employed as tea pluckers while the remainder (*n* 24) were housewives. The women were recruited into the study during the week after delivery. On three occasions, according to the age of the baby (3.5 months, 10 months and 13 months), detailed investigation of the mother's 24 h energy expenditure was performed at the same time as a measurement of her food intake was carried out. The energy expenditure was assessed by heart rate monitoring during 24 h and a calibration curve was established for each woman. Anthropometric measurements of the mother and child were recorded every month and a weekly questionnaire about menstrual and breastfeeding status was obtained from each mother.

	3.5 months				10 months				13 months			
	Non workers (*n* 14)		Workers (*n* 12)		Non workers (*n* 11)		Workers (*n* 15)		Non workers (*n* 14)		Workers (*n* 19)	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
Height (cm)	146.1	6.2	146.7	6.3	146.3	5.9	145.3	5.0	146.0	5.5	145.4	5.5
Weight (kg)	36.30	4.56	39.41	3.78	35.67	3.24	36.75	3.40	35.31	3.29	38.04	4.57
BMI (kg/m^2)	17.0	1.8	18.3 *	1.2	16.7	1.1	17.4	1.3	16.4	1.0	18.0 ‡	1.6
TEE (kJ/d)	7786	1700	9272 *	1469	8044	1591	9293 †	1620	8016	1507	9732 ‡	1101
EI (kJ/d)	7652	1260	8983	1553	8481	2143	9381	1616	9636	1143	9925	1160
EB (kJ/d)	-138	2235	-284	2286	456	2482	88	1980	1494	2034	188	1867
return of menses (*n*)	3		1		3		2		5		5	

TEE, total energy expenditure; EI, energy intake; EB, energy balance.
* Significantly different from non workers at 3.5 months post-partum ($P<0.05$).
† Significantly different from non workers at 10 months post-partum ($P<0.05$)
‡ Significantly different from non workers at 13 months post-partum ($P<0.01$).

The mean levels of energy expenditure were significantly higher in the workers compared with the non-workers at any time post-partum, while the mean levels of energy intake did not differ significantly. The mean BMI of the workers was also higher than that of the non-workers and this was significant at 3.5 months and 13 months post-partum, although it was in the lowest range for all of them reflecting a poor nutritional status. The mean energy balance was slightly negative in both groups at 3.5 months post-partum and tended to increase over time among the non-workers, but not the workers, who had mean energy balances close to zero. At any time post-partum, the proportion of women with a return of menses was higher among the non-workers.

We conclude that a higher level of daily energy expenditure observed among the workers seems to be associated with longer duration of post-partum amenorrhea, although they have a less detrimental nutritional status than the non-workers.

The effect of the 677C->T mutation on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase (MTHFR) thermolability in healthy volunteers. By A. Chango[1,2], S. Droesch[1], M. Pfister[1], F. Boisson[1], F. Barbé[1,2], M. Pariset[2], G. Nuel[2], S. Frémont[1,2], D. S. Rosenblatt[3], J. P. Nicolas[1,2]. *[1]Laboratoire de Biochimie Médicale et Pédiatrique INSERM U-308, [2]Laboratoire de Biochimie A, CHU Nancy Vandœuvre, France. [3]Division of Medical Genetics, Department of Medecine, McGill University, Montréal, Québec, Canada.*

Mild hyperhomocysteinaemia has been identified as an independent risk factor for vascular disease. Both sub-optimal folate status and genetic factors are implicated in the elevation of plasma homocysteine. We have studied the effect of the common mutation (677C->T) of the MTHFR gene in forty healthy volunteers, aged 27-47 years. The 677C->T mutation was investigated by PCR of genomic DNA and digestion with the restriction enzyme *Hinf*I (Frosst et al 1995). Plasma total homocysteine (tHcy) concentrations were measured (Araki & Sako 1989). Serum folate, vitamin B_{12} as well as the specific activity of MTHFR in lymphocytes before and after heat treatment (Kang et al 1988).

Homozygosity for the 677C->T mutation resulted in lower MTHFR activity and a thermolabile residual activity. Mean fasting tHcy was normal at 9.2 (SD 3.6) µmol/l for the entire sample. The mean serum folate and vitamin B_{12} concentrations were 11.8 (SD 5.2) nmol/l (normal range 6 - 30 nmol/l), and 377.3 (SD 194.5) pmol/l (normal range 156 - 674 pmol/l) respectively. The correlations between serum folate or vitamin B_{12} and tHcy concentrations for the entire sample were not significant. When subjects were divided into three groups (C/C, C/T, T/T) based on their MTHFR genotype (table), there was a significant correlation between low folate and high homocysteine concentrations (R^2 0.58, $P<0.05$) in homozygous mutant individuals (T/T), but not in heterozygous (C/T) or wild-type (C/C) groups. The homozygote mutant genotype was associated with higher levels of homocysteine when serum folate levels were in the low-normal range.

	677C/C (*n* 15)		677C/T (*n* 17)		677T/T (*n* 8)	
	mean	SD	mean	SD	mean	SD
Homocysteine (µmol/l)	7.8	2.5	9.4	3.2	11.3	5.5 ##
Folate (nmol/L)	11.2	4.6	12.2	5.9	11.9	4.4
Vitamin B12 (pmol/L)	368.6	126.9	436.7	256.7	267.1	72.5#

: $P<0.05$ when homozygote mutant genotype T/T was compared with normal C/C genotype.
: $P<0.05$ when homozygote mutant genotype T/T was compared with heterozygote C/T.

These results suggest that the 677C->T mutation leads to an increased requirement for folate, so that subjects with T/T genotype may be sensitive to a low folic acid intake.

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Frosst F, Blom HJ, Milos R, et al. (1995). *Nat Genet,* **10**, 111-113.
Kang SS, Zhou J, Wong PWK et al (1988) *Am J Hum Genet.* **43**, 414-421.

Thermoneutral temperature of Aston strain *ob/ob* mice acclimated to warm (30°) *v.* cold (20°) environments. By J.F. ANDREWS and S.M. McBENNETT, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

Genetically obese mice are characterized by having a lower resting metabolism and a lower deep body temperature (DBT) than their lean counterparts when measured at the same sub-thermoneutral temperature. However, these differences disappear at thermoneutrality (Trayhurn *et al.* 1979). The thermoneutral zone is that range of ambient temperatures over which the resting metabolic rate of an animal is minimal, and the bottom end of this range, the temperature at which thermoregulatory thermogenesis is first required to maintain homeothermy, is the lower critical temperature. The obese animals studied by Trayhurn *et al.* (1979) had been raised at normal animal house temperatures (about 20°) and, therefore, were permanently exposed to a sub-thermoneutral temperature and were acclimated to that temperature. We wished to investigate differences in feeding, and in metabolic and thermal responses to leptin injection, comparing obese animals that had been housed at thermoneutrality with animals cold acclimated to 20°. Therefore, it was necessary to investigate whether acclimation temperature had any effect on the lower critical temperature of the thermoneutral zone and the metabolic rate at that temperature. This was the purpose of the present study.

Four groups of six Aston strain mice were studied. A lean control group and an obese group were cold-acclimated (CA) for 3 weeks to an ambient temperature of 20±2°; a lean control group and an obese group were warm-acclimated (WA) to 30±2° for the same period. All animals had *ad libitum* access to the same laboratory chow and water and were maintained in the same 12h light-dark regimen. The metabolic rates of individual animals were determined at 2.5° decrements of temperature starting at a maximum of 35° in WA and 32.5° in CA, down to 20° in WA and 15° in CA animals. This design was adopted because previous experience led us to suspect that the CA animals would suffer hyperthermia at over 32.5° and the WA hypothermia at below 20°. Metabolic rate was determined by closed-circuit indirect calorimetry as O_2 consumption. To ensure a true resting value animals were placed in the chambers on several occasions before the study proper. Naive animals show a considerably enhanced metabolism on first experiencing the chambers.

The principal finding was that both WA and CA obese mice showed a relatively broad thermoneutral zone extending from 27.5 to 32.5°. However, metabolic rate at thermoneutrality was significantly reduced in WA *v.* CA animals (metabolic rate at 27.5°: WA 0.80(SE 0.20), CA 1.15(SE 0.15) ml/min; P<.0.05 and at 32.5°: WA 0.85(SE 0.05), CA 1.20(SE 0.01) ml/min, p<0.001). Therefore, 30° is a valid temperature at which to study both obese CA and WA groups at thermoneutrality.

A second interesting finding was that both WA and CA lean mice had a point thermoneutral temperature, 32.5°, at which their metabolic rates were indistinguishable (WA 0.80(SE 0.02), CA 0.82(SE 0.01) ml/min.

Trayhurn P, Thurlby PL, Woodward CJH and James WPT (1979]). In *Animal Models of Obesity*, pp. 191-203 [MFW Festing, editor]. London: Macmillan.

Magnesium and calcium absorption from a sulfate-rich natural mineral water in ileostomy subjects. By MAURICE J. ARNAUD [1], LENA NORMÉN [2] and HENRIK ANDERSSON [2], *[1]Water Institute Perrier Vittel, Vittel, France, [2]Department of Clinical Nutrition, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden*

Ileostomists offer a unique opportunity to study small-bowel absorption in man. The aim of the present study was to investigate the intestinal absorption of $MgSO_4$ and $CaSO_4$ from a natural mineral water which may represent a significant source of minerals in European countries. A controlled diet was served to six ileostomy subjects (four males and two females) in two periods of two consecutive days. Half a litre of a natural mineral water was served early in the morning to subjects in a fasting state. The order of two different brands was randomized. The investigated natural mineral water (Hépar®) contained 110 mg Mg/L, 555 mg Ca/L and 1477 mg sulfate/L, while another natural mineral water with a low concentration of minerals (Valvert®) was used as a control. Ileostomy effluents were sampled during the second day of each period (Langkilde *et al.* 1990). Diet and effluents were analysed for Mg, Ca and total sulfate. The net intestinal absorption was calculated as the difference between total intake and excretion. The data were tested statistically by Wilcoxon signed-rank test.

	Intake (mg/d)		Increased			
	Basal diet + Valvert	Basal diet + Hépar	intake (%)	Increased absorption (mg/d)	(%)	RDA (mg/d)
Magnesium	187	241	+29	19(0-32)	+30 ($p<0.05$)	Men (280)
				29(19-45)		Women (350)
Calcium	1429	1672	+17	334(267-546	+45 ($p<0.05$)	800
				595(334-816		
Sulfate	322	1051	+226	183(129-214	+197 ($p<0.05$)	---
				770(682-940		

RDA, recommended daily allowance.

Although considerable inter-individual variation in the absorption of Mg and Ca was observed, the Table shows that the replacement of water by a mineral-rich water such as Hépar®, significantly increased the intake of these two minerals. In addition, this mineral water with high levels of Mg and Ca associated with sulfate increased the absorption of both minerals, and might therefore be useful as a mineral supplement. The nutritional advantages of mineral waters over pill supplements are their regular consumption during the day, the absence of energy and their frequent use as part of established dietary habits in many countries. The inter-individual variation associated with the ileostomy model should, however, be further evaluated in larger groups of normal subjects in order to find factors that influence mineral absorption.

Langkilde AM , Andersson H , Schweizer TF & Torsdottir I (1990) *European Journal of Clinical Nutrition* 44, 5599-566

Effects of pine seed oil on hypercholesterolemic transgenic mice. By GAELLE ASSET[1], ERIC BAUGE[1], ROBERT L. WOLFF[2], JEAN-CHARLES FRUCHART[1] and JEAN DALLONGEVILLE[1],. *[1]Department of Atherosclerosis, Institut Pasteur de Lille, INSERM U325 & [2]Laboratory of Alimentary Lipid Chemistry, ISTAB, University Bordeaux I, France*

The aim of the present study was to assess the lipid-lowering potential of pine (*Pinus pinaster*)-seed oil. This oil contains a polyunsaturated fatty acid which has a chemical structure similar to eicosapentaenoic acid.

Twenty-four apolipoprotein (apo) E-deficient (KOE) mice, twelve LDL-receptor-deficient (KO-LDLrec) mice and twelve KO-LDLrec mice that overexpressed human apoB (KO-LDLrec/B) were used. Mice were treated for 4 weeks. Pine-seed oil and the control fat (lard) were incorporated into the diets at a level of 50g/kg. Plasma lipids were determined enzymatically using commercially available kits. Lipoproteins were separated by ultracentrifugation and gel permeation chromatography:

		cholesterol	VLDL-cholestérol	triglycerides
KOE	Lard	5.71 ± 2.12	5.62 ± 2.20	0.84 ± 0.25
	Pine-seed oil	4.52 ± 1.53	4.32 ± 1.62	1.78 ± 0.96*
KO-LDLrec	Lard	4.85 ± 1.13	2.43 ± 0.76	2.48 ± 1.46
	Pine-seed oil	3.89 ± 0.73*	2.10 ± 0.70	2.75 ± 1.14
KO-LDLrec/B	Lard	13.60 ± 3.68	7.96 ± 2.29	9.21 ± 4.35
	Pine-seed oil	8.61 ± 0.96*	4.29 ± 0.86*	7.08 ± 2.19

* Significantly different from Lard group

The table shows that in all three mouse strains the pine-seed oil regimen was associated with lower levels of total cholesterol and VLDL-cholesterol compared with control. Gel filtration chromatography showed lower LDL-cholesterol levels in the treated animals of the three strains. In contrast, the effect of pine seed oil on HDL-cholesterol was strain-dependent : KO-E no effect ; KO-LDLrec decrease ; KO-LDLrec/B increase. KOE mice treated with pine seed oil had higher levels of triglycerides. This effect was not found in KO-LDLrec and KO-LDLrec/B mice.

It is concluded that pine-seed oil has cholesterol-lowering potential in hypercholesterolemic mice. This effect is associated with a strain-dependent alteration of main lipoprotein and triglycerides levels.

Duodenal passage of α-conglycinin from soyabean in the preruminant calf. By RITA CABRAL, MICHELE FORMAL, LUCILE MONTAGNE, RENE TOULLEC and JEAN-PAUL LALLES, *Laboratoire du Jeune Ruminant, Institut National de la Recherche Agronomique, 65 rue de Saint-Brieuc, 35042 Rennes cedex, France*

Heated soyabean flour (HSF) is less digestible than skimmed milk powder (SMP) and can induce gut immune-mediated hypersensitivity reactions in preruminant calves (Lallès *et al.* 1996). Among storage globulins, β-conglycinin (βCG) is less well digested in the abomasum and is more allergenic than glycinin (GLY) (Lallès *et al.* 1996, 1998). α-Conglycinin (αCG), a minor globulin with anti-tryptic activity, is also allergenic and has been detected in ileal digesta of calves (Tukur *et al.* 1993). However its behaviour in the abomasum has not been reported.

Five Holstein calves were fitted with a T-piece cannula in the duodenum at 3 months of age. They were fed on a milk replacer containing 200 g of digestible protein / kg of powder, half provided by dairy products (SMP + whey) and half by HSF. Duodenal digesta samples were collected on preservatives at 0.5, 2, 4 and 7 h after the morning meal, frozen and freeze-dried. αCG was assayed in HSF and digesta using ELISA (Tukur *et al.* 1993). The concentration of αCG in digesta was expressed as a percentage of that in the diet on a protein basis.

Immunoreactive αCG amounted to 2.7 mg/g protein in HSF, that is 50- and 25-fold less than GLY and βCG respectively. The duodenal concentrations of αCG were 18 (SE 9), 10 (SE 3), 7 (SE 2) and 2.6 (SE 0.6) % at 0.5, 2, 4 and 7 h respectively. This contrasted with GLY and βCG concentrations which decreased from 209 to 0 % and from 185 to 25 % respectively, between 0 and 8 h. The mean retention time of αCG in the abomasum was estimated to be 173 (SE 13) min, as compared with 87 (SE 10) and 205 (SE 11) min for GLY and βCG respectively.

In conclusion, αCG lost a large part of its immunoreactivity in the abomasum. However, detectable amounts of αCG were still present in the small intestine up to 7 h post feeding and may be responsible for its immunogenicity in the calf.

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Tukur HM, Lallès JP, Mathis C, Caugant I & Toullec R (1993) *Canadian Journal of Animal Science* **73**, 891-905.

Compared with olfactory and gustatory stimuli, visual stimuli are operative cues for the estimation by healthy young men of the nutritional composition of all kinds of biscuits. By M. Chabert[1], B. Le Roux[2] and J. Louis-Sylvestre[1] *[1]EPHE, Faculté de médecine de Bobigny, 93017 Bobigny, France; [2]UFR Math-Info., Faculté de médecine des Saints Pères, 75006 Paris, France*

Food intake, especially meal size, is strongly controlled by conditioned mechanisms. The appearance and the flavour of foods are conditioned stimuli that provide information on its nutrient content. The aim of the present experiment was to evaluate the importance of the visual cues compared with the olfactory and gustatory ones.

Healthy young men (n 101), accustomed to eating an afternoon snack, came to the laboratory at 16.00 hours to test different commercial biscuits (items). Each one tasted thirty-nine different kinds of sweet biscuits, on three test days, 1 week apart. For each biscuit, they indicated if they were familiar with it (five levels); then they rated its flavour intensity and nutritional composition (moisture, fatness, sweetness, energy ("calories"))(nine-point scales), before and after tasting the biscuit.

However familiar the subjects were with the biscuits, moisture, fatness, sweetness and energy content evaluations rated before and after tasting were highly correlated (correlation coefficients were r = 0.83; r = 0.79; r = 0.79; r = 0.84 respectively). Correlation was lower between the pre and post-tasting ratings for the flavour intensity (r = 0.66).

Means and sampling errors of the differences (rating after tasting minus rating before) are presented below for two biscuits. Biscuit # 14, with a chocolate covering, provided very pertinent visual cues for the rating of its nutrient content and its sensory properties, whereas biscuit # 4, with a marmalade filling did not. Thus, differences in ratings were slightly more pronounced when subjects were unfamiliar with the biscuit than when they were familiar with it.

	Biscuit # 14 (chocolate covering)				Biscuit # 4 (marmalade filling)			
	Unfamiliar (n 20)		Familiar (n 30)		Unfamiliar (n 20)		Familiar (n 13)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
flavour	-0.20	0.91	-0.33	0.40	1.95**	1.12	-0.08	0.93
moisture	-0.20	0.42	-0.06	0.31	-0.24	0.47	0.23	0.43
fatness	0.05	0.62	-0.20	0.43	-0.38	0.65	-0.23	0.68
sweetness	-0.30	0.80	-0.46	0.46	1.90 **	0.72	0.00	0.63
energy	0.30	0.57	-0.17	0.39	0.86 **	0.45	0.08	0.83

* or ** Significantly different from zero, P<0.05 or P<0.01 (two-sided). SE = $t_{0.05}$ × uncorrected SD/$\sqrt{n-1}$

We have already shown (Abdallah *et al.* 1998) that tasting foods does not really lead to an agreement between estimated and actual values of their nutrient content. Thus, our results show that olfactory and gustatory cues improve the accuracy of the information provided by visual cues only for some unfamiliar biscuits. For common foods, and especially for familiar ones, visual cues are important for the unconscious estimation of the nutritional content of foods and consequently for their selection and the control of their intake.

Abdallah L, Chabert M, Le Roux B & Louis-Sylvestre J (1998) Appetite **30**, 309-324.

BILE ACID SYNTHESIS BY NEUTRAL (CHOLESTEROL-7α-HYDROXYLASE) OR ACIDIC PATHWAY (STEROL-27-HYDROXYLASE) IN HAMSTERS SENSITIVE OR RESISTANT TO DIET-INDUCED CHOLELITHIASIS. By M. COMBETTES-SOUVERAIN, M. SOUIDI, N. BOEHLER, J. FEREZOU and C. LUTTON, *Laboratoire de Physiologie de la Nutrition, Bat 447, Université Paris-Sud 91405 Orsay, France*

A sucrose-based diet (S), containing casein and fat (without added cholesterol), rapidly induces biliary cholesterol gallstones in young Syrian hamsters (incidence of 73 %) from the LPN strain, but not in hamsters from a commercial breeding unit (Janvier, France). Important variables of cholesterol metabolism were compared in the two strains of hamsters (*n* 6), fed on either the S diet or a commercial diet (basal dietary conditions). When fed with the S diet or commercial diet, LPN hamsters differed from Janvier hamsters in having a more active cholesterogenesis, a lower capacity for hepatic cholesterol storage, a higher lipid concentration in gall-blader bile and lower plasma cholesterol levels.

The present study focused on the hepatic activities of two key enzymes involved in the neutral (microsomal cholesterol 7α-hydroxylase ; C7OHase) or acidic (mitochondrial sterol 27-hydroxylase ; S27OHase) pathways of bile acid synthesis. The ratio S27OHase : C7OHase may reflect the relative amounts of hydrophobic and hydrophilic bile acids synthesized. We studied a strain-effect on the expression (determination of mRNA level by reverse transcriptase-polymerase chain reaction)) and activity of microsomal C7OHase (using [^{14}C]cholesterol carried on β-cyclodextrin-like substrate and a NADPH-regenerating system) and mitochondrial S27OHase (same assay).

Under basal conditions (commercial diet), the activities of C7OHase were similar in the two strains (98 SD 18) in LPN *v.* (99 SD 19) in Janvier, but LPN hamsters displayed higher activities of S27OHase (126 SD 10 *v.* 76 SD 10 in Janvier). The S diet reduced the two activities in the two strains. The variations of C7OHase activities were correlated with those of mRNA. The Janvier hamsters fed on the S diet displayed the lowest S27OHase : C7OHase value (0.16 SD 0.10) *v.* (1.04 SD 0.35) in LPN.

These results suggest a nutritional regulation of C7OHase transcription. Moreover they indicated that LPN hamsters differed from Janvier hamsters by a higher (6.5-fold) S27OHase : C7OHase value which may constitute a supplementary risk of predisposition to cholelithiasis.

Gastrin-stimulated pancreatic protein synthesis and activation of phosphatidyl inositol 3 (PI-3) kinase and p70-S6 kinase systems by gastrin via pancreatic cholecystokinin B/gastrin receptors. By C. DESBOIS[1], I. LE HUEROU-LURON[1], A. ESTIVAL[2], M. DUFRESNE[2], P.CLERC[2], V. ROME[1], F. CLEMENTE[2], P. GUILLOTEAU[1], and D. FOURMY[2], [1]*Laboratoire du Jeune Ruminant, INRA 35042 Rennes Cedex and* [2]*Laboratoire de Biologie et Pathologie Digestive, INSERM U151 31043 Toulouse Cedex, France*

The prevailing expression of the cholecystokinin B/gastrin (CCKB/G) receptors in the pancreas of higher mammals (man, calf, pig) but not in rodents is now clearly established. However, the role of these pancreatic receptors in the regulation of the exocrine pancreatic function has not been elucidated. Using a line of transgenic mice expressing the CCKB/G receptors in the exocrine pancreas we demonstrated gastrin-stimulated total protein synthesis. These results led us to search for transduction mechanisms involved in that response. We investigated whether gastrin could regulate the kinase activity and what mechanisms were responsible for this activation, assaying the p70-S6 kinase (p70S6K) activity which has as its main substrate the ribosomal protein S6.

Pancreatic acini were prepared by collagenase digestion. The whole experiment was carried out in the presence of SR27897 (1.8 μmol/l), an antagonist that blocks the CCKA receptors. The kinetics of activation of p70S6K with gastrin (1 nmol/l) showed a maximal stimulation after 10 min. At that time, the activity of p70S6K was stimulated in a dose-dependent way. The maximal effect corresponding to 160 % of the non-stimulated value was reached with 1 nmol/l-gastrin. This effect was reversed with rapamycin, an inhibitor specific to p70S6K. Moreover this effect was partially inhibited by wortmannin, an inhibitor specific to PI-3 kinase; this emphasizes the role of PI-3 kinase upstream of p70S6K.

To conclude, gastrin stimulates pancreatic total protein synthesis via CCKB/G receptors. Moreover, the mechanisms of the transduction of the signal linked with CCKB/G receptors require the activation of p70S6K protein partially mediated by the PI-3 kinase system.

Optimization of an *in vitro* gas production technique for measuring fibrolytic activity of equine intestinal contents. By C. DREVILLON[1], R.S. LOWMAN[2], C. DROGOUL[1], D. CUDDEFORD[2] and V.JULLIAND[1]. [1]*ENESAD, BP 1607, 21036 Dijon Cedex, France,* [2]*University of Edinburgh, Easter Bush, Roslin, EH25 9RG.*

In the equine hindgut, cellulolytic micro-organisms play a major role in degrading and fermenting plant polysaccharides. The extent of their fibrolytic activity can be estimated *in vitro*. The aim of the present study was to optimize an *in vitro* gas production technique.

Caecal contents and fresh faeces were collected from four caecally-fistulated ponies fed on, respectively, pasture or lucerne-orchard hay. They were homogenized, filtered (Blutex 100 µm) and inoculated (10 ml) in flasks (90 ml) containing Van Soest medium (VSM) or Lowe medium (LM) (pre-heated at 39° for 6 h) and 75 mg substrate. Four replicates were made for each substrate: wheat straw (WS), lucerne-orchard hay (LOH) or naked oat (NO). Flasks where closed hermetically, and pressure set at 0 Psi with a pressure transducer. The cumulative gas productions were measured and curves fitted to the data. pH and DM disappearance (DMd) were estimated after 72 h incubation.

The caecal inoculum caused more DM to be degraded than the faecal inoculum and the differences increased with the proportion of neutral-detergent fiber in the substrate (Table 1). With LM, the utilization of polysaccharides was more efficient, the lag time reduced (Table 2) and the correlation between asymptote and DMd increased (Table 3).

Table 1. Mean values of pH and DMd (%) of the substrates after 72 h

	Caecal content + LM		Caecal content + VSM		Faeces + LM		Faeces + VSM	
	pH	DMd	pH	DMd	pH	DMd	pH	DMd
	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
NO	6.81 0.4	92.5 1.0	6.99 0.3	94.2 0.5	6.79 0.4	91.2 0.9	6.84 0.9	91.2 1.6
LOH	6.83 0.2	56.2 1.1	6.95 0.1	57.1 0.1	6.75 0.5	51.5 1.4	6.96 0.3	45.5 1.2
WS	6.84 0.4	49.2 2.0	6.96 0.1	38.6 1.1	6.74 0.1	36.6 1.1	6.97 0.1	36.2 1.7

Table 2. Mean values of asymptote (A, ml) and lag time (T, h) of the substrates after 72 h

	Caecal content + LM		Caecal content + VSM		Faeces + LM		Faeces + VSM	
	A	T	A	T	A	T	A	T
NO	222	1.1	284	1.8	244	2.1	226	2.6
LOH	172	0.7	121	0.9	175	1.1	137	1.5
WS	153	3.2	103	3.5	125	4.8	107	5.1

Table 3. Correlation (r^2) between asymptote (A) and DMd of the substrates after 72 h

	NO		LOH		WS	
	LM	VSM	LM	VSM	LM	VSM
Caecal content	0.92	0.15	0.44	0.30	ND	0.89
Faeces	0.89	0.57	0.51	0.46	0.13	0.32

ND = non determined

LM optimized the *in vitro* degradation of the substrate, especially for plant polysaccharides. The LM probably offered a better environment to the horse microbial population than the VSM used in previous gas production experiments. With LM, the incubation time was reduced to 48 h, which is closer to feedstuffs flow rates in the hindgut. Caecal contents increased *in vitro* degradation of plant structural polysaccharides and provided more fibrolytic micro-organisms compared with faeces.

Fibrolytic activities in caecal and colonic microbial ecosystems in ponies: effect of the presentation of the forage. By C. DROGOUL, B. MUGNIER, E. MANSUY and V. JULLIAND, *ENESAD, BP 1607, 21036 Dijon, France*

This study was designed to determine the effect of the physical form of the hay on polysaccharidasic activities of solid-associated micro organisms (SAM) in the microbial ecosystem of ponies.

Four ponies, fitted with two cannulas (caecum, right ventral colon), used in a 2x2 Latin square design, were fed on a maintenance diet of lucerne-orchard hay given chopped (CH) or ground (1.5 mm) and pelleted (GPH). Five replicate samples of caecal and colonic digesta were taken before the morning feed. The SAM were separated anaerobically. SAM-enzymes were extracted by centrifugation (5000 g, 15 min, 4°) after a freezing/thawing/sonication treatment. Total (TotA) and specific (SpeA) activities of carboxymethylcellulase (CMC) and xylanase were measured by the amount of reducing sugars realeased from purified substrates by extracted enzymes after 70 min incubation at 39°. The rates of addition of SAM-enzymes to substrate solutions were: CMCase, 0.5 ml/2 ml (6 mg CMC/ml); Xylanase, 0.2 ml/2 ml (2 mg xylan/ml). TotA (nmol/g DM per h) and SpeA (nmol/mg proteins per h) were calculated. ANOVA was performed using GLM - SAS processing (1998).

Xylanasic activities (Table 1) were significantly ($P<0.005$) greater than CMCasic ones (Table 2). TotA in the hindgut was significantly greater with GPH than with CH, except for colonic fluid from one pony. With CH, variations between animals were greater than in animals fed on GPH. For both physical forms of hay, TotA values were not significantly different between caecal and colonic fluid, except for one animal fed on the CH diet. The effect of diet on xylanase SpeA was remarkable, whereas CMC-SpeA were low and showed no differences between diets or compartments. Variability was more important with SpeA, particularly for CH.

Table 1. Xylanasic activity

	TotA (nmol xylose/g DM per h)				**SpeA** (nmol xylose/mg of protein per h)			
	Caecum	Colon	Effect of compartment	Animal x compartment	Caecum	Colon	Effect of compartment	Animal x compartment
GPH	217	222	NS	*	33.2	39	NS	NS
CH	58.5	80.2	NS	**	14.9	19.2	NS	**
Diet effect	**	*			**	**		

(NS non significant - * P<0.05 -**P<0.01)

Table 2. CMCasic activity

	TotA (nmol glucose /g DM per h)				**SpeA** (nmol glucose /mg of protein per h)			
	Caecum	Colon	Effect of compartment	Animal x compartment	Caecum	Colon	Effect of compartment	Animal x compartment
GPH	24	25	NS	NS	3.7	5.7	NS	NS
CH	9.6	17.3	NS	**	14	19	NS	NS
Diet effect		NS			NS	NS		

(NS non significant - * P<0.05 -**P<0.01)

This study shows that grinding and pelleting forage increases fibrolytic activity in the equine hindgut when measured indirectly using an *in vitro* enzyme/substrate incubation technique. However, these results are limited to the activities of only two types of enzyme. Further analyses, with different substrates are being carried out.

Investigations should be continued using postprandial fluids to confirm that the caecum and the colon are equally involved in fibre degradation. Investigations with faecal SAM-enzymes would indicate if fibrolytic activity occurs in the distal part of the hind gut and, if it does, it would remove the need to use fistulated animals.

Effects of protected methionine in a fattening diet for culled cows and heifers. By M. EVRARD, C. VAN EENAEME, J.L. HORNICK, A. CLINQUART, P. RASKIN and L. ISTASSE, *Department of Nutrition, Veterinary Faculty, University of Liège, Liège, Belgium*

Methionine appears as the most limiting amino acid in meat production. A major problem in fattening culled cows is an excess of fat deposition. It is therefore of interest to develop strategies allowing fat mobilization and muscle deposition. In the present experiment it was postulated that energy undernutrition together with a high protein supply might result in achieving these goals. In this respect two types of diets, normal energy, normal protein (NENP) and restricted energy high protein (REHP), were compared in eight cows culled after two calvings, eight after four calvings and eight heifers. All animals were from the Belgian Blue breed. As methionine is limiting in soyabean meal, which was the main ingredient of the protein concentrate, half of the animals were given daily 3.5 g protected methionine, the other half being unsupplemented. In addition four cows in each age group were slaughtered at the start of the experiment in order to determine initial carcass composition (zero controls). Data were analysed in a 2x3x2 factorial ANOVA: diets, age and methionine. In this communication comments will be focused mainly on methionine supplementation.

Protected methionine increased daily gain by 0.2 kg/d ($P<0.01$) while intake rose equally, with as a result, a non-significant improvement of the net energy transformation index (0.042 *v.* 0.032 kg/Mcal). The fattening period was about 1 week shorter in the supplemented group ($P<0.05$). Carcass weight was increased but dressing rate was unaffected. Muscle and fat deposition values relative to zero controls were respectively 5.1 and 0.4 kg higher. The higher growth rate in the methionine-supplemented group may be related to a higher muscle protein (MP) turnover (*Van Eenaeme et al.*), as rates of MP deposition, synthesis and degradation were all increased. The efficiency of MP deposition, estimated as the ratio MP deposition : MP synthesis, was also slightly increased by methionine supplementation. In terms of meat quality few significant results were observed. Methionine tended to lead to a redder and darker meat. Meat from methionine-supplemented animals lost less water during cooking (28.60 *v.* 31.06 %, $P<0.01$).

	Methionine		Diet		Significance		
	Contr	Met+	NENP	REHP	Methionine	Diet	Age
Daily gain (kg/d)	0.341	0.530	0.582	0.289	+	**	NS
Daily net energy intake (Mcal/d)	10.81	12.62	14.78	8.66	*	***	*
Duration of fattening (d)	69.8	62.3	65.8	66.3	*	NS	NS
Composition of growth (relative to zero controls)							
Muscle (kg)	10.66	15.79	16.84	9.51	*	*	NS
Fat (kg)	3.87	4.27	6.91	0.99	NS	**	NS
MP Turnover							
MP deposition (g/d)	279	350	275	354	+	+	NS
MP degradation (g/d)	406	522	501	431	*	+	**
MP synthesis (g/d)	685	872	775	725	**	NS	**
Efficiency (%)	38.0	40.4	34.1	44.1	+	*	NS

+ $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

In conclusion, supplementation of culled cows and heifers with protected methionine improves daily growth rate. This may be related to a higher feed intake and higher MP turnover rates and efficiency.

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Van Eenaeme C, Evrard M, Hornick JL, Baldwin P, Diez M & Istasse L (1998) Nitrogen balance and myofibrillar protein turnover in double muscled Belgian Blue bulls in relation to compensatory growth after different periods of restricted feeding. *Canadian Journal of Animal Science 78, in press.*

Oxidative stress and vitamin E status in experimental diabetes. By CHRISTINE FEILLET-COUDRAY[1], EDMOND ROCK[1], KATARZYNA GRZELKOWSKA[2], ANNE PARTIER[1], DOMINIQUE DARDEVET[2], CHARLES COUDRAY[1] and ANDRE MAZUR[1],
CRNH d'Auvergne, [1]Unités Maladies Métaboliques et Micronutriments and [2]Unité d'Etude du Métabolisme Azoté, INRA Theix, 63122 St Genès Champanelle, France

Oxidative stress is frequently suggested as a mechanism underlying diabetes and diabetic complications in which deficiency in micronutrients has often been implicated. In the present study we evaluated, in streptozotocin-induced diabetes in rats (60 mg streptozotocin/kg), oxidative stress markers: plasma 8-epiprostaglandinF2α (8-epiPGF2α), stable end-product of lipid peroxidation (enzymic immunoassay, Cayman Chemicals, Ann Arbor, MI), plasma antioxidant capacity (chemiluminescence)(Whitehead *et al.* 1992) and erythrocyte sensitivity to oxidation (H_2O_2, NO_4)(Pryor *et al.* 1991). Observed mortality rates were 30 % and 72 % after 1 and 4 weeks of diabetes, respectively. Diabetic rats presented with hyperglycaemia and hyperlipidaemia. Plasma 8-epiPGF2α was not significantly modified compared with control after 1 week (46 (SE 4) v. 39(SE 3) pg/ml) and 4 weeks (9.8 (SE 1.1) v. 16.6 (SE 3.0) pg/ml). Plasma antioxidant capacity was not modified compared with controls after 1 week (117 (SE 15) v. 113 (SE 8) µmol/l Trolox equivalent) and tended to increase after 4 weeks of diabetes (200 (SE 57) v. 108 (SE 9) µmol/l Trolox equivalent). Plasma vitamin E concentrations were increased in diabetic rats compared with controls: at 1 week 17.7 (SE 2.2) v. 7.9 (SE 0.3)($P<0.005$); at 4 weeks 28.2 (SE 8.4) v. 8.3 (SE 0.7) (NS). After 4 weeks of diabetes erythrocytes were more resistant to oxidative stress. In conclusion, in streptozotocin-induced diabetic rats, accumulation of vitamin E in the blood compartment appears to be responsible for the reduced sensitivity to oxidative stress of plasma and erythrocytes induced in vivo.

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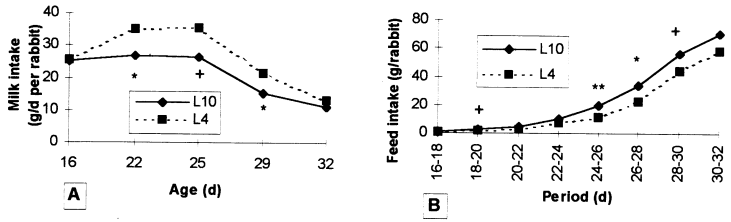
Feeding pattern of sucking rabbits. Effects of litter size. By LAURENCE FORTUN-LAMOTHE, THIERRY GIDENNE, and CLAUDIO SCAPINELLO, *INRA, Station de Recherches Cunicoles, BP 27, 31326 Castanet Tolosan, France*

Digestive disorders frequently observed after weaning in rabbit husbandry could be favoured by an incomplete maturation of digestive processes at weaning. Early intake of solid feed may improve such maturation since Maertens & De Groote (1990) showed that a delayed start of preweaning solid feed intake resulted in an increased post-weaning mortality. Milk intake level is thought to influence the pattern of solid feed intake. Therefore, the aim of the present work was to study how sucking rabbits adapt their feeding pattern (milk and solid feed) according to the availability of milk (modulated by litter size).

At birth (day 0), litter size was equalized to ten young, then on day 16, it was reduced to four rabbits (L4 group, *n* 18) or kept to ten rabbits (L10 group, *n* 20). Litters had free access to a commercial diet (10.7 MJ digestible energy/kg, 169 g crude protein/kg, 167 g acid-detergent fibre/kg). Live weight, individual milk intake and feed intake of litters were measured from birth (day 0) to weaning (day 32). Data were subjected to ANOVA (GLM, SAS, 1990) using the treatment (litter size), replicate (two levels) and interaction between treatment and replicate as fixed effects.



+ $P<0.1$; * $P<0.05$; ** $P<0.01$.

Fig. 1 . Evolution of milk intake (A) and solid feed intake (B) between day 16 and weaning of rabbits, according to litter size : ten rabbits (L10) or four rabbits (L4).

Weights of young rabbits at birth (50.8 g) and on day 16 (199.4 g) as well as milk intake before day 16 were similar in both groups. Milk intake between day 16 and weaning was higher in the L4 than in the L10 group (460 *v* 357 g ; P=0.05; Fig. 1A). Weak feed intake was registered before day 20 (<2 g/d ; Fig. 1B) as previously shown (Maertens & De Groote, 1990). During the period days 20-22, rabbits ate more than 5 g/2 d in ten litters in group L10 and two litters in group L4. From day 16 to weaning feed intake was lower in group L4 than in group L10 (128 g *v* 207 g ; $P<0.01$). Sucking rabbits gained more weight between days 16 and 32 in the L4 than in the L10 group (461 *v* 394 g ; $P<0.05$). Mortality during late lactation (16-32 d) was similar in both groups (9 %).

These results suggest that during competition for milk intake, the beginning of solid feed intake occurs earlier and the quantity consumed before weaning is enhanced. However, this compensatory effect is not sufficient to ensure an optimal growth rate.

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Maertens L & De Groote G (1990) *Journal of Applied Rabbit Research* **13**,151-158.

Influence of sucrose or fat on postprandial ^{15}N-labelled milk protein deamination in fasting subjects. By CLAIRE GAUDICHON[1], SYLVAIN MAHÉ[1], ROBERT BENAMOUZIG[2], CATHERINE LUENGO[1], HELENE FOUILLET[1], SOPHIE DARÉ[1] and DANIEL TOMÉ[1], [1]*Unité de Nutrition Humaine et de Physiologie Intestinale, INRA, INA-PG, 16 rue Claude Bernard, 75005 Paris, France,* [2]*Service de Gastro-entérologie, Hôpital Avicenne, 93009 Bobigny, France.*

The objective of the present study was to assess milk protein N retention during the postprandial repletion phase of the diurnal cycle and to evaluate the acute influence of energy nutrients, i.e. carbohydrate or fat, on this net postprandial protein utilization in human. Twenty-five subjects were each equipped with an ileal tube and ingested ^{15}N-labelled milk protein (30 g) alone or added to either lipid (43 g) or sucrose (100 g). The exogenous ^{15}N was followed for 8 h in the ileum, in the plasma and in the urine and the net postprandial protein utilization (NPPU) was determined.

	Protein (n 7)		Protein + sucrose (n 9)		Protein + fat (n 9)	
	Mean	SE	Mean	SE	Mean	SE
N ingested (mmol)	295		295		295	
N absorbed (mmol)	279.6^{a}	4.1	279.2^{a}	4.0	278.1^{a}	7.1
Digestibility (%)	94.7^{a}	1.3	94.6^{a}	1.3	94.3^{a}	2.4
N oxidized (mmol)	44.4^{a}	12.1	29.5^{b}	6.8	42.7^{a}	10.1
N retained (mmol)	235.1^{a}	13.1	249.8^{b}	7.6	235.4^{a}	10.7
NPPU (%)	79.7^{a}	4.4	84.7^{b}	2.6	79.8^{a}	3.6

Values with different letters within the same row were significantly different, P<0.05 (Tukey's studentized range test).

Sucrose, but not fat, delayed the digestive transit of milk protein, but the ileal digestibility was not influenced by any energy nutrient. The exogenous ^{15}N appearance in the body urea was lowered by sucrose for 4 h and the excretion of exogenous ^{15}N in the urinary urea was significantly reduced by sucrose but not by fat. The whole transfer of exogenous N to urea (i.e. the sum of the exogenous N excreted in the urinary urea and that present in the body urea) represented 14 % of the ingested N when protein was ingested either alone or supplemented with fat, whereas it was significantly reduced by sucrose (9 %). The N sparing effect of carbohydrates is well documented and is due both to an insulin effect and to a lesser participation of amino acids in the gluconeogenic pathways. In contrast, an acute postprandial sparing effect was not observed with fat which is mostly transferred to the adipose tissue and is then slowly mobilized. Lastly, the N retention, assessed by the NPPU, was 80 % when protein was ingested either alone or with fat and was significantly improved (by 5 %) in the presence of sucrose. This improvement, slight yet significant, must be taken into account since the scale of retention values of several proteins is not extremely large. Our study shows that the protein quality, assessed as the direct protein utilization, must be measured in optimal metabolic conditions.

Effects of increasing dietary lipid levels on lipogenic enzyme activities in liver of rainbow trout (*Oncorhynchus mykiss*). By A. GELINEAU, G. CORRAZE, L. LARROQUET and T. BOUJARD, *Laboratoire de Nutrition des Poissons, Unité Mixte INRA IFREMER, BP 3, 64310 Saint-Pée-sur-Nivelle, France*

In salmonid culture, the use of a high-lipid diet has become common practice; however, the effects of such a diet on fat deposition and lipogenesis remain unclear. So, the effects of four experimental diets containing increasing lipid levels were tested in immature rainbow trout reared at 17°c.

Diets	L15	L20	L25	L30
Crude protein (g/kg DM)	437	432	433	425
Crude fat (g/kg DM)	145	197	250	302
Starch (g/kg DM)	142	104	76	44
Gross energy (kJ/g DM)	202	218	224	233

Triplicate groups of 100 trout (initial body weight: 96 g) were fed by hand twice daily to visual satiation. At the end of the growth trial (9 weeks) and after 18 h of starvation, five fish per dietary treatment were sampled for whole-body composition analysis and livers from six fish per dietary treatment were withdrawn and immediately analysed for the activities of the following lipogenic enzymes: glucose-6-phosphate dehydrogenase (EC 1.1.1.99; GP6D), malic enzyme (EC 1.1.1.40; ME) and fatty acid synthetase (EC 2.3.1.85; FAS). The regulation of voluntary feed intake and the improvement in feed efficiency relative to the increase in dietary lipid level from 145 to 300 g/kg, allowed fish of all dietary treatment to reach the same final weight (final body weight: 200 g).

Diets	L15		L20		L25		L30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fat gain (g/kg/day)	1.32	0.03	1.44	0.03	1.77	0.01	1.63	0.01
PER	1.81	0.01	2.03	0.01	2.22	0.01	2.18	0.01
G6PD (UI/g liver)	28.0	6.1	23.7	5.1	17.4	2.9	13.5	2.0
ME (UI/g liver)	7.8	0.8	8.1	0.7	7.7	0.3	8.1	1.4
FAS (mUI/g liver)	6.9	1.9	7.3	3.9	4.8	1.8	2.1	1.0

The increase in dietary lipid level was revealed to influence fat gain and protein efficiency ratio (PER) directly. Of the two NADPH-generating dehydrogenases, the activity of G6PD was 3-fold higher than that of ME. The activity of G6PD was 2-fold higher with the 150 g lipid/kg diet than with the 300 g lipid/kg diet. The negative correlation (r^2 0.95, *n* 24) observed between G6PD liver activity and fat intake was lower than the strong positive correlation (r^2 0.98, *n* 24) observed between G6PD and starch intake. ME was not found to be affected by dietary treatment. FAS activity was significantly depressed when lipid level reached 250 g/kg and even more when it reached 300 g/kg.

In rainbow trout, high dietary lipid levels (up to 250 g/kg) still provided the advantage of protein sparing, while undesired fat deposition was increased. The strong inhibition of lipogenic enzyme activities with dietary lipid levels higher than 250 g/kg did not compensate the excessive supply in dietary supply.

Early nutrition modulates adipose tissue development in neonatal pigs. By V. GERFAULT, J. MOUROT, I. LOUVEAU and J. LE DIVIDICH, *Station de Recherches Porcines, INRA, 35590 Saint-Gilles, France.*

The neonatal period is characterized by numerous morphological and functional changes. Colostrum and milk contain hormones and bioactive peptides that stimulate cellular growth and differentiation both *in vivo* and *in vitro*. Insulin-like growth factor (IGF)-I, IGF-II and insulin concentrations are higher in colostrum than in milk (Simmen *et al.* 1988; Donovan *et al.* 1994). The present study aimed to determine whether the type of feeding (sow colostrum then milk (CM) v. artificial milk (AM) deprived of growth factors) influences the development of adipose tissue in neonatal pigs.

Twelve newborn pigs (Large White-Landrace x Pietrain) from three litters were used. They were removed from the sow before suckling and placed in individual cages in thermoneutral conditions. Pigs were bottle fed isoenergetically with CM or AM (supplemented with immunoglobulin for 24 h) at 2 h intervals. After 7 d they were killed and subcutaneous adipose tissue was aseptically removed. Pre-adipocytes were isolated by collagenase digestion and inoculated in culture wells to determine proliferation (day 4) and differentiation (day 6).

The number of pre-adipocytes per g adipose tissue was higher (+ 46 %) in AM than in CM pigs (Table 1). In culture, pre-adipocyte proliferation was higher (+ 59 %) whereas percentage of differentiating cells was lower (- 48 %) in AM than in CM pigs. Malic enzyme activities (ME; EC1.1.1.40) were similar in both groups.

Pre-adipocyte number in adipose tissue and potential of pre-adipocyte proliferation in primary culture were higher in AM than in CM neonatal pigs. It is possible that the lack of growth factors may induce a default in regulating proliferation. However, the mid- or long-term effect of early nutrition on adipose tissue development remains to be determined.

Table 1 . Effects of type of feeding on pre-adipocyte number in adipose tissue and on pre-adipocyte proliferation and differentiation in primary culture

	CM		AM		Effects
	Mean	SE	Mean	SE	
Pre-adipocytes/g tissue ($x10^6$)	6.9	0.5	10.1	1.6	$P < 0.01$
Proliferation (cpm $x10^4$: $[^3H]$-thymidine incorporation for 24 h)	17.8	6.3	28.4	15.4	$P < 0.05$
Differentiated cells (%)	67.4	15	34.5	14	$P < 0.01$
ME (nmol NADPH/min per mg protein)	220	46	199	71	NS

Donovan SM, McNeil LK, Jiménez-Flores R & Odle J (1994) *Pediatric Research* **36**, 159-168.
Simmen FA, Simmen RCM & Reinhart G (1988) *Developmental Biology* **130**, 16-27.

Effects of feed restriction during fattening on muscle lipid traits in the rabbit. By FLORENCE GONDRET[1,2], FRANÇOIS LEBAS[1] and MICHEL BONNEAU[2], *[1]Rabbit Research Station, INRA, 31326 Castanet-Tolosan cedex, France, [2]Pig Research Station, INRA, 35590 St-Gilles, France*

It is generally believed that delaying slaughter at a later age may result in a higher intramuscular fat content and could therefore improve the organoleptic qualities of rabbit meat. Restricting feed intake during fattening is sometimes carried out in order to increase age at a given slaughter weight. The aim of the present study was to determine the effect of a 30 % feed restriction during fattening on muscle lipid content and metabolism in the rabbit.

Male New-Zealand White rabbits were fed *ad libitum* from weaning (4 weeks) to 11 weeks of age. Thereafter, they were allocated to two groups (*n* 15 in each group). In the first group (15AL), the rabbits continued to have free access to the same diet, and were slaughtered at 15 weeks of age, at 2.9 kg body weight. In the second group (18R), the animals then received 70 % of the voluntary feed intake and were slaughtered at the same weight but 3 weeks older than the 15AL group.

	15AL group		18R group		
	Mean	SE	Mean	SE	Significance
Weight of perirenal fat (g)	40.2	3.3	11.7	1.4	***
Muscle lipid traits:					
Longissimus lumborum					
Lipid content (g/100 g)	1.2	0.06	0.9	0.04	**
Malic enzyme activity (UI/g)	1.5	0.05	1.3	0.03	**
Biceps femoris					
Lipid content (g/100 g)	1.6	0.09	1.1	0.07	***
Malic enzyme activity (UI/g)	1.6	0.04	1.4	0.06	**

Mean values were significantly different between 15AL and 18R groups *P<0.05, **P<0.01, ***P<0.001.

The results in the Table indicate that weight of perirenal fat was reduced by 70 % in restricted rabbits, compared with those fed *ad libitum*. Feed restriction also significantly decreased intramuscular fat content in *Longissimus lumborum* and *Biceps femoris*. In both muscles, limited feeding resulted in a significant decline in the activity of malic enzyme, as compared with the *ad libitum* regimen. Several reports have previously demonstrated that malic enzyme acts mostly in *de novo* lipogenesis in the muscles.

In conclusion, increasing age at slaughter at a given slaughter weight, via feed restriction, resulted in a lower intramuscular fat content, and could, therefore, be detrimental for the organoleptic qualities of the rabbit meat.

The technological treatment of bovine milk: influence on cell culture. By CHRISTIANE GUIMONT and GUY LINDEN. *Université Henri Poincaré-Nancy1, Laboratoire des Biosciences de l'Aliment, unité associée INRA (Dpt.TPA), BP 239, 54506, Vandoeuvre-les-Nancy, France*

Bovine milk contains about 2000 types of molecule, some of them exhibiting biological properties. For cell cultures *in vitro,* growth factors, adhesion factors, transport proteins are necessary in addition to the basic nutritional medium and these types of factors are present in bovine milk. Consequently, milk, fractions or proteins of milk and treated or not treated milk were studied as components or as substitute of foetal calf serum (FCS) in cell cultures. The table shows a review of results reported in the literature about factors found and originated from milk sources that could explain biological activity of bovine milk.

Factors	Milk Sources	Target cells/tissues
Insulin	mature milk or pasteurized milk or sweet whey	mammary, intestinal, bronchial epithelia, granulosa, ovocytes, mammary cells, foetus, chondrocytes
Bombesin	mature or boiled milk or whey and commercially instant non fat dry milk	gastric, pancreatic epithelium
Prolactin	mature milk or heated milk whey or sweet whey	mammary cells, hybridoma immunomodulation
Growth hormone	mature milk	pituitary gland, milk production
Adrenocorticotropin	sweet and acid whey	murine, human hybridomas
Progesterone	milk	murine hybridomas, mammary cells
Second messenger	sweet and acid whey or bacterial (yoghurt) whey	second cellular cell messenger
IGF-I	mature milk or heated milk	mammary, bronchial,intestinal epithelia, ovocytes, granulosa, fœtus, chondrocytes,
PDGF	mature milk	mammary cells, fibroblasts, smooth muscle,dental pulp, neural cells
aFGF	cheese whey	fibroblasts, mesodermis, neuroectodermis epithelial cells, prostate gland
bFGF	cheese whey	fibroblasts, vessels, mammary cells, chondrocytes, myoblasts
TGF β	pasteurized milk	fibroblasts, endothelium, B and T lymphocytes
NGF	mature milk	neural cells
EGF	mature milk or pasteurized milk	intestinal, pulmonary, mammary epithelia, ovocytes, foetal myoblasts, chondrocytes,
α,β,κ caseins	enzymatic or bacterial hydrolysates	fibroblasts, human hybridomas, colon cells, splenocytes, lymphocytes
Serum albumin	acid whey	lymphocytes, murine, human hybridoma
β-lactoglobulin	sweet and acid whey	T lymphocytes, murine hybridomas, mammary epithelial cells
Proteose peptone 3	sweet and acid whey	murine hybridomas
Lactoferrin	sweet and acid whey	mammary epithelia, lymphocytes, myoblasts,promonocytes, fibroblasts bacteria, human, murine hybridoma

This table shows that there is significant action on cell growth of mixtures such as industrial wheys, as well as that of proteins (caseins, proteose peptones, β-lactoglobuline, lactoferrine, and serum albumine) isolated after technological milk treatment. Such treatments include heating, acidification, bacterial fermentation, enzymatic action and ultrafiltration.
This raises the questions regarding the existence of growth factors in bovine milk or the role of the major proteins as well as the effect of biochemical changes or the suppression of inhibitory components obtained by milk processing.
Screening of the different components in milk likely to provide an explanation for this activity has not detected hormones or growth factors at levels comparable to those found in human milk or colostrum, but has suggested the role of molecules such as mature proteins, the structure of which would explain their function.
Thus, it is interesting to analyse protein changes undergoing the most common treatment in milk industry such as heating and the effect on the cell life. The first results on hybridoma cell growth were obtained with β–lactoglobulin whose modifications were characterized by mass spectrometry (poster n° 54).

Family resemblance in calcium intakes in the Stanislas Cohort. By BERNARD HERBETH, ANNE LLUCH, EDITH LECOMTE and GÉRARD SIEST, *Centre de Médecine Préventive, 2 avenue J. Parisot, F-54500 Vandoeuvre les Nancy, France*

This study was conducted on a sample of 422 families in order to assess the family aggregation of Ca intakes and the contributions of various factors to this resemblance.

The sample consisted of 422 nuclear families from the Stanislas Cohort who volunteered for a free health examination at the Centre for Preventive Medicine. Only families composed of two parents aged less than 65 years and two biological children aged between 10 and 25 years were included. Between 1994 and 1995, these families completed a 3 d food consumption diary and a questionnaire about eating style: the Dutch eating behaviour questionnaire (DEBQ).

For Ca intakes and nutritional density, correlation coefficients between relatives were between 0.23 and 0.36 ($P \leq 0.001$), child-child correlations being higher than spouse-spouse or parent-child correlations. Variance component analysis for Ca intakes showed that percent of shared common environment were similar between parents and children: between-generation component being 25.9 to 26.5% of global phenotypic variance, within-generation component being 30.8 to 32.2%. Identical results were observed for Ca nutritional density of the diet.

Ca intakes of children (both boys and girls) differed significantly according to education level of the mother, high Ca intakes being related to high education level. Eating behaviour scores of the mother were also related to Ca status of the children; for instance, maternal restriction level was negatively correlated with dairy product and cheese consumption while maternal external eating score was negatively correlated with milk consumption and positively with yoghurt and fresh uncured cheese consumption.

In conclusion, in this sample of Lorraine families, Ca intakes and nutritional density aggregated within families. As for macronutrient and energy intakes, family aggregations were determined by within- and between-generation components: cultural inheritance (parent-child environment component) being modulated by parent characteristics (education level and/or eating behaviours). This study provides additional justification for health promotion programmes that target the family as the unit of intervention.

Contribution of mineral waters to dietary calcium intake in the French SU.VI.MAX cohort. By SERGE HERCBERG[1], MAURICE ARNAUD[2], PAUL PREZIOSI[1], PILAR GALAN[1], MARJORIE ZAREBSKA[1], BERNADETTE FIEUX[1], and PIERRE VALEIX[1]
[1]*Institut Scientifique et Technique de la Nutrition et l'Alimentation, CNAM, Paris,* [2] *Institut de l'Eau, Vittel*

To be able to assess the relative contribution of mineral waters to daily dietary Ca intake in adults, we developed a case-control study in mineral water drinkers consuming mineral waters containing different concentrations of Ca. The sample population was selected in adults (women 35-60 years ; men 45-60 years) participating in the SU.VI.MAX study (Hercberg et al, 1998). Three groups of consumers were selected : regular drinkers of (1) highly mineralized water (Ca = 467 mg/l), (2) moderately mineralized water (Ca = 202 mg/l) and (3) poorly mineralized water (Ca = 9.9-67.6 mg/l). Groups were perfectly matched according to several sociodemographic items. A fourth group constituted only tap water drinkers, matched according to the same criteria. The sample population included 664 subjects distributed into four groups of 166 subjects each.

Dietary Ca intake provided by the various food groups did not differ between the four consumer groups, except for Ca provided by mineral water. Depending upon its Ca level, mineral water may contribute up to one-fourth of total daily Ca intake. Subjects who regularly drink highly mineralized water have a Ca intake which is significantly higher than that of drinkers of poorly mineralized or tap water.

Type of water	highly mineralized		moderately mineralized		poorly mineralized		tap	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Daily Ca intake (mg/d)								
men	1206	342	1087	408	944	320	1003	411
women	1111	363	948	329	854	279	881	395
Ca provided by water (mg/d)								
men	298	170	117	76	9	12	0 .6	0.3
	(26%)		*(11%)*		*(1%)*		*(0,1%)*	
women	308	171	120	74	8	9	0.5	0.4
	(28%)		*(14%)*		*(1%)*		*(0,1%)*	
Other sources of Ca (mg/d)								
men	908	335	971	385	935	319	1002	411
	(74%)		*(89%)*		*(99%)*		*(99,9%)*	
women	803	309	828	313	846	278	880	395
	(72%)		*(86%)*		*(99%)*		*(99,9%)*	

The amount of Ca provided by dietary products (the main source of Ca), like the total daily Ca intake (except for mineral water) did not differ between the four groups of water drinkers.

In conclusion, highly mineralized water may constitute a truly important supplementary contribution to the total Ca intake. This is of particular interest because consumption of highly mineralized water does not interfere with consumption of other sources of Ca and particularly dairy products.

Hercberg S, Preziosi P, Briançon S, Galan P, Paul-Dauphin A, Malvy D, Roussel AM & Favier A (1998), *Controlled Clinical Trials*, **19**, 336-351.

Metabolic differentiation of the bovine heart muscle during fetal development. By J.F. HOCQUETTE[1], C. PIOT[1], J.H. VEERKAMP[2], B. PICARD[1] and Y. GEAY[1], *[1]Laboratoire Croissance et Métabolismes des Herbivores, INRA, Theix, 63122 Saint-Genès-Champanelle, France, [2]Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands*

The metabolic differentiation of the bovine heart muscle starts during prenatal life. However, the induction of the different metabolic enzymes or nutrient transporters occurs at various stages of fetal development (Castiglia-Delavaud *et al.* 1997). Therefore, the aim of the present work was to study the ontogenesis of the heart's ability to catabolize fatty acids.

Thirty-seven bovine embryos from different genotypes and five 15-d-old Montbéliard calves were used. Fetuses were taken just after slaughter of their mother at 110, 180, 210 or 260 d of gestation. The capacity of the bovine heart to oxidize oleate was determined by measuring the oxidation rate of [1-^{14}C]oleate in homogenates of fresh tissues, and mitochondrial enzyme activities were measured as described (Veerkamp & van Moerkerk, 1985).

The weight of the heart represents 7 g/kg the whole body weight whatever the age of the animals. Cytochrome *c* oxidase activity was similar at all developmental stages (range 94 - 129 µmol oxidized substrate/min per g tissue wet weight). In contrast, citrate synthase activity increased slightly during fetal development and markedly around birth. Indeed, mean citrate synthase activities were 5.0 (SE 0.3), 9.7 (SE 2.5), 5.9 (SE 0.4) and 10.9 (SE 1.0) µmol liberated coenzyme A/min per g tissue wet weight at 110, 180, 210 and 260 d of fetal development respectively, and 42.5 (SE 6.5) µmol/min per g in 15-d-old calves. The mean capacity of the bovine heart to catabolize oleate was 95.5 (SE 11.8) µmol fatty acid oxidized/min per g tissue wet weight in 15-d-old calves. It increased gradually especially during the last third of gestation and around birth since it averaged 46.4 (SE 2.6), 57.4 (SE 4.4), 69.8 (SE 5.9) and 74.7 (SE 7.5) µmol fatty acid oxidized/min per g at 110, 180, 210 and 260 d of fetal development respectively, i.e. 48, 60, 73 and 78 % of that in 15-d-old calves.

In conclusion, this study provides evidence that the metabolic differentiation of the bovine heart occurs partly during the last third of gestation (from 180 d to birth) as previously described for bovine skeletal muscle (Gagnière *et al.* 1997). However, the differentiation process of the heart is characterized by different patterns of mitochondrial enzyme expression which induces an increase in the oxidative metabolism around birth.

Castigia-Delavaud C, Busset C, Hocquette JF, Bauchart D & Picard B (1997) *Journal of Muscle Research and Cell Motility* **18,** 210-211.

Gagnière H, Picard B, Jurie C & Geay Y (1997) *Meat Science* **45,** 145-152.

Veerkamp JH & van Moerkerk HTB (1985) *Biochimica et Biophysica Acta* **875,** 301-310.

***In vitro* digestibility of prickly pear protein.** By RADIA LAMGHARI EL KOSSORI[1], CHRISTIAN VILLAUME[1], ES-SADDIK EL BOUSTANI[2], MARIE-NOELLE MAUCOURT[3], YVES SAUVAIRE[4] and LUC MEJEAN[1], *Institut National de la Santé et de la Recherche Médicale (INSERM U308) Equipe de Recherches Aliment et Comportement, 38 rue Lionnois, 54000 Nancy, France, [2]Laboratoire de Biochimie Nutritionnelle et Métabolique, Faculté des Sciences Cadi Ayyad, Bd My Abdellah, 44000 Marrakech, Morocco, [3]Laboratoire de Physicochimie et Génie Alimentaire, ENSAIA, 2 Avenue Forêt de Haye, 54500 Vandoeuvre-lès-Nancy, France, [4]Laboratoire de Recherche sur les Substances Naturelles Végétales, UPR ES 1677, 24 Place Eugène Bataillon, 34095, Montpellier, France*

In order to assess protein quality, different *in vivo* and *in vitro* measures are used. Among these measures, digestibility is considered to be the main characteristic of food or feed proteins. The *in vitro* digestibility of a protein reflects its digestibility *in vivo*, as well as giving a measure of protein quality. Moreover, measurement of *in vitro* digestibility is a rapid, simple and inexpensive technique.

We evaluated the protein quality of three lyophilized fractions of the prickly pear fruit: pulp skin and seeds , each sample containing 40 mg N. We measured the protein digestibility over 6 h (Savoie and Gauthier, 1986) and compared the results with those obtained with 40 mg N from casein . From the second hour and the subsequent 5 h of protein digestion, the N released from the three fractions was significantly lower than that released from casein (Table).

Table. Proportion of nitrogen released (%) from prickly pear fractions during *in vitro* digestion for 6 h

Time (h)...	1		2		3		4		5		6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pulp	1.93	0.87	3.89	1.38	6.45	2.51	9.63	2.52	13.7	1.60	18.2	0.50
Skin	1.47	0.09	3.18	0.94	6.33	0.62	8.99	0.35	12.5	0.70	13.7	2.00
Seeds	2.12	0.40	4.00	0.69	7.35	1.38	9.50	2.10	11.8	2.40	14.6	3.10
Casein	2.89	0.60	10.5	1.00	16.5	0.60	26.4	4.40	41.2	3.40	54.8	4.70

Mean values for three determinations.

Despite the significantly different viscosity values of the three fractions (Pulp 700 x 10^{-3} Pa. s, Skin 2050 x10^{-3} Pa.s, Seeds 6.53 x 10^{-3} Pa.s) their digestibility values were comparable. The low digestibilities of the three fractions could be associated with the physicochemical interactions between protein and other components such as proteolytic enzymes or non-protein components of prickly pear fruit, rather than viscosity.

Savoie L & Gauthier S F (1986) *Journal of Food Science*, **51**, 494-498.

Composition and nutritional prospects of prickly pear fruit. By RADIA LAMGHARI EL KOSSORI[1], CHRISTIAN VILLAUME[1], ES-SADDIK EL BOUSTANI[2], YVES SAUVAIRE[3] and LUC MEJEAN[1], *[1]Institut National de la Santé et de la Recherche Médicale (INSERM U308) Equipe de Recherches Aliment et Comportement, 38 rue Lionnois, 54000 Nancy, France, [2]Laboratoire de Biochimie Nutritionnelle et Métabolique, Faculté des Sciences Cadi Ayyad, Bd My Abdellah, 44000 Marrakech, Morocco, [3]Laboratoire de Recherche sur les Substances Naturelles Végétales, UPR ES 1677, 24 Place Eugène Bataillon, 34095, Montpellier, France*

The nutritional evaluation of plants adapted to arid and semi-arid areas deserves a great deal of attention. One of these plants, the prickly pear, is a cactus that thrives in such a climate, and is of economic and nutritional interest.

We determined the crude composition (g/kg DM) of three lyophilized fractions of the prickly pear fruit: pulp, skin and seeds.

The ethanol-soluble carbohydrates measured according to Roe (1955), were the most abundant component of pulp and skin. In the pulp, glucose (350 g/kg) and fructose (290 g/kg) were the predominant sugars, whilst in the skin glucose (210 g/kg) was abundant. The protein contents (N x 6.25) of the three fractions (g/kg) determined by a Kjeldahl procedure were: pulp 51, skin 83, seeds 118.

The amino acid compositions of the three protein sources (determined by HPLC) showed a deficiency in several amino acids, threonine being the most limiting amino acid. Fatty acids were present in trace amounts in the pulp and skin, but constituted 67.7 g/kg seeds, of which linoleic and oleic acids represented 64 and 19.2 g/100 g respectively.

Starch, measured according to Beutler (1984), was present in all three fractions of the fruit. Fibers were measured according to Southgate (1969). Pulp was rich in pectin (703 g/kg total dietary fibre); cellulose was more abundant in the skin (714 g/kg) and seeds (832 g/kg) than in the pulp (14.2 g/kg). The skin was remarkably rich in Ca and K measured by flame emission.

The composition of the fruit of the prickly pear suggests that it is a potential source of energy which is often underestimated, its nutritional evaluation is of great importance. The pectin content of its pulp deserves detailed attention. Pectin could be of use as a therapeutic agent in diets requiring a high level of soluble dietary fibre, and as a food ingredient after determination of its physico-chemical properties.

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AFNOR (1984) NF-T-90-019

Influence of meal time and stress induced by mental load on postprandial energy expenditure. By CATHERINE LE FUR[1], JEAN LOUIS EDME[1], PATRICK DEVOS[2], ALAIN LANCRY[3], CLAIRE MOUNIER-VEHIER[4], LAURENCE GUEDON[5], MARGARITA KROUMOVA[6], and MONIQUE ROMON[7]. *[1]CERESTE, [2]CERIM université de médecine, [3]ECCHAT Amiens; [4]Service d'hypertension artérielle CHRU, [5]Service de pharmacologie hospitalière CHRU, [6]Centre d'Investigation Clinique CHRU, [7]Service de nutrition CHRU Lille, France*

In a previous study we found a circadian variation of the postprandial energy expenditure (EE) among subjects submitted to a mental load (Romon *et al* 1993). The postprandial thermogenesis has two components, an obligatory one due to substrate metabolism and a facultative one under sympathetic activation (Acheson, 1993). The aim of the present study was to evaluate the influence of a mental load on the postprandial energy expenditure and its circadian variation. Fourteen healthy males (BMI 22.42 (SE:2.17) kg/m²), were allocated in a counterbalanced order to four experimental sessions : day (meal 13.00 hours), night (meal 01.00 hour), with or without mental load (task of choice reaction (Lancry 1986)). The meal was the same for the four sessions (4.18 MJ, protein 16%, fat 34%, carbohydrate 50% energy). EE was measured by indirect calorimetry (Ferrannini E, 1988), for 1 h preceding and for the 6 h following the meal. Stress reaction was evaluated by the 7 h post-meal urinary catecholamines excretion (Moyer *et al* 1979), analysis of heart rate and blood pressure variations, and spectral analysis of heart rate variability. Comparisons were made by three-way (time post-meal, day/night, mental load) ANOVA for repeated measures.

	Time (T)	Day/night (D/N)	Mental load (ML)	TxD/N	TxML	D/NxML	TxD/NxML
Epinephrine		**<0.001**	**0.018**		0.82		
Norepinephine		0.285	0.384		0.667		
Heart Rate (HR)	**<0.001**	**0.005**	**<0.001**	0.241	**<0.001**	0.908	0.241
HF HRV	**<0.001**	0.395	**0.003**	0.565	**0.001**	0.535	0.53
LF HRV	**0.001**	0.134	**<0.001**	0.05	**0.029**	0.588	0.796
SBP	**<0.001**	**0.0316**	**0.0325**	0.841	**<0.001**	0.841	0.15
CV SBP	**0.0195**	0.867	**0.021**	0.099	**<0.001**	0.216	0.612
DBP	**0.017**	**0.025**	**0.019**	0.769	**<0.001**	0.927	0.571
CV DBP	**<0.001**	0.1915	**0.008**	**0.001**	**0.003**	0.455	0.933
EE	**<0.001**	**<0.001**	0.175	**<0.001**	**<0.001**	0.483	0.305

Abbreviations : HF HRV : High Frequency of HR variability, LF HRV : Low Frequency of HR Variability, SBP : Systolic Blood Pressure, CV SBP : Coefficient of Variation of SBP, DBP : Diastolic Blood pressure, CV DBP : coefficient of Variation of DBP, EE : Energy Expenditure.

The Table shows that the meal induced an activation of the autonomic nervous system, suggested by a decrease of heart rate and systolic blood pressure variability. This effect was enhanced by a mental load. The response of these variables to mental load was not modified according to the day/night factor, although baseline values had a circadian variation (Linsell *et al* 1985, Furlan *et al* 1990). The postprandial EE was lower after a night meal, this response was enhanced by a mental load, however, there was no interaction between these two factors. This study shows that mental stress has an influence on postprandial EE, this effect is not different between night and day and does not modify the circadian variation of diet-induced thermogenesis.

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Vegetarian meals which are proposed in collective catering may contribute to the recommended dietary allowances. By J. M. LECERF, C. BILA and C. RAKOTOFIRINGA, *Institut Pasteur de Lille, Liille, France*

Vegetarian lunches were proposed with the following criteria: (1) vegetable proteins containing foods; (2) milk products and possibly eggs; (3) fruits and vegetables; (4) fats. We wanted to assess their nutritional composition, (a) by chemical analysis of eight lunches, (b) by the analysis of twenty-one lunches with a food composition table.

The chemical analysis showed an energy content of 766 (SD 31) kcal with 31.5 (SD 2.11)% lipids, 52.2 (SD 2.0)% carbohydrates, 16.3 (SD 1.1)% proteins. The Fe amount is 8.9 (SD 0.5) mg, that of Ca is 513 (SD 56) mg, that of rough cellulosis is 7.85 (SD 0.75) g. In percent of the recommended dietary allowances (which must be equal to or greater than 40% of the daily recommended dietary allowances (DUPIN, 1992)), the Fe intake is 89% in men and 49.4% in women, the Ca intake is 57%.

The analysis from food composition table shows a caloric amount of 913 (SD 152) kcal, with 9.1% from saturated fatty acids. The cholesterol amount is 133 (SD 174) mg, that of NaCl is 1.5 (SD 0.77) g, of Mg is 234 (SD 88) mg, of Fe is 8.6 (SD 3) mg, of Zn is 4.3 (SD 1.7) mg, of vitamin B_{12} is 0.7 (SD 0.5) µg, of total fibre is 13.7 (SD 2.8) g. In percent of the daily recommended dietary allowances the Fe intake is 86% in men and 47.8% in women, the Mg intake is 55.7% in men and 70.9% in women, the Zn intake is 28.7% in men and 35.8% in women, the vitamin B_{12} intake is 23%. For essential amino acids, the absolute intake on one lunch is greater than the daily dietary requirements (FAO-OMS, 1985) calculated for an adult of 70 kg, except for Tryptophan(185 mg *v.* 210 mg).

In conclusion, the analysis of balanced vegetarian lunches which are proposed in collective catering may supply the recommended dietary allowances in energy, macronutrients and micronutrients which were analysed except for Zn and vitamin B_{12} for whom the amounts were insufficient and have to be completed on the other meals.

Physiological intake of chicory's fructo oligosaccharides does not change blood lipids and lipoproteins in healthy males. By J.M. LECERF [1], G. LUC [1], H.PARRA [1], and C.ECOCHART [2]
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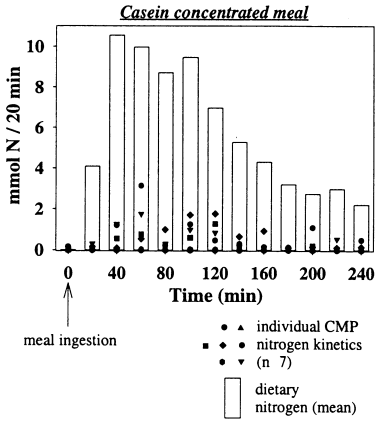
Non-digestible oligomers of fructose are found in many plant foods such as chicory. Chicory is used in drinks as a substitute for coffee. Lipid-lowering effects of high amounts of fructo-oligosaccharides (FOS) and inulin have been found in studies with rats. The number of studies of the effect of FOS on blood lipids in human subjects is limited. FOS are rapidly and completely fermented in the colon and the most common hypothesis is that the fermentation products, especially propionate reaching the liver by the portal vein, could modulate cholesterol synthesis. The present study concerned the effect of a physiological intake of 20 g soluble chicory (eight packs daily), supplying 4 g FOS, on healthy subjects. Twenty-eight normolipidaemic males were randomized, in a double blind-trial, into two groups with two sequences, A and B. (A), chicory for 6 weeks (V_2), then no chicory for 6 weeks (V_3) ; (B), no chicory for 6 weeks (V_2), then chicory for 6 weeks (V_3). Blood lipids were analysed before randomization and at the end of each period. Dietary intakes were not modified and each volunteer received a prescribed diet which was calculated from his initial recorded diet, during the last 2 weeks of each period. There were no significant changes in concentrations of total cholesterol, triacylglycerols, HDL-cholesterol, LDL-cholesterol, apoprotein A1, apoprotein B, lipoparticles Lp A1 and Lp A1A2 after each period, in sequence A or sequence B (Table 1). There were no significant changes in weight, cardiac frequency or blood pressure. In conclusion a physiological intake of FOS from soluble chicory does not modify blood lipids and lipid risk factors in healthy males.

Table 1
Results : blood lipids

	at V_2			at V_3		
(g/l)	Sequency A (chicory)	Sequency B (no chicory)	p	Sequency A (no chicory)	Sequency B (chicory)	p
	n = 14	n = 14		n = 14	n = 14	
Total cholesterol	1,62 ± 0,37	1,68 ± 0,26	ns	1,55 ± 0,34	1,77 ± 0,32	ns
Triglycerides	0,79 ± 0,35	0,72 ± 0,23	ns	0,75 ± 0,22	0,81 ± 0,24	ns
HDL chol	0,45 ± 0,071	0,51 ± 0,071	ns	0,44 ± 0,07	0,48 ± 0,08	ns
LDL chol	1,01 ± 0,31	1,03 ± 0,25	ns	0,96 ± 0,30	1,13 ± 0,28	ns
Apo A1	1,17 ± 0,13	1,29 ± 0,14	ns	1,23 ± 0,15	1,32 ± 0,17	ns
Apo B	0,69 ± 0,18	0,72 ± 0,16	ns	0,74 ± 0,20	0,83 ± 0,18	ns

Caseinomacropeptide release in human jejunum during digestion of a concentrated casein meal. By N. LEDOUX, S. MAHE, M. DUBARRY, M. BOURRAS, R. BENAMOUZIG and D. TOME, *INRA UNHPI, Institut National Agronomique, 16 rue Claude Bernard, 75005 Paris, France.*

Peptides released from protein digestion in the gastrointestinal tract may have regulatory effects on digestion. This is the case for caseinomacropeptide (CMP), a 64-amino acid peptide, which is the first product released after κ-casein hydrolysis (Shammet *et al.* 1992). *In vivo* studies in animals and human subjects have shown rapid release of CMP in the stomach (Yvon & Pélissier, 1987; Chabance *et al.* 1998). The present work investigated the release of CMP in human jejunum after ingestion of a concentrated casein meal intrinsically labelled with ^{15}N as a marker (Mahé *et al.*, 1994). Jejunal effluents were collected through a naso-jejunal tube, and analysed for ^{15}N-enrichment to evaluate the dietary N fraction. Detection and quantification of CMP was performed by an inhibition ELISA procedure (Picard *et al.*, 1994). The limit of detection was about 5-10 μg CMP/ml. The results (Fig. 1) showed that CMP appeared in effluents in the first 20 min after meal ingestion and was almost completely emptied from the stomach after 120 min. CMP recovered in the jejunum represented about 8 % of total dietary N. CMP concentration reached 10^{-4} M. These results demonstrate that CMP is released from the stomach during casein digestion and support the hypothesis that food-borne peptides could exert physiological function. The specific physiological activity of CMP in human subjects, particularly on the digestion process, requires further studies.



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Effects of fatty acids on proliferation and differentiation of cultured pig pre-adipocytes. By N. LOUAPRE, V. GERFAULT, and J. MOUROT, *Station de Recherches Porcines, INRA, 35590 Saint-Gilles, France*

Fatty acids (FA) have been found to be important modulators of adipocyte differentiation *in vitro* and *in vivo*. Dietary saturated fats induce expression of adipose tissue mass more effectively than polyunsaturated fats by acceleration of pre-adipocyte replication (Shillabeer & Lau, 1994). Long-chain FA have been shown to regulate expression of lipid-related genes in adipose cells (Ailhaud *et al.* 1996). Palmitate promotes Ob-1771 pre-adipocyte differentiation (Amri *et al.*, 1994). The present study was undertaken to investigate the action of FA on pig pre-adipocyte proliferation and differentiation in primary culture. The effects of short- and long-chain saturated FA (10:0 and 18:0) and long-chain polyunsaturated FA (18:2*n*-6) were examined.

Pigs were slaughtered at 7 d and subcutaneous adipose tissue was removed aseptically. Pre-adipocytes were isolated by collagenase digestion and inoculated in culture wells to determine proliferation (day 4) in serum-supplemented medium. Differentiation (day 6) was studied in serum-free medium and was estimated by cell counting after coloration with red oil and by measurement of malic enzyme (ME; EC1.1.1.40) activity. FA were added to the culture medium at the concentration of 100 μM in alcoholic solution on day 1.

The addition of FA in the medium decreased proliferation significantly compared with control culture (Table 1). The decrease was higher with 18:2*n*-6 than with 18:0 and 10:0. The percentage of differentiating cells was increased in the presence of 10:0 compare to control culture. ME activity, a later maker of adipose tissue differentiation, was increased in presence of 10:0.

This study shows that a short-chain FA may increase pre-adipocyte differentiation. Long-chain FA seem to decrease proliferation. It would be interesting to study *in vivo* the effect of dietary FA (length or saturation) on adipose tissue development in the neonatal period.

Table 1. Effects of FA on pre-adipocyte proliferation and differentiation in primary culture

	Control		10:0		18:0		18:2*n*-6	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Proliferation*	100[a]		43.1[b]	14.5	38.2[bc]	18.3	26.5[c]	8.1
Differentiation (%)†	54.3[b]	11.0	78.7[a]	6.7	69.8[ab]	10.9	< 5	
Malic enzyme‡	316[b]	68	415[a]	33	328[b]	66	ND	

a, b, means with different letters are significantly different, $P < 0.05$.
ND, not detectable.
*Incorporation of [^{3}H]-thymidine/24 h.
†Percentage of differentiated cells.
‡Activity (nmol NADPH/min per mg protein).

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Contribution of dietary glucose to milk protein synthesis in ruminants. By FRANÇOIS LUCAS, GUIDO RYCHEN, XIMO RUBERT-ALEMAN and GERARD BLANCHART, *INRA, INPL, UHP, Laboratoire de Sciences Animales, ENSAIA, BP172, F-54505 Vandoeuvre-lès-Nancy cedex, France*

Polysaccharides and their monomers, particularly glucose, are involved in the synthesis of lactose and milk fatty acids through the action of volatile fatty acids (VFA) produced in the rumen. The role of dietary glucose in milk protein synthesis is not well documented. The present study examined this role.

A single dose of glucose evenly labelled with ^{14}C (9.25 MBq) was infused (poured) via a cannula into the rumen of each of three lactating goats. Radioactivity was monitored over 24 h in different fractions of the rumen fluid (bacterial and liquid phases), and over 72 h in the main milk components: lactose, proteins and fat.

Within 2 h post-infusion, labelling decreased by more than 72 % in the total rumen fluid (Table 1). After 30 min, about 17 % of the total infused radioactivity was associated with bacteria, and this reached 75 % of the low residual labelling after 24 h.

Table 1. Time course and distribution of ^{14}C-labelling in the different fractions of the rumen fluid

Time (h)	Distribution of ^{14}C-labelling in the different fractions of rumen fluid (%)			Total residual labelling in rumen fluid (10^3.dpm/ml)
	Free bacteria	Bacteria associated to particles	Cell free fraction	
0.5	1.7	15.6	82.7	78
1	1.6	8.7	89.7	56
1.5	2.4	12.6	85.0	35
2	2.6	23.5	73.8	22
4	3.9	31.5	64.6	12
6	5.0	41.9	53.0	8
8	5.3	57.3	37.4	5
10	6.8	60.3	32.9	4
12	6.0	60.2	33.8	3
24	11.4	62.3	26.3	1

Table 2. Cumulative recovery of ^{14}C labelling in milk fractions (10^4.dpm)

Time (h)	Lactose	Milk proteins	Milk fat
6	1254	225	182
12	1607	396	396
36	1907	572	621
72	1982	642	696

The labelling of the different milk fractions was at its highest at the first milking, 6 h after the infusion of labelled glucose (Table 2). Cumulative protein radioactivity was on average equal to that of fat, and represented for the sampling period 1 % of infused radioactivity and 30 % of ^{14}C recovered in lactose.

These results seem to indicate that glucose contributes towards milk protein synthesis as much as towards fat synthesis probably through bacterial VFA's, since these compouds are the earliest to be absorbed during digestion. Furthermore, the additional involvement of glucose in milk protein synthesis through bacterial proteins cannot be ruled out, if we consider the level of early labelling of the ruminal bacteria.

Magnesium deficiency enhances immune stress response by altering cell calcium homeostasis. By CORINNE MALPUECH-BRUGERE, EDMOND ROCK, CATHERINE ASTIER, WOJCIECH NOWACKI†, ANDRZEJ MAZUR and YVES RAYSSIGUIER, *INRA - Centre de Recherches en Nutrition Humaine, Unité Maladies Métaboliques et Micronutriments, 63122 Saint Genès Champanelle, FRANCE*

The objective of the present study was to assess the potential mechanism underlying the enhanced inflammatory processes during Mg deficit. Weanling rats fed on a purified diet containing either 30 or 1000 mg Mg/kg for 2 or 8 d were used in these studies. Rats fed on the Mg deficient diet for 8 d were challenged either with live *E. coli* or platelet activating factor (PAF) as models for sepsis and anaphylaxis respectively (Keith & Fischer, 1992). In both cases higher mortality was found in Mg-deficient rats compared with controls. These results extend the concept that Mg deficiency greatly enhances the response of the immune system (Rayssiguier *et al.* 1997) whatever the stimulus used for that activation (Salem *et al.* 1995). To investigate the mechanisms by which Mg deficiency exacerbated the immune system, we studied the respiratory burst and intracellular Ca changes in stimulated peritoneal cells. The data showed that addition of Phorbol Myristate Acetate led to a 3 to 4-fold increase of superoxide anion production in cells from Mg-deficient animals as compared with controls: 9.5 (SE 1.3) *v* 2.25 (SE 0.5) nmol/min *per* 10^6 cells, $P < 0.001$. Changes of $[Ca^{2+}]_i$ induced by PAF were investigated after fura2-AM loading (Grynkiewicz *et al.* 1985) of peritoneal cells from control and Mg-deficient rats.

	Control (*n* 10) (nM $[Ca^{2+}]_i$)		2 d Mg-deficient (*n* 5) (nM $[Ca^{2+}]_i$)		2 d Mg-deficient (*n* 5) (nM $[Ca^{2+}]_i$)	
	Mean	SE	Mean	SE	Mean	SE
- PAF	69	3	70	3	90	10
+ PAF	160	24	243*	14	437*	80

Significantly different from control group, * $P < 0.05$.

The Table shows that the basal level of $[Ca^{2+}]_i$ did not differ significantly between the two groups. Addition of PAF induced the expected rise of $[Ca^{2+}]_i$; however, compared with controls, a 2.7-fold increase was observed in cells from Mg-deficient animals. Similar experiments were undertaken with peritoneal cells obtained from rats fed on a Mg-deficient diet for 2 d where no clinical signs had yet appeared. These cells also showed an abnormal rise in $[Ca^{2+}]_i$ induced by PAF indicating that transduction signal mechanism is affected at a very early stage of Mg deficiency. These studies are the first to show that abnormal Ca handling induced by extracellular Mg deprivation *in vivo* may be at the origin of the exacerbated inflammatory response.

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Intake and feed efficiency of brown trout selected for growth. By MURIEL MAMBRINI[1], EDWIGE QUILLET[1], LAURENT LABBE[2] and BERNARD CHEVASSUS-AU-LOUIS[1], *[1]Laboratoire de Génétique des Poissons, INRA, 78 352 Jouy en Josas Cedex, France, [2]SEMII, BP17, 29450 Sizun, France*

Brown trout (*Salmo trutta*) have been selected for enhanced growth for four generations by an improved individual selection process (PROSPER, Chevassus *et al.*, 1992). A control line having the same population size was also maintained. The response for a selection pressure of about 5 % was about 10 % per generation. The objectives of the present study were to compare intake and feed efficiencies of quadruplicate groups of control x selected (C) and selected x selected (S) offspring fed by self-feeders to satiety or limited to 80 % *ad libitum*. The C group was fertilized 10 d before the S group so that the growth period began when the offspring reached the same body weight (9.2 and 8.5 g for the C and S groups respectively). The growth period lasted for 173 d and was followed by 56 d of food deprivation. Data were analysed by means of a two-factor variance analysis, taking into account the effects of the selection, of the feeding level and the interaction between these two factors.

	S		C			*P*	*P*	*P*
	Ad lib	Limited	*Ad lib*	Limited	*SE*	Genotype	Feeding Level	Inter -action
Growth period								
FBW (g)	157.6	117.1	148.5	123.8	5.6	0.6823	0.0001	0.0159
DGC	1.66	1.38	1.59	1.42	0.04	0.4197	0.0001	0.0177
Intake*	4.41	3.15	4.19	3.27	0.23	0.6513	0.0001	0.1718
Gain/feed	1.12	1.13	1.09	1.14	0.02	0.3820	0.0088	0.1562
Fasting period								
FBW (g)	141.7	106.9	132.1	108.7	5.2	0.1775	0.0001	0.0589
DGC	-0.35	-0.27	-0.37	-0.39	0.04	0.0031	0.2398	0.0188

FBW, final body weight; DGC, daily growth coefficient : ($FBW^{1/3}$ - Initial $BW^{1/3}$)/duration (d); * g dry Matter/kg body weight per d

The effects of the selection on growth varied with the feeding level. For fish fed *ad libitum*, daily growth coefficient tended to be higher for the S than for the C groups because of a slightly higher intake of the S groups. The loss of body weight due to food deprivation was comparable among the two groups of fish. When feed amount was limited, daily growth coefficients were lower for S than for C groups, because feed efficiency was lower for the S groups during the first 6 weeks of the growth period. After the fasting period, the loss of body weight was lower for S than for C groups.

It can be concluded that the selection for growth, which has been realised on animals fed *ad libitum*, may have been beneficial to hyperphagic animals, which seem to be less efficient when feed is limited. However, the "metabolic efficiency" may be different between the two groups, because of the difference of mobilization when the restricted fish are starved. More studies are needed to analyse the proper effects of food restriction on the selected fish.

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Inhibitory effect of procyanidin-containing extracts on LDL oxidation *in vitro*. By ANDRZEJ MAZUR, DOMINIQUE BAYLE, CLAUDINE LAB, EDMOND ROCK and YVES RAYSSIGUIER, *Unité Maladies Métaboliques et Micronutriments, INRA, Theix, 63122 St Genès Champanelle, France*

Phenolic compounds of vegetable origin are of interest as dietary antioxidants. Some of them have been proposed as suitable for decreasing LDL oxidation and thus for the prevention of atherosclerosis. In the present study, we have evaluated the antioxidant activity of phenolic extracts obtained from grape seeds (*Vitis vinifera L.*) and from the bark of the marine pine tree (*Pinus pinaster Sol.*), in the model of LDL oxidation *in vitro*. These extracts are complex mixtures of phenolic substances, especially rich in procyanidins.

Grape seed extract (Vitaflavan, DRT) and extracts from the bark of the French marine pine tree (Pycnogenol®, Horphag Res. and Oligopin, DRT) were studied. Their phenolic content (catechin equivalents) was assessed by the Folin-Ciocalteu method. Oxidation of LDL was induced by Cu or 2,2'-azobis(2-amidinopropane)-dihydrochloride (AAPH) in the presence of various concentrations of extracts and conjugated dienes or thiobarbituric acid-reactive substances (TBARS) were measured (Vinson & Hontz, 1995). The median inhibitory concentration (IC_{50}) was calculated for each extract from the data of TBARS production after Cu-induced oxidation (Vinson & Hontz, 1995). Butylated hydroxytoluene (BHT), Trolox and rutine were used as reference substances. To evaluate if studied extracts could be associated to lipoprotein particles, they were added to the plasma, LDL was isolated by ultracentrifugation and oxidation experiments performed using Cu as prooxidant (Kerry & Abbey, 1997).

The phenolic contents in tested products were: 73 % for Vitaflavan, 52 % for Pycnogenol® and 43 % for Oligopin. All products tested presented inhibitory effects on LDL oxidation *in vitro*. IC_{50} values were 0.56, 0.68 and 0.67 µg/ml for Vitaflavan, Pycnogenol® and Oligopin, respectively. Values for reference products were: Trolox, 2.88, BHT, 0.35 and rutin, 0.73 µg/ml. In short, the extracts studied had an inhibitory effect on LDL oxidation. Some of components from these extracts could be associated to the LDL particle. From these results it appears that extracts from grape seeds and from the bark of French marine pine tree may be interesting sources of antioxidants.

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Reduced efficacy of leptin on weight reduction in *ob/ob* mice when administered at thermoneutrality. By S.M. McBENNETT, P.M. MANN and J.F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

Leptin has been shown to have profound effects in the *ob/ob* mouse, correcting in whole or in part the manifold deficits of the *ob* syndrome. Thus, leptin administration normalizes body weight through a combination of reduction in food intake, increased resting metabolism and activity (Pelleymounter *et al.* 1997); other systems are normalized including body temperature regulation and even, most strikingly, reproductive capacity. These effects of leptin were observed in *ob/ob* mice held at normal animal house temperatures, in the region of 20°. This is well below the thermoneutral zone of a mouse, therefore the animals in these studies were maintaining continuous sympathetic thermoregulatory drive.

A preliminary study (Swan, Andrews and McBennett, unpublished results) had suggested that leptin administration at thermoneutrality failed to elicit the dramatic reversal of the physiological deficits of the *ob/ob* mouse. The present study was done to further investigate that possibility. Twenty *ob/ob* mice were studied, divided into two equal groups, one held at 20° (sufficient to maximize thermoregulatory thermogenesis in the *ob/ob* mouse) and one at 30° (at or near thermoneutrality for *ob/ob* mice). Animals were held at their respective study temperatures for 3 weeks to achieve full thermal acclimation. The last week served as a control week when vehicle (0.1 ml sterile saline) was administered twice daily by intraperitoneal injection. Weight gain, food intake, deep body temperature (DBT) (by rectal thermister probe) and whole-body metabolic rate (MR) (indirectly as O_2 consumption in an open-circuit calorimeter) were determined. Animals were then injected intraperitoneally with leptin (PeproTech EC Ltd) twice daily, dissolved in sterile saline at a standard dose of 25 μg in 0.1 ml. The following results were obtained. The weight gains/losses (g) per week of 20° *v.* 30° maintained animals were control week: +0.2(SE 1.2) *v.* +3.55(SE 1.3), P<0.001; -6.18(SE 1.6) *v.* -0.8±1.4, P<0.001 over the first week of leptin injection and -7.10(SE1.1) *v.* -3.3(SE1.1), P<0.001 over the second week. MR (ml/kg per min) was significantly increased by leptin treatment in the cold-maintained animals rising from 30(SE±7) in the control week to 43(SE±3) in the first and 42(SE±7) in the second week of leptin treatment (both significant increases, P<0.001], whereas in the warm-maintained animals leptin treatment resulted in a small decrease in MR from 31(SE±10) in the control week to 21(SE±2) in the first (P<0.05) and 27(SE±3) in the second week of leptin treatment (NS). Leptin caused a significant decrease in food intake (g) in both groups, but greater in the 20° animals: 9.9(SE±1.9) *v.* 6.3(SE±0.9) in the control week to 4.5(SE±0.8) *v.* 3.8(SE±0.2) in the first week and 2.8(SE±0.2) *v.* 2.0(SE±0.4) in the second week of leptin treatment (P<0.001 with respect to control week). Finally, leptin treatment caused DBT (°) to increase, from 36.0 to 37.5 to 37.0 in cold-maintained but had no significant effect in the warm (36.7, 36.3, 37.0).

These data clearly show the expected effect of leptin at the colder sub-thermoneutral temperature of 20°: significant body weight loss, as a consequence of significantly increased MR and significantly decreased food intake. However, the same dose of leptin administered to the cohort of obese mice held at 30° showed a considerable blunting of these effects. We suggest that leptin requires parallel increased sympathetic activity, as during sustained cold thermal drive, for its function to be fully expressed.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F (1997) *Science* **269,** 540-543.

Expectations of patients in weight management. By MEYER L., MEJEAN L., GUERCI B., DROUIN P. and ZIEGLER O., *Diabétologie, Maladies Métaboliques et Nutrition, Hôpital Jeanne d'Arc, and INSERM U 308, Nancy, France.*

Obese people often have unrealistic expectations about the amount of weight they can lose and their ability to maintain their new weight. The present cross-sectional study was done on 218 patients (BMI > 25 kg/m^2), recruited at their first visit as outpatients. There were forty-six men and one hundred seventy two women (mean age 42.1 (SD 13.5) years, mean BMI 36.5 (SD 7.1) kg/m^2, mean peak weight 100.5 (SD 21.7) kg). The patients' weight loss goals ranged from 3 to 100 kg and from 4.5 to 53.9% (percentage of their current weight). The patient's weight loss goal was significantly positively correlated with his/her BMI, peak weight, lowest weight in adulthood, weight at 20 years, and delta weight (peak weight minus lowest weight) and inversely correlated with age (P < 0.0001 in all cases). A stepwise multiple regression analysis found that BMI accounted for 50 % of the variance of the patients weight loss goal as an independent predictor, whereas age (6 %), log delta weight (5.1 %) and lowest weight (2.9 %) were less important determinants. There was no correlation between the patient's weight loss goal and his/her profession or education, a past history of weight cycling or the duration of obesity. More than 90 % of the patients wanted to lose > 10 % of their current weight. It is thus important to prepare patients for therapy and describe the elements and results of a weight management programme. A weight loss of 5-15 % of initial weight seems to be a reasonable goal, as recently recommended by the International Obesity Task Force (World Health Organization. Obesity : preventing and managing the global epidemic. Report of a WHO consultation on Obesity. Geneva, 1998 : 276 p).

Optimization of anti-*Listeria monocytogenes* combinations of bacteriocins (nisin, curvaticin 13) and lactoperoxidase system in broth and milk. By ANNE BOUTTEFROY, NORA BOUSSOUEL, ANNE-MARIE REVOL-JUNELLES and JEAN-BERNARD MILLIERE, *Laboratoire de Fermentations et Bioconversions Industrielles, Institut National Polytechnique de Lorraine, Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (ENSAIA-INPL) 2, Avenue de la Forêt de Haye, BP 172, F 54505 Vandoeuvre- Les-Nancy Cedex, France*

Listeria monocytogenes is a foodborne pathogen responsible for listeriosis outbreaks or sporadic cases. This bacterial species is ubiquitous in nature and is often found in food-processing environments. Its physiological characteristics (psychrotrophic, tolerant to NaCl (11 %), and growth at pH 5.0) make it difficult to control its presence and growth in foods. Lactic acid bacteria bacteriocins (nisin, curvaticin 13) are anti-microbial peptides only active against Gram-positive bacteria. Curvaticin 13 is produced by *Lactobacillus curvatus* SB13 (Surdiman *et al.* 1993). The lactoperoxidase system (LPS) is known for increasing raw milk shelf-life due to its major action against Gram-negative bacteria. In the present study, the efficiencies of these three inhibitors and their combinations were analysed according to the behaviour of *L. monocytogenes* ATCC 15313 in broth and milk in terms of growth, survival or lethality.

Experiments in trypcase soy broth supplemented with 0.6% yeast extract (TSBYE) were made at pH 6.5, and at 22°c. Curvaticin 13 was obtained by the adsorption-desorption method (Yang *et al.* 1992). Nisin (Sigma, 50 IU/ml) and/or curvaticin 13 (160 AU/ml) were added at 0 h (t_0) and/or at 4 h of incubation.

Experiments in reconstituted skimmed milk (100 g/L) were made at pH 6.4 and 25°c. The LPS consisted of lactoperoxidase (35 mg/l), SCN^-(25 mg/l), glucose oxidase (1 mg/l) and glucose (200 mg/l). The nisin (200 IU/ml)/LPS combinations were: (i) nisin present at t_0, and LPS added at t_0, 4 or 12 h, (ii) LPS present at t_0, and nisin added at t_0, 24, 48 or 72 h. For experiments in broth or in milk, the initial population level was approximately 1.10^4CFU/ml.

In TSBYE, addition of nisin or curvaticin 13, at t_0 or after 4 h of incubation, always induced an immediate transitory bactericidal effect. The action of nisin depended on the physiological state of cells. The bactericidal effect of nisin added at t_0 (3 $\log_{(10)}$) was greater than that observed with nisin added at 4 h (0.8 $\log_{(10)}$), and re-growth rates were similar to those in the control test. Curvaticin 13, added at t_0 or after 4 h, induced the same bactericidal effect (1 $\log_{(10)}$), but re-growth was always at a less important rate. Re-growth was not due to the decrease of bacteriocin concentrations. All combinations of both bacteriocins induced greater inhibitory effects. The best one, nisin and curvaticin 13 added at t_0, led to the absence of *L. monocytogenes* cells in broth after 48 h. With the three other associations, a re-growth phase was always observed after 24 h.

In milk experiments, nisin gave a rapid but only transitory bactericidal effect. Then re-growth was observed at a rate similar to that of the control test. LPS induced a 48 h bacteriostatic phase, followed by re-growth at a rate less important than in the control. Simultaneous addition of both inhibitors at t_0 induced a bactericidal phase which led in 192 h to the reduction of *L. monocytogenes* population to no detectable level. Delayed addition of LPS to *L. monocytogenes* cells, which had already been in the presence of nisin for 4 h, gave a rapid bactericidal effect, with no detectable level within 48 h. When nisin was added to cells, which had already been in the presence of LPS for 24 h, a bactericidal phase immediately occurred with the absence of *L. monocytogenes* within 192 h. Other combinations, with an initial bactericidal effect, were always followed by a re-growth phase.

In dairy technology, nisin-curvaticin 13 or nisin-LPS combinations could be useful tools to control *L. monocytogenes* growth. These inhibitory systems could offer multiple potential applications, but toxicological investigations must be made.

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Yang R, Johnson MC & Ray B (1992) *Applied and Environmental Microbiology* **58**; 3355-3359.

Differential regulation of glutamine and glutamate transport during Caco-2 cell differentiation : Transcriptional regulation of the glutamate transporter EAAT1. By AGNES MORDRELLE[1], CYRILLE COSTA[1], JEAN-FRANÇOIS HUNEAU[1], ERIC JULLIAN[2] and DANIEL TOMÉ[1], [1]*INRA UNHPI, INA-PG, 16 rue Claude Bernard, 75005 Paris and* [2]*Laboratoire d'Histologie, Faculté de Médecine Cochin, 75014 Paris.*

Glutamate and glutamine are metabolic fuels for intestinal epithelial cells as well as precursors for amino acid and glutathione synthesis (Wu, 1998). Glutamate and glutamine utilization varies depending of cell proliferation and differentiation and on the amino acid supply. We used the Caco-2 line, which exhibits spontaneous enterocytic differentiation after confluency, to address glutamine and glutamate transport during this process. L-[^{3}H]-glutamate and L-[^{3}H]-glutamine uptake were measured at 37° on Caco-2 cells between day 7 and day 20 post-seeding.

At confluence, glutamate uptake occurred through a single Na^+-dependent high-affinity transport system. Glutamate transport was completely inhibited by L- and D-aspartate but was insensitive to D-glutamate, dipolar and dibasic amino acids. At least two Na^+ are cotransported with one glutamate. Altogether, these results are consistent with the involvement of system X_{AG}^- (Dall'Asta *et al.* 1983) In contrast, glutamine uptake involved a Na^+-independent saturable component and two Na^+-dependent transport systems with low and high affinity respectively.

Glutamate uptake	Kt		Vmax		EAAT1 transcript
	mmol/l		pmol/min per mg protein		arbitrary units
	Mean	SE	Mean	SE	
Confluent (day 7 post-seeding)	0.107	0.021	1443	118	20
Differentiated (day 20 post-seeding)	0.118	0.070	2666*	310	100

* significantly different from day 7 ($P<0.05$).

Cell differentiation resulted in a decrease in glutamine transport with a concomitant increase in glutamate transport rate (Table). Five different proteins (EAAT1-5) may account for the expression of the XA,G- transport system (Vandenberg, 1998). In the Caco-2 cell line, the up-regulation of glutamate transport was associated with an increase in the amount of EAAT-1 glutamate transporter transcripts between day 7 and 17 whereas EAAT-3 transporter mRNA was barely detectable at any time.

The present data support a transcriptional control of l-glutamate transport during differentiation that may reflect modification in metabolic substrate utilization.

Dall'Asta V, Gazzola GC, Franchi-Gazzola R, Bussolati O, Longo N & Guidotti GG (1983) *The Journal of Biological Chemistry* **258**, 6371-6379.
Vandenberg RJ (1998) *Clinical and Experimental Pharmacology and Physiology* **25**, 393-400.
Wu G (1998) . *Journal of Nutrition* **128**, 1249-1252.

The cholesterol-lowering effect of guar gum is accompanied by an increase of bile acid digestive pools and intestinal reabsorption By S. MORICEAU, C. BESSON, C. MOUNDRAS, C. RÉMÉSY, C. MORAND, C. DEMIGNÉ. *UMMM, INRA de Theix, 63122 St-Genès-Champanelle, France*

Various soluble fibres exert cholesterol-lowering effects, in human subjects as well as in animal models, especially hydrocolloids such as guar gum (GG). Altered viscosity of the digestive contents could depress cholesterol absorption and enhance spillover of bile acids (BA) into the large intestine, and hence their faecal elimination. This model implies that BA reabsorption in portal blood is reduced, thus limiting their down-regulatory capacity for liver cholesterol 7α-hydroxylase (C7H).

The present work was performed using 150 g rats adapted for 21 days to a semi-purified control (C) diet or a diet containing 50 g GG/kg, both supplemented with 2.5 g cholesterol/kg, in order to estimate the variables of enterohepatic BA cycling (especially digestive pool sizes and intestinal reabsorption).

The GG diet elicited a significant lowering of plasma cholesterol (–18 %) during the absorptive period. This was accompanied by a rise of biliary BA secretion and of both small-intestinal (from 64 to 74 μmoles) and caecal (from 20 to 38 μmoles) BA pools. In parallel, BA reabsorption in portal and caecal vein was enhanced (+ 48 % and + 73%, respectively).

[^{14}C]Taurocholate absorption capacity, determined in perfused ileal segments, was not significantly different in rats adapted to the C or GG diet; thus, in the latter, the rise of the ileal BA pool is probably a major cause of greater ileal BA reabsorption. Capacities of [^{14}C]cholate absorption in the caecum were higher in rats adapted to the GG diet, at pH 6.5 (+ 30 %). Acidification of caecal medium (from pH 7.1 down to 6.5 or 5.8) or addition of 100 mmol/l volatile fatty acids were found to stimulate caecal [^{14}C]cholate cecal absorption. These factors should contribute to accelerate caecal BA absorption in rats fed on the GG diet.

The effects of GG on faecal steroid (BA + sterols) excretion appear to be concomitant with a greater intestinal BA absorption and portal flux to the liver. The fact that cholesterol oxidation remains very active in the presence of high concentrations of bile acids in afferent blood suggests that the control of cholesterol 7α-hydroxylase activity is particularly complex ; changes in portal BA composition or other metabolic factors could also play a critical role.

Bioavailability of starch, rate of glucose absorption and intestinal metabolism in pigs. By LIONEL NOAH[1], GERARD LECANNU[1], PASCALE MAUGERE[2], MICHEL KREMPF[2] and MARTINE CHAMP[1], *[1]Institut National de la Recherche Agronomique, BP 71627, 44316 Nantes Cédex 03, France;[2]Centre de Recherche en Nutrition Humaine, Hôtel-Dieu, 44035 Nantes Cédex 01, France*

While a fraction of absorbed glucose is used by the gut, the effect of the rate of glucose absorption on intestinal metabolism has never been investigated.

The present study was undertaken in pigs (*n* 5) to investigate the intestinal metabolic fate of two maize starches (200 g glucose-equivalent) ingested in a mixed meal. The animals were fitted with catheters in the carotid artery, the jugular and portal veins and an ultrasonic flow probe was positioned around the portal vein. Starch, naturally ^{13}C-enriched, was either slowly (native) or rapidly (pregelatinized) digestible. Glucose absorption was followed by the use of the porto-arterial difference technique (Rerat *et al.* 1984), and the isotope dilution technique (infusion of [^{2}H]glucose in the jugular vein) was used to measure the rate of appearance of [^{13}C]glucose in the systemic blood (Steele 1959). Values are expressed as means with their standard errors.

In two pigs, each fitted with an ileal cannula, we found that the proportion of residual starch entering the colon was less than 1 % of the ingested amount for both starches. Moreover, less than 3 % of the oral starch was left within the gut when the animals were killed 720 min after the experimental meal. The concentration of short-chain fatty acids we measured in the ileal residues (<5 mmol/l) revealed a low microbial activity. For both diets, porto-arterial blood glucose difference returned to basal values after 720 min, indicating the end of absorption. It increased up to values of 2.28 (SE 0.17) mmol/l (at 75 min) and 2.78 (SE 0.28) mmol/l (at 60 min) for native starch and pregelatinized starch meals respectively. The rate of absorption was slightly higher for the pregelatinized starch during the first 120 min. At that time, the amounts of absorbed glucose were 56.3 (SE 3.3) g for the pregelatinized starch and 44.6 (SE 4.4) g for the native starch ($P<0.05$). However, no difference was observed between the cumulated amounts of absorbed glucose over the 720 min of the study (103.7 (SE 12.7) g for native starch and 108.7 (SE 9.1) g for pregelatinized starch, $P=0.39$). The amount of absorbed glucose converted to lactate by gut metabolism was 7.3 (SE 1.4) g for native starch and 6.7 (SE 0.7) g for pregelatinized starch ($P=0.35$). The exogenous [^{13}C]glucose systemic appearance overestimated by about 20 % the net glucose absorption at the end of the study. This indicated an important recycling of the absorbed glucose in the splanchnic area.

The findings indicate that the bioavailability of starch, for the starches we used, does not seem to affect the proportion of glucose reaching the portal vein, nor does it affect gut metabolism.

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Steele (1959) Influences of glucose loading and of injected insulin on hepatic glucose output. *Annals of the New York Academy of Science* **82**, 420-430.

Thyroid status affects the cellular action of vitamin A in adult mouse brain. By VERONIQUE PALLET[1], VALERIE ENDERLIN[1], SERGE ALFOS[1], DENISE HIGUERET[2], ROBERT JAFFARD[3] and PAUL HIGUERET[1], *[1]Laboratoire de Nutrition , Université Bordeaux I, 33405 Talence cedex, France, [2]Laboratoire de Biochimie Hôpital Pellegrin 33076 Bordeaux cedex, France, [3]Laboratoire de Neurosciences Comportementales et Cognitives (URA CNRS 339), Université Bordeaux I, 33405 Talence cedex, France,*

Triiodothyronine (T_3) and retinoic acid (RA) are important modulators of normal brain development and maturation. Less is known, however about their role in the adult brain. Evidence in recent years has provided the biological bases for the action of these molecules in mature neurones.

We examined the effects of hypothyroidism and subsequent acute repletion with (T_3) or (RA) on the expression of T_3 and RA nuclear receptors (TR, RAR and RXR) and that of target genes neurogranin (RC3) and tissue type transglutaminase (TGase) in adult mouse brain.

Mice received water containing 0.5g propylthiouracyl (PTU)/L. After 28 d of PTU administration mice were either maintained on PTU, or treated with RA or T_3 (150 µg/kg daily for 4 d). The amounts of mRNA in brain samples were determined by a semi-quantitative reverse transcriptase-polymerase chain reaction method, co-amplifying RARβ and TR$\alpha_1\beta$, RXRβγ and RC3, and TGase genes with the unrelated β-actin gene as internal control.

The hypothyroid state significantly reduced the expression of RAR and RXR mRNA and that of TGase and RC3. Administration of T_3 to hypothyroid mice up-regulated the expression of T_3 and RA nuclear receptor mRNA (TR +33 %, RXR +15 %, RAR +45 %). RA induced an up-regulation of the expression of its own receptors (RXR +22 %, RAR +30 %) but had no effect on TR. In hypothyroid mice the expressions of TGase and RC3 mRNA were up-regulated by RA or T_3 administration.

These results obtained *in vivo* indicate that the retinoid signalling pathway is affected by thyroid status. They show interplays between RA and T_3 signalling pathways. Moreover, the present data show that changes in nuclear receptor balances affect the expression pattern of RC3 and TGase genes, target genes involved in adult brain plasticity.

β-lactoglobulin modifications characterized by mass spectrophotometry lead to different activity on hybridoma cell culture. By PALUPI, N.S.[1,3], FRANCK P.[1], BELLEVILLE-NABET F.[1], NABET P.[1] DOUSSET B.[1], LINDEN G.[2] and GUIMONT C.[2], [1]Laboratoire de Biochimie, Faculté de Medecine, Université de Nancy 1, BP 184, Vandoeuvre-lès-Nancy Cedex, France. [2]Laboratoire des Biosciences de l'Aliment, UR 885 INRA, Université Henri Poincaré, Nancy 1, Domaine Victor Grignard, BP 239, 54506 Vandoeuvre-lès-Nancy Cedex, France. [3]Department of Food Technology and Human Nutrition, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, P.O. Box 220 Bogor 16002, Indonesia. (E-mail: guimont@scbiol.u-nancy.fr).

Milk contains macromolecules which can stimulate growth of cells *in vitro*. Critical review about the different components in bovine milk provides an explanation for this activity (Guimont *et al.* 1997). It was suggested that, either the role of protein maturation which was related to their function or the role of milk processing technology (e.g. heating) will promote structural changes which could affect their biological function. Previous research in our laboratory have demonstrated the selective activity on cell proliferation of different whey (Guimont & Badeche, 1994) and β-lactoglobulin (βLG) (Moulti-Mati *et al.* 1991; Capiaumont *et al.* 1994). In this study, we focus on the comparative activity between two βLG samples (commercial/*L*-βlg and laboratory preparation/*NL*-βLG) on the growth of hybridoma cells.

Biochemical characterization of the different βLG used in this study was determined by mass spectrometry. An established cell line of hybridoma (MARK-3) was cultured in serum-free medium supplemented with different concentrations of the two βLG's. Growth response of cells was analyzed using the [^{3}H]-thymidine uptake and flow cytometric analysis of DNA content.

Protein	M_r Theoritical[1]	*L*-βLG		*NL*-βLG	
		M_r	Modification	M_r	Modification
βLG_B	18,278.1	18,278.0	native (70%)	18,278.0	native (75%)
		18,604.0	+ 1 lactose (10%)	18,603.0	+1 lactose (3%)
		17,724.0	- peptide (20%)	18,469.0	+192 (7%)
				18,260.0	-18 (15%)
βLG_A	18,364.4	18,366.0	native (70%)	18,366.0	native (75%)
		18,690.0	+ 1 lactose (10%)	18,689.0	+ 1 lactose (3%)
		17,809.0	- peptide (20%)	18,558.0	+ 192 (7%)
				18,349.0	-18 (15%)

[1] The average molecular mass relative (M_r) of protein was calculated by summing the corresponding masses of all residues in the proteins according to the value of masses average of amino acid residues.

Mass spectra of βLG samples show molecules with different masses (presented in table above) due to the atoms or molecule(s) adduction (K^+, Na^+, lactose) or absence of a peptide. About 10% and 3% of lactosylated molecules were found respectively in *L*-βLG and *NL*-βLG samples. Compared with an equivalent protein basis of control medium, the cells proliferation was increased by the supplementation of βLG. These effects were 15% and 22% of positive control (medium supplemented with FCS 10%) and its were 30% and 70% of negative control (without FCS 10%), respectively for *L*-βLG and *NL*-βLG. The distribution activities among the two βLG were also measured by flow cytometry. The percentage of S-G2/M phase of *L*-βLG (38%) was lower than its for *NL*-βLG (50%). This last result is closed to the percentage of S-G2/M phase obtained with FCS (53 %). In the contrary, the percentage of sub-G1 (apoptose cells) of *L*-βLG (30%) was higher than its for *NL*-βLG (19%).

βLG samples obtained from different purification processes were selective in supporting growth of hybridoma. In all cases, the maximum cell proliferation was obtained with 3 mg/ml of βLG. The relation between proliferation activity of βLG and milk processing could be further discussed.

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Moulti-Mati F, Mati A, Capiaumont J, Belleville F., Linden G & Nabet P (1991) *Lait* 71, 543-553.
Capiaumont J, Legrand C, Dousset B, Parmentelot I, Linden G, Belleville F & Nabet P (1994) *Lait* 74, 127-137.

Global analysis of two-dimensional protein patterns of the lactic acid bacterium *Streptococcus thermophilus*. By CLARISSE PERRIN, PATRICE BRACQUART, JEAN LUC GAILLARD and CHRISTIANE GUIMONT, *Université Henri Poincaré-Nancy I, Laboratoire des Biosciences de l'Aliment, Unité associée INRA (Département TPA), Faculté des Sciences, BP 239, 54506, Vandoeuvre-les-Nancy, France*

Streptococcus thermophilus is a lactic acid bacterium widely used for the production of fermented dairy products. In the present study, two-dimensional gel electrophoresis (2-DE) coupled with an image analysis system was applied to determine: (1) the reference protein profile of a strain of S. *thermophilus* (PB18) originating from traditional cheese; (2) the extent of strain variation at the level of bacterial proteins by comparison with another strain (ST105) of commercial origin (yoghurt mixed starter); (3) the location of some major spots of *S. thermophilus* for futher sequencing and identification of corresponding proteins.

The reference image of the strain PB18 presents about 270 spots and their average molecular mass and isoelectric point were estimated to be 41 600 kDa and 5.2 respectively. The proportion of matched spots between the ST105 and PB18 images was calculated to be 75 % and the two strains did not exhibit very different profiles; however matching proportions were dependent on bacterial growth conditions.

Some major spots of the 2-DE protein pattern of *S. thermophilus* were well located and corresponding proteins were microsequencing and identified. Among them, five are prokaryotic ubiquitous proteins (β-galactosidase, fructose bisphosphate aldolase, phosphoglycerate kinase, GroEL, ribosomal protein L7/L12). In the *S. thermophilus* strain PB18, two proteins were detected in stress conditions: one 16 kDa acid shock protein (Asp16) previously described (Gonzalez-Marquez *et al.* 1997) and entirely sequenced (accession number P80485) was overexpressed under acidic conditions (pH 5) and one 21 kDa protein was overexpressed under cold stress conditions (15°c): it is still unknown.

The present work was undertaken to study protein homologies among strains of *S. thermophilus* with different technological history by 2-DE. It would also help to establish a two-dimensional map of proteins of *S. thermophilus*.

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Gonzalez-Marquez H, Perrin C, Bracquart P, Guimont C & Linden G (1997) *Microbiology UK*, **143**, 1587-1594.

Effects of genistein and daidzein on ovariectomy-induced bone loss in rats. By CHRISTEL PICHERIT, CECILE DESIR, VERONIQUE COXAM, SERAPHIN KATICOULIBALI, MARIE-JEANNE DAVICCO, PATRICE LEBECQUE and JEAN-PIERRE BARLET, *INRA Theix, 63122 Ceyrat, France*

Demonstrating a bone-loss preventing effect of naturally occurring substances in food, such as genistein or daidzein, could decrease the necessity for drug therapy in postmenopausal women. Thus, we performed an experiment on fifty-two rats fed on a diet devoid of vegetable proteins. At 12 months of age, thirteen were sham-operated (SH). The thirty-nine other were ovariectomized. Then they received orally genistein (G) (thirteen rats) or daidzein (D) (thirteen rats) (10 µg/g body weight), or acted as controls (OVX) (thirteen rats).

The animals were killed at day 90. The success of ovariectomy was confirmed by atrophy of the uterine horns (g) (0.21 (SE 0.02) *v.* 0.73 (SE 0.07) in SH). None of the treatments had any effect on this variable.

Body-weight composition (measured by densitometry) was not modified by any treatment.

Femoral total bone mineral density (BMD, g/cm^2) (densitometry, Hologic QDR 4500A) was decreased in OVX (0.1988 (SE 0.0044) *v.* 0.2263 (SE 0.0053)). This osteopenia was prevented by D (0.2197 (SE 0.0049)) but not G (0.2031 (SE 0.0047)), especially at the trabecular level, while G was effective on cortical bone (D: 0.1940 (SE 0.0024), G: 0.2005 (SE 0.0023), OVX: 0.1888 (SE 0.0016), SH: 0.2018 (SE 0.0013)). In the same way, bone Ca content was improved by D but not G.

The decreased femoral failure stress (MPa) (three-point bending test, Instron 4501) in OVX (4074 (SE 211) *v.* 4364 (SE 223) in SH) was corrected by both treatments. Vertebrae were also protected by D, while G was inefficient.

In conclusion, the present experiment highlights the possibility of preventing bone loss induced by ovarian deficiency by phyto-oestrogens, without uterotrophic effect. Furthermore, daidzein, at the dose used, is more efficient than genistein, especially at the trabecular level.

Role of cytokines in the decrease of food intake during human immunodeficiency virus (HIV) infection. By A. PRADIGNAC [1], M. KAZES [2], R. SAPIN [3], J.M. LANG [4], J.L. SCHLIENGER [1] and J.M. DANION [2], *1: Service de Médecine Interne & Nutrition, 2: Unité INSERM U405, 3: Laboratoire de Physique Biologie, 4: CISIH d'Alsace, Hôpitaux Universitaires de Strasbourg, 67 091 Strasbourg - Cedex, France.*

An increase in the secretion of cytokines, which can cause changes in eating behaviour, has been described in several infectious diseases. The aim of the present study was to look for a possible interaction between cytokine concentrations and the food intake of HIV-infected patients.

We report results concerning twenty-nine patients (fourteen seropositives, fifteen with acquired immunodeficiency syndrome) and sixteen age-paired controls. Each patient was free of any intercurrent major disease and had taken a stable anti-retroviral therapy for at least 2 months. Blood levels of tumour necrosis factor, interleukin-1, interleukin-6 (IL6) were determined with EIA Immunotech kits, interferon-α with IRMA Biosource and leptin with RIA Linco one. Food intake was monitored during a standardized meal. Simple linear regression demonstrated an inverse correlation between patients' IL6 blood level and energy (r -0.59; $P < 0.001$) or protein (r -0.84; $P < 0.001$) intake. There was a weaker correlation between leptin and the energy intake of patients (r -0.40; $P < 0.05$). This correlation desappeared if multiple regression was applied. This analysis reinforces correlations between IL6 and patients' energy or protein intake.

In conclusion, in HIV-infected patients, IL6 may contribute to the reductions in ernergy and protein intake. The role of other cytokines does not seem to be significant during the stable phase of HIV infection. If these results are confirmed in other studies, it would be of great interest to treat aggressively any illness that may increase IL6 levels in order to improve the food intake and prevent the wasting syndrome of HIV-infected patients.

Cross-population differences in Mauritian and French mothers' white adipose tissue (WAT) and milk and their influences on the erythrocyte phospholipid composition of their breastfed infants. By P. PUGO-GUNSAM[1,2], P. GUESNET[1], A.H. SUBRATTY[2], D.A. RAJCOOMAR[3], J. GORE[1], C. MAURAGE[1] and C. COUET[1], *[1]URA 'Lipides & croissance', Université-INRA, Tours, France. [2]University of Mauritius and [3]J. Nehru Hospital, Ministry of Health, Mauritius*

Although maternal milk is considered as the biological reference, there are cross-cultural differences in dietary habits which influence its lipid composition. The impact of these variations in lipid composition on the status of breastfed infants has not yet been assessed. The present study compared the milk and WAT lipid compositions of Mauritian and French mothers and the lipid status of their full term healthy infants after a 6-week period of exclusive breastfeeding.

Samples of WAT, colostrum and mature milk were obtained at day 5 and at day 42 postpartum from volunteer lactating Mauritian (n 13) and French (n 15) mothers. Anthropometric measurements and blood samples of their infants were obtained at birth and day 42. The fatty acid composition of biological material was determined by GC with flame ionisation detection. Two-way ANOVA and linear regression fit were used for statistical analysis.

Lower levels (g/100g fat) of saturated (23.64 (SE 1.54) $v.$ 29.75 (SE 0.67), $P<0.01$) and monounsaturated (MUFA 39.44 (SE 1.27) $v.$ 54.84 (SE 0.75), $P<0.001$) and higher levels of long chain polyunsaturated fatty acids (LCPUFA (n-6) series 32.47 (SE 1.31) $v.$ 14.32 (SE 0.47), $P<0.001$ and LCPUFA (n-3) series 2.87 (SE 0.49) $v.$ 0.8 (SE 0.07), $P<0.01$) were found in Mauritian WAT as compared with French. Lipid composition of WAT did not change over the study period in either population. Milk fat of the Mauritian population contained lower amounts of MUFA (g/100g fat) : (27.4 (SE 0.6) and 25.5 (SE 0.7) $v.$ 40.8 (SE 0.8) and 36.8 (SE 0.8), day 5 and day 42 respectively, $P<0.001$) and higher levels of parent essential fatty acids (linoleate 23.7 (SE 0.7) and 26.8 (SE 0.9) $v.$ 11.0 (SE 0.4) and 11.8 (SE 0.9); α linolenate 1.9 (SE 0.1) and 2.7 (SE 0.2) $v.$ 0.8 (SE 0.1) and 0.6 (SE 0.1); day 5 and day 42 respectively, all $P<0.001$). Arachidonate (0.5 (SE 0.0) $v.$ 0.2 (SE 0.0), $P<0.001$) and docosahexaenoic acid (0.4 (SE 0.0) $v.$ 0.1 (SE 0.0), $P<0.01$) were also more represented in Mauritian mature milk than in French. Significant correlations were observed for linoleate and linolenate between colostrum or mature milk and WAT (all $r^2>0.6$). The Table shows partial results of the infant's erythrocyte membrane phosphatidylethanolamine composition. Similar results were observed for phosphatidylcholine. Comparable growth was observed in both populations.

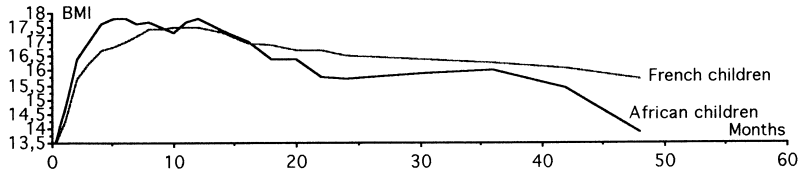
	Mauritian day 0 (n 13)		French day 0 (n 15)		Mauritian day 42 (n 13)		French day 42 (n 15)		Two-way ANOVA		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	T	P	T*P
18:2n-6	4.6	0.4	2.6	0.3	5.5	0.6	3.7	0.2	S	S	NS
20:4n-6	16.0	1.3	20.4	1.0	16.2	0.8	21.0	0.7	NS	S	NS
ΣPUFA(n-6)	30.1	1.7	32.5	1.3	30.6	1.4	32.5	1.2	NS	NS	NS
18:3n-3	0.3	0.1	0.1	0.0	0.2	0.0	0.1	0.0	NS	S	NS
22:6n-3	5.1	0.5	7.2	0.4	4.6	0.4	8.0	0.3	NS	S	NS
ΣPUFA(n-3)	6.9	0.7	8.6	0.4	6.7	0.4	10.3	0.4	NS	S	S

T , time ; P, population.

The milk and WAT lipid composition is consistent with a vegetarian type of diet in Mauritian compared with French mothers. Mauritian milk provides very high amounts of linoleic and α linolenic acids concomitantly and comfortable amounts of LCPUFA. However, high amounts of LCPUFA in human milk do not guarantee higher accretion in breastfed infants' membrane phospholipids when provided along with very high levels of parent essential fatty acids.

Food habits and growth of children of Subsaharan African ancestry born to immigrant parents and French children. By F. ROVILLÉ-SAUSSE and F. SOSSAH, *Laboratoire d'Anthropologie Biologique, Museum National d'Histoire Naturelle, Place du Trocadéro, 75116 - Paris, FRANCE.*

The objective of this study was to show the differences in food habits and their effects on growth rates of two communities of children up to the age of 6 years living in the Paris area. This study was composed of two stages: (1) A longitudinal growth study based on 848 medical files of children born in the Paris area (Val-de-Marne) and followed by the centres of"Protection Maternelle et Infantile" (PMI): 400 non-immigrant French children and 448 children of Subsaharan African ancestry born to immigrant parents. (2). A nutritional survey by face-to-face interview with 107 mothers, among the 848 families followed (sixty African and forty seven French mothers), who answered an interview about the way of feeding and food diversification. The nutrients of the diets were analysed and compared in both populations. Biometrical results revealed that growth rate of the African babies in the first months was faster than that of the French babies, prior to a significantly lower growth rate from 22 months.



French breast-feeding mean was 3 months (min. 0.5, max. 8 months), but 45% did not breastfeed (related to education level). African breast-feeding was longer (mean 6 months, min. 2, max. 18 months);only 10 % did not breastfeed (not related to education level, or date of arrival in France). However breast-feeding was distinctly less practised than in Africa (18 - 24 months, according to the mothers interviewed). At the time of weaning and diversified feeding, children of both communities received practically the same diet (commercial baby food). From 18-24 months, they shared family-diet, and then some differences began. According to the four showed diet -patterns, the needs of proteins, lipids, carbohydrates, Ca of the greater part of the 2 - 6 years aged children were supplied; 5 % of the African children and 4 % of the French children never consumed fruits or raw vegetables; nearly 90 % of children consumed many sweet things (drinks and candies).

Food	Milk	Cereals	Sugar	Cookies	Choco.	Nutella	Butter	Fruit	Raw veg.	Veg.	Beans	Carboh.	Meat	Candies
% of African children	100	54	86	35	31	11,5	34	83	20	81,5	4,5	100	100	14
% of French children	100	47,5	66	49	38,5	9,5	15	83	21	74,5	2,5	98,5	100	10

Drinks	Water	Soda	Juice	Water+ Cordial	Soda+ Juice	Cordial+ Juice	Sweet Dr total
% of African children	12	59	14	4	9	2	88%
% of French children	15	23	10	26	26	0	85%

Considering the Fe content and the absorption rate of the ingested food, the analysis of composition of the African families diets revealed an Fe deficit (diet-pattern 1 and 2), that would be partly supplied among the children eating school lunch (diet-pattern 4 providing a higher Fe rate).

Recommended iron rates for 1-6 years children	diet-pattern 1 (Maly-Senegal)	diet-pattern 2 (Togo-Benin)	diet-pattern 3 (Congo)	diet-pattern 4 (France)
0.7-1.0 mg/d	0.54	0.60-0.70	0.82	0.96

Socioeconomic status and cultural behaviours play a significant part in the nutritional supply, and in the growth of the children. The understanding of these habits is of primary importance for a better health education.

Nutritional value of a sorghum-based food complemented with soyabean proteins for African people. By MICHEL RUBAYIZA[1], MICHEL LINDER[1], RADIA LAMGHARI[2], LUC MEJEAN[2] and MICHEL PARMENTIER[1]. *[1] ENSAIA : Laboratoire de Physicochimie et Génie Alimentaires. 2, Avenue de la Forêt de Haye, 54500 Vandoeuvre-lès-Nancy, France. [2] INSERM U 308, 38 rue Lionnois, 54000 Nancy. France.*

Nutrional properties of sorghum spent-grain (SSG) and soyabean based food intended for infant's food formulations were investigated by means of chemical, *in vivo* and *in vitro* methods. Traditional sorghum-cooking processes generally greatly affect the nutritional potential of this cereal, e.g. the true digestibility (TD) decreases from 83 to 46 % after cooking (Knudsen *et al.* 1988). Combining soyabean protein and spent-grain improves the nutritional value of the mixtures (Table 1).

Results of *in vivo* (Block & Mitchell, 1946) and *in vitro* (Pedersen & Eggum, 1983) studies on the protein efficiency ratio (PER) and TD variations of sorghum and SSG blended with germinated soyabean (GS) and non-germinated soyabean (NGS) (1 : 3, w/w) revealed an improvement of the nutritional value of mixtures.

Table 1 : variations of the PER and TD values for the different tested-blends

	SSG	GS	GS+ sorghum	GS+ SSG	NGS	NGS+ sorghum	NGS+ SSG
PER	0.92	2.47	2.46	2.48	2.46	2.77	2.52
TD (%)	85.90	91.5	89.6	88.48	88.46	85.03	82.07

PER of casein was 2.5 and TD was 98.72 %

Mashing did not affect the protein quality of sorghum: TD of sorghum proteins in spent-grains was 85.9 %, compared with 46 % obtained with the traditional cooking methods. When SSG were blended with GS (Almeida-Dominguez *et al*, 1993), the TD value was 88.5 %. TD was monitored by an *in vitro* multi-enzymic method, based on pH-stat, and was correlated with *in vivo* digestibility (r^2 0.88).

Spent-grains are generally thrown in Africa and more than two hundred thousand tons of proteins are lost.

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Does dietary macronutrient composition relate to adiposity in young children? By C.H.S. RUXTON[1], T.R. KIRK[2] and N.R. BELTON[3], [1]*The Sugar Bureau, London SW1V 3PW;* [2]*Centre for Food Research, Queen Margaret College, Edinburgh EH12 8TS;* [3]*Department of Child Life and Health, University of Edinburgh, Edinburgh EH6 1UW*

Positive relationships between the dietary energy from fat and estimates of adiposity have been seen in adults (Bolton-Smith & Woodward, 1994) and 9-10-year-old children (Tucker *et al.* 1997). Conversely, inverse relationships have been found between adiposity indices and energy from dietary sugars in pre-school (Gibson, 1998) and adolescent children (Naismith *et al.* 1995). Such relationships were investigated in a sample of 136 Scottish children aged 7-8 years (boys *n* 65). Dietary intakes were assessed using the 7 d weighed method and the nutrition program COMP-EAT 4.0 (Nutrition Systems Ltd, London). Intakes of fat and non-milk sugars as a proportion of dietary energy were expressed as thirds of distribution. Height, weight and skinfold thickness (tricep, bicep, sub-scapula, supra-iliac) were measured and body fatness was estimated by BMI (kg/m^2) and percentage body fat from skinfolds. Pearson's correlation did not reveal any significant relationships between the macronutrient intakes and adiposity. When ANOVA was carried out on the thirds of macronutrient intake, there were no significant trends in measures of adiposity. The results are shown in the Table.

	Percentage energy from fat						Percentage energy from non-milk sugars					
	≤35.6		35.6-39.1		≥39.1		< 19.4		19.4-20.4		>20.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight SDS	0.04	0.8	0.06	1.2	-0.11	1.0	-0.01	1.0	-0.06	1.0	0.06	0.9
Body fat (%) (SK)	17.1	4.8	19.5	8.7	18.1	7.1	19.1	7.4	17.3	6.9	18.4	7.1
BMI SDS	-0.07	0.8	0.16	1.2	-0.04	1.1	0.14	1.1	-0.12	1.0	0.02	1.0

SDS standard deviation score; SK skinfolds; BMI body mass index

The results from this sample of young children showed no relationship between adiposity and dietary fat or sugars. This may be due to various reasons. It is possible that a relationship does not exist at this early age. In the pre-school population of Gibson (1998), the low r value (0.05) for the correlation between BMI and percentage energy from sugars was only significant due to the large sample size. There was no such relationship with percentage energy from fat. This is in contrast to the correlations in older children and adults which are stronger. Alternately, the relationship between macronutrient intakes and adiposity might only become significant when overweight starts to develop. Certainly, there were few children in this study who could be classified as overweight based on BMI SDS > 2.0 (*n* 10) or percentage body fat (SK) > 19.9 (*n* 6 boys) or > 20.8 (*n* 5 girls). Estimates for overweight in older children and adults are higher. This could explain the lack of agreement with Tucker *et al.* (1997) who reported percentage body fat from skinfolds of 19.3% for boys and 23.7% for girls. This is greater than the mean values in our children which were 16.5% and 18.9%. Another American study of 9-11 year olds which found relationships between adiposity and dietary fat pre-selected overweight children (Gazzaniga & Burns, 1993). To conclude, our data on 7-8 year old children do not indicate a link between adiposity and the macronutrient composition of their diets.

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Postprandial porto-arterial kinetics of ^{15}N, amino nitrogen, glucose and galactose in the growing pig after ingestion of milk, yoghurt and sterilized yoghurt. By G. RYCHEN[1], S. JURJANZ[1], J.M. ANTOINE[2], P.M. MERTES[3] and F. LAURENT[1], [1] *Laboratoire de Sciences Animales, ENSAIA, BP 172, 54505 Vandoeuvre cedex, France,* [2] *Danone Branche Produits Frais, 15 av Galilée, 92350 Le Plessis Robinson, France,* [3] *Laboratoire de Chirurgie Expérimentale, Faculté de Médecine, 54505 Vandoeuvre cedex, France*

The aim of the present experiment was to study [^{15}N], amino-N, glucose and galactose porto-arterial kinetics in the growing pig after ingestion of uniformly labelled [^{15}N] milk (0.2509 APE), yoghurt or sterilized yoghurt. This study was carried out with three castrated male Large White pigs (48-50 kg) fitted with two catheters, one placed in the portal vein and one in the brachiocephalic artery. At 7, 14 and 21 d postsurgery, 400 ml of either milk, yoghurt or sterilized yoghurt was assigned to the animals according to a Latin square design (3 X 3). Portal and arterial blood samples (10 ml) were collected simultaneously at 15, 30, 60, 90, 120, 150, 180, 210, 240, 300 and 360 min after ingestion of the milk products. The apparent absorption was estimated from the area between the portal and arterial concentrations.

The postprandial porto-arterial [^{15}N] enrichments were already observed 15 min after the start of milk, yoghurt and sterilized yoghurt ingestion. The [^{15}N] absorption profile indicated that the porto-arterial difference was maintained until 120 – 150 min. Absorption peaks were found at 30 min for milk and sterilized yoghurt and at 60 min for yoghurt. Thus, the use of intrinsically labelled [^{15}N] milk proteins made it possible to distinguish the specific absorption profiles of milk, yoghurt and sterilized yoghurt.

Table : Areas of porto-arterial concentration differences (mm^2) of amino nitrogen, glucose and galactose in the growing pig after ingestion of 400 ml labelled milk, yoghurt and sterilized yoghurt

	Milk		Yoghurt		Sterilized yoghurt	
	Mean	SD	Mean	SD	Mean	SD
Amino nitrogen	1604	280	2155	188	1604	296
Glucose	8443	4153	8746	628	7591	1148
Galactose	1719	593	2413	98	2029	226

Apparent absorption of amino-N was higher for yoghurt than for milk or for sterilized yoghurt. Apparent absorption of glucose was similar for the three products studied while apparent absorption of galactose was enhanced for yoghurt compared with milk and sterilized yoghurt.

This present work gives new insight into the kinetics of appearance of nutrients from milk products in the portal blood. Further studies should allow determination of the specific nutritional behaviour of milk proteins after different treatments and processes.

We thank Dr Simoes Nunes for conducting surgery

Cholesterol uptake and hydroxymethylglutaryl (HMG)-CoA reductase activity of Caco-2 cell-line clones. By SEVERINE SALVINI, MONIQUE CHARBONNIER, CHRISTIAN ALQUIER and DENIS LAIRON, *INSERM-U476 Nutrition Humaine et Lipides: biodisponibilité, métabolisme et régulations, 18 av. Mozart, 13009 Marseille, France.*

The human Caco-2 cell line is recognized as a valid model for studying lipid uptake by the enterocyte, intracellular trafficking and lipoprotein secretion. However, the parental cell line shows high heterogeneity. Recently, pure clones have been obtained from the parental cell line by Dr A. Zweibaum's group (Paris). The aim of the present study was to characterize some important aspects of cholesterol metabolism in three different clones (PD7, PF11 and TC7).

Cells were grown either in plastic dishes or on semi-permeable filter chambers in a conventional medium containing foetal calf serum (200 mL/L) at 37 °C, in a humidified atmosphere containing 10 % CO2. Mixed micelles of [^{3}H]cholesterol were prepared with egg phosphatidylcholine (2 mM), monoolein (0.03 mM), sodium oleate (0.25 mM), and taurocholic acid sodium salt (5 mM). Micelles were added at the apical side of enterocytes grown on semi-permeable filter. After different incubation times at 37 °C, the media was removed and the cells were washed twice with phosphate buffer saline. The [^{3}H]cholesterol was extracted from the cells with heptane/isopropanol (3/2, v/v) and counted for radioactivity. Proteins were determined by the method of Lowry *et al.*

The activity of HMG-CoA reductase was measured in cells every day for 10 d after seeding in plastic dishes, by the modified method of Goldstein *et al.*.

The results obtained showed that : (1) the uptake capacity of the apical side for cholesterol supplied as mixed micelles was time-dependent in a linear manner up to 60 min incubation time ; (2) the amount of cholesterol taken up by the apical side was concentration-dependent in the range 10-100 µmol/L ; (3) under optimal conditions (100 µ mol micellar cholesterol/L, 60 min incubation), the uptake rates of cholesterol in clones PD7, TC7 and PF11 were 673, 702 and 972 pmol/min per mg cell protein, respectively; (4) Similar patterns of HMG-CoA reductase activity were found for the three clones. A low activity level was measured on day 1 (17-34 pmol mevalonate/min per mg protein), then a marked increase was observed, with a maximum reached at day 4 (100-145 pmol mevalonate/min per mg protein). After cell confluence was reached on day 5-6, a dramatic drop in HMG-CoA reductase activity was observed, with baseline levels obtained on days 8-10. HMG-CoA reductase activity levels were higher after 10 d for all three clones when cells were grown on semi-permeable filters as compared to plastic dishes (PD7 +450%, PF11 +46%, TC7 +50%).

These preliminary results indicate that the Caco-2 clones studied exhibit different cholesterol uptake capacities at their apical side, and that the level of intracellular HMG-CoA reductase activity is influenced by the need of cholesterol for cell growth.

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Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) *Journal of Biological Chemistry* **193**: 265-275.

Effects of *Aspergillus fumigatus* phytase on phosphorus digestibility, phosphorus excretion, bone strength and performance in pigs. By CARLOS SIMÕES NUNES and PATRICK GUGGENBUHL, *Centre de Recherches en Nutrition Animale (CRNA), Société Chimique Roche, F-68128 Village-Neuf, France*

Phytases can improve phytic-P digestive utilization and consequently reduce P pollution by animal excreta. The aim of the present experiments was to evaluate the effects of *Aspergillus fumigatus* phytase (AFP) in swine.

Three experiments were performed. Four experimental mash diets were used: two phytic-P rich diets based on rapeseed, maize and barley (A and C) and these diets supplemented with 500 U AFP/kg (B and D, containing 490 (SE 30) and 525 (SE 28) U phytase/kg respectively).

Eight growing pigs (42 (SE 3.5) kg) were used under a Latin square design (diets C and D) for the measurement of faecal digestibility of DM, N, P and Ca (Expt 1). In the other experiments (2 and 3) seventy-two piglets (9.5 (SE 1.2) kg, groups A and B, 35 d of observation) and sixty growing pigs (35 (SE 4) kg, groups C and D, 63 d of observation) were used respectively for the evaluation of AFP effects on performance, and on the faecal excretion of P. Bone resistance (Newton at the breaking point) was determined on the right main external metacarpal and metatarsal withdrawn from animals of groups C and D at the end of the Expt 3.

	Expt 1				Expt 2				Expt 3			
	C		D		A		B		C		D	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood P (mg/dl)	6.7^a	0.69	8.3^b	0.93	4.1^a	0.50	6.2^b	0.94	6.5^a	1.21	7.8^b	0.65
Blood ALP (U/l)	196^a	36	152^b	34	214^a	30	156^b	37	208^a	39	164^b	27
Blood Ca (mg/dl)	11.9^a	0.91	10.6^b	0.77	9.5^a	0.76	8.4^b	0.87	11.4	0.61	10.9	0.44
PFC (% of DM)	2.8^a	0.19	1.9^b	0.18	2.2^a	0.07	1.9^b	0.06	2.5^a	0.11	2.0^b	0.07
Digestibility DM	83.2	1.4	84.3	1.2								
N	81.8	0.37	82.1	0.42								
P	30.8^a	2.03	52.8^b	1.87								
Ca	43.3	3.61	47.2	3.93								
DMG (g)					301^a	55	341^b	43	873^a	51	913^b	73
FCR (kg/kg)					1.8^a	0.09	1.6^b	0.07	2.7	0.13	2.6	0.18
Bone strength (N)												
Metacarpal									210^a	75	461^b	86
Metatarsal									161^a	67	526^b	79

^{a,b} Within each experiment, mean values within a row with unlike superscript letters were significantly different : $P<0.05$.

Diets A and C induced hypophosphataemia, hypercalcaemia and hyperphosphatasaemia. AFP restored the normal P, Ca and alkaline phosphatase (ALP) blood levels, and improved significantly the apparent digestibility of P by 22 percentage units and non-significantly that of Ca by 3.9 percentage units. Faecal digestibility of DM and N was not significantly modified. AFP significantly decreased the P concentration in faeces (PFC) by 33, 13 and 18 % in Expts 1, 2 and 3 respectively. AFP improved the daily mean gain (DMG) and the feed conversion ratio (FCR) in Expts 2 and 3. Bone resistance was significantly higher in AFP animals. The data obtained demonstrate that AFP is very efficient on the improvement of the availability of phytic-P and on the reduction of faecal excretion of P in the pig.

Effect of chronic heat exposure on liver insulin receptor number and affinity in broiler chickens. By SORAYA TEMIM, SOPHIE TESSERAUD, ROSA PERESSON, MICHEL DEROUET, CHRISTOPHE LEVEQUE and MOHAMED TAOUIS, *INRA, Station de Recherches Avicoles, 37380 Nouzilly, France*

In broiler chickens, chronic heat exposure suppressed growth and feed efficiency while enhancing fat deposition with a concomitant reduction in protein accretion. It also modified protein turnover, mainly by reducing protein synthesis (Temim *et al.* 1998). These changes may be the consequence of metabolic and hormonal modifications at high temperatures. Therefore, the impact of chronic heat exposure (32 v. 22°) on insulin responsiveness was examined in finishing male broiler chickens. Insulinaemia, glycaemia, liver insulin receptor (IR) number and affinity were measured at 5.5 weeks of age and in two nutritional states: fasted for 24 h or refed for 30 min after a 24 h fast (*n* 6). Glycaemia was measured by the glucose oxidase method (Glucose Beckman Analyser 2, Palo Alto, CA). Insulinaemia was determined as described by Simon *et al.* (1974). Binding of insulin to liver membranes was performed using ^{125}I-insulin as ligand as previously described by Taouis *et al.* (1993).

Ambient temperature	22°		32°	
(*n* 6)	Mean	SE	Mean	SE
Glucose (g/l)				
Fasted	2.03	0.09	1.90	0.04
Refed	3.32	0.11	2.74**	0.14
Insulin (ng/ml)				
Fasted	0.23	0.07	0.80	0.03
Refed	9.61	0.72	4.43**	1.11
B_0/T (%)†				
Fasted	13.7	1.2	15.5	1.7
Refed	12.5	0.9	15.9*	0.7
EC50 (ng/ml)‡				
Fasted	3.02	0.47	2.35	0.19
Refed	2.36	0.16	2.31	0.21

Means values were significantly different from 22°, * $P<0.05$; ** $P<0.01$.
†Maximal specific binding of ^{125}I-insulin (B_0) expressed as percentage of total radioactivity added (T).
‡EC50 represents the concentration of unlabeled insulin that inhibits ^{125}I-insulin binding by 50 %.

In the fasted state, glycaemia was not altered by heat exposure, whereas in the refed state, it was significantly lower at 32° compared with 22° ($P<0.01$). Plasma insulin levels were reduced by heat exposure irrespective of nutritional status ($P = 0.09$ for fasted birds; $P<0.01$ for refed birds). In the fasted state, hepatic IR number and affinity were similar at both temperatures, while in the refed state, IR number was significantly increased at the higher temperature (+28 %, $P<0.05$) with unmodified affinity.

In conclusion, in the refed state, insulinaemia of heat-exposed chickens was twofold lower than that of chickens maintained at 22°, which could at least partially explain the significant heat-related increase in IR number. These data indicate that changes induced by chronic heat exposure may not only be attributed to IR number variation, but could involve post-IR events or other hormones.

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Effects of dietary lipid source on membrane phospholipid composition and lipid mobilization in perirenal adipose tissue. By ANA I. TUEROS[1], M. TERESA MACARULLA[1], M. ISABEL TORRES[1], VALENTINA RUIZ[2], JAVIER SÁNCHEZ[2] and MARÍA P. PORTILLO[1], [1]*Department of Nutrition, University of País Vasco, Vitoria, Spain,* [2]*Instituto de la Grasa, CSIC, Sevilla, Spain*

Lipid mobilization is induced by the activation of β-adrenoceptors, located at the plasma membrane in adipocytes, which is followed by binding to adenylate cyclase. This process depends on the membrane lipid environment (Clandinin *et al.* 1994. The aim of the present work was to study the effects of dietary lipids on adipocyte phospholipid composition and its possible influence on adrenergic lipolysis.

Rats were fed on four different lipid sources for 1 month: olive oil (O), sunflower oil (S), palm oil (P) and beef tallow (BT). Isoproterenol (a β-adrenergic agonist) was used for the *in vitro* activation of lipolysis on isolated adipocytes (Langin *et al.* 1991). Phospholipid fatty acids were determined by gas chromatography. Statistical analysis was carried out using ANOVA.

The results obtained in this work are shown in the Table:

	O		S		P		BT	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Σ Saturated (%)	28.7^{a}	2.6	27.1^{a}	3.9	37.7^{b}	2.6	35.7^{b}	0.8
Σ Monounsaturated (%)	49.1^{a}	3.9	24.9^{b}	2.0	42.3^{a}	2.1	47.0^{a}	1.3
Σ Polyunsaturated (%)	19.3^{a}	1.2	49.0^{b}	1.2	19.3^{a}	2.8	15.1^{a}	1.4
Polyunsaturated:saturated	0.72^{a}	0.09	2.07^{b}	0.10	0.50^{c}	0.10	0.45^{c}	0.03
Maximal lipolysis (% of basal)	337^{a}	23	375^{a}	33	355^{a}	24	391^{a}	31

^{abc} Mean values within a row not sharing a common superscript letter were significantly different ($P<0.05$).

It can be concluded that dietary lipids modify the profile of fatty acids in adipocyte membranes (Suárez *et al.* 1996). The values for the polyunsaturated:saturated ratio suggest that membrane fluidity is greater in the adipocytes from rats fed on sunflower oil. These differences do not lead to modifications on lipid mobilization in perirenal adipose tissue.

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Suárez A, Ramírez MC, Faus MJ & Gil A (1996) *Journal of Nutrition* **126**, 887-897.

Food intake of lactating women in rural Bangladesh. By SOPHIE VINOY[1], C.G. NICHOLAS MASCIE-TAYLOR[2] and LYLIANE ROSETTA[1,3]. *[1]Laboratoire de Physiologie des Adaptations, Faculté Cochin Port-Royal, Paris, France, [2]Department of Biological Anthropology, University of Cambridge, Cambridge CB2 3DZ, [3]CNRS EP 1781, Paris, France.*

Twenty-six lactating women from a rural area of Bangladesh took part in a longitudinal survey between January 1996 and June 1997 to assess possible variation in energy balance during the breastfeeding period. For each woman a dietary survey was carried out in the same way on three occasions: during the monsoon, the winter and the dry season. Each time the total food and fluid intake was recorded on 3 d randomly chosen during the same week. Every ingredient was weighed before food processing, then, individual cooked foods consumed and the leftovers were weighed at each meal for each subject.

	Monsoon		Winter		Dry season	
	Mean	*SD*	Mean	*SD*	Mean	*SD*
Energy (MJ/d)	8.24	1.28	8.81	1.74	9.44 **	1.08
(kcal/d)	1970	306	2107	417	2259 **	258
Protein (g/d)	55	7	60	15	63 *	9
Carbohydrate (g/d)	401	67	427	78	462 *	54
Fat (g/d)	16	4	17	6	18	3
Fibre (g/d)	30	5	33	8	36 **	5
Liquid (litres/d)	1.6	0.5	1.6	0.3	1.6	0.3

n=26.
* $P<0.05$ (ANOVA with repeated measures).
** $P<0.01$ (ANOVA with repeated measures).

Mean fat and fluid intakes were not significantly different between seasons. Energy, protein carbohydrate and fibre intakes were significantly higher during the dry season. Nevertheless, the proportion of protein of animal origin was constantly low, 80 % of protein intake was from cereals, especially rice and wheat. In this sample, the proportions of energy intake from carbohydrate, fat and protein were respectively 76, 8 and 12 %. Protein intake, either absolute or as a proportion of energy intake, was within the recommended range (FAO/WHO/UNU, 1985), whilst there was an imbalance in the proportions of fat and carbohydrate. Fat intake being particularly low may cause deficiencies in fat-soluble vitamins.

In conclusion, over the period of investigation, there was no major variation in food consumption in this group of breastfeeding women in rural Bangladesh. Most of their diet is of vegetable origin, mainly cereals, with fat intake very much below the recommended requirements.

FAO/WHO/UNU (1985). Energy and Protein Requirements. WHO Technical Report Series no. 724, WHO: Geneva.

Specific under-reporting of "non-meals" and desserts by subjects suspected of under-reporting energy intake. By STEPHEN WHYBROW and TERRY R. KIRK, *Centre for Nutrition and Food Research, Queen Margaret College, Edinburgh EH12 8TS*

There is evidence that under-reporting of food intake affects the apparent macronutrient content of the diet (Voss *et al.* 1998), and it is believed that snacks and alcoholic drinks are under-reported more than meals (Livingstone *et al.* 1990). The present study compared the energy contribution of food groups for valid-reporters (VR) and suspected under-reporters (UR) (those with recorded energy intake:BMR values <1.1).

Subject details and data collection methods have been described in full previously (Drummond *et al.* 1998); in summary 7 d unweighed food records were collected from healthy Scottish adults (20-55 years), BMI 18-30 kg/m^2 for a cross-sectional study. Foods from each food diary were assigned to a food group according to when they were likely to be eaten based on information from the British Nutrition Foundation (1985). "Meal" foods comprised foods usually associated with main meals (e.g. meat and vegetables) while "non-meals" included all food and non-alcoholic drinks usually consumed between main meals ("snacks"). Foods that could not be clearly assigned were included in the "both" category.

	Percentage of energy intake from food groups.									
	Males					Females				
	VR (*n* 42)		UR (*n* 6)			VR (*n* 37)		UR (*n* 10)		
	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value
Meals	40.1	9.0	47.7	10.3	0.063	40.2	10.7	45.0	8.5	0.197
Non-meals	24.3	9.7	14.7	8.6	0.025	26.4	10.4	23.8	7.1	0.468
Both	19.7	6.9	20.4	13.7	0.912	20.7	7.2	19.5	7.1	0.626
Desserts	2.7	3.2	0.3	0.7	<0.001	3.4	4.1	1.9	2.3	0.275
Milk	4.7	3.7	4.7	5.8	0.999	3.8	2.4	4.3	1.8	0.517
Alcoholic drinks	8.4	5.2	12.3	8.7	0.366	5.5	5.7	5.4	7.8	0.980

P = 2-tailed t-tests.

When expressed as percentage of the energy intake, the contributions of "non-meal" and dessert food items were lower for UR than VR for males, but not significantly so for females. Surprisingly, there appeared to be no difference in reported consumption of alcoholic drinks.

The lower eating frequency of UR previously reported (Drummond *et al.* 1998) and these results suggest that, in this sample, under-reporting of food intake was biased towards "non-meals" and dessert foods. If these results are generally applicable, there are implications for dietary studies, especially those investigating relationships between eating frequency, diet composition and body weight status.

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Microencapsulation of marine oils for use in food fortification. By K.M. YOUNGER and M. E. NI NEIL, *Department of Biological Sciences, Dublin Institute of Technology, Kevin St, Dublin 8, Ireland*

Consumption of marine oils containing n-3 fatty acids is associated with health benefits, in particular the prevention and management of cardiovascular disease and autoimmune and inflammatory diseases (Fernandes *et al.* 1993). However, many people find marine oils unpalatable; also polyunsaturated oils are susceptible to oxidative deterioration, limiting their usage in foods. Microencapsulation is a process which masks flavours and odours, protects against oxidation and puts the active ingredient into a free-flowing form (Greenblat, 1993). The aim of the present project was to formulate a microencapsulated marine oil, using Irish ingredients where possible, that would be suitable for the fortification of foods at a level that would confer a health benefit (350 mg *n*-3 fatty acid/d).

Wall materials assessed included skimmed milk powder (SMP), sodium caseinate (NaC), whey powder (WP), whey protein concentrate (WPC), modified starch (N-Lok) and maltodextrin (MD). Core material was cod-liver oil. Aqueous solutions of wall materials were prepared and cod-liver oil was emulsified into the wall solutions in a ratio 4:1 (wall:core). Emulsions were spray-dried using an APV Anhydro A/S, Søborg, Denmark. Formulations with the highest WPC content had the best oil retention and encapsulation efficiency. Microencapsulation gave some protection against oxidative deterioration, and this was best in the formulations with the highest MD content. However, soda bread fortified with various of the formulations was not successful in triangular taste tests using an untrained panel of volunteers due to unacceptable sensory attributes. Other foods may be more successful.

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Effect of dietary energy sources and rearing temperature on lipogenesis in rainbow trout.

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The aim of the present study was to evaluate the effects of dietary energy sources (lipids, carbohydrates) on body lipids and lipogenesis in rainbow trout. The effect of water temperature (8 or 18°c) was also investigated. Fish being poïkilotherms, water temperature is a major regulator of energy needs.

Three experimental diets containing 450 g protein/kg DM combined with two lipid levels (90 or 180 g/kg DM) and two carbohydrate sources (gelatinized or crude starch) were tested on duplicate groups of fish (initial body weight : 65 g). Digestible energy (DE: 18-20 kJ/g DM) was mainly provided by digestible carbohydrate in diet A, by lipids in diet C and by both in diet B.

Fish were fed by hand, twice daily, near to satiation over 6 weeks. Two subsequent experiments was carried out at 8°c and at 18°c in a thermoregulated recirculated water system. At the end of experiment, whole liver was sampled from six fish of each group, for the measurement of the activities of lipogenic enzymes: glucose 6-phosphate dehydrogenase (G6PD) (Bautista *et al.* 1988), malic enzyme (ME) (Ochoa, 1955), and fatty acid synthetase (FAS) (Hsu *et al.* 1969). Six other fish were also withdrawn for determination of lipid content in muscle, digestive tract and liver. The results are presented in the Table below.

Experimental groups	**Tissue lipid level** (g/100g) Liver		Muscle		Digestive tract		**FAS** (mIU/g liver)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8° A	4.6 a	0.3	4.0 cd	0.3	33.9 ab	6.5	2.9 ab	0.6
8° B	4.7 a	0.4	5.5 ab	0.6	40.7 a	14.4	2.5 b	1.0
8° C	5.1 a	0.4	5.7 a	0.5	39.8 a	5.6	1.0 c	0.2
18° A	3.6 b	0.4	3.7 d	0.4	24.8 b	4.2	3.9 a	0.7
18° B	4.6 a	0.4	4.7 bc	1.0	28.0 b	4.3	2.9 ab	0.9
18° C	4.9 a	0.5	4.9 b	0.7	44.0 a	3.6	2.3 b	1.5

Different superscript letters indicate statistical differences (n=6), $P<0.05$

Hepatosomatic indices were lower in fish fed on diet C (low digestible carbohydrate content) whereas viscerosomatic indices were higher in fish fed on a high lipid level. An increase in dietary fat led to an increase in fat content of muscle and digestive tract at both temperatures whereas liver lipids were only increased at 18°c. The activity of FAS decreased with diets rich in lipids especially when dietary carbohydrate content was low (diet C). The G6PD activity was also reduced by the increase in dietary fat, but only at 18°c. No significant effect of diet or temperature was observed on ME activity. In all cases the activity of G6PD was about five times higher than that of ME, indicating that G6PD is the main enzyme providing the cytoplasmic reducing equivalents (NADPH). In all tissues, lipid content was higher at 8°c than at 18°c whereas lipogenic enzymes activities tended to be lower at 8° than at 18°c. These results suggest that, at low temperature, body lipid deposition mainly originates from dietary lipids.

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Effects of polyunsaturated fatty acids and peroxisome proliferators on stearoyl-CoA desaturase 1 gene expression in chicken hepatoma cells. By CHRISTIAN DIOT, PASCAL LEFEVRE, EDDY TRIPON, FRANCOIS DE QUELEN and MADELEINE DOUAIRE, *Laboratoire de Génétique Animale, INRA-ENSAR, 35042 Rennes cedex, France.*

Many authors have reported the inhibitory effect of dietary polyunsaturated fatty acids (PUFA) on the expression of genes involved in lipogenesis, including the stearoyl-CoA desaturase 1 (SCD1) gene. SCD1 enzyme catalyses the Δ9 desaturation of *de novo* synthesized fatty acids and is suggested to be involved in the regulation of triacylglycerol production, at least in the chicken (Legrand *et al.*, 1997). However, the mechanisms of the PUFA effect remain unclear. It has been suggested that this effect could be mediated by peroxisome proliferator activated receptors (PPAR) as has been described for the genes involved in peroxisomal β-oxidation (Lemberger *et al.*, 1996).

Regulation of SCD1 gene expression by arachidonic acid and peroxisome proliferators was analysed by transient transfection in chicken LMH hepatoma cells (Table) and by Northern blot analysis.

	CF (1 mM)*				ETYA (50 μM)*				AA (100 μM)*			
	-PPAR		+PPAR		-PPAR		+PPAR		-PPAR		+PPAR	
Expt	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	89	6.3	99	53								
2					32	7.8	35	9.0	60	11.2	57	14.0
3	97	3.9	116	3.5	29	12.2	56	17.4	48	12.4	39	17.0
4					25	6.7	42	10.0	26	6.0	25	2.0

CF, clofibrate ; ETYA, eicosatetrayonic acid ; AA, arachidonic acid.
*Luciferase activity ratio (in %) of effector-treated to untreated cells after transfer by electroporation of a plasmid containing the -375/+125 bp 5' part of the SCD1 gene upstream of the luciferase gene and with or without a rat PPARα expression vector.

SCD1 gene expression was inhibited by PUFA (AA) and ETYA (an AA analogue), as has been described in the rat. However, fibrates (CF) had no effect, although PPARα was expressed in the cells even when cells were cotransfected by rat PPARα. In this latter case, PUFA responsiveness was unaffected.

The results reported here indicate that chicken SCD1 gene expression is somehow different from that observed in the rat. Furthermore, they strongly suggest that PPARα is not involved in the response of SCD1 gene expression to PUFA. However, it is possible that the expression of lipogenic genes could be regulated by a common regulation mechanism.

Legrand P, Catheline D, Fichot M -C & Lemarchal P (1997) *Journal of Nutrition* **127**, 249-256.
Lemberger T, Desvergne B & Wahli W (1996) *Annual Review of Cell Developmental Biology* **12**, 335-363.

Hepatic lipogenesis in relation to fatty liver in the goose. By DOMINIQUE HERMIER[1], JACQUES MOUROT[2], GERARD GUY[3],and PHILIPPE PEINIAU[2]. *INRA, [1]Station de Recherches Avicoles, 37380 Nouzilly, [2]Station de Recherches Porcines, 35590 Saint-Gilles, [3]Station Expérimentale des Palmipèdes à foie gras, Artiguères, 40280 Benquet, France*

The variability of the response of geese to overfeeding relies on genetic differences in lipid metabolism (Fournier *et al*, 1997). Among genetic factors is level of lipid synthesis, that takes place mostly in the liver in avian species. Hepatic lipogenesis at the end of the overfeeding period was investigated in geese of the Landes breed, selected for fatty liver ("foie gras") production, and of the Poland breed, selected for meat production. Twenty 3-week-old male geese from each breed were overfed on a similar amount of boiled maize for 14 d, slaughtered and partly dissected. The potential of hepatic fatty acid synthesis was estimated by determination of the following enzyme activities: acetyl-CoA-carboxylase (ACX), malic enzyme (ME), glucose-6-phosphodehydrogenase (G6PDH), fatty acyl synthase (FAS).

The liver in the Landes breed was twice as heavy (791 g) as that in the Polish breed (359 g). Despite the fact that hepatic protein content is known to be similar in both breeds (Fournier *et al.*, 1997), the activity of lipogenic enzymes was significantly higher in the Landes goose than in the Polish goose.

Lipogenesis enzyme activities (units per liver)

	Landes geese		Poland geese		Statistical significance
Enzymes	Mean	SD	Mean	SD	of effect of breed
ACX (nmol HCO_3^-/min x 10^{-3})	18.2	4.8	13.6	3.4	$P < 0.05$
EM (μmol NADPH formed/min x 10^{-3})	456	111	220	109	$P < 0.001$
G6PDH (μmol NADPH formed/min x 10^{-3})	126	52	83	32	$P < 0.05$
FAS (μmol NADPH fixed/min x 10^{-3})	52.2	16.6	29.8	11.6	$P < 0.01$

Contrary to what is usually assumed in avian species, the contribution of G6PDH to providing NADPH necessary for fatty acid synthesis was far from negligible. Moreover, there was a positive significant correlation between the liver weight and the activity of ME (r +0.712; P = 0.0209) and FAS (r +0.879; P = 0.0008) in the Landes breed, and between the liver weight and the activity of ME (r +0.945; P = 0.0001) in the Poland breed.

These results indicate that, even after 14 d of intensive overfeeding, hepatic lipogenesis in the goose is still very active, and the liver does not appear to be exhausted from a metabolic point of view. The variation in susceptibility to liver steatosis among various breeds of geese relies, at least partly, on differences in hepatic lipogenesis, which are independent of food intake.

Fournier E, Peresson R, Guy G & Hermier D (1997) *Poultry Science* **76**, 599-607.

Differential liver lipid channelling in relation to susceptibility to hepatic steatosis in the goose. By MARIE-ROSE SALICHON[1], GÉRARD GUY[2], ROSARIA PERESSON[1] and DOMINIQUE HERMIER[1]. *INRA, [1]Station de Recherches Avicoles, 37380 Nouzilly, [2]Station Expérimentale des Palmipèdes à Foie gras, 40280 Benquet, France.*

In response to overfeeding, hepatic lipogenesis is dramatically increased in the waterfowl, which results in hepatic and extrahepatic steatosis. The grey Landes goose, which is highly susceptible to fatty liver, exhibits a greater accumulation of hepatic lipids than the white Poland goose, which exhibits a moderate hepatic steatosis together with higher concentrations of hepatic lipoproteins (VLDL and HDL) and a greater development of muscle and adipose tissue (Fournier *et al.*, 1997). The phospholipid (PL) (by TLC with flame ionization detection) and fatty acid (FA) (by GC) composition of liver was therefore studied in male Landes and Poland geese bred and overfed under the same conditions.

Control geese. Hepatic PL profile was very similar in control Landes and Poland geese, with phosphatidylcholine (PC) as a major component (~70 %) and phosphatidylethanolamine (PE) accounting for ≈25 % of total PL. PL composition was very similar in VLDL and HDL, with PC as their major component. The only marked difference was the considerable amount of lysoPC (LPC) in VLDL. In both VLDL and HDL, PL from the Landes geese contained more PC and less PE, and their PC: PE value was significantly higher (20.7 *v.* 12.6 % in VLDL and 33.8 *v.* 25.8 % in HDL).

Response to overfeeding. The increase in hepatic PL content concerned all PL classes, thus PL composition did not differ markedly from that in control geese, and the PC:PE value remained identical in the four groups (about 3).The Poland breed exhibited a higher concentration of HDL, as well as a higher proportion of PL in both VLDL and HDL. Overfeeding resulted in a dramatic decrease in LPC of both VLDL and HDL. VLDL-PL composition was identical in both breeds, but HDL-PL from the Landes geese contained more PE and less PC than in control, thus the PC:PE value decreased markedly in this group and was significantly lower than in the Poland group (13.6 *v.* 21.2%). The FA profiles of the fatty livers were very similar in both breeds : 18:1 *n*-9 (~50 %) and 16:0 (~30 %) predominated in neutral lipids, whereas PL exhibited also notable amounts of 18:0 (~20 %) and 20:4 *n*-6 (~13 %). Neutral lipids contained essentially no essential polyunsaturated FA (PUFA), which accounted for 25 % in PL. In both VLDL and HDL of overfed geese, neutral lipids were rich in 18:1*n*-9 (~40-50 %), and poor in long-chain PUFA (7-10 %), whereas 16:0 (~25-35 %) predominated in PL, which also exhibited high contents of 20:4*n*-6 (5-18 %). In the Poland breed, PL of both VLDL and HDL contained less monounsaturated FA (23 *v.* 30 %) and more *n*-6 PUFA (30 *v.* 15-25 %).

Conclusion. By contrast to the Landes goose, the Poland goose responds to overfeeding by an increased secretion of lipids, and especially PL, in plasma. In the Poland breed, the enhanced exportation of PUFA in VLDL- and HDL-PL can result in a relative deficiency in the liver, and impair membrane growth and cellular hypertrophy necessary for hepatic triacylglycerol accumulation during overfeeding.

Fournier E, Peresson R, Guy G, & Hermier D, (1997) *Poultry Science*, **76**, 599-607.

Effects of dietary coconut oil on the hepatic synthesis of apolipoprotein B, microsomal triacylglycerol transfer protein and LDL-receptor in the preruminant calf. By D. GRUFFAT, B. GRAULET, D. DURAND and D. BAUCHART, *Laboratoire Croissance et Métabolismes des Herbivores, INRA - Theix, 63122 Saint-Genès Champanelle, France*

In the young calf, dietary coconut oil (CO), rich in medium-chain fatty acids (FA), has a favourable effect on growth performance and protein accretion in muscles. However, when given as the sole source of FA, it induces a triacylglycerol (TG) accumulation in liver (Bauchart *et al.* 1997) which may be the result of : (a) a low capacity of TG to reach the secretory pool, (b) a low rate of apolipoprotein B (apoB) and/or microsomal TG transfer protein (MTP) synthesis and (c) a higher uptake of plasma TG from LDL via the LDL-receptor pathway.

Two groups of seven Friesian x Holstein male calves, aged 15 d, were given for 19 d a conventional milk replacer containing 22.4 g/kg DM as beef tallow (T) or CO. At the end of the experiment, liver samples were obtained under total anaesthesia. Hepatic homogenates were separated into microsomal and cytosolic fractions by sequential ultracentrifugation. The TG content in subcellular fractions was determined by HPLC from total lipids extracted according to the method of Folch *et al.* (1957). ApoB mRNA levels were determined by Dot blot analysis in liver homogenates. Hepatic biosynthesis and intracellular degradation of apoB were estimated by measuring apoB levels in cellular subfractions (endoplasmic reticulum (ER) and Golgi apparatus) by Western blot analysis. Hepatic amounts of MTP and LDL-receptor in microsomal fractions were determined by Western blot analysis.

The proportion of hepatic TG present in the microsomal subfraction (site of TG synthesis) was low compared with that of the cytosolic subfraction (storage pool) (23.3 and 8.7 % for T and CO diets respectively). In the CO group, we observed an increase in microsomal TG (+ 30 %), but a large accumulation in cytosolic TG (x 3.6). Hepatic amounts of apoB mRNA were similar for the two diets. By contrast, in the ER (site of apoB synthesis), apoB level tended to be higher (+ 69 %) in CO-fed calves than in T calves. In Golgi apparatus (apoB secretory pool), apoB level represented 85 % of that measured in ER for the two diets indicating a partial degradation of apoB (- 15 %) between these two cellular compartments. Hepatic levels of MTP and LDL-receptor were not significantly different between the two diets.

	Microsomal TG (mg/g liver)		Cytosolic TG (mg/g liver)		ApoB mRNA		ApoB in ER		ApoB in Golgi ($AU/10^6$ cells)		MTP		LDL receptor	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T	0.61	0.21	4.13	1.55	0.87	0.05	13.6	5.50	11.6	3.63	79.4	11.1	60.6	11.8
CO	0.80	0.26	14.8	4.77	1.00	0.09	22.9	10.8	19.1	5.64	90.1	14.4	62.6	16.1

Hepatic accumulation of TG in the liver of calves fed on the CO diet could not be explained by (1) an increase of TG uptake from LDL particles since hepatic content in LDL-receptor was unchanged, (2) an alteration of the VLDL assembly since hepatic amounts of apoB and MTP were not significantly reduced by the CO diet. However, there was greater TG accumulation in the cytosolic fraction corresponding to the storage pool. In the normal course of events, TG would be mobilized via a process of lipolysis and re-esterification in order to be available at the site of VLDL assembly. We might speculate that, in calves given the CO diet favouring FA esterification into TG (Graulet *et al.* 1997), this process was inadequate to stimulate the secretion of TG as part of VLDL particles by the liver.

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Folch J, Lees M & Sloane-Stanley GH (1957) *Journal of Biological Chemistry* **226**, 497-509.
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Contribution of peroxisomes to fatty acid oxidation in tissues from preruminant calves: effects of the nature of dietary fatty acids. By C. PIOT[1], J. H. VEERKAMP[2], D. BAUCHART[1] and J. F. HOCQUETTE[1], *[1]Laboratoire Croissance et Métabolismes des Herbivores, INRA, 63122 St-Genès Champanelle, France, and [2]Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands*

Fatty acid (FA) catabolism, which provides fuel for many tissues, occurs mainly in the mitochondrial matrix via the β-oxidation and the Krebs cycle reactions. However, FA can also be oxidized via the peroxisomal pathway which can be stimulated by nutritional or pharmacological treatments (Mannaerts & Van Veldhoven, 1995). The objective of the present study was to compare the effects of the incorporation in the milk diet of coconut oil (CN), rich in medium-chain fatty acids (MCFA, 12:0 and 14:0) and tallow (TA), rich in long-chain fatty acids (LCFA, 16:0, 18:0 and 18:1) on the contribution of peroxisomes to FA oxidation in tissues from calves.

Two groups of five 1-month-old preruminant Holstein-Friesian male calves were given, for 19 d, a high-fat milk replacer (224 g/kg diet DM) containing CN or TA. FA oxidation capacities of liver, heart and *longissimus thoracis* (LT) were determined by measuring oxidation rates of [1-^{14}C]oleate (18:1; 40 % of total FA in the TA diet) or [1-^{14}C]laurate (12:0; 42 % of total FA in the CN diet) in homogenates of fresh tissue as described by Veerkamp & van Moerkerk (1985). Peroxisomal FA oxidation was determined in the presence of inhibitors of mitochondrial oxidation, i.e. antimycin A and rotenone at 73 μM and 10 μM final concentrations respectively.

Tissue...		Liver				Heart				LT			
Diet...		TA		CN		TA		CN		TA		CN	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total FA oxidation	**12:0**	432^{a}	80.4	362^{ab}	102	187^{b}	21.0	204^{b}	45.3	26.3^{c}	5.38	20.9^{c}	3.81
rate (nmol/min per g)	**18:1**	218^{a}	23.0	163^{a}	19.4	147^{a}	20.8	170^{a}	20.8	18.8^{b}	4.78	14.7^{b}	3.99
Peroxisomal FA oxidation	**12:0**	169^{a}	29.0	189^{a}	26.4	46.5^{b}	7.00	57.2^{b}	4.43	8.64^{c}	4.43	5.95^{c}	2.98
rate (nmol/min per g)	**18:1**	33.2^{ab}	2.34	39.8^{b}	1.76	22.4^{a}	1.18	33.1^{b}	3.40	3.04^{c}	0.56	3.66^{c}	0.85
Peroxisomal / total	**12:0**	42.0^{ac}	5.86	67.9^{ad}	16.6	24.9^{bd}	2.58	35.8^{ab}	11.8	32.9^{ab}	12.2	22.9^{bc}	9.77
(%)	**18:1**	18.8^{ab}	3.82	32.1^{ab}	5.36	16.2^{ab}	2.01	20.7^{ab}	3.69	12.7^{b}	1.94	29.7^{a}	5.85

^{a,b,c,d} Means within the same row not sharing superscript letter were significantly different: $P < 0.05$.

Total and peroxisomal FA oxidation rates were 11- and 13-fold higher in the liver and in the heart than in LT ($P < 0.0003$) with both laurate and oleate used as substrates. However, relative contribution of peroxisomes to total FA oxidation was higher in the liver (55 *v.* 26 %, $P < 0.005$) and in the heart (31 *v.* 18 %, $P < 0.01$) with laurate than with oleate used as substrates but not in LT (25 % for each FA). The nature of dietary FA did not affect FA oxidation except in the heart in which a higher peroxisomal oleate oxidation rate was observed with calves fed on coconut oil (+48 %, $P < 0.05$).

We conclude that peroxisomal FA oxidation varies greatly with the nature of FA used as substrates and between tissues. Our results also show a possible enhancement of the activity of this pathway by MCFA especially in the liver and in the heart. This may explain at least in part, why the oxidation rate of MCFA is faster and greater than that of LCFA (Bach *et al.* 1996).

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Lipogenesis in isolated adipocytes from two subcutaneous adipose tissues in Large White and Meishan pigs . By M. KOUBA [1,2] and J. MOUROT [1], *[1] ENSA, 65, rue de St-Brieuc, 35000 Rennes, France ; Station de Recherches Porcines, INRA, 35590 St-Gilles, France*

In pigs, the existence of adipose tissues for lipid storage (subcutaneous neckfat) or lipid synthesis (subcutaneous backfat) has been demonstrated (Mourot *et al.* 1995). The aim of the present study was to show differences, if any, between adipocytes from theses two adipose tissues. One hundred castrated male pigs from two pure breeds, Large White (LW) and Meishan (MS), weighing 20, 40, 60, 80, 100 kg live weight (ten per breed and per weight group), were used. Adipocytes were isolated after collagenase digestion, from backfat and neckfat subcutaneous adipose tissues in both breeds of pig [^{14}C] Glucose incorporation was measured in 20 000 cells from each tissue. Adipocyte size was determined by image analysis after osmium fixation. Adipocyte size increased with live weight in both breeds (Table 1). It was higher in MS than in LW pigs and in neckfat than in backfat.

Table 1. Diameters of adipocytes (μm) from backfat and neckfat

Weight (kg)	Large White				Meishan			
	Neckfat	Backfat	Neckfat	Backfat	Neckfat	Backfat	Neckfat	Backfat
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	42.7	2.0	40.8	2.4	52.0	7.1	49.8	1.1
40	48.7	2.0	46.2	2.7	61.0	3.9	59.2	2.9
60	57.6	2.0	56.2	2.4	70.2	1.1	63.4	1.2
80	62.2	0.6	58.3	2.0	76.2	6.8	67.6	0.7
100	64.7	3.4	61.7	2.7	82.8	4.5	76.6	4.2

Glucose incorporation was higher in MS than in LW pigs at 20 and 40 kg (Table 2) (P<0.001). It was similar at 60 kg and became lower at 100 kg live weight (P<0.05). Glucose incorporation was higher in adipocytes from backfat than from neckfat whatever the weight (P<0.05).

Table 2. Glucose incorporation (μmol/h per 20 000 cells) in adipocytes from backfat and neckfat.

Weight (kg)	Large White				Meishan			
	Neckfat	Backfat	Neckfat	Backfat	Neckfat	Backfat	Neckfat	Backfat
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	77.7	2.0	86.8	13.4	110.5	6.3	136.4	16.1
40	84.4	14.9	93.6	14.7	139.4	17.9	174.5	18.4
60	144.4	17.6	167.6	16.0	168.9	10.2	183.9	10.5
80	144.8	12.6	156.5	15.9	124.6	17.1	143.6	24.9
100	137.1	10.1	159.9	11.0	118.5	14.1	138.9	16.1

These results showing differences between adipocytes from backfat and neckfat confirm those obtained previously (Mourot *et al.* 1995). This study shows that pig adipose tissues are not similar in their specific development and metabolism.

Mourot J, Kouba M, Peiniau P, (1995) *Comparative Biochemistry and Physiology* **III**, 379-384.

Comparison of adipocytes from subcutaneous and intramuscular adipose tissues in pigs. By J. MOUROT[1], G. SALVATORI[2] and M. KOUBA[1,3], *[1]Station de Recherches Porcines, INRA, 35590 Saint-Gilles, France, [2]Dimartemento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, 86100 Campobasso, Italia, [3]ENSA, Route de St-Brieuc, 35000 Rennes, France*

The aim of the present study was to compare glucose incorporation in isolated adipocytes from subcutaneous adipose tissue (backfat) and muscle *(Diaphragma)* in growing Large White (LW) and Meishan (MS) pigs. Thirty-two castrated male pigs from two pure breeds (LW and MS) weighing 20, 40, 60, 80 kg live weight (eight per breed and per weight group) were used. Adipocytes were isolated after collagenase digestion, from backfat and *Diaphragma* muscle. [^{14}C]Glucose incorporation was measured in 20 000 cells from each tissue. Adipocytes size was determined by image analysis after osmium fixation. Adipocyte size increased with live weight in both breeds and both tissues (Table 1). The cells were larger in backfat than in Diaphragma muscle (P<0.01), and in mS than in LW pigs (P<0.001).

Table 1. Diameter of adipocytes (µm) from backfat and *Diaphragma* muscle

Weight (kg)	Large White				Meishan			
	Backfat		*Diaphragma*		Backfat		*Diaphragma*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	35.8	1.9	24.9	3.7	44.6	0.9	37.6	2.6
40	42.2	3.7	34.4	3.1	52.2	5.1	48.6	2.9
60	48.1	2.8	41.4	3.7	56.9	4.9	54.4	3.2
80	52.6	2.6	44.4	3.1	65.4	7.8	56.1	5.7

In LW pigs, glucose incorporation (Table 2) was higher in backfat than in muscle at 20 kg live weight whereas it was lower in backfat from 60 kg live weight (P<0.01). In MS pigs, glucose incorporation was higher in *Diaphragma* muscle than in backfat from 40 kg live weight (P<0.01). Globally, the incorporation was higher in MS than in LW pigs, whatever the tissue, especially in young animals.

Table 2. Incorporation of glucose (µmol/h per 20 000 cells) in adipocytes from backfat or *Diaphragma* muscle

Weight (kg)	Large White				Meishan			
	Backfat		*Diaphragma*		Backfat		*Diaphragma*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	83	16	51	4	119	15	124	31
40	103	13	90	9	182	30	301	43
60	126	15	185	19	63	8	240	37
80	106	13	156	16	56	12	88	7

This study shows that adipocytes from *Diaphragma* muscle present a higher lipid synthesis activity (expressed by glucose incorporation) than adipocytes isolated from backfat, in LW and MS pigs.

Activity and transcription level of malic enzyme (EC1.1.1.40) in muscles of Large White and Meishan pigs. By C. RENARD [1], J. MOUROT [2], M. KOUBA [2,3] and G. SALVATORI [4], *[1] Laboratoire de Radiobiologie Appliquée, INRA, 78350 Jouy-en Josas, France; [2] Station de Recherches Porcines, INRA, 35590 Saint-Gilles, France; [3] ENSA, Route de St-Brieuc, 35000 Rennes, France; [4] Dipartemento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, 86100 Campobasso, Italy.*

The contribution of NADPH cofactor is essential in lipid synthesis. It is mainly produced by glucose 6 phosphodehydrogenase and malic enzyme (ME) in external adipose tissues. In muscle, only ME activity is of importance activity. The aim of the present study was to measure the correlation between the lipid content, the activity and the transcription level of ME in muscles of growing pigs.

Large White and Meishan pigs were slaughtered at 20, 40 and 60 kg live weight (eight per weight group). Samples of *Diaphragma* and *Semimembranosus* muscles were taken. Total lipid content (Chang *et al.*.) and ME activity (Hsu and Hardy) were measured. RNA was extracted from these muscles. Northern blots were hybridizated with a porcine ME probe to measure transcription level (Nunes *et al.*.)

The *Diaphragma* muscle was very rich in lipids. The lipid content in muscles increased with growth stage in both *Diaphragma* and *Semimembranosus* muscles. In Meishan pigs, the intramuscular lipid content was higher than in Large White pigs. In Large White pigs, ME activity increased with the development of tissues in both muscles (p<0.05) and it was constant in Meishan pigs. The activity was higher in *Diaphragma* than in *Semimembranosus* muscle as well as in Meishan than in Large White pigs. A high correlation exists between intramuscular lipid content and ME activity (r = 0.65 for Large White and r = 0.73 for Meishan pigs). The transcription level of ME in *Semimembranosus* muscle was half that in *Diaphragma* . In average, it increased with development of the two muscles in the two breeds as enzyme activity and total lipid content but in smaller proportion and with greater individual variations.

Table. ME activities and total lipid content in Large White and Meishan pigs

	Large White								Meishan							
	Diaphragma				*Semimembranosus*				*Diaphragma*				*Semimembranosus*			
body weight	malic enz.*		lipid+		malic enz.		lipid		malic enz.		lipid		malic enz.		lipid	
Kg	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	4.47	0.45	2.90	0.50	2.76	0.38	0.98	0.10	8.9	1.1	5.07	0.91	4.33	0.39	1.50	0.18
40	4.66	0.46	3.24	0.52	3.08	0.32	1.21	0.14	10.6	1.5	7.39	1.04	5.11	1.22	1.71	0.41
60	5.85	0.51	4.40	0.53	3.97	0.34	1.34	0.13	9.21	0.75	8.04	1.35	4.95	0.82	1.89	0.42

* UI / mg protein

\+ g / 100g fresh weight

The existence of a high correlation between intramuscular lipid content, ME activity and gene expression demonstrates the important role of malic enzyme in intramuscular lipid synthesis during muscle development.

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Effect of refeeding on two mRNA species of lipoprotein lipase in adipose tissue and cardiac muscle of sheep. By M. BONNET[1], Y. FAULCONNIER[1], C. LEROUX[2], F. BOCQUIER[1], P. MARTIN[2] and Y. CHILLIARD[1], [1]*Laboratoire Sous-Nutrition des Ruminants, INRA, 63122 Saint-Genès Champanelle,* [2]*Laboratoire Génétique biochimique et cytogénétique, INRA, 78352 Jouy-en-Josas cedex, France*

Lipoprotein lipase (*EC* 3.1.1.34 ; LPL), a key enzyme in lipid metabolism, is expressed predominantly in adipose tissues (AT) and oxidative muscles, where it makes available fatty acids, mainly for storage and oxidative metabolism respectively. In order to assess the effect of nutritional status on the regulation of LPL, we measured LPL activity as described by Faulconnier *et al.* (1994) and amounts of the two major LPL mRNA species (3.4 and 3.8 kb, due to an apparent alternative use of polyadenylation site) expressed in perirenal AT and cardiac muscle (CM) of ewes, either restricted or subsequently refed. Twenty adult, dry and non-pregnant ewes were restricted to 22 % of their maintenance energy requirements (MER) for 7 d. Ten were slaughtered whilst the remaining ten were refed to 190 % MER for 14 d before slaughter. Amounts of either both 3.4- and 3.8-kb LPL mRNA or only 3.8-kb mRNA were assayed by real time reverse transcription-polymerase chain reaction (Applied Biosystems Prism 7700 Sequence Detection System) using primer pairs and a probe chosen after characterization of the 3'untranslated region sequence by rapid amplification of cDNA end. The amount of 3.4-kb LPL mRNA was estimated by difference. Likewise, the amount of cyclophilin mRNA was assayed in AT and CM, to provide an internal control.

The 3.8-kb LPL mRNA was predominantly expressed in CM (56 %) but not in AT (39 %), suggesting a tissue-specific expression of LPL mRNA in ewe, as noticed in human tissues (Ranganathan *et al.* 1995). On the other hand, refeeding increased the ratio 3.8-kb LPL : cyclophilin mRNA both in AT (+583 %, $P < 0.01$) and in CM (+92 %, $P < 0.09$). Furthermore, variations with refeeding of the 3.4-kb LPL mRNA were similar to those of the 3.8-kb LPL mRNA, in AT as well as in CM, suggesting the lack of preferential regulation by refeeding for one of these two LPL mRNA species. Refeeding significantly increased LPL activity (expressed per gram of tissue) in AT (+288 %; $P < 0.001$) and, to a lesser extent, in CM (+34 %, $P < 0.001$). Thus, in contrast to observations made in the rat (Ong *et al.* 1994), but in agreement with our previous results in the ewe (Bonnet *et al.* 1998), refeeding up-regulated ovine LPL mRNA and activity both in AT and in CM.

These data reveal a tissue-specific differential expression pattern of the ovine LPL gene, and a nutritional regulation of its expression which is achieved in the same way in perirenal AT and CM (in contrast to the usual responses recorded in the rat). This occurs, at least partly, at a pretranslational level both for 3.4- and for 3.8-kb LPL mRNA.

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Pre- and postprandial changes in plasma leptin and insulin concentrations during underfeeding and refeeding in dry cows. By C. DELAVAUD, Y. FAULCONNIER, F. BOCQUIER and Y. CHILLIARD, *Laboratoire Sous-Nutrition des Ruminants, INRA, Theix, 63122 Saint-Genès Champanelle, France*

Leptin, a hormone produced by adipocytes, acts centrally on the regulation of body weight homeostasis through the control of appetite and energy expenditure. Conversely, fluctuations of energy supply, either short (h) or long term (weeks; Considine *et al.* 1996) may alter plasma leptin level. We analysed such effects of energy intake in nine dry, non-pregnant adult Holstein cows. Initially all animals were fed on a diet providing 130 % of maintenance energy requirements (MER) for 4 weeks and were thereafter underfed to 21 % of MER for 7 d. Four of these cows were then slaughtered, while the remaining cows (*n* 5) were refed to 237 % of MER for 21 d before slaughter. Jugular blood samples were drawn, before (at 09.00 hours) and after (at 14.00 hours) feeding time (10.00 hours), at the end of each experimental period. Plasma leptin and insulin levels were measured by radioimmunoassay (multi-species kit, Linco Research and INSI-PR kit, Oris Industry, respectively). Effects of treatments were evaluated by intra-animal changes as indicated in the Table.

	Initial (*n* 9)		Underfed - initial (*n* 9)		Refed - underfed (*n* 5)	
Sampling hour	09.00	14.00	09.00	14.00	09.00	14.00
Leptin (ng/ml)	2.43	1.97††	- 0.38**	- 0.14	+ 1.25**	+ 0.70*
Insulin (μIU/ml)	10.9	26.7††	- 2.30*	- 17.8††**	+ 1.65	+ 6.54†*

Mean values were significantly different from zero: * $P<0.05$, ** $P<0.01$.
Mean values were significantly different from the corresponding 09.00 hours values: $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$.

Preprandial (09.00 hours) plasma concentration of leptin was higher than (+23 %; Table), and positively related to, the postprandial (14.00 hours) value (r +0.84, n 23). Postprandial leptin level was less affected by underfeeding and refeeding (-7 and +47 % respectively) than preprandial level (-16 and +74 % respectively). However, both at 09.00 and 14.00 hours, leptinaemia rebounded above its initial values after the large refeeding of cows (237 % MER). As expected, plasma insulin level was altered by sampling hour and by feeding level (Table) but was, surprisingly, unrelated to leptin concentration (r 0.09, n 23). Furthermore, there was no significant correlation between absolute values or changes in plasma leptin and glucose (results not shown).

In the cow, plasma leptin is affected by changes in the feeding level, in agreement with recent observations in the ewe (Bonnet *et al.* 1997). No relationship was found between insulin and leptin levels, contrary to observations in human subjects (Havel *et al.* 1996). The significance of the postprandial decrease in plasma leptin concentration, observed 4 h after feeding the cow, remains to be elucidated.

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Nutritional regulation of lipoprotein lipase activity in bovine adipose tissues and muscles. By Y. FAULCONNIER, M. BONNET, J. FLECHET, F. BOCQUIER and Y. CHILLIARD, *Laboratoire Sous-Nutrition des Ruminants, INRA, Theix, 63122 St-Genès-Champanelle, France*

It is of interest to understand better the regulation of nutrient partitioning between adipose tissues (AT) and muscles in order to control the carcass lean:fat ratio. The utilization of circulating triacylglycerol (TG) by these tissues is regulated, at least partly, by lipoprotein lipase (*EC* 3.1.1.34; LPL) activity. This activity was measured (Faulconnier *et al.* 1994) in perirenal AT (PAT), subcutaneous AT (SAT), cardiac muscle (CM) and *Longissimus thoracis* muscle (LTM) of thirteen dry, non-pregnant adult cows. The control group (group C, *n* 4) was slaughtered after 4 weeks on a diet providing 130 % of maintenance energy requirements (MER). The other nine cows received this diet for 4 weeks and then were underfed to 21 % of MER for 7 d. Half the animals were slaughtered (group U, *n* 4), while the remaining were refed (237 % of MER; group R, *n* 5) for 21 d before slaughter.

Groups	PAT LPL *	SAT LPL *	CM LPL +	LTM LPL +
Control	241[a]	120[a]	108[a]	22[a]
Underfed	71[b]	72[a]	36[b]	20[a]
Refed	464[c]	223[b]	126[a]	31[b]

[a, b, c] Values within a column bearing unlike superscripts were significantly different, $P < 0.05$.
* nmol/min per 10^6 adipocytes.
+ nmol/min per mg DNA.

LPL activity was higher in PAT than in SAT, and in CM than in LTM (Table). The nutritional status significantly changed LPL activity in the four tissues. Underfeeding (U *v.* C) sharply decreased LPL activity in PAT (-71 %), CM (-67 %) and to a lesser extent in SAT (-40 %), but had no effect in LTM. Moreover, refeeding (R *v.* U) increased LPL activity by factors of 6.5, 3.0, 3.5 and 1.6 in PAT, SAT, CM and LTM respectively, to achieve levels significantly higher (PAT, SAT, LTM) or close (CM) to values measured in control cows. Similar trends were observed when the LPL activity was expressed either by gram of tissue or by protein. Hence, it appears that LPL activity of the internal AT (PAT) and the oxidative muscle (CM) is more affected by nutritional status than the activity of the external AT (SAT) and the glycolytic muscle (LTM).

This study shows that change in feeding level in cows alters AT and muscle LPL activities in the same way. These results are in agreement with previous observations in ewe PAT and CM (Bonnet *et al.* 1998), but they differ from observations in the rat showing that 6 - 12 h starvation increased or decreased LPL activity in rat muscles and AT respectively (Sugden *et al.* 1993). The differences in regulation of muscle LPL between ruminants and rat are probably linked to species differences in nutrient digestion and absorption, and liver lipogenesis.

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