STABLE ISOTOPE METHODOLOGY AND ITS APPLICATION TO NUTRITION AND GASTROENTEROLOGY.

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Summary

This report describes the activities of the Stable Isotope Laboratory in its function as a core resource facility for stable isotope applications in human nutrition research. Three aspects are covered: training of visitors, assessment of new instrumentation, and development of new methodology. The research achievements of the laboratory are indicated in the publications that appeared during this period.

1. TECHNICAL TRAINING ACTIVITIES

The Stable Isotope Laboratory has attracted the interest of scientists from many laboratories in the United States and abroad because of the diversity of its instrumentation, the isotope technologies used, and the variety of human investigative protocols generated. The Laboratory is emerging as a leading centre for advanced training in stable isotopes.

In addition to fulfilling the programmatic research needs of the Children's Nutrition Research Centre, the Stable Isotope Laboratory accepts a limited number of candidates for specialized training under specific conditions. The requirements are:

(a) institutional, grant, or agency support for the candidate's travel and living expenses,

(b) selection of a single area of training,

(c) a time commitment of at least three months, although a 6- to 12-month commitment is preferred, and

(d) the intention to apply the techniques learned upon return to the country of origin, i.e., the appropriate instrumentation will be available and accessible.

1.1. Individuals who have completed their specialized training during this period include:

Dr. Janet Dawson, Department of Animal Nutrition, University of Nottingham, Nottingham, U.K. Dr. Dawson is investigating the effect of growth hormone in cattle on protein synthesis and fatty acid utilization.

Dr. Peter Krumbiegel, Isotope and Radiation Institute, Leipzig, Germany. Dr. Krumbiegel received an orientation on recent developments in isotope ratio measurements and stable isotope applications.

Dr. Preeya Leelahagul, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. Dr. Leelahagul was an IAEA fellow for six months learning gas chromatographic-mass spectrometric techniques for measurement of protein turnover.

Dr. Bernhard Lembke, Division of Gastroenterology, Johann-Wolfgang Goethe University, Frankfurt, Germany. Dr. Lembke received training in breath test protocols and gas-isotope-ratio mass spectrometry.

Dr. Frans Stellaard, Department of Paediatrics, University of Groningen, Groningen, The Netherlands. Dr. Stellaard initiated studies of acetate metabolism and cholesterol synthesis in vivo using gas chromatography/combustion gas-isotope-ratio mass spectrometry.

Dr. Michael Verfurth, Department of Paediatrics, University of Munich, Munich, Germany. Dr. Verfurth developed a clinical protocol to measure peptide absorption from the small intestine using uniformly ^{13}C-labelled algal proteins.

1.2. Individuals currently in training and those who have arranged for future training include:

Dr. Monica Culea, Institute of Isotopic and Molecular Technology, Cluj Napoca, Romania. Dr. Culea is a current IAEA fellow developing protocols on serine and glycine interconversion in preterm infants. She is using gas chromatography-mass spectrometry.

Ms. Jane Murphy, Department of Nutrition, University of Southampton, Southampton, U.K. Ms. Murphy is expected to begin training in gas-isotope-ratio mass spectrometry and combustion analysis of labelled materials later this year.

2. TECHNOLOGICAL INSTRUMENT AND METHOD DEVELOPMENTS

We have published a number of articles which provide additional technological information on instruments and methods related to stable isotope applications. Included are:

A comparison of two elemental analyzer-gas-isotope-ratio mass spectrometer systems in the simultaneous measurement of $^{13}C/^{12}C$ ratios and carbon content in organic samples [22].

Rapid preparation of pyrogen-free $^{2}H_{2}{}^{18}O$ for human nutrition studies [20].

A new zinc product for the reduction of water in physiological fluids to hydrogen gas for $^{2}H/^{1}H$ isotope ratios [23].

Isotopic determination of organic keto acid pentafluorylbenzyl esters in biological fluids by negative chemical ionization gas chromatography- mass spectrometry [13].

Measurement of in vivo cholesterol synthesis from $^{2}H_{2}O$: a rapid procedure for the isolation, combustion, and isotopic assay of erythrocyte cholesterol [19].

Uniformly ^{13}C-labelled algal protein used to determine amino acid essentiality in vivo [11].

VLDL apolipoprotein B-100, a potential indicator of the isotopic labelling of the hepatic protein synthetic precursor pool in humans: studies using multiple stable isotopically labelled amino acids [17].

In addition, a report of the Interlaboratory Analysis of Reference Water Samples Enriched with Deuterium and Oxygen-18, carried out through the IAEA has been submitted for publication.

3. REFERENCES AND ANNOTATIONS

[1] BOUTTON, T.W., TYRRELL, H.F., PATTERSON, B.W., VARGA, G.A., KLEIN, P.D., Carbon kinetics of milk formation in Holstein cows in late lactation, J. Anim. Sci. **66** (1988) 2636-45.

Carbon transfer to milk in Holstein cows in late lactation was measured by introducing changes in the natural stable carbon isotope composition of the feed. Six Holstein cows in mid-lactation were placed on a diet naturally low in ^{13}C(-25.0 vs Pee Dee belemnite [PDB] an international carbon isotope standard), based on alfalfa/barley, and six others were placed on a diet naturally enriched in ^{13}C (-11.5 vs PDB), based on corn. After a 7-wk equilibration period on these diets, three cows were switched from alfalfa/barley to corn, and three were switched from corn to alfalfa/barley. The three other cows in each group served as controls. $^{13}C/^{12}C$ ratios were measured in daily morning milk samples during the week before and for 6 wk after the changes in diet. After the diets had been switched, milk isotope ratios rapidly approached the isotopic composition of the new diet, indicating rapid transfer of dietary carbon into milk. The data were consistent with a model whereby milk was synthesized from a single precursor pool that responded rapidly to dietary perturbation. The milk precursor pool had a half-life of approximately 0.9 d and had a mass of approximately 7 kg of carbon, which was renewed daily by the entry of 5 kg of digestible dietary carbon.

[2] BUTTE, N.F., WONG, W.W., PATTERSON, B.W., GARZA C., KLEIN, P.D., Human milk intake measured by administration of deuterium oxide to the mother: a comparison with test-weighing, Am. J. Clin. Nutr. **47** (1988) 815-21.

A comparison was made between the dose-to-the-mother deuterium-dilution method and the conventional test-weighing technique for determining human milk intake in five exclusively breast-fed infants and in four breast-fed infants who received supplemental foods. After administration of ^{2}H to the mothers, human milk and infant urine were sampled over 14 d and analyzed for $^{2}H:^{1}H$ ratios by

gas-isotope-ratio mass spectrometry. Infant total body water was determined by ^{18}O dilution. The test-weighing procedure was conducted for 5 d consecutively. The intake of human milk (mean ± SD) estimated by ^{2}H dilution was 648 ± 84 g/d. The mean difference between the two methods was not significantly different from 0. The ^{2}H-dilution and test-weighing techniques provide similar estimates of human-milk intake.

[3] IRVING, C.S., MALPHUS, E.W., THOMAS, R.M., MARKS, L., KLEIN, P.D., Infused and ingested labelled lysines: appearance in human-milk proteins, Am. J. Clin. Nutr. **47** (1988) 49-52.

Incorporation of two labelled forms of lysine into human-milk proteins was studied in fasted lactating subjects to determine whether highly labelled proteins could be produced for subsequent nutritional studies and whether the kinetics of milk synthesis could be studied in humans with stable isotope techniques. Five subjects, maintained on formula diets, received L-[$^{13}C_1$]lysine (27 μmol/kg) as an oral bolus 4 h postprandially. Milk samples were collected at 30, 45, 90, 150, 240, and 360 min. Tracer lysine levels in the hydrolysate of unfractionated milk protein were determined by gas chromatography-mass spectrometry isotope ratiometry. After a delay of at least 45 min, significant labelling of milk protein was detected and reached a maximum at 150 min with cumulative percent dose recovery over 6 h of 0.5%. Human-milk proteins can be labelled for nutritional investigations and *in vivo* kinetics of milk protein synthesis can be studied with stable isotope techniques.

[4] WONG, W.W., COCHRAN, W.J., KLISH, W.J., SMITH, E.O., LEE, L.S., FIOROTTO, M.L., KLEIN, P.D., Body fat in normal adults estimated by isotope dilution of oxygen-18 and deuterium and by anthropometry: a comparison, Eur. J. Clin. Nutr. **42** (1988) 233-42.

We estimated body fat in 20 normal adults (10 males and 10 females) from ^{18}O- and ^{2}H-dilution spaces and from the equations of Durnin and Womersley, and Pollock, Schmidt and Jackson based on skinfold thickness measurements. Differences between methods for body fat estimation were found to be sex-dependent: subsequent analysis indicated significant differences between methods within each sex. Regardless of sex, the highest fat estimates were obtained with the ^{18}O-dilution method, followed by those obtained with the ^{2}H-dilution method or the Durnin and Womersley equation. The lowest fat estimates were obtained using the Pollock, Schmidt, and Jackson equation. The ^{18}O-dilution method and the Durnin and Womersley anthropometric method are both suitable and appropriated for body fat estimation in adults studied under field conditions.

[5] WONG, W.W., COCHRAN, W.J., KLISH W.J., SMITH, E.O., LEE, L.S., KLEIN, P.D., *In vivo* isotope fractionation factors and the measurement of deuterium- and ^{18}O-dilution spaces from plasma, urine, saliva, and respiratory water vapour, and carbon dioxide, Am. J. Clin. Nutr. **47** (1988) 1-6.

In vivo isotope-fractionation factors were determined for hydrogen and oxygen between plasma water samples and samples of urine, saliva, respiratory water

vapour, and carbon dioxide in 20 normal adults. The isotope-fractionation factors ranged from 0.944 to 1.039 for ^{2}H in breath water vapour and for ^{18}O in breath CO_2, respectively. When corrected for isotope fractionation, the ^{2}H- and ^{18}O-dilution spaces determined from urine, saliva, respiratory water, and CO_2 were within -0.10 ± 1.09 kg (mean ± SD, n = 60) and 0.04 ± 0.68 kg (n = 80), respectively, of the values determined from plasma. In the absence of these corrections, we observed a 6% overestimation of ^{2}H-dilution space and a 1% overestimation of ^{18}O-dilution space from the use of respiratory water values. A 4% underestimation of the ^{18}O-dilution space was observed for breath CO_2 without correction for isotope fractionation.

[6] MOTIL, K.J., MONTANDON, C.M., HACHEY, D.L., BOUTTON, T.W., KLEIN, P.D., Whole body protein metabolism in lactating and nonlactating women, J. Appl. Physiol. **66(1)** (1989) 370-6.

The adaptive responses of body protein metabolism to lactation were characterized in women at 1, 5, and 12 mo postpartum and in nulliparous controls during a controlled diet of measured protein and energy intakes by nitrogen balance, a constant infusion of [^{13}C]bicarbonate, and a primed constant infusion of [1-^{13}C]leucine and [α-^{15}N]lysine. Dietary energy intakes in the lactating women were 27% greater than those in the nulliparous controls. Despite these differences, lactating women had significantly lower nitrogen balances compared with the nonlactating women (4.0 ± 37.8 vs +44.7 ± 30.8 mg·kg^{-1}·day^{-1}). No significant differences in amino acid flux, oxidation, or incorporation into protein were detected during fasting conditions in the two groups of women. However, significantly positive associations were noted between dietary intakes and the variables of protein metabolism in the lactating women. A more complete understanding of the mechanisms that regulate the disposition of dietary nutrients into maternal body stores or milk production will enhance the determination of nutrient requirements in lactating women.

[7] WONG, W.W., SHENG, H-P., MORKEBERG, J.C., KOSANOVICH, J.L., CLARK, L.L., KLEIN, P.D., Measurement of extracellular water volume by bromide ion chromatography, Am. J. Clin. Nutr. **50** (1989) 1290-4.

Extracellular body water can be determined from plasma bromide dilution. Plasma Br is separated from other anions by ion chromatography and is detected at an ultraviolet wavelength of 210 nm. Plasma proteins are removed by ultrafiltration, and interference by plasma chloride is minimized by dilution and the use of 5 mmol NaCl/L as the eluent. Human plasma samples were spiked with known quantities of Br (between 37.54 and 125.14 μmol/L) and were measured by ion chromatography. The results were reproducible to within 0.72 μmol/L (SD) and differed from the gravimetric values by -1.88 ± 4.27 μmol/L (mean ± SD). The difference, however, was not significantly different from 0 ($p = 0.19$). Extracellular water volumes of 10 newborn minipigs measured by Br dilution by using the chromatographic technique (400 ± 63 mL/kg) were comparable with literature values reported for premature infants.

[8] BUTTE, N.F., WONG, W.W., FERLIC, L., SMITH, E.O., KLEIN, P.D., GARZA, C., Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy, Pediatr. Res. **28** (1990) 631-40.

The energy intake, expenditure, and deposition of 40 breast-fed and formula-fed infants were investigated at 1 and 4 mo of age to explore possible differences in energy utilization between feeding groups. Energy intake was calculated from 5-d test-weighing records or pre- and post-weighing of formula bottles, in combination with bomb calorimetry of the milks. Total daily energy expenditure (TDEE) was determined by the doubly labelled water method. Sleeping metabolic rate (SMR) and minimal observable energy expenditure were measured by indirect calorimetry. Activity was estimated as the difference between TDEE and SMR. Energy deposition was estimated from dietary intake and TDEE. Energy intakes were significantly higher for the formula-fed than breast-fed infants at 1 mo (118 ± 17 vs 101 ± 16 kcal/kg/d) and 4 mo (87 ± 11 vs 72 ± 9 kcal/kg/d) ($p < 0.001$). TDEE averaged 67 ± 8 and 64 ± 7 kcal/kg/d at 1 mo and 73 ± 9 and 64 ± 8 kcal/kg/d at 4 mo for the formula-fed and breast-fed infants, respectively, and differed between feeding groups ($p < 0.005$). The energy available for activity and the thermic effect of feeding did not differ between feeding groups. Rates of weight gain (g/d) and energy deposition (kcal/kg/d) tended to be greater among the formula-fed infants at 1 and 4 mo ($p < 0.06$). Differences in weight gain, energy deposition, SMR, minimal observable energy expenditure, and TDEE partially accounted for the discrepancy in energy intake observed between breast-fed and formula-fed infants. The response to the varying levels of energy intake in infancy appear to be mediated through growth and basal-energy-requiring processes, but not through physical activity.

[9] MURRAY, R.D., BOUTTON, T.W., KLEIN, P.D., GILBERT, M., PAULE, C.L., MACLEAN, W.C. Jr., Comparative absorption of ^{13}C-glucose and ^{13}C-lactose by premature infants, Am. J. Clin. Nutr. **51** (1990) 59-66.

Oxidation of orally administered [^{13}C] glucose and [^{13}C]lactose and faecal recovery of malabsorbed substrates were determined in two groups of premature infants. Eighteen studies were performed with six infants at Johns Hopkins Hospital (JHH); 24 studies were performed with nine infants at Columbus Children's Hospital (CCH). The two groups differed in that JHH infants had shorter gestations but were older when studied. Faecal ^{13}C loss after [^{13}C]glucose administration did not differ between the two groups. Compared with glucose, the metabolism of lactose appeared to involve more malabsorption and colonic fermentation in JHH infants than in CCH infants and resulted in higher faecal losses of substrate carbon. Maturation appeared to involve increased proximal intestinal absorption and greater retention of absorbed carbohydrate. Simultaneous absorption of substrate from the small and large intestine may limit the usefulness of breath tests for ^{13}C in the premature infant.

[10] WONG, W.W., BUTTE, N.F., GARZA, C., KLEIN, P.D., Comparison of energy expenditure estimated in healthy infants using the doubly labelled water and energy balance methods, Eur. J. Clin. Nutr. **44** (1990) 175-84.

The doubly labelled water method was used to estimate energy expenditure in 20 formula-fed infants (10 aged 1 month and 10 aged 4 months). We then compared the energy expenditure values with energy balance values calculated from energy intake and energy cost of growth. Our purpose was to compare various published equations for calculating CO_2 expiration rates (and thus energy expenditure values) from the isotopic data. Those equations in which we used measured values for ^{18}O and ^{2}H isotope dilution spaces and estimated or measured values for insensible water losses yielded energy expenditure values (69.7 ± 8.4 kcal/kg/d) that agreed most closely with energy balance data (70.3 ± 11.9 kcal/kg/d). Equations in which we used a constant ratio of 1.03 between the ^{2}H and ^{18}O isotope dilution spaces resulted in energy expenditure values (66.3 ± 10.3 kcal/kg/d) lower than those predicted by the energy balance data. Data analysis by nonlinear curve fitting compared to logarithmic transformation did not alter the estimates of energy expenditure obtained in these infants.

[11] BERTHOLD, H.K., HACHEY, D.L., REEDS, P.J., THOMAS, O.P., HOEKSEMA, S., KLEIN, P.D., Uniformly ^{13}C-labelled algal protein used to determine amino acid essentiality in vivo, Proc. Natl. Acad. Sci. USA **88** (1991) 8091-5.

The edible alga *Spirulina platensis* was uniformly labelled with ^{13}C by growth in atmosphere of pure $^{13}CO_2$. The labelling biomass was then incorporated into the diet of a laying hen for 27 days. The isotopic enrichment of individual amino acids in egg white and yolk proteins, as well as in various tissues of the hen at the end of the feeding period, was analyzed by negative chemical ionization gas chromatography/mass spectrometry. The amino acids of successive eggs showed one of two exclusive enrichment patterns: complete preservation of the intact carbon skeleton or extensive degradation and resynthesis. The same observation was made in tissue proteins. These patterns were cleanly divided according to known nutritional amino acid essentiality/nonessentiality but revealed differences in labelling acids: most notable was that proline accretion was derived entirely from the diet. Feeding uniformly ^{13}C-labelled algal protein and recovering and analyzing *de novo*-synthesized protein provides a useful method to examine amino acid metabolism and determine conditional amino acid essentiality *in vivo*.

[12] BUTTE, N.F., WONG, W.W., KLEIN, P.D., GARZA, C., Measurement of milk intake: tracer-to-infant deuterium dilution method, Br. J. Nutr. **65** (1991) 3-14.

The tracer-to-infant deuterium dilution method for the measurement of milk intake was evaluated in twenty breast-fed and twenty formula-fed infants. The isotope method was compared with conventional direct-weighing techniques. Human milk intake was assessed by 5 d test-weighing. Intakes of formula, supplemental foods, and water were determined by pre- and post-weighing of feeding bottles. An oral dose of 200 mg $^{2}H_2O$/kg body-weight was given to each infant, and urine was sampled daily for 14 d. ^{2}H enrichment of the urine was measured by gas-isotope-ratio mass spectrometry. Milk intakes estimated from the deuterium dilution method were consistently higher than those from direct-weighing; the mean difference between methods was 106 (SD 47)g/d or 14% for the breast-fed group and 70(SD 155) g/d or 8% for the formula-fed group. Estimates of intake for some infants varied substantially between the two methods of measurement. When the estimated values of human milk intake were corrected for environmental water

influx and insensible water loss during breastfeeding, the relative bias decreased to 5%. Correction of the estimated values of formula intake for environmental water influx decreased the relative bias to 1-2%. The acceptability of the deuterium dilution method to determine milk intake depends on the goals and the tolerance for error in group and individual intake estimates of a given study.

[13] HACHEY, D.L., PATTERSON, B.W., REEDS, P.J., Isotopic determination of organic keto acid pentafluorobenzyl esters in biological fluid by negative chemical ionization gas chromatography/mass spectrometry, Anal. Chem. **63** (1991) 919.

A rapid, single-step procedure for the extraction and derivatization of organic α-keto acids from microlitre quantities of human plasma has been developed. The keto acids were analyzed as the pentafluorobenzyl (PFB) ester by methane negative chemical ionization gas chromatography/mass spectrometry. The PFB esters possess excellent chromatographic properties and required no further derivation to block the keto group. They fragment to produce intense carboxylate anions, often as the sole ion in the spectrum, and offer detection limits below 1 pmol. This derivative is suitable for isotopic analysis of organic keto acids because it does not introduce any additional isotopic complexity into the target molecule. Normal human plasma 4-methyl-2-oxopentanoic acid levels were 34.9 $\pm$ 5.3 μmol·L^{-1} and could be determined with 1.1% precision by isotope dilution GC/MS. We have used this procedure to study leucine and 4-methyl-2-oxopentanoic acid metabolism by using stable isotopically labelled tracers in a variety of normal and abnormal conditions.

[14] KLEIN, P.D., GASTROINTESTINAL PHYSIOLOGY WORKING GROUP, GRAHAM, D.Y., GAILLOUR, A., OPEKUN, A.R., SMITH, E.O., Water source as risk factor for *Helicobacter pylori* infection in Peruvian children, Lancet **337** (1991) 1503-1506.

Helicobacter pylori infection is widespread among Peruvian adults by age 30, but the age at which children become infected, the prevalence of disease, and the role of socioeconomic status in the epidemiology of infection are not known. We used the ^{13}C-urea breath test to study the prevalence of infection in 407 Peruvian children from Lima, aged 2 months to 12 years, from families of low and high socioeconomic status. Peruvian children acquire *H pylori* early in life and the number of infected individuals increases rapidly with age; overall prevalence was 48%. *H pylori* infection was independent of sex, but was highly correlated with socioeconomic status; prevalence of infection was higher among children from low-income families than from high-income families (56% vs 32%, $p = 0.001$). Children whose homes had external water sources were three times more likely to be infected than were those whose homes had internal water sources. Among families with internal water sources, there was no difference in *H pylori* infection associated with income. Children from high-income families whose homes were supplied with municipal water were 12 times more likely to be infected than were those from high-income families whose water supply came from community wells. The findings show that the prevalence of *H pylori* infection is high among young Peruvian children and that the municipal water supply seems to be an important source of infection among Lima children from families of both low and high socioeconomic status.

[15] LIFSCHITZ, C.H., TORUN, B., CHEW, F., BOUTTON, T.W., GARZA, C., KLEIN, P.D., Absorption of carbon ^{13}C-rice in milk by infants during acute gastroenteritis, J. Pediatr. **118** (1991) 526-30.

To determine whether rice cereal could be used to complement a cow milk-based diet in the nutritional management of infants with acute diarrhoea, we assessed its digestion and absorption in 8 affected male infants, 69 to 131 d old. They received cow milk formula with 5.4% lactose (diluted 1:1 with water and precooked rice cereal) 5 to 22 h after admission and rehydration. The first feeding consisted of milk diluted with ^{13}C-enriched rice cereal. A 48-h faecal collection and balance study was performed. Rice cereal was reasonably well absorbed (84.0% to 95.8%) by 7 of the 8 infants. The study was repeated in 7 of the infants after they had recovered. Our results indicate that rice cereal is well absorbed by young infants with a acute diarrhoea and that it is an adequate nutrient supplement for this patient population.

[16] MALATY, H.M., GRAHAM, D.Y., KLEIN, P.D., EVANS, D.G., ADAM, E., EVANS, D.J., Transmission of *Helicobacter pylori* infection. Studies in families of healthy individuals, Scand. J. Gastroenterol. **26** (1991) 927-932.

Helicobacter pylori is accepted as the commonest cause of type-B gastritis. Detailed information about the mode of transmission remains scanty. We investigated the frequency of *H. pylori* infection within families, defined as consisting of a husband and wife with at least one biologic child, all living in the same household. Inclusion criteria required that both the parents and the children had been born in the United States, had used no antibiotic or bismuth for the previous 2 months, had no recent major illness or surgical operation, and had no symptoms referable to the upper gastrointestinal tract. *H. pylori* infection was identified with a ^{13}C-urea breath test and an enzyme-linked immunosorbent assay for anti-*H. pylori* IgG. Forty-one families (151 healthy individuals) were enroled. Before the results of the *H. pylori* tests were known, one parent was selected as the index subject. *H. pylori* infection clustered; that is, 68% of spouses of *H. pylori*-infected index subjects were also *H. pylori*-infected, compared with 9% of spouses of *H. pylori*-negative index subjects ($p < 0.0001$). The children of infected index parents were also more likely to be infected than children of uninfected index parents—40% versus 3%, respectively ($p < 0.0001$)—and the results in the children were independent of whether the father or the mother was the index subject. Clustering of *H. pylori* infection within families suggests person-to-person transmission or common source exposure. The high frequency of *H. pylori* infection in spouses suggests that genetic factors are less important than living conditions of transmissions of *H. pylori* infection.

[17] PATTERSON, B.W., HACHEY, D.L., COOK, G.L., AMANN, J.M., KLEIN, P.D., Incorporation of a stable isotopically labelled amino acid into multiple human apolipoproteins, J. Lipid Res. **32** (1991) 1063-72.

Procedures are presented for the separation and determination of the isotopic enrichment of multiple human apolipoproteins labelled *in vivo* with a stable isotope amino acid. The isotopic enrichments of plasma lysine and plasma apolipoproteins were monitored for 16 days after a single intravenous dose of [4,4,5,5-$^{2}H_{4}$]lysine

(5 mg/kg body weight). The use of a multiply deuterated amino acid enabled the measurement of isotopic enrichments above background over the entire 16-day time course in all proteins. Individual apolipoproteins were separated on a specially designed gradient sodium dodecyl sulphate polyacrylamide gel electrophoresis system cast in a conventional slab gel apparatus which resolved apoB-100, apoE, apoA-I, apoA-II, apoC-I, apoC-II, apoC-III-1 and apoC-III-2 on a single gel. After staining with Coomassie blue, proteins bands (containing 5 to 30 μg of individual apolipoprotein) were excised from the gel. Amino acids were recovered from hydrolysed gel slices, derivatised, and analyzed by gas chromatography/mass spectrometry for determination of lysine isotopic enrichments. The utility of the method is demonstrated using examples of apolipoproteins B-100, A-I, A-II, C-I, C-II, and C-III from either total plasma $d < 1.21$ g/ml lipoproteins or selected lipoprotein subfractions. Lysine isotopic enrichments of proteins were generally determined with a precision of better than 5%. The isotopic enrichment profiles were consistent with literature reports of apolipoprotein metabolic kinetics based on the use of radioiodinated apolipoproteins. The procedures outlined can be used to separate and measure the isotopic enrichment of virtually any apolipoprotein from any chosen lipoprotein fraction. Thus, these procedures should find wide application in the study of apolipoprotein metabolic kinetics.

[18] THOMAS, M.R., IRVING, C.S., REEDS, P.J., MALPHUS, E.W., WONG, W.W., BOUTTON, T.W., KLEIN, P.D., Lysine and protein metabolism in the young lactating women, Eur. J. Clin. Nutr. **45** (1991) 227-242.

Five lactating and five postpartum nonlactating women of similar ages, times postpartum, body weight and height consumed a liquid formula diet that supplied 1.3 g protein and 32 kcal/kg per day (lactating subjects) and 1.1 g protein and 26 kcal/kg per day (nonlactating subjects). Their last meal supplied 25% of the daily intake and was consumed 4 h before they received L-[$^{13}C_1$]lysine (27 μmol/kg) by a single intravenous injection and L-[$^{15}N_2$]lysine (27 μmol/kg) orally. Frequent plasma and breath samples were collected for 6 h during which time they consumed no food. On a separate day, subjects received $NaH^{13}CO_3$ (10 μmol/kg) as a single intravenous dose and breath samples were collected for 6 h. Plasma tracer lysine levels were determined by gas chromatography-mass spectrometry isotope ratiometry, and breath $^{13}CO_2$ levels were measured by gas-isotope-ratio mass spectrometry. Averaged tracer data for the two groups were fitted to a multicompartmental model of lysine and protein metabolism which partitioned lysine kinetics between a central and two tissue compartments. The tissue compartments had characteristically fast and slow rates of lysine turnover. The results were compared with those previously obtained in nulliparous women. The postpartum state was associated with a reduction in protein turnover in a compartment with a rapid rate of protein turnover and postpartum women catabolized significantly less lysine than nulliparous controls. Lactating women catabolized slightly more lysine than the nonlactating postpartum subjects, especially when lysine catabolism was expressed as a proportion of lysine flux. Lactation was associated with smaller splanchnic and extracellular pools of free lysine and with an increase in the rate constant for absorption of orally-administered lysine. Lysine flux was significantly lower in the lactating subjects and this was associated with a decrease in the rate of lysine turnover in the slowly turning over lysine compartment. The results suggest that lactation is associated with a slower rate of protein turnover in a

peripheral tissue compartment. We conclude that an in take of 1.3 g protein/kg per day may be inadequate to support the protein needs of lactation and body protein metabolism and may result in metabolic adaptations that maintain lactation at the potential expense of other aspects of maternal protein turnover.

[19] WONG, W.W., HACHEY, D.L., FESTE, A., LEGGITT, J., CLARKE, L.L., POND, W.G., KLEIN, P.D., Measurement of *in vivo* cholesterol synthesis from ^2H_2O: a rapid procedure for the isolation, combustion, and isotopic assay of erythrocyte cholesterol, J. Lipid Res. **32** (1991) 1049-1056.

A rapid preparative scale purification of erythrocyte free cholesterol has been developed for measurements of *in vivo* cholesterol synthesis from ^2H_2O. The quantity and purity of cholesterol obtained is suitable for combustion, zinc reduction of the water formed, and determination of deuterium isotopic content by gas isotope ratio mass spectrometry. The ability to detect and to quantitate a range of cholesterol synthesis rates is illustrated by measurements on young pigs receiving diets without and with added dietary cholesterol.

[20] WONG, W.W., LEGGITT, J.L., CLARKE, L.L., KLEIN, P.D., Rapid preparation of pyrogen-free $^2H_2{}^{18}O$ for human nutrition studies, Am. J. Clin. Nutr. **53** (1991) 585-6.

We described a compact ultrafiltration system for the removal of pyrogens and bacteria from water labelled with the stable isotopes of deuterium and oxygen-18. The ultrafiltration system is constructed from readily available commercial components and can achieve complete removal of pyrogens and bacteria from 1 L contaminated water within 30 min. By use of our procedure loss of the isotopically labelled water by retention in the filtration system was minimal. The purified water is suitable for both oral and intravenous administration to healthy human subjects participating in nutrition studies.

[21] REEDS, P.J., HACHEY, D.L., PATTERSON, B.W., MOTIL, K.J., KLEIN, P.J., VLDL-Apolipoprotein B-100, a potential indicator of the isotopic labelling of the hepatic protein synthetic precursor pool in humans: studies with multiple stable isotopically labelled amino acids, J. Nutr. **122** (1992) 457-66.

Four adult men received a 48-h constant intravenous infusion of [$^2H_4$]lysine, [$^2H_3$]leucine, L-[ring-$^{13}C_6$]phenylalanine, and L-[1,2,3,-$^{13}C_3$]alanine. Subjects ingested hourly meals for two 12-h periods, separated by two 12-h fasting periods. The isotopic enrichments of free amino acids in venous plasma and in VLDL-apolipoprotein B-100 (apoB)-bound amino acids, plasma α-keto isocaproic acid α-KIC) and plasma pyruvic acid (PYR) were measured by negative chemical ionization gas chromatography-mass spectrometry. By 7 h of infusions, all four amino acids achieved an equilibrium isotopic enrichment (EIE) in plasma and in apoB. In the fed state, the EIE of the amino acids in apoB was lower than that in plasma free amino acids. The ratio EIE-apoB:EIE-plasma differed significantly among amino acids in the fed state (alanine 0.30; lysine 0.64; leucine the EIE-apoB:EIE-plasma ratio rose significantly compared with the fed state (alanine 0.38; lysine 0.73; leucine 0.94; phenylalanine 1.05). Plasma PYR and apoB-alanine were in isotopic equilibrium irrespective of nutritional state. The

EIE-apoB-leucine:EIE-plasma-α-KIC ratio rose from 0.75 in the fed state to near 1 in the postabsorptive state. We conclude that the contribution of systemic amino acids to apoB-100 synthesis is sensitive to nutritional state, and that systemic essential amino acids seem to be preferentially incorporated into apoB.

[22] WONG, W.W., CLARKE, L.L., JOHNSON, G.A., LLAURADOR, M., KLEIN, P.D., Comparison of two elemental-analyzer gas-isotope-ratio mass spectrometer systems in the simultaneous measurement of $^{13}C/^{12}C$ isotope ratios and carbon content in organic samples, Anal. Chem. **64** (1992) 354-8.

Two commercial elemental-analyzer gas-isotope-ratio mass spectrometer systems (Finnigan Heraeus-Delta E and VG Carlo Erba-PRISM) were evaluated for their ability to analyze $^{13}C/^{12}C$ isotope ratios ($\delta_{PDB}{}^{13}C$, -30.71-1433.67) and carbon content (36-100%) in a variety of organic samples (isotope standards, chemical standards, agricultural products, and physiological fluids). On average, the VG system produced better agreements with the accepted values than did the Finnigan system. Significant memory effects were observed in the Finnigan system but were negligible in the VG instrument. The minimal sample size necessary to produce acceptable $\delta_{PDB}{}^{13}C$ values was 0.05 mg of C in the Finnigan system and 0.03 mg of C in the VG system. Liquid nitrogen consumption by the Finnigan instrument was 4 times greater than that by the VG system. In our hands, the VG system is superior to the Finnigan system for carbon isotope ratio and content measurement.

[23] WONG, W.W., CLARKE, L.L., LLAURADOR, M., KLEIN, P.D., A new zinc product for the reduction of water in physiological fluids to hydrogen gas for $^{2}H/^{1}H$ isotope ratio measurements, Eur. J. Clin. Nutr. **46** (1991) 69-71.

When deuterium oxide ($^{2}H_2O$) is used for studies of body composition, energy expenditure, milk volume production, and lipid metabolism, measurements of ^{2}H enrichments in physiological fluids, e.g., urine, plasma, saliva or breastmilk must be accurate and precise. Measurements of ^{2}H abundances by gas-isotope-ratio mass spectrometry require that the water in physiological fluids be converted to hydrogen gas. The only zinc reagent that performed well in this method (AnalaR® zinc) has come from a single source, British Drug House Hopkins and Williams, who no longer produce AnalaR® zinc. We compared the hydrogen-isotope-ratio measurements in samples of 7 international water standards and 4 physiological fluids made using a new zinc reagent from the Biogeochemical Laboratory at Indiana University with measurements made using AnalaR zinc shot, the previous standard. Our results indicate that accurate and precise $^{2}H/^{1}H$ ratio measurements are obtained with the new zinc reagent prepared by the Biogeochemical Laboratory at Indiana University.