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National Community Nutrition Project in Senegal: changes in mothers' knowledge, attitudes and practices. C Méjean, A Gartner, Y Kameli, P Traissac, B Maire (Nutrition Unit, UR 106 – IRD Montpellier, France).

One of the main activities of the Community Nutrition Project in Senegal was to provide weekly nutrition education for groups of mothers during six-month periods. We studied the impact of this project on the changes in knowledge, attitudes and practices (KAP) of mothers from the city of St Louis about feeding their children (0–24 months). Changes over time were compared between two areas: the project target area (n = 150), and a neighbouring area not targeted at the start by the project (n = 150). Three representative cross-sectional surveys were carried out, in 1996 (baseline), 1998 and 2001. The answers to an open questions interview were coded (QSR NUD\*IST®) and quantitatively analysed using regression models. In both areas, knowledge and attitudes towards the use of colostrum improved for the first 2 years but failed to progress beyond, though practices continued to improve. Knowledge about exclusive breastfeeding also improved during the first 2 years particularly in the target area, but subsequently diminished; and, despite a continuing improvement in favourable attitudes towards exclusive breast-feeding, most mothers still have not adopted this recommended practice. Knowledge about weaning, at last, again diminished in the target area after 2 years; however, the age of complete weaning is approaching the recommended age (24 months). In definitive, no differences were found between the two areas in the way KAP changed. However, the comparison between the areas does not accurately reflect the actual exposure to the project given the fact that the project was spread to some extent beyond the limits of the target area. Changes in knowledge and attitudes were generally favourable during the first 2-year period, but the project did not succeed either in reinforcing knowledge further, or in improving all practices. It is consequently doubtful whether it will have a longterm impact in a social context characterised by well-anchored traditions. Renewed education methods should be searched for in order to significantly to improve mothers' practices on the long term.

Medi-RIVAGE study: Results after 3 months follow up. S Vincent<sup>a</sup>, M Gerber<sup>b</sup>, C Defoort<sup>c</sup>, R Planells<sup>a</sup>, MC Bernard<sup>d</sup>, P Vague<sup>d</sup>, D Lairon<sup>a</sup> (<sup>a</sup> INSERM U476, Faculté de médecine Timone, Marseille, France; <sup>b</sup> CRLC Montpellier, France; <sup>c</sup> Faculté de Pharmacie, Marseille, France; <sup>d</sup> CDPA, CHU Timone, Marseille, France).

The RIVAGE study is a diet intervention in which we compared a Mediterranean-type diet (MED) with the usual prescribed diet (CDPA/ AHA) in volunteers who already had one or more cardiovascular risk factors. The benefits of the diets were estimated by clinical evaluation, lipid parameters quantification and postprandial investigation. Gene polymorphisms of proteins associated to lipid metabolism or homeostasis were determined for each subject. After 3 months, 169 subjects (88 MED and 81 CDPA/AHA) were back for clinical and biochemical examination. Plasma concentrations of total cholesterol and LDL cholesterol significantly decreased (P < 0.05) by 7.4% and 9.9% respectively in the MED arm, and by 4.4% and 5.4% respectively (P < 0.05) in the CDPA/AHA arm. We did not observe any variation in HDL cholesterol. Fasting plasma TG significantly decreased in the MED arm (-13.0%; P < 0.05), and non significantly decreased in the CDPA/AHA arm (-7.9%); P = 0.1). During the postprandial state, after a standardized test meal, the area under the curve of TG decreased by 16.7% (NS) in the MED arm and by 9.1% (NS) in the CDPA/AHA arm. The area under the curve of apo-B48 significantly decreased by 65.6% in the MED arm and by 54.0% in the CDPA/AHA arm. The effects of apo-E and MTP polymorphisms on TG and cholesterol changes were also shown. In conclusion, both diets significantly improve biochemical parameters. The amplitude of the beneficial changes was less marked when changing for a prudent low-fat, low-cholesterol diet (CDPA/ AHA). Furthermore the MED diet is less restrictive (total lipid can reach 38% of total energy intake) and thus easier to follow.

Malnutrition, overweight and obesity in 425 General Medicine adult patients in the French region of Limousin. A Féblot<sup>a</sup>, JC Desport<sup>b,c</sup>, PM Preux<sup>c,d,e</sup>, H Belghiti<sup>b,c</sup>, F Burbaud<sup>a</sup>, P Duchez<sup>f</sup>, B Hervy<sup>f</sup>, D Buchon<sup>e,f</sup> (<sup>a</sup> ORS Limousin, Limoges, France; <sup>b</sup> Nutrition

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Twenty to fifty per cent hospitalized patients are malnourished, and 10–15% of the French population is concerned by obesity. The aim of the study was to evaluate the nutritional status of General Medicine patients in the French Haute-Vienne district. Adult patients of 3 General Practicionners (2 urban and 1 rural) were assessed during 6 weeks. Socioprofessional and nutritional (weight, weight variation, BMI) data were noted. Depressive status and autonomy score were assessed. Malnutrition was present if BMI was < 18.5 (age between 18-64.9 yrs), and if BMI was < 20 (age 65 yrs or more), or if the weight loss was > 10% in the last 6 months. Overweight or obesity was defined by the BMI (overweight if BMI was 25-29.9, obesity if BMI was > 30). Four hundred and twenty five patients were evaluated (sex ratio: 0.95, age: 55.3 ± 19.3 yrs, weight:  $69.3 \pm 14.7$  kg, weight variation:  $-0.2 \pm 2.7$  kg, BMI:  $25.4 \pm 4.8$ ). The patients (49%) were malnourished. In multivariate analysis, a bad depressive status multiplies the risk of malnutrition by 10 (CI: 1.3-74.5). An overweight was present in 32.9% of the patients. They were older than healthy patients (59.5  $\pm$ 17.6 vs.  $50.4 \pm 20.2$  yrs P < 0.0001), and there were professional differences: 54.2% of pensioners are overweight, 44.0% of intermediary professions and 38.5% of the class "others", vs. 22.0% of managers and 0% of craftmen. The obese concerned 15.5% of the patients. Obesity was only linked with age  $(61.5 \pm 12.8 \text{ vs. } 50.4 \pm$ 20.2 yrs P < 0.0001). In conclusion, there was a low percentage of malnutrition in the General Medicine population explored. A bad depressive status is a risk factor of malnutrition. Overweight and obesity are often encountered, linked with age, and for overweight also with profession: pensioners are a population at-risk. These results could guide public health actions.

Specific predictive equations for the estimation of body composition in African women using impedancemetry. A Gartner<sup>a</sup>, A Dioum<sup>b</sup>, B Maire<sup>a</sup>, F Delpeuch<sup>a</sup>, Y Schutz<sup>c</sup> (<sup>a</sup> Nutrition Unit, UR 106 (WHO Collaborating Centre for Nutrition), IRD (Institut de Recherche pour le

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In the developing world the nutritional status of women is a major concern. Simple methods are crucial for the measurement of body composition; however these indirect methods require preliminary validation. The objective of this study was to develop predictive equations to estimate lean body mass (LBM) and percentage body fat (%BF) in African women using impedancemetry. In a sample group of 170 Senegalese women living in Dakar (age = 30.6 (s.d. 8.7), weight = 63.4 kg (s.d.15.2); BMI 23.4 kg/m<sup>2</sup> (s.d. 5.2)), LBM and%BF measured by hand-tohand (Omron<sup>TM</sup>) or foot-to-foot (Bodymaster<sup>TM</sup>) impedancemetry were compared with reference measurements made by air displacement plethysmography. Predictions were established by linear regression, step by step, in a sub group (n = 112), validated in a cross-validation set (n = 58) then re-applied to the whole group (n = 170). With the standard algorithm supplied with the device, LBM was overestimated by respectively 5.8 kg and 2.4 kg, and %BF was underestimated by 8.8% and 3.5% using handto-hand and foot-to-foot impedancemetry, which justifies the use of new specific equations. It was not necessary to introduce anthropometrical parameters as independent variables in addition to estimates based on impedancemetry. The new equations predicted LBM with a precision to within 2.1 kg ( $R^2 = 0.84$ ; CV = 5.2%), and 2.6 kg ( $R^2 =$ 0.74; CV = 6.5%) respectively. %BF was predicted with a precision to within 3.6% ( $R^2 = 0.85$ ; CV = 10.3%) and 4.2% ( $R^2 = 0.80$ ; CV = 12.0%). The impedancemetry equations derived from Caucasian populations are not valid (marked bias) for the measurement of body composition in African women. After correction for the bias by specific equations, impedancemetry can be used for African populations with reasonable accuracy.

Homocysteinemia in 274 subjects with dyslipidemia. JM Lecerf<sup>a,b</sup>, F Allemon Coisne<sup>b</sup>

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Hyperhomocysteinemia is a new cardiovascular risk factor, but actually there is no clinical trial which could confirm that. Dyslipidemia are frequent atherogenic diseases. Few data are available on homocysteinemia in dyslipidemia. Homocysteinemia, plasma and erythrocyte concentrations of folate, lipid parameters, risk factors, clinical data and 7 days dietary record were evaluated in 274 subjects with dyslipidemia. Homocysteinemia is positively associated with age (P < 0.0001), with body mass index (P =0.0375), with triglycerides (P = 0.002) and negatively with vitamin B6 nutritional intake (P =0.04) and with plasma folate. Homocysteinemia is statistically higher in subjects with atherosclerosis (P = 0.008), with combined dyslipidemia (P = 0.003), with renal insuffisiency (P < 0.001)and in subjects with fibrate treatment. In multivariate analysis, only plasma folate is inversely correlated with homocysteinemia (OR 0.59 IC 95%: 0.46–0.74). The administration of 5 mg of folic acid in 52 subjects induces a significant decrease of homocysteinemia (P < 0.0001). In conclusion, this study shows that homocysteinemia should be taken into consideration in the investigation of dyslipidemia, and that it is influenced by metabolic, nutritional factors and by therapeutic agents; furthermore these results indicate that high plasma homocystein level is perhaps an important cardiovascular risk factor in this population.

Favourable association of breakfast with adiposity in adolescents. C Klein-Platat, JL Schlienger, D Arveiler, B Schweitzer, C Simon (Groupe d'Études en Nutrition, ULP 67085 Strasbourg, France).

Some studies suggest an inversed association between breakfast and overweight. However, since breakfast is often associated with a healthier diet and other beneficial health behaviour, the nature of this association remains unclear. This study was aimed at specifying the relation of breakfast with adiposity in adolescents from the sample of the ICAPS intervention study (Intervention Centered on Adolescents' Physical activity and Sedentarity). Analysis of ICAPS cross-

sectional data, which were collected in 2002, in sixth grade adolescents (n = 607), aged 11.5 years  $\pm$ 0.6 (m ± SD) old, from 8 drawn lot schools in the department of the Bas-Rhin. A food frequency questionnaire was used to collect food data. The percentage of fat mass (MG,  $m \pm SE$ ) was evaluated by impedancemetry (Tanita TBF-310) and the plasmatic leptin level (L, m  $\pm$  SE) was measured by RIA (DSL). Socio-economic status (SES) was estimated by the familial annual income tax. Data were analysed by T-tests and general linear models. Almost 15% of the subjects did not have any regular breakfast. Breakfast was associated with a higher consumption of vogurt, soft cheese, milk desserts (P = 0.02). milk (P = 0.01), reduced nibblings in front of the TV (P = 0.01), and after dinner ( $P < 10^{-3}$ ), with a lower MG (20.6  $\pm$  0.9% vs. 18.0  $\pm$  0.3%, P < $10^{-2}$ ) and a lower L (20.1 ± 1.5 ng·mL<sup>-1</sup> vs.  $14.7 \pm 0.6 \text{ ng} \cdot \text{mL}^{-1}$ ,  $P < 10^{-2}$ ). The relation of breakfast with MG or L was maintained after adjustment of different diet characteristics, physical activity, sedentarity and SES. In conclusion, breakfast is associated with a reduced adiposity in adolescents, independently of other health behaviours and diet characterisitics. The mecanisms remain, however, to be precised.

Body composition estimation using multifrequency Bio Impedance Analysis in children. C Michela, S Petitb, S Meeuwsenc, R Hankarda (a Inserm CIC 9202, Paris, France; b Antenne RePOP-IdF, Hôpital Robert Debré, Paris, France; c Body Stat Ltd, Isle of Man, UK).

Monofrequency-Bio-Impedance Analysis (BIA) is a simple and inoffensive method to estimate body composition. Multifrequency BIA (MFBIA) also provides estimates of body fluid spaces (intra and extra-cellular water). We compared resistance (R), reactance (Xc) and phase angle (PA) measured at 50kHz using a monofrequency BIA (BIA 101Q, RJL, USA) and calculated from impedance (Z) using a multifrequency (5, 50, 100, 200 kHz) BIA (Quadscan, Bodystat, UK). The measurements were performed the same day using a standardised procedure and without removing skin surface electrodes. Fat-free mass (FFM) was calculated using the HoutKooper equation and comparisons were performed according to Bland and Altman and using paired t-tests. Confidence (95%) interval is the mean of the difference between methods  $\pm 2$  SEM with ddl = 181. The measurements (182) were analysed (age < 8 y: n = 42, age < 12 y: n = 49, age < 16 y: n = 40, age < 1624, age > 16 y: n = 67). Mean differences between methods were  $-5.2 \Omega$  (95%CI: -7.0, + 3.4 $\Omega$ ), -2.9  $\Omega$  (95%CI: -4.2, -1.6 $\Omega$ ), -0.2° (95%CI: -1.4, +1.1), and +155 g ((95%CI: +93, -1.4))+ 217g) respectively for R, Xc, PA and FFM, all P < 0.01. In addition, the Z 5/200 ratio decreased significantly with age  $(Z5/Z200 = 0.85 - 0.04 \times$ age,  $r^2 = 32\%$ , P < 0.01). The differences between the two methods were not clinically significant. However, it is recommended to use the same method for clinical follow-up. The Z5/200 ratio might provide an index of whole body fluid compartments. Specific equations using MFBIA need to be validated in children for body composition as well as for fluid spaces.

Interest of measuring the muscle strength to assess the nutritional status in elderly patients. D Quinart, V Bige, E Bertin (Résidence des Capucins, CHU de Reims, France).

The means of assessing nutritional status are often unreliable in elderly subjects. Measuring muscle strength (MS) with a hand dynamometer represents an interesting approach, which has never been studied in elderly people. The aims of the present work were to test the accuracy of this technique, to precise its conditions of use and to check the relationship between the MS data and the Mini Nutritional Assessment (MNA) score. Seventy-six subjects from two private nursing homes for elderly people (57 females and 19 males; mean age  $\pm$  SD: 84.9  $\pm$  8.2 years; BMI:  $23.3 \pm 4.8 \text{ kg/m}^2$ ) were tested. Cognitive impairment was detected in 46% of the subjects. Malnutrition was present (MNA score < 17) or might happen (MNA score < 23.5) in 57% of the subjects. MS was measured at each arm, by using a baseline hydraulic hand dynamometer (Baseline, INC, Irvington, USA), with 3 consecutive handgrips at 10 s intervals (the forearm in a 90° flexion position on the arm). A second measurement was made two days later to calculate the coefficient of variation (CV), and then the accuracy of the technique. We found that the maximal MS (MMS) (the highest value from the 3 consecutive handgrip tests) did not significantly differ between the dominant and the nondominant arm  $(19.1 \pm 7.3 \text{ vs. } 18.4 \pm 6.4 \text{ kg})$ ; the MMS was obtained in 95% of the subjects after two handgrip tests (no impact of cognitive impairment); the CV for the MMS, at a 48 h interval, was  $1.7 \pm 2.3\%$ . MMS was significantly higher in men than in women and decreased with age in both sexes. It was positively correlated to the MNA score (r = 0.42; P < 0.001), this relationship being independent of age, sex and BMI. Therefore, the MS appears to be a simple, accurate, and usable technique in every elderly "reacting" person, and it should be more widely assessed in malnutrition detection.

Inefficiency of nutritional intervention in 82 adult patients in General Medicine in the French region of Limousin. A Féblot<sup>a</sup>, JC Desport<sup>b,c</sup>, PM Preux<sup>d,e,c</sup>, H Belghiti<sup>b,c</sup>, F Burbaud<sup>a</sup>, P Duchez<sup>f</sup>, B Hervy<sup>d</sup>, D Buchon<sup>e,f</sup> (<sup>a</sup> ORS Limousin, Limoges, France; <sup>b</sup> Nutrition Unit, Limoges, France; <sup>c</sup> EA 3174, Limoges, France; <sup>d</sup> Biostatistics, Limoges, France; <sup>e</sup> Medicine Faculty, Limoges, France; <sup>f</sup> General Practicionner, Limoges, France).

Nutritional problems are frequent in General Medicine. The intervention of a dietician is rarely possible, and General Practicionners (GP) have a low level of education in nutrition. The aim of the study was to evaluate the nutritional interventions of educated GP. Three GP (two urban and one rural) were first informed on malnutrition and obesity, then all their adult patients were followed during five months. Nutritional data (weight, weight variation, BMI, number of meals, dental diseases, type of diet) were noted at the 1st (V1) and 2nd visit (V2). Depressive status and autonomy score were assessed. Malnutrition was present if BMI < 18.5 (age 18-65 yrs), or < 20 (age > 65) or if weight loss is > 10%. BMI 25–30 defined overweight and > 30 obesity. At V1, a special diet, oral energetic supplements, enteral or parenteral nutrition could be prescribed. Albuminemia, transthyretinemia, CRP, thyroid hormonemia and dosage of urine albumin were proposed for malunutrition. Statistical analysis used the t test and Kruskal-Wallis test. Eighty-two patients were evaluated (sex ratio: 0.82, age:  $59.5 \pm 17.8$  yrs, weight:  $70.3 \pm 15.8$  kg, BMI:  $25.9 \pm 5.2$ ). Five per cent were malnourished, 34.2% overweight and 17.7% obese. Eight-and-a-half per cent were

depressed and 1.2% dependent. All malnourished patients and one obese (6.1%) had dietetic advices, 2.4% had oral energetic supplementation, and only one patient was biologically explored. No parenteral or enteral nutrition was prescribed. During the period V1-V2 (55.0 ± 31.9 days), there was no statistical variation of the data analysed (P > 0.05). In conclusion, although ≈ 50% of their patients are obese or overweight, and 5% malnourished, the nutritional interventions and biological examinations prescribed by the GP are scarce, and their efficiency is not adequate. When considering the possible consequences of malnutrition and the increasing incidence of obesity, nutritional information and actions of the GP have to be developed.

Relevancy of a computerised system of nutritional evaluation in a children's hospital. A LePort<sup>a</sup>, C Alberti<sup>b</sup>, S Petit<sup>c</sup>, JP Cézard<sup>d</sup>, R Hankard<sup>a</sup> (<sup>a</sup>CLAN, Paris, France; <sup>b</sup> Inserm CIC-EC, Paris, France; <sup>c</sup> Antenne RePOP-IdF, Paris, France; <sup>d</sup> Gastro-Entérologie-Mucoviscidose et Nutrition Pédiatriques, Hôpital Robert Debré, Paris, France).

We developed a computerised system evaluating the weight for age ratio for every child hospitalised in a children's hospital (medicine and surgery). However weight for age ratio does not take into account height. We evaluated the relevancy of this detection system over a two months period (May to June 2003). Fifty-one evaluations out of 106 children presenting W/A > +3SD or <-3 SD were checked by a trained student (physical exam and weight and height measurement) in five participating units (bone and visceral surgery, Paediatrics, Paediatric Nephrology and GastroEnterology). Fifty-five evaluations were excluded because of incomplete data (n =32), duplicated data (n = 20) or W/A index out of the range of the system (n = 3). Obesity was detected in 40/51 children (78%) and malnutrition in 11/51 children (22%). Obesity (according to the International Obesity Task Force criteria) was confirmed in 23/40 detected children and malnutrition (weight for height ratio < -2 SD) in 4/11 detected children. There were 16 error sin weight value data entries (15/24 evaluations that were not confirmed). Removing these errors,

obesity was confirmed in 22/28 detected children, and malnutrition in 4/7 children. The relevancy of such a system depends closely on the quality of data entry. The calculation of an index including height improves the detection, however, it also adds another source of error. The involvement of health professionals is mandatory for the overall relevancy of a systematic computerised evaluation system.

The management of nutritional care: a comparative analysis in hospital food and nutrition services both in Brazil and France. AA Sousa, RPC Proença (Departamento de Nutrição, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil).

This study had as its proposal a comparative analysis, with the support of anthropotechnology, of the work of the dietician/nutritionist and the management of nutritional care in hospital food and nutrition services both in Brazil and France. The research was carried out in two hospitals, one in each country. Data was obtained from documents, designed interviews and direct observation. The results suggest that the working conditions of the professionals focused on herein were directly affected by the characteristics of the operational environment: the background of the workers, the individual characteristics, the characteristics of the organisation in the hospital and the social, demographic and industrial contexts. In France, health professionals are assisted by higher investments and a better organization of the health system, resulting in improved conditions for the management of population ageing, changes in eating behaviour, low worker background, treatment of chronic non-transmissible diseases, and home hospitalisation. The educational background of dieticians is not easily recognized in the health area. A comparatively more challenging scenario is encountered in Brazil due to narrow investments in the health system, lack of education, limited access to consumption, deficient worker background, changes in eating behaviour and population ageing. However, the developing industrial context of the country in association with qualified background and working experience are positive aspects in favour of nutritionists.

The work of the dietician/nutritionist as a promotional agent of health in food service. RPC Proença<sup>a</sup>, MB Veiros<sup>b</sup> (<sup>a</sup> Departamento de Nutrição, Universidade Federal de Santa Catarina, 88040-900, Florianópolis – SC, Brazil; <sup>b</sup> Curso de Nutrição, UNIVALI, Balneário Camboriú – SC, Brazil).

This study analyzed the conditions of work of the dietician/nutritionist as a promotional agent of health in a food service unit in Brazil that produces around 7000 meals per day. The methodology consisted of the analysis of documents, interviews and direct comments, with the conditions of work of two dieticians being analyzed: one acting in management, with administrative activities concerning the general management of the unit, and the other acting in the sector of production, developing activities related to the supervision of the productive flow. The results concerning the Physical Conditions, show unsuitable conditions for acoustics (noise), luminosity and humidity. The nutritionists working equipment shows problems related to the computer and software used in the management of the unit (restaurant). The organization conditions include the management of the nutritionist, hygienicsanitary quality and process. In the Management of the Nutritional Quality, some points in the elaboration of the menu and the way foods are prepared. The analysis of the hygienic-sanitary question and process disclosed imperfections in the reception, storage and preparation of foods, that can be improved thus assuring a bigger food security of the meals. From the conditions analyzed, the performance of the nutritionist as the promotional agent of health in this unit was verified and was shown not to be effective in all professional aspects. Adoptions of strategies are recommended that produce organization changes in the management of the nutritional and hygienic-sanitary qualities, thus allowing more complete performance.

A review on dietician practices on a specific diet (S.D.) recommended for cancer patients with diarrhea during treatment with chemotherapy and/or radiotherapy. V Masdoua (Dietician-Master, Limayrac Institute, Toulouse, France).

This study assessed current dietician practices to define one "referent consensual practice". A lit-

erature review was undertaken and a survey of current practices was conducted through a postal questionnaire to 71 centers: 20 French cancer centers, 15 university hospital centers with a specialised radiotherapy staff, 36 dieticians trained in oncology and members of the French Dietetic Association (ADLF). The questionnaire was composed of the following questions: Who are the patients concerned by S.D.? When does it start? When and how is it developped? Is the S.D. is effective? The choice of foods recommended and not recommended. Scientific reasons of restriction. Nineteen centers replied (26.7%). This study indicates 222 foods that are avoided or permitted in this S.D. to reduce diarrhea. Ten centers approve that lactose not be banned and 11 centers recommend 5 to 10 g per day in the S.D. The results revealed that 89% of the dieticians give banana and 68% avoid milk (whole, skim, half-skim) in this S.D. An additionnal board presents the reasons why the foods are admitted or not. S.D. is started when diarrhea is tolerable (level 3 OMS). S.D. is developped when bowel movement becomes regular (3 times or less·day<sup>-1</sup>) and or consistency becomes normal. A normal diet is not necessarily recommended at the end of cancer treatment, but when the consistency and the number of the bowels (e.g.  $1 \cdot day^{-1}$ ) is equal to the normal habits of the patient. To evaluate the efficiency of the S.D., the dietician records each day the information about the number and the consistency of the bowels. In conclusion, for patients, diarrhea may interrupt cancer treatment. To decrease gastrointestinal symptoms the dietician's role is proved in its prevention and treatment. It seems very important that preventive nutritionnal advice must be given in each center where abdominal-pelvien radiotherapy is practised. For the moment only 7 centers out of 19 (37%) give this advice.

Supplementation of a weaning diet with bovine colostrum improves the health of the small intestine of weaned piglets. A Huguet, C Perrier, C Favier, J Le Dividich, I Le Huërou-Luron (UMRVP-INRA, Rennes, France (French project "Porcherie Verte" and EC project "Healthypigut" QLKS-CT2000-00522)).

In the intestinal piglets, weaning induces structural, functional and microbial disorders (villous atrophy and enzyme activity depression) which

facilitate the implantation of pathogenic bacteria. Colostrum has the potential to induce intestinal mucosa development and to control bacterial colonization. In addition, colostral preparation can reduce upper intestinal gut injury in humans. At 21 d of age, twelve piglets were weaned and fed either a standard diet (C group) or a standard diet supplemented with bovine colostrum (5% of dry matter, Col group) for 7 days. A third group of six piglets were sow's suckled up to 28 d of age (S group). The piglets were euthanazied at 28 d of age. The proximal small intestine was collected for histological analysis and brushborder enzyme activity analysis. Duodenal content was collected to count total aerobia flora. lactobacilli and coliforms. Data were analysed by variance analysis using the GLM procedure of SAS. Compared to the C group, supplementation with bovine colostrum enhanced the intestinal weight (33.6 vs. 31.4 g·k<sup>-1</sup> BW; P < 0.05). In the colostrum-treated piglets (Col group), villous height was 19% higher (P = 0.08) than in the weaned C group, but was 22% lower (P <0.05) than in the suckled pigs (S group). In weaned pigs, colostrum supplementation may prevent proliferation of pathogenic bacteria, as illustrated by the 17% decrease of coliform counts in the Col group compared to the C group (P < 0.05). No difference was observed in lactobacilli counts between the C and Col groups. The functional properties of the small intestinal mucosa did not seem to be affected by the colostrum supplementation. Indeed, lactase, maltase and aminopeptidase N activities, indicators of the digestive maturation of the small intestine, were not significantly different between the S, C and Col groups. In conclusion, supplementation of a weaning diet with a bovine colostrum may improve gut health around weaning in pigs.

Effect of oral supplementation with 13-cis retinoic acid on the kinetic activation of lymphocytes: a comparison of young and elderly subjects. G Mayot<sup>a</sup>, R Minet-Quinard<sup>a</sup>, E Thivat<sup>a</sup>, MC Farges<sup>a</sup>, J Ribalta<sup>b</sup>, B Winklhofer-Roob<sup>b</sup>, E Rock<sup>b</sup>, MP Vasson<sup>a,b</sup> (<sup>a</sup> LB2MN, Pharmacy School, Clermont-Ferrand, France; <sup>b</sup> European Vitage group, Austria, Spain, France).

Lymphocyte activation constitutes a key-stage in the immune response to aggression which is frequently altered in malnourished elderly subjects. Vitamin A and its metabolites i.e. retinoic acid, exert an in vitro stimulating effect on lymphocyte activation induced by various agents. The objective of this study was to define the effect of a nutritional supplementation with 13-cis retinoic acid (13cRA) on the kinetic activation of lymphocytes according to the age of the subjects. Lymphocytes were isolated from the peripheral blood of young (22  $\pm$  2 years, n = 6) and elderly  $(64 \pm 3 \text{ years}, n = 7)$  healthy subjects before and after an oral supplementation with 13cRA (0.5 mg·kg<sup>-1</sup>·day<sup>-1</sup>) during 28 days. Cells were incubated (10<sup>6</sup>⋅mL<sup>-1</sup>) with phytohaemagglutinin A (PHA) (5 μg·mL<sup>-1</sup>) during 6, 15, 24, 48 and 96 h. The percentage of stimulated lymphocytes expressing the CD25 receptor, well known as a marker of activation, was evaluated by flow cytometric analysis. Statistical analysis (mean  $\pm$  SEM) was performed by the ANOVA test followed by the Student-Newman-Keüls a posteriori test. The oral supplementation induced an increase in the percentage of CD25+ lymphocytes in young subjects at T48 (37  $\pm$  3% vs. No Supplemented (NS):  $21 \pm 5\%$ ,  $P \le 0.05$ ) and at T96 (45  $\pm$  5% vs. NS : 29  $\pm$  5%,  $P \le 0.05$ ). A similar effect was found in elderly subjects at T24 (26 ± 1% vs. NS :  $16 \pm 2\%$ ,  $P \le 0.05$ ), T48  $(38 \pm 3\% \text{ vs. NS} : 17 \pm 3\%, P \le 0.05)$  and T96  $(50 \pm 4\% \text{ vs. NS} : 33 \pm 4\%, P \le 0.05)$ . In conclusion, oral supplementation with 13cRA potentiates PHA-induced lymphocyte activation. This effect occured earlier (as soon as T24) in elderly subjects than in younger ones. These data suggest a benefice effect of RA supply particularly in immunodeficiency and consequently requires to be confirmed in immunodepressed elderly subjects.

Effects of the association hypertension/type 2 diabetes on polyunsaturated fatty acid biosynthesis. C Comte, S Bellenger, J Bellenger, C Tessier, J-P Poisson, M Narce (UPRES Lipides et Nutrition, Faculté des Sciences Gabriel, Dijon, France).

Polyunsaturated fatty acids (PUFA) are implied in various biological functions. Their biosynthesis is disturbed during hypertension and type 2 diabetes, because of interactions between lipid and glucid metabolisms. Since hypertension and type 2 diabetes are closely related, we generated an animal model showing these two pathologies,

in order to study the repercussions on PUFA biosynthesis. This model was obtained by a neonatal streptozotocin injection into spontaneously hypertensive rats (SHR). After installation of hypertension, fatty acid composition of total lipids, Δ6 desaturase activities (rate limiting-step of PUFA biosynthesis) as well as  $\Delta 6$  desaturase gene expression were estimated in the liver. The results were expressed as means ± SEM, statistical significance was determined by Student ttest. Dihommo-y-linoleic acid level was increased in the Experimental group, when compared with the Control SHR group (in molar %: 0.94  $\pm$  0.06 vs.  $0.58 \pm 0.04$ ), whereas the arachidonic acid level tended to decrease. The eicosapentaenoic acid level was increased  $(0.46 \pm 0.02 \text{ vs. } 0.32 \pm$ 0.01), whereas the docosahexaenoic acid level tended to decrease. Even if  $\Delta 6$  desaturase activities were inhibited during hypertension, the  $\Delta 6$ n-3 desaturation showed a less marked inhibition, when no modification was observed in the Δ6 n-6 desaturation process. Concerning the Δ6 desaturase gene expression, no difference appeared between the two groups. To conclude, this study showed perturbations of PUFA biosynthesis, different from those previously reported during hypertension. A pharmaconutritional study is now planned, in order to correct lipid metabolism alterations when hypertension is associated with type 2 diabetes.

Medium term effects of a high protein diet on glucose homeostasis in the growing rat. C Blouet, F Mariotti, M Lacroix, V Mathé, D Tomé, JF Huneau (UMR 914 de Physiologie de la Nutrition et du Comportement Alimentaire, INRA/INA-PG, Paris, France).

The consequences of a high protein intake on glucose tolerance and insulin sensitivity remain controversial. The aim of this work was to investigate the medium term effect of a high protein intake on glucose homeostasis in growing rats. Wistar rats, weighing  $181.83 \pm 4.95$  g, were assigned to a 14% (NP, n = 8) or a 55% (HP, n = 16) milk protein diet for 7 weeks. Growth and food intake were monitored and body composition was determined post mortem. Oral glucose tolerance, based on plasma glucose clearance rate after a  $1 \text{ g-kg}^{-1}$  oral glucose load, was tested before the introduction of the diets and 4 weeks later. An intravenous hypoglycaemic test, con-

sisting of the measurement of glucose decay over 16 minutes after an i.v. bolus of insulin (250 mU⋅kg<sup>-1</sup>), was performed before euthanasia. All results are expressed as means  $\pm$  SD. Data were analysed using mixed models (SAS system). Mean food intake was lower in HP rats than in NP rats (295  $\pm$  31 kJ·day<sup>-1</sup> vs. 331  $\pm$ 28 kJ·day<sup>-1</sup>, P < 0.05). After 8 weeks, HP rats had lower body weights than NP rats  $(343 \pm 22 \text{ g})$ vs.  $354 \pm 22$  g, P < 0.05), together with a lower total fat mass  $(20.8 \pm 2.5 \text{ g vs. } 28.9 \pm 6.6 \text{ g}, P <$ 0.05), but did not differ in their fat free mass. After 4 weeks, glucose tolerance was improved with the HP diet (AUC:  $28.33 \pm 9.21$  g·min·L<sup>-1</sup> at baseline vs.  $20.06 \pm 9.5$  g·min·L<sup>-1</sup> after 4 weeks of the diet, P < 0.05) and largely higher as compared to the NP rats (AUC:  $20.06 \pm 9.5 \text{ g} \cdot \text{min} \cdot \text{L}^{-1}$ in HP vs.  $30.5 \pm 8.9$  g·min·L<sup>-1</sup> in NP,  $\vec{P} < 0.05$ ). The plasma glucose decay after the insulin bolus was similar between the two groups. Taken together, these data suggest that a high protein diet favours a better glucose homeostasis in rats.

Mitochondrial free radical release increases in human muscle during aging. F Capel, V Rimbert<sup>b</sup>, A Diot<sup>a</sup>, P Patureau Miranda<sup>b</sup>, Y Boirie<sup>b</sup>, B Morio<sup>b</sup>, L Mosoni<sup>a</sup> (<sup>a</sup> UNMP, Centre INRA de Theix, France; <sup>b</sup> UMPE, LNH, Universite d'Auvergne; CRNH Auvergne, France).

The loss of muscle proteins that occurs during aging could be partly related to an activation of proteolysis induced by an accumulation of oxidized proteins. The mitochondria is the main cellular source of free radicals: an increased production with age could thus play a role in sarcopenia. We measured this production in vastus lateralis biopsies obtained from post-absorptive healthy sedentary men aged 18-35 years (n = 5)and 65–85 years (n = 6). Subsarcolemmal and intermyofibrillar mitochondria were isolated by differential centrifugation. H<sub>2</sub>O<sub>2</sub> release was measured with glutamate (5 mM), succinate (5 mM) and malate (2.5 mM) as substrates, with or without rotenone. Mean values (± SD) were compared by the t test with P < 0.05. Muscle H<sub>2</sub>O<sub>2</sub> release increased significantly with age, from  $0.64 \pm 0.36 \text{ nmol·mg}^{-1} \cdot \text{min}^{-1}$  in young men to  $1.76 \pm 0.81 \text{ nmol·mg}^{-1} \cdot \text{min}^{-1}$  in elderly men. Rotenone, which inhibits normal or reverse electron flow between complexes I and III of the respiratory chain, did not induce a significant change in  $\rm H_2O_2$  release in young (0.39  $\pm$  0.52 nmol·mg<sup>-1</sup>·min<sup>-1</sup>) as in elderly (1.35  $\pm$  1.55 nmol·mg<sup>-1</sup>·min<sup>-1</sup>) men. Rotenone led to an increase in  $\rm H_2O_2$  release in half of the subjects, and to a decrease in the other half, whatever the age. However, the range of variations was significantly higher in elderly men than in young men (1.28  $\pm$  0.68 vs. 0.41  $\pm$  0.39 nmol·mg<sup>-1</sup>·min<sup>-1</sup>). In conclusion, the mitochondria seem to be responsible for an increase in oxidative stress during aging in human muscle, and thus could play a role in sarcopenia.

Interactions between energy intake level, physiological state and methodology for determination of plasma leptin in bovine species. C Delavaud<sup>a</sup>, S Agenäs<sup>b</sup>, K Holtenius<sup>b</sup>, Y Chilliard<sup>a</sup> (<sup>a</sup> INRA-Theix, 63122, France; <sup>b</sup> Swedish Univ. Agric. Sci., Uppsala, Sweeden).

In dry non-pregnant adult cows, leptinemia is closely linked to adiposity and modulated, at constant adiposity, by the energy intake level. These results were obtained by using either a "multispecies" commercial RIA (Linco, RIAm), or a specific RIA (RIAs, developed at INRA). However, leptin concentrations measured by RIAm were much lower than those measured by RIAs. Therefore, the study of more acute regulations requiring a better specificity could be biased when using the RIAm. In order to verify this hypothesis, we compared these two methodologies in a study aimed at determining the interactions between energy intake level and physiological state on plasma leptin in the peripartum cow. 24 cows were allocated into three groups fed the same total mixed ration at low (L, 71 MJ·d<sup>-1</sup>), medium (M, 106 MJ·d<sup>-1</sup>) or high (H, 177 MJ·d<sup>-1</sup>) levels of metabolisable energy, during late pregnancy (8 weeks). After calving, the cows were fed ad libitum. Plasma leptin was measured at -8, -3, +1, +3 and +12 weeks (W) relative to parturition, by using both RIAs and RIAm. RIAs plasma leptin increased between W -8 and -3 (from 4.45  $\pm$  0.28 to 5.77  $\pm$  $0.44 \text{ ng} \cdot \text{mL}^{-1}$ ) and the response was related to the energy intake level; it decreased thereafter at W +1  $(3.32 \pm 0.29 \text{ ng} \cdot \text{mL}^{-1})$  and remained low until W +12. However, RIAm plasma leptin was neither modulated between W -8 (3.72  $\pm$  $0.24 \text{ ng} \cdot \text{mL}^{-1}$ ) and  $-3 (3.79 \pm 0.34 \text{ ng} \cdot \text{mL}^{-1})$ , nor at W +1 (3.74  $\pm$  0.30 ng·mL<sup>-1</sup>). A positive

curvilinear correlation was obtained between values measured by RIAs and RIAm during pregnancy (r = +0.51), whereas this correlation was negative during lactation (r = -0.39, P < 0.01). It is concluded that plasma leptin depends on both the energy intake level and physiological state, and that the "multispecies" RIA does not allow showing these interactions in the *peripartum* cow.

Interaction between lidocaine and the study of mitochondrial metabolism in skeletal muscle. F Capel<sup>a</sup>, V Rimbert<sup>b</sup>, A Diot<sup>a</sup>, D Lioger<sup>b</sup>, B Morio<sup>b</sup>, L Mosoni<sup>a</sup> (<sup>a</sup> UNMP, INRA Theix, France; <sup>b</sup> UMPE, LNH Clermont-Ferrand; CRNH Auvergne, France).

The study of mitochondrial physiology in human skeletal muscle is possible using muscular biopsies, necessarily performed after local anesthesia with bupivacaine, dibucaine, ropivacaine or lidocaine. However, these pharmacological agents can affect mitochondrial respiratory chain activity (at the level of complex I) and generation of reactive oxygen species. The aim of this study was to evaluate the impact of lidocaine on mitochondrial hydrogen peroxide release and respiration with several combinations of substrates. Intramuscular injection of lidocaine (xylocaine®) was performed in the right gastrocnemius muscle in young rats after general anesthesia. The results were compared with data obtained with mitochondria isolated from the left gastrocnemius. Data were analyzed using variance analysis and the student t-test (differences were considered significant at P < 0.05). Mitochondrial generation of H<sub>2</sub>O<sub>2</sub> in the presence of 5 mM glutamate (G), 2.5 mM malate (M) and 5 mM succinate (S) was significantly reduced after lidocaine injection (-40%) and also when the complex I was inhibited by rotenone (-50%). However, variance analysis detected only a significant interaction between the effects of rotenone and lidocaine. Succinate supported mitochondrial state 4 respiration was significantly enhanced by lidocaine (+35%), but the addition of rotenone abolished this effect. In the presence of GM and GMS, lidocaine had no effect. In the state 3, the only effect of lidocaine was a tendency to reduce GM supported mitochondrial respiration (P = 0.06). In conclusion, although this model induced a marked contact of lidocaine with muscular tissue, there were only moderate effects of lidocaine on mitochondrial respiration and hydrogen peroxide release (interaction with rotenone effect). In particular, there was no effect of lidocaine on GMS supported mitochondrial metabolism. However, it seems still safer to minimize the contact of muscular tissue with lidocaine when mitochondrial parameters are evaluated in human studies.

Effect of polysaccharides on peanut allergy after in vitro hydrolysis. J Mouécoucoua, S Frémont<sup>b</sup>, C Sanchez<sup>c</sup>, C Villaume<sup>d</sup>, L Mejean<sup>a</sup> (a Laboratoire des Sciences Animales, ENSAIA-INPL, USC INRA, 2, avenue de la Forêt de Haye BP 172, 54505 Vandoeuvre-lès-Nancy Cedex 5, France; <sup>b</sup> Laboratoire de Biochimie Médicale et Pédiatrique, Faculté de Médecine, 54500 Vandoeuvre-lès-Nancy, France; <sup>c</sup> Laboratoire de Physico-Chimie et Génie Alimentaires, ENSAIA-INPL, USC INRA, 2, avenue de la Forêt de Haye, 54505 Vandoeuvre-lès-Nancy Cedex 5, France; d Laboratoire de Pathologie Cellulaire et Moléculaire en Nutrition, EMI 0014 INSERM and URM 20 IFREMER. Faculté de Médecine. 54500 Vandoeuvre-lès-Nancy, France).

Allergy to the peanut is increasing in industrialized countries. Manufacturing processes used in food industries to improve the physicochemical properties of food-based peanut (stabilization, texturing, etc.), could lead to a modification of the digestibility of peanut proteins and, consequently, their allergenicity. In this study, peanut protein isolate (PPI) was hydrolysed in the presence of gum arabic, low methylated pectin (LMP) and xylan during a two-step in vitro hydrolysis by pepsin, then by pepsin followed by a trypsin/chymotrypsin (T/C) mixture performed in dialysis bags with molecular weight cut-offs (MWCO) of 1000 or 8000 Da. The SDS-PAGE electrophoresis pattern revealed that PPI was poorly digested by pepsin. Hydrolysis by all enzymes showed retention of some proteins in dialysis bags in the presence of gum arabic and xylan. Immunoblot assessed with serum from patient sensitised to peanut showed that after peptic digestion, the IgE did not recognize proteins with MW 17 and 15 kDa. The PPI hydrolysis products released through the dialysis bags were not immuno-reactive, while the retentates

were recognized by IgE, particularly peptides with MW < 20 kDa. The IgE-binding with peptides of retentate containing xylan from the dialysis bag with MWCO 1 000 Da was weak. The reduction of PPI hydrolysis was probably due to non-specific interactions between polysaccharides and proteins or peptides. IgE-binding epitopes were reduced by all digestive enzymes and by the presence of xylan. This work points out the possibility to modulate food allergy by an optimized formulation.

Influence of hypertension and vitamin Cenriched diet on the circulating and tissue levels of oxidative stress. P Sicard, A Oudot, J-C Guilland, C Vergely, D Moreau, L Rochette (LPPCE, IFR100, Faculty of Medecine and pharmacy, Dijon, France).

Pathophysiological-hypertension appears to be associated with an enhanced oxidative stress (OS). The aim of this study was to appreciate the effect of a short-term vitamin C-supplemented diet on (1) cardiovascular function, and (2) parameters of OS, in normotensive and hypertensive rats. For this study, 40 spontaneously hypertensive rats (SHR), and 40 normotensive rats (WKY) (12 weeks of age) were used. Some animals received a 7-day vitamin C-enriched diet in drinking water. Hearts were isolated and perfused according to the Langendorff method and subjected to 30 min of total global ischemia followed by 30 min of reperfusion (R). OS indexes were evaluated in plasma and tissue. Statistical analysis was performed using twofactor fully factorial ANOVA followed by Tukey inter-group comparisons. During the nonischemic perfusion, functional parameters deteriorated in SHR as compared to WKY hearts. During the reperfusion period, SHR hearts presented a decreased coronary flow (-40%) associated with an enhanced post-ischemic contracture  $(60 \pm 5 \text{ mmHg WKY vs. } 90 \pm 6 \text{ mmHg SHR},$ P < 0.001). Vitamin C supplementation did not affect the evolution of functional parameters. Plasma antioxidant capacity was decreased in hypertensive rats but not changed by the vitamin C-enriched diet. The use of the spin probe (CPH) associated with Electron Spin Resonance spectroscopy demonstrated that OS was increased in SHR aortic tissue (+ 40% vs. WKY). Moreover, the vitamin C-enriched diet prevented this

effect. Our results showed that hypertension is associated with an increase of OS in vascular tissue. Vitamin C supplementation did not modify cardiovascular function and circulating OS levels, but decreased vascular OS induced by hypertension. Our results questioned the relative importance of each antioxidant compound on the total levels of circulating and tissue defenses.

Effect of tyramine, a dietary amine, on glycerol and lactate release by isolated adipocytes from old rats. C Bairras, C Ferrand, C Atgié (Unité de Nutrition et Signalisation Cellulaire (laboratoire d'Agen), Université Bordeaux 1, avenue M. Serres, 47000 Agen, France).

Amine degradation by adipocyte amine oxidases leads to the production of metabolites that interact with lipid and glucose metabolisms and their hormonal regulations. To further investigate these interactions, we determined the effect of tyramine (TYR), a dietary amine, on glycerol and lactate releases, respectively taken as indices of lipolytic and glycolytic activities of isolated adipocytes. Old male Wistar rats were used to prepare adipocytes by collagenase dissociation of retroperitoneal fat pads. The two tested doses of tyramine (10 µM and 1 mM) had no effect on basal glycerol release. On the other hand, TYR, at the highest dose tested (1 mM), weakly but significantly increased basal lactate release (25.6% increase), which was elevated in adipocytes from old rats. Norepinephrine (NE), highly stimulated adipocyte lipolysis with a submaximal effect at 1 µM which was slightly but significantly inhibited by TYR 1 mM (30% decrease). Insulin (1 nM (INS)) poorly inhibited the NE-stimulated lipolysis (28.7% decrease) in adipocytes isolated from old rats. TYR was able to potentiate the poor antilipolytic efficiency of INS (60% decrease). Under similar conditions, a high dose of NE greatly reduced lactate production (72% decrease) and TYR (1 mM) partially reversed this inhibition of lactate release (50% decrease). INS was also able to totally reverse the inhibitory effect of NE on lactate release, but there was no potentiation between insulin and tyramine effects. It can be concluded that high doses of TYR interact with norepinephrine and insulin, at least on the control of glycerol and lactate release, by counteracting catecholamine effects and by mimicking insulin

actions. This work was supported by the « Communauté de travail des Pyrénées » (Conseil Régional d'Aquitaine).

Description of several transcripts for the gamma-butyrobetaine hydroxylase gene. C Rigault, F Le Borgne, B Georges, J Demarquoy (University of Burgundy, UPRES Lipids and Nutrition, Faculty of Science Gabriel, 6 boulevard Gabriel, 21000 Dijon, France).

L-carnitine is an essential molecule for the oxidative metabolism of long chain fatty acids by allowing their transport towards the mitochondrial matrix. The biosynthesis of carnitine is catalyzed by a hepatic enzyme: the gamma-butyrobetaine hydroxylase (BBH). We, recently, described the presence of two mRNA for the BBH gene in the rat. We looked at the origin and the nature of these transcripts. The analysis of the coding region showed that this region was identical for both transcripts but differences were observed for their 3'untranslated region. The BBH gene cloning and its sequencing allowed to identify several potential polyadenylation signals. These results as well as northern analysis of these transcripts suggest the intervention of an alternative polyadenylation mechanism. The site directed mutagenesis of the various polyadenylation signals, currently under progress, will make it possible to confirm this hypothesis. During this characterization, we also found an Adenylate uridylate-Rich Element (ARE) sequence upstream of the last signal of polyadenylation. These elements generally act on the post-transcriptional stages by influencing the stability of the messengers or the translation itself. The stability of these two transcripts was studied on rat hepatocytes treated with actinomycin D. The analysis of the gamma-butyrobetaine hydroxylase messengers did not show any substantial difference in the kinetics of disappearance of the two transcripts. If this ARE is active, it is possible that it interacts with the translation of the messenger.

Effects of a fructose-enriched diet on lipid metabolism and antioxidant status in spontaneously hypertensive rats. A Girard, J Prost, J Belleville (UPRES Lipides et Nutrition EA 2422, 6 boulevard Gabriel, 21000 Dijon, France).

Fructose consumption in Wistar and Sprague-Dawley rats induced type 2 diabetes in which hyperglycaemia, hyperinsulinemia and antioxidant status alteration were found. No similar experiment was realised on hypertensive rats. Twenty spontaneously hypertensive rats (SHR) were divided into two groups and fed a 60%fructose diet or a control diet (60% starch). Lipid metabolism and antioxidant status were studied after 3 months. An elisa method was used for insulinemia determination. Whole blood, erythrocytes and plasma antioxidant status were determined by a KRL<sup>TM</sup> test. Red blood cells (RBC) were subjected to a free radical generator, RBC antiradical resistance was expressed by the time to reach 50% hemolysis. Antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase and glutathione reductase) were measured with biochemical methods. In the fructose diet, glycaemia and insulinemia were significantly increased compared to the starch diet. Plasma and VLDL-LDL triacylglycerol and plasma total cholesterol concentrations were higher in rats fed the fructose diet than in controls (61, 56 and 39%, respectively). These changes were associated with an increase in plasma and VLDL-LDL TBARS levels in the fructose group. Moreover, the fructose diet alters the antioxidant status represented by enzymatic and non-enzymatic antioxidant defence systems: it diminished the whole blood antioxidant status associated with the decrease of erythrocyte glutathione concentrations and glutathione peroxidase activity. Also, the fructose diet lowered plasma vitamin C concentrations. The fructose-enriched diet consumption induced a type 2 diabetes in SHR. This is associated with an insulin resistance and an increased oxidative stress.

Regulation of intestinal cholesterol absorption by glucose and galactose. B Play<sup>a</sup>, S Salvini<sup>a</sup>, Z Haikal<sup>a</sup>, M Charbonnier<sup>a</sup>, A Harbis<sup>a</sup>, I Fromont<sup>a</sup>, M Roussel<sup>a</sup>, D Lairon<sup>a</sup>, D Jourdheuil-Rahmani<sup>a,b</sup> (<sup>a</sup> Unit 476- Human Nutrition and Lipids, National Institute for Health and Medical Research (INSERM), UFR de Médecine, Université de la Méditerrannée, 27 bd Jean Moulin, 13385 Marseille Cedex 05, France; <sup>b</sup> Laboratoire de Biochimie et Sémiologie Cliniques, UFR de Pharmacie, Université de la Méditerrannée, 27 bd Jean Moulin, 13385 Marseille Cedex 05, France).

Studies on the intestinal cholesterol absorption process are most generally performed by experiments where lipids are solubilized in micelle or vesicle structures without consideration for any other nutrient. However, dietary lipids are usually digested and absorbed in the small intestine in the presence of other nutrients ingested in the same mixed meal. Moreover, a high glucose level (25 mM) increases mRNA levels for several genes that are functionally important in HDL metabolism, including ABCA1 and SR-BI. Thus, we hypothesized that digestion products modulate the cholesterol intestinal absorption process. The aim of this study was to test this hypothesis, by exploring how membrane transporters involved in cholesterol or sugar handling by enterocytes can be implicated. A dose-dependent increase in cholesterol absorption was induced by glucose addition (0-75 mM) to the apical medium of TC7 cells, a well-characterized clone of Caco-2. The uptake into the cells and the secretion rate to the basolateral space were both enhanced by glucose and galactose. This up-regulation was suppressed by SGLT1 inhibition but not by GLUT2 inhibition. Cholesterol cell uptake was significantly decreased by PMA and increased by chelerythrine (protein kinase C effectors), with more pronounced changes in the presence of hexoses. Thus, the involvement of a protein kinase C signaling pathway was evidenced in the regulation processes of intestinal cholesterol absorption. In the presence of antibodies directed to hSR-BI, cholesterol absorption was reduced by 40% and glucose or galactose no longer enhanced it. We suggest that glucose or galactose, through an interaction with SGLT1, activates a protein kinase C pathway that regulates the activity of one of the intestinal cholesterol transporters, namely hSR-BI.

Influence of gender on intestinal lipid metabolism. A Morise<sup>a</sup>, P Weill<sup>b</sup>, E Fénart<sup>c</sup>, D Hermier<sup>a</sup> (<sup>a</sup> Endocrinologie de la Nutrition, Université Paris XI, Bâtiment 447, 91405 Orsay, France; <sup>b</sup> Valorex, La Messayais, 35210 Combourtillé, France; <sup>c</sup> ONIDOL, 75008 Paris, France).

In the hamster, the hypolipidemic effect of  $\alpha$ -linolenic acid (18:3n-3, ALA) is more pronounced in males than in females (Morise et al, 2001, Congrès commun AFERO, AFN & SNDLF « Nutrition-Obésité 2001 », Paris). To

evaluate the role of digestion/absorption processes on these gender-related differences, male (n = 18) and female (n = 18) adult hamsters were fed for 7 weeks diets containing 17% lipids and rich in either saturated fatty acids (SFA, based on butter) or in ALA (based on linseed oil). For lipid analysis, the feces were collected during the last 2 weeks and bile at the end of the 7 weeks. When compared to the respective diets, feces contained more SFA and less oleic acid (18:1n-9, OL), linoleic acid (18:2n-6, LA) and ALA, which indicates that the digestibility differed for each class of fatty acids. Indeed, when considering all groups together (n = 36), the apparent absorption of each fatty acid was very high (about 99%), but increased with unsaturation: SFA < OL = LA < ALA (P < 0.05). Moreover, there were differences between groups, as follows (n = 9, P < 0.05). Gender effects: PUFA were less completely absorbed in males (99.6 and 99.7 for LA and ALA respectively) than in females (99.7 and 99.8). Diet effects: with the butter diet, the absorption of SFA was higher (99.1 vs. 98.7 with the linseed oil diet), and that of ALA was lower (99.7 vs. 99.8 with the linseed diet). In parallel, biliary concentrations of cholesterol and phospholipids were significantly affected by gender (males > females) and by diet (linseed oil > butter). Since bile acid concentration was identical in the 4 groups ( $\pm$  90 g·L<sup>-1</sup>), it could not explain the differences in fatty acid absorption between males and females. In conclusion: in male hamsters, the bioavailability of beneficial fatty acids, such as ALA, was slightly decreased, which may constitute a long-term and additional cardiovascular risk factor. This was aggravated in males fed SFA. These observations have to be validated in the human, in standard dietary conditions.

Survival rate and bioconversion activity of genetically engineered yeasts in vivo in rats. G Garrait, JF Jarrige, S Blanquet, S Denis, E Beyssac, JM Cardot, M Alric (Équipe de Recherche Technologique « Conception, Ingénierie et Développement de l'Aliment et du Médicament » (ERT CIDAM), Université d'Auvergne, Clermont-Ferrand, France).

The "drug delivery" concept is based on the oral administration of genetically modified yeasts which catalyse a reaction of bioconversion or release active compounds directly in the digestive environment, in order to prevent or treat various diseases. Once the scientific feasibility of the concept was demonstrated in vitro, a rat experimental model was developped to study the survival rate and the activity of bioconversion of the recombinant model Saccharomyces cerevisiae WRP45073A1, transforming cinnamate into coumarate. The yeast survival rate was evaluated in Wistar rats fasted for 24 h after oral intake of 10<sup>9</sup> model yeasts. After sacrifice, the various parts of the digestive tract were sampled and the living yeasts were counted (n = 3). The bioconversion activity of the model yeasts was studied under the same experimental conditions but after oral administration of 10<sup>9</sup> yeasts and 70 μmoles of cinnamate. The urinary cinnamate and coumarate were quantified by HPLC. The statistical analysis implemented a variance analysis. The yeast survival rate in the stomach did not exceed 20% but it seemed to be stabilized in the small intestine. Eight hours following their ingestion, 0.01% of the initial yeasts were found alive in the large intestine. With regards to bioconversion activity,  $0.02 \pm 0.01\%$  (P < 0.05, n = 5) of initial cinnamate was converted into coumarate. Although this activity is weak, our experimental model has nevertheless allowed to show, for the first time, the ability of recombinant living yeasts to catalyse a reaction of bioconversion in the rat digestive tract.

Intestinal cholesterol absorption studies using transgenic mice over expressing SR-BI in the intestine. F Bietrix, Y Daoguang, C Ferrand, C Rolland, M Nauze, R Barbaras, F Tercé, B Perret, X Collet (INSERM U 563, Département Lipoprotéines et Médiateurs Lipidiques, Hôpital Purpan, Toulouse, France).

SR-BI (Scavenger Receptor class B type1) is a multiligand protein able to bind HDL (High Density Lipoproteins), native oxidized lipoproteins or anionic phospholipids. Essentially expressed in the liver and steroidogenic tissues, SR-BI is also present in the intestines according to a decreasing gradient along the cephalo-caudal axis and is expressed at the top of the villosities. These characteristics are in favor of a potential role as a cholesterol transporter in the intestine. In order to evaluate the importance of SR-BI in intestinal cholesterol absorption, transgenic mice were generated using the enhancer of

apolipoprotein CIII (apo CIII) associated with the promoter of apo AIV (apo AIV) linked to the mice SR-BI cDNA. This construct leads to the SR-BI over expression in the small intestine respecting natural distribution. With a regular diet, the transgenic mice presented no change in the plasma cholesterol content. Paradoxically, when transgenic mice were fed a diet enriched with cholesterol (1.25%), there was a dramatic decrease in plasma cholesterol (-50%) compared to the wild type and an increase in the intestinal cholesterol absorption (+15%). Furthermore, we found that expression of the SR-BI transgene was up regulated when the transgenic mice were fed the hypercholesterolemic diet; this evidenced that a specific region in the enhancer of apo CIII/promoter of apo AIV is sensitive to the diet.

Postprandial splanchnic metabolism of dietary nitrogen in piglets. C Bos<sup>a</sup>, B Stoll<sup>b</sup>, H Fouillet<sup>a</sup>, C Gaudichon<sup>a</sup>, X Guan<sup>b</sup>, MA Grusak<sup>b</sup>, PJ Reeds<sup>c</sup>, D Tomé<sup>a</sup>, DG Burrin<sup>b</sup> (<sup>a</sup> UMR 914 INRA-INAPG, Physiologie de la Nutrition et du Comportement Alimentaire, Paris, France; <sup>b</sup> USDA/ARS, Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030, USA, <sup>c</sup> Deceased).

It is still unclear how and to what extent the splanchnic zone (intestine and liver) contributes to the postprandial metabolism of dietary nitrogen, and this is due in part to methodological limitations. In the present work, surgery has been performed on 3-wk old piglets to implant a flow probe around the portal vein and catheters in the portal vein, carotid artery and jugular vein. One week later, piglets were studied while receiving a primed continuous iv. infusion of <sup>18</sup>O-urea and a single 15 N uniformly labelled mixed meal. Portal and arterial blood was sampled for 8 h after the meal and the animals were then sacrificed. A small fraction of dietary N was released in the portal vein partly in the form of protein. Dietary N in portal amino acids peaked 2 h 30 after meal ingestion. Dietary N distribution in splanchnic tissues reached 18.7% of the ingested amount (10.1% in intestinal walls, 5.4% in the liver and 3.2% in plasma protein) and attained 31% in muscle mass. Ammonia production by the intestine was 400 µmol·kg<sup>-1</sup>·h<sup>-1</sup>, of which most was of dietary origin for the first 2 h after the meal. Urea mass balance across the portal-drained viscera was zero throughout the postprandial period. Systemic urea production was maximal 3 h after the meal (1 mmolN·kg^-l·h^-l) and 40% of this flux was of dietary origin. Cumulated urea produced from dietary N reached  $4.7\pm1.5\%$  of the ingested N. These results illustrate the important role played by the small intestine in the uptake and metabolism of dietary N in this piglet model.

Nutritional quality of rapeseed protein in humans. C Gaudichon, C Bos, F Mariotti, R Ntounda, S Daré, C Luengo, J Evrard, E Fénart, S Bérot, R Benamouzig, D Tomé (UMR 914 INRA-INAPG, Physiologie de la Nutrition et du comportement alimentaire, Paris, France; Service de Gastro-entérologie, Hôpital Avicenne, Bobigny, France; CETIOM et ONIDOL, Paris, France).

Rapeseed proteins exhibit a well balanced amino acid composition regarding amino acid requirements in humans. On the contrary to most of the other vegetal proteins, they have no deficiency in any indispensable amino acids. However, this protein source has never been assessed in humans. To this end, healthy volunteers were equipped with intestinal tubes positioned in the jejunum (n = 5) or terminal ileum (n = 7) and studied after ingestion of a mixed meal containing 24 g of uniformly and intrinsically  $^{15}\mathrm{N}$ -labeled rapeseed protein added to an oral dose of <sup>13</sup>Cglycine. Blood was sampled and total urine was collected for the 8 h following the meal ingestion. This methodology allowed us to measure the half-time gastric emptying of the rapeseed meal (4 h 30) and the real ileal digestibility of rapeseed protein, which reached  $83.7 \pm 9.4\%$  of the ingested amount. This digestibility level was low compared to that usually measured for vegetal protein (~90 %). Urinary excretion of dietary N in the form of urea and ammonia was 5.6% of the ingested amount at 8 h, while 8.4% was still retained in the body urea pool. These results indicate a very low deamination of rapeseed protein and a subsequent high metabolic utilization of absorbed amino acids for protein synthesis, corresponding to a high postprandial biological value of  $83 \pm 5\%$  at 8 h. In conclusion, rapeseed postprandial protein retention at 8 h was  $69.5 \pm 10.6\%$  of the ingested amount. This

score was similar to that obtained after ingestion of soy proteins, which are considered as reference vegetal proteins.

Effects of raygrass preservation and linseed oil supplementation on goat milk yield and fatty acid composition. Y Chilliard<sup>a</sup>, Rouel<sup>a</sup>, JM Chabosseau<sup>b</sup>, P Capitan<sup>a</sup>, P Gaborit<sup>c</sup>, A Ferlay<sup>a</sup> (<sup>a</sup> INRA-Theix, 63122, France; <sup>b</sup> INRA-Lusignan, France; <sup>c</sup> ITPLC-Surgères, France).

The aim of this study was to evaluate the possibility to increase goat milk 18:3n-3 content, as well as side effects on other fatty acids (FA). Forty-eight multiparous mid-lactation goats were used indoor in a  $2 \times 2$  factorial design, including 2 ad libitum forages: fresh raygrass (FR) or raygrass hay (RH), and 2 lipid intakes  $(0 \text{ g} \cdot \text{d}^{-1}, \text{ control}, \text{ C}, \text{ or } 130 \text{ g} \cdot \text{d}^{-1} \text{ of linseed oil},$ LO, i.e. 5-6% of diet DM) for 5 weeks. The LO diet, compared to the C diet, did not modify milk yield, but increased (P < 0.05) milk fat  $(+4.8 \text{ g}\cdot\text{kg}^{-1})$ , protein  $(+1.5 \text{ g}\cdot\text{kg}^{-1})$  and lactose  $(+2.9 \text{ g} \cdot \text{kg}^{-1})$  content. The effects of LO on milk FA composition were large. The LO diet compared to the C diet, decreased (% of total FA) C6:0 to C16:0, branched-chain (BC) and oddnumbered (ON) FA, C18:2n-6, C20:3, C20:4n-6 and C22:2n-6, and increased C4:0, C18:0, trans9-C16:1 (+955%), trans6+7+8-C18:1, trans9 to trans13-C18:1 (+944% for trans11-C18:1), cis11 to cis15-C18:1, cis9,trans13-C18:2, cis9,trans11-C18:2 (+645%) and C18:3n-3 (+114%). The LO diet decreased the normalized delta-9 desaturation ratios for C14:0, C17:0, C18:0 and trans11-C18:1. Numerous significant interactions between raygrass preservation and lipid supplementation were observed: the effects of LO on trans 11, cis 15-C18:2 and the delta-9 desaturation ratios were more important with RH, whereas the effects on the isomers of C18:1 (particularly trans10 to trans13) and cis9,trans13-C18:2 were more important with FR. In conclusion, the LO diet largely improved production performances of goats, increased n-3 and trans-FA, particularly trans11-C18:1 and cis9,trans11-C18:2, and decreased short and medium-chain, and BC+ON FA. These effects were more or less important, according to raygrass preservation.

Hesperidin, a citrus flavonoid, can both affect bone acquisition and inhibit skeletal impairment in rats. MN Horcajada, C Morand, C Puel, J Mathey, MJ Davicco, V Coxam (INRA de Clermont-Theix, Unité des Maladies Métaboliques et Micronutriments, 63122 Saint-Genès-Champanelle, France).

Citrus fruits are rich in flavanones. Hesperidin (Hp), a hesperetin glycoside, is one of the main flavanones in oranges. We investigated the effect of hesperidin on bone metabolism in intact and ovariectomized Wistar rats. The study was carried out on forty 3 month-old Wistar female rats. Twenty were sham-operated (SH) while the others were ovariectomized (OVX). Among the two groups, 10 sham-operated (sSH) and 10 ovariectomized (sOVX) rats were fed a standard diet for 90 days following surgery while the 20 remaining animals received the same regimen, but added with 0.5% hesperidin (10 HpSH, 10 HpOVX). At necropsy, the ovariectomy-induced uterine weight decrease did not differ between sOVX and HpOVX rats. Hp consumption totally prevented the ovariectomized-induced loss of bone mineral density (sOVX: -6.2% vs. sSH; HpOVX vs. sSH: NS; HpOVX: +10.7% vs. sOVX) and also significantly improved femoral metaphyseal density (HpSH: +11.5% vs. sSH) in intact rats. The femoral diaphyseal density was also significantly enhanced by Hp in both SH and OVX rats, while an impact on bone strength was observed only in OVX animals. The effects on bone parameters could be partially explained by the changes observed in biomarkers of bone turnover. Plasma osteocalcin concentrations were higher (P < 0.05) and urinary deoxypyridinolin excretion was reduced (P < 0.01) in both HpSH and HpOVX rats vs. their controls, suggesting a parallel stimulation of bone formation and inhibition of bone resorption. Finally, the plasma concentration of hesperetin (aglycone) was  $12.53 \pm 2.48 \,\mu\text{M}$  in Hp rats, while no hesperetin was detectable in the plasma of the control rats. These results indicate that, under our experimental conditions, hesperidin consumption was able to improve both bone mass acquisition in intact rats and protection against OVX-induced bone impairment. Since citrus fruits are commonly consumed in the human diet, they could contribute to the nutritional management of osteoporosis, especially in Western countries where isoflavone intake is quite low.

Effect of omega 3 (ω3) fatty acids benefits on nutritional quality of pork meat. A Wilfart, JM Ferreira, A Mounier, J Mourot (UMRVP-INRA, 35590 St-Gilles, France).

Advised Nutritional Benefits (ANB) suggest an ω6/ω3 ratio near 5 in human food in order to decrease cardio vascular diseases. The fatty acid composition of breed influences the fatty acid content of pork tissues. Therefore,  $\omega$ 3 ratio can be increased in pork meat by feeding a diet supplemented with  $\omega 3$ . This study was aimed at determining the influence of ω3 fatty acid levels in the pig diet on total lipid level (TL) and fatty acids levels of cooked meat. Four groups of 12 pigs weighing between 45 and 105 kg were fed an isoenergetic and isolipidic diet containing different levels of n-3 PUFA: coconut oil (CO) (1g  $\omega 3 \cdot kg^{-1}$  of diet), sunflower oil (SO) (1.2 g  $\omega 3 \cdot kg^{-1}$  of diet), rapeseed oil (RO)(1.5 g  $\omega 3 \cdot kg^{-1}$ of diet) and extrudate linseed (5.1 g ω3·kg<sup>-1</sup> of diet)(EL). The total lipids levels in cooked meat were measured by the Folch procedure (1957) then the fatty acid composition was determined with a gas chromatograph. The data were analysed using a one way factorial analysis of variance. There was a relationship between the ω3 level in meat and the diet ratio. ω3 fatty acids in pork meat are resistant to cooking. In conclusion, is it possible to improve the nutritional quality of pork meat by introducing  $\omega 3$  in their diet complying with ANB.

Improvement beef fatty acids health value by dietary means. C Gladine<sup>a</sup>, D Durand<sup>a</sup>, L Leloutre<sup>b</sup>, D Bauchart<sup>a</sup> (<sup>a</sup> Unité de Recherches sur les Herbivores, INRA, CR Clermont/Theix, France; <sup>b</sup> Innovation en Nutrition et Zootechnie, Château-Thierry, France).

Bovine meat suffers from a negative health image for consumers. This is notably due to its abundance in saturated fatty acids (SFA) and in trans vaccenic acid (TVA) (considered as atherogenic FA) as well as its relative poverty in polyunsaturated FA (PUFA), especially of the n-3 family (anti atherogenic FA). In order to reduce these defaults, fattening Charolais steers received for 97 d, a high concentrate diet (C, 70%) or a high corn silage diet (CS, 60%), supplemented or not (C) with extruded linseed (L) (groups C, CL, CS, CSL, n = 7/group). Fatty acid analysis

of Rectus abdominis (express as % of total FA) was performed by GLC using a CP Sil88 column. The means of each variable were compared by ANOVA according to a factorial model (diet × linseed supplement). RA content in 18:2n-6 increased with C and CL diets in comparison with CS and CSL diets (+33%, P < 0.01) whereas 18:3n-3 content was deeply increased with the linseed supplement for both rations (×3 for CL,  $\times$ 5 for CSL, P < 0.001). RA muscles taken from C and CL animals showed a higher PUFA content (17 vs. 13%, P < 0.01) and a lower SFA content (41 vs. 45%, P < 0.01) leading to a higher PUFA/SFA ratio ( $\times 1.4$ , P < 0.01). Corn silage based diets (CS and CSL) favoured the deposition of more beneficial monounsaturated FA (18:1  $\Delta 9cis$ : +13%, P < 0.05; 18:1  $\Delta 11trans$ (TVA): -50%, P < 0.001). Linseed supplementation, however, led to a reduction of  $18:1\Delta9cis$ (-11%, P < 0.05) without affecting TVA content. Total CLA content was higher than those generally reported in the literature (0.9% vs. 0.4-0.7% total FA) but did not differ between diets with or without linseed. We conclude that the beef fatty acid health value can be improved in bulls given a concentrate based diet (C) for all indicators studied (except for TVA), and positive effects are accentuated with linseed supplementation.

Correlations between milk fat content and fatty acid composition in goats receiving different combinations of forages and lipid supplements. Y Chilliarda, J Rouela, P Capitana, JM Chabosseaub, K Raynal-Ljutovacc, A Ferlaya (a INRA-Theix, 63122, France; b INRA-Lusignan, France; c ITPLC-Surgères, France).

The *trans* unsaturated fatty acids (FA) originating from the rumen could play an important role in the decrease in milk fat content (MFC) and mammary de novo lipogenesis in the cow receiving vegetable oil supplementation. The aim of this study was to estimate if this is similar in the goat, despite the large differences between cows and goats which were observed previously in the response of MFC to vegetable oil supply. Eighty-four multiparous mid-lactation goats received one of 7 different diets indoor: fresh raygrass vs. raygrass hay vs. alfalfa hay, supplemented or not with  $130\,\mathrm{g}\cdot\mathrm{d}^{-1}$  of either linseed oil or high-oleic sunflower oil for 5 weeks. Milk

yield and MFC were 2.87-3.54 kg·d<sup>-1</sup> and 28.7-35.7 g⋅kg<sup>-1</sup>, respectively, for the different diets. Oil addition increased MFC significantly (P <0.05). Individual MFC values were  $32.3 \pm 5.2$ (mean  $\pm$  SD, n = 84). Correlations between MFC and FA percentages were significant (P < 0.05) and positive for C18:0 (0.34) and C20:0 (0.22). Significant negative correlations were observed for cis9-C14:1 (-0.35), cis9-C16:1 (-0.25), C16:0(-0.29), C15:0(-0.29), C14:0(-0.28) and for polyunsaturated n-6 FA (-0.32 to -0.25). Negative correlation coefficients were also observed between MFC and normalized delta-9 desaturation ratios (-0.49 to -0.27). High positive interindividual correlations between C18:0 and MFC were observed within each dietary lipid group (0.43, 0.49 and 0.57 for C, LO and OSO groups, respectively). In conclusion, this study suggests that the regulation of MFC differs between the goat and bovine species (no significant correlation was observed between MFC and any trans-18:1 or trans-18:2 isomer). Our working hypothesis is that C18:0 arising from the digestion of lipid supplements plays a central role in the positive regulation of goat mammary lipogenesis.

Effects of raygrass preservation and higholeic sunflower oil supplementation on goat milk yield and fatty acid composition. A Ferlay<sup>a</sup>, J Rouel<sup>a</sup>, JM Chabosseau<sup>b</sup>, P Capitan<sup>a</sup>, K Raynal-Ljutovac<sup>c</sup>, Y Chilliard<sup>a</sup> (a INRA-Theix, 63122, France; b INRA-Lusignan, France; c ITPLC-Surgères, France).

The aim of this study was to evaluate the possibility to increase goat milk oleic acid content, as well as side effects on other fatty acids (FA). Forty-eight multiparous mid-lactation goats were used indoor in a  $2 \times 2$  factorial design, including 2 forages ad libitum: fresh raygrass (FR) or raygrass hay (RH), and 2 lipid intakes (0 g·d<sup>-1</sup>, control, C, or 130 g·d<sup>-1</sup> of high-oleic sunflower oil, OSO, i.e. 5-6% of the DM diet) for 5 weeks. OSO diets, compared to the C diet, did not modify milk yield, but increased (P <0.05) milk fat ( $+3.0 \text{ g} \cdot \text{kg}^{-1}$ ), protein ( $+1.9 \text{ g} \cdot \text{kg}^{-1}$ with the RH diet) and lactose  $(+3.8 \text{ g} \cdot \text{kg}^{-1})$  content. The OSO diet compared to the C diet, decreased (% of total FA) C6:0 to C16:0, cis9-C16:1, branched-chain (BC) and odd-numbered (ON) FA, C18:2n-6, C18:3n-3, C20:4n-6, and

C20:5n-3 and increased C4:0, C18:0 (+148%), C20:0, C22:0, C24:0, *cis*9-C18:1 (+79%), trans9-C16:1, trans6+7+8-C18:1 (+1543%, up to 1.15% of total FA), trans9 to trans13-C18:1 (+666% for trans10-C18:1, up to 1.15% of total FA, +170% for trans11-C18:1), and cis9, trans11-18:2 (+79%). The OSO diet decreased the normalized delta-9 desaturation ratios for C14:0, C17:0, C18:0 and trans11-C18:1. The main significant interactions between raygrass preservation and lipid supplementation were that OSO decreased more cis9-C14:1, cis9-C16:1 and C17:1 with RH, whereas it decreased more the cis9,trans11-C18:2: trans11-C18:1 ratio with FR. In conclusion, the OSO diets largely improved production performances, increased stearic, oleic and some trans-18:1, and decreased delta-9 desaturation ratios, polyunsaturated FA, short-chain, medium-chain and BC+ON FA, with few interactions with raygrass preservation.

Effects of nature of forage and type of oil supplementation on kinetics of percentages of CLA and *trans*-fatty acids in cow milk. A Ferlay, P Capitan, A Ollier, Y Chilliard (INRA-Theix, 63122 Saint-Genès-Champanelle, France).

Dietary factors modify sharply the fatty acid (FA) composition of cow milk. The aim of this study was to analyze the kinetics of response of milk FA to the addition of different polyunsaturated FA in the diet in order to better understand the underlying digestive or metabolic mechanisms. Twelve primiparous Holstein cows received diets based on either grass hay (H, 60% of DM intake) or maize silage (M, 69% of DM intake) for 3 weeks. Then, the cows were fed these basal diets with either 5% sunflower oil (SO), 5% linseed oil (LO), or 2.5% fish oil (FO) for 3 weeks. Milk samples were collected at -4, -2, +3, +5, +7, +9, +13 and +20 days (d) relative to the addition of oil into the diet. Data were analyzed using the repeated-measures analysis of SAS (2000). Milk t10-18:1 increased (P < 0.05) slightly until +13 d, then it increased strongly between +13 and +20 d (up to 2.0 or 4.5% of total fatty acids for the H and M diets). The values with basal diets were 0.7 and 0.4% for t11-18:1 and c9-t11 18:2 (CLA), and their kinetics were curvilinear (P < 0.05) for the 3 oils. The maximal response (MR) of t11-18:1 and CLA to LO was at +13 d (11.7 and 5.3%) or +7 d (8.3 and 3.2%) for H or M diets, respectively. The t11-18:1 MR to SO was at +7 d (13.5%) or +13 d (11.8%) for H or M diets. The MR to FO was at +13 d (6.6 and 9.6%) for H and M diets. The CLA MR to SO and FO was at +13 d (5.9, 5.1, 3.4 and 3.7% for HSO, MSO, HFO and MFO diets). In conclusion, the kinetics of appearance in milk of t10-18:1 (which increased later), and FA belonging to the t11 series (t11-18:1 and CLA) were strongly different and variable according to the diet (forage-oil interactions).

Effects of the nature of forage and type of oil supplementation on cow milk fatty acids. Y Chilliard, A Ollier, P Capitan, A Ferlay (INRA-Theix, 63122 Saint-Genès-Champanelle, France).

The nutritional quality of milk depends in part on its fatty acid (FA) composition. The aim of this study was to analyze the effects of both the nature of the forage and the type of dietary polyunsaturated FA on milk FA having a nutritional interest. Twelve primiparous Holstein cows in 2 replicated  $3 \times 3$  Latin Square designs were fed diets based on either grass hay (H, 60% of DM intake) or maize silage (M, 69% of DM intake) with either 5% sunflower oil (SO), 5% linseed oil (LO), or 2.5% fish oil (FO). Milk samples were collected after 3 weeks. Data were analyzed according to the MIXED procedure of SAS (2000). The percentage of milk C18:0 or c9-C18:1 was higher (P < 0.05) for M (9.0 or 17.0% of total fatty acids) than for the H (7.4 or 14.6%) diets. In contrast, the percentages of C18:3n-3, C20:5n-3, t11-C18:1 (3.6 vs. 7.0%) and c9,t11-C18:2 (1.5 vs. 3.2%) were lower for the M than for the H diets. FO, compared to LO or SO, increased C4:0 to C16:0. The percentage of C18:0 or C18:2*n*-6 ranked by oil was FO (2.6 or 1.1%) < LO (9.8 or 1.2%) < SO (12.6 or 1.7%). The percentage of c9-C18:1 was much lower for FO (6.6%) than for LO or SO (20.4%). The percentage of t4- to t9-, and c12-C18:1 ranked by oil was FO < LO < SO. LO compared to SO decreased t10-C18:1 (3.5% vs. 5.4%). The percentage of C18:3n-3 ranked by oil was LO (0.5%) > FO (0.4%) > SO (0.3%). Significant oil-forage interactions were observed for t11,c15-C18:2 (an intermediate of C18:3n-3 hydrogenation), C20:4*n*-6, C20:5*n*-3, and C22:5*n*-3. In conclusion, the nature of the forage, in the presence of the studied oils, influenced milk C18:0, *c*9-C18:1, C18:3*n*-3, C20:5*n*-3, *t*11-C18:1 and CLA. The percentages of C18:0, C18:2*n*-6, C18:3*n*-3 and some isomers of C18:1 was influenced by the type of oil, whereas *t*11-C18:1 and CLA did not differ largely.

Combined effects of grazing and stall fattening on health promoting characteristics of muscle lipids in the lamb. B Aurousseau, X Faure, D Bauchart, D Micol, S Prache, A Priolo (URH, INRA Theix, 63122 Saint-Genès-Champanelle, France).

Several studies have already illustrated that meat from grazing lambs has high contents of fatty acids (FA), namely CLA and linolenic acid, i.e., C18:3n-3, which have health promoting effects on consumers. To study the effects of time on those FA in Longissimus Thoracis (L.T.) muscle in fattening lambs grazed during the growing period, 4 groups of 6 lambs were used: group G (grazed up to slaughter), group GSS (grazed and then stall-fattened for a short period, i.e. 22 days), group GSL (grazed and then stall-fattened for a long period, i.e. 41 days), group S (stall-fed and -fattened). The lambs were slaughtered when they reached 34.5 kg of bodyweight, at an age of 100 to 130 days. Compared to group S, in group G proportions among total FA was lower (P < 0.001) for 16:0 (-11%) and 18:1 9c (-22%) and higher (P < 0.001) for C18:0 (+ 23%), C18:1 11t (+ 123%), C18:3 (n-3) (+ 114%) and CLA 9c, 11t (+50%). Proportions were similar in both groups for C18:2 (n-6). The C18:2(n-6)/18:3(n-3) ratio was lower in group G (2.3 vs. 5.0, P <0.001). A short period of fattening (group GSS) proportions were not altered for CLA 9c, 11t, but decreased for C18:3 (n-3) (-37%; P < 0.01), so that the C18:2(n-6)/18:3(n-3) ratio (3.3 vs. 2.3) was slightly increased, compared to group G (P < 0.01). In the GSL group (long time of stallfattening after grazing) proportions of CLA 9c, 11t were lowered, but were not significantly different from the proportions observed in groups G and S. Other features matched those observed in the S group. This study confirms the potential health promoting effects for the consumers of meat from grazed lambs. It brings into evidence that a short period of stall-fattening alters only slightly the characteristics of the FA from the meat which follow grazing.

Comparison of the influence of different starch sources on the lipid synthesis in growing pigs. D Martinez-Puig<sup>a</sup>, J Pérez<sup>a</sup>, J Mourot<sup>b</sup> (<sup>a</sup> Depart. Ciència Animal i Aliments, Universitat Autòn. de Barcelona, 08193 Bellaterra, Spain; <sup>b</sup> UMRVP-INRA, 35590 St-Gilles, France).

In pigs, fatty acids synthesized in the adipose tissues represent more than 80% of the lipids. Thus, the main precursor for lipogenesis glucose is derived from starchy diets. In this work, we studied the influence of two different sources of starch on the lipogenic enzymes activities in adipose tissues. Two groups of 6 growing pigs averaging 30 kg live weight received, during 38 days, two diets with the same quantity of starch (250 g⋅kg<sup>-1</sup> food) of different origin: corn (CS) or raw potato (RPS) with respectively 19 and 64% of resistant starch. Growth performances were measured. The malic enzyme (ME) and Glucose 6P Deshydrogenase (G6PDH) activities were measured in the subcutaneous, abdominal and intermuscular (ham) adipose tissue. The data were analysed using a one way factorial analysis of variance. Backfat thickness tended to be greater in pigs fed CS than RPS (10 mm vs. 8.2). Malic enzyme (ME; µmol NADPH min<sup>-1</sup>·g<sup>-1</sup> protein) activity was higher for CS than RPS in subcutaneous adipose tissue (3.88 vs. 2.61, P < 0.01), abdominal adipose tissue (4.04 vs. 1.88, P < 0.01) and intermuscular adipose tissue (1.06 vs. 0.77, NS). The G6PDH activity (µmol NADPH min<sup>-1</sup>·g<sup>-1</sup> protein) was higher for CS than RPS in subcutaneous adipose tissue (1.59 vs. 0.90, P < 0.01), abdominal adipose tissue (1.70 vs. 0.87, P < 0.05) and intermuscular adipose tissue (0.43 vs. 0.34, NS), respectively. The lipogenic enzymes activities were stimulated by the CS diet, which explains the greater adiposity in animals fed CS. In conclusion, the resistant starch content in the diet can modify de novo fatty acid synthesis in the pig.

Dietary n-6 or n-3 PUFA and membrane fluidity of lymphocytes in finishing steers. V Scislowski<sup>a</sup>, D Durand<sup>a</sup>, C Motta<sup>b</sup>, D Bauchart<sup>a</sup> (<sup>a</sup> Unité de Recherches sur les Herbivores-INRA Clermont/Theix, France; <sup>b</sup> Laboratoire de Biochimie-CHU Clermont, France).

Diets supplemented with polyunsaturated fatty acids (PUFA) given to bovines to improve the nutritional quality of meat can be deleterious for their health via the peroxidation processes and

the risk of alteration of cell membrane fluidity. The fluidity of lymphocytes was evaluated in Charolais × Salers steers. Animals were distributed into 3 groups (n = 5/group) receiving, for 70 days, either a control diet (C<sub>S</sub>) consisting of hay (54%) and concentrate (46%), or the same basal diet supplemented with sunflower oil rich in n-6 PUFA (4%/DM) given either as crushed seeds  $(S_S)$  or as free oil directly infused into the duodenum (O<sub>S</sub>). A similar experimental scheme was used to test the effects of linseed oil rich in n-3 PUFA (diets  $C_L$ ,  $S_L$  and  $O_L$ ; n = 6/group). At the end of the experiments, the microviscosity values of the lymphocytes (opposite to fluidity) were determined by fluorescence polarization at 38.5 °C (bovine physiological temperature) and 24 °C (usually used for experiments). Fatty acid composition of total plasma was determined by GLC allowing PUFA/Saturated FA (SFA) ratio calculation since it is known to correlate positively with a higher fluidity. Compared to the C<sub>S</sub> diet, PUFA/SFA ratio was unchanged with the S<sub>S</sub> diet but increased with the  $O_S$  diet (×1.9, P < 0.05). The  $S_L$  diet increased the PUFA/SFA ratio compared to the C<sub>L</sub> diet  $(\times 1.5, P < 0.05)$  with the effect being more marked with the O<sub>L</sub> diet ( $\times 2.3$ , P < 0.05). Theoretically, supplementation with PUFA and particularly oil infusion would favour a higher membrane fluidity. However no significant differences between the diets were noted for microviscosity. This indicates that the fluidity of lymphocytes was maintained stable to an optimal level to ensure cellular functions whatever the type of dietary PUFA (n-6 or n-3) and their protection against biohydrogenation in the rumen (seeds in the diet or oil by duodenal infusion). Consequently, changes of plasma fatty acid composition induced in bovines by PUFA supplementation would not affect the health of the animals since they adapt in order to maintain metabolic functions of the cells.

Fish oil decreases skeletal muscle lactate production during hyperinsulinemia in healthy humans. J Delarue<sup>a</sup>, R Chioléro<sup>b</sup>, L Tappyc (a Laboratoire Régional de Nutrition Humaine & EA-948 (CHU de Brest), France; b Réanimation chirurgicale, Switzerland; c Institut de Physiologie de Lausanne, Switzerland).

Fish oil (FO) prevents high fat or high sucrose diet-induced insulin resistance in rodents. In healthy humans at rest, FO reduces the insulinemic response to oral glucose without altering plasma glucose utilization (Rd glucose) while during exercise FO reduces Rd glucose. Aim: in order to better characterize the effects of FO, we studied the effects of FO supplementation on Rd glucose, total carbohydrate (CHO) oxidation and muscle lactate production during hyperinsulinaemia in normal men. Seven men (23.4 ± 1.3 years;  $68.8 \pm 11.3$  kg; IMC:  $21.8 \pm 2.2$  kg) were studied before and after 7.2 g·d FO over 3 weeks. The test used was euglycaemic hyperinsulinaemic clamp (1 mU·kg<sup>-1</sup>·min<sup>-1</sup>) during 120 min. Total CHO oxidation was determined using indirect calorimetry. Muscle lactate production was obtained from the infusion of <sup>13</sup>C glucose into a microdialysis probe (CMA, Sweden) followed by the determination of <sup>13</sup>C lactate enrichment in the dialysate. During clamp, glycaemia and insulinaemia were similar without or with FO. Stimulation of Rd glucose (clamp:  $5.6 \pm 0.7$  vs.  $6.6 \pm 1.2$  mg·kg<sup>-1</sup>·min<sup>-1</sup>) and of CHO oxidation (clamp:  $3.1 \pm 0.3$  vs.  $3.7 \pm$  $0.2 \,\mathrm{mg \cdot kg^{-1} \cdot min^{-1}})$  were similar without or with FO. An increase in lactataemia was similar during hyperinsulinaemia without or with FO (1.24  $\pm$  $0.5 \text{ mM clamp vs. } 1.40 \pm 0.05 \text{ mM clamp}$ ). Muscle interstitial lactate and <sup>13</sup>C lactate were decreased by FO (both P < 0.05). The arterial lactate/interstitial lactate gradient was decreased by FO  $(0.60 \pm 0.21 \text{ vs.} 1.23 \pm 0.15 \text{ mM}; P < 0.05)$ . In conclusion, FO does not affect Rd glucose during hyperinsulinaemia but decreases muscle lactate production by 50%, which could be due to an inhibiting effect on Na+-K+-ATPase.

Tissue-specific regulation of phosphatidyl inositol 3'-kinase (PI3K) activity by fish oil in fed rats. C Corporeau<sup>a</sup>, C Le Foll<sup>a</sup>, M Taouis<sup>b</sup>, F Berthou<sup>a</sup>, J Delarue<sup>a</sup> (<sup>a</sup> EA-948 Faculté de Médecine, Brest, France; <sup>b</sup> Neuro-endocrinologie de la prise alimentaire-INRA, Jouy-en-Josas, France).

Fish oil (20% of total energy) when substituted for other fatty acids inside a high fat diet (60% of total energy) prevents insulin resistance (IR) induced by this diet; it prevents the decrease of PI3K activity induced by this diet in the muscle but not in the liver. In patients with type 2 diabetes, FO supplementation does not reverse insulin resistance. The aim was to study the effect of

a low FO intake inside a normolipidic diet on PI3K activity in rats treated by dexamethasone (D) known to induce IR and decrease PI3K in the muscle, liver and adipose tissue (AT). Sixteen rats were fed for 4 weeks with a diet containing 6.6% peanut-rape oil (PR) as the source of lipids (n = 8, control), or with 2.2% FO plus 4.4% PR (n = 8, n-3). 5 days before the end of the diet, the rats were divided into 2 groups, one treated subcutaneously with D (1 mg·kg<sup>-1</sup>·day<sup>-1</sup>) and the other with NaCl 9%. D induced hyperglycemia and hyperinsulinemia with both control and n-3 diets (P < 0.01). In the liver and muscle of control rats, D inhibits PI3K activity by 76% and 82% respectively. In FO rats, PI3K activity was inhibited by 53% and 54% in the liver and muscle respectively. In FO rats treated with D, inhibition of PI3K induced by D was amplified by 18% and 11% by FO. In adipose tissue (AT), D inhibits PI3K activity by 42% and by 51% in PR and in FO rats respectively. In contrast to other tissues, FO increases PI3K activity by 59% and did not amplify the inhibition induced by D. In conclusion a low level of FO inhibits PI3K activity in the liver and muscle but increases its activity in AT. FO amplifies the inhibitory affect of D on PI3K activity in the liver and muscle, but not in AT.

Effect of omega 3 (ω3) fatty acids benefits on size and number of pork backfat adipocytes. A Wilfart, G Robin, J Mourot (UMRVP-INRA, 35590 St-Gilles, France).

The amount and composition of dietary fatty acids influence the growth and the quality of fat tissue in pigs. This study was aimed at determining the influence of  $\omega$ 3 fatty acid levels in the pig diet on the size and number of pork backfat adipocytes. Three groups of 12 pigs weighing between 45 and 105 kg were fed an isoenergetic and isolipidic (3.5% of lipids) diet containing different levels of n-3 PUFA: coconut oil (CO) (1 g  $\omega 3 \cdot kg^{-1}$  of diet), rapeseed oi1 (RO)(1.5 g  $\omega 3 \cdot kg^{-1}$  of diet) and extrudate linseed (5.1 g  $\omega 3 \cdot kg^{-1}$  of diet) (EL). Adipocytes were fixed with osmic acid, and size was determined by image analysis. The adipocyte total number was calculated using the formula of Di Girolamo et al. (1971). The data were analysed using a one way factorial analysis of variance There was a relationship between the adipocyte size

reduction and an increase of the  $\omega 3$  in the diet (P < 0.05): 78.9  $\mu m$  (CO), 76.8  $\mu m$  (RO), 71  $\mu m$  (EL). The backfat weight decreased (P < 0.02) and therefore the adipocyte total numbers increased (NS): CO:  $9071 \times 10^6$  adipocytes – RO:  $9886 \times 10^6$  adipocytes – EL:  $10252 \times 10^6$  adipocytes. In conclusion, the amount and composition of dietary fatty acids may influence adipocyte proliferation. A high  $\omega 3$  level diet might induce the recruitment of new adipose cells.

Metabolisation of *trans* fatty acids in human intestinal cells. B Renaville<sup>a,b</sup>, E Mignolet<sup>b</sup>, T Sergent<sup>a</sup>, V Fievez<sup>a</sup>, J Pottier<sup>b</sup>, Y Larondelle<sup>b</sup>, YJ Schneider<sup>a</sup> (<sup>a</sup> Laboratoire de biochimie cellulaire, Université catholique de Louvain, Louvain-la-Neuve, Belgium; <sup>b</sup> Unité de biochimie de la nutrition ISV, Université catholique de Louvain, Louvain-la-Neuve, Belgium).

Trans fatty acids (TFA) are unsaturated fatty acids with at least one double bond in a trans configuration. Monounsaturated TFA has been associated with an increased risk of cardiovascular diseases, but recent data suggest that different isomers could have opposite effects. Trans-vaccenic acid (C18:1 trans-11), the main isomer of dairy products, is suspected to have positive effects on health after partial metabolisation to rumenic acid (C18:2 cis-9, trans-11), a conjugated linoleic acid (CLA). Indeed, rumenic acid has been proposed to be anticarcinogenic and antiatherogenic. The purpose of our study was to investigate intestinal absorption and metabolisation of trans-vaccenic acid using a polarised cell culture system (Caco-2 cells) as an in vitro model of the absorptive function of the human intestinal barrier. We studied  $\Delta 9$ desaturase suspected to desaturate trans-vaccenic acid into rumenic acid. First, we investigated the expression of  $\Delta 9$ -desaturase in Caco-2 cells. The transcription of the  $\Delta 9$ -desaturase gene was evidenced by RT-PCR. Then, the enzymatic activity was measured by the ability of cells to desaturate 14C-stearate into 14C-oleate, using TLC, and trans-vaccenic acid into rumenic acid, using gas chromatography. Rumenic acid was detected in the cellular lipids only if cells were cultured with trans-vaccenic acid. Currently, we are studying the modulation of this activity by different nutrients. Preliminary results suggest an inhibition of  $\Delta 9$ -desaturase, when Caco-2

cells are cultured with arachidonate ( $30\,\mu M$ ) and an activation when cultured with 55 mM glucose. This modulation of  $\Delta 9$ -desaturase was expected, since the enzyme has been described to be inhibited by PUFA and activated by carbohydrates. These results show that the cell culture system used as the in vitro model of the intestinal barrier, is relevant for the investigation of the effect of nutrients (poly-unsaturated fatty acids, carbohydrates, ...) and drugs (sterculic acid, fibrates, ...) on the expression and activity of  $\Delta 9$ -desaturase.

Alpha-linolenic acid: biomarkers of intake and bioconversion. C Boué-Vaysse<sup>a</sup>, A Morise<sup>b</sup>, N Combe<sup>a</sup>, B Delplanque<sup>b</sup>, E Fénart<sup>c</sup>, P Weill<sup>d</sup>, D Hermier<sup>b</sup> (<sup>a</sup> ITERG-Biochimie-Nutrition, Université Bordeaux I, Talence, France; <sup>b</sup> Laboratoire de Physiologie de la Nutrition, Université Paris-Sud, Orsay, France; <sup>c</sup> ONIDOL, Paris, France; <sup>d</sup> VALOREX, Javène, France).

Epidemiological studies generally prefer to use biomarkers of fatty acid consumption, such as blood lipids or adipose tissue (AT), rather than dietary survey. Nevertheless, there are no reference studies concerning the relationships between alpha-linolenic acid (ALA) consumption and the incorporation of ALA or its long chain derivatives, such as eicosapentaenoic acid (EPA), into these compartments. We assessed these relations in 4 groups of 6 male hamsters fed, during 7 weeks, diets providing 35% of total energy as fat, and supplying increasing doses of ALA as linseed oil (L1: 0.37% energy, L2: 3.5% energy, L3: 6.9% energy, L4: 14.6% energy) and equivalent levels of linoleic acid (LA) (LA, 8.5% energy), with an LA/ALA ratio varying from 22.5 to 0.6. The ALA content of adipose tissue increased from 0.85% (L1) of total fatty acids to 14% (L4). A very significant correlation between ALA intake (mg·day<sup>-1</sup>) and ALA level into adipose tissue ( $r^2 = 0.98$ ; P < 0.0001) was observed. In plasma, cholesteryl esters were the richest lipids in ALA (from 0.3% (L1) to 9.7% (L4) of total fatty acids). ALA level in cholesteryl esters was significantly correlated with ALA intake ( $r^2$  = 0.97; P < 0.0001). Moreover, ALA intake was strongly related to the EPA content of red blood cells ( $r^2 = 0.97$ ; P < 0.0001) and plasma phospholipids ( $r^2 = 0.93$ ; P < 0.0001). In conclusion, our data demonstrated that ALA and/or EPA content of adipose tissue, plasma (cholesteryl esters, phospholipids) and red blood cells were extremely reliable as biomarkers of ALA consumption and/or bioconversion, over a broad range of dietary supplies and LA/ALA ratio.

Olive or salmon oils affect differently the hepatic storage and transport of (n-3) polyunsaturated and (n-9) monounsaturated fatty acids by VLDL in growing rats fed a cholesterol-enriched diet and containing different proteins. S Bouderbala<sup>a</sup>, MY Lamri-Senhadji<sup>a</sup>, D Krouf<sup>a</sup>, M Bouchenak<sup>a</sup>, J Belleville<sup>b</sup> (a Université d'Oran Es-Sénia, Faculté des Sciences, Département de Biologie, Laboratoire de Nutrition Clinique et Métabolique, BP 1524, Es-Sénia, Algeria; b UPRES EA 2422, Lipides et Nutrition, Faculté des Sciences Gabriel, Dijon, France).

The aim of this study was to compare the effect of olive or salmon oils on the hepatic storage and transport of fatty acids by VLDL in growing rats fed a 0.5% cholesterol-enriched diet with either 20% casein (C) or chick pea (CP) protein combined to 10% olive (O) or salmon (S) oil for 28 days. Serum and hepatic total cholesterol (C)  $(4.62 \pm 0.64 \text{ mmol} \cdot \text{L}^{-1})$  and  $(85.91 \pm$ 12.70  $\mu$ mol·g<sup>-1</sup>), respectively were similar in all groups, whereas, VLDL-C was 1.9-, 2.88- 1.47and 2.22-fold higher in CO vs. CS, CPO vs. CPS, CO vs. CPO and CS vs. CPS, respectively. Serum triacylglycerol (TG) values were 1.76fold higher in the CPO group compared to the CPS group. VLDL-TG was 1.5-fold increased in the CPO vs. the CPS group and the CS vs. the CPS group and 1.8-fold lower in the CPO group vs. the CO group. Liver triacylglycerol fatty acid composition showed that C16:0 in the CPO and CPS groups represented, 14% and 74% of the values noted in the CO or CS groups, respectively. Indeed, C18:0 in the CPO and CPS groups represented, 29% and 20% of the values noted in the CO or CS groups, respectively. C18:1n-9 was twofold higher in the CS vs. CPS groups. VLDL-TG fatty acid contents showed that in the CPO group, C16:0 represented 48% of the values noted in CO. C18:1n-9 was twofold lower in CS than CPS. C18:3n-3 was 2- and 1.7fold higher in CPS vs. CPO and CS, respectively. In conclusion, these results suggest that a cholesterol-enriched diet containing casein or chick

pea proteins combined to olive or salmon oil affect hepatic storage and transport of (n-3) poly-unsaturated and (n-9) monounsaturated fatty acids by VLDL.

Increased fatty acid oxidation capacities in the steatotic liver of mice fed the *trans*-10, *cis*-12-linoleic acid isomer. P Degrace<sup>a</sup>, L Demizieux<sup>a</sup>, J Gresti<sup>a</sup>, JM Chardigny<sup>b</sup>, JL Sébédio<sup>b</sup>, P Clouet<sup>a</sup> (<sup>a</sup> UPRES Lipides et Nutrition EA2422, Université de Bourgogne, 6 bd Gabriel, 21000 Dijon, France; <sup>b</sup> INRA, Unité de Nutrition Lipidique, 17 rue Sully, 21034 Dijon Cedex, France).

Decreased body fat mass and liver steatosis have been reported in mice fed diets containing the conjugated linoleic acid trans-10,cis-12-C<sub>18:2</sub> (CLA2), but not the cis-9,trans-11-C<sub>18:2</sub> (CLA1). Since the alteration of fatty acid (FA) oxidation may cause fat accumulation, the question has been raised about the role of either CLA on enzyme activities and expressions related to liver FA oxidation. In C57BL/6j mice fed a diet containing 1% of CLA2 for 4 weeks, the percentages of this isomer in total FA of liver lipids were far lower than those of CLA1 in CLA1-fed ones under the same experimental conditions. The mitochondrial protein content per g liver was similar in either CLA-treated or control mice, but 1.8 fold greater in CLA2-fed mice when taking into account the total organ mass. Mitochondrial carnitine palmitoyltransferase I (CPT I) and carnitine-dependent palmitate oxidation activities were clearly greater in CLA2fed mice than in control and CLA1-fed ones. The sensitivity of CPT I to malonyl-CoA inhibition was hardly more marked in the CLA2-series than in the CLA1-one, with the amounts of malonyl-CoA per g tissue being practically similar in either CLA-series. Peroxisomal FA oxidation-related activities were increased in CLA1fed mice and to a greater extent in CLA2-fed ones. Concomitantly to the increased mitochondrial and peroxisomal FA oxidation activities found in CLA2-fed mice, an induction of mRNA expression of CPT I and acyl-CoA oxidase was also observed. The almost total disappearance of CLA2 from liver lipids and the total absence of impairments of FA oxidation capacities after CLA2 ingestion suggested that the liver steatosis was not likely to be due to low FA oxidation

rates, but rather to events initially occurring in extrahepatic tissues.

Dietary nitrogen transfer to metabolic pools and urea recycling after protein ingestion: a compartmental analysis. B Juillet, H Fouillet, C Bos, F Mariotti, C Gaudichon, D Tomé (Nutrition Physiology, INRA-INAPG, 16 rue C.

Bernard, 75005 Paris, France).

A 13-compartment model was developed to simulate the postprandial distribution of dietary nitrogen (N) in humans. It was built from the measurement of dietary N kinetics in different protein pools after the ingestion of a single mixed meal, either liquid and containing <sup>15</sup>Nlabeled milk (LM) or soy (LS) protein, or solid and containing <sup>15</sup>N-labeled wheat (SW) protein. The model integrated 6 sampled compartments (ileal effluents, plasma proteins and amino acids, body urea, urinary urea and ammonia) and 7 compartments not experimentally monitored. It enabled the simulation of the gastric emptying, intestinal absorption and subsequent partition kinetics of dietary N in both splanchnic and peripheral areas. After LM, LS and SW ingestion, the dietary N gastric emptying half-time reached 90 min, 70 min and 130 min, respectively. Splanchnic retention of dietary N decreased from 42% and 44% to 39% of ingested N 8 h after LM, LS and SW, respectively, while its peripheral retention reached 25%, 23% and 23%. Total (ileal and deamination) dietary N losses increased from 26% to 30% and 34% of ingested N 8 h after LM, LS and SW, respectively. Compared to LM, LS led to a faster conversion of dietary N to splanchnic amino acids, which induced a simultaneous increase in deamination and protein synthesis in the splanchnic bed. Moreover, dietary urea N reincorporated into splanchnic amino acids reached 1%, 7% and 10% of ingested N 8 h after LM, LS or SW. respectively. This entero-hepatic recycling may constitute an important N-sparing mechanism increasing with the deamination level of the dietary protein. This model provides a useful, explanatory tool describing the regional metabolic utilization of dietary proteins in humans under different nutritional and physio-pathological conditions. Its further development may require additional experimental data to distinguish between the relative contributions of the

gut and liver to the splanchnic metabolism of dietary N.

Signalling pathways involved in the activation of chicken muscle p70 S6 kinase (p70S6k). S Tesseraud, P Vaudin, K Bigot, J Dupont (INRA Tours, 37380 Nouzilly, France).

The Ser/Thr kinase p70S6k is a key enzyme involved in the control of protein synthesis. We have previously shown that this enzyme is activated by refeeding and insulin injection in chicken muscle despite a relative insulino-resistance in the early steps of insulin receptor signalling in this tissue. The aim of the present study was to further investigate the regulation of the PI3'-kinase (Phosphatidylinositol 3 kinase)/ Akt/p70S6k pathway in chicken muscle. Threeweek-old chickens were assigned to the following nutritional states: food-deprived for 16 h, refed for 30 min or intravenously injected with insulin (n = 4-5). The expression and/or the phosphorylation of p70S6K and Akt on Ser/Thr residues and PTEN were measured by Westernblot on muscle lysates. PI3'-kinase activity was determined after immunoprecipitation with an anti-phospho-Tyr antibody in the presence of labelled P33 ATP and a PI3'-kinase substrate. Statistical analysis was performed using ANOVA. Refeeding as insulin treatment (1 UI·kg<sup>-1</sup>) increased p70S6k phosphorylation on Thr389, Thr229 and Thr421/Ser424 (by 3 to 10-fold according to the residues, P < 0.05), as well as Akt phosphorylation on Ser473 (by 5-fold, P <0.01). Conversely, PI3'-kinase activity was not modified, whatever the treatment. Western blot analysis of proteins immunoprecipitated with an antibody directed against the regulatory subunit of PI3' kinase (p85) shows for the first time in vivo an association between the p85 and p70S6k. The function of this complex p85/ p70S6K remains to be determined. Lastly, the PTEN lipid and protein phosphatase, which is a potential inhibitor of the PI3' kinase pathway, is expressed in chicken muscle. In conclusion, the Akt-p70S6k pathway is activated by refeeding and insulin injection without stimulation of the PI3'-kinase. These results indicate some particularities of the insulin signalling in chicken muscle and suggest the involvement of pathways different from those described in mammals.

Phloridzin prevents ovariectomy/ inflammation – induced bone loss in rats. C Puela, J Matheya, C Obledb, A Mazura, MJ Daviccoa, P Lebecquea, MN Horcajadaa, V Coxama (a Unité des Maladies Métaboliques et Micronutriments, INRA Theix, 63122 Saint-Genès-Champanelle, France; b Unité du Métabolisme Protéique, INRA Theix, 63122 Saint-Genès-Champanelle, France).

Ageing and sex hormone related changes lead to inflammatory and oxidant conditions, which are involved in the pathogenesis of osteoporosis. We set up an animal model to mimic the influence of advancing age on osteopenia. Besides, recent studies have suggested that polyphenols may exert a protective effect. Since apples are considered as a staple in the Western diet, we assessed the effect of phloridzin, a flavonoid exclusively found in these fruits. Among fortyeight 6 month-old wistar rats, 16 were shamoperated (SH). The 32 others were ovariectomized (OVX) and allocated to 2 equal groups that received 0.25% phloridzin (P) for 80 days. Three weeks before necropsy, inflammation was induced by subcutaneous injections of talc in one half of each group. At necropsy, ovariectomy, checked by atrophy of the uterine horns, decreased both total (T-BMD) and metaphyseal (M-BMD) femoral bone mineral density. Concerning inflammation, it elicited an increase of the spleen weight and alpha-1-glycoprotein concentration in OVX rats, while intact SH animals seemed to be protected. Inflammation conditions exacerbated the drop in T-BMD (g/cm<sup>2</sup>) (and M-BMD, as well) observed in castrated animals (OVXinf: 0.2109 ± 0.0018 vs. OVX:  $0.2243 \pm 0.0031$  (P < 0.05), compared to SH:  $0.2378 \pm 0.0050$ , P < 0.01). Phloridzin consumption did not elicit any uterotrophic activity. A trend in T-BMD improvement under such a diet was observed, and the efficiency reached a significant level in conditions of inflammation (Pinf:  $0.2258 \pm 0.0020$ ; P < 0.001 vs. OVXinf). M-BMD followed the same pattern. These results could be explained by changes in bone remodelling as the increased urinary deoxypyridinoline excretion (bone resorption) in OVX and OVXinf animals was prevented by the polyphenol rich diet, while plasma osteocalcin concentration (bone formation) was similar in all groups. In conclusion, inflammation exacerbates ovariectomy-induced osteopenia. Phloridzin consumption may provide protection against

this effect, by inhibiting changes in the markers of inflammation and slowing down bone resorption.

Modulation of isoflavone-bone loss prevention by fructo-oligosaccharides in the ovariectomized rat. J Mathey, C Puel, MJ Davicco, P Lebecque, MN Horcajada, V Coxam (Groupe Ostéoporose, U3M, INRA Theix, 63122 Saint-Genès-Champanelle, France).

Isoflavones (IF) have been increasingly implicated in the prevention of osteoporosis. Since their bioavailability could be improved by modulating the intestinal microflora, the present study was undertaken to investigate whether IF and fructo-oligosaccharides (Fos), which are known to increase bifidobacteria, may exhibit a cooperative bone sparing effect. This work was carried out on ninety-six 3-month-old Wistar rats assigned to 12 groups of eight rats: 2 SH (sham-operated) and 10 OVX (ovariectomized). The animals received a soy-protein-free semipurified diet for 90 d containing at 0 (OVX and SH), 10 (IF10), 20 (IF20), 40 (IF40) or 80 (IF80) μg·g<sup>-1</sup> body weight per day. Fos were orally given to half of the groups, (OVX Fos), (IF10 Fos), (IF20 Fos), (IF40 Fos), (IF80 Fos) and (SH Fos) at 2.5% the first week, 5% the 2nd week, then 7.5% for the last 10 weeks. The animals were killed on day 91. Castration, validated by uterine atrophy, induced a marked change in bone metabolism as urinary deoxypyridinoline excretion (DPD, bone resorption) and plasma osteocalcin (OC, osteoblast activity) were increased. Furthermore, osteopenia was elicited by a lower femoral failure load and bone mineral density (BMD). Besides, concerning to IF, only the highest dose (IF80) induced a weak uterotrophic activity. However, IF exhibited a bone sparing effect when consumption reached 20  $\mu g \cdot g^{-1} \cdot d^{-1}$ . As far as the FOS diet is concerned, prebiotics addition significantly raised the efficiency of the IF protective effect on both femoral BMD and mechanical properties and the lowest IF dose (IF10) even reached a significant level. In each case, this effect could be explained by a reduced bone resorption (drop in urinary DPD). In conclusion, daily IF consumption prevented ovariectomy-induced osteopenia by depressing bone resorption activity, when given at 20, 40, or 80  $\mu g \cdot g^{-1} \cdot d^{-1}$ , while the lowest dose was only

efficient when given together with Fos. Consequently, simultaneous Fos consumption may improve the IF protective effect on the skeleton, probably by modulating IF bioavailability.

Nonheme iron absorption by the pig colon. P Vaugelade<sup>a</sup>, V Robert<sup>a</sup>, D Bouglé<sup>b</sup>, F Bureau<sup>c</sup>, D Neuville<sup>c</sup>, T Loise<sup>a</sup>, M Thomas<sup>a</sup>, E Volant<sup>a</sup>, I Kibangou<sup>b</sup>, F Blachier<sup>a</sup> (<sup>a</sup> Laboratoire de Nutrition et Sécurité Alimentaire, INRA de Jouy-en-Josas, France; <sup>b</sup> Laboratoire de Physiologie Digestive et Nutritionnelle, CHU de Caen, France; <sup>c</sup> Laboratoire de Biochimie A, CHU de Caen, France).

Less than 10% of the ingested nonheme iron is absorbed by the small intestine resulting in the presence of this element in the large intestine lumen. It has been proposed that this element through its prooxidative properties can exert deleterious effects on the colon epithelium. The aim of this study was to measure the large intestine absorptive capacity for iron. An intracecal can-

ula and two catheters were surgically implanted in the portal vein and in the carotid artery of twelve Large White pigs. During meal ingestion, iron sulfate was injected into the cecum and the blood was collected during 5 hours before serum iron analysis by atomic absorption. Before iron injection, the iron concentration in aqueous phases of the luminal contents averaged 36 ±  $6 \mu M$  in the cecum,  $98 \pm 16 \mu M$  in the proximal colon and  $111 \pm 37 \,\mu\text{M}$  in the distal colon. After intracecal injection of iron sulfate at 2.5., 5.0., 10.0 and 20.0 mg of elemental iron per kg body weight, rapid increases (respectively  $3.1 \pm 1.1$ ,  $4.4 \pm 2.6$ ,  $11.5 \pm 2.3$  and  $56.4 \pm 16.9 \mu M$ ) above the basal concentration (i.e.  $14.4 \pm 1.2 \,\mu\text{M}$ ) were measured in the portal vein blood. The injection of 5.0 mg of elemental iron per kg body weight in the duodenal lumen led to a serum iron concentration which was more than 2 times higher than the one measured after intracecal injection. Lastly, Western blot analysis allowed to detect the presence of ferritin in isolated colonocytes. The colonic epithelium is thus able to absorb luminal iron even if this absorption is less ample than the one measured in the small intestine.

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