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Biochemistry and nutrition

VITAMIN D OF HUMAN MILK: IDENTIFICATION OF BIOLOGICALLY ACTIVE FORMS. L.E. Reeve, R.W. Chesney, H.F. DeLuca (Dept. of Pediatrics, Univ. of Wisconsin Med. Schl. and the Dept. of Biochem., Univ. of Wisconsin-Madison, Madison WI 53706) *Am. J. Clin. Nutr. 36*(1):122-126 (1982). Human milk has been found to contain 40 to 50 IU/l of vitamin D activity. This was determined by measuring stimulation of intestinal calcium transport in the rat, an assay not subject to the errors inherent in the rat line test or calcification assay. Five vitamin D metabolites were than isolated using a combination of conventional chromatography on Sephadex LH-20 and Lipidex 5000 fillowed by high-performance liquid chromatography. 24,25-Dihydroxyvitamin D and 1,25-dihydroxyvitamin D were measured using binding protein assays and were found to be present at very low levels. These dihydroxylated metabolites do not contribute significantly to the total vitamin D activity. Vitamins D_2 and D_3 were found to be present at concentrations of 338 and 41 ng/l, respectively. This is equivalent to 14 to 16 IU/l of vitamin D activity. Human milk contains 163 ng/l of 25-hydroxyvitamin D_3, which gives about 33 IU/l of vitamin D activity. Thus 25-hydroxyvitamin D_3 accounts for about 75% of the biological activity observed in the calcium transport assay. Vitamin D_2, vitamin D_3, and 25-hydroxyvitamin D_3 are responsible for more than 90% of the total vitamin D activity present. This fails to support the idea that vitamin D-sulfate or any other unknown metabolites of vitamin D provide significant vitamin D activity in human milk.

ARACHIDONIC ACID METABOLISM IN GUINEA PIG SKIN. T. Ruzicka and M.P. Printz (Div. of Pharmacology, M-013, Dept. of Med., Univ. of California, San Diego, La Jolla, CA 92093) *Biochem. Biophys. Acta 711*(3):391-397 (1982). Studies were conducted to examine the metabolism of radioactively labelled arachidonic acid via the lipoxygenase and cyclooxygenase pathways and the metabolic conversions of radioactively labelled prostaglandin H_2 in the epidermal and dermal layers of the guinea-pig skin. Arachidonic acid was metabolized preferentially via lipoxygenase to hydroxyeicosatetraenoic acid (HETE). The major product of the cyclooxygenase pathway was prostaglandin D_2; prostaglandin E_2 was formed in lesser amounts. Epidermis exhibited much higher activities of these enzymes on a milligram protein basis than the dermis. In contrast, both skin layers showed the same very high activity of GSH-dependent prostaglandin H_2/prostaglandin D_2 isomerase; prostaglandin D_2 was virtually the only product formed by skin homogenates from prostaglandin H_2. Guinea-pig skin is a highly active site of arachidonic acid metabolism. These findings will provide the basis for pathobiochemical studies in inflammatory and hyperproliferative dermatoses.

THE MECHANISM OF α-KETOISOCAPROATE OXYGENASE. FORMATION OF β-HYDROXYISOVALERATE FROM α-KETOISOCAPROATE. P.J. Sabourin, L.L. Bieber (Dept. of Biochem., Michigan State Univ., East Lansing, MI 48824) *J. Biol. Chem. 257* (13):7468-7471 (1982). A soluble α-ketoisocaproate oxygenase from rat liver catalyzes the decarboxylation and hydroxylation of α-ketoisocaproate to form β-hydroxyisovalerate. The source of oxygen (O_2 or H_2O) enzymatically incorporated into β-hydroxyisovalerate was investigated using $^{18}O_2$ and $H_2{}^{18}O$. Greater than 92% of the carboxyl groups of β-hydroxyisovaleric acid contained 1 ^{18}O atom from $^{18}O_2$ and 15% of the β-hydroxyl oxygens of β-hydroxyisovaleric acid contained ^{18}O from $^{18}O_2$. Since some oxygen of the β-hydroxyl group is derived from O_2 and since others have shown a rapid $H_2O \longleftrightarrow ROH$ exchange for similar reactions, we conclude that both of the oxygens of β-hydroxyisovaleric acid are derived from O_2 and that exchange of water oxygen with the β-hydroxyl group of β-hydroxyisovaleric acid must occur with an intermediate of the reaction. Thus, the α-ketoisocaproate oxygenase would be a dioxygenase. A mechanism consistent with the ^{18}O experiments and other properties of the enzyme is proposed.

CHANGES IN VITAMIN A STATUS AFTER ACUTE ETHANOL ADMINISTRATION IN THE RAT. M. Sato and C.S. Lieber (Lab. of Liver Disease and Nutr. and Alcohol, Res. and Treatment Center, Bronx Veterans Admin. Med. Center and Mt. Sinai Schl. of Med. (CUNY), New York, NY 10468) *J. Nutri. 112*(6):1188-1196 (1982). To evaluate the effect of acute ethanol administration on vitamin A and retinol-binding protein (RBP) status, male Sprague-Dawley rats weighing 120-200 g were given an acute dose of ethanol (6 g/kg body weight, orally) or saline after a 15- to 18-hour fast. Hepatic vitamin A was decreased by 13% ($p<0.025$) 24 hours after ethanol administration. Serum vitamin A was increased 6 hours after the ethanol dose ($p<0.005$) with a 10-fold increase in retinyl ester concentration, whereas serum RBP was slightly decreased. Saline controls showed no changes. The increase in serum retinyl esters 6 hours after the ethanol dose was found in the lipoprotein fraction (density<1.21). When lipoprotein removal from plasma was blocked by Triton WR-1339, ethanol administration further enhanced serum retinyl ester concentration. In rats previously given [^{14}C]retinol, hepatic ^{14}C-labeled vitamin A was decreased, whereas in the kidney and adipose tissue it was increased after 24 hours after ethanol administration. Thus, an acute dose of ethanol increases serum vitamin A and decreases hepatic vitamin A, most likely because of increased release from the liver or decreased uptake by the liver of retinyl esters as part of the lipoproteins.

IDENTIFICATION OF DOMAINS OF PHOSPHATIDYLCHOLINE IN HUMAN ERYTHROCYTE PLASMA MEMBRANES. S.D. Shukla and D.J. Hanahan (Dept. of Biochem., The Univ. of Texas Health, Sci. Center at San Antonio, San Antonio, TX 78284) *J. Biol. Chem. 257*(6):2908-2911 (1982). Highly purified acidic (pI 4.9) and basic (pI 8.7) phospholipases A_2 from snake (*Agkistrodon halys blomhoffii*) venom hydrolyzed approximately 20% and 60%, respectively, of the phosphatidylcholine (PC) of intact human erythrocytes prior to hemolysis. Sequential use of the acidic enzyme followed by the basic phospholipase A_2 or vice versa manifested a characteristic PC hydrolysis pattern. For example, when acidic enzyme had hydrolyzed nearly 20% of this substrate, a subsequent treatment with the basic enzyme hydrolyzed only an additional 40% of the PC before hemolysis. On the other hand, in experiments where hydrolysis of about 20% of PC of erythrocytes was achieved by a short term incubation with the basic enzyme, then a further treatment of the same cells with the acidic enzyme caused only 10% additional PC hydrolysis before hemolysis. This demonstrated that the acidic enzyme hydrolyzed one domain of PC in the intact erythrocytes, whereas the basic enzyme hydrolyzed not only the same one but also another domain of PC in membranes. Analysis of fatty acids released by the action of these two phospholipases A_2 on erythrocytes indicated further characteristic differences. In particular, the ratio of released saturated to unsaturated fatty acids was significantly higher with the acidic enzyme as compared with the basic phospholipase A_2. These results provide firm support to the conclusion that there are different domains of PC in human erythrocyte membranes and that the acidic and basic phospholipase A_2 of *A. halys blomhoffii* can be used to identify them.

SUBCUTANEOUS FAT DISTRIBUTION IN MALES AND FEMALES FROM 1 TO 39 YEARS OF AGE. R.M. Siervogel, A.F. Roche, J.H. Himes, W.C. Chumlea, and R. McCammon (Fels Res. Inst., Dept. of Pediatrics, Wright St. Univ. Schl. of Med., Yellow Springs, OH 45387 and Wardenberg Student Health Service, Univ. of Colorado, Boulder, CO 80220) *Am. J. Clin. Nutr. 36*(1):162-171 (1982). Subcutaneous fat thicknesses measured in two longitudinal studies, the Denver Growth Study and the Melbourne Growth Study, were examined to determine 1) if one site of subcutaneous fat measurement is more representative than another of the body's subcutaneous fat layer, 2) if some measurements of subcutaneous fat from specific body areas are more representative of the subcutaneous fat layer than others, and 3) if there are sex and age differences in intersite relationships. Data from the Denver Study include skinfold thicknesses from 10 sites measured serially at annual ages from 4 to 39 yr and radiographic fat thickness measured at five sites at annual ages from 1 to 18 yr. In the Melbourne Study, five skinfold thicknesses were measured serially in children from 2 to 15 yr of age. Relatively low intersite communalities during the prepubertal years suggest a tendency in each sex for considerable site-to-site variation during this period. However, immediately before puberty and throughout adolescence, high communalities indicate that the thickness of subcutaneous fat at any site is highly related to thickness at all other sites. After puberty and into the mid-20's, there is a reoccurrence of greater site-to-site variability. This higher degree of variation continues into early middle age only in women, implying that more changes occur in their subcutaneous fat, that differentially affect various parts of the body, than in men.

SYNTHESIS, PROCESSING, AND SECRETION OF APOLIPOPROTEIN B BY THE CHICK LIVER CELL. P. Siuta-Mangano, S.C. Howard, W.J. Lennarz, and M.D. Lane (Dept. of Physiological Chem.,

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The Johns Hopkins Univ. Schl. of Med., Baltimore, MD 21205) *J. Biol. Chem.* 257(8):4292-4300 (1982). Estrogen-induced chick liver cells were used to investigate the synthesis, glycosylation, and secretion of apoprotein B. Pulse-chase experiments with [^{3}H]eucine showed that approximately 10 min are required for the synthesis of the apoprotein B polypeptide and an additional 20-25 min for [^{3}H] apoprotein B to be secreted into the medium. [^{3}H] Leucine-labeled apoprotein B nascent chains exhibited a molecular weight range of <30,000 to ~350,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis. The labeling pattern of apoprotein B nascent chains isolated from cells labeled with [^{3}H]mannose or [^{3}H]glucosamine showed that core oligosaccharide addition occurs co-translationally in at least two stages. That the nascent chains had acquired a characteristic "high-mannose" oligosaccharide chain was indicated by the release of all of [^{3}H] mannose label upon treatment with endo-β-*N*-acetylglucosaminidase H. After intracellular maturation and secretion, about 25% of the [^{3}H] mannose-labeled oligosaccharide chains of apoprotein B became resistant to cleavage by endoglycosidase H indicating the presence of both high mannose and complex oligosaccharide chains on secreted apoprotein B. The [^{3}H]galactose-labeled oligosaccharide of secreted apoprotein B was resistant to cleavage by endoglycosidase H. These results indicate that the apoprotein B polypeptide is synthesized de novo while attached to the ribosome and not by post-translational cross-linking or noncovalent aggregation of smaller peptide subunits as proposed by some investigators.

ASSOCIATION OF ACYLGLYCERIDE AND RETINYL PALMITATE HYDROLASE ACTIVITIES WITH ZINC AND COPPER METALLOPROTEINS IN A HIGH MOLECULAR WEIGHT LIPID-PROTEIN AGGREGATE FRACTION FROM CHICK LIVER CYTOSOL. D. Sklan and S. Donoghue (Faculty of Agriculture, Hebrew Univ., Rehovot 76-100, Israel) *Biochim. Biophys. Acta* 711(3):532-538 (1982). A lipid-protein aggregate fraction of molecular weight approx. 1.8 $\times 10^6$ was isolated by gel filtration from chick liver cytosol. This aggregate fraction had a hydrated density range of 1.06-1.13, was 45% lipid, contained zinc and copper and was associated with triolein, phosphatidylcholine and retinyl palmitate hydrolase activity. Hydrolytic activities were stimulated by albumin and cholate, but not by dihydroxy bile acids, and inhibited by serine esterase and sulphydryl inhibitors. Incubation of the aggregate with fatty acid-labelled acylglycerides resulted in protein-binding fatty acid fractions with molecular weights of 150 000, 60 000, 13 000 and approx. 2000. Incubation of the aggregate with [^{3}H]retinyl palmitate yielded retinol-containing fractions with molecular weights of 150 000, 60 000 and 15 000. The latter peak appears, on the basis of amino acid composition, to be similar to the cellular retinol-binding protein. In addition, on incubation of the aggregate fraction, zinc and copper peaks are found with molecular weights of 150 000, 60 000 and 12 000-8000. The latter were further purified to yield a copper-rich metalloprotein similar to "copper-chelatin" and a zinc-rich metalloprotein, possibly zinc-metallothionein. Both these metalloprotein fractions had acyl hydrolase activity which was depressed in zinc-depleted animals. This may provide a possible explanation for the documented nutritional interactions between zinc and retinol.

EFFECT OF RETINOL STATUS ON RETINOL-BINDING PROTEIN BIOSYNTHESIS RATE AND TRANSLATABLE MESSENGER RNA LEVEL IN RAT LIVER. D.R. Soprano, J.E. Smith, D.S. Goodman (Dept. of Med., Div. of Metabolism and Nutr., Columbia Univ. College of Physicians and Surgeons, New York, NY 10032) *J. Biol. Chem.*, 257(13):7693-7697 (1982). Studies were conducted to explore the role of retinol in the control of the rate of synthesis of plasma retinol-binding protein (RBP) in the liver of the rat. Previous studies have shown that nutritional retinol status strongly influences RBP secretion from the liver cell. Both the in vivo relative rate of RBP synthesis and the in vitro translatable level of RBP-specific mRNA were examined in normal vitamin A, retinol-depleted, and retinol-repleted rats. The relative rate of RBP synthesis was estimated by measuring the extent of incorporation of [^{3}H]leucine, [^{3}H]lysine, and [^{3}H]phenylalanine after a 12-min pulse label into immunoprecipitable RBP, relative to the incorporation of these amino acids into total liver trichloroacetic acid-precipitable protein. The level of translatable RBP-specific mRNA was quantitated in vitro by translation of rat liver poly(A$^+$)RNA in the rabbit retinculocyte lysate protein-synthesizing system. The amount of newly synthesized RBP was determined relative to the amount of newly synthesized total protein. Both the relative rate of RBP synthesis (approximately 0.26%) and the translatable level of RBP-specific mRNA (approximately 0.14%) were found to be constant regardless of the retinol status of the rats. These results indicate that retinol, the molecule that RBP specifically binds and transports, does not appear to control the rate of synthesis of RBP or the translatable level of RBP-specific mRAN in the liver of the rat. Regulation of plasma RBP levels by retinol must be exercised at a locus beyond that of RBP synthesis.

CHANGES IN PLASMA LIPID AND LIPOPROTEIN FRACTIONS AFTER ALTERATION IN DIETARY CHOLESTEROL, POLYUNSATURATED, SATURATED, AND TOTAL FAT IN FREE-LIVING NORMAL AND HYPERCHOLESTEROLEMIC CHILDREN. E. Stein, J. Shapero, C. McNerney, C. Glueck, T. Tracy, P. Gartside (Depts. of Med., Pathology, and Biostatistics, Univ. of Cincinnati Med. Center, Cincinnati, OH 45267) *Amer. J. Clin. Nutr.* 35(6): 1375-1390 (1982). To assess the effects of dietary cholesterol and the amount and type of fat on plasma lipid and lipoproteins, nutrient intakes were altered sequentially over 15 months in 11 normal children and 12 children with heterozygous familial hypercholesterolemia. After a 3-month baseline assessment period, on an ad libitum diet, the following diets were given sequentially for three months each: dietary cholesterol>450 mg/day, total fat<35% of total calories, and polyunsaturated fat to saturated fat ratio (P/S)>1.5 (diet 1); dietary cholesterol<160 mg/day, total fat<35% total calories and P/S>1.5 (diet 2); dietary cholesterol<160 mg/day, total fat 40% total calories, P/S=1 (diet 3), and dietary cholesterol>450 mg/day total fat>40% total calories, P/S<0.4 (diet 4). In normal and familial hypercholesterolemic children the high dietary P/S ratio lowered total and low-density lipoprotein cholesterol in the presence of high dietary cholesterol; sharp reductions in dietary cholesterol lowered the total and low-density lipoprotein cholesterol slightly in familial hypercholesterolemia subjects when P/S was high. High-density lipoprotein cholesterol was not affected by large changes in dietary cholesterol or amount or type of fat. Sustained dietary alteration which significantly lowers total and low-density lipoprotein cholesterol with commercially available products is achievable and practical in free-living children.

ANIMAL FATTY ACID SYNTHETASE. IDENTIFICATION OF THE RESIDUES COMPRISING THE NOVEL ARRANGEMENT OF THE β-KETOACYL SYNTHETASE SITE AND THEIR ROLE IN ITS COLD INACTIVATION. J.K. Stoops and S.J. Wakil (Marrs McLean Dept. of Biochem., Baylor Coll., of Med., Houston, TX 77030) *J. Biol. Chem.* 257(6):3230-3235 (1982). The chicken liver fatty acid synthetase is rapidly and irreversibly inhibited by the bifunctional reagent 1,3-dibromo-2-propanone. This inhibition results from the alkylation of sulfhydryl groups in the two β-ketoacyl synthetase sites of the multienzyme complex and these groups have been identified by chemical analyses as the pantetheine-SH and the cysteine-SH. The reaction of these two residues with 1,3-dibromo-2-propanone results in the cross-linking of the two subunits of the homodimer. The pantetheine residue of one subunit is juxtapositioned opposite a cysteine residue of the adjacent subunit and that there are two such arrangements in the complex which constitute this novel β-ketoacyl synthetase site. Malonyl-CoA does not protect the enzyme from inhibition by 1,3-dibromo-2-propanone though it prevents the cross-linking reaction. Chemical analyses indicate that the malonyl group forms an acyl enzyme intermediate with the pantetheine-SH but not the active cysteine-SH which is free to react with 1,3-dibromo-2-propanone. However, the resulting derivative of the cysteine residue is prevented from reacting with the malonyl-protected pantetheine-SH. Incubating the enzyme was also found to prevent 1,3-dibromo-2-propanone from cross-linking the two subunits though it was demonstrated that the reagent alkylates the active cystein-SH. The results suggest that a time-dependent conformational change occurs resulting in the movement of the reactive cysteine-SH and pantetheine-SH in the β-ketoacyl synthetase site. This conformation change renders the enzyme inactive; however, activity may be fully restored.

INACTIVATION OF HUMAN PLACENTAL 17β-ESTRADIOL DEHYDROGENASE AND 20α-HYDROXYSTEROID DEHYDROGENASE WITH ACTIVE SITE-DIRECTED 17β-PROPYNYL-SUBSTITUTED PROGESTIN ANALOGS. B. Tobias, D.F. Covey, and R.C. Strickler (Depts. of Obstetrics and Gynecology and Pharmacology, Washington Univ. Schl. of Med. St. Louis, MO 63110) *J. Biol. Chem.* 257(6):2783-2786 (1982). The steroids, 17β-[(*R*)-1-hydroxy-2-propynyl]androst-4-en-3-one (α-HPA) and 17β-(1-oxo-2-propynyl)-androst-4-en-3-one (OPA), were used to investigate the 17β-estradiol dehydrogenase and 20α-hydroxysteroid dehydrogenase activities which co-exist in the homogeneous enzyme purified from human placental cytosol. OPA is a substrate and a very rapid irreversible affinity alkylator which causes simultaneous inactivation of the 17β- and 20α- activities in a time-dependent manner which

follows pseudo-first order kinetics. Enzyme substrates, estrone, estradiol-17β, progesterone, and 20α-dihydroprogesterone, protect against inactivations by OPA. α-HPA does not inactivate the enzyme in the absence of NAD^+. However, in the presence of NAD^+, α-HPA is a poor substrate. Consequently, when α-HPA is enzymatically oxidized in the presence of excess NAD^+, identical, time-dependent, irreversible inactivation of both the 17β- and 20α-activities results with half-times of 138 min and 500 min. The β-isomer, 17β-[(1S)-1-hydroxy-2-propynyl]androst-4-en-3-one, is not oxidized by and, therefore, does not inactivate the enzyme. The simultaneous inactivation of both the major 17β-estradiol dehydrogenase and 20α-hydroxysteroid dehydrogenase activities by OPA and by enzymatic oxidation of α-HPA clearly demonstrates the bifunctional activity of the single enzyme active site.

ACYLTRANSFERASE-CATALYZED CLEAVAGE OF ARACHIDONIC ACID FROM PHOSPHOLIPIDS AND TRANSFER TO LYSOPHOSPHATIDES IN LYMPHOCYTES AND MACROPHAGES. J. Trotter, I. Flesch, B. Schmidt, E. Ferber (Max-Planck-Inst. for Immunbiologie, D-7800 Freiburg, West Germany) *J. of Biol. Chem.* 257:1816-1823 (1982). The cleavage of fatty acyl moieties from phospholipids was compared in intact cells and homogenates of mouse lymphocytes and macrophages. Liberation of free arachidonic acid during incubations of intact cells was only detectable in the presence of albumin. Homogenization of prelabeled thymocytes and further incubation of these homogenates at 37 C resulted in a pronounced decrease of phospholipid degradation and cleavage of arachidonoyl residues, while further incubation of homogenates from prelabeled macrophages produced a greatly increased phospholipid degradation. Homogenates of macrophages but not those of thymocytes contain substantial activites of phospholipase A_2 detectable using exogeneous radiolabeled substrates. These findings indicate that in thymocytes cleavage of arachidonic acid from phosphatidylcholine is an active process that is not catalyzed by phospholipase A_2 Addition of CoA and lysophosphatidylethanolamine to prelabeled thymocyte homogenates induced a fast breakdown of phosphatidylcholine and transfer of arachidonic acid to phosphatidylethanolamine. The transfer is restricted to arachidonic acid and does not require additon of ATP. Sodium cholate, completely inhibited this transfer reaction. These results suggest that the CoA-mediated, ATP-independent breakdown of phosphatidylcholine and transfer of arachidonic acid is catalyzed by the acyl CoA:lysophosphatide acyltransferase operating in reverse.

KINETICS OF THE INCORPORATION OF ADRENAL CYTOCHROME P-450_{SCC} INTO PHOSPHATIDYLCHOLINE VESICLES. R.C. Tuckey and H. Kamin (Dept. of Biochem., Duke Univ. Med. Center, Durham, NC 27710) *J. Biol. Chem.* *257*(6):2887-2893 (1982). The rate of incorporation of cytochrome P-450_{SCC} into artificial unilamellar phosphatidylcholine vesicles was measured from the spectral changes reflecting changes in the spin state of the cytochrome caused by dissociation of cholesterol from the cytochrome in the vesicle membrane. The incorporation was found to be a second order process dependent on both the cytochrome and vesicl concentrations. The rate of association of cytochrome P-450_{SCC} with phosphatidylcholine vesicles increased with decreasing vesicle size and lower catalytic activity was observed for the cytochrome incorporated into very large vesicles. Incorporation went to completion at a NaCl concentration of 500 mM while the maximum rate of incorporation was observed with 50 mM NaCl. The incorporation rate decreased markedly with decreasing temperature and a break was observed in the Arrhenius plot at the phase transition temperature of the phosphatidylcholine. The half-time decreased with increasing unsaturation of the fatty acyl chains of the phosphatidylcholine and increased linearly with the amount of cholesterol or stearic acid in the vesicle. From these effects, we conclude that the rate of incorporation of cytochrome P-450_{SCC} into phosphatidylcholine vesicles is largely determined by the fluidity of the vesicle membrane. Cholesterol analogues produced a smaller reduction in sucrose leakage from phosphatidylcholine vesicles than did cholesterol. This is in contrast to the report by other workers that, in liposomes, a reduction in permeability comparable to cholesterol is produced by these sterols. Of these sterols, only cholesta-5,7dien-3β-ol caused a small reduction while the other were without effect.

LACK OF EFFECT OF DIETARY FIBER ON SERUM LIPIDS, GLUCOSE, AND INSULIN IN HEALTHY YOUNG MEN FED HIGH STARCH DIETS. I.H. Ullrich, M.D., M.J. Albrink, M.D. (Dept. of Med., West Virginia Univ. Schl. of Med., Morgantown, WV 26506) *Amer. J. Clin. Nutr.* *36*(1):1-9 (1982). Eight healthy young men were fed a 72% carbohydrate high starch diet either high or low in dietary fiber for 4 days in a double cross-over design. Both groups showed a slight transient increase in plasma triglyceride level and a decrease in total and high-density lipoprotein cholesterol. There were few differences in glucose and insulin levels after glucose and meal tolerance tests after each diet. Fasting triglycerides and high-density lipoprotein cholesterol were inversely related at base-line; insulin response to oral glucose was inversely related to high-density lipoprotein cholesterol levels at the end of the study. We conclude that a high carbohydrate high starch diet, whether high or low in fiber, caused little increase in triglycerides, with little difference between the high and low fiber diets. Dietary fiber did not influence the fall in plasma cholesterol or high-density lipoprotein cholesterol concentrations over and above that seen after the low fiber diet.

INFLUENCE OF DIETS CONTAINING CASEIN, SOY ISOLATE, AND SOY CONCENTRATE ON SERUM CHOLESTEROL AND LIPOPROTEINS IN MIDDLE-AGED VOLUNTEERS. J.M.A. van Raaij, M.B. Katan, C.E. West and J.G.A.J. Hautvast (Dept. of Human Nutr., Agr. Univ., De Dreijen 12, 6703 BC Wageningen, The Netherlands) *Am. J. Clin. Nutr.* *35*(5):925-934 (1982). Fifty-seven healthy volunteers were fed for 45 days on diets containing 16% of energy as protein, 35% as fat and about 375 mg cholesterol/day. Of the protein in the diets 60% was provided as caseinate, as soy protein isolate, or as soy protein concentrate. After a control period of 17 days during which all the subjects received the casein diet, 17 subjects continued on this diet for the next 28 days. Serum cholesterol levels at the end of the control period were 207 ± 36, 205 ± 40, and 199 ± 35 mg/dl (mean ± SD) for the casein, isolate, and concentrate groups, respectively. Mean changes over the test period were -2 ± 10, -8 ± 12, and +1 ± 10 mg/dl, respectively. Compared with the casein diet, the isolate diet caused a small, nonsignificant decline in both serum total cholesterol and low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol. These effects may have been more obvious if there had been no differences between groups in weight loss. No correlation was found between the response and the initial cholesterol level. No differences in lipoprotein composition were found between the casein and soy concentrate groups. Our data suggest that soy protein preparations do not have dramatic effects on the serum total cholesterol concentration in healthy subjects. However, pure soy protein might have some beneficial effects on the distribution of cholesterol over the lipoproteins. The lack of effect of the less refined soy protein concentrate suggests the dietary fiber and other nonprotein components of soy concentrate do not have, at least in the short-term, a favorable effect on sserum cholesterol and lipoproteins in healthy adults.

A Ca^{2+}-BINDING LIPOPROTEIN FROM SUBMITOCHONDRIAL PARTICLES OF RAT SKELETAL MUSCLE OR BOVINE HEART. E.W. Yamada, N.J. Huzel, J.W. Burgess (Dept. of Biochem., Univ. of Manitoba, Winnipeg, Manitoba, Canada R3W 0W3) *J. of Biol. Chem.* 257:2087-2091 (1982). The ethanol-soluble protein that is released from submitochondrial particles of rat skeletal muscle or bovine heart by Ca^{2+} treatment at 0 C is also soluble in aqueous solutions. This lipoprotein is present in about 1.5-fold excess over the ATPase inhibitor protein. The molecular weight of the lipoprotein was estimated by electrophoresis under denaturing conditions to be about 6,500. Electrophoresis under these conditions showed that the lipoprotein dimerized at Ca^{2+} concentrations of 1 to 0.1 mM; dimerization decreased progessively as Ca^{2+} was lowered. The lipoprotein did not inhibit ATPase activity of inhibitor protein-depleted submitochondrial particles. Nor did it modulate the activity of the ATPase inhibitor protein. ATPase activity of Ca^{2+}-stripped submitochondrial particles was inhibited by *N,N'*-dicyclohexylcarbodiimide (DCCD) almost 2 times more than was the ATPase activity of the intact particles. The lipoprotein was responsible for this protection against DCCD in the intact particles; it prevented inhibition by DCCD of ATPase activity of the Ca^{2+}-stripped submitochondrial particles. Protection agsinst DCCD was reduced progressively as Ca^{2+} was increased to 10^{-4}M, indicating that the monomer is the active form of the lipoprotein. At low concentrations of DCCD and ATPase inhibitor protein, inhibition of ATPase activity of the Ca^{2+}-stripped submitochondrial particles was additive. F6, purified from rat skeletal muscle mitochondria, did not protect against inhibition by DCCD nor modulate the action of the ATPase inhibitor protein; similarly, other proteins such as lipovitellin had no effect.

EFFECT OF VITAMIN E AS AN IMMUNOPOTENTIATION AGENT FOR MICE AT OPTIMAL DOSAGE AND ITS TOXICITY AT HIGH DOSAGE. T. Yasunaga, H. Kato, K. Ohgaki, T. Inamoto, and Y. Hikasa (Second Dept. of Surgery, Faculty of Med., Kyoto Univ., 54 Kawaramachi Shogoin Sakyo-ku, Kyoto, 606 Japan) *J. Nutr.* *112*(6):1075-1084 (1982). Studies have been done to determine the optimal dosage of vitamin E. Vitamin E is generally con-

sidered to be relatively nontoxic at high dosage in spite of the fact that it is a fat-soluble vitamin. From our experiments using mice, when various dosages of *all-rac*-α-tocopherol were injected into the intraperitoneal cavity every day, 1) the body weight decreased when the dose was more than 100 IU/kg per day, and all the mice died within 3 days at 400 IU/kg per day; 2) immune responses investigated by lymphoproliferative assays with phytohemagglutinin, concanavalin A and lipopolysaccharide were enhanced significantly between 5 and 20 IU/kg per day, but were inhibited by 80 IU/kg per day. When the immunopotentiation effect of vitamin E was discernible, serum tocopherol levels were about twice the control values. From our results, the optimal dosage of vitamin E was between 5 and 20 IU/kg per day, and dosages over 80 IU/kg per day were toxic to mice. We then experimented similarly with vitamin K, which is fat soluble and possesses a quinone structure resembling vitamin E. When doses between 12.5 and 150 mg/kg per day of vitamin K were injected into the intraperitoneal cavity daily for 14 days, increase of body weight was generally inhibited. This did not depend on the dose, and there was no definite relationship between mitogen responses and vitamin K.

CONDITIONS WHICH CAUSE THE RIGHT-HANDED TO LEFT-HANDED DNA CONFORMATIONAL TRANSITIONS. EVIDENCE FOR SEVERAL TYPES OF LEFT-HANDED DNA STRUCTURES IN SOLUTION. W. Zacharias, J.E. Larson, J. Klysik, S.M. Stirdivant, and R.D. Wells (Univ. of Wisconsin, Dept. of Biochem., Coll. of Agric. and Life Sci., Madison, WI 53706) *J. Biol. Chem. 257*(6): 2775-2782 (1982). Various divalent metal ions in combination with a dehydrating organic solvent were tested for their ability to induce the transition of $(dG\text{-}dC)_n$ from a B-type structure to a left-handed Z-type conformation. By monitoring the CD properties of the polymer in these media, it was found that Co^{2+} or Mn^{2+} in the presence of ethylene glycol or ethanol were most effective. The metal ions and the dehydrating agents acted synergistically. The differences in shape suggested the existence of a family of left-handed conformations depending on the kind of salt and solvent used. Analysis of the CD changes showed that the two metal ions interacted in different ways, Mn^{2+} causing a monophasic transition and Co^{2+}causing a biphasic conversion. The dehydrating agent strongly increased the cooperativity of the transition. Several of the most effective environmental conditions were tested with a 157-base pair (bp) restriction fragment comprised of a 95-bp region of the *Escherichia coli lac* operator-promoter flanked by segments of (dCdG). The new environmental conditions caused a cooperative transition of the (dC-dG) regions into left-handed helices. Relaxation studies were performed on topoisomers of pRW751, a recombinant plasmid containing 58 bp of (dC-dG). As many as eight supercoil turns were lost when the electrophoretic determination was conducted in 5 mM $MnCl_2$ and 30% ethylene glycol. A variety of different cations and dehydrating solvent are effective in promoting the right-handed to left-handed DNA conformational transition, not only for the DNA polymer but also for tracts which are flanked by random sequence DNA.

CHOLESTEROL EXCRETION STUDIES IN FAMILIAL HYPERCHOLESTEROLEMIC CHILDREN AND THEIR NORMOLIPIDEMIC SIBLINGS. J.H. Zavoral, D.C. Laine, L.K. Bale, D.L. Wellik, R.D. Ellefson, K. Kuba, W. Krivit, and B.A. Kottke (Dept. of Pediatrics, Hennepin County Med. Center and the Univ. of Minnesota, Minneapolis, and the Atherosclerosis Res. Unit, Mayo Clinic and Foundation, Rochester, MN) *Am. J. Clin. Nutr. 35*(5):1360-1367 (1982). Twenty children ages 3 to 17 yr, eight with normal lipids and 12 with familial hypercholesterolemia were studied on a metabolic unit for 14 days to evaluate fecal bile acid and fecal neutral sterol excretion. The diet contained a moderately low cholesterol cotent, 180 to 200 mg/da6. Stools were collected in three separate, 3-day pools. Fecal bile acids and fecal neutral sterols were measured using two stool markers and thin-layer, and gas-liquid chromatography techniques. Fecal neutral sterol and fecal bile acid excretion were the same for normal and familial hypercholesterolemic children on a mg/kg basis. Fecal neutral sterols in familial hypercholesterolemic children decreased with age, $p<0.001$; fecal bile acid excretion also appeared to decrease with age, but less significantly, $p<0.07$. Although the familial hypercholesterolemic children have significantly increased plasma and potentially elevated tissue or total body cholesterol, the excretion of fecal bile acids and fecal neutral sterols did not differ between familial hypercholesterolemic and normal children.

STUDIES ON THE INCORPORATION OF $[1\text{-}^{14}C]$ARACHIDONIC ACID INTO GLYCEROLIPIDS AND ITS CONVERSION INTO PROSTAGLANDINS BY RABBIT IRIS. EFFECTS OF ANTI-INFLAMMATORY DRUGS AND PHOSPHOLIPASE A_2 INHIBITORS. A.A. Abdel-Latif and J.P. Smith (Dept. of Cell and Molec. Biol., Med. Coll. of Georgia, Augusta, GA 30912) *Biochim. Biophys. Acta 711*(3):478-489 (1982). The incorporation of arachidonate into glycerolipids and its conversion into prostaglandins were rapid and time-dependent. About 65% of the total radioactivity was recovered in triacylglycerol, followed by that in phosphatidylcholine (20%), diacylglycerol (6%), phosphatidylethanolamin (5%) and phosphatidylinositol (3%), respectively. Time-course studies on arachidonate release from glycerolipids of prelabelled tissue showed that triacylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol are the major source for arachidonate in prostaglandin synthesis in this tissue. Arachidonate release from glycerolipids was not blocked by indomethacin and the effects of the phospholipase A_2 inhibitors were nonspecific. *p*-Bromophenacyl bromide inhibited the labelling of glycerolipids in a dose-dependent manner. Mepacrine stimulated the labelling of phosphatidic acid, phospaticylinositol and diacylglycerol, and inhibited that of phosphatidylcholine, phosphatidylethanolamine and triacylglycerol. At concentrations under 0.25 mM it stimulated prostaglandin synthesis in microsomes and at concentrations over 0.25 mM it inhibited their synthesis in both muscle and microsomes. Indomethacin and aspirin moderately increased the labelling of glycerolipids; however, both drugs inhibited prostaglandin synthesis by iris and microsomes in a dose-dependent manner. Possible explanations for mechanisms underlying these effects were presented.

INFLUENCE OF FATTY ACIDS ON THE BINDING OF CALCIUM TO HUMAN ALBUMIN. CORRELATION OF BINDING AND CONFORMATION STUDIES AND EVIDENCE FOR DISTINCT DIFFERENCES BETWEEN UNSATURATED FATTY ACIDS AND SATURATED FATTY ACIDS. J.J. Aguanno and J.H. Ladenson (Div. of Lab. Med., Depts. of Pathology and Med., Washington Univ. Schl. of Med., St. Louis, MO 63110) *J. Biol. Chem. 257* (10):8745-8748 (1982). The influence of various fatty acids of 14-18 carbon chain lengths on the binding of calcium to human serum albumin was studied. The *cis*-unsaturated fatty acids (myristoleic, Palmitoleic, oleic, and linoleic) caused a much larger increase in the affinity and maximum binding of calcium for albumin than did saturated fatty acids (myristic, palmitic, and stearic). For the unsaturated fatty acids, the influence on calcium-albumin binding increased as the degree of unsaturation increased or the chain length decreased. With oleic or linoleic acid, increases in calcium binding were observed at ~2-3 mol of fatty acid-mol of albumin, while for myristic, palmitic, or elaidic acid, concentrations of 5-7 fatty acids/mol of albumin were required. The maximum fluorescence emission of albumin was found to change in parallel with the calcium binding for oleic, palmitic, and elaidic acids. We conclude that 1) *cis*-unsaturated and saturated or *trans*-unsaturated fatty acids have very different effects on the binding of calcium to human albumin, 2) the alteration in calcium binding is likely due to a conformational change in the albumin molecule, and 3) changes in calcium binding to albumin are observed at levels of *cis*-unsaturated fatty acids which are obtainable *in vivo*.

EFFECTS OF DIETARY FIBERS ON LIPID METABOLISM IN LIVER AND ADIPOSE TISSUE IN CHICKS. Y. Akiba and T. Matsumoto (Dept. of Animal Sci., Faculty of Agr., Tohoku Univ., Sendai 980, Japan) *J. Nutr. 112*(8):1577-1585 (1982). Two experiments were conducted to investigate the effect of dietary fibers on lipid metabolism with 14-day-old male chicks fed isonitrogenous and isocaloric diets with or without 4% cellulose or 8% alfalfa meal for 3 and 6 weeks. Feeding the cellulose or alfalfa meal resulted in significant reductions in hepatic lipid deposition and plasma lipid content. Hepatic de novo fatty acid synthesis from acetate, glucose and leucine was significantly reduced by feeding the cellulose or alfalfa meal. Triglyceride synthesis from palmitate in the hepatic tissue was significantly reduced by dietary alfalfa meal. Malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) activity in the hepatic tissue was not affected by feeding the cellulose or alfalfa meal for 3 and 6 weeks. Secretion of lipoprotein from the liver appears to be accelerated by the dietary fibers. Lipoprotein lipase activity in the adipose tissue and clearance rate of intravenously injected lipids were enhanced by feeding the alfalfa meal. Feeding the cellulose for 3 weeks reduced hormone-sensitive lipase activity in the adipose tissue.

EFFECTS OF DIETARY CHOLESTEROL AND TYPE OF DIETARY CARBOHYDRATE ON HEPATIC AND PLASMA GLYCERIDES AND PHOSPHOLIPIDS ON THE GERBIL. D.B. Andersen and B.J. Holub (Dept. of Nutr., Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1) *J. Nutr. 112*(7):1425-1436 (1982). The

influence of dietary cholesterol at levels of 0.01-0.5% and carbohydrate type on levels and composition of hepatic and plasma glycerides in the male Mongolian gerbil was studied. Hepatic and plasma triglyceride (TG) levels were generally higher with dietary cholesterol supplementation (0.1 and 0.5%), and animals fed sucrose diets exhibited very low levels of hepatic and plasma TG. The mixed carbohydrate did not affect glycerides in a way that could be interpolated from feeding starch or sucrose diets alone. The percent of 16:0 in liver TG was depressed with cholesterol-feeding, whereas the percent of 18:1 was elevated. Sucrose diets produced higher percentages of 18:0 in liver TG. Dietary cholesterol elevated concentrations of plasma but not liver phospholipid (PL). Percentages of 20:4ω6 and 22:6ω3 acids in liver and plasma PL were significantly depressed by cholesterol supplementation, whereas the percent of 18:2 in liver PL was higher when feeding cholesterol and starch. Dietary cholesterol produced higher levels of hepatic 1,2-diglyceride with lower percentages of 18:0 and 20:4ω6 and higher percentages of 18:1 and 18:2. Dietary cholesterol may possibly influence fatty acid elongation and desaturation reactions as well as transacylation reactions in phospholipid synthesis. In this regard, compositional data suggested a suppressing effect of dietary cholesterol on 22:6ω3 formation as exemplified by a 40% diminution in the docosahexaenate content of hepatic phosphatidylethanolamine in gerbils fed a 0.5% cholesterol diet for only 7 days.

HEPATIC CHOLESTEROL METABOLISM IN OBESITY: ACTIVITY OF MICROSOMAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE. B. Angelin, L. Backman, K. Dinarsson, L. Eriksson, and S. Ewerth (Depts. of Med., Surgery, and Pathology, Karolinska Inst. at Serafimerlasarettet and Huddinge Univ. Hosp., Stockholm, Sweden) *J. Lipid Res. 23*(5):770-773 (1982). Obesity is often associated with an elevated total body cholesterol synthesis. In order to evaluate the role of hepatic cholesterolgenesis in this phenomenon, we assayed the rate-limiting step in cholesterol biosynthesis, 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase in the microsomal fraction of liver biopsies obtained operatively from ten morbidly obese (relative body weight > 155%) subjects. Eighteen normalweight patients (relative body weight < 120%) with cholesterol gallstones served as controls. Hepatic HMG-CoA reductase activity, expressed as pmol·min^{-1}·mg protein^{-1}, was 60% higher in the obese subjects compared to the gallstone patients ($P < 0.05$). Microsomal protein concentration was lower in the obese patients, so that enzyme activity calculated per gram liver was not significantly different between the two groups. However, mevalonate formation, expressed in terms of total organ activity, was higher in the obese than in the nonobese group. The results suggest that the liver is a major contributor to the increased cholesterol production seen in obesity.

APOPROTEIN E SUPPRESSES PHYTOHEMAGGLUTININ-ACTIVATED PHOSPHOLIPID TURNOVER IN PERIPHERAL BLOOD MONONUCLEAR CELLS. E.M. Avila, G. Holdsworth, N. Sasaki, R.L. Jackson, and J.A.K. Harmony (Inst. Venezolano de Investigaciones Cienfificas, Centro de Biofisica y Bioquimica, Lab De Lipoproteinas, APDO 1827, Caracas 1010A, Venezuela and the Div. of Lipoprotein Res. Depts. of Pharmacol. and Cell Biophys. and Biol. Chem., Univ. of Cincinnati, College of Med., Cincinnati, OH 45267) *J. Biol. Chem. 257*(10):5900-5909 (1982). Plasma lipoproteins with hydrated densities less than 1.063 g/ml, very low density, intermediate density, and low density lipoproteins, suppress mitogen-activated inductive biochemical events in lymphocytes. The ability of these lipoproteins to inhibit phytohemagglutinin (PHA)-enhanced incorporation of ^{32}P into lymphocyte phospholipids correlates directly with the amount of associated apoprotein E(apo-E). Apo-E purified from the very low density lipoproteins of subjects with Types III, IV, and V hyperlipoproteinemia also inhibits PHA-induced ^{32}P-phospholipid formation. With all of the apo-E preparations, 50% suppression occurs at approximately 2-4 μg/ml. Moreover, delipidated apo-E is as inhibitory as apo-E complexed with dimyristoyl phosphatidylcholine or with sphingomyelin. Other apoproteins, apo-AI, -AII, -CI, and -CII, do not inhibit PHA-activated phospholipid turnover, indicating that suppression is due to a specific structural feature of apo-E. Suppression by apo-E is not due to a shift in the optimum concentration of PHA, nor is it the result of competition with PHA for mitogen receptors at the cell surface. These data, taken together with the results of previous studies, indicate that lipoprotein-associated apo-E serves as a recognition determinant for lymphocyte surface receptors *and* as a suppressive lipoprotein constituent.

COMPARISON OF DIFFERENT METHODS OF ISOTOPICALLY LABELLING THE ESTERIFIED CHOLESTEROL IN HUMAN HIGH DENSITY LIPOPROTEINS. P.J. Barter, L. Gorjatschko, G.J. Hopkins (Unit of Clin. Biochem., Flinders Univ. Schl. of Med., Bedford Park, South Australia, 5042, Australia) *Biochim. Biophys. Acta 710*(3):349-358 (1982). Methods of incorporating esterified [^{3}H]cholesterol into human high density lipoproteins (HDL) have been assessed in terms of influence on the subsequent rate at which esterified [^{3}H]cholesterol was transferred from HDL to other plasma lipoproteins in 37°C incubations containing tracer amounts of the labelled preparations added to aliquots of a common pool of unlabelled human plasma. The HDL esterified cholesterol was labelled by (a) the action of lecithin:cholesterol acyltransferase in incubations of plasma containing exogenous [^{3}H]cholesterol and (b) by exchange in incubations of plasma containing preparations of pre-labelled LDL. The exchange labelling approach was also used to examine the effects of various HDL compositional changes on the subsequent behaviour of its esterified [^{3}H]cholesterol. The source of the label had no influence on the subsequent behaviour of HDL esterified [^{3}H]cholesterol. Modification of the HDL composition had profound effects. In preparations in which the esterified cholesterol content of the labelled HDL was increased by the action of lecithin:cholesterol acyltransferase, there was a reduced rate of transfer of esterified [^{3}H]cholesterol out of the HDL fraction in subsequent incubations of the labelled HDL with fresh, unlabelled plasma. When the esterified cholesterol content of the labelled HDL had been decreased, the subsequent rate of transfer of esterified [^{3}H]cholesterol out of the HDL fraction was increased. To obtain a biologically valid, labelled tracer of human HDL esterified cholesterol involves labelling by exchange in incubations in which lecithin:cholesterol acyltransferase is inhibited in plasma from which VLDL has been removed.

EARLY EFFECT OF *MYO*-INOSITOL DEFICIENCY ON FATTY ACID SYNTHETIC ENZYMES OF RAT LIVER. D.C. Beach, P.K. Flick (Dept. of Biol. Chem., Wright State Univ. Schl. of Med., Dayton, OH 45435) *Biochim. Biophys. Acta 711*(3):452-459 (1982). Young rats (100 g) were fed either a purified *myo*-inositol-deficient balanced diet or a control diet containing 0.5% by weight *myo*-inositol, ad libitum, for up to 2 weeks following a 48 h fast. Weight gain was the same for animals in both groups. Liver triacylglycerol levels in the deficient animals were 1.8-, 3.5- and 3.0-fold higher than the corresponding levels in the control animals after 4, 8 and 14 days of feeding, respectively. In the *myo*-inositol-deficient group the specific activities of liver fatty acid synthetase and acetyl-CoA carboxylase were elevated 1.5-2.0-fold over controls, reaching a maximum after 3-4 days of feeding. Subsequently, activities declined to control levels. Rates of fatty acid synthetase synthesis in the deficient group, as measured by [^{3}H]eucine incorporation into immunoprecipitable fatty acid synthetase polypeptide, were significantly higher (1.5-2.0-fold) than controls after 12-18 h of feeding and then declined to control levels by 1 day. No difference was noted between groups in either the rate of total, soluble liver protein synthesis or the half-life of fatty acid synthetase over this time period. These results suggest that the liver lipodystrophy observed during *myo*-inositol deficiency in rats may be due in part to elevated levels of lipogenic enzymes in this tissue in the early stage of the deficiency.

EVIDENCE FOR ALDEHYDES BOUND TO LIVER MICROSOMAL PROTEIN FOLLOWING CCl_4 OR $BrCCl_3$ POISONING. A. Benedetti, H. Esterbauer, M. Ferrali, R. Fulceri, and M. Comporti (Instituto di Patologia Generale dell'Universita di Siena, Via del Laterino 8, 53100 Siena, Italy) *Biochim. Biophys Acta 711*(2):345-356 (1982). Since it has been demonstrated in previous studies that peroxidation of liver microsomal lipids leads to the production of aldehydes provided with cytopathological activities—namely 4-hydroxyalkenals—evidence was searched for aldehydes bound to microsomal protein in in vivo conditions (CCl_4 and $BrCCL_3$ intoxications) in which peroxidation of lipids of hepatic endoplasmic reticulum had been demonstrated previously. Experiments carried out to ascertain the reliability of the spectrophotometric detection of protein-bound alkenals showed that in the in vitro system in which liver microsomes are allowed to react with 4-hydroxynonenal there is a good agreement between the binding value that can be calculated from the absorption spectrum and the binding value obtained by using labelled 4-hydroxynonenal.

EFFECTS OF ORAL GLYCEROL ON FOOD INTAKE IN MAN.

Abstracts

H. Bjorvell and S. Rossner (Obesity Univ. Dept. of Internal Med., Karolinska Hospital and Kung Gustaf V:s Res. Inst., Stockholm, Sweden) *Am. J. Clin. Nutr. 36*(2):262-265 (1982). The amount of food ingested was measured in 12-non-obese volunteers after preloads with glycerol, glycose, and water, administered in a double-blind design. A palatable soup was served in concealed containers and on different plates and the volume, consumed on each occasion, was registered. Administration of 7.5 g glycerol 20 min before the meal reduced the mean energy intake by about 10% compared to both an equicaloric amount of glucose solution or the same volume (30 ml) of water. At the start of the meal 20 min after the preload, the plasma glycerol concentration was about 10 times higher than base-line values. The free fatty acid/glycerol ratios did not correlate to the reduction of energy intake.

TISSUE SITES OF DEGRADATION OF NATIVE AND REDUCTIVELY METHYLATED $[^{14}C]$SUCROSE-LABELED LOW DENSITY LIPOPROTEIN IN RAT. CONTRIBUTION OF RECEPTOR-DEPENDENT AND RECEPTOR-INDEPENDENT PATHWAYS. T.E. Carew, R.C. Pittman, and D. Steinberg (Div. of Metabolic Disease, Dept. of Med. MO13D, Univ. of California, San Diego, La Jolla, California 92093) *J. Biol. Chem. 257*(14);8001-8008 (1982). Low density lipoprotein (LDL) is catabolized by both receptor-dependent and receptor-independent pathways; methylated LDL (MeLDL) is catabolized only by receptor-independent mechanisms. Rats were injected with either LDL or MeLDL labeled with $[^{14}C]$sucrose and the tissue sites of degradation were determined 24 h later. On degradation, the ^{14}C-labeled ligand remains trapped intracellularly as a cumulative measure of degradation. The fractional catabolic rate (FCR) of $[^{14}C]$sucrose-MeLDL was lower than that of $[^{14}C]$sucrose-LDL. Liver was the predominant site of catabolism of both LDL and MeLDL. The fraction of the plasma LDL pool "cleared" per tissue weight per unit of time was determined for individual tissues. The differences in these rates for LDL and MeLDL are an approximation of receptor-mediated uptake of LDL. In other studies, rats were continuously infused with LDL to down-regulate and saturate receptors prior to injection of labeled LDL or MeLDL. Rats infused with LDL exhibited a lower FCR for $[^{14}C]$sucrose-LDL compared to controls; the FCR for sucrose MeLDL was unchanged by LDL infusion. The fractional degradation rate of $[^{14}C]$sucrose-LDL by individual tissues was lowered by LDL infusion in liver, adrenal, ovary, and intestine. The determination of receptor dependency by this independent approach supports the conclusions reached using $[^{14}C]$sucrose-MeLDL in normolipemic animals.

ALTERATIONS IN LIPOPROTEIN COMPOSITION ASSOCIATED WITH GALACTOSAMINE-INDUCED RAT LIVER INJURY. C.K. Cartwright, J.B. Ragland, S.W. Weidman, and S.M. Sabesin (Div. of Gastroenterology, Dept. of Med., Univ. of Tennessee Center for the Health Sci., Memphis, TN 38163) *J. Lipid Res. 23*(5):667-679 (1982). The apoprotein and lipid composition and the morphology of lipoproteins was determined in rats with D-(+)-galactosamine (GalN) hepatitis. Single intraperitoneal injections of GalN at several dose levels and postinjection exsanguination times resulted in depressed levels of cholesteryl esters, an index of plasma lecithin: cholesterol acyltransferase (LCAT) activity, and increased levels of phospholipids, unesterified cholesterol, and triglycerides. Plasma withdrawn from rats 24 hr after injection of 1000 mg/kg GalN was most deficient in cholesteryl ester and was studied further by sequential isolation of VLDL, LDL, HDL_1, HDL_2, and HDL_3. The increased plasma triglyceride (TG) after GalN treatment accumulated in TG-rich VLDL which contained two types of particles: a large and rough-edged particle, and a smooth one with a mean diameter similar to control VLDL. The increased phospholipids and unesterified cholesterol were predominantly in LDL, HDL_1, and HDL_2 which were largely rouleaux of flattened vesicles. Density gradient ultracentrifugation of $d > 1.006$ g/ml lipoproteins confirmed these results. GalN hepatitis appeared to decrease the larger $apoB_{335K}$ subspecies and the $apoC\text{-}III_0$ and $apoC\text{-}III_2$ content of VLDL. However, total apoB concentration as GalN VLDL was increased 2.6-fold over control. LDL and HDL were markedly enriched in apoE. LDL apoB concentration was decreased by 41% while HDL was deficient in apoA-I, A-II, and A-IV, and C.

STEROL SYNTHESIS BY THE OCULAR LENS OF THE RAT DURING POSTNATAL DEVELOPMENT. R.J. Cenedella (Dept. of Biochem., Kirksville Col. of Osteopathic Med., Kirksville, MO 63501) *J. Lipid Res. 23*(4):619-626 (1982). Great amounts of plasma membranes are formed during early postnatal development of the ocular lens as lens epithelial cells differentiate into fiber cells. Little information is available on the source of the lipids, and particularly cholesterol, required for formation of these plasma membranes. The present study measured the capacity of the lens of the rat to synthesize cholesterol during this dynamic period of growth. Incorporation by lens of ^3H_2O into total fatty acids was also examined. Absolute rates of cholesterol synthesis per whole lens were estimated in vitro from incorporation of ^3H from ^3H_2O into digitonide precipitable sterols (DPS) by intact lenses of 6-to 30-day old rats. Rates of cholesterol synthesis were calculated which were adequate to furnish from either 50-100% or 20-40% of the cholesterol required by the lens for growth, depending upon the animal's age and upon whether on considered NADPH to be generated by the pentose phosphate pathway or by oxidative enzymatic processes (NADPH from the pentose pathway is not labeled from ^3H_2O). Generation of the NADPH necessary for cholesterol synthesis principally by the pentose pathway would support the higher percent contribution of synthesis to the total growth requirement. The pentose pathway was clearly active in the young rat lens, since between 7.5 to 9.0 times more $[1\text{-}^{14}C]$glucose than $[6\text{-}^{14}C]$glucose was oxidized in vitro to $^{14}CO_2$ by 6- and 22-day old lenses. Incorporation of ^3H_2O into DPS decreases sharply after 2 weeks of age in spite of a constant rate of cholesterol accumulation by the lens.

INCREASED URINARY EXCRETION OF GLYCOSPHINGOLIPIDS IN FAMILIAL HYPERCHOLESTEROLEMIA. S. Chatterjee, C.S. Sekerke, P.O. Kwiterovich, Jr. (Depts. of Pediatrics and Med., Lipid Res. Atherosclerosis Unit, Johns Hopkins University Schl. of Med., Baltimore, MD 21205) *J. Lipid Res. 23*(4):513-522 (1982). The content of glycosphingolipids (GSL) was studied in the urinary sediments (24-hr specimens) from seven normal subjects, a patient with Fabry's disease and five homozygotes with familial hypercholesterolemia (FH). Normal urinary sediments contained very small amounts of GalCer, GlcCer, $GaOse_2Cer$, LacCer, $GbOse_3Cer$, and $GbOse_4Cer$. In Fabry urinary sediment, the levels of $GaOse_2Cer$ and of $GbOse_3Cer$ were 389 and 550. In urinary sediments from the FH subjects, the mean contents of GlcCer, GalCer, and LacCer were 2.7, 1.9, and 15.8 times higher than in normals. The mean contents of total cholesterol and phospholipid in the urinary sediments of FH and normals were similar. The mean contents of Glc Cer, GalCer, and LacCer, expressed in terms of the cholesterol content of urinary sediment were increased 3.4-, 1.6-, and 5.4-fold in the FH homozygotes. Of the five FH homozygotes, only one, which had undergone a portacaval shunt and was also receiving lipid-lowering therapy, had a normal value of LacCer. The other FH homozygotes had levels of LacCer that were 3- to 55-fold higher and 5.5- to 7.3-fold higher than the mean of the normals. One homozygote underwent plasma exchange therapy that reduced both the baseline urinary and plasma LacCer levels from 86 to 7 and from 1491 to 852. Eleven days after plasma exchange, the urinary LacCer levels approached pre-exchange levels. The data indicate that there is an abnormality of GSL metabolism associated with familial hypercholesterolemia and that the urinary excretion of GSL can be modified by plasma exchange therapy.

DIRECT ESTIMATION OF DOLICHYL PHOSPHATE IN RAT LIVER BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. N. Chaudhary, D.J. Freeman, J.W. Rip and K.K. Carroll (Dept. of Biochem., Univ. of Western Ontario, London, Ontario N6A 5C1, Canada) *Lipids 17*(8):558-560 (1982). A method involving reverse-phase high pressure liquid chromatography has been developed for determining the concentration of dolichyl phosphate (Dol-P) in tissues. Individual Dol-P homologs are resolved and amounts as small as 50 ng can be detected. Rat liver was found to contain 2.4 μg Dol-P/g wet weight, or ca. 4% of total liver dolichol. In contrast, rat liver microsomes contained 64 ng Dol-P/mg protein, which is about 40% of total microsomal dolichol. This enrichment in Dol-P is consistent with the role of microsomes as the major site of Dol-P-mediated glycoprotein biosynthesis in liver.

CHARACTERIZATION OF PROTEOLIPOSOMES CONTAINING APOPROTEIN A-I: A NEW SUBSTRATE FOR THE MEASUREMENT OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE ACTIVITY. C.-H. Chen and J.J. Albers (Dept. of Med., Harborview Med. Center, Univ. of Washington Schl. of Med., Seattle, WA 98104) *J. Lipid Res. 23*(5):680-691 (1982). Proteoliposome vesicles containing apoA-I, lecithin, and cholesterol (including labeled cholesterol) were prepared from various molar ratios of the three components by the cholate dialysis technique. Comparative studies on the sensitivity and efficiency of these proteoliposomes to serve as substrate for lecithin:cholesterol acyltransferase (LCATase) indicated that the proteoliposomes with apoA-I:lecithin:cholesterol molar ratio of 0.8:250:12.5 was ideal for assaying LCATase activity of both plasma and purified enzyme. This proteoliposome was

shown to be comparable in size by gel filtration (radius, 131.9 4.8 Å, n = 6) and by electron microscopy (radius, 123.4 5.1 Å, n = 100). The proteoliposome preparation was stable as LCATase substrate for at least 3 and 5 weeks, respectively, when stored at 4°C and -20°C, and was a better substrate for the enzyme activity assay than were lecithin-cholesterol liposomes incubated with apoA-I. Under the standardized assay system LCATase activity was a linear function of plasma enzyme added and was independent of the amount of plasma cholesterol added to the proteoliposomes in the range of 3 to 20 l of plasma. The mean LCATase activity by this method was 95.1 14.0 (range 76.5-122.5) nmol/hr per ml of plasma from fifteen normal human subjects. This method of substrate formation using the cholate dialysis technique permits the preparation of large amounts of stable, efficient, homogeneous, and well-defined substrate that is suitable for measuring low levels of enzyme activity, comparative studies, and large scale investigations of plasma LCATase, as well as studies of the mechanism and regulation of LCATase reaction.

DISTRIBUTION OF HIGH DENSITY LIPOPROTEIN PARTICLES WITH DIFFERENT APOPROTEIN COMPOSITION: PARTICLES WITH A-I AND A-II AND PARTICLES WITH A-I BUT NO A-II. M.C. Cheung and J.J. Albers (Northwest Lipid Research Clinic and Dept. of Med., Harborview Med. Center, Univ. of Washington Schl. of Med., Seattle, WA 98104) *J. Lipid Research 23*(5):747-753 (1982). High density lipoproteins (HDL) were subfractionated by equilibrium CsCl gradient centrifugation of the d 1.063-1.21 g/ml HDL fraction isolated from two men and two women. The various HDL subfractions were analyzed for their apoproteins (apo) A-I, A-II, B, D, and E and the major lipid contents. ApoA-I and A-II were found throughout the density gradient with the maximum concentration between the d 1.105 and 1.120 g/ml fractions. ApoE was found in all HDL fractions with the higher concentration in the lower density fractions. Conversely, the concentration of apoD increased as the density of the HDL fraction increased. Each density subfraction underwent quantitative precipitation with anti-A-I and anti-A-II immunoglobulin. Essentially all A-II in all density subfractions was precipitated with either immunoglobulin. Particles from each density subfraction precipitated with anti-A-II immunoglobulin had an A-I/A-II molar ratio of approximately 2.0 (range 1.9-2.3). However, particles precipitated with anti-A-I immunoglobulin had A-I/A-II molar ratios identical to the A-I/A-II ratio of the subfraction (range 2.1-7.1). The subfractions (d 1.105-1.149 g/ml fractions) with A-I/A-II molar ratios of about 2 had the least proportion of A-I in particles containing A-I but no A-II. Conversely, the subfractions (d 1.063-1.075 g/ml fractions) with the highest A-I/A-II molar ratio had the greatest proportion of apoA-I in particles containing A-I but not A-II. These data indicate that HDL contains at least two types of particles: particles with both A-I and A-II in a 2:1 molar ratio, and particles containing A-I but no A-II.

FURTHER CHARACTERIZATION OF A CHINESE HAMSTER OVARY CELL MUTANT REQUIRING CHOLESTEROL AND UNSATURATED FATTY ACID FOR GROWTH. J. Chin and T.-Y. Chang (Dept. of Biochem., Dartmouth Med. Schl., Hanover, NH 03755) *Biochemistry 21*(13):3196-3202 (1982). In lipid-depleted medium, a Chinese hamster ovary cell mutant requires cholesterol and unsaturated fatty acid for growth was found to have a much smaller increase in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and low-density lipoprotein (LDL) binding activity than normal, wild-type cell. We now characterize this mutant further by using compactin which is known to induce an increase in the synthesis of HMG-CoA reductase by a mechanism distinct from the regulatory mechanism controlled by sterol. We found that the mutant, grown in various media, responded to compactin in a manner identical with that of the normal cell. Results from assays of stearoyl-CoA desaturase from normal, revertant, and mutant cells implied that the requirement for unsaturated fatty acid in the mutant may be a secondary consequence of the primary defect in the regulation of cholesterol biosynthesis.

THE INFLUENCE OF THE SIDE-CHAIN ON STEROL SIDE-CHAIN CLEAVAGE IN RAT ADRENAL GLANDS. I.F. Craig, J.I. Mason, K.E. Suckling, and G.S. Boyd (Dept. of Biochem., Univ. of Edinburgh Med. Schl., Edinburgh, EH8 9XD, U.K.) *Biochim. Biophys. Acta 711*(1):123-127 (1982). The cholesterol sidechain cleavage enzyme system of rat adrenal cortex, the enzyme catalyzing a rate-limiting step of adrenal steroidogenesis, was shown to metabolize a series of cholesterol analogues to pregnenolone. In the presence of Ca^{2+}, rat adrenocortical metochondria converted the analogue with two less methylene groups (C_{25}) than cholesterol into pregnenolone at a faster rate than cholesterol. The analogues with one or three less methylene groups (C_{26} or C_{24}) were metabolized at a similar rate to cholesterol. Lengthening the non-polar side chain produced analogues that did not appear to be metabolized. Studies of the metabolism of these analogues in isolated rat adrenocortical carcinoma cells showed that the C_{24} and C_{25} analogues were converted into pregnenolone much more efficiently than was cholesterol or the C_{26} sterol. The experimental findings are explained in terms of the differing ability of each exogenously added sterol to gain access to the active site of the sterol side-chain cleavage enzyme by passage through the membranes of the adrenal cell.

HYDROGEN TRANSFER BETWEEN C_{19} STEROIDS DURING OXIDOREDUCTION AT C-17 IN VIVO. T. Cronholm, U. Rudqvist (Dept. of Physiol. Chem., Karolinska Institutet, S-104 01 Stockholm, Sweden) *Biochim. Biophys. Acta 711*(1):149-158 (1982). The metabolism of intravenously infused 3-sulphates of 5α-androstane-3β,17β-diol and 3β-hydroxy-5α-androstan-17-one was studied in female rats by analysis of metabolites in bile. Using ^{3}H-labelled steroids, the main metabolites were found to arise from extensive interconversion of these two steroids and to an essentially complete sulphurylation of the 17β-hydroxyl group. In addition, 15-hydroxylated metabolites were formed. Hydrogen transfer at C-17 was studied by simultaneous infusion of the 3-sulphates of 5α-[1,1,4,4-$^2H_4$ androstane-3β,17β-diol and 5α-[17α-^{2}H androstane-3β,17β-diol. Gas chromatography-mass spectrometry of metabolites from bile indicated transfer of the 17α-deuterium. After 3 h, 45-50% of the excreted androstandediol had undergone oxidoreduction. The incorporated hydrogen was derived from C-17 of the administered steroids to an extent (25-50%) that was dependent on the rate of infusion. This indicates that the dissociation of reduced coenzyme was slow and that the enzyme was not saturated. The excreted 17-oxosteroid had a constant deuterium excess, indicating absence of isotope effects in the oxidation at C-17. The extent of deuterium transfer was dependent on the rate of infusion of the former steroid. The results indicated that the administered 17-oxosteroid accumulated in a pool, and that molecules from this pool mixed completely with molecules derived from the 17β-hydroxysteroid, before direct excretion and reduction followed by sulphurylation and excretion.

EFFECTS OF ETHANOL METABOLISM ON OXIDOREDUCTION AND INTERMOLECULAR HYDROGEN TRANSFER AT C-17 IN STEROID 3-SULPHATES IN VIVO. T. Cronholm and U. Rudqvist (Dept. of Physiological Chem., Karolinska Institutet, S-104 01 Stockholm, Sweden) *Biochim. Biophys. Acta 711*(1):159-165 (1982). Steroid sulphates were infused intravenously in female rats, and metabolites were isolated from bile. Infused 3β-hydroxy-5α-androstan-17-one 3-sulphate was excreted together with 5α-androstane-3β-diol disulphate, which formed a larger part after ethanol administration. Results from infusions of the 3-sulphates of 5α[17α-^{2}H] androstane-3β, 17β-diol and 3β -hydroxy-5α [2,2,4,4-$^2H_4$] androstan-17-one indicated that ethanol decreased the extent of transfer of the 17α-deuterium and increased the reduction of 17-oxosteroid without affecting the oxidation of 17β-hydroxysteroid. Ethanol metabolism decreased the deuterium transfer from 17α-^{2}H estradiol 3-sulphate to C-17 of 3β-hydroxy-5α-androstan-17-one 3-sulphate. The results indicate that NADH from ethanol metabolism increased the concentration of oxidoreductase-NADH complex without affecting the corresponding complex with NAD^+. The effects of ethanol on steroid reduction were dependent on the initial redox state of the enzyme-coenzyme complex. This redox state was modified by substrates for the enzyme, indicating slow dissociation of the complex. Thus, ethanol metabolism may interfere with the interactions between steroid oxidoreductions.

EFFECT OF FATTY ACID MODIFICATION ON PROSTAGLANDIN PRODUCTION BY CULTURED 3T3 CELLS. G.M. Denning, P.H. Figard, and A.A. Spector (Dept. of Biochem., Univ. of Iowa, Iowa City, IA 52242) *J. Lipid Res. 23*(4):584-597 (1982). We have investigated the extent to which modifications in the essential fatty acid content of mammalian cells can affect prostaglandin production. Swiss mouse 3T3 cells stimulated with the calcium ionophore A23187 produced 1.7 to 7 times more prostaglandin E_2 (PGE_2) when the cultures were supplemented with linoleic acid. Increases in PGE_2 production as a result of linoleic acid supplementation occurred under all culture conditions except during the first 24 hr after attachment, when prostaglandin production was very high. Arachidonic acid supplementation produced a similar enhancement in the capacity of the cells to produce PGE_2, but no appreciable increase occurred when the cultures were supplemented with oleic acid. The phospholipids of the cells exposed to the linoleate-enriched medium contained 4 times more arachidonic acid

and twice as much linoleic acid as compared with the corresponding controls. The choline phosphoglycerides were most highly enriched in arachidonic acid, but 2- to 3-fold increases also occurred in the inositol and ethanolamine phosphoglycerides. When cultures initially enriched with the linoleic acid were transferred to an unsupplemented medium, the fatty acid composition as well as the capacity of the cells to produce PGE_2 reverted almost to control values. The amount of exogenous arachidonic acid converted to PGE_2 as measured by radioimmunoassay also was greater when the cells were enriched with linoleic acid. Studies with radioactive arachidonic acid indicated that the distribution of prostaglandin metabolites was not affected appreciably by linoleic acid enrichment.

A STUDY OF DIET, SERUM LIPIDS, AND FECAL CONSTITUENTS IN SPOUSES. M.A. Eastwood, W.G. Brydon, D.M. Smith, and J.H. Smith (Wolfson Gastrointestinal Labs. Univ. Dept. of Med., Western General Hosp., Edinburgh, Scotland) *Am. J. Clin. Nutr. 36*(2):290-293 (1982). The diet, serum cholesterol, and fecal constituents of 23 healthy spouses have been compared. The pattern of food intake between couples was similar, but of dietary constituents, only dietary fat and fiber intake showed a significant correlation. Of fecal constituents, only fecal fat correlated significantly between couples.

METABOLIC HETEROGENEITY OF LOW DENSITY LIPOPROTEIN-APOB PRODUCTION FAMILIAL HYPERCHOLESTEROLEMIA: AN ANALYTICAL MODEL SOLUTION OF TRACER DATA. R.P. Eaton, R.C. Allen, and D.S. Schade (Depts. of Med. and Math, Univ. of New Mexico, Albuquerque, NM 87131) *J. Lipid Res. 23*(5):738-746 (1982). Six subjects with heterozygous familial hypercholesterolemia (FH) (three males, three females) formed the basis for an investigation of the pathways of production of apoprotein B within plasma low-density-lipoprotein (LDL-apoB). Following the intravenous injection of $[^{75}Se]$ selenomethionine as an amino acid tracer, incorporation of the radioactive isotope into the putative precursor of LDL, intermediate density lipoprotein (IDL-apoB), was examined over a 9-day period. The resulting tracer data provided the precursor profile for both the IDL catabolic conversion of LDL as well as for direct synthesis of LDL from amino acids. The fractional conversion rates, (α) for IDL and (β) for amino acids to LDL-apoB, were determined utilizing the two compartment model for LDL involving both the intravascular and the extravascular pools of apoB. This LDL model was resolved analytically and the parameters α and β were determined so as to give the least squares fit to the LDL tracer data. In this solution, the fractional conversion rates of IDL and of amino acids to LDL-apoB were resolved with a mean fractional residual of 20 ± 6%, which was randomly distributed within the LDL-apoB data throughout the 216 hours of the studies.

AN ASSAY FOR BETA-ADRENERGIC RECEPTORS IN ISOLATED HUMAN FAT CELLS. P. Engfeldt, P. Arner, H. Wahrenberg, and J. Ostman (Dept. of Med. and the Res. Center, Huddinge Hospital, Karolinska Inst., S-141 86 Huddinge, Sweden) *J. Lipid Res. 23*(5):715-719 (1982). The beta-adrenergic receptors have been characterized in isolated human adipocytes using apotent beta-adrenergic antagonist (-)-$[^3H]$ dihydroalprenolol. Binding of (-)-$[^3H]$ dihydroalprenolol to isolated fat cells was stereospecific and saturable, the maximum number of binding sites calculated being 7.8 ± 2.2 pmol of bound ligand/10^7 cells, corresponding to 450,000 binding sites/cell. The dissociation constant was estimated to be 2.7 ± 1.1 nM. The results with competition-inhibition experiments using beta-adrenergic agonists and antagonists indicated that the binding sites in isolated adipocytes were predominantly of the $beta_1$-subtype; about 80% of the receptors were of this type. With the present method, specific beta-adrengic receptor number and affinity in isolated human adipoctyes could be determined in about 1 g of human adipose tissue.

COMPARISON OF CHANGES IN INOSITIDE AND NONINOSITIDE PHOSPHOLIPIDS DURING ACUTE AND PROLONGED ADRENOCORTICOTROPIC HORMONE TREATMENT IN VIVO. R.V. Farese, M.A. Sabir, R.E. Larson (Veterans Administration Hospital and the Dept. of Med., Univ. of South Florida Coll. of Med., Tampa, FL 33612) *Biochemistry 21*(14):3318-3321 (1982). To further evaluate the potential role of phospholipids in the steroidogenic action of adrenocorticotropic hormone (ACTH), we compared ACTH-induced increases in rat adrenal inositide and noninositide phospholipids during acute (1-4 h) and more prolonged (48 h) ACTH treatment in vivo. From our findings, we conclude the following: (a) major adrenal phospholipid changes are similar in acute and prolonged ACTH treatment; (b) percent increases in inositides are greater than those of noninositide phospholipids during acute and prolonged ACTH treatment; (c) ACTH-induced increases in adrenal noninositide phospholipids are as dependent upon protein synthesis as are increases in the inositides; (d) protein synthesis dependence of induced increases in adrenal steroidogenesis and phospholipids is equally apparent in acute and prolonged ACTH treatment; and (e) although the present findings continue to support the possibility that inositides (?PPI) may play an important role in the steroidogenic action of ACTH, it now seems clear that noninositide phospholipids may also participate in this action.

CHOLESTEROL TURNOVER IN HUMAN PLASMA LIPOPROTEINS: STUDIES WITH STABLE AND RADIOACTIVE ISOTOPES. J. Ferezou, J. Rautureau, T. Coste, E. Gouffier, and F. Chevalier (Laboratoire de Physiologie de la Nutrition, Equipe associee au C.N.R.S.: ERA 415, Unite de Recherche I.N.S.E.R.M.: U231, Universite de Parid-Sud 91 405 Orsay, Cedex, France) *Am. J. Clin. Nutr. 36*(2):235-244 (1982). The kinetics of cholesterol labeling was studied in the plasma lipoproteins of three subjects who had received an oral dose of octadeuterated cholesterol and an intravenous administration of ^3H-cholesterol and ^{14}C-mevalonate or ^{13}C-acetate. After each labeled cholesterol pulse into the plasma (absorption, exchange or synthesis), the isotopic concentrations of free and esterified cholesterol became identical after 120 h. Before this time, very low-density lipoprotein free cholesterol appeared to more easily exchangeable than high-density and low-density lipoprotein free cholesterol, high-density lipoproteins were shown to be a source for very low-density lipoprotein cholesterol esters and the role of very low-density lipoproteins associated with chylomicrons was demonstrated in the initial transport of dietary cholesterol. The rates of the various processes involved in cholesterol turnover were calculated. The total cholesterol inflow into the plasma by absorption and synthesis, determined by long-term kinetic data (18 or 28 wk) was consistent with the result obtained by sterol balance for the total cholesterol outflow from the plasma (fecal excretion and conversion into bile acids).

POSTNATAL DEVELOPMENT OF PALMITATE OXIDATION AND MITOCHONDRIAL ENZYME ACTIVITIES IN RAT CARDIAC AND SKELETAL MUSCLE. J.F.C. Glatz and J.H. Veerkamp (Dept. of Biochem., Univ. of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen The Netherlands) *Biochim. Biophys. Acta 711*(2):327-335 (1982). 1. The palmitate oxidation rate was measured in intact diaphragm and m. flexor digitorum brevis and in whole homogenates of heart, diaphragm and m. quadriceps of developing rats between late foetal life and maturity. Activities of the mitochondrial enzymes cytochrome *c* oxidase and citrate synthase were also determined. 2. Immediately after birth the palmitate oxidation rate increases markedly in both intact diaphragm and m. flexor digitorum brevis and falls gradually after day 1 to adult values which are about 35% of those at birth. 3. The oxidation capacities of diaphragm and m. quadriceps, but especially of heart, increase steadily during development, starting before birth and reaching adult values at 15-20 days postnatally. The activities of the mitochondrial enzymes show a similar developmental pattern. 4. In heart the increase of oxidative capacity is the result of an increase of both mitochondrial content and mitochondrial activity. The mitochondrial contents of diaphragm and m. quadriceps, on the other hand, decrease with age and the increase of their oxidative capacities is due to a large rise of the mitochondrial activity.

EFFECT OF THE MATERNAL VITAMIN D STATUS AT PARTURITION ON THE VITAMIN D STATUS OF THE NEONATAL CALF. J.P. Goff, R.L. Horst, E.T. Littledike (Natl. Animal Disease Center, Agric. Res. Serv., U.S. Dept. of Agric., P.O. Box 70, Ames, IA 50010) *J. Nutr. 112*(7):1387-1393 (1982). The plasma concentrations of calcium, phosphorus, magnesium, hydroxyproline, vitamin D, and vitamin D metabolites were determined in cows and their colostrum-deprived calves. At birth, calf plasma calcium, phosphorus, and hydroxyproline concentrations were not correlated ($P > 0.05$) with the maternal plasma concentrations of these substances. There was a high degree of correlation between maternal and neonatal calf plasma concentrations of 25-hydroxyergocalciferol (r=0.733), 25-hydroxycholecalciferol (r=0.888), 24,25-dihydroxyergocalciferol (r=0.770), 24,25-dihydroxycholecalciferol (r=0.629), and 25,26-dihydroxycholecalciferol (r=0.840). Neonatal calf plasma concentrations of 1,25-dihydroxyvitamin D were low (41.2 ± 3.4 pg/ml) and had no correlation with maternal concentrations (r=0.219, $P > 0.05$). Neonatal plasma calcium and inorganic phosphorus concentrations were correlated ($P < 0.05$) with maternal plasma concentrations of 1,25-dihydroxyvitamin D (r=0.559 and 0.525, respectively). Vitamin D status of the dam, therefore,

appears to be important in determining neonatal calf plasma concentrations of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25,26-dihydroxyvitamin D, and, in addition, the plasma calcium and inorganic phosphorus status of the neonatal calf is apparantly dependent on maternal concentrations of 1,25-dihydroxyvitamin D.

LYSOPHOSPHATIDYLCHOLINE METABOLISM IN THE RABBIT HEART. R.W. Gross, B.E. Sobel (Washington Univ. Schl. of Med., St. Louis, MO 63110) *J. Biol. Chem.* *257*(12):6702-6702 (1982). Metabolism of lysophosphatidylcholine (LPC) implicated in arrhythmogenesis was characterized in rabbit ventricular homogenates. Activities of four enzymatic pathways were distinguishable after subcellular fractionation and DEAE-Sephacel chromatography including microsomal lysophospholipase, microsomal acyl coenzyme A/LPC acyltransferase, cytosolic lysophospholipase, and cytosolic lysophospholipase-trans-acylase. Microsomal lysophospholipase activity was attenuated 81% by acidosis comparable to that in ischemic myocardium and was inhibited by substrate. LPC acyltransferase was identified in the microsomal fraction based on CoA-dependent phosphatidylcholine synthesis, the positional specificity of acylation of LPC, and identical reaction celocities with both of its labeled co-substrates. LPC acyltransferase had a V_{max} of 5.1 nmol/mg/min, a broad pH optimum centered at pH 7, and an apparent K_m for LPC and palmitoyl-CoA of 14 μm and 7 μM. Cytosolic lysophospholipase was separated from lysophospholipase-transacylase by DEAE-Sephacel chromatography and distinguished from microsomal lysophospholipase by its broad pH activity curve, Michaelis-Menten kinetics and lack of substrate inhibition. Lysophospholipase-transacylase was identified in the cytosolic fraction by CoA-independent phosphatidylcholine synthesis and purified 4885-fold from homogenate by ammonium sulfate precipitation, DEAE-Sephacel, hydroxylapatite, gel filtration, and polylysine chromatography. The partially purified enzyme had a transacylase/losophospholipase activity ratio of 0.6, and transacylation of LPC was prominent at submicellar concentrations of substrate.

EFFECTS OF 12-*O*-TETRADECANOYLPHORBOL 13-ACETATE ON GLYCEROLIPID METABOLISM IN CULTURED MYOBLASTS. R.I. Grove and S.D. Schimmel (Dept. of Biochem., Univ. of South Florida, Col. of Med., Tampa, FL 33612) *Biochim. Biophys. Acta* *711*(2):272-280 (1982). We recently reported that treatment of differentiated chick embryo myoblasts in culture with the potent tumor promoter 12-0-tetradecanoylphorbol 13-acetate (TPA) caused a 2-fold increase in the level of 1,2-diacylglycerol in the plasma membrane fraction within 15-30 min (Grove, R.I. and Schimmel, S.D. (1981) Biochem. Biophys. Res. Commun. 102, 158-164). This system has been characterized further and the metabolic origin and fate of the stimulated diacylglycerol have been investigated. The stimulation of 1,2-diacylglycerol was insensitive to alterations of Ca^{2+} concentration in the medium and to the presence of inhibitors of Ca^{2+} flux, protein synthesis and prostaglandin synthesis. The fatty acid composition of the newly formed diacylglycerol was similar to that of phosphatidylcholine. In addition, the glycerol moiety of the diacylglycerol was shown to be derived from a lipid with metabolic turnover similar to that of phosphatidylcholine. The tumor promoter was also found to stimulate rapidly synthesis of phosphatidic acid, phosphatidylinositol and phosphatidylcholine. A possible model is proposed, therefore, in which the tumor promoter stimulates a membrane-associated phospholipase C which generates 1,2-diacylglycerol via the hydrolysis of phosphatidylcholine. The newly formed diacylglycerol is then metabolized back to phosphatidylcholine or to phosphatidic acid and phosphatidylinositol.

HMG-CoA REDUCTASE KINASE: MEASUREMENT OF ACTIVITY BY METHODS THAT PRECLUDE INTERFERENCE BY INHIBITORS OF HMG-CoA REDUCTASE ACTIVITY OR BY MEVALONATE KINASE. H.J. Harwood, Jr., and V.W. Rodwell (Dept. of Biochem., Purdue Univ. Biochem. Program, West Lafayette, IN 47907) *J. Lipid Res.* *23*(5):754-761 (1982). Assay of HMG-CoA reductase kinase activity requires HMG-CoA reductase free of associated reductase kinase. Microsomal reductase insensitive to inactivation by Mg-nucleotides alone may be prepared by heating microsomes at 50°C for 15 min. The reductase in these microsomes may subsequently be inactivated by Mg-nucleotides only after addition of reductase kinase. Inactivation is a linear function of time and of cytosol protein concentration and may be reversed by treatment with a phosphoprotein phosphatase. The extent of inactivation observed under standard conditions provides an assay for reductase kinase activity. Factors present incytosol that hinder measurement of either reductase or reductase kinase activity must be removed or inhibited. Reductase phosphatase is inhibited by 50 mM NaF. Reductase kinase kinase activity is not expressed under the assay conditions used. Mg-Nucleotide-independent inhibitors of reductase activity are removed by chromatography on DEAE-Sephacel or Blue Sepharose. Mevalonate kinase and reductase kinase are separable by chromatography on DEAE-Sephacel or Sepadex G-2000. We describe a rapid chromatographic procedure for separating reductase kinase of crude fractions from mevalonate kinase and from Mg-nucleotide-independent inhibitors of reductase activity. The 1.0 M KCl eluate from DEAE-Sephacel contains all of the cytosol kinase activity. This method is applicable to measurement of reductase kinase activity in cytosol or more purified fractions.

ACYL COA:RETINOL ACYLTRANSFERASE IN RAT SMALL INTESTINE: ITS ACTIVITY AND SOME PROPERTIES OF THE ENZYMIC REACTION. P. Helgerud, L.B. Petersen, and K.R. Norum (Inst. for Nutr. Res., Univ. of Oslo, Norway) *J. Lipid Res.* *23*(4):609-618 (1982). The present study was conducted to examine whether the intestinal esterification of retinol could be due to a microsomal acyl-CoA transferase. When the 'microsomal fraction' of rat mucosa was incubated with $[^3H]$ retinol and palmitoyl-CoA or oleoyl-CoA, $[^3H]$ retinyl esters were formed as identified by alumina column chromatography and reverse phase high-pressure liquid chromatography (HPLC). Unlabeled retinol and $[1-^{14}C]$ palmitoyl-CoA yielded retinyl $[1-^{14}C]$ palmitate. The esterifying activity was lost when microsomes were heated at 60°C for 30 min. Only negligible activity was observed without exogenous acyl-CoA while 10-20 μM gave optimum activity provided that 2-5 mg/ml of albumin was present. Replacement of acyl-CoA by palmitate gave no esterification, indicating that the activity was not a reversed hydrolase reaction. Optimum pH was 7.1-7.6 and optimal concentration of retinol was 15 μM. With palmitoyl-CoA, the formation of retinyl ester was 1.00 0.26 nmol·mg $protein^{-1} \cdot min^{-1}$ ($\bar{x} \pm SD$, n=4) in rats killed postprandially versus 2.06 ± 0.66 (n=5) after 36 hr of fasting. Oleoyl-CoA gave lower activity: 0.52 ±0.14 and 1.41 ± 0.36, respectively. The variation with feeding and fasting was significant ($P < 0.05$) and corresponded to that of the intestinal acyl-CoA: cholesterol acyltransferase (ACAT). Inhibition of retinol esterification was observed with taurocholate and the thiol-blocking agent 5,5′-dithiobis (2-nitrobenzoic acid).

UPTAKE, TRANSPORT AND DISTRIBUTION OF DL-α-TOCOPHERYL ACETATE COMPARED TO D-α-TOCOPHEROL IN RAINBOW TROUT (*SALMO GAIRDNERI*). S.S.O. Hung, T.W. Moon, J.W. Hilton, and S.J. Slinger (Department of Nutrition, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1) *J. Nutr.* *112*:1590-1599 (1982). The appearance of radioactivity after the oral administration of 3 μ Ci D-α-[5-*methyl*-^3H] tocopherol and 10 μCi DL-α-$[3',4'-^{14}C]$ tocopheryl acetate in plasma, liver, kidney, spleen and heart of rainbow trout showed an exponential increase up to 32 hours, followed by a plateau or slight decline from 32 to 64 hours. Radioactivity in the skeletal muscle increased exponentially up to 8 hours followed by a slower linear increase up to 64 hours. Comparisons of plasma ^3H and ^{14}C radioactivity suggested that the uptake of D-α-tocopherol (EOH) was 6 to 18 times greater than DL-α-tocopheryl acetate (EAc) in the first 4 hours and 2 to 3 times greater between 8 and 64 hours. At the plateau, the amount of ^3H and ^{14}C radioactivity incorporated per unit wet weight of tissue decreased in the order liver>kidney>plasma>spleen>heart>skeletal muscle. More than 87% of the ^3H and ^{14}C radioactivity after 16 hours was found to be free α-tocopherol in both plasma and liver. The radioactively labeled vitamins were bound primarily to plasma low-density lipoprotein (density 1.015 to 1.085). These studies support the hypothesis that the uptake, transport and distribution of EAc after hydrolysis in the gastrointestinal tract of trout follows a pattern similar to that of EOH.

SYNTHESIS OF SERUM AND CYTOSOL VITAMIN D-BINDING PROTEINS BY RAT LIVER AND KIDNEY. M. Imawari, Y. Matsuzaki, M. Mitamura, and T. Osuga (Div. of Gastroenterology, Dept. of Med., Inst. of Clinical Med., Univ. of Tsukuba, Sakura-mura, Niiharigun, Ibaraki 305, Japan) *J. Biol. Chem.* *257*(14):8153-8157 (1982). The synthesis of vitamin D-binding proteins in rat was examined using liver and kidney slices, isolated hepatocytes, and isolated renal tubules. Rat liver synthesized both serum vitamin D-binding protein (DBP) and a tissue DBP-binding component and secreted serum DBP. Rat kidney also synthesized a tissue DBP-binding component and cytosol vitamin D-binding protein (CDBP) which was immunologically related to serum DBP, but secretion of renal CDBP was not observed. Renal CDBP had a higher molecular weight (approximately 65,000) than that of serum DBP (54,000) and was heat-labile, while serum DBP was heat-stable (60°C, 60 min). Renal

Abstracts

CDBP was considered to exist in cytosol, forming a complex with a tissue DBP-binding component. These results indicate that liver is a site of synthesis of serum DBP and that CDBP, which is immunologically related to but physicochemically different from serum DBP, is synthesized *in situ* in kidney.

LACK OF EFFECT OF TOCOPHEROL ON PLASMA LIPIDS AND LIPOPROTEINS IN MAN. Y.A. Kesaniemi, S.M. Grundy (Veterans Admin. Med. Center and the Univ. of California, San Diego, CA 92161) *Am. J. Clin. Nutr. 36*(2):224-228 (1982). The influence of D,L-α-tocopherol (vitamin E) on the plasma total and very low-density lipoprotein, low density lipoprotein, and high-density lipoprotein cholesterol and triglyceride was studied in one normolipidemic and four hypertriglyceridemic subjects. Overall vitamin E caused no decrease in plasma total, very low-density and low-density lipoprotein cholesterol and triglyceride concentrations and no increase in high-density lipoprotein cholesterol level. D,L-α-Tocopherol does not seem to have any consistent effect on plasma lipids and lipoproteins in these patients.

CORRELATION OF LOW AND HIGH DENSITY LIPOPROTEIN BINDING *IN VIVO* WITH RATES OF LIPOPROTEIN DEGRADATION IN THE RAT. A COMPARISON OF LIPOPROTEINS OF RAT AND HUMAN ORIGIN. H.R. Koelz, B.C. Sherrill, S.D. Turley, and J.M. Dietschy (Dept. of Internal Med., Univ. of Texas Health Sci. Center at Dallas, Dallas, TX 75235) *J. Biol. Chem. 257*(14):8061-8072 (1982). These studies were done in the rat to correlate the ability of low and high density lipoproteins of rat (rLDL and rHDL) and human (hLDL and hHDL) origin to bind *in vivo* to specific tissues with the rates at which these same lipoprotein fractions were cleared from the circulation. The adrenal gland and liver manifested the greatest amounts of rLDL binding *in vivo*, but activity also was found in spleen, lung, kidney, ovary, and intestine. In contrast, little or no such binding was found utilizing either methyl-rLDL or hLDL. rHDL containing E apoprotein bound to the same group of tissues although in lesser amounts, except in the case of ovary and adrenal gland which bound disproportionately greater amounts of rHDL than rLDL.

COMPOSITION, CONCENTRATION, AND SIZE OF LOW DENSITY LIPOPROTEINS AND OF SUBFRACTIONS OF VERY LOW DENSITY LIPOPROTEINS FROM SERUM OF NORMAL MEN AND WOMEN. Z. Kuchinskiene and L.A. Carlson (King Gustaf Vth Research Inst. and the Dept. of Internal Med., Karolinska Inst. and Karolinska Hosp., Stockholm, Sweden) *J. Lipid Res. 23*(5):762-769 (1982). Low density lipoprotein (LDL) and four subfractions, A,B,C, and D of very low density lipoprotein (VLDL), characterized by the following decreasing S_f values, >400, 175-400, 100-175, and 20-100, respectively, were isolated by density gradient preparative ultracentrifugation from serum of normal men and women and analyzed for lipids, total protein, and apoB. Our results fit the following hypothesis for the VLDL to LDL cascade. The large VLDL particle successively loses molecules of triglycerides (core) and of soluble protein, free cholesterol, and phospholipids (surface). In this process, VLDL retains its molecules of apoB, and cholesteryl esters are lost from large VLDL but not from medium- or small-sized VLDL.

DIET-INDUCED AND PHYSIOLOGICALLY OCCURRING HYPERCHOLESTEROLEMIAS IN THE SPONTANEOUS HYPOTHYROID EUROPEAN BADGER (*MELES MELES L.*): A DENSITY GRADIENT STUDY OF LIPOPROTEIN PROFILE. P.M. Laplaud, L. Beaubatie, and D. Maurel (Laboratoire de Biochimie medicale, Faculte de medecine et de pharmacie, 2 rue du Dr. Marcland, 87032 Limoges Cedex, France) *J. Lipid Res. 23*(5):782-794 (1982). As previously shown in this laboratory, the European badger is, with regard to its plasma lipid transport system, an original and complex animal of great potential interest to lipoprotein research. In an effort to study the response of this animal to cholesterol feeding, we gave a diet supplemented with 1% cholesterol to six male badgers (group H) during the late fall period when spontaneous hypercholesterolemia and hypothyroidism occur. Six more male animals of similar age received the standard diet (group C) and were simultaneously used as controls. Plasma lipids were measured using enzymatic methodologies, while the use of a recently described density gradient ultracentrifugation technique allowed detailed examination of lipoprotein composition and polyacrylamide gell electrophoresis of lipoproteins and tetramethylurea-soluble apoproteins in the fractions. The results suggest the superimposition, in H badgers, of the spontaneous and diet-induced hyperchoelsterolemias, maximum levels being reached in December in both C and H groups.

ISOLATION AND CHARACTERIZATION OF GLYCOSPHINGOLIPIDS FROM HUMAN LEUKOCYTES. A UNIQUE GLYCOSPHINGOLIPID PATTERN IN A CASE OF ACUTE MYELOMONOBLASTIC LEUKEMIA. W.M.F. Lee, M.A. Westerick, J.C. Klock, and B.A. Macher (Dept. of Med., Cancer Res. Inst., Univ. of California, San Francisco, CA 94143) *Biochim. Biophys. Acta 711*(1):166-175 (1982). Neutral glycosphingolipids and gangliosides were isolated from the malignant cells of a patient with acute myelomonoblastic leukemia. Structural analyses were performed by gas-liquid chromatography and by high-performance liquid chromatography combined with enzymatic hydrolysis of glycosphingolipids using glycosidases. We found that, in contrast to normal leukocytes and chronic leukemia cells which have only a single tetraosylceramide species, these acute myelomonoblastic leukemia cells have approximately equal amounts of both globo- and neolactotetraosylceramide. This is the first population of human leukocytes in which we found two families of neutral glycosphingolipids to be present. The ganglioside fraction was composed of appreciable quantities of both NeuAcα2→3Galβ1→4Glcβ1→1Cer (GM3, hematoside) and NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer (sialoparagloboside). These cells did not have the 'leukocyte-specific' *N*-acetylneuraminosyllactotriaosylceramide found in normal human lymphocytes and neutrophils. These results are discussed in relation to normal leukocyte differentiation and acute leukemia. The present study also illustrates the usefulness of combining enzymatic degradation with high-performance liquid chromatography for glycosphingolipid structural determination.

BIOCHEMICAL AND ULTRASTRUCTURAL ALTERATIONS IN PLATELETS, RETICULOCYTES, AND LYMPHOCYTES FROM RATS FED VITAMIN E-DEFICIENT DIETS. J. Lehmann and M. McGill (Agr. Res. Service, Beltsville Human Nutr. Res. Center, Lipid Nutr. Lab., Beltsville, MD 20705) *J. Lipid Res. 23*(2):299-306 (1982). Effects of vitamin E-deficiency, dietary fat (corn oil versus lard), and incubation on ultrastructure of platelets and plasma were studied. Platelets from vitamin E-deficient rats had no obvious morphological defects, but reticulocytes and lymphocytes had swollen and deformed mitochondria. Fatty acid and glycogen levels of platelets were not affected by the deficiency but total lipid levels in plasma were decreased or increased depending upon the type and level of dietary fat. In comparison with supplemented controls, the proportion of stearate increased in the phospholipid fraction of plasma from vitamin E-deficient, lard-fed but not corn oil-fed rats. In platelets, total fatty acids per mg protein were 8-12% lower with lard than with corn oil as fat source. Oleate and linoleate were higher and lower, respectively, although not to the same degree, and arachidonate was not affected. With incubation of platelet-rich plasma at 37°C for 6 hr, there were no obvious morphological changes in platelets from control or from deficient, lard-fed rats, but platelets from deficient, corn oil-fed rats contained mitochondria that were swollen and deformed. Incubation did not affect fatty acid, glycogen or tocopherol levels of platelets or tocopherol levels of plasma, regardless of type or amount of dietary fat.

DE NOVO FATTY ACID SYNTHESIS IN DEVELOPING RAT LUNG. W.M. Maniscalco, J.N. Finkelstein and A.B. Parkhurst (Div. of Neonatalogy, Dept. of Pediatrics, Univ. of Rochester, Schl. of Med., Rochester, NY 14642) *Biochim. Biophys. Acta 711*(1):49-58 (1982). The rate of de novo fatty acid synthesis in developing rat lung was measured by the rate of incorporation of ^3H from ^3H_2O into fatty acids in lung slices and by the activity of acetyl-CoA carboxylase in fetal, neonatal and adult lung. Both tritium incorporation and acetyl-CoA carboxylase activity increased sharply during late gestation, peaked on the last fetal day, and declined by 50% 1 day after birth. In the adult, values were only one-half the peak fetal rates. In vitro regulation of acetyl-CoA carboxylase activity in fetal lung was similar to that described in adult non-pulmonary tissues: activation by citrate and inhibition by palmitoyl-CoA. Incubation conditions that favored enzyme phosphorylation inhibited acetyl-CoA carboxylase activity in lung while dephosphorylating conditions stimulated activity. Incorporation of U-^{14}C glucose into lung lipids during development was influenced heavily by incorporation into fatty acids, which paralleled the rate of tritium incorporation into fatty acids. The utilization of acetyl units from exogenous glucose for fatty acid synthesis was greater in adult lung than in fetal or neonatal lung, suggesting that other substrates may be important for fatty acid synthesis in developing lung. In fetal lung explants, de novo fatty acid synthesis was inhibited by exogenous palmitate. These data suggest the de novo synthesis may be an important source of saturated fatty acids in fetal lung but of less importance in the neonatal period; the regulation of acetyl-CoA carboxylase activity and fatty acid synthesis in lung may be similar

to non-pulmonary tissues.

THE EFFECTS OF FASTING AND STREPTOZOTOCIN DIABETES ON THE TRIGLYCERIDE LIPASE ACTIVITY OF RAT LIVER PLASMA MEMBRANES. T. Nomura, A. Iguchi, H. Matsunaga, and N. Sakamoto (Third Dept. of Internal Med., Nagoya Univ. Schl. of Med., Nagoya, 466, Japan) *Lipids 17*(8):573-575 (1982). The activity of hepatic triglyceride lipase (H-TGL) of plasma membranes isolated from rat liver is shown to be reduced by fasting. Refeeding restores the enzyme activity. The suppressed activity of H-TGL in streptozotocin diabetic rats is restored by insulin treatment. The behavior of the enzyme activities in both situations coincides with that of plasma insulin levels. The results suggest that the H-TGL of rat liver plasma membranes is under hormonal regulation by insulin.

CHOLESTEROLEMIC RESPONSES OF RATS TO HUMAN-TYPE DIET INGREDIENTS. B.C. O'Brien and R. Reiser (Dept. of Biochem. & Biophys., Texas Agricultural Experiment Station, Texas A & M University, College Station, TX 77843) *J. Nutr. 112*:1490-1497 (1982). The average serum cholesterol level of rats fed a human-type diet that contained 28% (wt/wt) beef fat and 0.5% added cholesterol and that was low in non-digestible constituents was lower than that of rats fed an equivalent diet prepared from purified ingredients, 94 mg/dl and 163 mg/dl, respectively. In order to identify the hypocholesterolemic ingredients in the human-type diet, the sources of protein and carbohydrate in the purified diet, casein and cornstarch, were substituted for the corresponding sources in the human-type diet, beef tenderloin, bread, and rice. These prepared diets were fed to female Sprague-Dawley rats for 10 weeks. Diets containing casein were hypercholesterolemic compared to stock rat diet, but rats fed the bread with the casein had the lowest serum cholesterol level, 112 mg/dl, of any casein diet group. The cholesterolemic response of rats fed the beef and bread diet was the same as that of stock-fed rats, 94 mg/dl and 86 mg/dl, respectively. The level of cholesterol in the liver of rats fed the beef and bread diet was lower than that of rats fed the purified diet, 7.7 mg/g and 28.5 mg/g, respectively, but it was higher than that of rats fed the stock diet, 3.8 mg/g. The hypocholesterolemic effect of beef and bread may be related to reduced cholesterol absorption. These data demonstrate the sensitive control on cholesterol metabolism of nonlipid diet ingredients.

OCCURRENCE OF OCTADECENOIC FATTY ACID ISOMERS FROM HYDROGENATED FATS IN HUMAN TISSUE LIPID CLASSES. J.B. Ohlrogge, R.M. Gulley, and E.Z. Emken (Northern Regional Res. Center, Agric. Res. Service, U.S. Dept. of Agric., Peoria, IL 61604) *Lipids 17*(8):551-557 (1982). The level of *trans*-18:1 isomers in several isolated lipid classes of human liver, heart, red blood cells and plasma was determined. Phospholipids contained substantially fewer *trans*-18:1 isomers than triglycerides. The double bond distribution of the *cis* and *trans* octadecenoate fraction of triglycerides and phosphatidylcholines from human liver and heart was determined. Whereas the double bond distribution of the triglycerides correlated closely with the pattern found in dietary hydrogenated vegetable oils, the phosphatidylcholine fraction showed evidence of selective incorporation or metabolism of specific *trans* positional isomers. In general, isomers with double bonds near the methyl terminus were present at levels higher than expected from their relative abundance in the diet. Refinements in methodology needed to analyze octadecenoate double bond configuration and location in human tissues are presented.

IDENTIFICATION OF A NEW C-23 OXIDATION PATHWAY OF METABOLISM FOR 1,25-DIHYDROXYVITAMIN D_3 PRESENT IN INTESTINE AND KIDNEY. N. Ohnuma and A.W. Norman (Dept. of Biochem. Univ. of California, Riverside, California 92521) *J. Biol. Chem. 257*(14):8261-8271 (1982). Evidence is presented for the existence of a new C-23 oxidation pathway for the metabolism of the hormonally active form of vitamin D_3 ($1,25(OH)_2D_3$). It is proposed that the newly discovered C-23 pathway for metabolism of $1,25\text{-}(OH)_2D_3$ by the target intestinal mucosa and kidney may play a prominent role under physiological conditions of controlling the tissue levels of this hormonally active form of vitamin D.

SYNTHESIS OF THE DIASTEREOISOMERS OF 1,2-DIPALMITOYL-*SN*-GLYCERO-3-THIOPHOSPHORYLETHANOLAMINE AND THEIR STEREOSPECIFIC HYDROLYSIS BY PHOSPHOLIPASES A_2 AND C. G.A. Orr, C.F. Brewer, and G. Heney (Depts. of Molecular Pharmacology and Microbiol. and Immunology, Albert Einstein Coll. of Med., Bronx, NY 10461) *Biochemistry 21*(13):3202-3206 (1982). A convenient three-step synthesis of the phosphorothioate analogue of phosphatidylethanolamine is described. The reaction pathway involves the conversion of 1,2-diacyl-*sn*-glycerol to its corresponding thiophosphoric acid dichloride by using $PSCl_3$ in the presence of a tertiary base. Treatment of the dichloride with ethanolamine results in the formation of a cyclic thiophosphoramidate which, upon acidification, undergoes P-N cleavage, giving rise to 1,2-diacyl-*sn*-glycero-3-thiophosphorylethanolamine. ^{31}P NMR reveals that both diastereoisomers are present in equivalent amounts. It is not possible, however, to separate the two isomers by high-pressure liquid chromatography. ^{31}P NMR and high-pressure liquid chromatography are used to show that phospholipases A_2 and C exhibit absolute and opposite stereoselectivity in the hydrolysis of the pair of diastereoisomers.

RECEPTOR-DEPENDENT AND RECEPTOR-INDEPENDENT DEGRADATION OF LOW DENSITY LIPOPROTEIN IN NORMAL RABBITS AND IN RECEPTOR-DEFICIENT MUTANT RABBITS. R.C. Pittman, T.E. Carew, A.D. Attie, J.L. Witzum, Y. Watanabe, and D. Steinberg (Div. of Metabolic Disease, Dept. of Med., Univ. of California, San Diego, La Jolla, California 92093) *J. Biol. Chem. 257*(14):7994-8000 (1982). Low density lipoprotein (LDL) catabolism was studied using WHHL rabbits, an inbred strain deficient in LDL receptor activity and, thus, an animal model for homozygous familial hypercholesterolemia. WHHL and normal rabbits were injected with [^{14}C] sucrose-LDL and the tissue sites of LDL degradation were determined 24 h later. Kupffer cells, a major component of the reticuloendothelial system, do not play a major role in LDL catabolism in WHHL rabbits. Despite receptor deficiency, the relative contribution of various tissues to overall LDL degradation was not greatly altered and the absolute rate of delivery of LDL to all tissues was increased with the exception of the adrenal. Thus, there was no evidence that the increased degradation occurred in any special subset of "scavenger" cells. Nevertheless, local scavenger cell uptake may be critically important, especially in atherogenesis. If it is assumed that receptor-independent degradation occurs at the same rate in the tissues of WHHL and normal rabbits and that catabolism in the absence of receptors is a linear function of concentration, then one can estimate the fraction of uptake in normal tissues mediated by receptors. The difference in the fraction of the plasma LDL pool cleared per unit of time in normal and WHHL rabbits would reflect the contribution of receptors to fractional clearance.

A PHOSPHOLIPID REQUIREMENT FOR DOLICHOL PYROPHOSPHATE *N*-ACETYLGLUCOSAMINE SYNTHESIS IN PHOSPHOLIPASE A_2-TREATED RAT LUNG MICROSOMES. P.L. Plouhar and R.K. Bretthauer (Dept. of Chem., Univ. of Notre Dame, IN 46556) *J. Biol. Chem. 257*(15):8907-8911 (1982). The role of phospholipids in the activity of UDP-GlcNAc:dolichol phosphate GlcNAc-1-phosphate transferase of rat lung microsomes has been investigated. Treatment of microsomes with phospholipase A_2 in the presence of delipidated bovine serum albumin resulted in a time-dependent loss of 65 to 75% of the enzyme activity and approximately 30% of the phospholipids. Addition of phosphatidylglycerol to the enzyme assay system containing phospholipase A_2-treated microsomes restored activity to that obtained with native microsomes and phosphatidylglycerol. Addition of phosphatidylinositol, phosphatidylcholine, or cardiolipin resulted in only partial restoration of activity, whereas phosphatidylserine and phosphatidylethanolamine were without effect. Triton X-100 was not by itself capable of restoring activity, but was required for the phospholipid effect. Measurements of the phospholipase A_2 hydrolysis products released from the microsomes during digestion, and other control experiments of adding fatty acids and lysophospholipids to the enzyme assay system, indicated that the loss of UDP-GlcNAc: dolichol phosphate GlcNAc-1-phosphate transferase activity was not due to product inhibition.

MECHANISM OF DISSOCIATION OF HUMAN APOLIPOPROTEIN A-I FROM COMPLEXES WITH DIMYRISTOYLPHOSPHATIDYLCHOLINE AS STUDIED BY GUANIDINE HYDROCHLORIDE DENATURATION. D.-J. Rejingoud and M.C. Phillips (Dept. of Physiol. and Biochem., Med. Coll. of Pennsylvania, Philadelphia, PA 19129) *Biochemistry 21*(12):2969-2976 (1982). The reversibility of the binding of human apolipoprotein A-I (apo A-I) to phospholipid has been monitored through the influence of guanidine hydrochloride (Gdn-HCl) on the isothermal denaturation and renaturation of apo A-I/dimyristoylphosphatidylcholine (DMPC) complexes at 24°C. There is an intermediate state in the denaturation pathway of apo A-I/DMPC complexes which is not present in the renaturation; the intermediate comprises partially unfolded apo A-I molecules still associated with the complex by some of their

apolar residues. Complete unfolding of the α-helix and subsequent desorption of the apo A-I molecules from the lipid-water interface involve cooperative exposure of these apolar residues to the aqueous phase. The energy barrier associated with this desorption step makes the binding of apo A-I to DMPC a thermodynamically irreversible process. Consequently, binding constants of apo A-I and PC cannot be calculated simply from equilibrium thermodynamic treatments of the partitioning of protein between free and bound states. Apo A-I molecules do not exchange freely between the lipid-free and lipid-bound states, and extra work is required to drive protein molecules off the surface. The required increase in surface pressure can be achieved by a net mass transfer of protein to the surface; in vivo, increase in the surface pressure of lipoproteins by lipolysis can cause protein desorption.

SPECIFIC, SATURABLE BINDING AND UPTAKE OF RAT CHYLOMICRON REMNANTS BY RAT SKIN FIBROBLASTS. T.G. Redgrave, N.H. Fidge, J. Yin. (Dept. of Physiol., Univ. of Melbourne, Parkville 3052, Victoria, and Baker Med. Res. Inst., Prahran 3181, Victoria, Australia) *J. Lipid Res.* *23*(4):638-644 (1982). To investigate the possible contribution of chylomicron remnants to the accumulation of cholesterol in nonhepatic tissues, rat chylomicron remnants were incubated with rat skin fibroblasts. The binding of remnants was saturable and specific. Native, undegraded chylomicrons were almost as effective as unlabeled remnants in displacing the uptake of labeled remnants. Rat low density and high density lipoproteins were relatively ineffective in displacing the uptake of labeled remnants. Accumulation of radioactive unesterified fatty acids occurred in proportion to the uptake of labeled remnants, indicating probable internalization and degradation of the particles after binding. The incorporation of added $[^{14}C]$acetate into cell non-saponifiable lipids was not significantly suppressed by added remnants, indicating an apparent lack of feedback regulation of cholesterol biosynthesis after remnant uptake. Our results show that the physiological mechanism underlying uptake of remnants by hepatic parenchymal cells might be accounted for by tissue or cellular specificity, but may perhaps arise because of the lower capillary permeability of extra-hepatic sites compared with the hepatic sinusoid.

FATE OF PROSTAGLANDIN E_2 SUCKLING RATS AFTER INTRAGASTRIC ADMINISTRATION. B. Revsin, R.J. Lemen, and O. Koldovsky (Dept. of Pediatrics and Physiology, Arizona Health Sci. Center, Tucson, AZ 85724) *Biochim. Biophys. Acta* *711*(1):101-106 (1982). $[^3H]$Prostaglandin E_2 was administered intragastrically to suckling rats at 10 μg and 0.1 μg doses. At the higher dose, 91% of the radioactive label was recoverable at zero time, decreasing to 29% at 5 h. At the lower dose, 40% of the dose was recoverable at zero time, decreasing to 8% at 5 h. With time, the radioactivity in the stomach showed a steady decrease whereas it increased in the tissues. At the 10 μg dose, the amount of radioactivity showed a steady increase in the small intestine lumen and small intestine wall. In liver and kidney the maximum amount of radioactive label was found at 1 h. The radioactivity began to decline in the liver, while the kidney remained at the same level for the entire 5-h period. At the 0.1 μg dose, the radioactivity in the small intestine lumen reached a maximum 3 h after gavage and then declined. The amount of label in the small intestine wall increased for the entire 5 h. In liver and kidney the radioactivity peaked at 1 h, remained at the same level until the 3rd h, then declined. Quantitation of the unmetabolized prostaglandin E_2 was possible 30 and 60 min after administration of the 10 μg dose. At 30 min 42.9% of radioactive label present in the liver could be shown to be authentic prostaglandin E_2. This corresponded to 0.64% of the original dose. At 60 min only 22.8% of the radioactive label found in the liver could be shown to be authentic prostaglandin E_2, which corresponded to 0.46% of the administered dose. Similar results were found in the small intestine lumen, the small intestine wall and in the kidney. At 3 and 5 h, none of the radioactivity found in these organs could be identified as authentic prostaglandin E_2.

REGULATION OF COLLAGEN SYNTHESIS IN FETAL RAT CALVARIA BY 1,25-DIHYDROXYVITAMIN D_3. D.W. Rowe and B.E. Kream (Depts. of Pediatrics and Med., Univ. of Connecticut Health Center, Farmington, CT 06032) *J. Biol. Chem.* *257*(14):8009-8015 (1982). 1,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) inhibited type I collagen synthesis in the central bone but not the periosteum of fetal rat calvaria maintained in organ culture. The central bone synthesized primarily type I collagen, whereas the periosteum synthesized both types I and III collagen. Enhanced degradation of newly synthesized collagen seemed an unlikely mechanism for the observed decrease in collagen synthesis since 1,25-$(OH)_2D_3$ reduced both the labeling of intracellular procollagen and total $[^3H]$hydroxyproline formation in fetal rat calvaria. As measured by cell-free translation of RNA extracted from calvaria, 1,25-$(OH)_2D_3$ decreased the level of functional procollagen mRNA and collagen synthesis to the same extent. Both collagen synthesis and procollagen mRNA levels were decreased as early as 3 h after exposure to 1,25-$(OH)_2D_3$ and were 50% of the control level by 24 h. Upon removal of 1,25-$(OH)_2D_3$ from cultures, collagen synthesis and procollagen mRNA remained depressed for 24 h but returned to control levels by 48 h. A reduction in collagen synthesis and procollagen mRNA was also observed in calvaria excised from 6-day-old rat pups given a single subcutaneous injection of 1,25-$(OH)_2D_3$ (1.6 ng/g body weight). We conclude that 1,25-$(OH)_2D_3$ inhibits the synthesis of type I collagen in the differentiated osteoblast by reducing the level of functional procollagen mRNA.

REGULATION OF DIACYLGLYCEROL METABOLISM AND ARACHIDONIC ACID RELEASE IN HUMAN AMNIONIC TISSUE. N. Sagawa, T. Okazaki, P.C. MacDonald, and J.M. Johnston (Cecil H. and Ida Green Center for Reproductive Biol. Sci. and the Depts. of Biochem. and Obstetrics-Gynecology, Univ. of Texas Southwestern Med. Schl., Dallas, TX 75235) *J. Biol. Chem.* *257*(14):8158-8162 (1982). We reported previously the finding of phosphatidylinositol-specific phospholipase C activity as well as diacylglycerol kinase activities in human fetal membranes and uterine decidua vera tissues. Some of the properties of diacylglycerol lipase and diacylglycerol kinase in human fetal membranes and uterine decidua vera tissues were defined. In the present study, certain of the kinetic properties of the diacylglycerol lipase and diacylglycerol kinase of human amnion and decidua vera tissues obtained from term pregnancies were measured and compared in order to evaluate the kinetics of the reactions involved in the regulation of arachidonic acid release from diacylglycerol in these tissues. The effect of Ca^{2+} on the multiple steps involved in the release of arachidonic acid from phosphatidylinositol for prostaglandin formation is discussed.

EFFECT OF A SINGLE ORAL DOSE OF DDT ON LIPID METABOLISM IN PROTEIN-CALORIE MALNOURISHED MONKEYS. S. Sanyal, N. Agarwal, P.K. Dudeja, A. Mahmood and D. Subrahmanyam (Depts. of Biochem. & Gastroenterology, Postgraduate Inst. of Med. Education & Res., Chandigarh 160 012) *Indian J. Biochem. & Biophys.* *19*(2):111-114 (1982). Effects of oral administration of DDT (150 mg/kg body wt) on lipid metabolism in various tissues of rhesus monkeys with a prior history of malnutrition have been studied. Protein-energy-malnutrition was induced in monkeys by feeding low protein/low energy diet for 12 week regimen. A significant accumulation of total lipids, cholesterol, phospholipids and triacylglycerols in plasma and adipose tissue and a considerably elevated level of triacylglycerol in liver were obtained in the treated animals. $[1\text{-}^{14}C]$Acetate incorporation into lipids *in vitro* revealed enhanced lipid synthesis in liver of treated animals. Lipoprotein-lipase activity was depressed but there was no change in the lipid-peroxidation ability of liver in pesticide-exposed malnourished monkeys compared to control group.

EFFECT OF PGE_1 ON LIPID MOBILIZATION FROM PUPAL FAT BODIES OF THE SILKWORM, *BOMBYX MORI*. B.N.Y. Setty and T.R. Ramaiah (Dept. of Post-graduate Studies & Res. in Biochem., Univ. of Mysore, Manasagangothri, Mysore 570 006) *Indian J. Biochem. & Biophys.* *19*(2):115-118 (1982). Effects of PGE_1 and PGE extract on lipid mobilization from fat bodies of *Bombyx mori* pupae were studied. Activities of esterase and lipase decreased in the fat bodies of PGE_1 and PGE extract treated pupae. The total lipid content of haemolymph decreased and that of fat body increased in PGE_1 and PGE extract treated pupae. There were significant increases in the triglyceride and total cholesterol contents of the fat bodies and significant decrease in free fatty acid content of both haemolymph and fat bodies. PGE_1 elevated triglyceride content in the fat bodies through antilipolytic action and agumentation of lipid synthesis in these tissues.

UPTAKE OF $[^3H]$CHOLESTEROL FROM LOW DENSITY LIPOPROTEIN BY CULTURED HUMAN FIBROBLASTS. R.B. Shireman and J.F. Remsen (Dept. of Food Sci. and Human Nutr. and Dept. of Biochem. and Molec. Biol., Univ. of Florida, Gainesville, FL 32611) *Biochim. Biophys. Acta* *711*(1):281-289 (1982). The uptake of $[^3H]$cholesterol from low density lipoprotein (LDL) was studied in LDL receptor-positive and receptor-negative human fibroblasts. In both cell lines the uptake depended upon temperature, time of incubation and the concentration of LDL in the medium.

Abstracts

Although the incorporation of ^{125}I-labeled LDL was minimal after 2 h of incubation in the receptor-negative (homozygous familial hypercholesterolemia, FH) cells, the uptake of [^{3}H] cholesterol was only slightly less than that of the receptor-positive (WI-38) cells. With longer periods of incubation, a larger difference in labeled cholesterol incorporation was observed; this appeared to be due to a continued accumulation of the steroid in the WI-38 cells. After 8 and 24 h of incubation, some of the [^{3}H] cholesterol was present as the ester in the WI-38 cells, but not the FH cells. Modified (reduced and methylated) LDL did not enter WI-38 cells by the receptor-mediated pathway during 2 h of incubation, as indicated by ^{125}I uptake. [^{3}H]-Cholesterol uptake, however, was not significantly different from modified and unmodified LDL. While experiments indicated that significant amounts of cholesterol moved rapidly from LDL to cultured cells with a dependence on time and LDL concentration, no increase in total cell cholesterol was detected in either cell line. FH cells contained less total cholesterol and had a higher ^{3}H specific activity than the WI-38 cells. These data suggest that there may be important mechanisms in addition to the LDL pathway for the movement of lipids into cells.

BIOSYNTHESIS OF CHOLESTANOL FROM INTESTINAL 7α-HYDROXY-4-COLESTEN-3-ONE. S. Skrede and I. Bjorkhem (Inst. of Clinical Biochemistry, Rikshospitalet, Univ. of Oslo, Norway and Dept. of Clinical Chem. and Research Centre, Huddinge Univ. Hospital, Karolinska Institutet, Stockholm, Sweden) *J. Biol. Chem. 257*(14):8363-8367 (1982). Using isotope dilution-mass spectrometry, it was shown that human bile contains significant amounts of 7α-hydroxy-4-cholesten-3-one, an intermediate in the major pathway for bile acid biosynthesis. In bile from 14 healthy subjects, the concentration was 0.14 ± 0.01 μg/ml (mean ± S.E.). Four bile samples collected from two patients with cerebrotendinous xanthomatosis contained considerably higher amounts of this steroid, 0.47-1.32 μg/ml. After oral administration of [4-^{14}C] 7α-hydroxy-4-cholesten-3-one to rabbits, ^{14}C-labeled cholestanol could be isolated from the intestinal wall, liver, and blood after 24 h. The label incorporated into the intestinal wall was about 10% of that obtained with [4-^{14}C] cholesta-4,6-dien-3-one or [4-^{14}C] 4-cholesten-3-one as precursors. Labeled cholesta-4,6-dien-3-one and 4-cholesten-3-one could be isolated from the intestinal contents 12 h after feeding [4-^{14}C] 7α-hydroxy-4-cholesten-3-one to rabbits. It is proposed that cholesta-4,6-dien-3-one and 4-cholesten-3-one are formed from 7α-hydroxy-4-cholesten-3-one by the same mechanism as that involved in 7α-dehydroxylation of primary bile acids. We suggest that biliary 7α-hydroxy-4-cholesten-3-one may be a physiological precursor to cholestanol. The possibility is discussed that part of the increased formation of cholestanol in patients with cerebrotendinous xanthomatosis is due to excess biliary 7α-hydroxy-4-cholesten-3-one or some metabolite of this steroid.

EXERCISE INTENSITY, DIETARY INTAKE, AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL IN YOUNG FEMALE COMPETITIVE SWIMMERS. M.P. Smith, J. Mendez, M. Druckenmiller, P.M. Krin-Etherton (Nutrition Program and Noll Human Performance Laboratory, The Pennsylvania State University, University Park, PA) *Am. J. Clin. Nutr. 36*(2):251-255 (1982). Plasma-high-density and low-density lipoprotein cholesterol (HDL-C and LDL-C) and dietary intake were compared in female competitive swimmers (CS, n = 7), synchronized swimmers (SS, n = 11), and sedentary controls (C, n = 6). CS tended to be taller, heavier, and leaner than C; SS were intermediate. The caloric intake of CS was 21.5% greater than C (2468 ± 534 versus 2030 ± 668, $p < 0.001$). Dietary composition for all groups was 49% carbohydrate, 35% fat, and 15% protein. Total plasma cholesterol (TC) and LDL-C were not significantly different among groups. HDL-C, and HDL-C/TC were increased in CS compared with SS and C. HDL-C was 17 and 22% higher for CS than SS or C (82.0 ± 14.6 versus 70.0 ± 10.9 versus 67.2 ± 14.0 mg/dl, $p < 0.05$, respectively). The results of the current study indicate that plasma HDL-C is significantly elevated in young women who participate in an intensive, but not moderate, exercise regimen.

THE STEROL SUBSTRATE SPECIFICITY OF ACYL COA: CHOLESTEROL ACYLTRANSFERASE FROM RAT LIVER. D.M. Tavani, W.R. Nes, and J.T. Billheimer (Dept. of Biol. Sci., Drexel Univ., Philadelphia, PA 19104) *J. Lipid Res. 23*(5):774-781 (1982). Rat liver microsomes were incubated with various sterols suspended in Triton WR-1339, and the extent of esterification of these sterols by acyl CoA:cholesterol acyltransferase was determined. A 3β-hydroxyl group was required for esterification to occur. Furthermore, the rate of ester formation of campesterol was only 20% that of cholesterol, and the rates for sitosterol and stigmasterol were below detectable limits indicating that the structure of the alkyl side chain plays an important role in the interaction between substrate and enzyme. Additional evidence concerning the importance of the side chain was obtained by following the esterification of a series of linear side chain analogues of cholesterol. Maximal ester formation was obtained when the longest chain on C-20 had five carbons (the same as cholesterol) and either an increase or decrease in the number of carbons reduced the amount of ester formed. Sterols containing a 4-*gem*-dimethyl group were not esterified, while 4α-methyl-cholest-7-en-3β-ol showed a significant esterification. Lathosterol, cholestanol, and desmosterol were esterified 41%, 70%, and 62%, respectively, as well as was cholesterol. The relationship between the specificity of acyl CoA:cholesterol acyltransferase and the occurrence of sterol esters in tissues is discussed.

BIOSYNTHESIS OF CHOLESTEROL, LANOSTEROL, AND Δ^{7}-CHOLESTENOL, BUT NOT CHOLESTANOL, IN CULTURED FIBROBLASTS FROM NORMAL INDIVIDUALS AND PATIENTS WITH CEREBROTENDINOUS XANTHOMATOSIS. G.S. Tint, and G. Salen (Gastroenterology Section, VA Med. Center, East Orange, NJ 07019, Dept. of Med., College of Med. and Dentistry of New Jersey-New Jersey Med. Schl. Newark, NJ 07103, and Gastroenterology Dept., Cabrini Med. Center, New York, NY 10003) *J. Lipid Res. 23*(4):597-603 (1982). The cholesterol and cholestanol biosynthetic pathways and the control of cholesterolgenesis were investigated in skin fibroblasts, from patients with cerebrotendinous xanthomatosis (CTX) and from normal subjects, grown in a lipoprotein deficient (d < 1.25 g/ml) medium. [^{3}H] Acetate was added to the culture medium and its incorporation into sterols was assayed by both argentation and reversed-phase thin-layer chromatography (TLC). The labeling patterns were similar in both CTX and control cells with ^{3}H being found, in order of increasing activity, in lanosterol, Δ^{7}-cholestenol, and cholesterol. No ^{3}H-labeled material at all, however, could be detected in the TLC mobility region corresponding to cholestanol. The ratio of cholestanol to cholesterol in the low density lipoprotein (LDL) subfraction from the plasma of individuals with CTX ranged from 1.4 to 5.3%, which is equal to or slightly greater than the ratio in whole plasma. Approximately 65-70% of the total plasma and LDL cholestanol and cholesterol were esterified. Since CTX-LDL added to incubates of normal cells and normal LDL added to CTX fibroblasts suppressed HMG-CoA reductase activity and stimulated cholesterol esterification equally, and since ^{125}I-labeled control LDL was degraded with normal kinetics from the surface of CTX fibroblasts, both CTX-LDL and CTX fibroblast LDL membrane receptors appear to be biologically normal.

DIETARY INTAKE AND SERUM TOTAL CHOLESTEROL LEVEL: THEIR RELATIONSHIP TO DIFFERENT LIFESTYLES IN SEVERAL JAPANESE POPULATIONS. H. Ueshima, M. Iida, T. Shimamoto, M. Konishi, M. Tanigaki, M. Dio, N. Nakanishi, Y. Takayama, H. Ozawa, and Y. Komachi (Department of Public Health, Osaka University Medical School, 4-3-57 Nakanoshima, Kita-ku, Osaka 530, Japan) *Circulation 66*(3):519-526 (1982). Serum total cholesterol level and dietary intake were surveyed 1975-1977 in six Japanese population groups with different lifestyles, including groups in both rural and urban areas. Clerical workers in Osaka, who had the most westernized lifestyle of all the study groups, had the highest mean serum total cholesterol level (202 mg/dl for men ages 40-49 and 50-59 years), while farmers in Akita had the lowest mean serum total cholesterol level (163 mg/dl for men 40-49 years old, 159 mg/dl for men 50-59 years old, 165 mg/dl for men 60-69 years old). Nutrient intake data for men ages 40-59 years showed 23% of calories from fat for clerical workers in Osaka, the highest among the study groups, whereas farmers in Akita showed a low level of 14%. The ratio of dietary polyunsaturated to saturated fatty acids was over 1.1 for all groups. Cholesterol intake was 339-487 mg/day. Total carbohydrate as a percentage of calories was 53-65%; 75-80% of carbohydrate energy was ingested from cereals. Sugar accounted for less than 3.5% of total calories. In the cross-group correlation analysis between dietary lipid intake and serum total cholesterol, a significant strong positive correlation was found between the dietary lipid factor of Keys et al. and the mean serum total cholesterol level. A weak but significant correlation was observed between the dietary lipid factor and serum total cholesterol for individual inhabitants of Osaka.

INTRAVASCULAR METABOLISM OF AN ARTIFICIAL TRANSPORTER OF TRIACYLGLYCEROLS. ALTERATIONS OF SERUM LIPOPROTEINS RESULTING FROM TOTAL PARENTERAL NUTRITION WITH INTRALIPID. S.H. Untracht (Dept. of Biophys. and Theoretical Biol., Univ. of Chicago Pritzker Schl. of

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Med., Chicago, IL 60637) *Biochim. Biophys. Acta 711*(1):176-192 (1982). As a model for transport in the bloodstream of exogenous triacylglycerols, we have studied the intravascular metabolism of Intralipid, an artificial, cholesterol- and protein-free 'chylomicron'. Two patients with Crohn's disease and one patient with intermittent abdominal pain received total parenteral nutrition including between 50 and 100 g of Intralipid per day. Samples of serum were analyzed chemically for lipids, assayed for lecithin:cholesterol acyltransferase (EC 2.3.1.43), and analyzed by isopycnic density-gradient ultracentrifugation and electron microscopy. Two normal subjects were studied during and after a single 4-h infusion of 50 g of Intralipid. Although Intralipid contains no steroids, unesterified cholesterol progressively entered the serum after the vesicles appeared. During these short-term studies, the levels of HDL and lecithin:cholesterol acyltransferase did not change. The observations indicate that vesicles, derived from a variable fraction of the Intralipid phospholipids extract unesterified cholesterol into plasma. Inert and slowly cleared, the resulting mixed vesicles accumulate in the blood. The levels of HDL and lecithin:cholesterol acyltransferase decrease only when the intestine is bypassed for long periods of time, probably because the lipoprotein and enzyme are synthesized more slowly than usual. Our results suggest that in chylomicrons other mechanisms prevent phospholipids from being released into the bloodstream as vesicles.

PURIFICATION AND PROPERTIES OF LIPOPROTEIN LIPASE IN GUINEA PIG MILK. L. Wallinder, G. Bengtsson, and T. Olivecrona (Dept. of Physiol. Chem., Univ. of Umea, S-901 87 Sweden) *Biochim. Biophys. Acta 711*(1):107-113 (1982). Lipoprotein lipase was purified from guinea pig milk by chromatography on heparin-Sepharose followed by chromatography on an immobilized preparation of heparin that had been N-desulphated and then acetylated. This second step was necessary to separate a plasma protein, presumably antithrombin, from the lipase. The guinea pig enzyme turned out to be quite similar to lipoprotein lipase from bovine milk with respect to composition and molecular size. Furthermore, the specific activities and the dose-response relations for activation by apolipoprotein C-II were quite similar for the two enzymes. Antibodies raised against the guinea pig milk enzyme inhibited not only this enzyme but also the lipoprotein lipase activity in post-heparin plasma and in homogenates from adipose tissue and heart.

(1-PYRENEBUTYRYL)CARNITINE AND 1-PYRENEBUTYRYL COENZYME A: FLUORESCENT PROBES FOR LIPID METABOLITE STUDIES IN ARTIFICIAL AND NATURAL MEMBRANES. P.E. Wolkowicz, H.J. Pownall, and J.B. McMillin-Wood (Dept. of Med., Sec. of Cardiovascular Sci., Baylor Coll. of Med., Houston, TX 77030) *Biochemistry 21*(12):2990-2996 (1982). Membrane properties of fatty acyl coenzyme A (CoA) and acylcarnitine have been studied with 1-pyrenebutyryl-CoA and (1-pyrenebutyryl)carnitine (PBC). These molecules have the spectroscopic properties of pyrene and its derivatives and exhibit biological and chemical characteristics related to the acyl esters. PBC is more soluble in nonpolar solvents than 1-pyrenebutyrul-CoA (PB-CoA), and critical micelle concentrations of both compounds resemble the medium-chain fatty acyl esters. PB-CoA inhibits phosphorylating (ADP-stimulated) respiration in rat liver mitochondria noncompetitively (K_I = 2 μM) and carnitine palmitoyl-CoA and octanoyl-CoA transferases competitively (K_I = 2.1 μM and 15 μM, respectively). PBC does not inhibit carnitine palmitoyl-CoA transferase or mitochondrial respiration when glutamate-malate or succinate (+rotenone) is used as the respiratory substrate. PBC is a potent inhibitor of phosphorylating repiration with either palmitoylcarnitine (I_{50}=1.4 μM) or octanoylcarnitine (I_{50}=40 nM) as the respiratory substrate. The mitochondrial carnitine-acyl-carnitine translocase is competitively inhibited by PBC with a K_1=0.6μM for palmitoylcarnitine exchange and 23 nM for carnitine exchange. PBC and PB-CoA exhibit excimer and monomer fluorescence, the relative intensities of which are functions of their microscopic concentrations. PB-CoA is accessible only to the outer half of artificial lipid vesicles while PBC may cross lipid vesicle bilayers. PBC in the inner half of the bilayer appears "trapped", i.e., not easily removed by exogenous bovine serum albumin, which binds PBC.

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