OBESITY: TOWARDS A MOLECULAR APPROACH

Organizers: George Bray, Daniel Ricquier and Bruce Spiegelman

April 17-23, 1989

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Poster Session

Clinical Types of Obesity

Q 001 GENETICS OF HUMAN OBESITY, Claude Bouchard, Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec G1K 7P4, Canada.

Three different lines of evidence will be used to demonstrate the role of the genotype in human variation for total body fat, and particularly abdominal fat. First, based on data obtained in about 1700 individuals from 9 different kinds of relatives by descent or by adoption, and using the analytical methods from the field of genetic epidemiology, we concluded that the additive genetic effect was quite low (less than 10%) for subcutaneous fat but reached about 25% to 30% of the age and gender adjusted fat mass, percent fat and regional fat distribution. We could not find any evidence for a larger maternal or paternal effect, a specific X- or Y-linked effect, or a sex-limited effect. From data obtained in pairs of parent-child, dizygotic (DZ) and monozygotic (MZ) twins, we also reported that the heritability for resting metabolic rate, thermic response to a standardized meal and energy cost of submaximal exercise was significant and reached at least 40% of the variance after adjustment for the proper concomitants. Second, using an experimental genetic strategy, we were able to conclude that the genotype was an important determinant of the human adaptive response to chronic overfeeding, in both short-term (22 days) and long-term (100 days) studies. In the latter case, 12 pairs of young adult male MZ twins were overfed by a total of 84 000 kcal over 100 days. We found 3 times more variance between pairs than within pairs in the changes observed in subcutaneous fat, total fat mass and body energy gain as well as for the various components of energy expenditure. However, the genotype-environment interaction effect was much stronger (F ratio $\geq$ 6) for the changes in visceral fat deposition. Third, we have undertaken the search for genetic markers of the obese state and of the sensitivity to chronic overfeeding. Thus far, we have identified 10 proteins of the adipose tissue from 2-D gel electrophoresis which exhibit genetic variation. Two of these are particularly interesting: variants for the 8D2 protein being primarily observed in the MZ twins gaining less fat with overfeeding, while 10DE variants were seen in the high gainers. These studies have been extended to nuclear and mitochondrial DNA using RFLP technology. One interesting observation is that obese women exhibit more mtDNA polymorphism than lean controls with a panel of 22 restriction enzymes. Several promising avenues are currently explored.

Q 002 OVERVIEW OF OBESITY, George A. Bray, Dept. of Diabetes and Clinical Nutrition, University of Southern California, Los Angeles, CA 90033

The elements of a feedback system for regulating body fat stores are reviewed. The effects of cholecystokinin has been used as an example of a molecular mechanism involved in the afferent limb of the feedback system. In the central controller, the increase in body weight following the chronic infusion of norepinephrine into the ventromedial nucleus is used as an example of a molecular mechanism for inducing obesity since similar infusions into the paraventricular nucleus do not produce obesity, but will produce acute increases in food intake. A second example of molecular mechanisms regulating food intake and body weight within the central nervous system will come from the effects of adrenalectomy and corticotrophin releasing factor. Efferent controls for this system are illustrated with two examples. First, the reciprocal relationship of activity of the sympathetic nervous system to food intake is discussed. Second, the role of acetylation of MSH in the development of the obese yellow mouse is presented in detail. The percentage of desacetyl-MSH in pituitary extracts from yellow obese (A^{vy}/a) mice was twice as high as in the pituitaries from lean black (a/a) mice of the same strain. Treatment of yellow obese mice with desacetyl-MSH significantly increased body weight and food intake, but treatment with alpha-(N-acetyl)-MSH did not. Alpha MSH produced more black pigmentation of regrowing hair whereas desacetyl MSH produced less pigmentation. Desacetyl-MSH increased food intake in yellow obese mice, but alpha-(N-acetyl)-MSH did not. Corticosterone levels in yellow obese mice treated with desacetyl-MSH were increased, but alpha-(N-acetyl)-MSH was without effect. The higher ratio of desacetyl MSH to alpha-(N-acetyl)-MSH may provide a biochemical mechanism for the yellow coat color, hyperphagia and obesity in the yellow obese mouse.

Adipocyte Differentiation

Q 003 REGIONAL OBESITY AND STEROID HORMONE INTERACTIONS IN HUMAN ADIPOSE TISSUE, Per Bjorntorp and Marielle Rebuffe-Scrive, Department of Medicine I, Wallenberg Laboratory, Sahlgren's Hospital, University of Goteborg, Sweden

Abdominal distribution of body fat is an independent risk factor for cardiovascular and cerebrovascular disease, as well as non-insulin dependent diabetes mellitus. Genetic and environmental factors particularly steroid hormones, regulate fat distribution. The tight statistical associations to disease makes it important to understand these mechanisms.

Glucocorticoids are important for the induction of lipoprotein lipase activity (LPL), the main enzyme regulator of lipid uptake. The density of the glucocorticoid receptor seems to be higher in intraabdominal than other adipose tissues. Progesterone seems to increase LPL specifically in the gluteal-femoral region. Progesterone binding is very low in human adipose tissue and of uncertain significance for progesterone function.

Progesterone interacts with LPL induction via competition over the glucocorticoid receptor while testosterone seems to inhibit LPL via another mechanism. Corticosteroids apparently interact with sex steroid hormone receptor density.

The currently available knowledge is compatible with a balance between the effects of hormones produced by the hypothalamic-adrenal cortical or gonodal axes, determining lipid accumulation in either intraabdominal or peripheral adipose tissues. With corticosteroid excess, with or without a decrease in the sex steroid hormones, lipid accumulation is favoured in the intraabdominal regions.

Functional cortisol overproduction is seen in subjects with abdominal excess of fat, probably on the basis of prevalent smoking, stress, and alcohol overconsumption, which also cause a decrease in sex steroid production. The intraabdominal distribution of fat in these subjects might thus be due to this endocrine profile combined with the distribution of steroid hormone receptors in different adipose tissue regions.

Q 004 HORMONAL CONTROL OF ADIPOCYTE DIFFERENTIATION, Gordon M. Ringold, Marc Navre and Hans-Michael Wenz, Cancer and Developmental Biology, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304.

The environmental cues that control developmental decisions more often than not take the form of hormonal signals. We have been studying the mechanisms by which glucocorticoids, fibroblast growth factor (FGF) and tumor necrosis factor (TNF) regulate the conversion of adipoblasts to adipocytes. The differentiation of the adipogenic cell line TA1 is accelerated by glucocorticoids and requires high cell density. In contrast either FGF or TNF will block and under appropriate conditions reverse the differentiation of these cells.

We have recently focused our attention on the observation that the effects of FGF on adipocyte differentiation are independent of a mitogenic response. Moreover, using selective modulators of protein kinase C we have determined that there are two FGF-sensitive pathways in TA1 cells: one, associated with initiation of differentiation can be blocked by either TPA or FGF whereas the other, required for maintenance of differentiation can be inhibited by FGF but not TPA. This latter pathway is also regulated by second messengers signalling systems influenced by both protein kinase C and Ca^{2+}.

Lastly, we have recently identified an FGF-repressible gene encoding a 27 kDa protein (AP27) that appears to be required for triggering differentiation. The expression of this gene is induced by increasing cell density and glucocorticoids but repressed by FGF or TPA. Production of AP27 anti-sense RNA in either 3T3-L1 or TA1 cells markedly inhibits their ability to undergo adipogenic conversion. This effect is specific since expression of β-actin or ferritin heavy chain anti-sense RNA does not compromise these cells' differentiative ability. We conclude that AP27 is required though not necessarily sufficient for the initiation of differentiation of adipoblasts to adipocytes.

Control of Gene Expression Related to Energy Balance

Q 005 REGULATION OF ADIPSIN EXPRESSION IN OBESITY,Jeffrey Flier,Brad Lowell, Antonella Napolitano, Abdul Dulloo, Pat Usher, Barry Rosen and Bruce Spiegelman. Dept. of Medicine, Beth Israel Hospital, and Dana Farber Cancer Center, Boston, MA 02165. Adipsin is a circulating, adipocyte derived serine protease whose expression we have shown to be profoundly impaired in several rodent models of genetic (ob/ob and db/db) and acquired obesity (monosodium glutamate induced,MSG). We have sought to define some of the factors that regulate adipsin expression in vivo in normal mice and in mice with syndromes of obesity. In each of the mouse models of obesity characterized by reduced adipsin expression, sympathetic nervous system activity is impaired. We therefore assessed the effect of treatment with the sympathomimetic cocktail of ephedrine (1 gm/kg chow) and caffeine (1.36 gm/kg chow) upon both adiposity and adipsin expression in MSG and ob/ob mice. Ephedrine plus caffeine (E+C) therapy given as a supplement to chow completely reversed obesity in MSG mice over 4 weeks and increased adipsin mRNA abundance in epidydimal fat and brown adipose tissue (BAT) to levels at or above normal. The concentration of adipsin in the plasma as measured by radioimmunoassay was also restored. In contrast, E+C treatment had little effect on adiposity, adipsin mRNA abundance or circulating adipsin concentration in ob/ob mice. Ephedrine did increase adipsin expression in lean littermates of ob/ob mice . Thus, the ability of E+C to mobilize fat correlated well with the ability of the treatment to induce adipsin expression. The ability of E+C to induce adipsin expression suggested that diminished SNS activity might be responsible for some component of adipsin deficiency in obesity. However, neither cold exposure (4 C) for up to 3 days which markedly stimulates SNS activity nor treatment with 6OH-dopamine, which destroys sympathetic nerve terminals, affected adipsin expression in WAT or BAT or changed circulating adipsin levels. Furthermore, regulated expression of adipsin during these manipulations (ie E+C, cold exposure) diverges from that of brown fat uncoupling protein. Thus, adipsin expression appears not to be regulated by the SNS and the mechanism whereby E+C induces adipsin expression remains to be determined. Another in vivo factor that might be implicated in altered adipsin expression is the level of glucocorticoid hormones, since these are increased in ob/ob, db/db and MSG mice. Evidence that glucocorticoids suppress adipsin expression in vivo and account in part for the reduced expression in obesity will be presented. Finally, in vivo and in vitro evidence that insulin plays a role in the regulation of adipsin will be discussed.

Q 006 MOLECULAR MECHANISMS INVOLVED IN THE NUTRITIONAL AND HORMONAL REGULATION OF THE AVIAN GENE FOR MALIC ENZYME, Alan G. Goodridge, Dominic A. Fantozzi, Steven A. Klautky, Xiao-jun Ma, David A. Mitchell, Lisa M. Salati, and Julian Swierczynski, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.
The activity of hepatic malic enzyme, one of a set of lipogenic enzymes, is high in well-fed chickens and low in starved chickens. In chick-embryo hepatocytes in culture, insulin and triiodothyronine (T3) are positive effectors, and glucagon acting via cyclic AMP is a negative effector. Hormone concentrations in the blood are consistent with insulin and T3 laying major positive roles, and glucagon a major negative role, in regulating hepatic malic enzyme activity during the transitions between the fed and the starved states. Nutrition- and hormone-induced changes in malic enzyme activity are due to altered concentrations of malic enzyme protein which, in turn, are due to altered rates of synthesis of malic enzyme. Synthesis of malic enzyme is controlled by regulating the abundance of malic enzyme mRNA. Based on kinetic and inhibitor experiments, both starvation and glucagon stimulate degradation of malic enzyme mRNA, but not sufficiently to account for the decrease in mRNA level caused by these treatments. The main regulatory mechanism appears to involve initiation of transcription. The T3-induced accumulation of malic enzyme mRNA in culture is blocked by puromycin, an inhibitor of protein synthesis, and by H8, H7 and HA1004, inhibitors of protein kinases. T3 also stimulates the activity of a nuclear protein kinase in our hepatocytes in culture. Thus, the intracellular signalling pathway by which T3 regulates transcription of the malic enzyme gene may include a protein intermediate and phosphorylated protein intermediate or require the activity of a rapidly turning over protein(s) and a phosphorylated protein(s). Supported in part by grant DK21594 from the National Institutes of Health.

Q 007 THE INTRODUCTION OF GENES OF METABOLIC INTEREST INTO CELLS AND ANIMALS, R.W. Hanson, M. Hatzoglou, A. Wynshaw-Boris, M.M. McGrane, F.J. Rottman, J. Yun and T. Wagner, Department of biochemistry, Case Western Reserve Univ., Cleveland, OH 44206 and the Edison Animal Biotechnology Center, Ohio Univ., Athens, OH.

A series of chimeric genes containing the P-enolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) promoter ligated to the structural gene for bovine growth hormone (bGH) were introduced into hepatoma cells or into the germ-line of mice and pigs in order to determine factors which regulate the expression of the promoter in vivo and the effect(s) of the expression of bGH on metabolic processes in the animals. The PEPCK promoter (-450/+73) contained elements responsive to cAMP and insulin as well as tissue-specific sequences which direct the expression of the chimeric gene to the liver. A murine retroviral vector was also used to introduce the chimeric PEPCK/bGH gene into hepatoma cells and into the livers of 20 day fetal rats infected with the virus in utero by interperitoneal injection. Bt_2cAMP markedly increased (within 2 hrs) and insulin decreased the transcription of bGH from the PEPCK promoter in hepatoma cells infected with the retrovirus. Rats infected in utero with the retrovirus, containing the PEPCK/bGH gene, expressed the gene in the liver and bGH was detected in the blood several months after birth. Transcription of the chimeric gene was controlled by the administration of hormones and by diet in the predicted manner. In transgenic mice expressing the PEPCK/bGH gene, diets high in carbohydrate reduced the concentration of bGH in the serum to 5% of that noted in control animals and subsequent refeeding the diet high in protein but devoid of carbohydrate resulted in a 20-fold increase in serum bGH within a week. Pigs containing the PEPCK/bGH transgene introduced into the germ line by microinjection had a 50% reduction in back fat and a 30% increase in feed efficiency. The potential usefulness of these methods of introducing genes of metabolic interest into cells and animals will be discussed and the effects of altered bGH levels on carbohydrate and lipid metabolism outlined.

Animal Models of Obesity

Q 008 GENETIC OBESITY SYNDROMES IN MICE, D.L. Coleman, Jackson Laboratory, Bar Harbor, ME 04609

Several different rodent models are available for metabolic studies on the development of obesity. Although the abnormalities associated with each obesity type have many features in common, the documentation of several different genes being involved makes it unlikely that the various syndromes will be reduced to a single disturbance in one metabolic pathway. The severity of the diabetes/obesity syndrome produced depends on the interaction of the individual mutation with genetic factors in the inbred background of the host. Establishing the nature of these gene-host interactions in rodents should aid us in understanding similar interactions that occur in human diabetes/obesity. The development of the syndrome in most models is similar and includes hyperinsulinemia, hyperphagia, and attempts at increasing insulin supply by β-cell hyperplasia and hypertrophy in the early stages. Hyperglycemia, obesity, and severe diabetes are secondary features that result from a combination of insulin resistance and a failure to sustain the secretion of the large amounts of insulin. Most models utilize ingested food and stored food reserves more efficiently. This increased metabolic efficiency extends to heterozygotes that are normal in all respects having only one dose of the deleterious gene. Establishing this increased metabolic efficiency in heterozygotes lends credence to the thrifty gene hypothesis of diabetes/obesity and suggests a mechanism whereby some deleterious diabetes/obesity genes may be favored in the human population. The best studied mouse models, and those for which the most complete information is available, are those caused by single genes, e.g., yellow, obese, diabetes, tubby, and fat. In the other models, the mode of inheritance is either polygenic or otherwise unclear, features which interfere with the interpretation of the data. This talk will briefly summarize the developing syndrome in each model, point out differences, and suggest areas where future research should be most productive in the light of contemporary studies.

Q 009 DIETARY OBESITY, Janis S. Fisler and George A. Bray, Department of Medicine, University of Southern California School of Medicine, Los Angeles, CA 90033.

Obesity has been produced in experimental animals by providing sucrose solutions to drink, by providing a varied and palatable diet, or by feeding high fat diets with or without added sucrose. Genetic susceptibility to a high fat diet occurs in the yellow mouse with the A^y gene and in some strains of rats. Feeding rats a high fat diet produces the greatest increase in body fat in Osborne-Mendel rats, an intermediate level of increase in animals of most other strains, and only a very small weight gain in S 5B/Pl rats. This difference in response to a high fat diet implies that the mechanisms controlling energy balance differ between the strains. We have developed a model utilizing the dietary fat resistant S 5B/Pl strain and the dietary fat sensitive Osborne-Mendel strain. Our model is based on the concept that energy balance is a regulated system with afferent feedback signals about the state of nutrient stores and a central integrating system for translating messages into efferent signals which regulate food intake and energy expenditure. The metabolism of fat through 3-hydroxybutyrate (3-OHB) provides an afferent signal which differs between S 5B/Pl and Osborne-Mendel rats. That 3-OHB is involved in the regulation of energy balance is suggested by the following: 1) Oxidation of 3-OHB in the liver reduces food intake; 2) S 5B/Pl rats have a higher transport of 3-OHB into the brain and higher brain 3-OHB levels than do Osborne-Mendel rats; 3) The chronic infusion of 3-OHB into the brain reduces both food intake and body weight; and 4) Injection of 3-OHB into the hypothalamus increases the firing rate of the sympathetic nerves to brown adipose tissue in a dose dependent manner. Thermogenesis in brown adipose tissue, regulated by sympathetic nerves, is a major means of energy wastage during overfeeding. A high fat diet eaten chronically depresses the firing rate of sympathetic nerves to brown adipose tissue. Thus, dietary fat induced obesity may be the result of defective nutrient stimulation of the sympathetic nervous system in genetically susceptible strains.

Glucose Metabolism

Q 010 INSULIN RESISTANCE, Jose F. Caro, Madhur K. Sinha and G. Lynis Dohm, Departments of Medicine and Biochemistry, East Carolina University School of Medicine, Greenville, NC 27858

Insulin resistance is a universal finding in obesity and NIDDM. Morbidly obese patients with and without NIDDM entered into a gastric bypass program comprise an ideal human model to study *in vivo* and *in vitro* the mechanism(s) of insulin resistance. To this end we have developed methods to freshly isolate and culture insulin responsive human hepatocytes (*JCI* 78:249, 1986), adipocytes (*JCI* 80:1073, 1987), and muscle fibers (*JCI* 82:486, 1988) from intraoperative biopsies.

The *in vivo* data generated by the euglycemic insulin clamp indicate that morbidly obese patients without NIDDM have a lesser degree of insulin resistance in liver and peripheral tissues than those with NIDDM, which is consistent with the *in vitro* data from liver and adipose tissue. In contrast, human muscle fibers from obese patients with and without NIDDM are equally resistant to insulin. Insulin resistance in the skeletal muscle may serve as a mechanism to initiate and perpetuate obesity by shuttling substrates to the adipose tissue. Since obese patients are euglycemic, it is likely that the quantitative role of the adipose tissue to dispose glucose has been underestimated.

At the cellular level we have demonstrated that insulin receptor kinase activity is markedly decreased in liver, adipose tissue, and muscle from NIDDM and in muscle from obese patients, and may offer an explanation for the insulin resistance. The mechanism(s) of decreased insulin receptor kinase activity remains to be elucidated; however, we have serendipitously found that PIP_2-specific phospholipase C (PLC) activity is increased in the liver from NIDDM patients. Increased PIP_2-PLC might result in increased diacylglycerol and activation of protein kinase C, which will phosphorylate the insulin receptor at serine residue, resulting in inactivation of the insulin receptor kinase. Thus, the increased PIP_2-PLC might be involved in a cascade of events that result in insulin resistance.

Q 011 CELL BIOLOGY OF INSULIN ACTION ON GLUCOSE TRANSPORT, Samuel W. Cushman and Ian A. Simpson, Experimental Diabetes, Metabolism and Nutrition Section/MCNEB, NIDDK, NIH, Bethesda, MD 20892

The acute regulation of glucose transport in the rat adipose cell is mediated by various distinct but interactive hormonal systems. Insulin, the principal stimulatory hormone, can induce a 20-40-fold increase in the maximum rate (V_{max}) of glucose transport. The primary mechanism by which insulin elicits this enhanced rate of glucose transport is by inducing the translocation of glucose transporters (glycoproteins of M_r≈45 kDa) from a large intracellular pool to the plasma membrane. This translocation is rapid, reversible, and energy-dependent and results in a 5-10-fold increase in the concentration of glucose transporters in the plasma membranes with a corresponding 50-60% decrease in the intracellular pool. The lipolytic agents/hormones isoproterenol, glucagon, and ACTH represent the major inhibitors of insulin-stimulated glucose transport activity. These agents stimulate lipolysis through the activation of adenylate cyclase. Conversely, the antilipolytic agents adenosine, prostaglandin E_1, and nicotinic acid, inhibitors of adenylate cyclase, enhance insulin-stimulated glucose transport. In contrast to insulin, these lipolytic and antilipolytic agents achieve their modulation of glucose transport activity by altering the intrinsic activity of those glucose transporters residing in the plasma membrane, rather than modulating the translocation of glucose transporters. Furthermore, despite their actions on adenylate cyclase, these agents appear to achieve their regulatory action by a cAMP-independent mechanism. Studies with cholera and pertussis toxins, which selectively interact with the guanine nucleotide-binding proteins G_s and G_i, respectively, suggest that the latter proteins may mediate the regulatory action of these various agents. A further regulatory action of these agents is to alter the insulin sensitivity, with the lipolytic agents decreasing the sensitivity 3-5-fold and the antilipolytic agents enhancing the sensitivity 2-fold. Oxytocin and vasopressin represent a third hormonal group, whose stimulatory actions on glucose transport appear to be mediated by protein kinase C. These hormones stimulate glucose transport activity in the absence of insulin and augment insulin-stimulated activity by a translocation mechanism distinct from that achieved by insulin. Integration of these various hormonal responses to regulate glucose transport in the adipose cell serves to fine tune the balance between lipolysis and lipogenesis.

Q 012 REGULATION OF ADIPSIN GENE EXPRESSION IN DIFFERENTIATION AND OBESITY, Bruce M. Spiegelman*+, Barry S. Rosen*+, Kathleen S. Cook*+, William O. Wilkison*+, John S. Volanakis**, Jeffrey S. Flier+, Deborah Damm++, Tyler White++, Ken Platt‡ and Susan R. Ross‡, Dana-Farber Cancer Institute* and Harvard Medical School+, University of Alabama at Birmingham**, Metabolic Biosystems++ and University of Illinois Medical School‡.

We have shown that expression of the adipsin gene is markedly reduced in certain forms of genetic and acquired obesity in rodents. We have now studied the mechanisms regulating the adipsin gene in cultured adipocytes and intact animals. In lean (db/+) transgenic mice carrying the adipsin gene promoter (⁻950 - ⁺35) linked to a reporter gene (bacterial CAT), CAT activity is expressed exclusively in adipose tissue. In genetically obese (db/db) littermates, CAT activity is strongly suppressed, indicating that the adipsin promoter region contains an element that responds to the homozygous obesity allele. When a heterologous enhancer from the AK leukemia virus is included in the adipsin construction, CAT expression in lean mouse tissues is broadened to include salivary gland, spleen and thymus. However, in obese animals carrying this construction, CAT activity is suppressed in all of these tissues, indicating that the trans-acting factors controlled by the obesity gene can operate in tissues other than fat. Deletion analysis of the adipsin promoter in cultured cells suggests that the region between ⁻114 to ⁻38 is important for fat-specific expression and protein binding assays suggest that novel single-stranded DNA binding factors may be involved. The enzymatic activity of the adipsin protein and its relation to complement Factor D and the alternative pathway of complement activation will also be discussed.

Nervous System and Obesity

Q 013 MICRODIALYSIS MEASUREMENTS OF DOPAMINE RELEASE IN THE MESOLIMBIC SYSTEM AS A FUNCTION OF INGESTIVE BEHAVIOR AND BODY WEIGHT,
B. G. Hoebel, L. Hernandez, G. P. Mark, G. A. Hunter and M. Pothos, Department of Psychology, Princeton University, Princeton, NJ 08544-1010

An increase in hypothalamic self-stimulation is a common outcome of several procedures which cause hyperphagia and obesity[1,2,3]. The role of dopamine in feeding and self-stimulation was tested by measuring extracellular dopamine (DA) and its metabolites at 20 min intervals during ongoing behavior. The use of microdialysis in the study of feeding reward is illustrated by the following experiments in which DA *increased* in the nucleus accumbens: 1) during free feeding at 80% body weight[4], 2) during bar-pressing for food or hypothalamic self-stimulation at a normal weight[4,5], 3) during hypothalamic stimulation with or without food to eat[4], 4) during water intake in response to deprivation or intraventricular angiotensin[6,7], 5) during salt intake in response to sodium depletion[7], and 6) during systemic or local infusion of amphetamine, cocaine, nicotine and related stimulants[8,9]. On the other hand, mean basal extracellular DA *decreased* in rats reduced to 80% body weight. In these underweight animals DA release in response to amphetamine was dampened. In sum, the results suggest that the rewarding properties of food are somehow related to mesolimbic DA release, and that this DA system is less active and less reactive in underweight animals.

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Q 014 OBESITY AND THE SYMPATHETIC NERVOUS SYSTEM (SNS). Lewis Landsberg, Department of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215. The SNS is now recognized to be the major regulator of dietary thermogenesis. Broadly defined, the latter encompasses the regulatory component of heat produced in response to meal ingestion (distinct from the obligatory component or specific dynamic action) as well as alterations in resting metabolic rate that reflect the impact of antecedent diet. The SNS thus is the effector limb of a system that relates energy intake to metabolic heat production. Brown adipose tissue (BAT), regulated by the SNS is the major heat producing organ in laboratory rodents and of possible, but unproved, importance in man. The role of the SNS was elucidated in part by the effect of dietary intake on SNS activity: fasting suppresses while overfeeding stimulates the SNS in animals and humans. The stimulatory effect of overfeeding depends upon carbohydrate and fat since the latter two nutrients stimulate the SNS even when caloric intake is not increased. Evidence from a variety of sources indicates that insulin-mediated glucose metabolism within neurons of the ventromedial hypothalamus is intimately involved in the coupling of dietary intake and SNS activity. Decreases in insulin-mediated glucose metabolism in these cells, induced by fasting, suppresses sympathetic outflow while increased insulin-mediated glucose metabolism in association with carbohydrate feeding, or peripheral insulin resistance, increases SNS activity. This link between dietary intake and sympathetically mediated thermogenesis, therefore, provides a potential buffer against the development of obesity and raises the possibility that genetic or acquired deficiencies in the capacity for sympathetically-mediated thermogenesis may be involved in the pathogenesis of obesity. In genetically obese rodents such as the ob/ob mouse deficient sympathetic activation of BAT appears to play a role in the efficient metabolic state characteristic of these animals despite the fact that dietary changes in SNS activity are preserved. In the gold thioglucose-treated mouse, in contrast, sympathetic activity is increased and dietary changes in sympathetic outflow obliterated. Chronically overfed rodents, obese or not, have increased SNS activity. In human obesity the functional state of the SNS has not been defined since methods for assessing SNS activity in humans are insensitive and regional differences in sympathetic outflow difficult to detect. Human obesity, furthermore, is a heterogeneous disorder, with variable underlying pathophysiology in different subtypes. Preliminary evidence suggests that in hypertensive obese with upper body obesity increased SNS activity may be present as a compensatory mechanism. Despite the difficulties involved, continued study of sympathetically mediated thermogenesis in the obese is important since therapeutic strategies may evolve from knowledge of the underlying pathophysiology.

Q 015 CHEMICAL AND NEURONAL FACTORS AFFECTING FEEDING BEHAVIOR, Yutaka Oomura, Department of Central Nervous Function Control System, Toyama Medical and Pharmaceutical University, Toyama Japan

Certain neurons are described as glucoreceptor neurons [GRN] if their activity increases dose dependently upon direct exposure to glucose. Other neurons are described as glucosesensitive [GSN] if their spontaneous activity is diminished by direct exposure to glucose. GSNs can now be found in many discrete, identifiable neuronal centers although they are apparently most dense in the lateral hypothalamic area [LHA], GRNs in a similar way are probably most dense in the ventromedial nucleus of the hypothalamus [VMH]. These neurons are, in fact, not specific to glucose but respond to most of the metabolites and hormones that have been found to either control feeding behavior or change in level with result of feeding. Recently, a group of sugar acids have been found in the blood of rats and monkeys, sugar acids 2[s], 4[s], 5-trihydroxypentanoic acid γ-lactone induces feeding, and 3[s], 4-dihydroxybutanoic acid γ-lactone and 2-buten-4-olide suppress feeding. The action of all three of these substances is mediated by GSNs and GRNs in the hypothalamus and medulla. A phasic increase in activity of acidic fibroblast growth factor (aFGF) was detected in the cerebrospinal fluid of rats and monkeys after feeding or after an intraperitoneal injection of glucose. Intracerebroventricular microinfusion of aFGF suppressed food intake in rats. Central infusion of inactivated aFGF, or peripheral administration of aFGF, were without effect. Electrophoretically applied aFGF specifically suppressed the activity of GSNs. This inhibition was due to the activation of protein kinase C in the GSNs. GRNs were unaffected by aFGF. Immunohistochemical study by aFGF antibody revealed that neurons contained aFGF after feeding in the LHA, zona inserta, amygdala, hippocampus, globus pallidus etc but not in the VMH. In situ hybridization study indicated that aFGF produced in the ependimal cells in the cerebroventricular wall and the neurons in the above nuclei took in aFGF which was released from the epenimal cells. The results suggest that, the endogenous chemical factors participates in the central regulation of feeding.

Q 016 AFFERENT SIGNALS FOR EATING AND METABOLISM, Gerard P. Smith, Bourne Laboratory, New York-Hospital Cornell Medical Center, White Plains, NY 10605

Afferent signals for the control of food intake and metabolism are initiated at the mucosal surface of the gut from the tip of the tongue to the end of the small intestine and from the liver. The afferent signals from the mouth travel to the central nervous system through chemosensory and somatosensory fibers of the fifth, seventh, ninth and tenth cranial nerves. The afferent signals from the abdomen travel through visceral afferent fibers of the vagus and of the dorsal roots of the thoracic and lumbar segments of the spinal cord. Afferent signals from ingested food acting in the mouth provide primarily positive feedback signals for eating; those from the stomach, small intestine and liver provide primarily negative feedback signals for eating. Central processing of these afferent signals begins in the hindbrain and continues in widespread and ill-defined networks of the forebrain. Although there is some knowledge concerning the adequate chemical and mechanical stimuli for these afferent neurons, their receptors, mechanisms for activation and central transmitters are poorly understood. This ignorance is the major obstacle to a molecular analysis of their information transmitting function in the control systems for the intake, digestion, and metabolism of food.

Fatty Acid Metabolism

Q 017 RECENT DEVELOPMENT IN HUMAN FAT CELL ADRENERGIC RECEPTORS CHARACTERIZATION AND FUNCTION. Max Lafontan, Jean Galitzky, Pascale Mauriège, Jean Sébastien Saulnier-Blache, Michel Berlan. I.N.S.E.R.M. - Unité 317, Institut de Physiologie Université Paul Sabatier, Rue François Magendie - 31400 Toulouse, France.

Three adrenoceptors are involved in the control of lipolysis (β1, β2 and α2-A). For α2-adrenoceptors, two major labeled antagonists are usable for identification of total number of α2A-sites : [^{3}H]-yohimbine and [^{3}H]-RX821002. The full agonist [^{3}H]-UK-14,304 revealed the high affinity forms of the α2-adrenoceptor; the "tight agonist binding" observed with the full agonist is not seen with the partial agonist clonidine. In addition to the β1-adrenoceptor, functional assays with procaterol and zinterol (β2-agonists) clearly revealed the existence of β2-adrenoceptors in human fat cells. The evaluation of β1 and β2 adrenoceptors density based on the inhibition of binding of a non-selective antagonist radioligand by increasing concentrations of an unlabeled, subtype-selective competing ligand causes distorsion of the estimated binding parameters (inhibitory constants of the competitor and proportion of β-subtypes) since all the β-adrenergic antagonist radioligands used are slightly (2.5-8-fold) subtype selective: (^{125}I)-iodocyanopindolol, [^{3}H]-dihydroalprenolol are β2-selective while [^{3}H]-CGP12177 is β1-selective. A truly non-selective β-antagonist radioligand is still required. Recent studies involving animal models (hamster, dog, rabbit) indicate that diversity of expression and differential regulation exists among β1, β2 and α2-A fat cell adrenoceptors. The adrenergic receptor subtypes are encoded by genes which present an homology with a large family of guanine nucleotide-binding protein (G-protein)-coupled receptors. Human β1, β2 and α2-A adrenoceptors have been recently cloned. Preadipocytes from human and hamster adipose tissue undergo a lesser degree of differentiation of adenylyl cyclase system than expected by their lipid accumulation ability (absence of α2-adrenoceptors and reduced Gi-mediated inhibition of the enzyme). The ability of differentiated preadipocytes to fully express all the characteristics of the adipocyte in culture is an issue which may ultimately be reached for elucidation of adrenoceptor subtype genetics and regulation. Suitable cell culture systems (adipocyte precursors and/or preadipose cell lines) should provide a perequisite system to investigate how key hormones may regulate adrenoceptor genes expression in fat cells.

Energy Regulation and Thermogenesis

Q 018 THE ROLE OF S14 AND LIPOGENESIS IN THE THERMOGENIC EFFECTS OF THYROID HORMONE, Hedley C. Freake and Jack H. Oppenheimer, Division of Endocrinology and Metabolism, Department of Medicine, University of Minnesota, Minneapolis, MN 55455.

Thyroid hormone is well known to regulate metabolic rate, but the underlying mechanisms are obscure. Though numerous energy-utilizing pathways are thyroid hormone-regulated, it is unclear which are primary targets for the hormone and to what extent they quantitatively contribute to the thermogenic process. In rat liver, the mRNA encoding S14 is an extremely early responder to triiodothyronine (T3) administration, suggesting a direct effect of the hormone. The nucleotide and amino acid sequences of S14 bear no homologies to known structures, but accumulating evidence suggests a role for the protein in some aspect of fatty acid synthesis. mRNA S14 is abundantly expressed only in lipogenic tissues, namely liver, white and brown adipose tissue (BAT) and lactating mammary gland. In liver and epididymal fat, its responses to T3, carbohydrate feeding and other physiological interventions parallel those of known lipogenic enzymes. In contrast to liver, thyroid hormone withdrawal stimulates mRNA S14 in BAT, and a similar increase in lipogenesis also occurs. Physiological activation of BAT by cold exposure or overfeeding also stimulate both parameters. In liver, T3 treatment of hypothyroid animals resulted in a 10-15 fold stimulation of both mRNA S14 and fatty acid synthesis, measured by tritiated water incorporation. The rise in S14 preceded that in lipogenesis, raising the possibility of a regulatory role for S14. Fatty acid synthesis rose following a 12-16 hour lag time and reached a maximum after 3-5 days. This time course is consistent with the slow response of oxygen consumption to T3 administration. The energy costs of fatty acid synthesis are substantial (57 moles ATP/mole palmitate) and thus this process appears to be a likely contributor to thyroid thermogenesis. Hepatic lipogenesis was quantitated every 4 h for 24 h and the integrated rates were 0.063, 0.213 and 0.531 μmoles palmitate/min in hypo-, eu- and hyperthyroid rats respectively. The T3-dependent increment in hepatic lipogenesis accounts for 6-9%, of thyroid-dependent whole body oxygen consumption, depending on which published values for this parameter are used. Total body fatty acid synthesis was also measured and amounted to 0.72, 1.13, and 1.98 μmoles palmitate/min in hypo-, eu-, and hyperthyroid rats. Thus when all tissues are considered, T3-stimulated fatty acid synthesis utilizes 16-25% of the additional ATP generated in the hyperthyroid state. The precise role of S14 in lipogenesis remains to be determined, but it is clear that lipogenesis is a major contributor to T3-regulated thermogenesis.

Q 019 THE UNCOUPLING PROTEIN OF BROWN ADIPOSE TISSUE : PHYSIOLOGICAL AND MOLECULAR ASPECTS, Daniel Ricquier, Frédéric Bouillaud, Louis Casteilla, Anne-Marie Cassard, Serge Raimbault, Odette Champigny, Eliane Hentz and Susanne Klaus, Centre de Recherche sur la Nutrition, Centre National de la Recherche Scientifique, F-92190 Meudon. Thermogenesis in brown adipose tissue results from uncoupled respiration related to a high proton conductance of the inner mitochondrial membrane. This unique proton translocation is due to a characteristic membraneous component, the Uncoupling Protein or UCP, of which the activity is triggered by free fatty acids and inhibited by purine nucleotides. Following acute interaction of norepinephrine with B-adrenoceptors of brown adipocytes, the metabolic cascade results in UCP activation and heat production. In other respects, chronic stimulation of brown adipocytes by norepinephrine induces cell hyperplasia, mitochondriogenesis and synthesis of various components including UCP. This trophic response is also controlled by T3. We have developed molecular studies on UCP to investigate both the activity of UCP itself and the regulation of UCP gene expression. cDNAs for rat and bovine UCP were isolated. Sequencing of rat UCP cDNA confirmed previous cell-free synthesis experiments showing that synthesized UCP has no N-terminal targeting extension. UCP is made of six hydrophobic segments interpersed with hydrophilic loops. UCP is partially homologous to other ubiquitous mitochondrial carriers such as the ADP/ATP and the phosphate carriers. Diagon analysis identified a domain related to an ADP binding site of the ADP/ATP carrier. Surprisingly, this domain is also homologous to DNA binding domain of hormone receptors. In order to understand the structure of UCP and residues or domains involved in proton translocation or regulation by nucleotides, experiments of expression of UCP in E. coli, yeasts and Xenopus oocytes have been undertaken. Rat and human UCP genes have been isolated. The complete exonic/intronic sequence of rat UCP gene has been determined as well as the organization of human gene. A long 4.5 kb fragment of the 5' upstream region flanking the start site of transcription has been sequenced and shown to contain Nuclease sensitive sites as well as possible regulatory elements. Analysis of promotor and nuclear factors is under progress. Moreover the human gene of UCP has been localized in chromosome 4 in q31. Northern blot experiments indicated that UCP mRNA could be strongly induced by cold exposure, B-agonist drug dosing or refeeding after starvation. Run on transcription experiments were used to demonstrate a transcriptional control of UCP gene. Basal initiation rate of UCP gene transcription is lowered in Fa/Fa obese rats. Genomic probes were used to detect UCP mRNA in human samples. Northern blot experiments in developing calves raised the question of a possible transformation of brown adipose tissue into white adipose tissue.

Q 020 ROLE OF LOCAL T_4 5'-DEIODINASE ON THE RESPONSE OF BROWN ADIPOSE TISSUE TO ADRENERGIC STIMULATION, J. Enrique Silva and Antonio C. Bianco, Thyroid Unit, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215. Two types of T_4 5' deiodinating activities, I and II, that differ in enzyme kinetics, inhibitor sensitivities, tissue distribution and physiological responses have been described. The Type II (5'D-II) has been found in the anterior pituitary, brain and brown adipose tissue (BAT), but only in the latter is stimulated by the sympathetic nervous system via α_1-receptors. This suggested an important role for T_3, and hence for 5'D-II, in the function of BAT. The following results obtained by us are consistent with this view. BAT has a high concentration of nuclear T_3 receptors (NTR). NTR are about 75% saturated in rats maintained at room temperature (23 C) and over 50% of this T_3 is produced by 5'D-II. The uncoupling protein (UCP) concentration and its response to adrenergic stimulation are severely reduced in hypothyroid rats. These rats become hypothermic when exposed to 4 C for 48 hours. Replacement of T_3 for a week restored the plasma T_3 levels and liver α-glycerophosphate dehydrogenase (GPD) but it neither normalized the UCP response to cold nor did it prevent the cold-induced hypothermia. In contrast, replacement of T_4 for just two days normalized the UCP response and prevented the hypothermia, but did not normalize either plasma T_3 or liver GPD. The response to T_4 was blocked by iopanoic acid, an inhibitor of 5'D-II. Experiments where increasing doses of T_3 were given revealed that the normalization of UCP required near saturation of NTR. Since the mere replacement of T_4 achieved the same effect as a receptor saturating dose of T_3, we hypothesized that the adrenergic stimulation of 5'D-II resulted in NTR saturation in the T_4-treated hypothyroid rats and, by extension, in euthyroid rats. Kinetic studies in euthyroid rats showed that cold exposure for as long as 3 weeks did not change the number of NTR and that indeed as soon as 4-6 hours after placing the animals at 4C, NTR were >95% occupied. The extra T_3 was all locally generated. Actually, the contribution of plasma T_3 to NTR occupancy was reduced, a logical consequence of the expansion of the intracellular pool of T_3. The contribution of BAT 5'D-II to NTR was not only prevented but reduced by pretreatment with prazosin below basal levels suggesting that the saturation was the result of the adrenergic activation of 5'D-II and, further, that even at room temperature the adrenergic tone contributes via 5'D-II to NTR occupancy. These results, along with others that show that norepinephrine-induced stimulation of UCP gene transcription is amplified by T_3 and that the response of several enzymes to adrenergic stimulation also requires the 5'D-II-generated T_3 for a full response underscore the physiological role of T_3 and 5'D-II for the function of BAT and hence for temperature regulation and energy balance.

Q 021 INSULIN REGULATION OF GAPDH GENE EXPRESSION IN LIPOGENIC TISSUES

N. Nasrin, M. Denaro, I. Kang, L. Ercolani, M. C. Alexander-Bridges, Howard Hughes Medical Institute, Wellman 3, Massachusetts General Hospital, Boston, MA 02114

Glyceraldehyde-3-phosphate dehydrogenase [GAPDH] gene expression is enhanced 10-fold by insulin in fat and liver tissue isolated from rats fasted and refed a high-carbohydrate, low-fat diet. This nutritional manipulation is known to induce enzymes involved in lipogenic pathways. Conversely, GAPDH gene expression is reduced in the adipose tissue of diabetic rats and is induced 4-fold above control levels when insulin is replaced.

We have used two cultured cell lines, the 3T3 adipocyte and the H35 hepatoma cell line to study the specific effect of insulin on expression of this gene. We have previously reported that a construct containing the 5'-flanking region of the human GAPDH gene fused to the chloramphenicol acetyl transferase gene [CAT] is regulated 4- to 8-fold in H35 hepatoma cell lines and 3-fold in 3T3 adipocyte cell lines.[1] Two independent cis-acting elements in the 5'-flanking region of the GAPDH gene confer insulin inducibility on the GAPDH promoter as well as the RSV promoter. Using the gel-mobility shift assay, two distinct insulin-sensitive DNA-binding proteins have been identified. Of interest, both DNA-binding proteins are detected predominantly in lipogenic tissues. This finding correlates with the fact that insulin does not regulate GAPDH in 3T3 preadipocytes.

Using the methylation interference assay we have identified the contact points for the interaction of protein with one of the binding domains. This sequence is present in a number of insulin-sensitive genes. We are in the process of defining the minimal element sufficient to confer insulin inducibility to a heterologous promoter. This sequence may represent a "consensus sequence" for insulin action on gene expression.

1. Alexander, et al., (1988) Proc. Natl. Acad. Sci., Vol. 85, pp. 5092-5096.

Molecular Regulation of Adipose Cells

Q 022 GLYCEROL-3-PHOSPHATE DEHYDROGENASE: HORMONAL AND DEVELOPMENTAL MARKER OF THERMOGENESIS AND LIPOGENESIS, Leslie Kozak, Ulrike Kozak, Joseph Jerry, and James Wells, The Jackson Laboratory, Bar Harbor, Maine 04609.

There is a strong correlation between active utilization of the glycerol phosphate pathway for lipid synthesis and high levels of glycerol-3-phosphate dehydrogenase. This correlation is observed during development of the epididymal fat pad, during glial cell development and during cold-induced thermogenesis. Since several hormones, including growth hormone, insulin, thyroid hormone, catecholamines and glucocorticoids, have been implicated in controlling the levels of glycerol-3-phosphate dehydrogenase (GPDH), it is likely that the promoter and regulatory regions which control expression of the GPDH gene are complex. The transcriptional region itself is complex. In addition to the Gdc-1 gene two other transcriptional units have been localized in this region. The D15Kz1 gene, located 3.5 kb upstream of Gdc-1, produces two transcripts of 3.5 and 3.7 kb which are differentially expressed among various tissues. The D15Kz2 gene, located about 1.5 kb downstream of Gdc-1, has a major transcript of 0.75 kb which is also differentially expressed among tissues. Some important features in the expression of the three genes are apparent. First, the fact that they are ubiquitously expressed has lead us to suggest that this may be a chromosomal domain for housekeeping genes. Second, the pattern of expression among tissues suggests that a mechanism exists to achieve independent regulation of each transcriptional unit. Third, coordinate regulation may also be possible since the expression of all three transcripts is modulated in brown fat by cold induction.

Clinical Obesity

Q 100 ADIPOSE TISSUE PROTEIN VARIANTS, BODY FAT AND ADAPTATION TO OVERFEEDING. Monique Chagnon and Claude Bouchard. Physical Activity Sciences Laboratory, Laval University, Ste- Foy, Québec, Canada G1K 7P4.

In the course of a study on the effects of long-term overfeeding on body fat deposition, genetic polymorphism of adipose tissue proteins was investigated. The adipose tissue was obtained by needle biopsies from twelve pairs of males monozygotic [MZ] twins overfed a total of 353 MJ over 100 days. Adipose tissue was also obtained from fourteen obese women [BMI>30] and fourteen non-obese female controls [BMI<26]. Tissues from the abdominal or the femoral sites were used. The proteins were extracted in a denaturing buffer and analysed by the two-stage electrophoresis method of O'Farrell followed by silver-staining. Ten polymorphic loci were detected among all those that were vizualized. Two of these variant proteins, whose inheritance was confirmed by gene-dosage effect and segregation in two families, showed a relationship with changes in body mass , fat mass , and the sum of 11 skinfolds in the MZ experiment. In the four pairs with the highest fat gains, the genetic charge variant 10D2 was present while those who gained less fat tended to have the 8D2 genetic charge variant. No high-gainers had the 8D2 variant. Such a trend was not found in the comparaison between obese and non-obese women. The frequency of the 10D2 high-gainer marker 10D2 was the same in both groups. From these observations, we suggest that the 10D2 and 8D2 variant proteins might be useful markers of the sensitivity to long-term overfeeding. However, they do not discriminate between obese and lean individuals measured only on one occasion.

Supported by NIH.

Q 101 CHARACTERIZATION OF BROWN FAT CELL LINES DERIVED FROM a-AMYLASE-SV40 T ANTIGEN TRANSGENIC MICE HIBERNOMAS, Rosanne M. Crooke and Barbara B. Knowles, The Wistar Institute, Philadelphia, Pennsylvania, 19104.

Several lineages of transgenic mice containing the mouse a-amylase (Amy-1^a) liver promoter fused to the SV40 T antigen (Tag) early coding region have been developed. Mice containing this hybrid gene developed hibernomas, rare brown adipose tissue tumors. Multiple cell lines were derived from these tumors using the collagenase, preadipocyte enrichment procedure. We characterized 4 of the 27 established cultures (734,988,1285,1366) since these cells continuously produce multioculate fat droplets. These cells have centrally localized nuclei, multiple lipid vacuoles and numerous, densely packed pleiomorphic mitochondria within the cytoplasm. SV40 Tag and appropriate major histocompatibility complex (MHC) class I gene products were expressed in all 4 cell lines. High levels of B-adrenergic receptors were identified on the 2 brown adipocyte lines tested (988,1366) by radioligand binding studies using ^{125}I-cyanopindolol. Preliminary immunoprecipitation experiments using antibodies raised in sheep against rat thermogenin suggest that thermogenin is expressed in at least one of the brown adipocyte lines. These cells are valuable models for investigating brown fat differentiation, lipid metabolism, and thermogenesis as well as the mechanisms underlying neoplastic transformation in brown adipose tissue.

Q 102 LONG TERM EFFECTS OF AN ANTISERUM TO ADIPOCYTES ON BODY FAT AND PROTEIN DEPOSITION IN THE RAT, Deborah Panton, Steven C. Kestin* and David J. Flint, Hannah Research Institute, Ayr KA6 5HL, Scotland and *Institute of Food Research, Bristol Laboratory, Bristol BS18 7DY, UK

We have previously described long-term reduction of adiposity in rats using antibodies to adipocytes[1]. We now report effects of a similar antiserum raised against rat adipocyte membranes where hyperphagia and increased body weight gain were evident. Rats ($\sim$ 140g) were injected for 4 consecutive days with sheep anti-(rat adipocyte plasma membrane) antibodies and remained untreated subsequently. Control rats received equivalent amounts of non-immune sheep γ-globulin. During the first 4 weeks treated rats showed a 15% increase in body weight gain with no effect on food intake whilst between 4 and 7 weeks they ate 20% more and showed a 40% increase in body weight gain compared with controls. This resulted in an approximate 15% increase in food conversion efficiency. At the end of this period treated animals were 25g heavier than controls but showed a 54, 31 and 10% reduction in parametrial, subcutaneous and perirenal fat respectively. Subsequently food intake and body weight gain returned to normal. 6 months after treatment body weight difference was maintained, total fat content was identical and body protein was increased by approximately 30%.

Reference. Futter, C.E. and Flint, D.J. (1987) Recent Advances in Obesity Research V, Chapter 27 (Ed E.M. Berry), John Libbey and Co. London.

Q 103 GROWTH HORMONE REGULATES INSULIN-LIKE GROWTH FACTOR-I GENE EXPRESSION IN PORCINE PREADIPOCYTES IN VITRO. H.R. Gaskins***, J.W. Kim**, G.J. Hausman* and J.T. Wright*. *USDA-ARS and Depts. of **Poultry Sci. and ***Foods and Nutrition, Univ. of GA, Athens, GA 30613.

The effect of human growth hormone (GH) on insulin-like growth factor-I (IGF-I) mRNA accumulation and IGF-I peptide secretion by stromal-vascular (SV) cell cultures from postnatal (2-d-old) porcine adipose tissues was studied. Northern blot analysis using a human IGF-I cDNA probe (Dr. G.I. Bell, Univ. of Chicago) indicated the presence of three major IGF-I RNA transcripts (4.5, 2.2 and 1.0 kb) in adipose SV cultures. A decrease in the abundance of the 4.5 kb RNA transcript and accumulation of the 2.2 and 1.0 kb transcripts occurred within 90 min after adding 1 nM GH to SV cultures maintained on medium containing 2% porcine serum (PS). Concentrations of IGF-I in the medium (measured by RIA) were not detectable at 0 and 90 min after adding GH but increased to 6 ± 2, 26 ± 3, 54 ± 7, and 102 ± 3 pg/ug total RNA after 3, 9, 24, and 48 h exposure, respectively. Ontogeny of IGF-I secretion by adipose SV cells was examined in cultures from 72 and 110-d-old fetal pigs. Addition of 1 nM GH did not alter IGF-I secretion by SV cultures from 72-d-old fetal pigs. In contrast, a 1.5-fold increase ($P<0.01$) in IGF-I secretion was observed after adding 1 nM GH to PS-supplemented SV cultures from 110-d-old fetal pigs. The gestational age at which IGF-I secretion by adipose SV cells is responsive to GH corresponds to periods of lipid accumulation in fetal porcine adipose tissue. However, GH antagonizes porcine preadipocyte development in vitro suggesting that locally secreted IGF-I may be a negative regulator of adipogenesis.

Q 104 **LONG-TERM EFFECT OF INSULIN ON GLUCOSE TRANSPORTERS IN SUBCELLULAR MEMBRANES FROM 3T3-F442A ADIPOCYTES. M.GUERRE-MILLO, B.HAIMQUE, N.MOUSTAID, I.HAINAULT, L.WARDZALA and M.LAVAU. U 177 INSERM, 15 rue de l'Ecole de Médecine, Paris 75006, FRANCE.**

We have adressed the question of a long term effect of insulin on adipocyte glucose transporter (GT) content, by using the 3T3-F442A pre-adipocyte cell line.

At confluence, cells were made to differentiate in DMEM + 20 mM glucose, without (A) or with (B) 10nM insulin, for 15 days. Fully differentiated adipocytes (B cells) were then insulin depleted for 4 days, before being re-exposed (C) or not (D) to the hormone, for 4 days. The GT concentration in plasma (PM) and microsomal (LDM) membranes was assessed by both cytochalasin B binding and immunoblotting, with an antibody against a C terminal peptide of GT (kind gift of Dr S.CUSHMAN). Insulin during differentiation (B vs A cells) increased GT concentration in PM (63 vs 18 pmol/mg) and in LDM (29 vs 18 pmol/mg). Insulin deprivation of B cells for 1 day induced GT translocation from PM (-40%) to LDM (+80%). Thereafter, GTs decreased gradually in the two pools. Conversely, long-term rechallenge with insulin (C vs D cells) increased GTs in both PM (by 2.2 as assessed by cytochalasin B binding to 5.8 as assessed by immunoblotting) and LDM (by 1.7 whatever the method). Changes in 2-deoxyglucose uptake (x4 in B vs A cells and x5 in C vs D cells) paralleled closely changes in GT concentration in PM. Neither the kd of cytochalasin B, nor the Mr of GT on immunoblots were affected by insulin.

These data support a direct long-term regulatory role of insulin on GT content in either differentiating or mature 3T3-F442A adipocytes. This study points out the role of insulin in the control of glucose transport capacity in adipose cells.

Q 105 DIFFERENTIATION OF HUMAN ADIPOCYTE PRECURSOR CELLS INTO FAT CELLS. Hans Hauner, Gero Entenmann, Martin Wabitsch, Danielle Gaillard[§], Raymond Negrel[§] and Ernst Friedrich Pfeiffer. Medizinische Klinik und Poliklinik, Universität Ulm, D-7900 Ulm, West Germany and [§]Centre de Biochimie du CNRS, Universite de Nice, Parc Valrose, 06034 Nice cedex, France.

The stromal-vascular fraction of human adipose tissue contains an undefined number of cells capable to develop into fat cells. Aim of this study was to further characterize the adipocyte precursor pool in man. Adipose tissue samples of adults were completely disaggregated by collagenase digestion. The isolated stromal-vascular cells were inoculated in a serum-containing medium. After 16 hours, cells were refed a serum-free medium according to Deslex et al. (Int. J. Obesity 11:19). In the presence of supraphysiological concentrations of insulin up to 20 % of the cells developed adipocyte morphology. When a combination of insulin and cortisol was added, a differentiation rate of up to 70 % was observed resulting in GPDH activities of up to 1600 mU/mg protein. The adipose conversion strongly depended on the culture conditions and on the age of the donors. Prolonged presence of serum was found to reduce the number of differentiating cells and enzyme activities. This improved culture system may help to study the role of adipocyte precursor cells during the development of human obesity and its regulation by adipogenic and antiadipogenic factors.

Q 106 EFFECT OF HUMAN GROWTH HORMONE ON PREADIPOCYTE DEVELOPMENT IN SERUM SUPPLEMENTED AND SERUM FREE CULTURES OF STROMAL-VASCULAR CELLS FROM PIG ADIPOSE TISSUE, G. J. Hausman* and R. J. Martin**, *Animal Physiology Research Unit, USDA-ARS, R. B. Russell Agricultural Research Center, Athens, GA 30613 and **Department of Foods & Nutrition, University of Georgia, Athens, GA 30602

The influence of human growth hormone (hGH) on the differentiation of preadipocytes was examined in primary cultures of stromal-vascular cells from porcine adipose tissue. In these experiments cells were exposed to test media for 7-8 days after seeding in fetal bovine serum. In serum free (insulin, transferrin and selenium) cultures hGH (1 and 10 nM) reduced the number and size of fat cell clusters ($P<.05$) by fifty percent relative to controls (no hGH). Differentiation of preadipocytes was assayed by labelling dividing cells with tritiated thymidine and then exposing cultures to test media for seven days. Fat cells were then separated from other cells and radioactivity determined in each fraction. In serum containing (2% pig serum) cultures hGH (10 nM) inhibited ($P<.05$) the differentiation of labelled preadipocytes. One and ten nM hGH reduced ($P<.05$) the levels of glycerol phosphate dehydrogenase (GPDH) specific activity by approximately fifty percent in cultures supplemented with serum plus insulin (1 µm) and in serum free cultures. However, hGH (1 and 10 nM) had no effect on GPDH activity in serum-supplemented cultures without added insulin. These studies indicate that hGH significantly impedes preadipocyte development in vitro. Therefore, the decreased rate of adipose tissue growth observed in animals chronically treated with GH could be due in part to impaired preadipocyte growth.

Q 107 EXISTENCE OF A NEW KIND OF SITES IN FAT CELL MEMBRANES: "IMIDAZOLINE PREFERRING BINDING SITES", Dominique Langin, Jean-Sébastien Saulnier-Blache, Christian Carpéné and Max Lafontan, I.N.S.E.R.M. - Unité 317, Université Paul Sabatier, Rue François Magendie - 31400 Toulouse,France

The imidazoline α2-antagonist [3H]idazoxan has been used as probe for α2-adrenoceptor identification in different tissues and species. In fat cell membranes from rat, hamster and rabbit, there is a specific binding of [3H]idazoxan to non α2-adrenoceptor sites. The binding of [3H]idazoxan was not displaced neither by 200mM epinephrine or norepinephrine nor by yohimbine, prazosin, propranolol, piperoxan and histamine. However, it was displaced by a large variety of imidazoline compounds. Scatchard plots were linear and Hill coefficients revealed the existence of a single class of [3H]idazoxan binding sites. Ligand specificity of [3H]idazoxan binding (naphazoline defined) was explored in rabbit fat cell membranes. Determination of the Ki values of more than 20 imidazoline compounds showed that their affinity was closely related with the chemical structure of the non-imidazoline part of molecules. Curiously, amiloride (inhibitor of Na+/H+ exchanger and Na+ channels) strongly competed with [3H]idazoxan binding (Ki=48nM). These data provide evidence of high affinity [3H]idazoxan binding sites in fat cells which are not α2-adrenoceptors. Recently, such "imidazoline preferring sites" have also been described in brain, urethra and kidney membranes of the rabbit, but never in other species. The isolated fat cells, with their larger number of [3H]idazoxan binding sites and their homogeneity could be of interest for further studies on these sites and their putative transducing systems.

Q 108 FAT CELL ALPHA2-ADRENERGIC RECEPTOR IDENTIFICATION. A NEW TOOL TO LABEL ALPHA2-ADRENOCEPTORS : [3H]RX821002. Jean Sébastien Saulnier-Blache, Dominique Langin, Christian Carpéné, Jean Galitzky, Dominique Larrouy and Max Lafontan, I.N.S.E.R.M. - Unité 317, Université Paul Sabatier, Rue François Magendie - 31400 Toulouse, France.

[3H]-yohimbine and [3H]rauwolscine are suitable ligands for α2-site identification in human and dog fat cell membranes but cannot be used in some animal models (hamster, rabbit, rat). A compound RX821002, (2-(2-methoxy-1,4-benzodioxan-2yl)-2-imidazoline with a higher potency and selectivity than yohimbine for α2-adrenoceptors was used for α2-sites identification. It was an excellent α2-antagonist in biological assays. [3H]RX821002 ([3H]RX) binding was performed on fat cell membranes at 25°C, in 50mM Tris-HCl buffer (pH=7.5) containing 0.5mM Mg^{2+}. Non-specific binding was defined with 200µM epinephrine. [3H]RX binding was rapid, saturable and reversible. Equilibrium dissociation constants ranged between 0.5 to 4.1nM, and maximal binding (B_{max})were from 20fmol/mg protein (rat) to 300fmol (rabbit) and 700fmol/mg protein (hamster, human). The rank order of various agonists and antagonists in displacing [3H]RX from its binding sites was typical of that defined for α2-adrenoceptor definition in biological assays. It is concluded that [3H]RX is a highly valuable radioligand for α2-adrenoceptors identification and quantification in all the species tested. [3H]RX821002 offers a major improvement in the species the α2-adrenoceptors of which cannot bind yohimbine with high affinity (hamster, rabbit, rat).

Q 109 MALE ADIPOSE TISSUES, Marielle Rebuffe-Scrive, Department of Medicine I, Sahlgren's Hospital, Gothenburg, Sweden

In contrast to a number of studies on morphology and function of female adipose tissue in relation with fat patterning, very little is known about adipose tissue in men.
Men consistently have 2 to 3 times more intraabdominal fat than women (Kvist et al 1988). As judged from fat cell size, the enlargement seems to include portal (mesenteric and omental) and non portal (subcutaneous and retroperitoneal) tissues.
In non obese men, portal adipocytes are more lipolytically sensitive than any other fat tissues in men or in women.
There are no characteristic differences in lipoprotein lipase (LPL) activity between any subcutaneous and intraabdominal tissues in men.
However subcutaneous abdominal LPL activity is higher in older men than in younger. Injection of one dose of 250 mg testosterone enanthate tends to decrease LPL specifically in this region. Furthermore, administration of testosterone undecanoate (in moderate dose, during 6 weeks) causes a marked decrease of LPL and tends to stimulate norepinephrine lipolysis, in subcutaneous abdominal fat.
These results suggest that abdominal adipose tissues function is regulated by testosterone.

Q 110 OLEIC ACID INFLUENCE INSULIN BINDING, DEGRADATION AND ACTION IN RAT HEPATOCYTES, J. Svedberg, P. Lönnroth, U. Smith, P. Björntorp. Department of Medicine I, Sahlgren's Hospital, 413 45 Göteborg, Sweden.
Abdominal obesity is associated with hyperinsulinemia and an impaired insulin sensitivity in the peripheral tissues. One possible mechanism of action is that the increased amount of highly lipolytic active abdominal fat cells release, into the portal vein system, a large amount of free fatty acids which may reduce the insulin uptake and action in the liver and hence contribute to a peripheral hyperinsulinemia. In order to investigate this hypothesis rat hepatocytes were isolated and the experiments were performed in a 3% bovine serum albumin - hepes - Krebs-Ringer bicarbonate-butter.
Results: The binding of ^{125}I-insulin was direct proportionate to the degradation of the tracer as measured with TCA-precipitation. Oleic acid (0.4mM) added to the cells rapidly reduced insulin binding and degradation (40% and 30% respectively), ($p < 0.001$, n=7) as well as the basal and insulinstimulated cellular 14C-aminosobutyric uptake (59%). The inhibitory effect of oleic acid was dose-and energydependent.
Conclusions: The impairment in insulin binding and action may reduce the hepatic insulin clearance and contribute to the hyperinsulinemia seen in obesity as well as the insulin resistance.

Q 111 MONOCLONAL ANTIBODIES AGAINST CYTOPLASMIC DETERMINANTS OF PORCINE ADIPOCYTES. J.T. Wright, N.A. Stocks and G.J. Hausman, USDA-ARS, Russell Research Center, Athens, GA 30613.
This report describes the preparation and characterization of monoclonal antibodies (MAbs) produced by fusion of myeloma cells and lymph node cells of mice immunized with porcine adipocyte cellular protein. Two of the MAbs (designated CB6 and IB4) exhibited reactivity towards adipocyte cytoplasmic determinants on cryostat sections of sc tissues, whereas the CB6 MAb also detected muscle fibers containing lipid droplets. In Vitro, the CB6 and IB4 MAbs each detected cytoplasmic determinants only in cells containing lipid droplets: no cells devoid of lipid were not stained by either MAb, whereas reactivity was minimal or absent in cells containing the smallest lipid droplets (presumably cells at the onset of cytodifferentiation). CB6 and IB4 immunoreactivity increased as preadipocytes became larger due to lipid accumulation with larger adipocytes exhibiting the strongest immunoreactivity. Addition of 10 nm GH to cultures resulted in decreases in both adipocyte size and number within 48 hours. Reactivity towards the IB4 MAb was diminished after treatment of cultures with GH but exhibited the same pattern of staining observed in untreated cultures [i.e., fluorescence intensity paralleled fat cell (lipid droplet) size with no detectable reactivity in cells containing little or no lipid]. On the other hand, the CB6 MAb exhibited reactivity towards some lipid-free cells as well as cells containing the smallest lipid droplets after 48 hours of GH treatment, possibly representing detection of cells in the process of losing lipid due to the action of GH.

Gene Expression, Animal Models

Q 200 DIFFERENTIAL EXPRESSION AND REGULATION OF pOb24 AND LIPOPROTEIN LIPASE (LPL) GENES DURING ADIPOSE CONVERSION.

DANI, C.; AMRI,E.Z.; BERTRAND, B.; ENERBACK,S.+; BJURSELL, G.+; GRIMALDI, P.; AND AILHAUD,G.- Laboratoire du Developpement du Tissu Adipeux , CNRS (UPR37)06034 Nice FRANCE- + Department of Medical Biochemistry, University of Gothenburg, S-400 33 Gothenburg SWEDEN.

It has been shown recently that, during adipose conversion, the expression of early genes(pOb24 and LPL genes), defining the preadipose state, is coupled to growth arrest at the G1/S stage of the cell cycle and does not require hormones. In contrast the expression of late genes(glycerophosphate dehydrogenase and adipsin genes), defining the adipose state, is triggered by growth hormone and triiodothyronine and is coupled to a critical growth resumption which leads to lipid accumulation .

Further studies on the expression of pOb24 and LPL genes during adipose conversion of Ob17 cells as well as in the stromal-vascular cells and adipocytes of mouse adipose tissues show that pOb24 mRNA (6 kb)is specifically expressed in the preadipose state whereas LPL mRNAs(3.3 and 3.7 kb species) are preferentially expressed in the adipose state: 90% of pOb24 mRNA recovered in the stromal-vascular fraction and 80% of LPL mRNAs recovered in the adipocyte fraction. Thus these two early genes appear to be differentially regulated as a function of differentiation. This assumption is supported by the inhibitory effects of TNF and TGF-β on the accumulation of pOb24 and LPL mRNAs: both factors regulate the expresssion of LPL gene in preadipose and adipose cells whereas the expression of pOb24 gene is regulated in preadipose cells only.Thus pOb24 mRNA appears to be both in vivo and in vitro a unique marquer of cell commitment during adipose cell differentiation.

Q 201 MITOCHONDRIAL DNA SEQUENCE VARIATION AND ADAPTATION TO CHRONIC OVERFEEDING IN MONOGYGOTIC TWINS, France T. Dionne, Lucie Turcotte and Claude Bouchard. Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec, Canada G1K 7P4.

Previous studies from our laboratory have shown that changes in fat deposition and components of energy expenditure in response to positive energy balance are largely determined by the genotype. In an attempt to identify genetic markers of sensitivity to overfeeding, mitochondrial (mt) DNA variation was studied using RFLPs in 12 pairs of male monogygotic (MZ) twins, overfed by 4.2 MJ per day, 6 days a week, for 100 days. Southern blot analyses were performed on DNA extracted from white blood cells using 22 restriction enzymes. The mtDNA fragments were visualised by hybridization with a ^{32}P-labeled mtDNA probe. Nine RFLPs were detected among 6 of the pairs with the endonucleases AvaII, BamHI, BstNI, HaeII, KpnI and PstI implying base substitutions in the D-Loop region, in regions coding for the subunit 6 of ATPase, subunits 2 and 5 of NADH dehydrogenase and in a short non-coding region on the mt genome. The only MZ pair exhibiting polymorphism with BstNI (D-Loop) and PstI (ATPase 6) had also a considerable increase (about 30 %) in the thermic effect of a 4.2 MJ meal test while the others generally showed a decrease. In addition, mutations in the D-Loop region detected with AvaII, BamHI and KpnI were generally associated with very low overfeeding-induced changes in the energy cost of submaximal exercise. These data suggest that mtDNA sequence variation may be associated with the response of the energy expenditure components to chronic overfeeding, even though a causal mechanistic link cannot be established at this time. Supported by NIH and NSERC.

Q 202 MECHANISM OF THE INCREASED FATTY ACID SYNTHETASE ACTIVITY IN ADIPOSE TISSUE OF GENETICALY OBESE ZUCKER RAT. Dugail, I., Guichard, C., Lavau, M. INSERM U177, 15 rue de l'Ecole de Médecine, Paris. France.

The activity of fatty acid synthetase (FAS) is increased in adipose tissue of obese Zucker rats fa/fa (50%, 4 and 20 fold as compared to lean Fa/fa at 7, 16 and 30 days of age respectively). The aim of this study was to investigate the molecular mechanism responsible for this increased FAS activity.

Molecular weight (PAGE-SDS), Km values for enzyme substrates and immunological properties of purified FAS were identical in the 2 genotypes, ruling out a structural alteration of the protein in the mutant rat. The determination of absolute amounts of FAS protein in adipose tissue (Western blot) showed that, at 16 days of age, the amount of FAS was 4.4 µg per adipose tissue in lean versus 22 µg in obese rats. At 30 days of age, the lean values were 83 µg versus 1700 µg in the obese. The increased FAS content in adipose tissue from obese rats could be accounted for, at least partly, by a stimulation of FAS biosynthesis (measured by immunoprecipitation of in vivo ^{14}C labelled FAS) which was 8 fold higher in obese than in lean rats at 30 days of age. Northern blot analysis of FAS mRNA (cDNA FAS 18) in 30 day-old rats showed a very high level of FAS mRNAs in the obese rats (at least 10 fold over the lean values).

This work shows that increased FAS activity in obese Zucker rat adipose tissue can be ascribed to a corresponding increase in the amount of FAS ,due to a stimulation of FAS gene expression occurring at the pretranslational level.

Q 203 GROWTH HORMONE mRNA IN GENETICALLY OBESE RATS, Judith A. Finkelstein, Iqbal Ahmad and Alan W. Steggles, Departments of Anatomy and Biochemistry, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.
Genetically obese Zucker rats have lower plasma growth hormone (GH) levels than their lean littermates. In order to understand the role of GH synthesis in this abnormality, levels of GH mRNA in the pituitaries of groups of lean (L) and obese (O) male rats (littermates) were measured. Following decapitation, pituitaries were homogenized in Tris-EDTA buffer with Nonidet P-40, the supernatants collected by centrifugation, aliquots serially diluted and applied to nylon membranes for cytoplasmic dot blot analysis. Membranes were hybridized to a GH ^{32}P cDNA probe. The resulting autoradiographs were quantitated by densitometry and the results expressed in scanning units (S.U.). At fourteen weeks of age there was a significant reduction of GH mRNA levels in the obese rat pituitaries in comparison to the lean animals (O: 84 S.U. vs L: 329 S.U., at 1:320 dilution). In an attempt to investigate the etiology of this difference, animals were also studied at three weeks of age. No significant difference was observed between the two groups of younger animals (O: 178 S.U. vs L: 185 S.U., at 1:40 dilution). These data indicate that obesity-associated depression of GH mRNA is under developmental control. Further studies will focus on the exact age at which this difference first appears and its correlation with factors (e.g. insulin, growth hormone releasing factor, thyroid hormone) which regulate GH mRNA in lean animals and are abnormal in genetically obese Zucker rats. Supported by funds from the Office of Gerontology and Geriatric Medicine, NEOUCOM, and the Ohio Board of Regents.

Q 204 LOCALIZATION OF AN INSULIN-RESPONSIVE ELEMENT IN THE PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK) GENE, Claude D. Forest, Richard M. O'Brien, Mark A. Magnuson, and Daryl K. Granner, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232
Transcription of the PEPCK gene in liver and H4IIE hepatoma cells is stimulated by cAMP and by the glucocorticoid analog, dexamethasone. Insulin inhibits transcrition of the gene and this effect is dominant. The DNA adjacent to the transcription start site of this gene contains the cis-acting response elements required for the regulation of this gene by cAMP and dexamethasone. In order to locate the insulin responsive element(s) (IREs), chimeric PEPCK-CAT genes have been cotransfected with pSV2NEO into H4IIE cells and transfected colonies were selected using the antibiotic geneticin, (G418). The ability of these stable PEPCK-CAT transformants to respond to dexamethasone and/or to 8-CPT cAMP (an analog of cAMP) by an increase in CAT activity, and the ability of insulin to override these effects were measured. Results obtained with numerous 5', 3' and internal deletions of the PEPCK promoter region strongly suggest the presence of an IRE between -401 and -271 bases relative to the transcription start site of the gene.

Q 205 INDUCTION OF DIABETES BY THE EXPRESSION OF MHC-ANTIGENS ON PANCREATIC ISLET CELLS IN TRANSGENIC MICE, Juergen Goetz, Hermann Eibel & Georges Koehler, Max-Planck-Institute for Immunology, Freiburg, F.R.G.
As we are interested in analyzing the establishment of self-tolerance against antigen expressed by non-lymphoid tissues, we directed the expression of the MHC-antigens E_α^k, E_β^b and K^k to the β-cells of the pancreas. In order to generate transgenic mice we cloned the rat insulin II promoter in front of these genes and microinjected them into eggs of F_1 (BL/6 x SJL) eggs. Here we present our analyses of three founder mice and their offspring. E_α^km50 has integrated 3-4 copies of the transgene, E_β^bm1 two copies and E_β^bm3 contains more than 60 copies/genome. Diabetic offspring were obtained from crosses of E_α^km50 X E_β^bm1 founders. Offspring of either single transgenic mice remained healthy. For the E_β^bm3 offspring, however, diabetes was observed already with the single transgenic line with high glucose levels (> 500mg/dl at 4 - 5 w of age). Immunofluorescence analysis of cryostat sections of the pancreas obtained from both types of diabetic mice revealed an almost complete depletion of insulin in the islets. Lymphocyte infiltration was not observed. This suggests, that the MHC-antigen expression itself in these cells interferes with the insulin production. At present we are analyzing the underlying mechanisms by *in situ* hybridization studies and the state of tolerance to the transgenic gene products by mixed lymphocyte reactions.

Q 206 **ADIPSIN mRNA AND PROTEIN LEVELS IN THE GENETICALLY OBESE fafa RAT.** Patricia R Johnson, Bruce Spiegelman, Barry Rosen, Iris Turkenkopf, Todd Kirschgessner, Monica Tacinelli and M.R.C. Greenwood, Department of Biology, Vassar College, Poughkeepsie, N.Y. and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston MA.

Obesity in the genetically obese Zucker rat is inherited as a single mendelian recessive trait (**fa**). Like the **obob** and **dbdb** mouse, its associated pathology is modulated by genetic background. This laboratory has demonstrated over many years that **fafa** obesity is highly refractory to diet, and both pharmacological and surgical interventions. Nonetheless, in spite of extensive knowledge regarding the developmental physiology of the **fafa** rat, little is known about the specific gene which leads to the development or refractoriness of the obesity. Adipsin has been described by Spiegelman and his associates as absent or present in very low quantities in some strains of genetically obese mice. Since there are large differences in cellularity between obese and lean rats and 80% or more of DNA in adipose tissue is contained in non adipose cells, we prepared isolated fat cells using a modification of the procedure described by Rodbell. In initial experiments, all rats were 10 weeks old. RNA was extracted from isolated cells using the thiocyanate -phenol-chloroform method, blotted on nylon filters and probed with c-DNA's for adipsin, tubulin and actin. Serum protein levels of circulating adipsin were assessed using a high titer rabbit antibody against whole mouse adipsin protein that reacts with rat adipsin in immunoblots. Both adipsin mRNA and adipsin serum protein levels were approximately 4-6 fold greater in the lean compared to obese rats. Further experiments on age and hormonally specific effects are ongoing. Supported by NICHD 12637.

Q 207 IDENTIFICATION OF A PORCINE SUBCUTANEOUS ADIPOCYTE PLASMA MEMBRANE PROTEIN BY MONOCLONAL ANTIBODY UNIQUE TO ADIPOSE TISSUE, John Killefer and C.Y. Hu, Department of Animal Science, Oregon State University, Corvallis, OR 97331. Polyclonal antibodies have been previously produced which appeared to be specific to adipocytes. We have been able to produce a monoclonal antibody which recognizes a plasma membrane protein that is immunologically unique to adipose tissue. Adipocytes were isolated from the subcutaneous backfat of 35 Kg barrows. Plasma membranes were purified using a self-forming Percoll gradient. Balb/c mice were hyperimmunized by intraperitoneal immunization with an emulsion of subcutaneous adipocyte plasma membranes(APM) in Freund's Incomplete Adjuvent using standard protocols (100µg/20µg/5µg APM/mouse). Splenocytes were harvested and fused to Sp 2/0-AG 14 myeloma cells. An ELISA assay was used to identify those hybridomas producing antibodies directed towards APM. Cross-reactivity was assayed using liver and erythrocyte plasma membranes and subcutaneous belly and perirenal fat plasma membranes. A McAb (designated LA-1) was isolated having specific activity towards a porcine subcutaneous APM component. SDS-Polyacrylamide Gel Electrophoresis was utilized to determine the protein profiles of the individual plasma membrane sources. Immunoblotting with LA-1 indicated a single protein of approximately 64 kD which is unique to APM. Attempts to deglycosylate possible carbohydrate groups from the protein by digestion of the APM with Endoglycosidase-F indicate that the protein is not glycosylated. Presence of this protein may be linked to normal adipose tissue function and may act as a critical component in adipose tissue differentiation and development. (Funded by NIH Biomedical Research Support Grant RR07079.)

Q 208 THE EXPRESSION OF BRAIN-AND LIVER-TYPE GLUCOSETRANSPORTER mRNA IN OBESE AND INSULIN RESISTANT MICE. Laszlo I.Koranyi, Alan M.Permutt and Mike Mueckler. Metabolism Division and Cell Biology. Washington Univerity School of Medicine, St. Louis. MO 63110.

The connection between insulin resistance and levels of brain-,or HepG2 (BGT.) and liver-type glucose-transporter(LGT.)mRNAs in mice tissues was investigated. Male C57BL/KsJ db/db mice with hyperphagic obesity and diabetes and +/db mice, C3H/HeJ controls and DBA/2J(hyperinsulinemic, nondiabetic)mice , five in each groups, were studied at age 40 days. The BGT.mRNA levels in brain and heart tissue and LGT.mRNA levels in liver tissue were evaluated by dot-blot hybridization comparing the ratio of glucose-transporter mRNA/actin mRNA. The HepG2 and LGT genes were isolated from a HepG2 cDNA library, subcloned into Bluescript(Stratagene)plasmids and their cRNA transcripts were used as probes. The BGT.mRNA levels were reduced in db/db mice compared either to C3H/HeJ strains(brain:76.9%, $P<0.01$, heart:55.1%, $P<0.01$) or to +/db group(brain:68.5%, $P<0.02$, heart:86.2%, $P<0.05$)as well as the LGT.mRNA levels(59.6%, $P<0.01$ versus C3H and 63.9%, $P<0.02$ versus +/db mice). These decreased levels of glucose-transporter mRNAs we observed may be responsible for the known insulin resistance and decreased glucose transport in this mouse model of spontaneous obesity and diabetes.

Q 209 **ADIPOSE CONVERSION OF 3T3-L1 CELLS IN A CHEMICALLY DEFINED MEDIUM**, Georg A. Löffler, Gisela Pöll-Jordan and Wilfried Schmidt, Department of Biochemistry, University of Regensburg, Regensburg, FRG

A culture system for 3T3-L1 preadipocytes based on a serumfree chemically defined medium is described. In this medium adipose conversion depends on addition to the culture medium of corticosterone together with methyl-isobutylxanthine (MIX) in the presence of high insulin concentrations (10^{-6} M). MIX may be replaced by forskolin or cyclic AMP analogues. At a low insulin concentration (10^{-9} M) IGF-I together with EGF have to be present as a medium supplement together with corticosterone and MIX to get maximal adipose conversion. Adipose conversion depends on postconfluent mitoses, as inhibition of DNA-replication with aphidicolin, hydroxyurea or a thymidine block inhibits adipose conversion. Neither PDGF nor basic FGF are able to replace EGF, IGF-I however may be replaced by growth hormone.

Q 210 S14 - A NOVEL DIFFERENTIATION DEPENDANT GENE PRODUCT IN 3T3-L1 ADIPOCYTES, Ulrich Loos, Joachim Clement, Siegfried Hausdorf, Dept. of Internal Medicine I, University of Ulm, D-7900 Ulm, Germany

Protein S14 has been detected in liver and adipogenic tissues of the rat. Expression of S14 gene is markedly influenced by T_3 and carbohydrates. In rat liver the gene is transcribed into two mRNAs with 1.3 Kb and 1.47 kb, both coding for a single protein with 150 aa and M_r 17010 Da (1). We studied the expression of S14 gene during differentiation of 3T3-L1 preadipocytes. The cells were grown to confluence, kept in a resting state and then allowed to differentiate in adipogenic medium containing 10% FCS, 1 nM biotin, 10 g/ml insulin, 0.25 mM dexamethasone and 0.5 mM MIX. Northern blots and hybridization studies with a specific S14 cDNA (pS14-C2) showed a single S14 mRNA band with 1100 bp. Time course experiments revealed a striking increase of S14 mRNA beginning at day three. S14 mRNA was neither detectable in preadipocytes nor during the first 48 hrs of differentiation. Recombinant plasmid was used for hybrid-selected translation of S14 mRNA. 2-D gel electrophoresis demonstrated identical migration properties of 3T3-L1 adipocyte S14 compared to rat liver. 1-D analysis showed a M_r of about 17000 Da. No protein spot was detectable in preadipocytes.

(1) Oppenheimer, J.H., Kinlaw, W.P., Wong, N.C., Schwartz, H.L., Mariash, C.N. Horm. Metabol. Res., Suppl 17, 1-5, 1987

Q 211 METABOLIC ABNORMALITIES OF THE HYPERGLYCEMIC OBESE ZUCKER RAT, Michael L. McCaleb and Janet Sredy, Wyeth-Ayerst Research, Princeton, NJ 08543 In a cross-sectional study, we evaluated the metabolic profiles of lean (Fa/?) and obese (fa/fa) Zucker male rats at 4 to 8 months of age. Although all of the obese rats (N = 43) demonstrated glucose intolerance, most of the obese rats exhibited only mild elevations of fasted and fed plasma glucose. Only 14 of the obese rats were severely hyperglycemic, which resulted in substantial elevations of GHb levels. The nerve and lens levels of glucose, sorbitol and fructose were elevated and the myoinositol was depleted in all hyperglycemic obese rats, but not in the euglycemic obese rats. With the duration of hyperglycemia, the neural myoinositol level approached normal while the lenses became cataractous. All obese rats had increased urinary albumin excretion (UAE), which was dependent upon age ($r = 0.45$, $p < 0.02$) and independent of hyperglycemia, glucosuria and polyuria. In conclusion, although the euglycemic obese rat exhibited some abnormalities, the hyperglycemic obese Zucker rat more closely resembled the altered metabolic profile associated with Type II diabetes mellitus.

	GHb (%)	Nerve (nmol/mg)		Lens (nmol/mg)		UAE
	(Mean ± SE)	Sorbitol	Myoinositol	Sorbitol	Myoinositol	(mg/d)
Normal Lean	5.6 ± 0.5	0.17 ± 0.02	2.77 ± 0.15	0.97 ± 0.17	1.43 ± 0.23	0.5 ± 0.2
Euglycemic Obese	6.3 ± 0.2	0.21 ± 0.01	2.71 ± 0.12	1.58 ± 0.44	1.20 ± 0.15	14.8 ± 2.3
Hyperglycemic Obese	14.0 ± 0.4	1.81 ± 0.21	1.61 ± 0.09	35.40 ± 4.25	not detected	20.7 ± 3.4

Q 212 **SHEEP AS AN ANIMAL MODEL FOR HYPERINSULINEMIA AND INSULIN RESISTANCE IN DIETARY OBESITY,** Joseph P. McCann, Dorothy L. Aalseth & Emmett N. Bergman, Department of Physiological Sciences, Oklahoma State University, Stillwater, OK 74078; and Department of Physiology, Cornell University, Ithaca, NY 14853.

Sheep become dietary obese by consuming ad libitum intake of the same diet as fed at maintenance to lean sheep. Feed intakes (g/Kg body weight) become equivalent in both groups of sheep during the static phase of obesity, which is attained in obese sheep after 10-12 mo of ad libitum intake. Adult sheep with static dietary obesity are much fatter than lean sheep, are hyperinsulinemic and hyperglycemic (Table), and are insulin resistant. The peripheral hyperinsulinemia was caused by a greater pancreatic secretion of insulin (mU/min) in obese Suffolk (12 ± 1 vs 5 ± 1) and Dorset (18 ± 2 vs 5 ± 2) ewes than in their lean counterparts, as assessed directly in conscious fasted sheep instrumented with splanchnic blood-sampling catheters.

	Dorset ewes		Suffolk ewes		Rambouillet ewes	
Item[a]	Lean (7)	Obese (8)	Lean (6)	Obese (6)	Lean (5)	Obese (5)
Body weight, Kg	50 ± 2	97 ± 3	67 ± 2	93 ± 3	48 ± 3	76 ± 3
Carcass lipid, %	23 ± 2[b]	50 ± 4[b]	25 ± 2	41 ± 2	ND	ND
Plasma insulin, μU/ml	9 ± 1	34 ± 4	9 ± 1	15 ± 2	6 ± 1	15 ± 2
Plasma glucose, mg/dl	49 ± 1	60 ± 1	54 ± 3	63 ± 2	39 ± 1	48 ± 3

[a]Values for an item differ within each breed ($P < 0.05$). [b]N=3 animals.

Q 213 **DEXAMETHASONE DECREASES GLYCEROPHOSPHATE DEHYDROGENASE GENE EXPRESSION IN FULLY DIFFERENTIATED 3T3-F442A CELLS,** **Naïma Moustaïd, Bernard Hainque, Annie Quignard-Boulangé, Jacques Pairault",INSERM U177, Paris 75006, "INSERM U282, Créteil 94200. FRANCE.**

We have previously shown that dexamethasone(DEX) inhibited the terminal differentiation of 3T3-F442A preadipocytes. In this work, we have studied the DEX regulation of glycerophosphate dehydrogenase (GPDH) gene expression in fully differentiated 3T3-F442A cells.

GPDH activity as well as its mRNA content were measured after 1 or 2 days of treatment by DEX(20nM), insulin(10nM) and/or both hormones. The rate of GPDH mRNA degradation was determined by inhibiting mRNA synthesis with 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and subsequently measuring the loss of these mRNA by Northern blotting. Northern blots of total RNA were performed using GPDH, actin and 28K(adipsin) cDNA probes. In insulin deprived cells, DEX elicited a 50% decrease in the GPDH mRNA content within 24h whereas the decay of enzyme activity was delayed (48h). This treatment by DEX did not modify both the actin and 28K mRNA levels. In insulin-supplemented cells, DEX treatment did affect neither GPDH activity nor its mRNA throughout the experimental period. When compared to control cells, the rate of GPDH mRNA degradation was accelerated by DEX whereas no changes could be observed for 28K mRNA. By contrast, insulin exerted a stabilizing effect on GPDH mRNA.

These data strongly suggest that, in differentiated adipocytes, DEX regulates the GPDH gene expression at post-transcriptionnal level by increasing the rate of degradation of its mRNA.

Q 214 ALTERNATIVE POLYADENYLATION OF RAT FATTY ACID SYNTHETASE mRNA, Juergen Naggert, Brenda Williams and Stuart Smith, Children's Hospital Oakland Research Institute, 747 52nd Street, Oakland, CA 94609.

The mammalian fatty acid synthetase, a 250 kDa multifunctional polypeptide is coded for by a single gene in humans, rats, mice and rabbits. Unlike in human and mouse, the rat gene is transcribed into two mRNA species, 8.3 and 9.1 kb in size. Both mRNA's can be observed in northern blots with liver, mammary gland, adipose, lung and brain polyA$^+$ RNA, but neither is transcribed at a detectable level in heart and spleen. The two mRNA's arise through differential utilization of two polyadenylation signals, a ATTAAA sequence 785 b upstream of a canonical signal sequence. In a preliminary fasting / refeeding experiment, the 8.3 kb RNA appeared to accumulate faster than the 9.1 kb species. Studies have been initiated to determine whether the existence of two mRNA's is necessary for the regulation of the fasting / refeeding response in rat.

Q 215 HYPOTHALAMO-PITUITARY-ADRENAL FUNCTION IN LEAN AND OBESE ZUCKER RATS.

Shigeo Nakaishi, Yoshikatsu Nakai, Yoshiyuki Naitoh, Junichi Fukata, Tomoko Tominaga, Norihiko Murakami, Toshihiko Tsukada, Takeshi Usui, Hiroo Imura, Hitoshi Ikeda, Takao Matsuo, Second Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto and Biological Research Laboratories, Central Research Division, Takeda Chemical Industries, Osaka, Japan. Morning plasma corticosterone concentrations have been reported to be elevated in obese Zucker rats compared with lean rats. It has been suggested that the elevated corticosterone is an important factor in the expression of obesity in the Zucker rats. To clarify the mechanisms that control plasma corticosterone concentrations in obese Zucker rats, we measured the contents of ACTH and the mRNA contents of proopiomelanocortin (POMC) in the anterior and intermediate pituitaries. We further measured the contents of corticotropin-releasing hormone (CRH) in the different parts of the brain and also in the individual nuclei of the hypothalamus. The mRNA contents of POMC were measured by Northern blotting or dot hybridization method. The contents of CRH and ACTH in tissue extracts were measured by radioimmunoassay. The mRNA contents of POMC in the anterior pituitary in obese rats were significantly lower than those in lean rats. CRH contents in the mediam eminence (4603 vs. 2787 pg), paraventricular nucleus (101 vs. 61 pg) and periventricular nucleus (139 vs. 96 pg) in obese rats were significantly lower than those in lean rats. These results suggest that the hypothalamo-pituitary-adrenal functions in obese rats are disturbed.

Q 216 CONTROL OF PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE EXPRESSION IN THE ADIPOSE TISSUE, H. Nechushtan., L. Eisenberger., H. Cohen., N. Benvenisty., M. Shani, and L. Reshef. Dept. Developmental Biochemistry, *Hebrew University-Hadassah Medical School, Jerusalem and Volcani Center*, Rehovot, Israel. 91010. Cytosolic Phosphoenolpyruvate carboxykinase (PEPCK) gene, is expressed in several tissues (mainly liver, kidney and adipose tissue), and glucocorticoids regulate this gene transcription in a tissue specific manner, by stimulating it in the liver and kidney and inhibiting it in the adipose tissue. A. We have established two lines of transgenic mice, harboring the entire rat PEPCK gene with 2.2 kb of 5' and 0.5 kb of 3' flanking region, in which the transgene is expressed in just the liver, kidney and adipose tissue and its opposite control of expression by glucocorticoids is reproduced. B. In parallel, to examine cis regulatory elements that confer specific PEPCK gene expression in several tissues, we have transfected various cell lines, including rat hepatoma cells (H4IIEC3) and mouse adipocytes (3T3F442A), with PEPCK-CAT chimeric genes. Thus, while 597 bp of the PEPCK promoter are sufficient for exclusive gene expression in PEPCK-expressing cell lines, separate elements within the promoter are used by the two cell types to confer enhanced expression. The hepatocytes-specific domains reside upstream of position -362 and downstream of position -98, while the adipocytes-specific region resides in between these two domains.

Q 217 CONTROL OF TERMINAL DIFFERENTIATION OF ADIPOSE PRECURSOR CELLS BY CORTICOSTEROIDS, **Danielle Gaillard*, Martin Wabitsch+, Martine Cazalès* and Raymond Négrel*- *Centre de Biochimie (CNRS), Faculté des Sciences, Parc Valrose, 06034 Nice cédex, France and + Medizinische Klinik Universität Ulm, D-7900 Ulm, West Germany.**

The expression of the terminal stage of the adipose conversion program (characterized by the emergence of late markers such as glycerol-3-phosphate dehydrogenase and triacylglycerol accumulation) is concomitant with a critical mitosis which is dependent on the presence of extracellular adipogenic-mitogenic stimuli. Among them, we have characterized arachidonic acid **(ARA)** and two of its metabolites, PGI_2 and $PGF_{2\alpha}$, which should act in a paracrine/autocrine manner through the activation of intracellular mitogenic signaling pathways: cyclic AMP elevation and inositol-phospholipid breakdown, respectively (Biochem.J., 1989, 257, 389-397 and 399-405). Corticosteroïds at physiological concentrations (10^{-10}M-10^{-7}M) do not lead to an immediate activation of these signaling pathways but are able to promote a similar adipogenic-mitogenic effect to that induced by ARA or prostaglandins in the Ob17 preadipocyte clonal line. Potentiations between corticosterone and ARA are clearly observed. Similar data are obtained in primary culture using adipose precursor cells of various species including man. Using [^{3}H]ARA prelabelled Ob17 cells, corticosterone was shown to enhance within 24h ARA mobilization and metabolism and to lead to increased PGI_2 (6-keto-$PGF_{1\alpha}$) production. It is proposed that corticosteroïds control adipose conversion via their ability to trigger PGI_2 production, allowing early marker-containing cells (lipoprotein lipase-positive cells) to enter mitosis and to terminate differentiation.

Q 218 INSULIN EFFECTS DURING THE DEVELOPMENT OF RAT VMH-OBESITY: PHYSIOLOGICAL AND MOLECULAR DATA., L. Pénicaud, P. Ferré, C. Coupé, D. Perdereau and L. Picon. Lab. Physiol. Develop. CNRS UA 307, Paris and Centre Rech. Nutr. CNRS LP 1511, Meudon, France.

Insulin resistance in rat made obese by lesion of the ventromedial hypothalamus (VMH) follows a time-related pattern. Using the euglycemic hyperinsulinemic clamp coupled with an injection of ^{3}H-2deoxyglucose, an in vivo insulin resistance in glycolytic muscles together with an hypersensitivity to insulin in white adipose tissue, were shown one week after the lesion. This differential insulin sensitivity preferentially channels glucose towards adipose tissue and contributes to the development of obesity One of the earliest event after VMH lesion are changes in the activity and mRNA levels of key lipogenic enzymes in white adipose tissue. Six weeks after the lesion all the tissues studied are resistant to the action of insulin. It is speculated, on the basis of in vivo data, that insulin hypersecretion which is present as early as two days after the lesion, could play a crucial role in the regulation of these phenomena.

Q 219 ADIPSIN HAS COMPLEMENT D ACTIVITY: AN IMMUNE-RELATED DEFECT IN GENETIC AND ACQUIRED OBESITY, Barry S. Rosen, Kathleen S. Cook, Julia Yaglom, John Volanakis*, Deborah Damm**, Tyler White** and Bruce M. Spiegelman, Dana-Farber Cancer Institute and the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, *University of Alabama at Birmingham, Birmingham, Alabama and **Metabolic Biosystems, Mountain View, California.

Adipsin is a serine protease of unknown physiological function that is secreted by adipocytes and reduced in several models of rodent obesity. To approach the function of adipsin in normal adipose tissue biology and obesity, we have purified adipsin from baculovirus and mammalian expression systems. N-terminal sequence analysis reveals that the baculovirus-produced protein has the structure of the predicted zymogen while the mammalian expressed material is isolated as the activated protease. Activated adipsin has little proteolytic activity toward most peptide and protein substrates but has an activity qualitatively identical to human factor D of the alternative pathway of complement, a protein with which it shares extensive sequence homology. Adipsin will cleave complement factor B zymogen only in the presence of an activated form of complement factor C3. Serum factor D activity is markedly reduced in several models of rodent obesity compared to that of lean controls, including genetically obese ob/ob and db/db mice, the Zucker fa/fa rat, and the chemically-induced MSG mouse. These results suggest that adipsin/Factor D and the alternative pathway of complement may play an unexpected but important role in the regulation of energy balance.

Q 220 CLONING AND EXPRESSION OF A GENE ENCODING 6-PHOSPHOFRUCTO-2-KINASE/FRUCTOSE 2,6-BISPHOSPHATASE, Guy G. Rousseau, Martine I. Darville, Karine M. Crepin and Louis Hue, University of Louvain Medical School, B-1200 Brussels, Belgium.

Glycolysis provides not only ATP but also C-3 units for lipid and cholesterol synthesis. The most potent stimulator of glycolysis is fructose 2,6-bisphosphate which is synthesized by 6-phosphofructo-2-kinase (PFK-2) and degraded by fructose 2,6-bisphosphatase (FBPase-2). PFK-2/FBPase-2 is controlled by hormones, growth factors, and oncogenes. In liver, a single polypeptide bears the two catalytic activities but there is biochemical evidence for distinct PFK-2/FBPase-2 isozymes in different tissues. We have characterized from a rat liver cDNA library two full-length cDNA clones which encode PFK-2/FBPase-2 polypeptides that differ in amino terminal sequence. Clone 22c codes for the liver isozyme which is phosphorylated on Ser-32 by cAMP-dependent protein kinase. In clone 5c, the sequence upstream from amino acid 33 differs from that in 22c. Northern hybridization with probes from these clones suggests that a 6.8-kb mRNA codes for the heart isozyme, a 2.1-kb mRNA codes for the liver isozyme, and a 1.9-kb mRNA codes for the muscle isozyme. The latter was expressed in E. Coli and displayed PFK-2 and FBPase-2 activities. The transcription initiation sites of the liver and muscle isozymes were identified by primer extension of mRNA and by S1 mapping of genomic clones. Study of the latter suggests that the gene is at least 55 kb-long, contains at least 12 exons, and is located on the X-chromosome.

Q 221 **MOLECULAR GENETIC MAPPING OF MOUSE OBESE(ob) GENE BY BACKCROSS SYSTEM OF MUS MUSCULUS**, Takashi Sasaki*,Masami Nemoto*,Yoshio Ikeda*, and Masahiko Nishimura+, *3rd Department of Internal Medicine, Jikei University School of Medicine,Tokyo, +Hamamatsu University,Hamamatsu,Japan. In order to study the involvement of genes that develop obesity and hyperglycemia, we have mapped the mouse obese (ob) gene with DNA probes on mouse chromosome 6. Homozygous ob/ob mice are sterile after development of the disease, and old laboratory mice rarely show the genetic polymorphisms (RFLPs). To overcome these difficulties, we have developed the unique backcross-system between C57BL/6J-ob/ob(B6-ob/ob) and MOA, which is an inbred strain derived from Japanese wild mice Mus musculus molossinus, with ovarian transplantation. Male mice of MOA were crossed to (B6xC3H)F1 female that had been transplanted B6-ob/ob ovary, and the F1 male mice were backcrossed to B6-ob/ob in the same way. Phenotypic analysis of these progenies about ob and Agouti locus revealed their Mendelian segregation in this cross system. Contamination from the ovary of host mice were ruled out by using ribosomal RNA gene as probe. Because of the long evolutionary distance between B6 and MOA, DNA probes frequently show RFLPs in BC and F2 mice. BC mice were typed by Southern blot analysis by a series of DNA probes closely linked to the ob gene. One of these genes (Cpa; kindly provided by Dr. W.J.Rutter) was assigned 2cM distal to the ob gene. We are now undertaking fine genetic mapping of these genes on mouse chromosome 6.

Q 222 HORMONAL CONTROL OF ADIPOCYTE PRECURSORS DIFFERENTIATION IN PRIMARY CULTURE IN DEFINED MEDIUM. Ginette Serrero and Dianne Mills.
W. Alton Jones Cell Science Center, Lake Placid, NY 12946.

An insulin-independent variant cell line which has lost the ability to undergo adipose differentiation produces in its culture medium inhibitors of adipose differentiation and identified as TGF-α and TGF-β. In order to determine if both types of factors could act as physiological regulators of differentiation, their effect on the differentiation of adipocyte precursors in primary culture was investigated. For this purpose, a defined medium able to support the proliferation and differentiation of adipocyte precursors isolated from inguinal fat pads of 2-day old NBR rats, was developed. It consists of DME-F12 medium supplemented with fibronectin, insulin, transferrin and FGF. In these conditions after 8 days differentiation has reached an optimal level and 90% of the cells have accumulated triglycerides. The effect of TGF-α/EGF and TGF-β on proliferation and differentiation of adipocyte precursors was then examined. EGF stimulated proliferation with an ED_{50} of 3 x 10^{-10} M whereas it inhibited differentiation with an ED_{50} of 6 x 10^{-11} M. TGF-α acted in a similar fashion as EGF. In contrast TGF-β inhibited proliferation and differentiation of adipocyte precursors with an ED_{50} of 5 to 20 pM according to the source of TGF-β. Results about TGF-α and TGF-β effect will be discussed in detail here. These data suggest that both types of factors could play a role as physiological regulators of adipose tissue development in vivo.

Q 223 HORMONAL REGULATION OF FATTY ACID SYNTHASE AND OTHER SPECIFIC GENE TRANSCRIPTION IN MOUSE LIVER, Hei Sook Sul, Joseph D. Paulauskis, Nicholas Gekakis, Dong-Hoon Shin and Carl P. Verdon, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115

Insulin administered to streptozotocin-diabetic mice and refeeding previously starved mice markedly increased liver mRNA levels for fatty acid synthase(FAS), liver phosphofructokinase(L-PFK) and another specific mRNA (7.2 kb mRNA). And dibutyryl cAMP abolished the increase in mRNA levels caused by refeeding. Run-on transcription with isolated nuclei showed 4-fold increase in the rate of FAS gene transcription after 30 min and 7-fold increase after 2 h of insulin administration to diabetic mice, and the rate was maintained at maximum level to 12 h. For the 7.2 gene, transcription increased 4-fold at 30 min, and reached maximum of 9-fold at 2 h. The transcription rates of FAS, L-PFK and 7.2 genes were also increased when starved mice were refed a high carbohydrate diet. Refeeding increased FAS gene transcription 39-fold by 6 h and this level was maintained through 16 h. The increase in 7.2 gene transcription was slower, reaching a 3-fold after 6 h and 23-fold after 16 h. Moreover, streptozotocin-diabetes or dibutyryl cAMP administered to normal mice during refeeding abolished the nutritional effects on FAS, L-PFK and 7.2 gene transcription. Furthermore, the effect of insulin on FAS and 7.2 gene transcription was abolished by cycloheximide indicating that ongoing protein synthesis or a labile protein is necessary for the transcriptional activation of these genes by insulin. (Supported in part by NIH grant DK-36264)

Q 224 NEUROPEPTIDE Y CONCENTRATIONS IN BRAIN AREAS AND TISSUES OF ZUCKER RATS, Gary Truett and Roy J. Martin, Department of Foods and Nutrition, College of Home Economics, University of Georgia, Athens, GA 30602

Neuropeptide Y (NPY) injected into the central nervous system stimulates feeding behavior, insulin release, and the hypothalamo-pituitary-adrenal axis, but inhibits growth hormone release and sexual behavior. The obese (fa/fa) Zucker rat displays disturbances in these systems and does not overeat in response to doses of NPY which stimulate feeding in lean (Fa/fa and Fa/Fa) Zucker rats. NPY is also present in autonomic nerves in peripheral tissues. We measured levels of NPY in six brain areas and seven tissues of obese and lean Zucker rats. Obese rats had higher levels of NPY in medial basal hypothalamus (MBH) compared to lean rats. No significant differences were found in the paraventricular nucleus, ventromedial nucleus, dorso-medial nucleus, lateral hypothalamus, and area postrema-nucleus of the solitary tract. NPY concentrations in hearts of obese rats were lower than those of lean rats. No significant differences in NPY concentrations were found in spleen, kidney, and adrenals. The increased NPY in the MBH may represent increased synthesis, primarily in the arcuate nucleus. The decreased amount of cardiac NPY in obese rats may represent an adaptation to obesity or decreased sympathetic activity in this organ.

Q 225 INCREASED ADENOSINE RECEPTOR SENSITIVITY IS ASSOCIATED WITH DECREASED G_i IN ADIPOCYTES FROM YOUNG OBESE ZUCKER RATS, Vannucci, S.J., Klim, C.M., Martin, L.F. and LaNoue, K.F., Departments of Physiology and Surgery, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033

Hormone stimulated lipolysis is characteristically reduced in obesity. In adipocytes from young female Zucker (fa/fa) rats, this is accompanied by a reduction in cAMP production. We have previously reported that this is due, at least in part, to an increased level of inhibition of adenylate cyclase mediated by the adenosine A_1 receptor-G_i transmembrane signalling system. Adipocyte plasma membranes prepared from 5-7 week old Zucker rats contain comparable A_1 receptor number as compared with lean controls but the obese receptors exhibit an increased sensitivity to activation by guanine nucleotides. This correlates well with the observed enhancement of agonist induced biochemical response in obese adipocytes. To determine whether the difference between the lean and obese A_1 receptor response is due to altered G protein levels, G_i was measured in these membranes. Pertussis toxin labelled 49% less G_i protein in fa/fa membranes, as compared with lean controls. Quantitation of G_i by immunblot analysis demonstrated a 40% decrease in G_i in obese vs. lean membranes. This surprising reduction in G_i in the face of increased adenosine receptor activity may represent an attempt to down-regulate the receptor system in genetic obesity.

Q 226 INCREASED HYPOTHALAMIC NEUROPEPTIDE Y mRNA IN OBESE ZUCKER FATTY RATS, Jeffrey D. White, Gerard Sanacora and Maryann Kershaw, Div. Endocrinology, SUNY Stony Brook, Stony Brook, NY 11794

Neuropeptide Y (NPY) is a 36 amino acid peptide that potently stimulates carbohydrate feeding when injected into the hypothalamic paraventricular nucleus in rats. NPY immunoreactive cell bodies have been localized to the arcuate nucleus and project to the paraventricular nucleus. In this study, we investigated the possibility that NPY mRNA levels are increased in the arcuate nucleus of obese Zucker "fatty" rats compared to their lean littermate controls. Total RNA was isolated from whole hypothalamic dissections from obese and lean Zucker male and female rats. NPY mRNA content was measured using RNase protection analysis followed by urea/ PAGE and quantitation of autoradiographic bands using 2-D laser densitometry. This analysis revealed an approximate 3-fold increase in NPY mRNA levels in the samples from obese male and female rats. In situ hybridization analysis confirmed that the increase in hypothalamic NPY mRNA was detectable in the arcuate nucleus. This increase in NPY mRNA was not generalized to the entire brain but was localized to the hypothalamus and olfactory bulb, as determined by both nuclease protection and in situ hybridization analyses. These data are consistent with the hypothesis that regulation of hypothalamic NPY expression is disturbed in obese Zucker rats and that this disturbed regulation may play a role in the etiology of obesity in these animals.

Q 227 ALTERATIONS IN THE HEPATIC INSULIN RECEPTOR KINASE IN GENETIC AND ACQUIRED OBESITY IN RATS, DG Hurrell, O Pedersen, CR Kahn, Joslin Diabetes Center, Boston, MA
In the present study, we have characterized hepatic insulin receptor function in two animal models of obesity: the Zucker Fatty Rat (ZFR), a model of genetic obesity with severe hyperinsulinemia and the dietary obese Sprague-Dawley (SD) Rat, a model of acquired obesity. ZFRs were also treated with streptozotocin (STZ) in an effort to examine the acute effects of relative insulin deficiency and hyperglycemia in the setting of obesity. Using hepatic WGA partially purified insulin receptor, no significant difference in insulin binding was identified in either model of obesity. Beta-subunit autophosphorylation was significantly decreased in both obese models relative to control (72% in the obese ZFR and 49% reduction in the overfed SD model). Kinase activity, as measured by phosphorylation of the 1142-1153 synthetic peptide, was also decreased in both models of obesity, 22% and 64%, respectively. In the ZFR, STZ treatment led to an 80% increase in receptor concentration and a further 70% increase in beta-subunit autophosphorylation per receptor, yet activity was not altered. We also determined the fraction of autophosphorylated (activated) receptors versus nonphosphorylated (inactivted) receptors by unsing antiphosphotyrosine antibody to precipitate receptors bound with [125I]-insulin. There was no significant difference in the percent of activated insulin receptors in the obese, STZ-treated, and control models which ranged from 32-46%. These data suggest that in these models of genetic and acquired obesity, autophosphorylation of the beta-subunit is the limiting factor in insulin receptor function and that beta-subunit activation occurs in a similar fraction of receptors. However, full activation of the receptor does not occur, resulting in a decrease in kinase activity. This block in autophosphorylation may account for significant reductions in insulin receptor function.

Glucose Metabolism, Nervous System; Fatty Acid Metabolism, Energy Regulation and Thermogenesis

Q 300 A NOVEL GLUCOSE TRANSPORT PROTEIN EXPRESSED PREDOMINANTLY IN INSULIN-RESPONSIVE TISSUES, Maureen J. Charron[1], Frank C. Brosius, III[1,2], Seth L. Alper[1,2] and Harvey F. Lodish[1]. [1]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, [2]Department of Molecular Medicine, Beth Israel Hospital, Boston, MA 02115. Insulin stimulated glucose transport has been studied extensively and results of recent publications raise the possibility that the "basal" and "insulin-stimulated" transport activities may represent distinct molecular species of glucose transporters (GT) or different modifications of the same polypeptides. Using low stringency hybridization to the rat brain GT a novel 2490 base pair cDNA clone was isolated from a rat soleus λgt10 cDNA library. It encodes a 509 amino acid protein whose sequence and predicted membrane structure is very similar to those of the rat brain and liver GT's. The muscle GT-like protein is 65% identical in amino acid sequence to the rat brain GT and 52% identical to the rat liver GT; the major differences are in the NH_2- and COOH-terminal hydrophilic segments. This novel GT-like mRNA is expressed predominately in tissues where glucose transport is sensitive to insulin, including striated muscle, cardiac muscle and adipose tissue; low level expression is also detected in smooth muscle and kidney mRNA. This GT-like cDNA is the fourth member of the mammalian GT-related gene family identified to date. We propose that it encodes an insulin-sensitive GT. Currently we are expressing this clone in various systems to identify its function and modifications that take place in response to insulin. Results of these experiments will be discussed.

Q 301 GENETIC SELECTION OF ANIMALS DIFFERING IN OBESITY USING A BIOCHEMICAL PARAMETER. Susan M. Francis, Roy Bickerstaffe, Andrew P. Parratt. Biochemistry Department, Lincoln College, Canterbury, New Zealand and Ministry of Agriculture and Fisheries, Ruakura Research Centre, Hamilton, New Zealand. Two lines of sheep selected on the basis of an intravenous glucose tolerance test differ in body fatness. Progeny of rams with high T-half cleared glucose more slowly (sires: 130, progeny: 76 minutes) than progeny from rams with a low T-half (sires: 50, progeny: 60 minutes). The high T-half lambs were heavier (2.3 kg at 30 weeks of age) and had significantly less subcutaneous (8.4 versus 9.2 mm) and internal (210 versus 232 g) fat depots than the low T-half lambs. The insulin status of the animals is being investigated with the objective of studying the molecular mechanisms of inheritance of obesity in lambs.

Q 302 THE ANTI-OBESITY AND ANTI-DIABETIC ACTIVITIES OF CIMATEROL, Jo Alene Goidl, Michael Burns, Thomas H. Claus, Elwood Largis and Helen Meunkel, Medical Research Division, American Cyanamid Company, Pearl River, NY 10965. Numerous reports have described the anti-obesity effects of the B-adrenergic agonist, cimaterol. Anti-diabetic effects of this agent, however, have not as yet been described. The present study confirms the anti-obesity effects of cimaterol at a high dose, 0.02% of the diet, in obese (ob/ob) and diabetic (db/db) mice. Weight loss was observed over the course of the first three weeks of a one month test in both strains of mice. Fat pad weights were also reduced as was total body fat. These effects were apparent only at the high dose but not at lower doses of the compound. In addition to the anti-obesity effects, blood glucose levels were normalized in the mildly hyperglycemic ob/ob mouse and in the severely hyperglycemic db/db mouse not only at 0.02% but also at 0.005% of the diet. At the next lower dose of 0.002% of the diet, however, a hypoglycemic response was observed following one week of treatment but was not sustained throughout the month-long study. An improvement in glucose tolerance was observed in mice treated for 10 days with 0.002% cimaterol. The findings presented support the premise that cimaterol is more efficacious as an anti-diabetic agent than an anti-obesity agent. Furthermore, the hypoglycemic effects may be due to an improvement in insulin sensitivity.

Q 303 GLUCOSE TRANSPORT IS ALTERED IN SKELETAL MUSCLE OF OBESE MICE IN RESPONSE TO INSULIN BUT NOT IN RESPONSE TO TPA. Y Le Marchand-Brustel, J-F Tanti, N Rochet, T Grémeaux, E Van Obberghen INSERM U145, Faculté de Médecine, 06034 Nice Cedex France.
In isolated soleus muscle of lean mice, insulin and phorbol ester (TPA) acutely stimulated glucose uptake 3- and 2-fold, respectively (control: 1.78 $\pm$ 0.11; insulin: 4.84 $\pm$ 0.34; TPA: 3.43 $\pm$ 0.16; means $\pm$ SEM of 26 muscles). In order to look for a possible implication of protein kinase C (PKC) in insulin stimulation of glucose transport, we used two different approaches. First, a long-term pretreatment of muscles with TPA, which down-regulated PKC activity, not only abolished TPA-stimulated glucose transport but in addition, prevented insulin-stimulated glucose uptake. Second, an inhibitor of PKC, polymyxin B, blocked the insulin stimulation of glucose transport without altering basal glucose uptake or insulin activation of glycogen synthase. These results suggest that PKC is involved in insulin effect on glucose transport in muscle but PKC activation only explains part of this insulin stimulation.
In muscles of obese, insulin-resistant mice, basal glucose uptake (1.45 $\pm$ 0.11 nmol / mg protein) was slightly but significantly decreased compared to lean mice (1.78 $\pm$ 0.11) probably as a consequence of a decrease in the number of glucose transporters. Furthermore, insulin and TPA stimulated glucose transport to the same extent (insulin: 2.71 $\pm$ 0.29; TPA 2.41 $\pm$ 0.20). No difference was observed when PKC activation by TPA was measured in muscle from lean and obese mice. We propose that the translocation step of glucose transporters under insulin stimulation is a PKC dependent mechanism and is unaltered in obesity while the activation step, only induced by insulin, would be altered.

Q 304 TRANSPORT AND METABOLISM OF OLEATE BY BFC-1 CELLS, A CLONAL LINE FROM MOUSE BROWN ADIPOSE TISSUE, BEFORE AND AFTER DIFFERENTIATION INTO ADIPOCYTES. Nada Abumrad, Claude Forest, Usha Barnella and Samir Melki, Department of Molecular Physiology and Biophysics, Vanderbilt Univ. School of Medicine, Nashville, TN 37232. The BFC-1 cell line is characterized by a high degree of adipose conversion and sensitivity to β adrenergic agonists (Experimental Cell Research 168 (1987) 218-232). Exponentially growing, confluent and differentiated cells exhibited a similar high affinity (K_m = 0.3-0.4 μM) membrane system for the transport of oleate. Transport capacity (V_{max}) was altered by cell differentiation and averaged about 0.3 and 0.9 nmoles of oleate per 10^6 cells per min respectively for confluent and differentiated cells. Catecholamines and cyclic AMP analogues stimulated influx and efflux of labelled oleate only in differentiated cells and beginning at around 3 days following confluence. Responsiveness was optimal at 10-12 days post confluence with an EC_{50} value, for norepinephrine of 6 nM. Oleate transport in exponentially growing and confluent cells was insensitive to catecholamines and cAMP analogs in spite of demonstrable stimulation by these agents of the activity of cAMP dependent protein kinase. Norepinephrine also inhibited incorporation of the label into diglycerides and triglycerides in differentiated cell . Studies are underway to define the interrelationships between the effects of norepinephrine on the transport and metabolism of oleate.

Q 305 MOLECULAR CLONING AND SEQUENCING OF cDNAS ENCODING THE ENTIRE RAT FATTY ACID SYNTHETASE, Christopher M. Amy, Andrzej Witkowski, Jurgen Naggert, Brenda Williams, Zafar Randhawa, and Stuart Smith, Children's Hospital Oakland Research Institute, Oakland, CA 94609. In animals and fungi the enzymes of *de novo* fatty acid synthesis are integrated into complex multifunctional polypeptides. Overlapping cloned cDNAs representing the entire sequence of the rat fatty acid synthetase mRNA have been isolated from a λgt11 cDNA library and sequenced. Authenticity of the cDNA clones was supported by hybridization to fatty acid synthetase mRNA, by Western immunoblotting, and by amino terminal sequencing of 39 fatty acid synthetase CNBr fragments. The full-length fatty acid synthetase mRNA is 9156 nucleotides long and includes an 84 nucleotide 5' noncoding region, a 7515 nucleotide coding sequence and a 1537 nucleotide 3' noncoding region; a second mRNA species containing a shortened 3' noncoding sequence is also transcribed in the rat. The encoded fatty acid synthetase subunit contains 2505 amino acids and has a molecular mass of 272,257 Da. Active site sequences for the condensing (TAC*SS), transferase (IIGHS*), ACP (GLDS*L) and thioesterase (AGYS*FGA) domains and two nucleotide binding-site motifs (GXGXXG) were identified; one of the motifs was close to the pyridoxal phosphate binding site (GSAEK*R) characteristic of the enoyl reductase and the other was therefore assigned to the ketoreductase. Thus, the order of domains within the multifunctional animal fatty acid synthetase has been established as condensing enzyme - transferase - dehydrase - enoyl reductase - ketoreductase - acyl carrier protein - thioesterase.

Q 306 MASSIVE WEIGHT LOSS IN OBESE ZUCKER RATS CAUSED BY CHRONIC PERIPHERAL ADMINISTRATION OF NEUROPEPTIDE Y (NPY) AND NOREPINEPHRINE (NE) IN COMBINATION. J.F. Andrews and A. Al-Arabi , Department of Physiology , University of Dublin, Trinity College Dublin 2, Ireland. NPY has been reported to co-exist within catecholaminergic neurons of the central and peripheral nervous systems. Its functional significance in noradrenergic neurons has been related to the vasomotor effects of NPY which complement and interact with NE which is known to have central and peripheral effects on resting metabolic rate (RMR), food intake and body weight of rats. We have studied the effect of chronic peripheral administration of NPY on the metabolic action of NE in obese adult male rats.Twenty adult male obese (*fa/fa*) Zucker rats were acclimated to 28°C (thermoneutrality), divided into 5 groups: (1) untreated controls; (2) Carrier-treated Controls; (3) NPY treated; (4) NE treated and (5) NPY+NE treated. In groups 2-5 , Alzet™ (2002) osmotic minipumps were implanted under the skin in the interscapular region. Pumps were filled with carrier alone (group 2) plus NPY (group 3), or NE (group 4), or both (group 5). Delivery rates were calculated to be 0.5 µg/h NPY ; 20 µg/h NE, extending over a period of 14 days. The RMR of the animals was measured on days 2, 8, and 14 (indirectly as minimal oxygen consumption) . Food intake and changes in total body weight were measured every two days starting from day 2. Specimens of brown adipose tissue (BAT) were histologically analysed for the effect on the size of triglyceride droplets (TGD) in the adipocytes as an indirect measurement of the thermogenic activity of BAT. NE alone stimulated RMR, enhanced food intake, but a balanced body weight though maintained, failed to increase. NPY alone had no sustained effect on RMR, enhanced food intake and therefore body weight gain occurred. Combined treatment, enhanced RMR, suppressed food intake which led to massive weight loss. Histological analysis of BAT showed TGD significantly reduced by combined treatment, indicating increased thermogenic activity. We suggest that BAT of obese animals may be deficient in NPY which could be necessary for full thermogenic activity.

Q 307 THE GENE OF RAT AND HUMAN BROWN FAT UNCOUPLING PROTEIN, Frédéric Bouillaud, Serge Raimbault, Anne M. Cassard and Daniel Ricquier, Centre de Recherche sur la Nutrition, Centre National de la Recherche Scientifique, F92190 Meudon, France.
Thermogenic brown adipocytes are characterized by unique and strong expression of Uncoupling Protein (UCP) gene. We have isolated rat UCP gene as well as human gene. The complete nucleotide sequence of rat UCP gene including 4.5 Kb of the 5' domain upstream the transcription start site was determined. Rat and human UCP gene contains six exons scattered over a 13 Kb genomic region. A presumptive TATA box and two DNAse 1 sensitive sites were detected in 5' region of rat UCP gene. This region contains sequences partially homologous to described glucocorticoid, thyroid and cAMP-regulatory elements. Identification of regulatory sequences are under progress. At the 3' end of rat UCP gene, two polyadenylation sites were predicted from the sequence and confirmed using S1 nuclease mapping. They explain the two mRNAs for rat UCP detected in Northern blot analysis. Human UCP gene was localized on the chromosone 4 in q31 using in situ hybridization. UCP gene results from the triplication of an ancestral gene and each repeat corresponds to 2 exons. Moreover, limits between exons and introns never cut predicted trans-membranous helices.

Q 308 THERMAL INSULATION OF THE ABDOMINAL WALL REDUCES MEAL-INDUCED THERMOGENESIS

Tomas Brundin & John Wahren; Department of Clinical Physiology, Karolinska Institute, Huddinge University Hospital, S-141 86 Stockholm, Sweden.

Heat produced in internal organs is drained mainly by heating of the effluent venous blood. However, hepatic venous blood drains only little heat in relation to the splanchnic oxygen consumption. Consequently, the aim of this study was to find out whether leakage of splanchnic heat occurs directly across the abdominal wall.
In 15 healthy men thermistor catheters were inserted into the abdominal aorta, pulmonary artery and a hepatic vein. Pulmonary gas exchange, splanchnic blood flow and splanchnic arterio-venous differences for oxygen and heat were measured before and at timed intervals at 0-2 h after oral ingestion of a mixed meal. In 7 of the subjects extra insulation of the abdominal wall was carried out by blankets and heat-reflecting aluminium foil prior to the meal.
Results: In the non-insulated group the hepatic venous heat drainage was low in the fasting state (12 ± 1 J heat/ml O_2 consumed) and fell to 5 ± 2 J/ml O_2 at 2 h after the meal. Thermal insulation of the abdominal wall increased the blood-drained splanchnic heat to 18-19 J/ml O_2, reduced the splanchnic O_2-uptake by 18-31% and also diminished the meal-induced increase in pulmonary O_2-uptake by 27-33%. The heat leakage through the abdominal wall amounted to 11-17 watts corresponding to 80-90% of the total meal-induced thermogenesis. - It is concluded that heat dissipation through the anterior abdominal wall is an important factor in the regulation of human energy expenditure.

Q 309 MACROPHAGE-DERIVED MODULATORS OF ADIPOCYTE LIPOPROTEIN LIPASE ACTIVITY-SPECIES SPECIFIC EFFECTS OF TNF-α, Simon C.Butterwith and Harry D.Griffin, AFRC Institute for Grassland and Animal Production, Poultry Department, Roslin, Midlothian, EH25 9PS, Scotland.Macrophage-derived products in particular TNF-α have been shown to have dramatic effects on lipid metabolism in cell lines such as 3T3-L1.We have been studying their effects on primary cell cultures of chicken adipocytes and hepatocytes by measurement of lipogenic and lipoprotein lipase activities.Adipocyte lipoprotein lipase was decreased by 86% whereas lipogenesis appeared to be stimulated by about 100% after addition of 2ml of medium from endotoxin stimulated chicken macrophages.Hepatocyte lipogenic activity was unaffected after incubation with the same macrophage medium.hrTNF-α when incubated with chicken adipocytes failed to decrease lipoprotein lipase activity, and when incubated with hepatocytes had no effect on lipogenesis.The findings with chicken adipocytes incubated with hrTNF-α are in contrast to experiments in 3T3-L1 adipocytes which showed a marked reduction of both lipoprotein lipase and lipogenic activity in response to TNF.These results are discussed in relation to the possible species specificity of TNF-α, and to the role of macrophage-derived products in the overall control of lipid metabolism in the whole animal.

Q 310 BROWN ADIPOSE TISSUE DEVELOPMENT IN BOVINE SPECIES AND RELATIONSHIP TO WHITE ADIPOSE TISSUE, Louis Casteilla, Frédéric Bouillaud, Odette Champigny, Francesc Villaroya and Daniel Ricquier, Centre de Recherche sur la Nutrition, CNRS F-92190 Meudon, France, Universitat de Barcelona, Spain, and INRA, Clermont-Ferrand, France.
We have studied the development of brown adipose tissue (BAT) in bovine species and its relationship to white adipose tissue (WAT). A cDNA for bovine uncoupling protein (UCP) which is unique to BAT was cloned. The homology between UCP and other mitochondrial carriers was confirmed and we propose a new folding model for UCP. We have studied the expression of UCP mRNA in comparison with that of mRNA for other mitochondrial proteins (cytochrome oxidase subunits III and IV and ADP/ATP carrier) in different adipose depots during the fetal and perinatal life of the calf. Concomitantly T4-5' Deiodinase (DI) activity was measured. This enzyme seems to be involved in the control of UCP expression. DI activity is detectable very early in pregnancy and exhibits a high peak just before the appearance of UCP mRNA (last third of pregnancy) in perirenal tissue. During fetal life, BAT acquires gradually its specific characteristics. At birth, UCP mRNA is present in all adipose depots except the subcutaneous one. After birth contrary to COX III and IV, UCP mRNA disappears quickly. The rapid disappearance of UCP mRNA after birth is in agreement with an apparent transformation of BAT into WAT in some fat pads. It can be concluded that DI activity is an earlier marker of BAT differentiation than UCP and we propose a sequence of events for the development of BAT in vivo. The characterization and the cloning of markers of BAT differentiation is the next step in the study of the development and involution of this tissue.

Q 311 ATROPHY OF BROWN ADIPOSE TISSUE (BAT) IN CAPSAICIN-DESENSITIZED RATS: ROLE FOR SENSORY NEUROPEPTIDES IN BAT FUNCTION ? J. Himms-Hagen, J. Cui, S. L. Sigurdson, and G. Zaror-Behrens. Department of Biochemistry, University of Ottawa, Ottawa, ONT K1H 8M5 Canada. Capsaicin (CAP) is the pungent compound in hot chilli peppers. Capsaicin-desensitization (CAP-DES) of rats destroys a subpopulation of sensory nerves that contains substance P and calcitonin gene-related peptide (CGRP). CAP-DES rats can thermoregulate in the cold but not in the heat. Our objective was to study BAT in CAP-DES rats subjected to two types of stimulus that promote BAT growth, a cafeteria diet (CAFE) for 1 or 3 wk or exposure to cold for 1 or 7 days. Rats were studied 2 wk after a series of s.c. CAP injections (12.5 mg/kg under halothane anesthesia, day 1; 25 mg/kg twice, day 2; 62.5 mg/kg, day 3). Control rats (CON) were injected with vehicle. BAT of CAP-DES rats was initially small (low protein content) and thermogenically inactive (low mitochondrial GDP binding). Thyroxine 5'-deiodinase (TD) activity was normal. No growth occurred after 1 or 3 wk of CAFE diet. No thermogenic activation occurred after 1 wk of CAFE diet but some occurred by 3 wk. Food intake was doubled by the CAFE diet and food selection was similar in CON and CAP-DES rats. Body weight gain was increased by the CAFE diet in all rats, more in the CAP-DES rats during the first week; both groups became obese (doubled weight of gonadal white adipose tissue). Cold-exposure increased BAT mitochondrial GDP binding and TD activity normally in CAP-DES rats. Growth of BAT was delayed, but had occurred by 7 days. Noradrenaline (NA) content (by HPLC) of BAT was not altered by CAP-DES but depletion of CGRP (immunofluorescence) occurred. We suggest that sensory neuropeptides play a role in control of trophic response of BAT to diet and to temperature. (Supported by MRC Canada).

Q 312 THE NORADRENALINE (NA) STIMULATED INCREASE IN BROWN ADIPOSE TISSUE (BAT) THYROXINE 5'-DEIODINASE (T5'D) ACTIVITY IS MEDIATED BY BOTH α- AND β-ADRENERGIC MECHANISMS IN LEAN AND GENETICALLY OBESE (ob/ob) MICE.
Anna-Lisa Kates and Jean Himms-Hagen, Department of Biochemistry, University of Ottawa, 451 Smyth Rd., Ottawa, Ontario, K1H 8M5, CANADA.
Genetically obese (ob/ob) mice do not activate BAT thermogenesis in response to cold exposure. BAT thermogenesis and the activity of T5'D are controlled by NA. The objective of this study was to examine the effect of acute injection of NA and other adrenergic agents on BAT T5'D activity in lean and ob/ob mice. Female C57BL/6J lean (+/?) and obese (ob/ob) mice (8 weeks old) were housed in individual cages at 28 °C with a 12:12 lighting schedule. The animals were injected at 9:00 am with saline, NA, isoproterenol (ISO), phenylephrine (PHE), both ISO and PHE, yohimbine (YOH), NA & propranolol (PROP), NA & prazosin (PRAZ), NA & YOH and killed 3 hours later. The results of this experiment show that ob/ob mice are able to increase BAT T5'D activity in response to acute injection of NA to the same degree as lean mice. Both lean and ob/ob mice need the presence of both PHE and ISO to increase BAT T5'D activity. The increase in BAT T5'D can be blocked by both PROP and PRAZ but not YOH. In conclusion, both lean and ob/ob mice can increase BAT T5'D in response to acute NA injection through both α- and β-adrenergic mechanisms. Thus ob/ob mice do have the capacity to respond to the cold stimulated increase in NA turnover (Zaror-Behrens and Himms-Hagen, 1983) but we cannot rule out the possibility that another component of the cold response other than NA is responsible for the defective response of ob/ob BAT T5'D to acute cold exposure.

Q 313 INDUCTION OF ENZYMES BY THE THERMOGENIC HORMONES, TRIIODOTHYRONINE AND DEHYDROEPIANDROSTERONE. Henry Lardy, University of Wisconsin, Madison, WI 53705.
We have sought the enzymatic basis for the thermogenic and antiobestiy activity of dehydroepiandrosterone (DHEA). Thyroid hormone, a classic thermogenic agent, induces the formation of mitochondrial sn-glycerol 3-phosphate dehydrogenase (G3PDH) in all of those tissues that respond to the hormone with an increased rate of oxygen consumption (Lee and Lardy, J. Biol. Chem. 234, 3051 and 240, 1427). From the work of H. and J. Tepperman and, more recently, of others, we know that liver cytosolic malic enzyme is increased by both thyroid hormone and DHEA. We find that DHEA also induces G3PDH but only in liver.
G3PDH carries electrons from cytosolic NADH to ubiquinone, thus bypassing the first phosphorylation step in the mitochondrial energy-coupling pathway. Because only one sixth of the electrons removed from carbohydrate are liberated in the cytosol, this pathway can account for only a 5.6% decrease in metabolic efficiency. We postulate that malate produced in liver mitochondria moves to the cytosol where malic enzyme converts it to pyruvate and CO_2 and reduces NADP to NADPH. By an unknown pathway NADPH brings about the reduction of dihydroxyacetone phosphate to G3P which is oxidized by the G3PDH. Such a process could decrease efficiency of total oxidative metabolism by one third.
Evidence in support of this hypothesis has been obtained with isolated hepatocytes from normal and thyroidectomized rats fed DHEA or given triiodothyronine.

Q 314 THE ANTI-INSULIN EFFECT OF S-OXALYLGLUTATHIONE ON PALMITOYL-COA METABOLISM IN ADIPOCYTE MITOCHONDRIA, K.H. Moore, C. Czajkowski, D.M. Dandurand, and F.L. Kiechle, Department of Chemistry, Oakland University, Rochester, MI 48309 and Department of Clinical Pathology, Wm. Beaumont Hospital, Royal Oak, MI 48072.
Oxalyl thiolesters, a novel group of metabolic effectors, have been shown to inhibit malic enzyme and phosphorylase phosphatase, two activities which are stimulated by insulin (Biochem. 26:1, 1987). In this study, the effects of S-oxalylglutathione (GS-Ox) on mitochondrial palmitoyl-CoA hydrolase and β-oxidation activities were determined and compared to the previously determined insulin effects (Diabetes 37:185A, 1988). GS-Ox was synthesized via S-oxalyl-p-thiocresol, purified, and quantitated in our laboratory. Purified mitochondria were isolated from adipocytes released by collagen digestion of rat epididymal fat pads. After preincubation of mitochondria with GS-Ox (0 to 1.0mM), substrates and cofactors were added for radioisotopic assay of either palmitoyl-CoA hydrolase or β-oxidation. Control palmitoyl-CoA hydrolase activity was 5.51±0.93 nmol/min/mg; this activity was increased 27% ($p<0.01$) in the presence of 0.3mM GS-Ox and 71% ($p<0.01$) in the presence of 1.0mM GS-Ox. In contrast, preincubation of adipocytes with insulin (400μU/ml for 10min) had decreased hydrolase activity by 14% ($p<0.025$). The control rate of β-oxidation was 4.91±0.90 nmol/min/mg; this rate was inhibited by as little as 50μM GS-Ox. Palmitate oxidation was decreased to 65% ($p<0.01$) of control rate by 0.3mM GS-Ox and to 49% ($p<0.01$) of control rate by 1.0mM GS-Ox. Again, the GS-Ox effect is in contrast to the 7% ($p<0.05$) increase in β-oxidation observed subsequent to insulin treatment. The effects of GS-Ox on the adipocyte mitochondrial acyl-CoA pool are opposite to those observed for insulin and support the concept that GS-Ox may function as a negative effector for insulin.

Q 315 TWO β-ADRENOCEPTOR mRNAs ARE EXPRESSED IN RAT INTERSCAPULAR BROWN ADIPOSE TISSUE. Patrick Muzzin, Jean-Pierre Revelli, Françoise Assimacopoulos-Jeannet, Jean-Paul Giacobino, J. Craig Venter and Claire M. Fraser. Département de Biochimie médicale, CMU, et Laboratoire de Recherches Métaboliques, 1211 Genève 4, Switzerland, and Section of Receptor Biochemistry and Molecular Biology, LMCN NINCDS, National Institutes of Health, Bethesda, MD 28902.
Brown adipose tissue (BAT) thermogenesis is under the control of the sympathetic nervous system which acts through β-adrenergic receptors (β-AR). The subtype specificity of BAT β-AR is controversial. Most recent studies suggest that BAT contains either a mixed population of β_1-AR and β_2-AR or an atypical β-AR that does not conform to the β_1/β_2-AR classification. β-AR mRNA levels in interscapular brown adipose tissue (IBAT) were determined by Northern blot analysis of poly A^+ mRNA using a β_1-AR DNA probe isolated from a human genomic DNA library and a rat heart β_2-AR cDNA probe. Two species of poly A^+ mRNA were detected in IBAT; a 3.0 kb species that hybridized with the β_1-AR receptor probe and a 2.2 kb species that hybridized with the β_2-AR receptor probe, suggesting that BAT contains both subtypes of β-AR. A novel thermogenic β-AR agonist, Ro 16-8714, which selectively stimulates BAT thermogenesis in rats increased the density of β-AR in obese (fa/fa) Zucker rat IBAT as determined by [^{125}I]iodocyanopindolol binding to plasma membranes. The effects of Ro 16-8714 on the levels of β-AR mRNA in IBAT have been studied.

Q 316 LONG-TERM EFFECTS OF EARLY CARBOHYDRATE INTAKE ON LIPID METABOLISM IN THE RAT, Mulchand S. Patel, Thomas J. Thekkumkara, Peter M. Haney and Lap Ho, Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.
To test the hypothesis that a high-carbohydrate diet during early life can influence the development of obesity in adulthood, a high-carbohydrate, low-fat milk formula (56% and 20% calories from carbohydrate and fat, respectively) was fed to artificially reared male rat pups via intragastric cannula from day 4 to 18, and fed ad libitum the same liquid diet to day 24). Naturally reared pups served as controls. Artifically reared pups were hyper-insulinimic (2-3 fold higher) while they were fed the high-carbohydrate milk formula. At the time of weaning on day 24 the two groups of pups had similar body weights. Pups were weaned to laboratory chow and were fed a high-sucrose diet (48% calories from sucrose) for one month before killing on day 100 or 130. Compared to the controls, the artificially reared animals showed: i) a higher weight gain during the post-weaning period (20-25% heavier at the end of the experiment), ii) significantly higher activities of several lipogenic enzymes (e.g. fatty acid synthetase and glucose-6-phosphate dehydrogenase) in liver, iii) significantly (about 2-fold) higher rates of fatty acid synthesis in vitro in liver and adipose tissue, and iv) significantly (2-fold) higher rates of synthesis of non-saponifiable lipids in vitro in liver. These results demonstrate that early and prolonged exposure to a high-carbohydrate milk formula in rats during the suckling period contributes to long-term alterations of lipid metabolism. (Supported by NIH grant HD 11089).

Q 317 HYPOTHYROIDISM ENHANCES THE α_1/β ADRENERGIC SYNERGISTIC STIMULATION OF THE IODOTHYROININE DEIODINASE IN BROWN ADIPOCYTES, Atso Raasmaja and P. Reed Larsen, Howard Hughes Medical Laboratory and Thyroid Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115.
Norepinephrine stimulates the Type II iodothyronine 5'-deiodinase activity in rat dispersed brown adipocytes by an mRNA synthesis-dependent mechanism which requires several hours. Stimulation and maximum activities are 3-4 fold greater in cells from hypothyroid than in those from euthyroid rats. We now show a synergistic effect of α_1 and β adrenergic catecholamines to increase deiodinase activity which leads to 2 (euthyroid) to 4 fold (hypothyroid) higher deiodinase activities in the presence of both agonists than can be accounted for by the independent effect of either. The α_1 synergism is also found in combination with cAMP analogues or forskolin and α_1 agonists do not increase forskolin stimulated cAMP in euthyroid or hypothyroid cells. Since α_1 agonists cause minimal stimulation alone this response is due to an enhancement of the effect of cAMP but there is no alteration in the EC_{50} for its action. We conclude that activation of the deiodinase in brown adipocytes requires cAMP and its effect is amplified by α_1 agonists especially in hypothyroid cells. While the marked α_1/cAMP synergism in hypothyroidism is salutory for the thermogenic function of this tissue, it contrasts with the attenuation of β adrenergic effects typically observed in thyroid hormone deficiency.

Q 318 OXYGEN UPTAKE AND HEAT PRODUCTION IN TISSUES DRAINED BY THE AZYGOS VEIN IN MAN, John Wahren and Tomas Brundin, Department of Clinical Physiology, Karolinska Institute, Huddinge Hospital, Stockholm, Sweden.
This study was undertaken to examine the possible metabolic role of brown adipose tissue in intact humans. Oxygen uptake and heat generation in intrathoracic and interscapular tissues in healthy man were studied in the basal state, after a mixed meal and after infusion of norepinephrine. Catheters were placed percutaneously in the azygos vein, the pulmonary artery and a brachial artery in 11 healthy men in the postabsorptive state. Azygos vein blood flow was determined by thermodilution, blood temperatures were recorded and samples for oxygen content were collected in the basal state and at timed intervals after ingestion of a mixed meal or during i.v. infusion of norepinephrine (0.1 ug/kg BW/min). Basal oxygen uptake in the area drained by the azygos vein was 7±1 ml/min or 3% of the total. Azygos vein blood flow was 94±10 ml/min and its blood temperature was consistently higher than that in the brachial artery. The local heat production was 0.54±0.11W. After ingestion of a mixed meal azygos vein blood flow increased to a peak of 163±22 ml/min, but the arterial-azygos vein blood temperature difference and the regional heat production decreased. Local oxygen uptake rose from 7±1 to 9±2 ml/min while total oxygen uptake increased from 261±7 to 320±11 ml/min at 120 min after the meal. Norepinephrine infusion for 10-15 min resulted in 7% rise in total oxygen uptake but unchanged blood flow and oxygen uptake in the azygos region while local heat production tended to decrease. These data do not support an important role for intrathoracic or interscapular brown adipose tissue in meal-stimulated or catecholamine-induced rises in energy expenditure in humans.