THE ADIPOSE CELL

Organizers: Constantine Londos, Samuel Cushman and Vincent Manganiello

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Keynote Address (Joint)

CZ 001 HOW G PROTEINS TRANSMIT SIGNALS, Henry R. Bourne, Departments of Pharmacology and Medicine, Cell Biology Program, and Cardiovascular Research Institute, University of California School of Medicine, San Francisco, CA 94143.

Heterotrimeric ($\alpha\beta\gamma$) G proteins transmit signals from cell surface receptors for hormones and sensory stimuli to effector molecules — enzymes and ion channels — that initiate cellular responses. Among recent advances in understanding this signaling system, this lecture focuses on work in many laboratories that has provided a plausible, consistent, and testable model of the relations between structure and function of Gα subunits. Now that 3D crystal structures of Gα subunits have been solved, Gα is arguably the best understood protein signaling machine in biology. We can now identify in Gα the four key functional regions of a generic signaling machine, and begin to describe in detail how they work. Experiments with antibodies, mutations, synthetic peptides, and chimeric Gα subunits all point to the extreme carboxy (C) terminus of Gα as the location of the Gα signal detector region, which interacts with and discriminates among ligand-activated receptors, and then initiates a change in conformation that leads to release of GDP from α·GDP. Work in several labs seeks to pinpoint specific contact points between receptor and Gα. Similar techniques have located the Gα signal generator region, which interacts with and activates effectors such as adenylylcyclase and cGMP-phosphodiesterase (respective targets of the α subunits, α_s and α_t, of G_s and retinal transducin). Evidence from studies of α_s and α_t locates the amino acids that make up the signal generator region in loops situated on the "back" face of Gα, opposite to the face that includes the guanine nucleotide binding site. Mutations, biochemical probes of Gα structure, and analogies with homologous GTPases all point to the Gα region that corresponds to loop 4 and helix α2 of $p21^{ras}$ as the principal switch region, responsible for translating the presence of a γ-phosphate on GTP into a conformational change that enables the signal generator region to activate the appropriate effector. Finally, each Gα subunit possesses a sophisticated built-in timing device that turns off its active conformation by catalyzing hydrolysis of bound GTP, just as GTPase Activating Proteins (GAPs) do for small GTPases like $p21^{ras}$ and Elongation Factor Tu. Recent evidence from this laboratory shows that this timer-turnoff region is composed of an inserted domain of ~120 amino residues, which serves as a built-in GAP and catalyzes GTP hydrolysis via a conserved arginine residue (R201 in α_s).

Mechanisms for Adipocyte Differentiation

CZ 002INTERACTION OF THE NUCLEAR PROTEIN CHOP WITH TRANSCRIPTIONAL FACTORS DURING ADIPOCYTE DIFFERENTIATION, Joel F. Habener and David Ron, Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA.

During the hormonally-induced differentiation of 3T3-L1 preadipocytes (adipoblasts) to adipocytes (6 days), at least three isoforms of C/EBP (α, β, δ) are expressed in temporally distinct patterns. C/EBPα is expressed late in the differentiation program (4-5 days) concurrent with, and required for, the expression of adipocyte-specific genes, such as fatty acid binding protein 422 and glycerol-3-phosphate dehydrogenase. C/EBPs β and δ are expressed much earlier (1-3 days) and are believed to perform important regulatory functions early during adipocyte differentiation. To identify additional members of the C/EBP family of DNA-binding transcriptional proteins that may participate in the interplay of factors involved in the regulation of genes in adipocyte differentiation, a mouse adipocyte cDNA expression library was screened with a ^{32}P-labeled peptide consisting of the DNA-binding and dimerization domain (bZIP) of C/EBPβ. Two unique recombinant proteins that dimerize with the bZIP probe were identified. One protein is a new member (C/ATF) of the CREB/ATF family of bZIP proteins that dimerizes with C/EBPs α and β, and transactivates gene transcription by binding to cAMP-response elements. The other protein is a C/EBP-related bZIP protein, CHOP10 (CHOP), identical to GADD-153, a protein induced in cells by growth arrest and DNA-damaging agents. CHOP has an unusually structured DNA-binding basic region, a glycine and two prolines are substituted for invariant asparagine, arginine and aliphatic residues, respectively, known to be critically required for DNA recognition and binding. As a consequence of the intact leucine zipper domain and apparently defective basic region, CHOP readily dimerizes with C/EBPs α and β, but abrogates binding of CHOP-C/EBP heterodimers to C/EBP DNA-binding sites in gene promoters, e.g., the APRE site of the rat angiotensinogen gene. Co-transfection of C/EBP and CHOP in HepG2 cells inhibits transactivation from a APRE-CAT reporter plasmid. Thus CHOP functions as a dominant negative inhibitor of C/EBP-mediated transactivation of transcription on C/EBP binding sites, such as the APRE. During differentiation of preadipocytes to adipocytes, the expression of CHOP parallels that of C/EBPα. Further, dedifferentiation of mature adipocytes by TNF decreases expression of CHOP and C/EBPα. Thus, CHOP is envisioned to be a negative regulator of C/EBP functions that may modulate over-expression of C/EBPα during critical stages in the cascade of gene expression during terminal adipocyte differentiation. It remains possible, however, that CHOP-C/EBP heterodimers and bind to a specialized subset of DNA elements that are distinct from the conventional C/EBP binding sites and thereby may exert unique controlling influences on a subset of genes during adipocyte differentiation apart from the inhibitory effects of CHOP on C/EBP.

Regulation of Gene Expression in the Adipocyte (Joint)

CZ 003 METABOLIC INTEGRATION OF GENE EXPRESSION IN ADIPOSE CELLS, Gérard Ailhaud[1], Nada Abumrad[2], Ez-Zoubir Amri[1], Irina Safonova[1], Christian Darimont[1], Georges Vassaux[1], Danielle Gaillard[1], Raymond Négrel[1], and Paul Grimaldi[1], [1]Centre de Biochimie (UMR 134 CNRS), Faculté des Sciences, Parc Valrose, 06108 Nice cédex 2, France, and [2]Dept. Physiology & Biophysics, School of Medicine, Health Sciences Center, SUNY, Stony Brook, NY 11794-8661.

At growth arrest, adipoblasts become committed to preadipose cells which are able to express various early genes, among which *clone* 9, a member of the pentaxin family and A2COL6/pOb24. Genes encoding for lipoprotein lipase (**LPL**) and a fatty acid transporter (**FAT**) are also activated, allowing fatty acids to enter efficiently preadipose cells. At that stage, a lack of Ca^{2+} mobilization is needed to observe a positive, growth hormone-dependent modulation of LPL gene expression. Subsequent events leading preadipose cells to enter terminal differentiation implicate the involvement of a few adipogenic hormones acting in concert by means of cell surface receptors to insulin, IGF-I and growth hormone as well as nuclear receptors to triiodothyronine, glucocorticoids and retinoids. Recent evidence indicates that prostacyclin plays in a transient manner a critical role as a cAMP-elevating and a Ca^{2+}-mobilizing agent, allowing terminal differentiation of preadipose cells from various origin. More recent evidence indicates also that both metabolized and non-metabolized long-chain fatty acids are able to activate in preadipose cells the expression of lipid-related genes, *i.e.* FAT, adipocyte lipid-binding protein (**ALBP**) and acyl-CoA synthetase (**ACS**) genes, and to modulate the expression of LPL gene. These events take place primarily at a transcriptional level. As new members of the steroid hormone receptor superfamily can confer fatty acid responsiveness (**PPARs**) and as mPPARα is not detected in mouse Ob1771 preadipocytes, a new nuclear *trans*-acting factor has been cloned and sequenced from a cDNA library of fatty acid-treated Ob1771 cells. Therefore, in addition to a few adipogenic hormones which have to be present at the same time about threshold concentrations, it is proposed that fatty acids act as signal transducing molecules and that any metabolic event raising their intracellular concentration should favor terminal differentiation of preadipose cells. Recent data showing that both natural and non-metabolized fatty acids induce the formation of new fat cells *in vitro* support this hypothesis and will be presented.

CZ 004 TRANS-ACTING FACTORS IN ADIPOGENIC DIFFERENTIATION. M. Daniel Lane, Fang-Tsyr Lin, Mireille Vasseur-Cognet and Peter Cornelius, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

During differentiation of preadipocytes into adipocytes transcription of adipose-specific genes is coordinately activated. We have sought to identify regulatory elements common to a limited set of differentiation induced genes and then to identify the trans-acting factor(s) that interact with these elements. It was discovered that C/EBPα is the differentiation-induced nuclear factor which binds specifically to elements of similar nucleotide sequence within the promoters of several differentially expressed adipocyte genes, e.g. the 422(aP2), GLUT4 and SCD1 genes. We further demonstrated both by transfection of chimeric promoter-reporter gene constructs into 3T3-L1 preadipocytes and by cell-free transcription that C/EBPα transactivates these chimeric genes and that mutation of the C/EBP binding site within the promoters blocks transactivation. To obtain definitive proof for its involvement in the transcriptional activation of the endogenous 422(aP2), GLUT4 and SCD1 genes, expression of C/EBPα was blocked selectively during preadipocyte differentiation with an antisense C/EBPα RNA (~400 bp) expression vector. Vector-directed expression of antisense C/EBPα RNA in 3T3-L1 preadipocytes effectively blocked expression of C/EBP mRNA and protein, as well as several adipose-specific mRNAs and also prevented cytoplasmic triglyceride accumulation. Rescue of the "adipocyte phenotype" was accomplished by transfection of cells expressing antisense RNA with a modified vector which directs transcription of the complementary sense C/EBPα RNA. Taken together, these findings indicate that C/EBPα functions in coordinate gene activation during preadipocyte differentiation.

To investigate the basis for the transcriptional activation of the C/EBPα gene *per se* during differentiation, we cloned the mouse gene and identified two relevant DNA sequences within the promoter: 1. a C/EBP binding site at which C/EBPα appears to autoactivate transcription, and 2. a bi-partite site that binds a nuclear protein present in preadipocytes, but not adipocytes. We have purified and characterized the latter protein, (CUP, i.e. C/EBP undifferentiated protein) which binds to a bipartite element in the promoter. CUP interacts more strongly with its binding site in the presence of an Sp1-like GT-box binding protein which also binds to the bipartite element. During the differentiation of preadipocytes, expression of CUP activity decreases concomitant with increased expression of C/EBPα. Moreover, it was found that cell-types that express CUP do not express C/EBPα and vice versa. Evidence suggests that a CUP-containing protein complex bridges between the CUP and C/EBPα (autoactivation) elements in the promoter and may serve to repress expression prior to differentiation.

In addition to full-length (42 kDa) C/EBPα, a 30 kDa isoform was identified in 3T3-L1 adipocytes, mouse adipose tissue and rat liver. Mutational analysis established that 30 kDa C/EBPα is an alternative translation product initiated at the third in-frame methionine of the C/EBPα message. Like $p42^{C/EBP\alpha}$, $p30^{C/EBP\alpha}$ transactivates the 422(aP2) and C/EBPα gene promoters. Unlike $p42^{C/EBP\alpha}$, however, $p30^{C/EBP\alpha}$ is not anti-mitogenic. Thus, the N-terminal 12 kDa segment of full-length C/EBPα contains amino acid sequence required for its anti-mitotic activity. During differentiation of 3T3-L1 preadipocytes and during hepatocyte development, the cellular $p42^{C/EBP\alpha}/p30^{C/EBP\alpha}$ ratio changes raising the possibility of a regulatory role.

CZ 005 MOLECULAR CHARACTERIZATION AND HORMONAL REGULATION OF ADIPOSE DIFFERENTIATION RELATED PROTEIN (ADRP), Ginette Serrero, Hui-Ping Jiang*, Dominic Eisinger and Hong Ye, W. Alton Jones Cell Science Center Inc., 10 Old Barn Road, Lake Placid, NY 12946, *present address: Division of Biology, Calif. Inst. Technology, Pasadena, CA 91125.

Elucidation of molecular events occurring during adipose differentiation has been greatly facilitated with the isolation and characterization of genes that are activated during the process of differentiation. In our laboratory, we have been studying adipose differentiation by using the adipogenic cell line 1246 which can proliferate and differentiate in defined medium. Recently, we have constructed a cDNA library of fully differentiated 1246 cells cultivated in defined medium which was screened by differential hybridization. By this approach, several cDNAs were isolated. Among them, we particularly focused on a cDNA hybridizing to a 1.7 kb mRNA which is differentiation dependent in the adipogenic cell line 1246 and in adipocyte precursors in primary culture and which is called adipose differentiation related protein (ADRP). ADRP mRNA is induced shortly after differentiation is triggered and one day before lipoprotein lipase mRNA is induced, thus making ADRP an early marker of differentiation. Nucleic acid sequence and deduced amino-acid sequence share no homology to any reported sequence in the data banks. ADRP mRNA expression is adipose tissue specific in vivo. Immunocytochemical localization of ADRP in 1246 cells and in rat adipocytes indicates that it is a 50 kDa membrane associated protein. Complete mouse ADRP gene was sequenced and characterized. Details of this characterization will be provided. Hormonal regulation of ADRP expression in adipocytes as well as studies of its expression in vivo in animal models of acquired and genetic obesity will be discussed.

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Growth Factors and Cytokines in Adipocyte Differentiation and Function

CZ 006 GROWTH HORMONE RECEPTORS IN ADIPOCYTES, H. Maurice Goodman, G. Peter Frick, Lih-Ruey Tai, Tova Bick, and Jack L. Leonard, Department of Physiology, University of Massachusetts Medical School, Worcester, MA 01655

Adipocytes are a major target for the diverse metabolic actions of growth hormone (GH) which include both stimulation and inhibition of glucose metabolism, acceleration of lipolysis, and maintenance of intracellular calcium concentrations. Rat adipocytes have ~10^4 binding sites that turn over with a half life of about 45 min. The gene for the rat GH receptor gives rise to 2 isoforms by alternate splicing of mRNA. The long form (GHR_L) is a 620 amino acid glycoprotein with a single membrane-spanning domain and a long cytosolic tail. The short form (GHR_S) has the same sequence as the circulating GH binding protein and consists of the extracellular domain of GHR_L with a hydrophilic 17 amino acid carboxyl tail replacing the transmembrane and cytosolic portions. Both isoforms sediment with membrane fractions of adipocyte extracts. GHR_S is about 5X as abundant as GHR_L, but the two isoforms are equally abundant on the cell surface as judged by loss after mild digestion of adipocytes with trypsin. GHR_L turns over with a $t_{1/2}$ of ~30 min, but little GHRS is synthesized under *in vitro* conditions. Although total GHR_S declined with a $t_{1/2}$ of ~2 h, its abundance on the cell surface remained constant at the expense of the intracellular pool. No change in mRNA for either form was seen for at least 2 h after inhibition of RNA synthesis, suggesting separate post-transcriptional regulation of GHR_L and GHR_S. Quantitative considerations and data from crosslinking experiments strongly suggest that GHR_S participates in cellular binding of GH. Overexpression of GHR_S in 3T3-L1 preadipocytes resulted in 3-4X increase in GH binding without altering the abundance or turnover of endogenous GHR_L. GH is thought to bind sequentially to 2 GHR to form heterotrimeric complexes that signal biological responses by activating intracellular tyrosine kinase(s). GH mutated in its second binding site competitively inhibits the metabolic actions of GH in adipocytes. We suggest that different combinations of GHR_L and GHR_S in these complexes may trigger different signalling pathways and thus account for the diversity of effects of GH in adipocytes.

CZ 007 G-PROTEINS: ROLES IN REGULATING DIFFERENTIATION AND DEVELOPMENT, Hsien-Yu Wang[1], Ping Gao[3],Yaacov Hod[2], Christopher M. Moxham[3], Hui-Ling Su[1], David C. Watkins[3], and Craig C. Malbon[3], [1]Department of Biochemistry, National Defense Medical Center, Taipei 10764 Taiwan, R.O.C., [2]Department of Physiology & Biophysics, Health Sciences Center, University at Stony Brook, Stony Brook, NY 11794, and [3]Department of Molecular Pharmacology, Diabetes & Metabolic Diseases Research Program, Health Sciences Center, University at Stony Brook, Stony Brook, NY 11794-8561.

G-proteins mediate transmembrane signaling from a populous group of cell surface receptors to a smaller group of effectors that includes adenylylcyclase, phospholipase C, and ion channels. Fully-differentiated mouse 3T3-L1 fibroblasts display an adipocyte-like phenotype characterized by accumulation of lipid when induced by insulin, dexamthasone and methylisobutylxanthine, as well as other agents. G_s levels decline precipitously upon differentiation. Oligodeoxynucleotides antisense to the $G_{s\alpha}$ subunit accelerate the rate of differentiation induced by other agents as well as act as inducers of differentiation themselves[4]. Conversely, activation of $G_{s\alpha}$ by cholera intoxication blocks the ability of inducers to provoke differentiation, although elevation of cyclic AMP itself does not block differentiation. Increased expression of a constitutively active mutant form (Q205L) of $G_{i\alpha 2}$, whose activity counterregulates $G_{s\alpha}$, also promotes terminal differentiation of fibroblasts to adipocytes. These data, which parallel studies in mouse F9 teratocarcinoma stem cells[5], and studies of suppression of $G_{i\alpha 2}$ *in vivo* in transgenic mice[6], highlight a critical role of G-proteins in differentiation and development. The linkage between G-proteins and the MAPkinase regulatory network is under investigation.

[4]Wang *et al.* (1992) *Nature* **358**, 334-337.
[5]Watkins *et al.* (1993) *Science* **258**, 1373-1376.
[6]Moxham *et al.* (1993) *Science* **260**, 991-995.

CZ 008 GROWTH HORMONE SIGNALLING IN ADIPOCYTES, Hans Tornqvist[1], Hans Eriksson[2], Martin Ridderstråle[2], and Eva Degerman[2], Departments of [1]Pediatrics and [2]Physiological Chemistry, University of Lund, Lund, Sweden.

The effects of growth hormone on adipocytes can under different circumstances either be diabetogenic or insulin-like. New data on the underlying molecular mechanisms have evolved from studies of the acute insulin-like effectssuch as increased lipogenesis and antilipolysis. Knowledge of the details in the cascade of events leading to the anti-lipolytic effect of insulin has facilitated these studies.

Growth hormone inhibits noradrenaline-stimulated lipolysis within 2-3 minutes and the maximal effect is about 40-50% of that seen with insulin. There is a net decrease in hormone-sensitive lipase activity and phosphorylation. This is accomplished by decreased cAMP levels since selective inhibition of the cGMP-inhibited cAMP phosphodiesterase completely blocks the anti-lipolytic effect of growth hormone. GH induces a serine phosphorylation of this enzyme presumably leading to its activation. To date it is not known which GH-sensitive kinase is responsible for this phosphorylation. Up to this point growth hormone uses the same signalling mechanism as insulin indicating that the point of divergence towards separate receptor events lies at or upstreams this level.

A central question in GH signalling in general has been to identify molecular mechanisms that convey the signal at the receptor level. We have investigated whether tyrosine phosphorylation is involved in the primary adipocyte. After binding to its receptor GH induces a rapid and dose-dependent tyrosine phosphorylation of a separate 114-kD microsomal protein. The identity of this protein is not known. Several lines of evidence indicate that this tyrosine phosphorylation is part of the molecular mechanism mediating the rapid insulin-like metbolic effects.

Lipid Uptake and Storage in Adipocytes

CZ 009 STRUCTURE AND FUNCTION OF THE ADIPOCYTE LIPID-BINDING PROTEIN David A. Bernlohr, Dept. of Biochemistry and Institute of Human Genetics, University of Minnesota, St. Paul Minnesota 55126

The Adipocyte Lipid-Binding Protein (ALBP or aP2 protein) is a small, cytoplasmic, 15 kDa, fat cell specific fatty acid binding protein that belongs to a large multigene family of cellular hydrophobic ligand binding proteins. Members of this family include, but are not limited to, the fatty acid binding proteins from liver, intestine, ileum, heart muscle, Schwann cell and a number of retinoid binding proteins. ALBP is expressed in a wide variety of species from cattle and pigs to chickens, mouse and man; the amino acid similarity of the proteins between these species is greater than 90%. The protein is extremely abundant with cellular concentrations approaching 250 μM. The protein is also present in a number of adipocyte cell lines including the 3T3-L1, 3T3-F442A and Ob 17 lines. The role of the protein is presumed to be that of facilitating the intracellular solubilization and metabolism of fatty acids either from uptake during the lipogenic process or efflux during lipolysis. Other fatty acid binding proteins such as MAL-1 are expressed in adipose cells, however at a much lower level. As a consequence of its abundance and fatty acid binding properties, the protein establishes a large solubilized pool of fatty acid that may be used by the cell to regulate a variety of fatty acid regulated metabolic events such as gene expression. Because of its ability to bind fatty acids (K_d= 0.5 μM), the protein has been used as a model for the analysis of protein-lipid interactions on both a structural and mechanistic basis.

The X-ray structure of ALBP has been solved to 1.6Å in both its apo- and holo protein forms. Holoprotein structures have been solved with bound stearate, palmitate, oleate, arachidonate (monovalent anions) and hexadecylsulfonic acid, a divalent anionic lipid. Overall, the structure of the protein can be best described as a 10-stranded antiparallel β-barrel in which the ith strand forms a hydrogen bonding network with the (i + 1)th strand. In three dimensions, the first and last strands are situated so that they also form a ladder of hydrogen bonds. The result is a barrel with two open ends along the axis and with the β-strands running up-and-down the surface. At one end of the β–barrel the sidechain packing density is very high, closing the end and generating a hydrophobic core or backbone that is conserved in all hydrophobic ligand binding proteins. At the other end of the protein the first two β-strands are connected by a helix-turn-helix motif which closes off the other end of the barrel. The packing density at the helix-turn-helix end is quite loose, generating an interior water filled cavity of nearly 750 $Å^3$. Fatty acids are found bound within the interior cavity, totally engulfed by the polypeptide. Surprisingly, there is no discernible opening from the exterior of the protein into the cavity. The fatty acid carboxylate forms a hydrogen bonding network with two arginine and a tyrosine residue at the base of the cavity. Except for a single residue, Phe^{57}, which resides at a position hypothesized to serve as a portal to the cavity, there is little discernible difference between the apo-and holoprotein structures. The rates of fatty acid exchange from the protein are exceptionally high, with a transfer rate of 25 ± 1.0 sec^{-1} at 25°, (pH = 7.0, I = 0.15). Interestingly, analysis of the mechanism of transfer indicates a collisional process. Physical contact between donor and acceptor are necessary for optimal transfer of a bound ligand. Current studies are focusing on the interaction(s) between the holoALBP and cellular proteins involved in adipocyte lipid metabolism (such as the adipocyte fatty acid transporter) that may serve as either the donor or acceptor of fatty acids with the binding protein. Supported by NSF/DMB 9118658

CZ 010 REGULATION OF LIPOPROTEIN LIPASE, Robert H. Eckel, University of Colorado Health Sciences Center, Denver, CO 80262.

Lipoprotein lipase (LPL) is an early gene marker for adipose tissue (AT) differentiation and rate-limiting for the uptake of triglyceride-rich lipoprotein triglyceride fatty acids by adipocytes. Because the large majority of fatty acids incorporated into AT triglyceride stores are derived from circulating lipoproteins, LPL is important to the biology of AT. In addition to increases in transcription of the LPL gene during AT differentiation, ATLPL is subject to regulation by hormones, nutrients and cytokines. During adipogenesis, LPL is one of the early genes to be activated. Although c-fos and CCAAT-enhancer binding protein (C/EBP) play important roles in adipocyte differentiation, only anti-sense c-fos inhibits the differentiation-dependent expression of LPL mRNA. Enerback *et al* have identified two *cis*-acting elements, LP-α (-702 to -666) and LP-β (-466 to -430), that also contribute to the differentiation-dependent increase in transcription of the LPL gene. These regions, however, resemble consensus sequences for HNF-3 and forkhead, respectively, not c-fos. Regulation of LPL gene expression at the level of transcription has also been demonstrated in fully differentiated adipocytes for tumor necrosis factor-α (TNF-α) and variably for beta-adrenergic agents, both of which decrease transcription. In ongoing experiments, transient transfection of 3T3-L1 cells with LPL promoter deletions suggest that the DNA region responsible for the TNF-α effect is located downstream of -180 bp. Furthermore, using a -115 to +44 LPL promoter probe, nuclear extracts from TNF-α treated cells enhanced the migration of the probe vs. non-treated cells. This region of the LPL promoter contains numerous binding sites for *cis*-acting elements including Oct-1 and NF-Y. Most of the regulation of LPL in AT is, however, post-transcriptional. Insulin-mediated increases in LPL activity in AT appear to be associated with increases in LPL synthesis and LPL mRNA. However, similar to other AT proteins, insulin appears to decrease the rate of mRNA degradation rather than increase LPL gene transcription. Although glucocorticoids may also increase ATLPL gene transcription during differentiation, in adipocytes glucocorticoids decrease LPL synthesis and LPL mRNA. Catecholamines decrease ATLPL synthesis, but have variable effects on LPL gene transcription and LPL degradation. The nutritional regulation of ATLPL is macronutrient-dependent. Diets high in carbohydrate increase ATLPL activity, but have inconsistent effects on protein synthesis and LPL mRNA. These variations likely reflect the period of food restriction prior to refeeding. High fat diets when controlled for caloric excess have no effect on ATLPL. Finally, cytokines other than TNF-α, e.g. interleukins -6, -11 and interferon-γ inhibit ATLPL activity. Although these cytokines in general inhibit ATLPL enzyme activity, interferon-γ also inhibits ATLPL synthesis and mRNA. Because cytokines such as TNF-α are produced in adipocytes, they become excellent candidates for mediators of basal expression of the lipase. Overall, the regulation of ATLPL by hormones, nutrients and cytokines is relevant to lipid fuel partitioning and related disorders such as obesity.

CZ 011 EXPRESSION CLONING AND CHARACTERIZATION OF A NOVEL ADIPOCYTE LONG CHAIN FATTY ACID TRANSPORT PROTEIN, Jean E. Schaffer[1] and Harvey F. Lodish[2], [1]Whitehead Institute for Biomedical Research, Cambridge, MA 02142; Cardiovascular Division, Brigham & Women's Hospital, Boston, MA, [2]Whitehead Institute for Biomedical Research, Cambridge, MA; Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Long chain free fatty acids are an important energy substrate which are taken up by many cells, particularly cardiac myocytes and adipocytes, but the mechanism whereby these molecules cross the plasma membrane is poorly understood. We used an expression cloning strategy and a cDNA library from 3T3-L1 adipocytes to identify a cDNA which, when expressed in cultured cells, augments uptake of long chain fatty acids. This cDNA encodes a novel 646 amino acid fatty acid transport protein (FATP) with 6 predicted membrane-spanning regions and which is integrally associated with membranes. We propose that FATP is a plasma membrane transporter for long chain free fatty acids.

CZ 012 REGULATION OF GENE EXPRESSION DURING FAT SYNTHESIS AND ADIPOCYTE DIFFERENTIATION, Hei Sook Sul, Naima Moustaid, Cynthia Smas, Shaw-Fang Yet, R. Scott Beyer and Ann Jerkins. Harvard School of Public Health, Boston.

To start examining transcriptional control during triacylglycerol biosynthesis, we have cloned fatty acid synthase (FAS) that catalyzes all the steps in the synthesis of palmitate from malonyl CoA and acetyl CoA and mitochondrial glycerol-3-phosphate acyltransferase (GPAT) which is not well characterized but catalyzes the committed step in triacylglycerol synthesis. Mitochondrial GPAT was identified by immunoprecipitating with antibodies generated with the fusion protein expressed in E.coli and correlating with the GPAT activities using CHO cells transfected with the putative open reading frame. These genes are regulated similarly in that the transcription rates are increased by insulin or by carbohydrate in vivo. By transfection of various FAS promoter-reporter plasmids into 3T3-L1 adipocytes and gel shift and footprinting assays, we have defined a cis-acting element for up-regulation by insulin present at the proximal promoter and a more upstream sequence that is necessary for carbohydrate regulation of the FAS gene.

By differential hybridization screening, we have also cloned cDNA sequences coding for a novel member of EGF-like family of proteins. Pref-1 is synthesized as a transmembrane protein with six tandem EGF-like repeats. Pref-1 is coded by a single copy gene. However, in preadipocytes, multiple discrete forms of pref-1 protein of 45-60 kd are present, owing to glycosylation and alternative splicing. The expression of pref-1 decreases by transcriptional mechanism during adipose differentiation of 3T3-L1 cells and is not detectable in mature adipocytes. Moreover, constitutive expression of pref-1 in preadipocytes, which in effect blocks its down-regulation, drastically inhibits adipose conversion. Pref-1 functions as a negative regulator of adipocyte differentiation, possibly in a manner analogous to EGF-like proteins that govern cell fate decisions in invertebrates.

Lipid Mobilization

CZ 013 CELLULAR AND MOLECULAR ASPECTS OF LIPID DROPLET-ASSOCIATED PERILIPINS, Constantine Londos, NIDDK, National Institutes of Health, Bethesda, MD 20892

Perilipins are highly insoluble, adipocyte proteins that reside exclusively on the surface of the lipid storage droplets (LSD); these proteins are associated with the first detectable lipid depositions in differentiating adipocytes, i.e. nascent LSDs. Immunogold electron microscopy indicates that perilipins are either on or within the LSD surface monolayer. Phosphorylation of perilipins by protein kinase A in concert with lipolytic stimulation of cells suggests a role for these proteins in lipid hydrolysis. Rat cDNAs have been isolated for a major (perilipin A; 56 kDa) and a minor species (perilipin B; 46 kDa), which exhibit limited but significant homology to one other known protein, ADRP[1], also found in adipocytes. Murine 3T3-L1 adipocytes express perilipins A and B as well as mRNAs for two additional species (C, 42 kDa and D, 47 kDa). Although previous tissues scans indicated that perilipin expression was limited to adipose cells, more recent studies with immunofluorescence as well as Western and Northern analyses show perilipins to be present in adrenal cortical cells. Perilipin C is as abundant as perilipin A in cultured murine Y-1 adrenal cortical cells, where perilipins are bound to cholesteryl ester droplets. This finding is especially intriguing in view of the similar lipolytic mechanisms employed by adipocytes and adrenal cortical cells. Since cellular studies with adipocytes suggest that translocation of hormone-sensitive lipase (HSL) to the surface of adipocyte LSDs is a critical event in lipolysis, we have attempted to reconstruct this event in vitro with LSDs purified from 3T3-L1 adipocytes. Purification of LSD-associated proteins indicates that the perilipins are the only detectable proteins that are located exclusively on the droplets. Perilipins on isolated, morphologically intact LSDs are readily phosphorylated by protein kinase A. The phosphorylated LSDs exhibit increased HSL binding, thus providing an experimental system for molecular dissection of lipolytic processes and perilipin function. Another approach to address function is through sense and antisense expression of perilipin mRNAs in 3T3-L1 cells. Antisense constructs reduce perilipin expression but not lipid accumulation during differentiation. Conversely, inhibition of lipid synthesis reduces perlipin expression in differentiating adipocytes, and sense constructs appear to lead to precocious lipid accumulation, which suggests participation of perilipin in lipogenesis. Other possible functions for perilipin and experimental approaches will be discussed.

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CZ 014 MOLECULAR STUDIES OF INSULIN REGULATION OF LIPOLYSIS, Vincent C. Manganiello*, M. Taira*, M.J. Leroy*, S. Kedev*, A. Rascon*, E. Degerman*, H. Tornqvist*, and Per Belfrage*, *Laboratory of Cellular Metabolism, NHLBI, NIH and *Dept. Physiological Chemistry, U. Lund, Sweden.

Activation of a membrane-associated adipocyte Type III (cGMP-inhibited) Cyclic Nucleotide Phosphodiesterase (cGI PDE) is an important component in the antilipolytic action of insulin. Two subfamilies of the Type III cGI PDE gene family have been cloned from both rat and human cDNA libraries. R(rat)cGIP1 mRNA is highly expressed in rat adipocytes and is dramatically increased during differentiation of 3T3-L1 adipocytes. RcGIP1 PDE has been stably expressed in NIH 3006 fibroblasts which overexpress the human insulin receptor; incubation of these cells with insulin resulted in an increase in RcGIP1 PDE activity. RcGIP2 mRNA is highly expressed in rat heart, aorta and lung. The H(human)cGIP1 gene is apparently located on chromosome 11; HcGIP2, on chromosome 12. The four cGI PDEs exhibit a common domain structure, with a conserved catalytic domain in the C-terminal portion and N-terminal regulatory domains which contain putative hydrophobic membrane-association domains and several consensus cAMP-dependent protein kinase (A-PrK) phosphorylation sites. The deduced amino acid sequences of RcGIP1 and HcGIP1 are very similar to each other, and differ, especially in the N-terminal regulatory domains, from those of RcGIP2 and HcGIP2 which are similar. These and other results indicate that cGIP1 and cGIP2 cGI PDEs are products of distinct but related genes and that RcGIP1 PDE represents the isoform expressed in rat adipocytes.

Activation of the rat adipocyte cGI PDE by both cAMP and insulin is associated with serine phosphorylation of the enzyme by A-PrK and a recently identified insulin-sensitive protein kinase (ISK) (Aparacio et al., BBRC, 192, 1137, 1993). After phosphorylation by A-PrK in vitro, a comparison of the chromatographic and immunological properties of ^{32}P-peptides released by trypsin from authentic adipocyte ^{32}P-cGI PDE and a synthetic ^{32}P-peptide (based on the deduced sequence of RcGIP1 PDE) indicates that Ser 427 is the site most likely phosphorylated on the adipocyte cGI PDE by A-PrK in vitro; studies to identify the site(s) phosphorylated by ISK are in progress.

Adrenergic Function in Brown Fat

CZ 015 WHY DO ADIPOCYTES MAKE THE β_3-ADRENERGIC RECEPTOR?, James G. Granneman, Archana Chaudhry and Kristine N. Lahners, Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48210.

Rodent and human adipocytes express both β_1- and β_3-adrenergic receptor subtypes. At first glance it is unclear why these subtypes are co-expressed given that activation of either subtype leads to the elevation of intracellular cyclic AMP. We have therefore begun to examine the pharmacological and biochemical properties of β_1 and β_3 receptors, as well as their regulation, in order to understand the distinct roles these subtypes play in adrenergic signal transduction in adipocytes. In ligand binding and adenylyl cyclase assays, catecholamines display a much higher affinity for the β_1 versus β_3 receptor. The difference in potency of catecholamines at the β_1 versus the β_3 receptor is less when cyclic AMP accumulation is monitored in intact cells, apparently owing to the greater coupling efficiency of β_3 receptors in intact cells. Nevertheless, β_1 receptors appear to mediate the actions of low concentrations of catecholamines, while β_3 receptors can mediate responses at higher concentrations. In isolated rat adipocytes, acute adrenergic stimulation rapidly desensitizes β_1, but not β_3, receptors. Despite resistance to acute desensitization, chronic agonist exposure down-regulated β_3 receptor mRNA levels and reduced β_3 receptor activity. Together, these data indicate that β_1 receptors mediate acute responses to low-level catecholaminergic stimulation, while β_3 receptors mediate responses to sustained, high-level stimulation.

In rats, the β_1 receptor is expressed in numerous tissues, whereas high levels of β_3 receptor mRNA are found only in brown and white adipose tissues. β_3 receptor mRNA is also detected in the rodent gastrointestinal tract, but levels there are at least 50 times lower than in adipocytes. Analysis of the mRNA from rodent tissues demonstrates that the initiation sites of β_3 receptor gene transcription differ in adipose and gastrointestinal tissues, suggesting tissue-specific regulation of β_3 receptor gene expression. In humans, only low levels of β_3 receptor mRNA were found in adipose tissue and intestine, suggesting that the elements which confer abundant fat-specific expression in rodents may not be conserved in man. Studies are in progress to identify the genetic elements and trans-acting factors responsible for tissue-specific expression of the β_3 receptor gene.

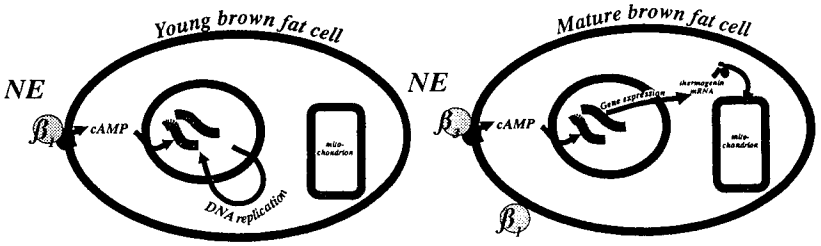
CZ 016 GENES OF ENERGY BALANCE: MODULATION IN TRANSGENIC MICE, Leslie P. Kozak[1], Ulrike Kozak[1], and Jan Kopecky[2], [1]The Jackson Laboratory, Bar Harbor, ME, [2]Czech Academy of Sciences, Prague.

A high frequency of non-insulin dependent diabetes has been found in primitive cultures that have rapidly acquired the life style and diet of developed, industrialized cultures. It has been hypothesized that the highly efficient metabolism essential for survival in a foraging, physically-active primitive culture leads to obesity and diabetes in a sedentary life style supported by a high fat diet. We seek to understand the genetic basis of energy efficiency with transgenic mouse models. Two genetic models have been developed: 1) Over-expression of the cytoplasmic NAD-linked glycerol-3-phosphate dehydrogenase generates a futile ATPase which burns off energy and results in a depression of non-shivering thermogenesis and a reduction in white fat lipid stores. These effects are observed in normal transgenic mice and in transgenic mice also homozygous for the diabetes (*db*) gene. The results indicate that modulations in the basic pathways of carbohydrate and lipid metabolism can have profound effects on energy efficiency. 2) The second model has attempted to alter energy efficiency by modulating expression of the mitochondrial uncoupling protein (UCP), a key step in the pathway of non-shivering thermogenesis. A transgene of the *Ucp* coding sequence under control of the fat-specific aP2 gene promoter results in increased *Ucp* mRNA and protein levels in brown fat and constitutive expression in white fat. These transgenic mice on a C57BL/6J background appear normal with respect to body weight and fat composition. The aP2-Ucp transgene has also been combined with A^{vy} to determine its effects on the progression of obesity in a genetically obese model.

CZ 017 ADRENERGIC CONTROL OF CELL PROLIFERATION AND DIFFERENTIATION, Jan Nedergaard, Anders Jacobsson, Stefan Rehnmark, Håkan Thonberg, Pertti Kuusela, Ulf Andersson, Petr Tvrdik, Josef Houstek, and Barbara Cannon, The Wenner-Gren Institute, The Arrhenius Laboratories, Stockholm University, S-106 91 Stockholm, Sweden

The cellular effects of adrenergic stimulation were long supposed to consist only of acute metabolic responses, but today a much wider array of cellular effects are recognized. In the brown adipocyte system, we see a fourfold series of effects: in young cells, a cAMP-mediated stimulation of proliferation; in the mature cell, an increased expression of genes associated with thermogenesis, such as lipoprotein lipase and the uncoupling protein thermogenin; in both cell stages, an alteration in the expression of transcription factors such as c-fos and C/EBPα and C/EBPβ, supposedly associated with control of cell differentiation; and in the mature cell, the classical acute metabolic stimulation, leading to heat production; this capacity for heat production is also associated with an altered supply of enzymes for mitochondrial ATP synthesis.

However, the adrenergic receptors involved in these responses diverge: in the young cells, the β_1 receptor is the only expressed and coupled β-receptor; in the mature cells, only the β_3 receptor is coupled to a response. Most interestingly is the participation of the α_1-adrenergic receptors, which seem to be necessary for the regulation of the expression of certain transcription factors. Thus, whereas the first steps in the signal transduction pathway are undoubtedly identical in the immature and in the mature cells, the inherent differentiation process apparently alters the transcriptional responses to the intracellular messengers. The two basic states of differentiation are summarized in the figures.



CZ 018 MOLECULAR STUDIES OF THE MITOCHONDRIAL UNCOUPLING PROTEIN OF BROWN ADIPOCYTES, Anne-Marie Cassard-Doulcier, Bruno Miroux, Serge Raimbault, Chantal Gelly, Susanne Klaus, Odette Champigny, Corinne Levi-Meyrueis, Valérie Frossard, Frédéric Bouillaud, and Daniel Ricquier, Centre National de la Recherche Scientifique, Centre de Recherche sur l'Endocrinologie Moléculaire et le Développement, Meudon, France.

The uncoupling protein (UCP) of brown adipose tissue is a regulated proton carrier which uncouples respiration and induces dissipation of energy as heat. Whereas it is uniquely present in brown adipocyte mitochondria, it is a member of the evolutionary related mitochondrial membrane transporters family. We are studying (i) the mechanisms that restrict UCP gene transcription to brown adipocytes, (ii) the functional organisation of the UCP in the inner membrane. To identify cis-acting regulatory elements controlling rat UCP gene transcription, a region encompassing 4.5 kb of DNA upstream the transcription start site was analyzed using DNA-CAT constructs, cell transfection and transgenic mice. Evidence was obtained that this 4.5 kb DNA contains most cis-acting elements regulating the tissue-specific expression of UCP in brown adipocytes, as well as elements mediating the stimulation by cold exposure and norepinephrine. Moreover, using deletion analyses, a strong enhancer located at -2.4 kb was identified and a proximal silencer was suspected (1). To study UCP structure, we have generated a library of bacterial clones randomly expressing short subsequences of the UCP fused to the MalE periplasmic protein of *E. coli*. The availability of fusion proteins to select antibodies directed against a short subsequence of the UCP, was used to study the topological organisation of the UCP. The orientation of 5 out of 6 predicted α-helices was determined (2). In collaboration with E. Rial (Madrid) we have also undertaken a study of the expression of wild or mutated UCP in yeasts.

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Early Events in Hormone and Lymphokine Action II (Joint)

CZ 019 CD45 TYROSINE PHOSPHATASE REGULATION OF TYROSINE KINASES THAT INTERACT WITH THE T CELL ANTIGEN RECEPTOR, Arthur Weiss[1], Andrew Chan, Makio Iwashima, David Straus, Monica Sieh, Jan Sap[2], Joseph Schlessinger[2], and Dev M. Desai[1], [1]Howard Hughes Medical Institute, The University of California, San Francisco, CA 94143, [2]Department of Pharmacology, New York University Medical Center, New York, NY 10016

Stimulation of the T cell antigen receptor (TCR) induces the protein tyrosine phosphorylation of several proteins, some of which have been implicated in downstream signaling pathways leading to cellular response. The TCR, which does not have intrinsic protein tyrosine kinase (PTK) activity, sequentially interacts with two classes of cytoplasmic PTKs. The PTKs implicated in TCR signal transduction include members of the Src family, Lck and Fyn, as well as members of the Syk/ZAP-70 family. The interaction of ZAP-70 with the TCR in a T cell line requires Lck. Studies in heterologous systems suggest that Lck and ZAP-70 interact synergistically to induce PTK activity. In addition to these PTKs, expression of CD45, a transmembrane protein tyrosine phosphatase (PTPase), is required for the induction of protein tyrosine phosphorylation by TCR stimulation. In CD45 deficient cells, Lck is hyperphosphorylated at a negative regulatory site of tyrosine phosphorylation suggesting a possible physiologic target and control point of CD45 function.

The cytoplasmic portion of CD45 contains 2 invariant PTPase domains, whereas the extracellular domain of CD45 is structurally heterogeneous due to alternative splicing of three consecutive exons (exons 4, 5 and 6). CD45 isoforms are expressed in a cell activation, developmental and tissue specific manner. Studies suggest that CD45 isoforms differentially associate with a number of T cell surface molecules on distinct T cell subsets. To examine the function of the extracellular domain of CD45, we constructed a chimeric molecule, in which the extracellular and transmembrane domains of CD45 were replaced with those of the epidermal growth factor receptor (EGFR). We demonstrate that the EGFR/CD45 chimera restores TCR signal transduction in a CD45-deficient cell line. Thus, expression of the cytoplasmic domain of CD45 is necessary and sufficient for normal TCR signaling. The addition of EGFR ligands before or during TCR signal transduction inhibits TCR-mediated signaling. This modulation of TCR signaling by the EGFR/CD45 chimera is the result of functional inactivation of the chimera, since ligands to the chimera have no effect on TCR signaling in cells which express both the chimera and wild-type CD45. Moreover, ligand-mediated inhibition of EGFR/CD45 chimera function requires dimerization of the chimeric protein. These studies demonstrate that CD45 plays a critical and active role in TCR signal transduction and suggests a very dynamic interaction of proteins involved in the signaling pathway with PTKs and PTPases whose functions can be regulated. These results also suggest that ligand mediated regulation of receptor-PTPases share mechanistic similarities with that of receptor PTKs.

CZ 020 INTERACTIONS BETWEEN IRS-1, RAS AND RAS-RELATED PROTEINS IN INSULIN ACTION, C. Ronald Kahn[1], Lee-Ming Chuang[1], Christine Reynet[1], and Morris J. Birnbaum[2], [1]Joslin Diabetes Center, and [2]Harvard Medical School, Boston, MA 02215.

Insulin and IGF-1 produce a wide variety of metabolic effects and stimulate cell growth and differentiation. The immediate downstream substrate of the insulin/IGF-1 receptors is the protein IRS-1. Analysis of the sequence of IRS-1 has revealed over 20 potential tyrosine phosphorylation sites, nine of which occur in the sequence motif YMXM or YXXM. Following insulin-stimulated phosphorylation, IRS-1 serves as a docking protein which binds to the 85 kDa subunit (p85) of PI 3-kinase, GRB2 (which is believed to link IRS-1 to ras activation) and other proteins possessing src homology-2 (SH2) domains. To investigate the interaction of IRS-1, SH2-containing signaling molecules and Ras in insulin action, we have microinjected IRS-1 and GST fusion proteins linked to different SH2 domains, as well as ras proteins, into *Xenopus* oocytes to study the pathway(s) by which insulin/IGF-1 mediate their effects. We find that insulin actions are mediated by IRS-1 through two independent but convergent pathways involving p85 of PI 3-kinase and GRB2. Thus, microinjection of GST-fusion proteins of either p85 or GRB2 inhibited IRS-1 dependent activation of MAP and S6 kinases and oocyte maturation, although only the GST-SH2 of p85 reduced insulin-stimulated PI 3-kinase activation. Microinjection of activated [R12, T59] Ras increased basal MAP and S6 kinase activities and also sensitized the oocytes to insulin-stimulated maturation without altering basal or insulin-stimulated PI 3-kinase. The insulin-stimulated Ras enhanced oocyte maturation response, but not the MAP and S6 kinase activation was partially blocked by the SH2-p85, but not SH2-GRB2, whereas all components of this pathway were blocked by injection of a dominant negative Ras (S17N). Our data strongly suggest that ras activation of MAP and S6 kinases alone is not sufficient for the maximal response of insulin on oocyte maturation. Rather, IRS-1 mediated cooperation between the PI 3-kinase pathway and the GRB2/ras pathway is necessary to achieve maximal insulin-stimulation of oocyte maturation.

A second approach which has indicated potential involvement of ras-related proteins in insulin action has come from studies of insulin resistance. Tissue resistance to insulin action is a central feature and the earliest detectable defect in Type II (non-insulin dependent) diabetes mellitus. In an effort to identify gene(s) associated with this insulin resistance, we prepared two subtraction libraries from skeletal muscle of normal and Type II diabetic humans and screened them using subtracted probes. Only one clone out of the 4000 screened was selectively over-expressed in Type II diabetic muscle as compared to muscle of non-diabetic or Type I diabetic individuals. This clone encodes a new 29 kDa member of the *ras*/GTPase superfamily with unique structural features. We have termed this novel *ras*-associated with diabetes *rad*. *Rad* is expressed primarily in skeletal and cardiac muscle, and is increased by an average of 8.6-fold at the mRNA level in the muscle of Type II diabetics. In view of the potential roles of *ras*-related proteins in insulin signaling, *rad* may play a pathogenetic role in the insulin resistance of Type II diabetes or serve as a marker for the disease.

GTP-Binding Proteins in Signal Transmission (Joint)

CZ 021 RAS IN T LYMPHOCYTE ACTIVATION, Bengt Hallberg, Patricia Warne, Sydonia Rayter and Julian Downward Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK.

In T lymphocytes, Ras proteins are activated in response to stimulation of the antigen receptor, stimulation of protein kinase C by phorbol esters and by cytokines such as interleukin 2. Phorbol ester stimulation of Ras is almost unique to T cells, indicating that regulation of Ras in this system may differ from other cell types. We have reported previously that phorbol ester treatment leads to a reduction in the level of GTPase activating protein (GAP) activity in T cell lysates. This appears to occur through the activation of a competitive inhibitor of GAP. The stimulation of Ras by antigen receptor agonists occurs through both PKC-dependent and PKC-independent, tyrosine kinase mediated pathways. Permeabilisation of T cells using streptolysin O confirms these findings and also shows that the rate of exchange of nucleotide on Ras is constitutively very high.

We have studied guanine nucleotide exchange factors for Ras in this system. Sos 1 and 2 are present and active. There is some binding of Sos to a 36K membrane tyrosine phosphoprotein in response to T cell receptor stimulation. This interaction is mediated by the Grb2 adaptor protein. In addition Shc is phosphorylated and associates with Grb2 on T cell receptor stimulation. In comparison to fibroblasts, only a small proportion of Sos associates with phosphotyrosine containing proteins. The role of the putative Ras exchange factor Vav has also been studied in this system. Vav shares homology with the Rho family exchange factor dbl, but has been reported to act on Ras in activated T cells. Vav fails to interact with Sos. The role of Vav in the Ras signalling pathway will be discussed.

Downstream effectors of Ras include the serine/threonine kinase Raf. This can be shown to be activated in T cells in response to a number of stimuli that activate Ras. Other potential effectors of Ras are being studied in this system. Ras has been shown to be essential, but not sufficient, for activation of IL2 gene transcription in response to phorbol esters and T cell receptor stimulation. Ras therefore clearly occupies a central role in T cell activation mechanisms.

CZ 022 G-PROTEIN REGULATION OF PHOSPHOLIPASES, John H. Exton, Howard Hughes Medical Institute and Vanderbilt University, Nashville, TN 37232

G-proteins mediate the actions of many agonists to induce the hydrolysis of phosphoinositides (PI) in their target cells. The G-proteins are either sensitive or insensitive to pertussis toxin. The toxin-insensitive G-proteins are now recognized to be members of the G_q family, and the specific targets of their α-subunits are certain β-isozymes ($\beta 1$, $\beta 3$) of PI-specific phospholipase C (PI-PLC). Recently, it has been found that some β-isozymes ($\beta 2$, $\beta 3$) of PI-PLC are activated by G-protein $\beta\gamma$ subunits. There is evidence that this is the mechanism by which the toxin-sensitive G-proteins act. Modification of PI-PLC by proteolysis or mutagenesis indicates that the sites of interaction of α_q and $\beta\gamma$ subunits on the enzyme are distinct. In vitro studies indicate that much higher concentrations of $\beta\gamma$ subunits are required for activation relative to α_q, but this difference can be ascribed in part to the use of GTPγS to activate α_q. A limited comparison of the effects of different combinations of β- and γ- subtypes has not shown striking differences except for a low potency of $\beta_1\gamma_1$.

The findings that $\beta\gamma$-subunits can activate certain PI-PLC isozymes, but can inhibit the stimulatory effect of α-subunits of the G_q family on other isozymes means that there may be considerable cross-talk between G-proteins in the regulation of PI hydrolysis. Although $\beta\gamma$ subunit subtypes do not seem to show in vitro the high degree of effector specificity observed for α-subunit subtypes, studies with intact cells indicate that both β- and γ-subunits are important determinants of receptor specificity in G-protein-mediated cell signalling. Different cells may thus respond differently to the same agonist depending on their complement of receptor subtypes, G-proteins (and their specific α-, β- and γ-subtypes) and specific isoforms of effector proteins.

CZ 023 MUTATIONS IN G PROTEINS AND IN G PROTEIN-COUPLED RECEPTORS IN HUMAN DISEASE, Allen M. Spiegel, Molecular Pathophysiology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Heterotrimeric GTP-binding proteins (G proteins) couple receptors for extracellular first messengers such as hormones, neurotransmitters, growth factors, light and odorants to effectors including enzymes of second messenger metabolism and ion channels. Mutations in either G proteins or in G protein-coupled receptors can impair signal transduction and lead to clinically significant disease. "Loss of function" mutations manifest as "resistance" to extracellular first messengers. Resistance to vasopressin action in X-linked nephrogenic diabetes insipidus for example is due to mutations that block synthesis or function of the V2 vasopressin receptor. Other mutations lead to constitutive activation of receptor or G protein. Such mutations activate the signalling process even in the absence of first messengers. Autonomous endocrine hyperfunction and benign neoplasia in the McCune-Albright syndrome for example is caused by constitutively activating mutations of the Gs protein that stimulates cAMP formation. Receptor and G protein mutations provide unique insights into both structure and function of these key signalling components. The potential implications of receptor and G protein mutations for regulation of islet β cells will also be discussed.

Pathophysiology of Obesity

CZ 024 CELL BIOLOGY OF INSULIN ACTION ON GLUCOSE TRANSPORT, S. W. Cushman, S. Satoh, S. J. Vannucci, I. A. Simpson, and G. D. Holman*, Diabetes Branch, NIDDK, NIH, Bethesda, MD 20892, U. S. A. and *Department of Biochemistry, The University of Bath, Bath BA2 7AY, U. K.

Insulin stimulates glucose transport in rat adipose cells through the translocation of GLUT4 glucose transporters from a large intracellular pool to the plasma membrane. We have now used an impermeant glucose transporter photolabel, ATB-BMPA, to specifically tag cell surface glucose transporters in the insulin-stimulated state and then to follow the subcellular trafficking of GLUT4 under various hormonal conditions. The following have thus been observed: 1) During insulin removal and restimulation, the kinetics of tagged GLUT4 endocytosis and exocytosis, respectively, are indistinguishable from those of untagged GLUT4. 2) During restimulation following removal of the initial insulin, only 45% of the originally tagged GLUT4 are restored to the plasma membrane at steady state despite full restoration of total GLUT4 and full stimulation of 3-O-methylglucose transport. 3) Tagged GLUT4 are internalized even in the continuous presence of insulin despite retention of plasma membrane total GLUT4 and stimulated glucose transport activity; the new steady state plasma membrane level of tagged GLUT4 is 45% of the initial level. 4) The rate of endocytosis of tagged GLUT4 following insulin removal is similar to that in the continuous presence of insulin. 5) A distinct sequence of events is observed in the plasma membrane during the response to insulin: a) the appearance of GLUT4 by Western blotting, b) the accessibility of GLUT4 to cell surface photolabeling, and c) the detection of stimulated glucose transport activity. 6) Adenosine augments and isoproterenol inhibits insulin-stimulated glucose transport activity and simultaneously, the accessibility of GLUT4 to cell surface photolabeling with little change in plasma membrane total GLUT4. These results focus attention on exocytosis, and specifically docking and fusion, as primary sites of hormone action.

CZ 025 MOLECULAR GENETICS OF OBESITY/DIABETES, Wendy K. Chung, David Markel, Streamson C. Chua Jr., Rudolph L. Leibel, The Rockefeller University, New York, New York 10021. The majority of individuals with non-insulin dependent diabetes (NIDDM) are obese; the diabetes in such individuals is usually ameliorated or eliminated by modest weight reduction. Although several genes (e.g. glucokinase), or genetic regions(ADA @ 20q) have been linked to MODY (a special NIDDM phenotype), the molecular bases for the strong association between obesity and NIDDM are unknown. To identify the genes which are most important in mediating the influence of obesity on diabetes-susceptibility, we areusing rodent models of NIDDM in which an obesity-producing mutation is segregated between strains of divergent susceptibility to the diabetogenic effects of obesity.For example, the autosomal recessive mutation, ob (mousechromosome 6) results in early onset, severe obesity due toincreased food intake and enhanced energy efficiency. The development of diabetes in these animals is dependent uponpolygenes of the background strain on which the ob mutation is carried. The C57BL/6J strain is relatively diabetes-resistant; DBA/2J and CAST/Ei are diabetes-susceptible. Identical patterns of susceptibility are seen in animals homozygous for the obesity-producing mutation db (mouse chromosome 4), indicating that the effect of obesity on *diabetessusceptibility* is not obesity mutation-specific. Mouse genetic crosses segregating for ob in either F1 ob/+ B6/DBA or F1 ob/+ B6/CAST intercrosses were used to generate obese ob/ob F2 offspring which demonstrated extensive variability in diabetes phenotype as characterized by body mass index, plasma glucose, and plasma insulin concentrations. Using a set of 80 polymorphic molecular markers (microsatellites) spaced at 20cM intervals throughout the mouse genome, a small number of the most severely affected animals were genotyped to identify areas of the genome associated with a diabetic phenotype (high plasma [glucose], low plasma [insulin]). Using this strategy, a locus on chromosome 10 was shown to have a significant deviation from expected Mendelian ratios. Genotypic characterization of all F2 ob/ob offspring in each cross with this marker on chromosome 10 demonstrated identical relationships between plasma insulin concentrations and genotype at this locus in the two crosses. Similar approaches are now being used to characterize other genetic loci which determine susceptibility to diabetes within the setting of obesity.

CZ 026 TRIACYLGLYCEROL METABOLISM IN OBESITY. Ulf Smith, Department of Medicine, University of Göteborg, Gothenburg, Sweden

Lipoprotein lipase and hormone-sensitive lipase are the key enzymes involved in triacylglycerol turnover in human fat cells. This presentation will mainly focus on lipolysis in man and its regulation at the molecular, cellular and integrated organ level. Activation of lipolysis is dependent on the cellular cAMP levels and, thus, the corresponding rates of formation and degradation. Catecholamines are the most important activators of lipolysis in man - their effects being dependent on the α/ß-receptor balance, coupling and activation of the adenylate cyclase. Insulin is the most important antilipolytic hormone in man, exerting its effect by activating the cGMP-inhibitable phosphodiesterase (cGIPDE). Since elevated cellular cAMP levels elicit resistance to many effects of insulin, including glucose transport, activation of cGIPDE is also a critical step for regulating cellular insulin sensitivity.
The insulin mimicker, vanadate, is a powerful activator of cGIPDE in fat cells from animal models. However, human fat cells are completely unresponsive to vanadate while a peroxide, pervanadate, exerts a pronounced insulin-like effect. In contrast to insulin, however, pervanadate also exerts a marked inhibitory effect on adenylate cyclase and, thus, cAMP formation.
By using the unique microdialysis method combined with an in situ calibration technique developed by us, it becomes feasible to study adipose tissue metabolism in vivo in man. When combined with blood flow measurements, local production rates (Ra) can be calculated. Using this technique, we have recently shown that lipolytic rate in situ is a function of both tissue region and fat cell size. Furthermore, local adipose tissue production rate of glycerol (a measure of local lipolysis) is similar per unit weight in lean and obese individuals.
Thus, the increased Ra for glycerol (and FFA) in obesity is a function of the expanded adipose tissue mass.
Adipose tissue blood flow increases in lean individuals during an oral glucose tolerance test. However, no such effect is seen in obese, insulin-resistant subjects. A similar perturbation in response to insulin is seen in skeletal muscle in insulin-resistant states. This differential effect to insulin thus seems to involve at least two different organs. It also has repercussions on the effects of insulin to stimulate glucose uptake and to inhibit lipolysis in vivo.

CZ 027 STRUCTURE, FUNCTION AND REGULATION OF β3-ADRENERGIC RECEPTORS. A.D. Strosberg, Institut Cochin de Génétique Moléculaire, Paris, France

The cloning, sequencing and expression of a third subtype of β-adrenergic receptor (βAR), now generally designated as the β_3AR, has been described for man (1), mouse (2) and rat (3). The detailed pharmacologic characterization of the human β_3AR expressed in CHO cells after transfection of the gene (1) has clearly established obvious differences from the β_1 and β_2 human subtype (4) and has confirmed striking similarities with the rodents "atypical" βAR (5). Most potent β_1/β_2AR antagonists thus act as selective agonists on human and murine β_3AR. β_3AR are mostly expressed in brown or white adipocytes but may also been found in gall bladder and several other regions of the gastro-intestinal system (6). Several regulatory mechanisms distinguish the β_3 from the β_1 and β_2AR subtypes. β_3AR is resistant to agonist-induced short-term desensitization mediated by phosphorylation by PKA or βARK (7). Murine β_3 (and β_1) transcription is severally reduced after treatment of adipocyte-like 3T3-F442A cells with dexamethasone which upregulates transcription of β_2AR (8). Similar reduction is induced by treatment with butyrate (9) but in that case both β_1 and β_2AR are upregulated β_3AR appears to be an early marker of brown adipocyte differentiation and may act as a growth factor receptor for this type in dogs and mice.(10).

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Adipocyte Differentiation, Gene Expression, and Function I

CZ 100 CLONING OF A RAT ADIPOCYTE MEMBRANE PROTEIN IMPLICATED IN FATTY ACID TRANSPORT THAT IS INDUCED DURING PREADIPOCYTE DIFFERENTIATION, Abumrad N.A.#, El-Maghrabi M.R.#, Amri E.Z.*, Ibrahimi A#. and Grimaldi P.A.* #Department of Physiology and Biophysics, State University of New York at Stony Brook, NY 11794 and *Centre National de la Recherche Scientifique, UMR-134, Nice Cedex 2, France.

A cDNA for an adipocyte membrane protein, implicated in fatty acid transport was isolated by screening with a synthetic oligonucleotide derived from the amino terminal sequence of the protein. The 88-kDa protein was previously identified by covalent labeling with N-sulfosuccinimidyl esters of long-chain fatty acids which inrreversibly inhibited fatty acid transport by 75%. The cDNA (FAT) contained 70 bp of 5'-untranslated sequence, an open reading frame encoding a 472-amino acid protein with a predicted molecular mass of 52466 and 940 bp of 3'-untranslated sequence with two polyadenylation signal sequences but with no polyadenylation tail. Extensive glycosylation most likely explains why the molecular mass of the isolated protein differs from that deduced from the cDNA sequence. An antipeptide raised against the deduced carboxyl-terminal sequence immunoreacted with an 88 kDa protein band in adipocyte plasma membranes. Protein sequence was 85% homologous to that of human CD36 and to bovine mammary PAS IV. Probing of RNA from rat tissues, using FAT cDNA, identified two major transcripts (4.8 and 2.9 kb) which were abundant in heart, intestine, fat, muscle and testis but not in kidney and liver. The mRNAs were not detectable in cultured preadipocytes but were strongly induced by differentiation or by treatment with dexamethasone. Induction of the protein was paralleled by an increase in cellular fatty acid transport. Preliminary data indicate that transfection of 3T3-C2 cells, which lack the protein, with a vector containig FAT cDNA leads to an increase in fatty acid transport.

CZ 101 OBESITY ASSOCIATED MUTATIONS OF THE MOUSE *AGOUTI* LOCUS IDENTIFY A NOVEL SIGNALLING MOLECULE THAT AFFECTS ADIPOCYTE DIFFERENTIATION. Greg Barsh, Michael Ollmann, Lana Kang, Harry Vrieling, and David Duhl. Department of Pediatrics and HHMI, Stanford, California 94305-5428

Mutations of the mouse *agouti* (*A*) gene were identified as a cause of genetic obesity more than 90 years ago. Recently, we and others have cloned the *agouti* gene, which encodes a signalling molecule expressed in hair follicles. We have now discovered the basis for genetic obesity observed with four different *agouti* alleles, and have developed an in vitro assay that may help to understand *agouti* gene action in vivo.

Mice heterozygous for the alleles *lethal yellow* (A^y), *viable yellow* (A^{vy}), *sienna yellow* (A^{sy}), or *intermediate yellow* (A^{iy}) have a yellow coat color, and, in addition, develop adult onset obesity, insulin-resistant hyperglycemia, and premature infertility. Analysis of the structure and expression patterns of cDNAs arising from these alleles indicates that A^y is a 115 kb deletion that results in the production of a chimeric RNA containing a ubiquitously expressed non-coding first exon fused to the coding region of *agouti*. A^{vy} results from the insertion of an intracisternal A particle element into the non-coding first exon of *agouti*, and A^{sy} and A^{iy} result from the insertion of ubiquitously expressed novel DNA sequences into the first intron of *agouti*. Thus, in every case, obesity observed with these *agouti* mutations is a consequence of ectopic expression of the normal agouti protein in nearly every tissue of the body.

The agouti protein contains a basic amino terminus and a cysteine-rich carboxyl terminus that exhibits sequence similarity to a group of molecules that act at voltage-gated calcium channels. In melanocytes, the protein antagonizes the effects of the G_s-coupled melanocortin receptor. Expression of the agouti protein in preadipocytes affects their morphology and blocks hormonally induced adipogenic differentiation. Because neither melanocortin nor its receptor are present in preadipocytes, our results suggest a model for agouti signalling in which the agouti ligand can interfere with other G_s-coupled pathways by activating its own receptor. Our results suggest that this receptor may be involved in adipocyte differentiation and the physiologic regulation of body weight.

CZ 102 GF-I IS A MITOGEN INVOLVED IN DIFFERENTIATION RELATED GENE EXPRESSION IN FETAL RAT BROWN ADIPOCYTES, Manuel Benito, Angela M. Valverde, Teresa Teruel and Margarita Lorenzo, Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad Complutense, 28040-Madrid, Spain.

Fetal rat brown adipocytes at time zero of culture constitute a population of cells of broad spectrum, as estimated by cell size, intrinsic fluorescence and lipid content, and show an intrinsic mitogenic competence. They express constitutively early growth-regulated genes such as c-myc, c-fos and b-actin, tissue specific-genes such as the uncoupling protein (UCP) and the lipogenic marker malic enzyme (ME). Fetal brown adipocytes bear a high expression of insulin-like growth factor receptor (IGF-IR), and show a high affinity IGF-I specific-binding to its receptor, and a high number of binding-sites per cell. After cell quiescence, insulin-like growth factor I (IGF-I) was as potent as 10% fetal calf serum (FCS) in inducing DNA synthesis, cell number increase, and the entry of cells into the cell-cycle. In addition, IGF-I or 10% FCS for 48 h increased the percentage of ^{3}H-thymidine labelled nuclei as compared to quiescent cells. Single cell autoradiographic microphotographs show typical multilocular fat droplets brown adipocytes, resulting positive to ^{3}H-thymidine labelled nuclei in response to IGF-I. IGF-I increased mRNA expression of the early-response genes c-fos (30 min), c-myc (2 and 24 h) and H-ras (4 and 24 h). 10% FCS also increased c-fos and c-myc, but failed to increase H-ras as an early event. IGF-I or 10% FCS, however, similarly increased the mRNA late expression of c-myc, H-ras, c-raf, b-actin and glucose 6-phosphate dehydrogenase (G6PD) at 72 h, as compared to quiescent cells. IGF-I or FCS maintained at 24 h or increased at 48 and 72 h UCP mRNA expression. The results demonstrate that IGF-I is a mitogen for fetal rat brown adipocytes, capable of inducing the expression of early and late growth-regulated genes, and of increasing the lipogenic marker ME and the tissue-specific gene UCP, suggesting the involvement of IGF-I in the differentiation as well as in the proliferation processes.

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CZ 103 REGULATION OF ADIPOCYTE DIFFERENTIATION BY PEROXISOME PROLIFERATOR AND RETINOID NUCLEAR SIGNALLING PATHWAYS, Ajay Chawla and Mitchell A. Lazar, Departments of Medicine and Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The conversion of 3T3-L1 cells into adipocytes has provided an important model of fat cell differentiation. However, the mechanism by which fetal calf serum, dexamethasone, insulin and isobutylmethylxanthine initiate the differentiation cascade is unknown. We are interested in the role of members of the thyroid/retinoid receptor superfamily in adipose conversion. We have found that multiple activators of the peroxisomal proliferator activated receptor (PPAR) cause adipocyte differentiation in the absence of other agents. Adipose conversion due to PPAR activators, including clofibric and pirinixic acid, has a similar time course (5-7 days) and yields a similar percentage of lipid accumulating cells as those observed with standard differentiation conditions. Two PPARs, PPARα and Nuc-I, are induced during this process. PPARs heterodimerize with retinoid X receptor (RXR) *in vitro* and transactivate gene expression maximally when ligands for both receptors are present. Interestingly, two RXRs (α and γ) are also induced during adipogenesis. RXRα is an early response gene induced within 4 h of exposure to differentiation conditions. Others have previously shown that retinoic acid (RA), which activates retinoic acid receptors (RARs), can inhibit adipocyte conversion by standard differentiation conditions. 3T3-L1 cells were found to express RARα and RARγ, but very little RARβ mRNA. RARα gene expression changed insignificantly during adipogenesis, whereas RARγ gene expression was down regulated. This reduction in RARγ expression was rapid (within 24h), and primarily due to a decrease in RARγ1 isoform. The disappearance of RARγ1 correlated well with the development of refractoriness to the anti-differentiation effects of RA. Thus, adipocyte conversion is controlled by peroxisome proliferator and retinoid signalling pathways.

CZ 104 CHARACTERIZATION OF TWO SMALL GENOMIC SEQUENCES CAPABLE OF COMMITTING MOUSE FIBROBLASTS TO ADIPOGENESIS, Suzie Chen, Jeffrey Martino, Gregg Davis and Anthony Accardi, Department of Chemical Biology & Pharmacognosy, College of Pharmacy, Rutgers University, Piscataway, N.J. 08854

Differentiation to a particular cell type requires ordered and coordinate expressions of large numbers of genes. The specific set of genes and the levels of expression determine the unique differentiated phenotype. Cells usually become "committed" to differentiate long before any actual morphological change is apparent. Therefore, commitment corresponds to the expression of a control gene, while differentiation is the consequences of that decision. We have chosen adipocyte differentiation as our model system to study commitment and differentiation at the molecular level. The mouse Swiss 3T3-F442A/3T3-C2 cell system is well suited for the isolation of genes involved in commitment and the study of their mechanisms of action. 3T3-F442A preadipocytes convert to adipocytes with high efficiency in response to confluence and insulin. The sister clonal line 3T3-C2 does not respond to either confluence or insulin but can convert to adipocytes when transfected with DNA from 3T3-F442A preadipocytes or from human fat tissue. A human fat tissue biopsy (FO46) transfected into 3T3-C2 gave rise to fat foci after two rounds of transfection and selection. To isolate adipose commitment (AC) genes, we screened a cosmid library of a subclone of secondary transfectant 3T3-C2/FO46-1 with the human repetitive Alu sequence. Five out of eight Alu+ recombinant clones committed 3T3-C2 cells to adipogenesis. The adipose commitment (AC) activity of one recombinant cosmid, p18A4, resides in two small subcloned sequence designated as Clone A (1.2 kb) and B (2.0 kb). Clones A and B are each separately able to commit precrisis mouse and rat fibroblasts and the multipotential C3H10T1/2 cell line to adipogenesis. By Southern blots and sequencing data there is no homology between them nor to previously sequenced genes or domains the Genbank and EMBL databases. These AC DNA clones we have isolated are active in the bioassay as functionally intact genes. No external regulatory regions are provided. We conclude that commitment to adipogenesis can be brought about *in vitro* by transfection of specific sequences. Molecular characterization of these two genomic sequences will be discussed. Such sequences provide a useful tool for the study of the molecular mechanisms that give rise to the committed state *in vitro* and perhaps also *in vivo*.

CZ 105 RAPAMYCIN SUPPRESSION OF MURINE ADIPOCYTE DIFFERENTIATION: OKADAIC ACID COUNTERACTS A SUBSET OF RAPAMYCIN ACTIONS INDICATING THAT DISTINCT PATHWAYS REGULATE ADIPOCYTE GENE EXPRESSION AND OTHER DIFFERENTIATION-DEPENDENT EVENTS. Van Cherington, Departments of Physiology, Anatomy and Cell Biology, and Pathology, Tufts Univ. Schl of Med. and New England Medical Center Hospital, Boston, MA. 02111.

The murine preadipocyte cell line, 3T3-L1, terminally differentiates into functional adipocytes, following treatment with glucocorticoid, when cultured at confluence in medium containing fetal calf serum and insulin. The macrolide immunosuppressive drug, rapamycin (RAPA), suppresses adipocyte differentiation in 3T3-L1 cells when present continuously from the time of glucocorticoid induction. In addition, the following results show that the protein phosphatase inhibitor, okadaic acid (OA), prevents RAPA suppression of adipocyte gene expression, but not other differentiation-dependent events, when added simultaneously with RAPA.

Cell proliferation following the induction of adipocyte differentiation, expression of differentiation-dependent gene products including glycerophosphate dehydrogenase (GPD) enzyme activity and mRNA, and adipocyte P2 (aP2) mRNA, and triglyceride (TG) production are suppressed by RAPA (ED_{50}=0.8nM). Preadipose cell proliferation is, however, little effected by RAPA. RAPA prevents growth factor/serum induced phosphorylation of the 70kDa S6 kinase (70S6K) in preadipocytes, as has been reported in other cell types. The related immunophilin ligand, FK506, which is known to bind to one or more RAPA targets but which fails to suppress RAPA-sensitive signal transduction and cell proliferation in a variety of cell types, fails to suppress GPD activity or TG production in adipocyte differentiation. Nanomolar OA (2-5nM), a concentration at which OA is fully inhibitory for protein phosphatase 2A but at which it has little or no effect on protein phosphatase 1, prevents RAPA suppression of GPD activity and GPD and aP2 mRNA, but fails to prevent RAPA suppression of 70S6K phosphorylation, differentiation-associated proliferation, or TG production. This indicates that the RAPA-sensitive signalling event(s) which regulate adipose gene expression and proliferation/TG production are either distinct from one another, or the event that is reversed by OA is on a branch of a signalling pathway, downstream from the RAPA sensitive step, which is specific for adipocyte gene expression, but which is not involved in 70S6K phosphorylation, proliferation, or TG production.

CZ 106 MORPHOLOGICAL CHANGES OF HUMAN PREADIPOCYTES DURING IN VITRO ADIPOCONVERSION WITH SPECIAL ATTENTION TO THE CYTOSKELETON, Gero Entenmann, Erika Junger, F. Arnold Gries and Hans Hauner, Diabetes Research Institute, Auf'm Hennekamp 65, D-40225 Düsseldorf, Germany.

It is now well established that adipose precursor cells, capable of undergoing conversion into adipocytes, are present in the adipose tissue of human adults. Aim of this study was to characterize the adipocyte precursor cells obtained from human adipose tissue before and during in vitro differentiation into adipocytes. The stromal-vascular cells of human subcutaneous adipose tissue isolated by collagenase digestion were cultured in a chemically defined serum-free medium. In the presence of 0.2 nM triiodothyronine, 10 nM insulin and 0.1 μM cortisol, up to 70% of the cells developed the adipocyte phenotype within 16 days. When examined immediately after inoculation, cells were characterized by a stellate or spindle-like shape with several thin tenous processes protruding from the cells. More than 90% of the cells contained small intracytoplasmatic lipid droplets as revealed by Oil red O staining. Corresponding electron microscopy revealed cells of the mesenchymal type with prominent nuclei and poorly developed endoplasmatic reticulum and Golgi system. Bundles of filaments extended over most of the cytoplasma. Immunhistological investigations showed that two components of these filaments were vimentin and actin. Four days after initiation of differentiation by insulin and cortisol, an extensive enlargement of the lipid content started to occur. With the ongoing accumulation of lipids, the lipid droplets fused together and the shape of the cells became more and more spherical. The intracytoplasmatic fibrils were reduced and located around the lipid globules forming microfilamentlike structures. The stromal-vascular cells not undergoing terminal differentiation, showed a widespread fibroblastlike cytoplasma with small lipid droplets remaining detectable.

This study suggests that the stromal fraction of human adipose tissue is almost exclusively composed of preadipocytes. The initiation of terminal adipoconversion is accompanied by a substantial and characteristic change of the cytoskeleton, which may be one of the factors that control differentiation.

CZ 107 **Isolation of a gene related to both Rb and C/EBP**

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The retinoblastoma gene, Rb, is a prototype growth suppressor gene, the inactivation of which is associated with tumor development. In our attempt to identify proteins that are associated with the Rb protein, we made the surprising discovery that an anti-Rb antibody Rb1-Ab-16-2 , which was raised against a Rb peptide, P16, can independently immunoprecipitate the SV40 large T antigen . In addition to this antigenically homologous domain , H1, detailed comparison of the sequence of the Rb protein and the SV40 large T antigen revealed the amino acid sequence homology at several other domains. Antibodies raised against a peptide , P9, corresponding to another such domain (H2) of the Rb protein also independently recognize the SV40 large T antigen. That these domains are functionally important is shown by the fact that mutants of the Rb protein at these two domains failed to suppress the growth of cells . Although the function at the molecular level is unknown, we surmise that these structural domains conserved between Rb and the SV40 large T antigen may also be present in cellular proteins that are structurally and /or functionally related to either the Rb protein and /or the SV40 large T antigen. Indeed, immunoprecipitation studies show that the anti-RB antibody Rb1-Ab-16-2 recognizes at least eight cellular proteins of different molecular weights. Candidate cDNA clones corresponding to these cellular proteins were isolated from lambda gt11 libraries using the anti-RB antibody Rb1-Ab-16-2. Initial sequence analysis of three of the clones revealed the existence of structural motifs that are conserved in SV40 large T, Polyoma large T, Rb and the Rb related protein, P107. Since the common thread between these clones is the H1 domain, we propose a model in which the Rb protein (and related proteins) and the CHLAs (cellular homologues of the large T antigen) regulate cell growth by competing for binding to a common target protein(s) through their conserved homology domains.

CZ 108 ISOLATION OF cDNAs FOR RAT AND HUMAN PERILIPINS, A.S. Greenberg, J.J. Egan, S.A.Wek, M.C. Moos, Jr., Y.H. Kang, C. Londos, and A.R. Kimmel, NIDDK, National Institutes of Health, Bethesda, MD 20892

The major cAMP-dependent protein kinase (PKA) substrate in adipocytes is perilipin, a protein found exclusively at the surface of the lipid storage droplets. Although far more abundant in adipocytes, perilipin(s) has been found recently on lipid droplets in adrenal cortical cells (See other abstracts in this symposium). Using anti-perilipin serum we have isolated two related classes of full-length coding cDNAs, designated perilipin A and B, from a rat adipocyte cDNA expression library. The two cDNAs apparently derive from two mRNA species, 3.0 and 3.9 kb for perilipin A and B, respectively, that arise by differential splicing of a common precursor. The mRNAs are predicted to encode perilipins A and B, of 517 amino acids (56,870 Daltons) and 422 amino acids (46,420 Daltons), respectively, that share a common 406 amino acid N-terminal sequence. Modeling of predicted secondary structures fails to reveal an underlying basis for the tenacious association of perilipins with lipid droplets. Other than phosphorylation, we find no evidence for posttranslational modification of perilipin A. Analysis of a cDNA clone from a human adipocyte library reveals that human and rat perilipin A proteins are 85% identical and 95% similar, showing greatest divergence at their NH-termini. The perilipins exhibit a significant sequence relationship (~65% similarity through 105 aa) with only one other known protein, the adipocyte differentiation-related protein (ADRP). Their common cellular expression suggests that perilipin and ADRP may interact in a related intracellular pathway. The molecular probes for perilipins A and B described here provide the basis for detailed analyses of possible role(s) for these proteins in lipid metabolism.

CZ 109 CHARACTERIZATION OF MURINE GENOMIC AND cDNA SEQUENCES FOR PERILIPINS, J. Gruia-Gray, D.A. Servetnick, A.S. Greenberg, C. Londos, and A.R. Kimmel, NIDDK, National Institutes of Health, Bethesda, MD 20892

Perilipins are abundant phosphoproteins associated with the triacylglycerol droplets of adipocytes and the cholesterol ester droplets of steroid producing Y-1 adrenal cortical cells (See other abstracts in this Symposium). We have isolated **murine** genomic and cDNA perilipin sequences and characterized their organization and expression patterns. The gene is single-copy and spans >9.5kb. Several perilipin mRNA species, which encode different forms of perilipin, are distinguishable by their splice patterns. Perilipin A (~62 kDa) is encoded by a 3kb mRNA, whereas perilipin B (~46 kDa) derives from a 3.9kb mRNA. The NH-terminal 406 aa of the A and B proteins are identical; thereafter they diverge. The presence or absence of a single 0.9kb accounts for the difference between perilipin A (3.0kb) and B (3.9kb) mRNAs. Seven introns (~0.04-2.3kb) interrupt the protein coding regions of perilipin A; the seventh intron (~0.9kb) is retained in the B mRNA. Two additional perilipin mRNAs are expressed in murine adipocytes and Y-1 adrenal cortical cells. Preliminary data indicate that 1.8kb and 1.5kb perilipin mRNAs encode an ~47 kDa perilipin D and an ~42 kDa perilipin C, respectively. Although, both cells express all four perilipin mRNA species, total perilipin mRNAs are 20-30 times more abundant in adipocytes than in the steroid producing adrenal cortical cells. In addition, the relative abundance of the various mRNA species differ between the two types of cells. The Y1 cells exhibit high levels of the A and C mRNAs but relatively low levels of B and D, whereas the perilipin A and D mRNAs are the most abundant in differentiated 3T3-L1 adipocytes and in primary murine white and brown adipocytes. By contrast, primary rat adipocytes express primarily mRNAs for perilipins A and B. Differentiated 3T3-L1 adipoblasts that express antisense perilipin A mRNA are suppressed in perilipin protein expression, but not in lipid accumulation, whereas sense constructs appear to lead to precocious lipid deposition. Such cells form a basis for probing for perilipin function.

CZ 110 IMPAIRED CYTODIFFERENTIATION OF THE ADIPOCYTE ASSOCIATED VASCULATURE ASSOCIATED WITH ENHANCED ADIPOCYTE DIFFERENTIATION, Gary J. Hausman and J. Thomas Wright, USDA-ARS, Russell Research Center, Athens, GA 30613

We have utilized fetal hypophysectomy (hypox) to elucidate endocrine regulation of porcine adipose tissue differentiation. Hypophysectomy per se markedly enhanced adipocyte differentiation as evidenced by fat cell hypertrophy ($P<.05$) and 10 to 40 fold increases ($P<.01$) in do novo lipogenesis and levels of lipoprotein lipase (LPL) and lipogenic enzyme activities. The monoclonal antibody designated AD-1 was raised against adipocyte surface antigens and can "mark" or identify preadipocytes prior to lipid deposition in vitro and in vivo. Adipocyte immunoreactivity for AD-1 was markedly enhanced by hypox per se and was further increased by hydrocortisone (HC) and thyroxine (T_4) treatment (21 days) following hypox. Conventional measures also showed that T_4 and HC enhanced ($P<.05$) differentiation. Capillaries associated with differentiating adipocytes are immunoreactive for the AD-1 antibody. Immunoreactivity for AD-1, laminin and Type IV collagen on capillaries in large fat cell clusters was considerably reduced by hypox and lectin binding by capillaries was also diminished with a complete loss of the binding of the soybean agglutinin lectin. Reactivity for cytosolic markers (actin, non muscle myosin) in capillaries (large fat cell clusters) was also reduced by hypox. HC and T_4 treatment did not influence lectin binding and immunoreactivity for surface and cytosolic markers. On a qualitative basis the cytodifferentiation of capillaries in skin were not affected by hypox. These studies demonstrate that the coordinate differentiation of adipocytes and associated capillaries is complex and in vivo studies are necessary to ultimately determine the endocrine regulation of adipocyte differentiation.

CZ 111 FAT CELL DETERMINATION AND DIFFERENTIATION: IDENTIFICATION OF GENES NECESSARY FOR FAT CELL GENE EXPRESSION, Deborah K. Hoshizaki, Thomas Blackburn, Kathy Miles, and Rami Sweis, Department of Biochemistry, University of Illinois, College of Medicine, Chicago, IL 60612

We have used a P-element enhancer trap (29D) to trace the fat cell lineage in Drosophila to 9 bilateral clusters of cells within the mesoderm. These presumptive fat cells are identified within the mesoderm at the same developmental stage at which nau positive cells are first detected.

The temporal expression within the fat cell lineage was determined for four genes, Adh, DCg1, and the steroid hormone receptor genes svp and HNF4-D. Adh and DCg1 are expressed in the fat cell tissue or fat body in later stages of embryogenesis (stage 15) and serve as fat cell terminal differentiation markers. svp gene expression is detected in precursor fat cells at stage 12 and, in contrast to the results of Zhong et al., (1993), we do not detect expression of HNF4-D within the fat cell lineage.

The site of P-element insertion for 29D has been determined and a small synthetic deficiency which includes the site of insertion has been identified. To determine whether the gene associated with the 29D enhancer is necessary for fat cell development, mutant embryos bearing the synthetic deficiency were examined for the presence of the fat body. We find in mutant embryos the specific loss of Adh and DCg1 expression within the fat body. We suggest that the loss of Adh and DCg1 expression is due to the absence of fat cells. We have also examined svp mutant embryos and find that svp function is necessary for expression of Adh and DCg1 within the fat body.

Reference: Zhong et al., (1993). EMBO. 12:537-544.

CZ 112 ROLE OF HORMONE-INDUCED CHANGES IN REDOX EQUILIBRIUM IN THE REGULATION OF FAT CELL DIFFERENTIATION, Horst Kather, H.Ingrid Krieger-Brauer, Klinisches Institut für Herzinfarktforschung an der Medizinischen Universitätsklinik, Bergheimer Str.58, 69115 Heidelberg, FRG.

Human fat cells possess a stimulus-sensitive NADPH oxidase producing 1 mol H_2O_2/mol NADPH oxidation. This system is under antagonistic control by various hormones and cytokines that typically act through several distinct receptor families. Insulin, oxytocin and TNF-a acted as stimulators of NADPH-dependent H_2O_2 formation, whereas isoproterenol, a ß-adrenergic agonist, had inhibitory effects. Surprisingly, the acidic and basic isoforms of FGF as well as the AA- and BB-homodimers of PDGF had antagonistic stimulatory and inhibitory effects on NADPH-dependent H_2O_2 generation. The agents tested acted at discrete ligand-specific receptors and their mechanisms of action were membrane-delimited and occurred in the absence of ATP. These findings placed the stimulus-sensitive H_2O_2-generating system of human fat cells in a position comparable to adenylate cyclase and demonstrated that this plasma membrane-bound redox system meets all criteria of a universal signal transducing system for hormones and cytokines that may link ligand binding to cell surface receptors to changes in the intracellular redox equilibrium.

3T3 L1 cells, which undergo adipose conversion in vitro, also possess a stimulus-sensitive H_2O_2-generating system, and its properties are virtually identical with those of the enzyme of primary fat cells. This cell line made it possible to gain first insights into the role that might be played by ligand-induced redox changes in normal fat cell physiology. $PDGF_{BB}$ and bFGF (which inhibit NADPH-dependent H_2O_2 generation) prevented the conversion of 3T3 L1 preadipocytes to adipocytes, and this effect could be partially reversed by H_2O_2. Conversely aFGF and $PDGF_{AA}$, which stimulate H_2O_2 generation, augmented adipose conversion in the presence of insulin and their effects were reversed by antioxidants, such as ascorbate or N-acetylcysteine. Thus, the H_2O_2 produced in response to hormones and cytokines may contribute to the development and maintainance of the differentiated state.

CZ 113 REGULATION OF GLUCOSE TRANSPORTER mRNA STABILITY IN 3T3-L1 ADIPOCYTES BY TUMOR NECROSIS FACTOR-ALPHA (TNF), Sheree D. Long and Phillip H. Pekala, Department of Biochemistry, East Carolina University School of Medicine, Greenville, NC 27858

Previous data from our laboratory demonstrated that exposure of 3T3-L1 adipocytes to TNF resulted in both an attenuation of GLUT4 transcription and a destabilization of the GLUT4 mRNA (*J. Biol. Chem.* **267**: 13580). The effect on mRNA stability was marked with control adipocytes exhibiting a $T_{1/2}$ of approximately 8.75 h for the GLUT4 message, while the $T_{1/2}$ in TNF treated cells decreased to 3 h. The control of mRNA stability is thought to reside, at least in part, in the 3'untranslated region (UTR) of the message and may be mediated through specific sequences within this region. The GLUT4 mRNA has an extensive 834 nucleotide 3'UTR which is GC-rich and contains one copy of the purported destabilizing motif, -AUUUA-. To examine for presence of TNF-induced GLUT4 3'UTR binding proteins, a riboprobe was prepared corresponding to the full length GLUT4 3'UTR. Gel shift assays performed using cytosolic extracts prepared from both control and TNF-treated adipocytes indicated that treatment of the cells with TNF did not result in induction of a GLUT4 3'UTR binding protein. Gel shift assays performed with these same extracts using a riboprobe prepared from pT3/T8-AUUUA (four AUUUA repeats in an 80 base sequence) indicated the presence of AUUUA binding protein in control adipocytes; however binding activity was absent in the extracts prepared from adipocytes that had been exposed to TNF. These data suggest that although the adipocytes have an AUUUA-binding protein it does not appear to be involved in the TNF-induced destabilization of the GLUT4 mRNA. Examination of polysome profiles indicated that GLUT4 mRNA was predominately associated with polysomes in control cells; while both polysome content and GLUT4 mRNA associated with this fraction decreased markedly in the TNF-treated adipocytes. In the same cells actin mRNA remained associated with the polysomes. These data suggest that dissociation of the GLUT4 message from polysomes may be the first step in TNF-induced stabilization.

CZ 114 RECIPROCAL REGULATION OF C/EBP α AND δ BY GLUCOCORTICOIDS IN ADIPOSE TISSUE AND 3T3-L1 ADIPOCYTES, *Ormond* A. MacDougald, Peter Cornelius, Sylvia S. Chen, Fang-Tysr Lin and M. Daniel Lane, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Glucocorticoids are known to affect adipocyte metabolism. Glucocorticoid receptor agonists, i.e. dexamethasone (DEX) or triamcinolone acetonide (TRIAM), induce expression of C/EBPδ and repress expression of C/EBPα in mature 3T3-L1 adipocytes. DEX induces C/EBPδ protein ≥100-fold in 4 h with a detectable rise within 30 min. C/EBPα protein decreases within 1 h after DEX treatment and the effect is maximal by 4 h (~90% suppression). Both responses are transient, with partial to full recovery by 24 h. Consistent with mediation by the glucocorticoid receptor, the ED_{50} for both processes is in the nM range. The glucocorticoid effects on C/EBPδ and α appear to be on the expression of their mRNA's with little effect on C/EBPα protein turnover.

Within 4 h of administration of DEX or TRIAM to adult rats, C/EBPδ mRNA and protein levels in epididymal fat are induced while C/EBPα mRNA and protein is repressed. Deoxycorticosterone acetate, or estradiol-17β have no effect on C/EBPα or δ message or protein levels in this adipose depot. These findings suggest that glucocorticoid-induced expression of C/EBPδ may be responsible for repression of the C/EBPα gene.

CZ 115 INFLUENCE OF ADIPOSE TISSUE CONDITIONED MEDIA FROM OBESE ZUCKER RATS ON THE PROLIFERATION OF RAT PREADIPOCYTES IN CULTURE. **Brenda G. Marques, Dorothy B. Hausman and Roy J. Martin. Department of Foods and Nutrition, University of Georgia, Athens, GA 30602.**

It had been suggested that growth and development of adipose tissue may be regulated by autocrine and/or paracrine factors. This study investigates the effect of media conditioned by adipose tissue from lean and obese rats on the proliferation of preadipocytes in culture. Conditioned media was prepared by incubating pieces of inguinal adipose tissue from lean and obese 12 week old male Zucker rats in Dubelcco's Modified Eagles' Media (DMEM) for 4 h. Stromal-vascular cells isolated from male Sprague-Dawley (60g) rats were plated at a density of 1 x 10^4 cells/mL using DMEM supplemented with 10% fetal bovine serum. At 24 h the plating media was replaced with media containing 5, 10 or 25% conditioned media, 0.5, 1.0, or 2.5% pig serum and 0.05 uCi/mL ^3H thymidine. After 48 h the label was removed and fresh treatment media applied. On day 5 of culture treatment media was replaced with a differentiation enhancing media. Fresh differentiation media was applied every 48 h until day 15 when cells were enzymatically harvested. Preadipocyte and non-preadipocyte fractions were separated by density gradients and centrifugation. Results from two experiments indicate that the effect of conditioned media was most evident when the lowest percentage of pig serum was used. There was a significant increase ($P<0.05$) in proliferation of the preadipocytes in cultures treated with 0.5% pig serum and 25% obese conditioned media compared to those treated with lean conditioned media or a DMEM control. Under these conditions there was no significant difference in stimulation of the non-preadipocytes between lean, obese, and control treatments. These data suggest that adipose derived growth factors from obese Zucker rats enhance proliferation of preadipocyte cells in culture.

CZ 116 ARACHIDONIC ACID DOWN-REGULATES THE INSULIN-DEPENDENT GLUCOSE TRANSPORTER GENE (GLUT4) IN 3T3-L1 ADIPOCYTES BY INHIBITING TRANSCRIPTION AND ENHANCING mRNA TURNOVER, Kevin M. McGowan, Paul W. Tebby, Jacqueline M. Stephens, Thomas Buttke and Phillip H. Pekala, Department of Biochemistry, East Carolina University School of Medicine, Greenville, NC 27858

Chronic exposure of fully differentiated 3T3-L1 adipocytes to 50 μM arachidonic acid (20:4) resulted in the development of an insulin-resistant state based upon a diminished ability of insulin to stimulate glucose entry into the cells. Western blot analysis revealed that 20:4 was specifically reducing the insulin-responsive glucose transporter (GLUT4) in both plasma and intracellular membrane. Furthermore, the inhibitory effect of 20:4 coincided with a reduction (~87%) in GLUT4 mRNA after a 48 h exposure, without similarly affecting the mRNA content of the ubiquitous glucose transporter, GLUT1. Subsequent investigations revealed that transcription of the GLUT4 gene was reduced by ~50% in response to 20:4 treatment and the half-life of GLUT4 mRNA decreased from 8.75 h to 4.8 h. By contrast, 20:4 increased the accumulation of GLUT1 mRNA 2-fold, by a mechanism that also involved regulation at both transcriptional and mRNA stability levels. The results presented in this study indicate that 20:4 can partially mimic the effects of both tumor necrosis factor-α (TNF) and insulin which when chronically supplied to 3T3-L1 adipocytes also down-regulate GLUT4 gene expression. Therefore, these data may also implicate 20:4 in the insulin-resistance associated with non-insulin-dependent diabetes mellitus, (NIDDM).

CZ 117 LIPOPROTEIN LIPASE DNA:PROTEIN INTERACTIONS ARE MODIFIED BY TUMOR NECROSIS FACTOR-α IN 3T3-L1 ADIPOCYTES AT THE PROXIMAL PROMOTER, Catherine L. Morin, Isabel R. Schaepler and Robert H. Eckel, Department of Endocrinology, University of Colorado Health Science Center, Denver, Colorado 80262

Adipose tissue lipoprotein lipase activity (LPL) and mRNA are decreased by TNFα predominantly through transcriptional mechanisms as shown by nuclear run-on experiments (Zechner 1988). The mechanism of this inhibition has not been fully elucidated. Using differentiated 3T3-L1 adipocytes, we have shown that an 18 hr treatment with TNFα (10 ng/ml) results in a decrease of 97% of the heparin-releasable LPL activity and 60% of LPL mRNA. Additionally, this inhibition of LPL activity was found to be dose-dependent. To locate the TNFα transcriptional effect transfections were preformed by electroporation on recently differentiated 3T3-L1 cells. Plasmids containing murine LPL promoter deletions, including a 1.8 kb, 1.2 kb and 225 bp (-181 to +44) fragment, attached to a luciferase reporter gene were co-transfected with B-galactasidase plasmids for internal control of transfection. These experiments suggested that a DNA region downstream of -180 bp confers the TNFα effect. Electrophoretic mobility shift assays were then used to decern any possible LPL promoter:protein interaction. ^{32}P-labeled LPL promoter probes, based on the 225 bp fragment, were used to compare DNA:protein interactions in nuclear extracts from TNFα-treated (10 ng/ml, 18 hrs) versus non-treated differentiated 3T3-L1 cells. These experiments have shown the loss of a LPL promoter-bindng protein in the TNFα-treated cells. We conclude that TNFα transcriptionally regulates adipose LPL at the proximal promoter.

CZ 118 THE ANALYSIS OF GENE EXPRESSION PATTERNS IN LIPOSARCOMA, MESENCHYMAL TUMORS RESEMBLING VARIOUS STAGES OF ADIPOCYTE DIFFERENTIATION. Ola Myklebost[¶], Anna Elisabeth Stenwig[†] and Øystein Fodstad[¶]. Departments of Tumor Biology[¶] and Pathology[†], The Norwegian Radium Hospital, Montebello, N-0310 OSLO, Norway.

Liposarcomas are malignant tumors of mesenchymal origin which show varying levels of adipocyte-like differentiation, from almost benign, well differentiated tumors resembling mature fat to aggressive tumors in which only a minor fraction of the cells contain lipid vacuoles. There is an inverse correlation between differentiation and prognosis. We have studied the expression of general tumor markers and genes induced at various stages during adipocyte differentiation in these tumors.

In our study adipocyte genes like lipoprotein lipase, aP2 and adipsin are expressed in human liposarcomas. Preliminary results indicate that expression of some of these genes is inucible in cultured tumor cells by agents inducing adipocyte differentiation in model systems. We hope that cell lines can be established to provide a model system for human adipocyte function and differentiation.

CZ 119 MITOGENIC,ADIPOGENIC and ANTIADIPOGENIC ROLES OF VARIOUS EFFECTORS ON RAT ADIPOSE PRECURSOR CELLS, R. Négrel, D. Gaillard, G. Vassaux, and G.Ailhaud. Centre de Biochimie (UMR 134 CNRS), Faculté des Sciences, Parc Valrose, 06108 Nice cedex 2, France

Rat adipose precursor cells in primary culture, maintained in a chemically defined medium supporting their differentiation and containing insulin , transferrin and T3 (ITT medium), have been used to investigate the abilities of several agents to modulate their proliferation and differentiation.These agents,include EGF,FGF,a kallikrein from submaxillary gland (SMGK),TGFβ, TNFα,fetuin,PGF2α and carbaprostacyclin (cPGI2), a stable analogue of prostacyclin.SMGK,TGFβ and TNFα behave as growth-promoting agents and elicit within 9 days a strong dose-dependent inhibition of differentiation, whereas EGF and PGF2α fail to affect proliferation at concentrations which exert a maximal inhibitory effect on differentiation.Resumption of differentiation occurs upon removal of the antiadipogenic agents from ITT medium,except for TGFβ.In contrast FGF and fetuin,which exhibit also and respectively a weak and a strong growth-promoting ability,do not behave as inhibitors of differentiation. Their presence in ITT medium leads to an increase in the number of triacylglycerol-containing cells without significant changes in the specific activity of GPDH. Contrarily to the above agents,cPGI2 behaves as a true adipogenic factor leading to a 3-fold increase in GPDH activity with no significant growth effect. Altogether these data allow to distinguish growth-promoting factors with or without inhibitory effect on adipose cell differentiation and emphasize the specific adipogenic role played by prostacyclin.

CZ 120 EXPRESSION OF THE GLUCOSE TRANSPORTER TYPE 2 (GLUT2) MARKS PROGENITOR CELLS OF THE DEVELOPING RAT PANCREAS, Kevin Pang, Chengeto Mukonoweshuro and Gordon G. Wong, Embryonic Growth and Regulatory Proteins, Genetics Institute, Cambridge, MA 02140

We have used an antibody to the glucose transporter type 2 (Glut2) to examine beta cell ontogeny in the developing rat pancreas. Although in the adult Glut2 is specific to the beta cells of the islets of Langerhans, we find that Glut2 is expressed very early in pancreas formation by almost all cells of the initial pancreatic epithelium. This spatial expression pattern is distinct from the early glucagon, pancreatic polypeptide, and insulin expression patterns previously described by others. At day 17 of embryonic development clusters of cells expressing both Glut2 and insulin can be found in the ductal network, which we believe form the basis of the beta cell cores in the islets of Langerhans. From this data we propose a model which postulates the existence of at least two discrete endocrine cell lineages.

CZ 121 TCDD INHIBITS ADIPOSE DIFFERENTIATION OF 3T3-L1 CELLS, Marjorie Phillips, Essam Enan, and Fumio Matsumura, Department of Environmental Toxicology, University of California, Davis, CA 95616

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most potent of the halogenated aromatic hydrocarbons. In animals at low doses, it exerts a variety of adverse symptoms, including a wasting syndrome and inhibition of glucose transport in adipose tissue. Because adipocytes appear to be a particularly susceptible tissue for the toxic actions of TCDD, we investigated its effect on adipose differentiation using 3T3-L1 as a model system. Treatment of cells before differentiation or during the 2 day induction with dexamethasone (dex) and isobutylmethylxanthine (MIX) led to a drastically reduced number of fat cells as evidenced by Oil Red O staining. Northern blots of RNA harvested 10 days after initiation of differentiation confirmed that TCDD treatment greatly reduced amounts of RNA encoding several adipocyte markers. If TCDD treatment was delayed until after removal of dex and MIX, its effect was greatly diminished, suggesting that it is affecting the decision to differentiate rather than subsequent events. Dose response and structure activity experiments are consistent with an Ah-receptor mediated process. These results suggest TCDD may interfere with glucocorticoid or cAMP signalling during the differentiation process.

CZ 122 RAPID ONSET OF HEAT SHOCK PROTEIN 72A EXPRESSION DURING IN VIVO AND IN VITRO PREADIPOCYTE DIFFERENTIATION, Timothy G. Ramsay and Srinivas V. Rao*, Laboratory of Developmental Biology, Pennington Biomedical Research Center, Baton Rouge, La. 70808 and *Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH 43699

In an attempt to identify and analyze specific gene products regulating differentiation of adipocyte precursors, two cDNA libraries of sizes $1.62x10^9$ and $1.49x10^9$ cfu were developed from porcine adipocytes and stromal vascular cells, respectively. Subtractive hybridization was performed using these libraries to obtain adipocyte enriched, cDNA clones of differentially expressed mRNAs. Random selection, phagemid DNA isolation and sequencing resulted in five clones of interest. Primary screening by Northern analysis of the five clones revealed that four (clones 5, 6, 10 and 12) were preferentially expressed in the adipocytes. Secondary screening by Northern analysis of the clones with total RNA from fetal, neonatal and adult pigs revealed that clone 6 has a rapid and transient expression during fetal development, suggestive of a gene with a critical role in adipose tissue growth. The other clones were expressed at all stages of adipose tissue development. Sequence analysis of clone 6 demonstrated that it was homologous to porcine Heat Shock Protein 72A (HSP72A). More direct analysis of the role of HSP72A was performed utilizing primary cultures derived from porcine adipose tissue. At confluence, cultures were treated with media (medium 199 + 2.5% pig serum) supplemented with no additions; 5 mM sodium butyrate; 5 mM sodium butyrate and 10 ug insulin/ml medium; or 150 uM sodium arsenite. Total RNA was isolated from cells at the end of various treatment periods. Induction of differentiation was verified by northern analysis for porcine lipoprotein lipase (pLPL). Exposure of confluent cultures for as little as one hour to these treatment media resulted in a six fold increase in expression of porcine hsp72A. Similarly, LPL expression was increased twofold by the treatment media during the same time frame. These data indicate that heat shock proteins are expressed in a rapid and transient manner during the earliest phase of preadipocyte differentiation.

CZ 123 STIMULATORY ROLE OF RETINOIDS ON ADIPOSE CELL DIFFERENTIATION, I.Safonova+, C.Darimont+, E.Amri+, D.Gaillard+, U.Reichert°, B. Shroot°, R. Négrel+, P. Grimaldi+ and G.Ailhaud+

+ Centre de Biochimie CNRS, Université de Nice-Sophia Antipolis, Nice, France and ° CIRD-GALDERMA, Sophia-Antipolis, France.,

Adipose tissue is known to be a target organ for all-trans retinoic acid (tRA) and the occurrence of mRNAs encoding for RARs and RXRs has been recently reported both in rat adipose tissue and mouse 3T3-L1 cells. At supra-physiologic concentrations, tRA has been reported to prevent the differentiation of preadipose cells (ST13, 3T3-L1 , 3T3-F442A and Ob17 clonal lines) and, under the same conditions, our studies performed on Ob 1771 cells lead to similar results. However, using tRA, 9-cis RA and synthetic retinoids at concentrations close to the Kd values of RARs and RXRs for these various ligands (1pM-10nM range), we show that retinoids behave as potent and positive effectors of adipose cell differentiation (as assayed by fat cell cluster formation and GPDH activity) both in serum-supplemented and serum-free medium. These effects involve primarily RARα as shown by the use of receptor subtype selective ligands. Exposure of preadipose cells for three days appears sufficient to trigger terminal differentiation whereas no significant effect of retinoids on cell growth can be seen. Additional experiments on the regulation of the expression of early and late genes are underway and will be presented. Thus, at low concentrations, retinoids appear to act as strong adipogenic agents.

CZ 124 STIMULATION OF TGF-α mRNA AND PROTEIN EXPRESSION BY PROSTAGLANDIN F2α IN ADIPOCYTE PRECURSORS IN PRIMARY CULTURE, Ginette Serrero and Nancy M. Lepak, W. Alton Jones Cell Science Center, Inc., 10 Old Barn Road, Lake Placid, New York 12946

Transforming growth factor-α (TGF-α) and prostaglandin F2α are potent inhibitors of adipose differentiation. We report here the presence of TGF-α mRNA transcripts in adipose tissue. TGF-α mRNA expression was also found in adipocyte precursors in primary culture before and after they had undergone differentiation. We have found that TGF-α mRNA expression is increased by treatment of the cells with $PGF_{2\alpha}$. In addition to stimulating TGF-α mRNA expression, PGF2α also stimulated the level of TGF-α found in the conditioned medium of adipocyte precursors in primary culture. Both adipocyte precursors and adipocytes were responsive to $PGF_{2\alpha}$ by stimulation of TGF-α mRNA expression. Removal of $PGF_{2\alpha}$ from culture medium of adipocyte precursors led to a down regulation of TGF-α mRNA level indicating that the effect of $PGF_{2\alpha}$ on TGF-α expression was reversible. Other prostaglandins such as PGE2 and PGD2 that do not affect adipose differentiation did not stimulate the level of TGF-α mRNA expression in the adipocyte precursors. Moreover, other inhibitor of differentiation such as transforming growth factor-β (TGF-β) did not have affect TGF-α mRNA expression. These data suggest that stimulation of TGF-α mRNA is specific to PGF2α and may be correlated to its ability to block adipose differentiation. These results would suggest the existence of paracrine feedback regulatory pathway between two adipose differentiation inhibitors within the adipose tissue which could play an important role in the physiological control of adipocyte function. This work was supported by grant DK38639 from the National Institutes of Health.

CZ 125 PREF-1, A NOVEL MEMBER OF THE EGF-LIKE FAMILY OF PROTEINS, INHIBITS ADIPOCYTE DIFFERENTIATION. Cynthia M. Smas and Hei Sook Sul, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115

With the aim of identifying novel regulators of adipocyte differentiation, by differential screening, we isolated a cDNA sequence coding for a novel member of the epidermal growth factor (EGF)-like family of proteins, which we designated pref-1. Pref-1 is synthesized as a transmembrane protein with six tandem EGF-like repeats at the putative extracellular domain. Pref-1 is encoded by a single copy gene. However, multiple discrete forms of pref-1 protein of 45-60 kDa are present in preadipocytes, owing to N-linked glycosylation and alternative splicing. RT-PCR and cDNA cloning demonstrate that 3T3-L1 cells express at least 5 alternatively spliced pref-1 transcripts. Pref-1 mRNA is highly restricted in adult tissues and present only in adrenal gland. Pref-1, however, is present in variety of tissues in developing embryo, most prominent in pituitary and in developing vertebra. Pref-1 is first detected in E.8.5 mouse embryo and continues until E. 18.5. While pref-1 mRNA is abundant in preadipocytes, its expression is completely abolished, by decrease in pref-1 gene transcription, during differentiation of 3T3-L1 preadipocytes to adipocytes. Differentiation defective 3T3-C2 cells express 3-fold higher pref-1 mRNA levels as compared to 3T3-L1 cells. Fetal calf serum decreases pref-1 mRNA levels by 70%. Moreover, constitutive expression of pref-1 in preadipocytes, which in effect blocks its down-regulation, drastically inhibits adipose differentiation. This indicates that pref-1 functions as a negative regulator of adipocyte differentiation, possibly in a manner analogous to EGF-like proteins that govern cell fate decisions in invertebrates.

CZ 126 IDENTIFICATION OF A NOVEL NEGATIVE ELEMENT IN THE MOUSE GLYCEROPHOSPHATE DEHYDROGENASE LOCUS, Claire M. Steppan, Helena M. Madden and Deborah E. Dobson. Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118

During adipocyte differentiation, the mouse adult glycerophospate dehydrogenase (GPD) gene is transcriptionally activated. In an attempt to elucidate the mechanisms underlying this activation, we have identified a novel negative regulatory element. Functional analysis of the GPD negative element indicate that it is a strong transcriptional silencer. Transient transfections with several constitutively expressed chimeric viral promoter-CAT gene constructs show significant downregulation in both 3T3F442A and 3T3L1 preadipocytes and adipocytes. Electrophoretic mobility shift assays reveal the formation of multiple sequence-specific DNA-protein complexes. Similar patterns were seen with preadipocyte and adipocyte nuclear extracts. Molecular dissection of the GPD silencer indicates that minimal element required to maintain activity resides within a 39 base pair region which has no known homology to characterized negative elements. Further experimentation to identify the cognate nuclear proteins is ongoing.

These results suggest a model where the GPD silencer is active in both preadipocytes and adipocytes; whereas, upon differentiation, the GPD locus is transcriptionally activated by as yet unidentifiedpositive element(s) that overwhelms any repression.

CZ 127 Isolation and partial characterization of porcine serum borne factor that inhibit adipose development in culture. A. Suryawan, D.P. Froman and C.Y. Hu. Dept. of Animal Sciences, Oregon State University, Corvallis, OR. 97331.

Some serum borne factors that regulate the development of adipose cell lines and primary culture have been identified in rat and bovine serum. To date however, there have been no reports of "antiadipogenic factors" in porcine serum. We have established a serum-free culture for porcine preadipocytes (isolated from dorsal subcutaneous adipose tissue) that supports a high rate of adipose differentiation. This system was used as a bioassay to test our serum fractions. We have partially purified and characterized a porcine serum borne factor that inhibited the adipose development. The inhibiting factor was purified by preparative isoelectric focusing, DEAE-Sephacel, Affi-Gel Blue chromatography and gel filtration chromatography (Ultrogel ACA 44). The molecular weight of the inhibitor is $\pm$ 70 Kd and has an isoelectric point (pI) value of 4.79. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis indicated that the inhibitor coeluted with porcine serum albumin. In our cell culture system the inhibitor suppressed approximately 40% of Sn-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) activity, an indicator of adipose differentiation. However, cellular DNA analysis indicated that the inhibitor did not affect cell proliferation. In order to verify the uniqueness of the inhibitor, further purification and comparison with other known growth factors which inhibit adipose development will be conducted. (Supported in part by NIDDK Grant DK42536)

CZ 128 INHIBITION OF ADIPOCYTE DIFFERENTIATION BY TEMPERATURE-SENSITIVE SV40 T-ANTIGEN

Andrew G. Swick and Richard S. Carroll, Department of Metabolic Diseases, Pfizer Central Research, Groton, Connecticut, 06340

It is fundamental to understand the regulation of adipocyte differentiation and gene expression in order to make progress in the study of obesity. A number of agents have been demonstrated to either accelerate or inhibit the differentiation of preadipocytes into adipocytes in cell culture. Constitutive expression of the SV40 large T-antigen effectively blocks the ability of preadipocytes to differentiate (Cherington et al., Mol. Cell. Biol. 8:1380-1384). We investigated whether T-antigen expression altered the preadipocyte and thus rendered it unable to differentiate or whether T-antigen specifically blocked differentiation. To this end, an expression vector containing the temperature sensitive (ts) T-antigen was stably introduced into 3T3-L1 preadipocytes. The ts T-antigen contains a mutation that results in the T-antigen protein being labile at 38°C but stable at 33°C. Therefore we were able to "turn on" and "turn off" the T-antigen protein during the differentiation process. Our basic finding is that the T-antigen must be present during the induction period in order to block differentiation, as assessed by oil red o staining and expression of adipocyte-specific genes. Presence of T-antigen only during the period prior to induction did not have a significant effect on the differentiation process. Therefore, T-antigen does not irreversibly block the ability of preadipocytes to differentiate. These results also provide information about the difference between white and brown adipocytes, since SV40 T-antigen expression is not compatible with white adipocyte differentiation, while T-antigen expressing brown preadipocytes have been reported to differentiate normally.

Adipocyte Differentiation, Gene Expression, and Function II

CZ 200 TEMPORAL EFFECTS OF SOMATOTROPIN (STH) TREATMENT AND WITHDRAWAL ON ADIPOSE TISSUE CELLULARITY AND METABOLISM IN THE PIG. M.J. Azain and K.C. Lee, Animal and Dairy Science Dept., University of Georgia, Athens, GA 30602.

Two experiments were conducted to examine the effects of STH on subcutaneous adipose tissue cellularity and lipogenesis in growing pigs. In a 10 wk study, pigs were treated with STH (3 mg/d) for the first 5, the last 5 or all 10 wk and compared to a non-injected control. STH resulted in a cessation of lipid filling in adipocytes as evidenced by a decrease in cell diameter (64 μm) relative to the control (82 μm) at the end of the study. Average cell diameter and distribution of cell sizes was similar to that at the initiation of treatment. This effect was reversed upon withdrawal of STH. The time-course for the inhibitory effects of STH on lipogenesis in adipose tissue and the recovery from this inhibition upon withdrawal of treatment were investigated in the second study. Tissue biopsies were obtained from control pigs or 24 hr after the last injection in pigs treated with STH (4 mg/d) for 1, 2, 4, and 7 days. Similarly, biopsies were obtained 1, 2, 3 and 8 days after withdrawal from STH in pigs treated for 7 consecutive days. Tissue slices were prepared and incubated in 5 mM ^{14}C-glucose. The maximal inhibition of lipogenesis was 30% of control and occurred after 4 days of treatment. Lipogenesis was decreased 28% after a single injection and began to recover within 2 days of the last injection. Lipogenic activity was not different from control values after 8 days of withdrawal. Results indicate that STH results in a relative decrease subcutaneous adipose thickness through an inhibition of the lipid filling in adipocytes that normally occurs as pigs grow. This inhibition is partially accounted for by an inhibition of lipogenesis. Withdrawal of treatment is followed by normalization of lipogenic activity and cell size.

CZ 201 PERILIPIN IS ON THE SURFACE LAYER OF INTRACELLULAR LIPID DROPLETS IN ADIPOCYTES AND ADRENAL CELLS, E.J. Blanchette-Mackie, N.K. Dwyer, T. Barber, R.A.Coxey, C.M. Rondinone and C. Londos, NIDDK, National Institutes of Health, Bethesda, MD 20892.

We have examined the intracellular location of perilipin in cells and tissues involved in lipid metabolism using confocal light microscopy and immunogold staining of cryosections for electron microscopy (EM). Perilipin is located on the surface phospholipid monolayer surrounding lipid droplets. Freeze-fracture EM revealed that the hydrophobic face of the surface monolayer that apposes the triacylglycerol (TG) core contained particles identical in size and distribution to intramembranous particles (IMPs), an exclusive feature of the hydrophobic faces of bilayered membranes. This structural evidence is consistant with the concept that TG, synthesized by enzymes bound to the endoplasmic reticulum, "oils out" as a separate phase of hydrophobic lipid droplets between leaflets of endoplasmic reticulum (EM) membrane bilayers. Lactating mammary gland (LMG) and liver of newborn mice contain cells other than adipocytes that synthesize and store TG in intracellular lipid droplets. Although perilipin was at the surface of lipid droplets in LMG adipocytes, none was associated with milk lipid droplets in alveolar epithelial cells, nor was the protein located on lipid droplets in hepatocytes. However, perilipin was present on the surface of cholesteryl ester droplets in steroidogenic Y-1 mouse adrenal cells, but not in non-steroidogenic SW13 human adrenal cells, which also contain cholesteryl ester droplets. Thus perilipin is present in cells which have the capacity to hydrolyze TG or cholesteryl esters via a lipase similar, if not identical, to hormone sensitive lipase. While these results point to a role for perlipin in lipid hydrolysis, they do not preclude participation of the protein in structural or lipogenic functions.

CZ 202 PERILIPINS AT THE SURFACE OF CHOLESTERYL ESTER DROPLETS IN Y-1 ADRENAL CORTICAL CELLS, D.L. Brasaemle, D.A. Servetnick, J. Wolff, and C. Londos. NIDDK, National Institutes of Health, Bethesda, MD 20892

Perilipins are proteins located on the surface of triacylglycerol storage droplets in adipocytes. We speculate that perilipins may play a role in lipolysis since they are phosphorylated by cAMP-dependent protein kinase in concert with the lipolytic response. Lipid droplets in adrenal cortical cells contain cholesteryl esters (CE), and steroidogenesis is initiated with CE hydrolysis by cholesteryl esterase (CEase), an enzyme thought to be similar, if not identical, to the hormone-sensitive lipase (HSL) of adipocytes. These similarities between adipocytes and adrenal cells prompted an investigation as to whether perilipins are associated with the CE droplets of murine Y-1 adrenal cortical cells. Western blotting of adrenal cell fractions with affinity purified antibodies raised against rat full length perilipin A show that: 1) adrenal cells contain two major forms of perilipin, one of which appears to be perilipin A, the major form in adipocytes. This species migrates as a 62 kDa protein by SDS-PAGE, but upon stimulation of cells with ACTH or forskolin to elevate cAMP, the protein migrates as a 65 kDa species; these data are in accord with those for the adipocyte. Moreover, this species is recognized by antibodies specific for the COOH-terminus of perilipin A. 2) a second major form of ~42 kDa, which we designate as perilipin C, is at least as abundant as perilipin A. Perilipin C appears to lack the COOH-terminus found in perilipin A. Additionally, adrenal cells contain minor amounts of other perilipins, B (46 kDa) and D (47 kDa). 3) as in adipocytes, perilipins are found exclusively in the fat fraction. Western blotting of adrenal cell extracts with affinity purified antibodies raised against rat HSL reveals a major band at 86 kDa, in contrast to adipocytes, which have a major band at 84 kDa and a minor band at 86 kDa. Hence, the two cells differ in the relative amounts of HSL variants expressed. Following stimulation of Y-1 adrenal cells with cAMP-elevating agents, a significant fraction of the adrenal HSL/CEase is translocated from the cytosol to the CE droplet surface, again in accord with the adipocyte findings. Thus, other than differences in the lipid content of their storage droplets, adipocytes and adrenal cells exhibit remarkable similarities both in the composition of their lipid droplet surfaces and in the mode by which they metabolize lipids. Differences that point to tissue specificities include the expression of perilipin C in adrenal cells, but not fat cells, and the expression of a lipase species in adrenal cells that is a minor species in the adipocyte.

CZ 203 ANALYSIS OF IGF-BINDING PROTEIN PRODUCTION BY CHICKEN ADIPOCYTE PRECURSOR CELLS AND MOUSE 3T3-L1 PREADIPOCYTES. REGULATION BY IGF-I AND TGF-ß. Simon.C.Butterwith., Diana Peddie and Chris Goddard. AFRC Roslin Institute Edinburgh, Division of Developmental Biology, Roslin, Midlothian EH25 9PS. UK.

The insulin-like growth factors are potent stimulators of preadipocyte proliferation and differentiation *in vitro*. IGF-I is expressed in preadipocytes *in vitro*, and in adipose tissue *in vivo*. Despite this evidence that the IGF peptides may be important regulators of adipogenesis, very little is known about IGF binding protein (IGFBP) production by adipose tissue. This study was designed to characterise and compare the patterns of binding protein production by chicken adipocyte precursors (CAP) and mouse 3T3-L1 preadipocytes during proliferation and differentiation and to identify potential regulators. Proliferating CAP produced major IGFBP's of 32.5, 30.5, 27 and 24kD and minor ones of 45 and 38.5kD. In contrast differentiating cells lacked the 27kD binding protein. 3T3-L1 cells produced IGFBP's of 38.5, 26 and 20kD and there was no difference in the pattern between proliferating and differentiated cells. IGF-I stimulated the production of IGFBP's in proliferating CAP, an effect which was antagonised by TGF-ß1. However in differentiated cells, although IGFBP production was stimulated by IGF-I, addition of TGF-ß had no effect. IGF-I stimulated the production of all IGFBP's in both proliferating and differentiated 3T3-L1 cells.

These results demonstrate that a range of IGFBP's are produced by CAP and 3T3-L1 cells with a different pattern produced in each cell type. Whether this represents the presence of different IGFBP's between the two will require the identification of each of the IGFBP's. We have also demonstrated that IGF-I regulates the production of IGFBP's in both cell types. The pattern of IGFBP's between the proliferating and differentiated states differs in CAP but not in 3T3-L1 cells. The significance of this is not known at present.

CZ 204 IDENTIFICATION AND PARTIAL CHARACTERIZATION OF AN INGUINAL ADIPOCYTE-SPECIFIC GENE Chu-Liang Chen and Ching Yuan Hu, Department of Animal Sciences, Oregon State University, Corvallis, OR 97331.

Evidence suggests that adipose tissue is not a homogeneous entity, but rather, that it differs functionally and morphologically according to its anatomic location. In order to study adipocyte development and differentiation and to eliminate adipose tissue at a specific site in vivo, a monoclonal antibody was desired. After cyclophosphamide selection, the monoclonal antibody (RI-1) was obtained which recognized a specific protein in the inguinal adipose tissue of Sprague-Dawley rats. The protein was identified in the inguinal plasma membrane preparation and the molecular mass was determined to be 25 kDa by using SDS-PAGE and western blot analysis. When epididymal and perirenal plasma membrane preparations were similarly tested, the 25-kDa protein was not found. Results from indirect-immunofluorescence demonstrated that the antigen is located on the plasma membrane of inguinal adipocytes. RI-1 monoclonal antibody was used to screen a cDNA expression library constructed from mRNA purified from rat inguinal adipose tissue. A positive cDNA clone of approximately 2,000 bp has been obtained. Characterization of the tissue distribution and differential expression of the mRNA will be performed by using the cDNA probe.

CZ 205 THE ADIPSIN-ACYLATION STIMULATING PROTEIN SYSTEM IN HUMAN ADIPOCYTES: REGULATION OF TRIACYLGLYCEROL SYNTHESIS, Katherine Cianflone, Magdalena Maslowska, Allain Baldo, Allan Sniderman, Cardiology, McGill University, Montreal, Quebec, Canada

This study presents evidence that the process of triacylglycerol synthesis can be modulated in human adipocytes by the adipsin/Acylation Stimulating Protein (ASP) pathway. We have previously characterized an activity from human plasma that markedly stimulates triacylglycerol synthesis in cultured human skin fibroblasts and adipocytes named ASP. The molecular identity of the active serum component is identical to C3adesArg and the components of this pathway are identical to those in the proximal portion of the alternate complement pathway. The data demonstrate: 1) that human adipocytes contain mRNA for the specific serine protease, adipsin and the two precursor proteins, C3 and factor B, all required to interact for the production of ASP; 2) that the extent to which cultured differ-entiating adipocytes produce ASP is proportional to the degree to which they have accumulated triacylglycerol mass during differentiation (r^2=0.7523, $p<0.0005$); 3) that ASP stimulates triacylglycerol synthesis within such cells but this occurs to a greater extent in differ-entiating than undifferentiated cells (242% ±32% $p<0.025$ and 168% ±11% $p<0.0005$ respectively at an ASP concentration of 88 ng/mL); and 4) that when ASP is generated *in vitro* through incubation of its precursor proteins under appropriate conditions, triacylglycerol synthesis increases to the same extent as when plasma-purified ASP is added to the medium. These findings provide the first evidence that the adipsin-ASP system is present in human adipocytes. If this system is indeed of physiologic importance, our understanding of the processes which regulate triacylglycerol clearance from plasma will be considerably enhanced.

CZ 206 ADIPOSE FATTY ACID BINDING PROTEIN (a-FABP) GENE: A TARGET FOR THE INSULIN SENSITIZER, PIOGLITAZONE. Steven D. Clarke, Xiouming Sha, Rolf Kletzien, and Philippe Thuillier, Program of Human Nutrition, Colorado State University, Fort Collins, CO 80523

PIOG is an orally active, anti-diabetic agent that accelerates glucose utilization by peripheral tissues. The mode of action for PIOG involves an intracellular mechanism whereby the insulin and IGF-1 signals are amplified which in turn enhances the events of fat cell differentiation. The PIOG amplification of gene expression involves several glucose and lipid metabolizing enzymes, including Glut 4 and lipoprotein lipase. However, the transcript which displays the earliest and most extensive induction by PIOG is the a-FABP mRNA, i.e. PIOG induced a-FABP mRNA levels 10-15 fold. The PIOG-dependent induction of a-FABP gene expression is largely the result of accelerated gene transcription. When 3T3-L1 preadipocytes were stably transformed with a CAT vector containing -7.6 kb of 5'-flanking sequence for the a-FABP gene, PIOG induced the differentiation dependent expression of CAT by 8-10 fold. Deletion analysis of the 5-flanking region for a-FABP revealed that the cis-acting element for PIOG resided in the region of -5.4 to -4.9 kb. The enhancer activity of the PIOG element was preserved when the 500 bp sequence was ligated to the heterologous thymidine kinase promoter. Gel shift assays indicate that this region contains a differentiation dependent element that may be the target for PIOG action. Finally, treatment of differentiating 3T3-L1 preadipocytes with genistein (an inhibitor of tyrosine kinase signalling) completely blocked PIOG's ability to induce a-FABP. Thus, the mechanism by which PIOG accelerates fat cell differentiation appears to require an intact insulin and/or IGF-1 signalling system. (Supported by NIH grant DK 46363)

CZ 207 USE OF 5-[^{125}I]IODONAPHTHYL-1-AZIDE TO LABEL ADIPOCYTE PROTEINS, Neile K. Edens[1] and Constantine Londos[2], [1]Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742 and [2]Section on Membrane Regulation, Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD 20892

Adipocyte triglyceride synthesis is catalyzed by enzymes that have not been purified to homogeneity or cloned in a mammalian system. Our goal was to identify these enzymes and other adipocyte proteins involved in TG synthesis by use of 5-[^{125}I]iodonaphthyl-1-azide (INA). INA is a hydrophobic compound that can be activated directly by illumination with UV light (314nm) or indirectly by collision with excited fluorophores such as (N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)) lipid analogs (NBD-lipids). When taken up by cells and activated, INA labels cellular proteins by covalent binding. 3T3-L1 preadipocytes and adipocytes were labelled in this way, and INA labelling of adipocyte proteins was detected by autoradiography of SDS-PAGE gels. Several adipocyte proteins were better labelled by NBD-dodecanoic acid mediated INA activation than by direct activation with UV light. NBD-phosphatidic acid induced INA labelling of an overlapping, but nonidentical, set of adipocyte proteins. Proteins from undifferentiated 3T3-L1 cells were labelled relatively poorly by NBD-dodecanoic acid mediated INA activation. Centrifugation of adipocyte homogenates showed that most of the proteins preferentially labelled by NBD-dodecanoic acid mediated INA activation were found in the supernatant, rather than the pellet or fat fractions. INA, in conjunction with NBD-lipids, may be a useful tool for identifying adipocyte proteins involved in lipid metabolism. Supported by NIH DK41380.

CZ 208 MULTIHORMONAL REGULATION OF PEPCK GENE TRANSCRIPTION IN 3T3-F442A ADIPOCYTES

Claude Forest, Sylvie Franckhauser, Jocelyne Antras-Ferry, *Daryl K. Granner, Pierre Robin and Danielle Robin

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The enzyme phosphoenolpyruvate carboxykinase (GTP) (PEPCK) catalyzes the rate limiting step in hepatic gluconeogenesis and is involved in glyceroneogenesis in adipose tissue. The hormonal control of PEPCK gene expression was studied in 3T3-F442A adipocytes maintained in a serum-free medium. A radiolabeled PEPCK cDNA was used as a probe to monitor the hormone-induced variations in PEPCK mRNA content. A ß-actin cDNA was used as a control probe. The ß-agonist isoproterenol (ISO), an analog (8-CPT-cAMP) of its second messenger (cAMP) and retinoic acid (RA) increased whereas dexamethasone (DEX) reduced PEPCK mRNA concentration. Maximal inductions were 3 fold at 2 hours for ISO and 5 fold at 4 hours for RA. The DEX-induced decrease was of 80% in 4 hours. Half maximum effects were obtained with about 2 nM, 50 nM and 1 nM of ISO, RA and DEX respectively. Actinomycin D, 4 µg/ml, totally prevented ISO, RA and DEX effects. Cycloheximide, 10 µM, did not affect ISO or RA stimulations but hindered DEX-induced decrease. DEX was able to counteract in a dominant and cycloheximide-independent manner the inductions produced by ISO, RA or 8-CPT-cAMP. Nuclear run-on transcription experiments showed that ISO and RA stimulations as well as DEX-induced inhibition of ISO action were transcriptional. Transient and stable transfections were performed using a plasmid (pPL1-CAT) containing -2100 to +69 base pairs of the PEPCK gene fused to the chloramphenicol acetyltransferase (CAT) gene. In both transiently and stably transfected adipocytes, ISO and RA stimulated CAT expression. DEX had no effect on basal CAT activity whereas it inhibited the stimulation induced by ISO. We conclude that, in adipocytes, these hormones modulate PEPCK gene transcription *via* cis-acting sequences located in the regulatory region. The cAMP or RA effects are similar in adipose tissue and liver whereas DEX exerts an opposite action in these two tissues. In hepatoma cells, DEX stimulates PEPCK gene transcription *via* a complex glucocorticoid responsive unit (GRU), part of which responds also to RA. Whether this GRU also mediates RA and DEX actions in fat cells remains an open question.

CZ 209 DIFFERENTIAL EFFECTS OF DEXAMETHASONE AND INSULIN ON LIPOPROTEIN LIPASE IN CULTURED RAT ADIPOCYTES COMPARED TO ADIPOSE TISSUE. S.K Fried and T D'Souza Dept. of Nutritional Sciences, Rutgers Univ., New Brunswick NJ 08903

Adrenalectomy decreases rat adipose tissue(AT) lipoprotein lipase (LPL) activity, but reported effects of dexamethasone (dex), a synthetic glucocorticoid, on isolated adipocyte and AT LPL activity are conflicting. Rat epididymal AT fragments or collagenase-isolated adipocytes were cultured for 24 hrs in serum-free medium 199 without or with dex (2.5nM or 25nM), insulin (ins, 7nM) or dex (2.5nM or 25nM) plus ins (7nM). In isolated adipocytes, ins increased LPL activity compared to basal, but dex added in the presence of ins did not produce a further increase in LPL activity. In contrast, in cultured AT fragments, ins plus dex increased LPL activity over ins alone, similar to our results in cultured human AT. In cultured rat AT fragments, in the absence of hormones, LPL activity decreased, but ins maintained LPL activity at levels similar to fresh tissue (~10-fold over basal). Dex plus ins further increased heparin releasable LPL activity ~3 fold ($p < 0.02$) at 2.5nM and ~2 fold ($p < 0.01$) at 25nM respectively, to levels that were 2-3-fold higher than fresh tissue. Dex alone had no consistent effect. These variations in LPL activity were not explained by changes in LPL synthesis (determined by biosynthetic labelling and immuno- precipitation) or levels of LPL mRNA. However, 25 nM dex decreased LPL synthesis. Thus, in AT fragments in the presence of insulin, low concentrations of glucocorticoids increase LPL activity by acting at the post-translational level. The lack of responsiveness of isolated fat cells to effects of dex plus ins remains to be elucidated, but paracrine mechanisms may be involved. (supported by NJAES)

CZ 210 ENERGY DEPENDENT PROTEIN-TRIACYLGLYCEROL INTERACTION IN A CELL-FREE SYSTEM FROM 3T3-L1 ADIPOCYTES, James F. Hare, Karyn Taylor, and Audrey Holocher, Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201.

Triacylglycerol is synthesized from the precursors sn-1,2-diacylglycerol and palmitoyl CoA in a reaction catalyzed by the microsomal enzyme diacylglycerol acyl transferase (EC 2.3.1.20). Isolated 3T3-L1 adipocyte microsomal vesicles from cells pulse-labeled with L-[^{35}S]methionine were found to release microsomal proteins into a low density form during the synthesis of triacylglycerol. The proteins released, which represent a subset of those present in the labeled microsomes, include a 62 kD protein found in high concentration in mature fat droplets. The formation of the triacylglycerol/protein complexes was dependent on time and temperature, was not stimulated by cytosol, and required ATP as well as diacylglycerol and palmitoyl CoA. Only nucleoside triphosphates and not non-hydrolyzable analogues could replace ATP in the reaction. Unlike the enzyme reaction that measures the synthesis of triacylglycerol, the formation of low density membrane is thus dependent on ATP hydrolysis as well as enzyme substrates. The newly formed, low density particles are selectively enriched in triacylglycerol synthesized during the reaction as well as that synthesized prior to the reaction. The cell-free system described thus appears to represent an early adipogenic event leading to the lipid vacuoles found in mature adipocytes.

CZ 211 EFFECTS OF TNF-alpha IN HUMAN ADIPOSE TISSUE, Hans Hauner, Thorsten Petruschke, Martina Russ, Karin Röhrig, Jürgen Eckel, Diabetes Research Institute, Heinrich-Heine University of Düsseldorf, D-40225 Düsseldorf, Germany

Studies in preadipocyte cell lines and adipocytes from rodents suggested that TNF-alpha exerts a variety of metabolic effects on fat cell formation and adipose tissue function. Aim of the present study was to investigate the effects of this cytokine on the differentiation of human adipocyte precursor cells and on metabolic functions of newly developed human fat cells. Primary cultures of human preadipocytes were obtained by collagenase digestion from subcutaneous adipose tissue samples from young women. Presence of TNF-alpha during the induction of the differentiation process by adipogenic hormones inhibited adipose conversion in a dose- and time-dependent manner. The expression of the kex enzymes glycerol-3-phosphate dehydrogenase and lipoprotein lipase as well as the cellular accumulation of lipids was dramatically reduced. When TNF-alpha was added to newly formed fat cells a marked suppression of lipogenic enzymes was observed. Chronic exposure of fat cells to 1 nM TNF-alpha resulted in a considerable delipidation within 10 to 14 days. In order to elucidate the mechanisms by which TNF-alpha causes lipid depletion of the cells we studied the effect of the cytokine on lipolysis and glucose transport. TNF-alpha was found to have a strong lipolytic activity which became evident after a 24-hour incubation increasing basal lipolysis by 3 to 4fold at the maximum concentration. In addition, TNF-alpha increased basal 2-deoxy-D-glucose uptake but abolished the stimulatory action of insulin on glucose transport. Northern blot analysis revealed that TNF-alpha dramatically reduced the cellular content of the insulin-regulated Glut-4 mRNA after a 24-hour exposure.

In conclusion, our experiments indicate that TNF-alpha exerts multiple effects in human adipose tissue which may contribute to the metabolic changes characteristic of cachexia in man.

CZ 212 TNF-α INHIBITS SIGNALING FROM THE INSULIN RECEPTOR: IMPLICATIONS FOR DIABETES MELLITUS. G.H . Hotamisligil, D. Murray, L. Choy and B.M. Spiegelman, Dept. of Biological Chemistry and Molecular Pharmacology, Dana-Farber Cancer Institute, Boston, MA

Insulin resistance is a common problem associated with diseases such as infection and cancer, and most importantly, the central component of non-insulin dependent diabetes mellitus (NIDDM). Although a role has been suggested for cytokines as potential mediators of insulin resistance during severe infections and certain cancers, it has not been possible to directly demonstrate this link. However, in NIDDM, we have recently demonstrated that tumor necrosis factor (TNF)-α is a key mediator of insulin resistance through its expression in adipose tissue of obese-diabetic animals. Here, we examine adipose expression of TNF-α in human obesity and investigate how TNF-α interferes with insulin action. Chronic exposure of adipocytes to very low concentrations of TNF-α strongly inhibits insulin-stimulated glucose uptake without a significant decrease in cellular insulin-sensitive glucose transporter (Glut4). Concurrently, TNF-α treatment causes a moderate decrease in the insulin-stimulated autophosphorylation of the insulin receptor (IR) and a dramatic decrease in the phosphorylation of IRS-1, the major substrate of the IR in vivo. The degree of inhibition of these tyrosine phosphorylations depends upon the concentration of both TNF-α and insulin. The IR isolated from TNF-α treated cells is defective in the ability to autophosphorylate and phosphorylate IRS-1, in vitro. These results show that TNF-α directly interferes with the signaling of insulin through its receptor and consequently block biological actions of insulin.

CZ 213 DIFFERENTIAL REGULATION OF C/EBPα AND C/EBPβ IN BROWN ADIPOSE TISSUE: A shift in intracellular mediation during differentiation.

Anders Jacobsson, Per Antonson*, Kleanthis G. Xanthopoulos* and Stefan Rehnmark, The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, S-106 91, Stockholm, Sweden and * Karolinska Institute, Center for Biotechnology, NOVUM, S-141 57 Huddinge, Sweden

Two members of the C/EBP family of transcriptional activators, C/EBPα and C/EBPβ seem to be involved in adipocyte differentiation and proliferation. However, the mechanisms of C/EBPα and C/EBPβ activation and their function are not well understood.

Our present study shows that cold stress is a physiological regulator of C/EBPα and C/EBPβ expression in brown adipose tissue in mice. Less than one hour of cold exposure leads to dramatic changes in expression of both genes. C/EBPα steady-state mRNA and protein levels are drastically and rapidly reduced whereas C/EBPβ mRNA and protein levels are induced several fold. The expression pattern for C/EBPα found here in the cold-exposed mice is a mirror image of the rate of DNA synthesis under the same conditions. The reduced C/EBPα mRNA level during the first week of cold exposure correlates inversely to the peak activity of the cold-induced DNA synthesis.

In adition, preconfluent brown fat primary cultures respond to norepinephrine, which is the main physiological activator, in a similar way to the early response of brown fat to cold stress in vivo, whereas confluent primary brown fat cells display a pattern similar to that found in stimulated tissue with low proliferation rate.

This is, among other things, caused by a change in the receptor complex on the cellsurface of the brown fat cell which leads to a shift in intracellular mediation during differentiation.

CZ 214 MODULATION OF ANDROGEN METABOLISM IN CULTURED HUMAN ADIPOSE STROMAL CELLS, D. W. Killinger, D. A. Roncari, B. Strutt and M. W. Khalil, Department of Medicine, St. Joseph's Health Centre, University of Western Ontario, London, Ontario, N6A 5A5, and Department of Medicine, University of Toronto.

The metabolism of dehydroepiandrosterone (DHEA) in cultured human adipose stromal cells was studied under culture conditions which influenced the formation of specific steroid metabolites. Cells grown in the presence of fetal bovine serum (FBS) converted DHEA to 7α-hydroxydehydroepiandrosterone (7αOHDHEA) during the early phases in culture. The degree of 7αOHDHEA formation varied in cells from different subjects and ranged from 5 to 60 per cent. As the cells reached confluence, there was a fall in the formation of 7αOHDHEA and a progressive rise in the formation of androstenedione (Δ^4 dione) and estrone (E_1). Under these conditions, up to 50 per cent of substrate is converted to Δ^4 dione. Dexamethasone added to the culture medium prior to confluence resulted in a 2-4 fold increase in the formation of 7αOHDHEA and when added after confluence resulted in an increase in the formation of E_1. Cells that had acquired the capacity to convert DHEA to Δ^4 dione (3ß-hydroxysteroid dehydrogenase, 3ß HSD) in primary culture retained this activity in subculture. Cells grown under serum free conditions did not acquire 3βHSD activity. Formation of 7αOHDHEA in the early phase of culture was greatest under serum free conditions with a progressive decrease with increasing concentrations of serum from 1 to 10 per cent.

These studies show that metabolism of DHEA in human adipose stromal cells is controlled by factors in serum which direct its metabolism to products of differing biological activity.

CZ 215 Protein Kinase C in rat adipocytes : influence of ovarian status and fat localization. Danièle Lacasa, Brigitte Agli, Mireille Mur, Jean-Pierre Dausse and Yves Giudicelli, Department of biochemistry, faculty of Medicine Paris-Ouest, Centre Hospitalier 78303 Poissy Cedex FRANCE.

Protein Kinase C (PKC) intervenes in the insulin activation of lipogenesis. In some cells, PKC is regulated by sex steroid hormones. These hormones are responsible for some sex- and site-related specificities of fat cell metabolism. To see if ovarian status could influence PKC site-specifically, insulin- and phorbol ester-stimulated lipogenic responses, phorbol ester-specific binding to PKC and immunoblots quantified ß- and ϵ-PKC isoforms levels were compared in subcutaneous (SC) and parametrial (PM) fat cells from sham-operated (SHAM), ovariectomized (OVX) and OVX treated by estradiol plus progesterone (OVX+E2+PG) rats.

In SHAM rats, the cytosolic PKC content was lower in SC than in PM fat cells. This parameter was blunted in both SC and PM cells in OVX rats and restored to normal in OVX+E2+PG rats. Immunoblot experiments showed a reduction of the major isoform of fat cells, ß-PKC, in SC and PM cells after ovariectomy and restoration to normal by the hormone treatment. The ϵ-PKC isoform which is also translocated by insulin remained insensitive to ovarian status in SC and PM cells. The lipogenic response to TPA was unaltered by the ovarian status in both fat deposits. In contrast, the lipogenic response to insulin was increased in PM cells and restored to normal in OVX+E2+PG rats. This response was unaltered in SC cells.

These results showing important site-related differences in the fat cell PKC content, reveal that ovarian status modulates PKC according to the fat localization.

CZ 216 CULTURED MAMMARY EPITHELIAL CELLS SECRETE A FACTOR THAT SUPPRESSES LIPOPROTEIN LIPASE PRODUCTION BY 3T3-L1 ADIPOCYTES, Margaret C. Neville and Sean J.P. Gavigan, University of Colorado HSC, Dept. of Physiology, Denver CO 80262.

Lipoprotein Lipase (LPL) activity within the mammary gland is low during pregnancy and high during lactation. In contrast, the activity in peripheral adipose tissue is high during pregnancy and low during lactation, although adipocytes in both tissues are responsible for LPL synthesis. In order to investigate the role of mammary epithelial cells in this reciprocal regulation, primary mammary epithelial cells or cells from a mammary epithelial cell line, COMMA 1D, were co-cultured with 3T3-L1 adipocytes. The heparin-releasable LPL activity of 3T3-L1 adipocytes was substantially decreased in the co-cultures. Conditioned medium from Comma 1D cultures produced the same effect. The decrease was not the result of direct inhibition of enzyme activity since conditioned medium had no effect upon the assay itself. Twelve hours preincubation of the adipocytes with the conditioned medium was required for maximum effect. The effect was reversible: LPL activity of adipocytes recovered to normal levels 24 hours after removal of the conditioned medium. The factor is heat-stable, can be precipitated by ammonium sulphate (350 mg/ml) and is retained by both dialysis tubing and a filter with 10kd molecular weight cut-off. Preliminary findings from gel filtration experiments suggest the factor has a molecular weight between 15,000 and 20,000. These observations suggest the factor is a peptide; its role in suppressing LPL activity suggests it may act within the mammary gland during pregnancy. Supported by NIH grant HD19547.

CZ 217 **PERCUTANEOUS ESTROGEN ADMINISTRATION DECREASES LIPOPROTEIN LIPASE ACTIVITY IN GLUTEAL ADIPOSE TISSUE**

Susan N. O'Brien, Richard L. George, Thomas M. Price, Greenville Hospital/Clemson University Research Cooperative, Greenville, SC 29605

Lipoprotein lipase (LPL) plays a major role in energy storage. The enzyme hydrolyzes triglycerides in circulating chylomicrons and very low density lipoproteins (VLDL) to free fatty acids and glycerol. Control of LPL gene expression is regulated by hormones and is tissue specific. Estrogen appears to influence the pattern of deposition of adipose tissue since changes in fat distribution parallel changing levels of circulating estrogen during menarche and menopause. In premenopausal women, LPL activity levels are higher in the lower body (gluteal) adipose tissue compared to that of upper body (abdominal). In postmenopausal women, LPL activities are similar in these two areas. We have demonstrated in our laboratory the presence of mRNA for the estrogen receptor (ER) in human adipose tissue. We have also detected significant differences in ER mRNA from adipose tissue of the abdominal and gluteal regions. In this study, we measured the effect of percutaneous estrogen administration on LPL activity in subcutaneous adipose tissue. Healthy premenopausal women in the early follicular phase of the menstrual cycle were administered 0.2 mg/d of 17-ß estradiol via percutaneous patches applied to the upper gluteal region. A placebo patch was applied to the contralateral gluteal region as a control. After 6 days, subcutaneous adipose tissue was obtained from under the patches by needle aspiration biopsy. A radiometric assay involving heparin induced elution of extracellular enzyme from adipose tissue was used. LPL activity was measured by the conversion of radiolabeled triolein to oleic acid. Using this elution method of extraction, estrogen decreased LPL activity from 9 to 70% of the control value. These data suggest that estrogen negatively regulates adipose tissue LPL activity. We suggest that localized estrogen production via aromitization may regulate regional adipose tissue LPL activity.

CZ 218 ANTI-DIABETOGENIC EFFECT OF GROWTH HORMONE ANTAGONISTS, *Shigeru Okada, John J. Kopchick, Edison Biotechnology Institute, Department of Biological Sciences, and Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701*

Growth hormone (GH) induces an insulin resistant state in 3T3-F442A adipocytes. For example, glucose uptake in mature adipocytes is stimulated 5- to 6-fold above basal levels by 1 μM insulin. However, 48 hours exposure of the adipocytes to 1 nM GH reduces basal glucose uptake levels by approximately one half. Under these conditions 1 μM insulin can not stimulate glucose uptake above untreated control levels. Cells treated with a bovine or human GH antagonist do not develop this insulin resistant state and these GH antagonists inhibit the diabetogenic effect of GH. For example, 0.3 nM GH treatment induces insulin resistance, however, when adipocytes are co-incubated with 0.3 nM GH and increasing concentrations of GH antagonists, the development of the insulin resistant state is progressively inhibited. Cells treated with 10 nM GH antagonist are fully insulin responsive. Thus, GH antagonists inhibit the diabetogenic effect of GH in 3T3-F442A adipocytes.

CZ 219 EFFECT ON TRANSPORTER FUNCTION OF NATURAL AND ENGINEERED CHANGES IN THE C-TERMINUS OF GLUT4, Brent C. Reed, Robin Dauterive, and Stephen Laroux, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130

We have identified in 3T3-L1 adipocytes a 2.4 kb mRNA, designated GLUT4B, that encodes a variant of GLUT4 in which the C-terminal amino acids 443-509 are replaced by a unique 33 amino acid sequence possessing a potential site for geranylgeranyl addition. The GLUT4B message is likely an alternatively spliced form of GLUT4 in which the 3' Exon XI of GLUT4 has been replaced. It does not appear to represent an isolated intermediate of normal processing of GLUT4 message as the sequence of the Exon X-Exon XI boundary of GLUT4B does not match that of the Exon X-Intron boundary of GLUT4. Northern analysis of differentiated 3T3-L1 adipocytes using a probe to a 5' domain common to both GLUT4 and GLUT4B message detects both a 2.7 kb GLUT4 mRNA and the predicted 2.4 kb GLUT4B mRNA. GLUT4 is not expressed in undifferentiated 3T3-L1 adipocytes and neither GLUT4 or GLUT4B message was detected. This is consistent with the production of GLUT4B being dependent upon the transcription of GLUT4 precursor mRNA. Northern analysis of polyA$^+$ mRNA isolated from differentiated 3T3-L1 adipocytes identified a single 2.4 kb message when a probe unique to the 3'-end of GLUT4B was utilized. Xenopus oocytes expressing GLUT4B transported 3-O-methylglucose at a rate higher than water injected oocytes, but substantially lower than oocytes expressing GLUT4. Quantitation of expressed GLUT4 and GLUT4B protein indicated the levels of GLUT4B were low relative to GLUT4. This suggests that GLUT4B may be capable of transporting sugar at rates comparable to GLUT4, in spite of the altered C-terminal domain. To assess further the requirement of the C-terminal domain for functional transport by GLUT4, two mutants of GLUT4, designated GLUT4M1 and GLUT4M2 were constructed which terminated after amino acid 471 and 489, respectively. When expressed in Xenopus oocytes, both GLUT4M1 and GLUT4M2 exhibited transport rates comparable to or exceeding those of native GLUT4. In contrast to GLUT1, transport studies using native GLUT4B and truncated forms of GLUT4 produced by in vitro mutagenesis, indicate that functional transport of glucose by GLUT4 is not absolutely dependent upon the presence of the C-terminal domain.

CZ 220 ON THE RELATIONSHIP BETWEEN PERILIPINS AND TRIACYLGLYCEROL ACCUMULATION IN DIFFERENTIATING 3T3-L1 ADIPOCYTES, C. Rondinone, T. Takeda, T. Barber, E.J. Blanchette-Mackie, A.R. Kimmel, A.S. Greenberg, and C. Londos. NIDDK, National Institutes of Health, Bethesda, MD 20892.

We have examined the relationship between triacylglycerol (TG) accumulation and perilipin expression in differentiating 3T3-L1 adipocytes, which express perilipins A and B. Adipoblasts contain no perilipin, but 2-3 d after induction of differentiation both perilipin and TG appear, and both are co-localized as revealed by Nile red and immunofluorescence staining, indicating that perilipin is associated with nascent lipid storage droplets. Over the course of 2 to 3 weeks, perilipin accumulation occurs in concert with lipid deposition. A comparison of the expression of several differentiation-associated genes reveals two general expression patterns, one biphasic (LPL, aP2, hormone-sensitive lipase) and the other monophasic (perilipin, adipsin). Removal of biotin from a defined medium produces a brief lag in perilipin expression, but a pronounced lag in TG deposition. This apparent dissociation between perilipin and TG may be explained by the fact that in the biotin deprived condition the lipid appears in numerous small droplets, suggesting that perilipin expression is related to the total droplet surface area. Biotin removal plus the addition of avidin strongly inhibits TG accumulation, reduces the amount of perilipin and hormone-sensitive lipase, but has no effect on most other proteins. Northern analysis reveals that the (-)biotin+avidin protocol inhibits expression of perilipin mRNA during differentiation. On the other hand, performing the (-)biotin+avidin manipulation late in differentiation (16 d) has no effect on perilipin expression. In summary, perilipin appears concomitantly with the earliest TG depositions and its accumulation approximately parallels and is dependent on TG synthesis and accumulation. The biotin deprivation studies suggest that an event secondary to biotin action, such as fatty acid synthesis, may be a controlling factor in perilipin expression.

CZ 221 In vitro TRANSLOCATION OF HORMONE-SENSITIVE LIPASE TO ADIPOCYTE LIPID STORAGE DROPLETS. D.A. Servetnick, J.L. Theodorakis, D.M. Brasaemle, and C. Londos. NIDDK, National Institutes of Health, Bethesda, MD 20892.

In primary rat adipocytes, lipolysis occurs secondary to cAMP elevation, activation of cAMP-dependent protein kinase (PKA), and phosphorylation of hormone-sensitive lipase (HSL). Also, in intact cells HSL is translocated from the cytosol to the lipid droplet surface, perhaps the critical event in lipolytic stimulation since phosphorylation alone produces only a minimal increase in HSL catalytic activity. In order to explore the molecular basis for translocation, we have purified lipid droplets from 3T3-L1 adipocytes by hypotonic lysis, homogenization, centrifugal floatation and filtration on low-protein-binding filters. Purified droplets retained their native shape and size, and were stable in albumin-containing buffers for several hours. In thoroughly washed and filtered droplets, perilipin A and B appeared to be the only detectable proteins specific to the droplets. Western blotting of homogenate fractions with affinity-purified anti-HSL serum detected proteins of 84 and 86 kDa, presumably isoforms of HSL, in the cytosolic fraction. In vitro translocation experiments involved incubation of droplets with the cytosolic fraction, followed by filtration on 0.65 μ filters to harvest the droplets. In the presence of 4 mM $MgCl_2$, the addition of cAMP+ATP increased the HSL associated with the droplets in the absence, but not the presence, of a specific PKA inhibitor (PKI). The cytosolic fraction contained sufficient endogenous PKA to polyphosphorylate perilipin A, which occurred in parallel with HSL movement to the droplets. On the other hand, in the absence of Mg^{2+}, cAMP+ATP led to significant HSL translocation which was not inhibitable by PKI. The data indicate that it is possible to mimic in vitro a PKA-dependent translocation of HSL to intact lipid droplets, providing a system for further dissecting the lipolytic process of adipocytes.

CZ 222 COMPLEX RECIPROCAL REGULATION OF ADIPOSE ACYL-COA SYNTHETASE GENE EXPRESSION; INSULIN ENHANCES RECOVERY FROM DOMINANT TNF-ALPHA INHIBITION, Pamela Smith, Mala Kansara, Laura Caprario, John Nitting,and Jean von Hagen, Dept. of Medicine, UMDNJ/NJMS, VA Medical Center, E. Orange, NJ 07019

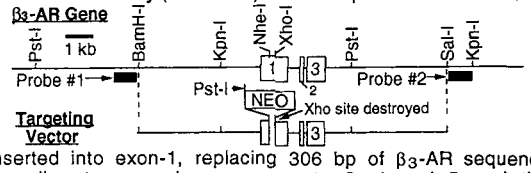
Acyl-CoA synthetase (ACS) catalyzes the activation of long chain fatty acids, the first step in the synthesis of triacylglycerols from free fatty acids. We previously demonstrated that ACS, which is not expressed in preadipocytes, is transcriptionally sensitive to insulin and TNF-a; ACS transcription is rapidly induced by .5 nM insulin and strongly inhibited (90%) by TNF-a. We sought to delineate the issue of signal dominance in the interaction between these reciprocal regulators of ACS gene expression. 3T3-L1 adipocytes were maintained in standard medium supplemented with fetal calf serum or in serum-free medium. Cultures were treated for 24 hours with TNF-a + or - .5 nM or 1 uM insulin and harvested or were incubated another 24 hours. Other sets of cultures were pretreated with insulin, or were treated with insulin for 24 hours after removal of the cytokine. RNA was prepared and Northern hybridization was performed using [32-P] labeled ACS cDNA. Results demonstrated that insulin did not block or reduce TNF-a inhibition of ACS mRNA levels but did accelerate recovery from TNF-a; little or no spontaneous reaccumulation of ACS mRNA occurred for 48 hours, but .5 nM insulin induced ACS mRNA to or above pretreatment levels. Significant spontaneous recovery was not observed until 6 days, and did not approach pretreatment levels in the absence of insulin treatment even when adipocytes were maintained in serum containing medium. Thus the TNF-a signal is acutely dominant, and exposure to the cytokine for 24 hours exerts long term effects on ACS gene expression which are reversed by insulin. We have isolated 5' flanking genomic sequences in order to characterize mechanisms for the reciprocal regulation of ACS by insulin and TNF-a.

CZ 223 TRANSGENIC MICE WITH ALTERED ADRENERGIC RECEPTOR LEVELS IN ADIPOSE TISSUE, Veronika Soloveva[1], Reed A. Graves[2], Bruce M. Spiegelman[3] and Susan R. Ross[1],[1]Dept. of Biochemistry, University of Illinois School of Medicine, Chicago, IL 60612; [2]Dept. of Medicine, University of Chicago Medical School, Chicago, IL 60637; [3]Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Body fat has different metabolic properties depending on its anatomical location, which is thought to be due in part to the level of expression of the different members of the adrenergic receptor (ARs) family. We are studying the action of ARs in adipose tissue using transgenic mice. The promoter-enhancer region of the adipocyte P2 gene (aP2; fat-specific lipid-binding protein) has been used to direct expression of the α_2-AR, β_1-AR and β_3-AR to white (WF) and brown fat (BF). Several strains of mice with the α_2-AR transgene have been analyzed. Transgenic mice that express high levels of the α_2-AR transgene in different fat-pads show no differences in either total body or fat pad weight from their non-transgenic littermates up to 5 months of age. Monosodium glutamate (MSG) was used to induce obesity in the α_2-AR transgenic mice, to analyze the role of the receptor during adipose tissue hypertrophy; again, such mice resembled their non-transgenic littermates. The MSG-treated mice were placed on a thermogenic caffeine-containing diet and our preliminary results indicate that the transgenic animals are less sensitive to this treatment than non-transgenic mice. The β_1-AR and β_3-AR transgenic mice will also be discussed.

CZ 224 GERM-LINE TRANSMISSION OF A DISRUPTED β_3-ADRENERGIC RECEPTOR GENE FOLLOWING MICROINJECTION OF DNA INTO MOUSE ZYGOTES. Vedrana S. Susulic, Joel A. Lawitts, Jeffrey S. Flier and Bradford B. Lowell, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

The β_3-adrenergic receptor (β_3-AR) is expressed most heavily in brown and white adipose tissue where it is thought to play an important role in the regulation of thermogenesis and lipolysis. However, its function has been difficult to define since β_3-specific antagonists do not exist. To address this issue, we have used gene targeting to create mice which lack β_3-ARs. The β_3-AR targeting vector was constructed using genomic DNA cloned from a 129 mouse library (see below). A *neo* expression vector was



inserted into exon-1, replacing 306 bp of β_3-AR sequence encoding transmembrane segments 3, 4 and 5 and the intervening loops. The targeting vector was electroporated into ES cells and genomic DNA from 34 G418 resistant clones was analyzed by Southern blotting (*Pst-I* digest hybridized to probe #1, *Kpn-I* + *Xho-I* digest hybridized to probe #2). Out of 34 clones, 17 had undergone homologous recombination. Prompted by the high targeting frequency observed in ES cells, the DNA knockout vector was then microinjected into mouse zygotes of two inbred strains (129, FVB) and one hybrid strain (F1 of FVB X 129). Twenty transgenic founders were generated: 129 (n=1), FVB (n=9) and F1 of FVB X 129 (n=10). Using the two Southern blot detection schemes described above, 2 of the FVB transgenic animals were found to have a disrupted β_3-AR gene. The null allele has been passed to offspring and 8 week old heterozygotes are being mated to obtain homozygous, β_3-AR deficient mice. Direct microinjection of DNA into mouse zygotes is an effective method of generating mice with targeted mutations.

CZ 225 SOLUBILIZATION AND PURIFICATION OF PERILIPIN, A LIPID DROPLET-ASSOCIATED ADIPOCYTE PROTEIN, J.L. Theodorakis and C. Londos, National Institutes of Health, Bethesda, MD 20892

Perilipin A is an adipocyte protein located exclusively at the surface of the lipid storage droplet. The protein is multiply-phosphorylated *in vivo* in response to lipolytic agents, suggesting a role in the regulation of lipid metabolism. Although the predicted size is 57 kDa, perilipin A migrates on SDS-PAGE with an apparent size of 62-67 kDa depending on its phosphorylation state. Whereas minimal hydrophobicity is anticipated based on the predicted amino acid sequence (from the rat cDNA), initial studies revealed a great resistance to solubilization. Indeed, from among a wide variety of detergents and chaotropic agents tested, only SDS solubilized perilipin A. The purpose of these studies was to solubilize and chromatographically purify perilipin A in the absence of SDS. We now find that perilipin A can be extracted directly from fresh, but not frozen, fat cakes in non-denaturing aqueous media with non-ionic detergents in the presence of 50 mM dithiothreitol. Alternatively, following extraction of lipid from the fat cake fraction of primary rat adipocytes or cultured mouse 3T3-L1 adipocyte homogenates with 98% acetone, perilipin A can be solubilized in 8M urea with 1% Triton X-100 in a neutral buffer. The solubilized perilipin A is purified by cation exchange chromatography in solutions containing 8 M urea and 1% Triton X-100 to $\geq$80% purity with near quantitative recovery. A lower concentration of either reagent greatly reduces protein recovery, presumably by reducing the solubility of perilipin A and the other extracted proteins in aqueous medium. Following SDS-PAGE, perilipin A appears as five discreet bands between 62 and 67 kDa, a multiplicity due, at least in part, to differences in phosphorylation. A 46 kDa variant, perilipin B, exhibits similar solubility and chromatographic elution properties. These studies lay the groundwork for physical chemical and biochemical characterization of perilipin and for examination of protein interactions at the lipid droplet/cytosol boundary in adipocytes.

CZ 226 SKELETAL MUSCLE-SPECIFIC OVEREXPRESSION OF GLUCOSE TRANSPORTER 4 (GLUT4) IN TRANSGENIC MICE, Tsu-Shuen Tsao and Maureen J. Charron, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461

GLUT4 is the predominant glucose transporter expressed in skeletal muscle, the major site of insulin-mediated glucose disposal. In an attempt to understand the role of GLUT4 in the regulation of glucose uptake by skeletal muscle and also in overall glucose metabolism, we have overexpressed murine GLUT4 specifically in skeletal muscle. Muscle-specific overexpression of GLUT4 is achieved by placing the murine GLUT4 gene under the control of the promoter and enhancer sequences from rat myosin light chain (MLC) 1/3 locus. MLC 1/3 promoter is turned on specifically in fast skeletal muscles of verterbrates. The degree of overexpression of the GLUT4 protein in hindlimb muscles of mice carrying the transgene ranges from 2-fold to 7-fold over their normal non-transgenic littermates. No overexpression of GLUT4 is observed in the heart, adipose tissue, and liver of these transgenic animals. Preliminary studies on skeletal muscle plasma membrane vesicles isolated by fractionation of hindlimb skeletal muscles using discontinous sucrose gradients have shown no significant difference in GLUT4 protein levels between overexpressors and normal controls under basal metabolic conditions. This suggests that there is normal cellular distribution of these overexpressed GLUT4 proteins. Consequently, basal glucose uptake assays performed on these plasma membrane vesicles under conditions of equilibrium flux exchange also revealed no significant difference in glucose transport rate between the two experimental groups. Further studies will be done to show the cellular distribution of the GLUT4 transgene product under different metabolic conditions. Other physiological studies such as glucose and insulin tolerance tests will be carried out to examine the effects of GLUT4 overexpression on glucose homeostasis.

CZ 227 BIOLOGICAL EFFECTS OF HUMAN GROWTH HORMONE ON RAT ADIPOCYTE PRECURSOR CELLS IN PRIMARY CULTURE, Martin Wabitsch[1,2#], Mapoko M. Ilondo[1], Eberhard Heinze[2], Ronald M. Shymko[1], Walter M. Teller[2], and Pierre De Meyts[1], Hagedorn Research Institute[1], DK-2820 Gentofte, Denmark, and Department of Pediatrics 1, University of Ulm[2], D-87075 Ulm, Germany

The effects of growth hormone (GH) on the differentiation and the proliferation of primary adipocyte precursor cells isolated from rat epididymal fat pads were studied under chemically-defined conditions using a serum-free culture medium. In the presence of both triiodothyronine and insulin, up to 90% of the initially fibroblast-like cells differentiated within 8 days into mature fat cells. ^{125}I-hGH binding to the cells increased significantly during adipose conversion. In newly differentiated cells (day 6), the binding kinetics reached steady-state after 180 min at 22° C. Scatchard analysis yielded linear plots with an apparent K_d of 0.16 nM and 8400 sites/cell. Human GH (hGH) at 0.5-50 nM dose-dependently inhibited the differentiation and decreased glycerophosphate dehydrogenase activity. In cultures treated with 5nM hGH, insulin-stimulated glucose uptake and lipogenesis were significantly reduced (61 ± 3 and 89 ± 4 % of control, respectively, $p<0.01$), whereas a 5-fold stimulation of lipolysis was observed. All these hGH effects were not affected by an anti-IGF I monoclonal antibody (IGF I-MAb), suggesting a direct GH action, and were observed even in the presence of corticosterone which by itself stimulated adipocyte differentiation, glucose uptake and lipogenesis. In undifferentiated cells, 5 nM hGH markedly stimulated IGF I production (12-fold) and slightly increased both cell number and ^{3}H-thymidine incorporation (1.3 and 2.6-fold, respectively). At variance with the direct GH action on cell differentiation, the cell proliferation effects were suppressed by the IGF I-MAb. Taken together, these data show that, in contrast to established preadipocyte cell lines, hGH directly inhibits the adipose conversion in primary cultures of rat adipocyte precursor cells while indirectly promoting cell proliferation through an increase in IGF I production. #was recipient of an ESPE Research Fellowship.

CZ 228 IN VITRO IMMUNOCYTOTOXICITY OF PORCINE ADIPOCYTES, J. Thomas Wright and Gary J. Hausman, USDA-ARS, Russell Research Center, Athens GA. 30605

Monoclonal antibodies (MAbs) were used to demonstrate complement-mediated cytotoxicity of porcine adipocytes and preadipocytes in primary stromal-vascular (SV) cultures. Cytotoxicity was dependent upon several factors including growth medium, MAb concentration and the particular MAbs used. Under most conditions, adipocytes in cultures supplemented with a panel of six of adipocyte-specific MAbs in the presence of complement exhibited cytotoxicity within 15 minutes of treatment. After 24 h, replicate cultures stained for lipid exhibited greater than 50% reduction in adipocyte number without any effect on the other cells (non-preadipocyte numbers) in cultures. On the other hand, immunocytotoxicity using a single MAb to target preadipocytes and adipocytes was successful only for some MAbs and only under certain growth conditions, such as dexamethasone supplementation of differentiation medium. Dexamethasone enhances expression of several of the receptors identified by the MAbs suggesting that receptor density is one factor in the ability of some combinations of MAbs to elicit preadipocyte or adipocyte complement-mediated cytotoxicity. In conclusion, selective removal of preadipocytes from primary stromal-vascular cultures was demonstrated. The cells remaining in cultures can be utilized as a new primary cell culture model system for examining determination of cells into the adipocyte lineage.

CZ 229 THE LIPOLYTIC ACTION OF GROWTH HORMONE (GH) IN RAT ADIPOCYTES DEPENDS UPON SELECTIVE TRANSLOCATION OF THE INHIBITORY G PROTEIN, G_i. Rupert G. C. Yip and H. Maurice Goodman, Department of Physiology, University of Massachusetts Medical School, Worcester, MA 01655

In the presence of glucocorticoid (eg. dexamethasone, DEX), and after a delay of 1-2 h, GH produces a sustained increase in lipolysis in rat adipocytes. Although no direct coupling between the GH receptor and adenylyl cyclase (AC) has been found, this response is accompanied by increased activity of protein kinase A and is blocked by a competitive antagonist of cyclic AMP (RP-CAMP-S), suggesting that GH acts through the same CAMP-dependent pathway as other lipolytic agonists. Since inactivation of G_i by pertussis toxin increases lipolysis in the absence of stimulatory agonists, we investigated the possibility that the lipolytic action of GH might also result from decreased inhibitory input of G_i to AC. Adipocyte homogenates prepared 4h after treatment with 30ng/ml GH and 1μg/ml of DEX were centrifuged, subjected to polyacrylamide electrophoresis (PAGE) and Western analysis. GH caused a partial redistribution of G_i, but not the stimulatory G protein G_s, from the 16K x g pellet to the supernate. GH/DEX also activated AC recovered in the 16K pellet, but not the AC recovered in the supernate. Fractionation of adipocyte membranes on linear sucrose gradients followed by PAGE revealed a selective shift of G_i from heavier (plasma membrane) to lighter (microsomal membranes) as revealed both by Western blots and autoradiography of pertussis or cholera toxin catalyzed ^{32}P NAD ribosylated proteins. Treatment of adipocytes with 100 μM colchicine blocked both the increase in lipolysis and the shift of G_i. The inactive analog lumicolchicine had no effect. We propose that stimulation of lipolysis by GH/DEX results from actions exerted on the adipocyte cytoskeleton that selectively decrease the tonic inhibitory influence of G_i on cAMP production.

CZ 230 CREATING TREANSGENIC MICE EXPRESSING *RAS* SELECTIVELY IN ADIPOSE TISSUE, Andrew X. Zhu and Jeffrey S. Flier, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

p21*ras*, a family of GTP binding proteins, has been implicated as an important intermediate in insulin signal transduction. Most of the data supporting this derives from experiments using cultured cell lines. To assess the function of *ras* in a fully differentiated cell type in the *in vivo* context, we have used a transgenic approach. A fat specific promoter/enhancer segment from the fatty acid-binding protein, aP2, was used to drive the expression of either the wild type H-*ras* or a dominant inhibitory mutant of H-*ras*, which changes Ser-17 to Asn-17, generating two transgenic constructs AP2-*ras* wt and AP2-*ras* mu respectively. The transgenes were injected into the pronuclei of fertilized zygotes from FVB mice and transferred to pseudopregnant females. Up to this point, six founders that carry the AP2-*ras* wt transgene and seven founders that carry the AP2-*ras* mu transgene have been obtained. These mice are currently in breeding and the fat specific expression of *ras* will soon be assessed. The availability of these mice will potentially shed light on the role of *ras* in insulin action in adipose cells and in adipose tissue development.

Brown Fat; Obesity

CZ 300 ß3-ADRENERGIC RECEPTOR mRNA EXPRESSION AND *ADENYLATE CYCLASE* ACTIVITY ARE DECREASED IN BROWN ADIPOSE TISSUE OF 14-DAY OLD PREOBESE ZUCKER RATS (fa/fa), Raymond Bazin, Christine Charon, *Stéphane Krief, *Laurent Emorine, *Donny Strosberg & Marcelle Lavau, INSERM U 177 and * ICGM, Paris, France

In obese Zucker rats (fa/fa), brown adipose tissue (BAT) is the first known site of the fa gene expression. Abnormalities that characterize BAT of fa/fa neonates (decrease in both the expression of UCP gene and the activity of thyroxine 3'-5' monodeiodinase) are totally corrected by administration of a ß3 adrenoreceptor agonist (BRL 35135), suggesting that a change in the expression of this ß adrenoreceptor subtype might be involved in the development of this obesity.

This study was undertaken to determine whether ß3 mRNA levels and ß adrenergic activation of adenylate cyclase (AC) activity were altered in BAT of preobese fa/fa rats.

RNA level was assessed by quantitative RT-PCR and activation of AC by several ß agonists (Norepinephrine, BRL 37344, CGP 12177A) was studied by measurement of ^{32}P-cAMP production in membrane homogenate (dose response curves). As early as 14 days of age, ß3 mRNA concentration was 3.5-fold lower in fa/fa than in Fa/fa. Basal AC activity was decreased by 30% in fa/fa compared to Fa/fa pups and maximal response to NE (10^{-7}M) was two fold lower in fa/fa than in Fa/fa. Kact for NE was 3 fold lower in fa/fa than in Fa/fa. Maximal stimulation by ß3 selective agonists (BRL 37344 and CGP 12177A) was observed for 100µM and 1µM in Fa/fa and fa/fa respectively. In both the lean and the preobese pups, BRL but not CGP was as potent as NE to stimulate AC activity. In fa/fa pups, due to the very low efficiency of CGP to stimulate AC, Kact could not be determined.

In conclusion, these results clearly establish that ß3 adrenergic receptors mRNA expression and AC activity are reduced in BAT of preobese fa/fa rats. However, the role of these abnormalities in the onset of this genetic obesity has to be defined more accurately.

CZ 301 A NOVEL BONE MARROW DERIVED PREADIPOCYTE CELL LINE EXPRESSES β-ADRENERGIC RECEPTORS.

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Studies of adipocyte gene expression have been hindered by the fact that the immortalized preadipocyte cell lines available such as the widely used 3T3-L1 cells, do not express the full complement of genes found in mature cells or adipose tissues. Several short term primary cultures of brown adipose tissue (BAT), stromal cells, and recently a line derived from a hibernoma in a transgenic (aP2) mouse have been described. Here we present the development of a preadipocyte cell line derived from normal rat bone marrow. The preadipocytes are fusiform in appearance after plating and become cobblestone shaped upon reaching confluence. The cells are maintained in the preadipocyte state in the presence of acidic FGF, with rat acidic FGF being a more potent stimulator of cell growth than human acidic FGF. The cells differentiate upon addition of insulin, dexamethasone, and IBMX, and become fully differentiated (>90%) after 8-10 days in culture. The differentiated adipocytes are multilocular in appearance, thus resembling brown, rather than white adipose cells and express thermogenin which is a characteristic of brown adipose tissue. Both the preadipocytes and the fully differentiated adipocytes respond to the non-selective β-adrenergic agonist isoproterenol with increases in intracellular cAMP. The differentiated adipocytes, but not the undifferentiated preadipocytes, also respond to β3 selective agonists. These β-adrenergic agonists stimulate lipolysis in the differentiated adipocytes, with EC 50's indistinguishable from those observed in freshly isolated adipose tissue. The OM-RBM cell line has been differentiated successfully up to passage 49 with no decrease in the percentage of cells that become adipocytes. Thus OM-RBM is an immortalized cell line, which differentiates into adipocytes, can be carried to high passage levels, and express all three β-adrenergic receptor subtypes. These cells should prove useful in the characterization of the activity of β-adrenergic receptors and adipocytes.

CZ 302 APPARENT LACK OF β_3-ADRENERGIC RECEPTORS AND OF INSULIN-RESPONSIVE GLUCOSE TRANSPORT IN BROWN ADIPOCYTES OF WARM- AND COLD-ADAPTED GUINEA PIGS, Jean Himms-Hagen, Nicole Bégin-Heick, Anna-Lisa Kates, Joan Triandafillou and Masoud Ghorbani, Department of Biochemistry, University of Ottawa, Ottawa, ONT K1H 8M5 Canada.

Noradrenaline (NA)-induced thermogenesis was large in adipocytes from brown adipose tissue (BAT) of cold-adapted guinea pigs (GPs) and absent in adipocytes from BAT of warm-adapted GPs. Its magnitude was correlated with the content of mitochondrial uncoupling protein in BAT. No thermogenic response to any β_3-adrenergic agonist (CL 316,243, D7114, BRL 26810, CGP 12177) occurred in either type of adipocyte. The receptor for stimulated thermogenesis is characterized as a β_1-adrenergic receptor (relative potencies isoproterenol (ISO) > adrenaline (A) = NA). Adenylate cyclase in membranes from BAT of both warm- and cold-adapted GPs was stimulated by adrenergic agonists with the relative potencies ISO > A = NA > salbutamol (also β_1). There was no response to any β_3-adrenergic agonist. Adipocytes of GP BAT, like adipocytes of GP white adipose tissue (WAT), apparently lack β_3-adrenergic receptors. Glucose transport by adipocytes of BAT of warm-adapted GPs was not stimulated by either NA or insulin (INS). In adipocytes from BAT of cold-adapted GPs, NA stimulated glucose transport, in association with stimulated thermogenesis, but there was no response to INS. GLUT4 is present in plasma membranes (PM) and microsomes (MIC) of GP BAT. The proportion in the PM was 36.7 ± 3.6 % (n = 25) of the sum of PM and MIC concentrations and this increased slightly after 1-4 weeks of cold-adaptation to 52.7 ± 5.1 % (n = 18). It is known that adipocytes of both BAT and WAT of GPs are sensitive to the antilipolytic effect of insulin (EC_{50} 10^{-9} M). There is a selective resistance to INS of glucose transport in GP BAT, as is known for GP WAT, and this is not improved by cold-adaptation. Thermogenesis in BAT needs glucose and in GPs this appears to be controlled by NA. GPs have highly mutated forms of some proteins, e.g. insulin and L-gulono-γ-lactone oxidase. They might also have mutated forms of both the β_3-adrenergic receptor and a component of the translocation mechanism for GLUT4. (Supported by the Medical Research Council of Canada).

CZ 303 **$G_{i\alpha}$ LEVEL IS REDUCED IN BROWN ADIPOSE TISSUE MEMBRANES OF RATS FED HIGH ENERGY DIETS, Yael Kenan[1], Martha Levinson[1], Mark Pines[2] and Michael Naim[1], [1]Department of Biochemistry, Food Science and Nutrition , The Hebrew University of Jerusalem and [2]Institute of Animal Science, Volcani Center, Bet Dagan, Israel**

Previous studies indicated that post-receptor responses of adenylate cyclase system were modified in the liver, white adipose tissue (WAT) and brown adipose tissue (BAT) of both genetically and dietary obese rodents. In the present study, we evaluated the level of G-proteins in order to investigate their role in the dietary fat-induced post receptor modifications. Membranes of WAT and BAT were prepared from rats fed a high-energy diet (EXP group), and from those fed a nutritionally-balanced one (CON group), for 2, 6 and 10 weeks. Immunoblotting analyses of BAT membranes from EXP rats showed significant lower level of $G_{i\alpha}$ after 6 and 10 weeks of feeding compared with the CON rats. No differences were found in the content of $G_{s\alpha}$ between the EXP and CON rats. Furthermore, the ratio of $G_{s\alpha}/G_{i\alpha}$ was significantly higher in membranes of EXP group than in CON group. In contrast to BAT membranes, in WAT membranes, there were no differences in both $G_{s\alpha}$ and $G_{i\alpha}$ levels between the two groups. It is hypothesized that the increased sensitivity of guanine-nucleotide-induced adenylate cyclase activity in BAT during dietary obesity, is related to the reduced levels of $G_{i\alpha}$.

CZ 304 REGULATION OF ß3 ADRENERGIC RECEPTOR mRNA LEVELS IN BROWN ADIPOCYTES DIFFERENTIATED IN VITRO, Susanne Klaus, Serge Raimbault and Daniel Ricquier, CEREMOD (C.N.R.S.) 9, rue J. Hetzel, F-92190 Meudon, France

Brown fat can play an important function in mammalian energy metabolism by its thermogenic, i.e. energy dissipating function which is due to the uncoupling protein (UCP), uniquely expressed in brown adipocyte mitochondria. Thermogenic function of brown fat is mainly mediated by adrenergic stimulation, acting on atypical, ß3 adrenergic receptors (ß3-AR).

Using PCR amplification of genomic DNA from the Siberian hamster, we generated a partial cDNA probe which was used in Northern blot analysis. Siberian hamster brown preadipocytes, differentiated in primary cell culture show high expression of UCP, whose adrenergic stimulation was found to be mainly mediated by ß3 ARs. In this cell model, ß3-AR mRNA levels were found to increase with differentiation, obtaining steady state levels higher than in brown fat in vivo, which were easily detectable by Northern blot analysis in total RNA extracts.

When cellular transcription was blocked by addition of actinomycin D, ß3-AR mRNA levels decreased very rapidly with a half life of about 50 minutes, suggesting a high turnover rate of this mRNA, i.e. a strong transcriptional regulation of its gene activity.

Stimulation of brown adipocytes with different adrenergic agonists (norepinephrine, isoproterenol, D7114, CGP 12177 at 1µM) as well as cAMP (1 mM) resulted in a rapid slight increase of ß3-AR mRNA levels after 0.5 to 1 h, followed by a subsequent decrease to about 20-30% of control values within 4 hours, with levels staying low for at least 24 hours. Dose-response experiments revealed a slight upregulation of ß3-AR mRNA at agonist concentrations of 1 nM, and a distinct downregulation by concentrations higher than 10 nM. No difference could be detected between the action of general ß-AR agonists (norepinephrine, isoproterenol) and specific ß3-AR agonists (D7114, CGP 12177). Dopamine, a catecholamine that has no ß3 adrenergic action and no effect on UCP expression, increased ß3-AR mRNA levels to approximately 200 % of control values at concentrations between 10 nM and 10 µm.

CZ 305 AN UPSTREAM ENHANCER REGULATING BROWN FAT SPECIFIC EXPRESSION OF THE MITOCHONDRIAL UNCOUPLING PROTEIN GENE, Ulrike C. Kozak, Jan Kopecky, Jan Teisinger, Sven Enerback, Bert Boyer and Leslie P. Kozak, The Jackson Laboratory, Bar Harbor, ME.

Previous studies on the regulation of a *Ucp* minigene in transgenic mice demonstrated that the sequences necessary for brown fat specific expression and inducibility by norepinephrine were located in the 5'-flanking region between 1 and 2.8 kb from the transcriptional start site. We have investigated this region in more detail in cultured mouse brown adipocyte tumor cells. Deletion analysis of two types of chloramphenicol acetyltransferase reporter gene constructs under control of either the *Ucp* promoter or a heterologous HSV-tk promoter defined an enhancer in a 220 bp Hind III/Xba I fragment which was essential for both brown fat specificity and norepineophrine inducibility. Site-directed mutagenesis of the reporter gene constructs established that independent mutations to a cAMP responsive element (CRE-2) or one of two TTCC motifs, all within 17 bp, eliminated transient expression. Competitive DNA mobility shift assays with probes of the CRE and the TTCC motifs (BRE, brown fat regulatory element) indicate that nuclear proteins interact with these motifs in a manner suggestive of heterodimeric transcription factors While these CRE-BRE probes do not show changes in binding which is dependent on norepinephrine treatment, a third TTCC motif located 130 bp downstream of BRE-1 does show this dependency. The results indicate that a complex interaction of the CRE and BRE motifs, which cannot be functionally separated, control *Ucp* expression.

CZ 306 MEMBRANE CONDUCTANCES OF CULTURED RAT BROWN FAT CELLS: EFFECTS OF NOREPINEPHRINE AND TIME IN CULTURE, Pamela A. Pappone, Sherwin Lee and Sonia I. Ortiz-Miranda, Section of Neurobiology, Physiology and Behavior, Division of Biological Sciences, University of California, Davis CA 95616

We are studying the role of cell membrane conductances in the hormonal and developmental responses of brown fat cells. To this end we have made patch voltage clamp measurements in acutely isolated and cultured brown adipocytes from neonatal rats. Five different membrane conductance mechanisms can be present in these cells; a voltage-gated, delayed rectifier type K conductance ($I_{K,V}$), a Ca-activated K conductance ($I_{K,Ca}$), a transiently activated Cl conductance, a nonspecific cation conductance, and a depolarizing conductance carried by very large (~250 pS) channels. All of these conductances can be modulated by adrenergic stimulation. The Cl conductance and the large channel conductance are present only in the first 1-3 days following isolation of the cells, and are apparently absent after longer times in culture. In contrast, $I_{K,Ca}$ was seen only in cells cultured for more than one day. $I_{K,V}$ was present in all cells we recorded from, but in many cells could be inactivated by brief exposure of the cells to norepinephrine or isoproterenol. We have previously shown that block of functional voltage-gated K currents prevents the proliferation and/or differentiation of brown fat cells from preadipocytes in culture. Thus, the hormone-induced inactivation of $I_{K,V}$ may be a part of the switch from a proliferative to a differentiative state that occurs in vivo in response to norepinephrine stimulation. These results suggest that membrane conductances in brown fat cells are modulated as a part of the long term hormonal control of adipocyte properties. Supported by NIH GM44840.

CZ 307 CCAAT-ENHANCER BINDING PROTEINS IN RAT BROWN ADIPOSE TISSUE AND THE REGULATION OF THE UNCOUPLING PROTEIN GENE PROMOTER. Francesc Villarroya, Pilar Yubero, Carles Manchado, Anne-Marie Cassard-Doulcier*, Roser Iglesias, Octavi Viñas, Teresa Mampel and Marta Giralt. *CEREMOD, CNRS, Meudon, France and Unitat de Bioquímica i Biologia Molecular B, Dpt Bioquímica i Fisiologia, Universitat de Barcelona, Barcelona, Spain.

The molecular mechanisms responsible for the establishment of the characteristic phenotype of the brown adipocyte are poorly known. To get further insight in this field, studies upon expression and functional role of the CCAAT-enhancer binding proteins (C/EBP) in brown adipose tissue were undertaken. C/EBP*α*, C/EBP*β* and liver inhibitory protein (LIP) were found to be expressed in rat brown fat. During development, peak values of expression were attained in the late fetal life, when the transcription of the uncoupling protein (UCP) gene, the molecular marker of terminal brown fat cell differentiation, is switched on. A specific increase in CEBP*β* expression was observed in brown fat from cold-exposed rats, in concurrence with tissue hyperplasia and rise of UCP gene expression. Mice primary brown adipocytes in culture were transiently co-transfected with C/EBP expression vectors and plasmids where the CAT reporter was driven by the rat UCP gene 5' region. C/EBP*α* and C/EBP*β* trans-activated the UCP gene promoter. Mutant deletion analysis indicated that the cis-acting elements responsible for this trans-activation were present in the proximal 5' non-coding region of the UCP promoter. DNAse I footprint analyses of that region indicated the presence of two sites capable to interact with C/EBP*α* and CEBP*β* as well as with C/EBP proteins present in nuclear extracts from brown adipose tissue. Both sites of the UCP gene showed affinities for C/EBP*α* and C/EBP*β* in the range of functionally relevant C/EBP binding sites from other genes, as assessed by gel-shift competition analyses. When two copies of each site were cloned upstream the enhancerless SV1-CAT, they conferred C/EBP responsiveness to that promoter. Results indicate that the UCP gene transcription is regulated by C/EBP proteins and suggest that this family of transcription factors may be involved in the differentiation of the brown fat cell.

CZ 308 BETA$_3$-RECEPTOR AND ADENYLYL CYCLASE ACTIVITY IN WHITE ADIPOSE TISSUE OF LEAN AND OBESE (OB/OB) MICE, Nicole Bégin-Heick, Department of Biochemistry, Faculty of Medicine, University of Ottawa, Ottawa, Canada, K1H 8M5

Adenylyl cyclase (AC) activity in response to β_3-adrenergic activation was assessed in white epididymal adipose tissue (WAT) of lean and obese mice. In WAT of lean mice, the maximal AC activity elicited by the β_3 agonist (B3A) was the same as that elicited by isoproterenol (INA) (~ 5-10 fold of the activity obtained with GTP alone, depending on the concentration of GTP) but its potency was more than 10 times greater (EC_{50} = 1μM for B3A compared to 20 μM for INA). In the WAT from obese mice, under conditions that allow maximal activation by INA, the relative activity elicited by INA was more than that elicited by B3A (2 vs 0.5 fold). Under these conditions, B3A was less potent than INA (EC_{50} = ‹ 100 μM vs 2μM). These data show that the β_3 receptor is predominant in WAT from lean mice but it is less abundant than the β_1 and β_2 receptors in WAT from obese mice. Experiments with antagonists confirmed these findings. To explore the possibility that the hyperinsulinemic or hypercorticoid status of the obese mice was responsible for the suppression of β_3 receptor activity the effect of adrenalectomy (ADX), which corrects both, was assessed. When compared with preparations from sham-operated (SHM) obese mice, the ADX were not significantly different in either the potency or the activity of INA and B3A., which were both lower than the values obtained with control lean mice. To determine whether the progress ion of the obesity syndrome influences the expression of β-adrenergic receptor function, experiments were done with young (4-6) wks and older (10-12 wks) obese mice. There were no differences between the two age groups in the response to INA or B3A.

Supported by the Medical Research Council of Canada.

CZ 309 EFFECT OF OBESITY AND AGE ON MUSCLE FATTY ACID BINDING PROTEIN, Julie O. Carey, Patricia L. Dolan, G. Lynis Dohm, Department of Biochemistry, East Carolina University, Greenville, NC 27858

Fatty acid binding proteins (FABP) transport fatty acids from the plasma membrane to the mitochondria where they are subsequently oxidized. FABP protein concentration increases during conditions of increased fat oxidation, thus FABP may be an important component in the regulation of fat oxidation. Obesity in Zucker rats has been reported to increase (Morrow et al. Fed. Proc. 38:280, 1979) or have no effect (Malewiak et al. Int. J. Obesity 12:543-546, 1988) on liver fatty acid binding protein. The effect of obesity on muscle fatty acid binding protein (H-FABP) has not been reported. Because age can effect protein expression, we measured H-FABP protein in gastrocnemius muscles from 9 and 28 week old lean and obese Zucker rats. H-FABP protein levels were higher in lean than obese Zuckers at 9 weeks, but not significantly so. At 28 weeks of age, H-FABP protein levels were 10-fold higher in the obese rats compared to lean controls. These findings reinforce the trend observed by McNamara et al. (Am. J. Physiol. 243:R258-R264, 1982) where soleus muscles from obese Zuckers oxidized less palmitic acid than muscle from lean rats at 6 weeks but oxidized more at 12 weeks of age. This obesity-linked increase in fat oxidation with age, coupled with the present observation that H-FABP increases in older obese rats supports the hypothesis that H-FABP plays an important role in fat oxidation.

Supported by NIH grant DK38416.

CZ 310 IMPAIRED EXPRESSION AND FUNCTIONAL ACTIVITY OF THE β_3- AND THE β_1-ADRENERGIC RECEPTORS IN ADIPOSE TISSUE OF CONGENITALLY OBESE (C57Bl/6J **ob/ob**) MICE. Sheila Collins, Kiefer W. Daniel, Elizabeth M. Rohlfs, Ian L. Taylor# & Thomas W. Gettys# Depts of Medicine (GI) and #Cell Biology. DUMC Durham, NC 27710. Adipocytes from genetically obese (**ob/ob**) mice display an impaired response to β-adrenergic stimulation, but the molecular defects are not unequivocally identified. The mRNA expression and functional activity of the β_1-, β_2-, and β_3-adrenergic receptor (AR) subtypes in white and brown adipose tissue from genetically lean (L) and obese (Ob) mice were compared. β_3AR mRNA levels were dramatically reduced (> 350-fold) in 12-week old Ob mice compared to L animals. β_1AR mRNA levels were also reduced (4-fold) in Ob mice, while β_2AR mRNA levels were not significantly changed. β-agonist stimulated adenylyl cyclase (AC) activity was measured in adipocyte plasma membranes with βAR subtype-selective agonists and antagonists. Dose-response curves with epinephrine (EPI) from L mice were best fit to a 2-component model of 20% high-affinity ($K=1.48 \times 10^{-7}M$) and 80% low-affinity ($K=1.86 \times 10^{-5}M$) components, corresponding to activation of β_1AR and β_2AR conjointly, and β_3AR, respectively. The β_1AR-selective antagonist CGP20712A (CGP) reduced the high-affinity component to ~10%, while the non-selective β-antagonist propranolol eliminated the high-affinity component. The β_3AR-selective agonist BRL37344 stimulated AC in L membranes to a slightly lesser extent than EPI, but was more potent ($K_{act} = 3.61 \times 10^{-8}M$). In Ob mice, stimulation of AC by all agonists was severely blunted and was best fit to a single class of sites. Studies with CGP or the β_2AR-selective antagonist ICI118,551 show this residual response to be predominantly β_2AR. Expression of βAR subtypes in both brown and white adipose tissue of weanling Ob mice (4-5-wks age) was also affected, but to a lesser extent, consistent with the progressive severity of the obesity with age. Together the reduction in expression of the β_3AR and β_1AR impair the β-agonist-stimulated AC response over a broad concentration range by greatly lowering the maximum stimulation and shifting the adrenergic sensitivity at low concentrations from a mixed β_1AR/β_2AR response to predominantly β_2AR.

CZ 311 ACTIVITY OF GAPDH PROMOTER IN TRANSFECTED RAT ADIPOCYTES. EFFECT OF GENETIC OBESITY.
Dugail, I, Rolland, V, Le Liepvre, X, Lavau, M.
INSERM U177 Institut biomédical des cordeliers Paris, FRANCE.
We have shown that the adipose tissue of young obese Zucker rats was characterized by an over transcription of a subset of genes linked to fat storage activity such as GAPDH, FAS and ME, suggesting that the fatty mutation affected a regulatory step of adipocyte transcriptional activity. As an approach to this question, we examined the activity of GAPDH promoter, using the well-characterized 500bp promoter fragment of human GAPDH (gift of M. Alexander-Bridges), fused to CAT, in transiently transfected adipocytes. Mature adipocytes isolated from 30 day-old lean (Fa/fa) and obese (fa/fa) rats were co-transfected by electroporation with 25µg of p(-486+21hGAPDH-CAT) and 2µg of pRSVBgal to normalize for efficiency of transfection. Results were expressed as the ratio of CAT to Bgal activity measured 2 days after transfection. In agreement with experiments on HepG2 cells, which defined an insulin responsive element on this fragment, transfection of p(-486+21hGAPDH-CAT) in mature adipocytes from lean rats resulted in a 3 fold increase in CAT/Bgal activity in the presence of insulin. In transfected adipocytes from obese rats compared to lean rats, there was a 2.6 fold increase in CAT/Bgal ratio. The study of nuclear protein interactions with GAPDH promoter fragments by gel retardation assay did not allow to detect any difference in band pattern or intensity between lean and obese adipocyte nuclei.
In conclusion this study suggests that -486+21 region of GAPDH promoter contains DNA sequences that binds trans-acting factor(s) responsible for GAPDH overexpression in the genetically obese rat. Their characterization deserves further investigation.

CZ 312 MAPPING CANDIDATE GENES FOR OBESITY USING SINGLE STRANDED CONFORMATIONAL POLYMORPHISM ANALYSIS AND ALLELE-SPECIFIC OLIGONUCLEOTIDE HYBRIDIZATION, Fred T. Fiedorek, Jr., Philippa Charlton, Eric S. Kay, Poorvi J. Shah, and Bryan S. Vinik, Departments of Medicine & Pediatrics and Curriculum in Genetics & Molecular Biology, University of North Carolina School of Medicine, Chapel Hill, NC 27599.
The genetic basis underlying the high degree of heritability for human obesity is unclear. Putative candidate genes for these common polygenic human disorders include cDNAs encoding neuropeptides and their receptors, enzymes of nutrient intermediary metabolism, and cellular proteins involved in growth factor and hormonal signal transduction cascades. PCR-based cloning has been used to examine candidate cDNAs for DNA sequence alterations in the coding, intronic, and 3′ untranslated regions. Single stranded conformational polymorphism (SSCP) and allele-specific oligonucleotide (ASO) variants have been identified for such candidate genes in genomic DNA from a variety of inbred mouse strains and somatic hybrid cell lines. The segregation of these SSCP and/or ASO variants has been analyzed in several genomic mapping resources including an F_1(C57Bl/KsJ X *Mus m. musculus* Czech II) X C57Bl/KsJ intrasubspecific backcross, recombinant inbred lines, and panels of human/rodent and mouse/rodent somatic hybrid cell lines and radiation-reduced cell lines. Through these genetic approaches, chromosomal localizations of several candidate genes on human and mouse genetic maps have been established. An improved understanding of genetic determinants involved in obesity and diabetes inheritance should be gained as genetic mapping and mutation screening of putative disease loci is accomplished. [Supported by NIH Grant DK-44074.]

CZ 313 PROTECTION AGAINST INCREASED ADIPOSITY DUE TO HIGH FAT FEEDING IN TRANSGENIC MICE OVEREXPRESSING GLUT4 SELECTIVELY IN FAT, Luigi Gnudi, Effie Tozzo, Peter R. Shepherd, Judy L. Bliss and Barbara B. Kahn, Diabetes Unit, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.
We recently reported adipocyte hyperplasia and enhanced glucose disposal in transgenic (T) mice overexpressing the human GLUT4 gene driven by the aP2, fatty acid binding protein, promoter/enhancer. Now we report the effects of high fat feeding on adiposity and glucose tolerance in male T mice compared to wildtype (W) littermates. Mice were caged individually and fed either a control (Con) diet (calories: 10% from fat, 69% carbohydrate, 21% protein) or a high fat (Hifat) diet (55% fat, 24% carb, 21% pro) from 3 wks of age to 16-18 wks. All groups had a similar calorie intake. W on Con diet was the lightest; T on Con diet was ~5 g heavier. W and T on Hifat diet were similar in weight. I.P. glucose tolerance tests showed enhanced glucose disposal in T on Con diet compared to W on the same diet. Both T and W on Hifat diet had decreased glucose disposal compared to littermates on Con diet. Carcass analysis showed a doubling of body lipid in W on Hifat compared to W on Con diet. In T on Con diet, lipid was increased 60-80% compared to W on Con diet but T on Hifat tended to be leaner than T on Con diet. Adipose cell size was increased 2-fold in W on Hifat compared to W on Con diet while no difference was detected between T on both diets. Basal and insulin-stimulated glucose transport in isolated adipocytes was markedly decreased in W on Hifat diet while T on Hifat showed no down regulation due to persistent overexpression of the transgene; basal transport was 14-fold and insulin-stimulated 4.3-fold above W on Con diet. Thus, while overexpression of GLUT4 selectively in fat induces obesity in T on a Con diet, the T is protected against a further increase in adiposity due to high fat feeding. T on Hifat diet show in vivo insulin resistance most likely due to decreased glucose uptake by skeletal muscle since adipose cells are protected against insulin resistance. We speculate that the decreased adiposity in T on Hifat diet may be due to enhanced partitioning of glucose to fat resulting in increased oxidation of fatty acids by muscle rather than fat storage in adipocytes.

CZ 314 DEVELOPMENT OF SEVERE INSULIN RESISTANCE IN TRANSGENIC MICE WITH BROWN FAT DEFICIENCY, Andreas Hamann, Heike Benecke, Vedrana S.-Susulic, Bradford B. Lowell and Jeffrey S. Flier, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

We created transgenic mice in which the uncoupling protein promoter drives expression of diphtheria toxin A-chain in brown adipose tissue (BAT), thus causing ablation of BAT. Transgenic animals have primary BAT deficiency, severe obesity, insulin resistance and late onset hyperphagia. The goal of the present study was to further define the insulin resistant state of BAT deficient animals. Transgenic mice, 20 - 30 weeks old, were massively obese (> 50% body fat). In the fed state, older transgenic females had a mild increase (40%) in blood glucose and a 13 fold increase in plasma insulin levels while older transgenic males had a 2.6 fold increase in glucose and a 70 fold increase in plasma insulin levels. Fasted female mice responded to glucose tolerance tests (GTT, 1 mg glucose/g mouse i.p.) and insulin tolerance tests (ITT, 0.6 U insulin/kg mouse, i.p.) with the following glucose values (mg/dl):

GTT:	0	30	60	90	150	210 min
Trans.	105±10	265±26	253±32	267±40	177±31	117±16
Cont.	92±5	174±7	160±13	123±6	84±7	81±8

ITT:	0	15	30	45 min
Trans.	137±9	169±22	164±30	160±34
Cont.	113±7	119±9	71±9	70±9

Insulin receptor number and tyrosine kinase activity were determined in older female mice using an solid phase, microtiter plate immunoassay. In transgenic animals total insulin receptor number was decreased by 36% in muscle and 59% in adipose tissue. Insulin receptor tyrosine kinase activity, which was assessed following maximal insulin stimulation *in vivo*, was reduced in transgenic animals to a degree proportional to the decline in receptor number. In conclusion, primary BAT deficiency results in the development of severe insulin resistance with both receptor and post-receptor components. These animals should be a useful model for studies of obesity-induced NIDDM.

CZ 315 LIPOLYTIC RESPONSE IS NOT DECREASED IN NEWLY DIFFERENTIATED ADIPOCYTES FROM OBESE ZUCKER RATS, Dorothy B. Hausman, Brenda G. Marques and Roy J. Martin, Department of Foods and Nutrition, University of Georgia, Athens, GA 30602

This study was designed to determine if the reduction in lipolytic sensitivity and responsiveness previously observed in isolated adipocytes from obese Zucker rats is also present in adipose cells grown in culture from obese Zucker rats. Adipocytes and stromal vascular cells were isolated from inguinal adipose tissue of 7.5 week old male lean and obese Zucker rats via collagenase digestion. Adipocytes were washed in a Krebs-ringer bicarbonate buffer containing 5 mM glucose and 4% bovine serum albumin, pH 7.4 (KRBC) and incubated for 2 h at 37° C in KRBC containing various concentrations of isoproterenol. The media was subsequently collected for determination of glycerol release. Alternatively, stromal vascular cells were plated (9 x 10^4 cells) for 24 h in 10% fetal bovine serum and cultured in serum free media supplemented with insulin, transferrin, selenium and triiodothyronine. On day 8 of culture, a time when the newly differentiated adipocytes contained an abundance of lipid droplets, culture media was removed and the cells were washed with KRBC and exposed to various concentrations of isoproterenol for determination of lipolytic response. Maximum lipolytic response was similar in newly differentiated adipocytes from lean and obese rats. Lipolytic sensitivity was greater in newly differentiated adipocytes from both phenotypes relative to the freshly isolated adipocytes. Consistent with previous results, lipolytic sensitivity and responsiveness were both significantly lower in freshly isolated adipocytes from obese as compared to lean Zucker rats. These results suggest that the alterations in lipolysis observed in the obese Zucker rat may be due to an altered hormonal and metabolic milieu rather than intrinsic defects within the adipose cell. *Supported in part by a grant from the Weight Watchers Foundation.*

CZ 316 CHRONIC ADMINISTRATION OF A_1 ADENOSINE RECEPTOR ANTAGONISTS TO OBESE AND LEAN ZUCKER RATS, K.F. LaNoue and D. Luthin, Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA 17033 and Department of Physiology, University of Virginia, Charlottesville, VA 22908

Previous studies of isolated adipocytes and adipocyte plasma membranes from lean and obese Zucker rats have indicated that the A_1 adenosine receptors are particularly active in the obese adipocytes. Excessive activity results in inhibition of adenylate cyclase and low hormone-stimulated lipolysis. Therefore the effect of chronic systemic administrative of adenosine receptor antagonist 1,3 dipropyl-8-p-acrylate phenylxanthine (BW 1433) which does not cross the blood brain barrier was tested on glucose tolerance, glycerol production and weight gain. When the antagonist was administered chronically over a 7 day period using subcutaneously implanted osmotic minipumps (0 and 0.4 mg/kg/hr) glycerol levels of lean animals were not altered by the drug but they increased by 72% in obese animals, peaking at day 2 and declining back to normal at day 7. At day 7, the areas under glucose tolerance curves of untreated animals were 50% larger in obese than lean animals. The drug lowered these areas 50% in lean animals and 30% in obese. BW1433 also significantly altered weight gain from day 1 through day 3 but in opposite directions for lean vs. obese animals.

In order to determine the molecular basis for the adaptation to chronic administration of A_1 adenosine receptor antagonists, levels of A_1 adenosine receptors, β-adrenergic receptors, and G-proteins were quantitated. G-proteins were quantitated using specific antibodies in combination with pure recombinant standards. Antagonist binding studies of isolated fat cell membranes obtained from antagonist treated and vehicle treated animals after 7 days of treatment revealed a 50% increase in A_1 adenosine receptors in obese treated, but no change in lean treated, animals relative to vehicle treated. Neither group of animals exhibited a change in β-adrenergic receptors, but the level of Gsα (52 kDa) was decreased to 47±15% of vehicle control by antagonist treatment in obese but not lean animals' membranes. (PHS 1 R01 DK44070-01 and AHA Natl. 891212)

CZ 317 HORMONE-SENSITIVE LIPASE DEFICIENCY IN A PATIENT WITH ABDOMINAL OBESITY, Dominique Langin[1], Signy Reynisdottir[2], Peter Arner[2] and Cecilia Holm[3], [1]INSERM U-317, CHU Rangueil, Bât. L3, 31054 Toulouse Cedex, France, [2]Department of Medicine, Huddinge University Hospital, Karolinska Institute, Sweden and [3]Department of Medical and Physiological Chemistry, Lund University, Sweden

Hormone-sensitive lipase (HSL) has a critical role in the control of energy homeostasis by catalyzing the hydrolysis of adipocyte triacylglycerol and thereby releasing free fatty acids for transport to energy-requiring tissues. HSL has been suggested to play a role in lipid metabolism disorders, but up to date there has been no report on a direct involvement of HSL. A 72-year old male caucasian patient with abdominal obesity and impaired adipose tissue lipolysis was investigated in comparison with 8 healthy elderly subjects (65-75 years). Lipolysis studies performed on adipocytes isolated from subcutaneous abdominal adipose tissue revealed that the patient presented a dramatically impaired lipolytic response at the level of the protein kinase A/HSL complex since neither isoproterenol (a β-agonist), nor forskolin (a direct activator of adenylyl cyclase) and dibutyryl cyclic AMP were able to stimulate glycerol release. HSL activity was determined using a highly specific substrate and known inhibitors of HSL. HSL activity of the patient was 7-11% of the activity of the healthy subjects. A comparable decrease of the amount of HSL protein was observed in Western blot analyses. The HSL mRNA level of the patient was 33% of that of the controls.

This study is the very first to describe a patient with HSL deficiency. The data indicate that the absence of detectable stimulation of lipolysis is due to the very low level of HSL in the tissue. HSL mRNA level was also reduced although to a lesser extent than HSL activity and protein amount. This difference could indicate that the mechanism underlying the low HSL expression is dual with both transcriptional and post-transcriptional effects.

CZ 318 GLUCOSE MODULATION OF LIPID STORAGE-RELATED ENZYMES IN PREADIPOCYTES FROM OBESE ZUCKER RATS.

Quignard-Boulangé A, Briquet-Laugier V, Rolland V, Ardouin B, Lavau M, INSERM U177, 75006 Paris, FRANCE.

Previously we showed that both mature adipocytes and preadipocytes from genetically obese Zucker rats displayed an hyperactive lipid storage capacity after long term culture suggesting an intrinsic alteration in these cells. In order to test whether nutritional regulation of adipose-related enzymes could be affected by genotype, glucose effect on expression of these enzymes was compared in obese and lean preadipocytes differentiated *in vitro*.

Preadipocytes were isolated from inguinal adipose tissue of 4-wk old obese and lean Zucker rats. Cells were differentiated in DMEM containing 10% FCS, 5.5mM glucose, 33µM biotin and 15nM insulin. At day 12, dishes were exposed or not to high glucose concentration (20mM). Activities of fatty acid synthase (FAS), malic enzyme (ME), glycerol 3-phosphate dehydrogenase (GPDH) and lipoprotein lipase (LPL) were determined. Amount of LPL, Glut4 and actin mRNAs were also estimated from Northern blots. In basal conditions, obese preadipocytes exhibited significantly more active adipose-related enzymes than those from lean. When cells were cultured for 7 days in 20mM glucose, there was no change in enzyme activities in lean cells except for LPL that was 2-fold increased. By contrast, in obese preadipocytes, 20mM glucose provoked 50 to 120% increase in ME, FAS, LPL activities amplifying genotype effect. Similar increase in amount of LPL and glut4 mRNA was observed in obese cells. The increased FAS activity mediated by the presence of 20mM glucose was observed after 24h of treatment. Such increase was prevented by the addition of actinomycin D suggesting that glucose could act at a transcriptional level.

In conclusion, preadipocytes from obese rats exhibited *in vitro* high sensitivity to glucose that could explain the emergence of genotype differences in this cultured adipocyte system and argues for an adipocyte intrinsic expression of the fatty mutation.

CZ 319 EXPRESSION AND FUNCTION OF THE AGOUTI GENE PRODUCT, A GENETICALLY DEFINED LESION INVOLVED IN OBESITY/DIABETES, William Wilkison, Inder Patel, Laurie Overton, Bruce Wisely, Sue Kadwell, and *Rick Woychik. Glaxo Research Institute, Inc. 5 Moore Drive, Research Triangle Park, NC, 27709 and *Oak Ridge National Labs, Oak Ridge, TN 37831.

The agouti locus has been recently shown to be involved in the development of a non-insulin dependent diabetic phenotype in animals which ectopically overexpress this gene. Recently, the gene encoding the agouti locus has been cloned and sequenced and shows no obvious homology to any other known coding sequence. In an attempt to understand the function of this polypeptide, we have recently expressed the agouti protein in both Cos cells and a baculovirus/insect cell line. Using tagged constructs, the protein expressed in Cos cells has an apparent molecular weight slightly larger than that predicted. Furthermore, we have developed a functional assay for the agouti protein, based on its ability to lower α-MSH-induced cAMP levels. These assays show that introduction of an agouti expression vector into either COS or the insect cells allows the production of a secreted factor which antagonizes cAMP levels. Detailed characterization of this antagonism, and the implications of this activity will be discussed.

Late Abstracts

PERILIPIN IS ON THE SURFACE LAYER OF INTRACELLULAR LIPID DROPLETS IN ADIPOCYTES AND ADRENAL CELLS, E.J. Blanchette-Mackie, N.K. Dwyer, T. Barber, R.A.Coxey, C.M. Rondinone and C. Londos, NIDDK, National Institutes of Health, Bethesda, MD 20892.

We have examined the intracellular location of perilipin in cells and tissues involved in lipid metabolism using confocal light microscopy and immunogold staining of cryosections for electron microscopy (EM). Perilipin is located on the surface phospholipid monolayer surrounding lipid droplets. Freeze-fracture EM revealed that the hydrophobic face of the surface monolayer that apposes the triacylglycerol (TG) core contained particles identical in size and distribution to intramembranous particles (IMPs), an exclusive feature of the hydrophobic faces of bilayered membranes. This structural evidence is consistant with the concept that TG, synthesized by enzymes bound to the endoplasmic reticulum, "oils out" as a separate phase of hydrophobic lipid droplets between leaflets of endoplasmic reticulum (EM) membrane bilayers. Lactating mammary gland (LMG) and liver of newborn mice contain cells other than adipocytes that synthesize and store TG in intracellular lipid droplets. Although perilipin was at the surface of lipid droplets in LMG adipocytes, none was associated with milk lipid droplets in alveolar epithelial cells, nor was the protein located on lipid droplets in hepatocytes. However, perilipin was present on the surface of cholesteryl ester droplets in steroidogenic Y-1 mouse adrenal cells, but not in non-steroidogenic SW13 human adrenal cells, which also contain cholesteryl ester droplets. Thus perilipin is present in cells which have the capacity to hydrolyze TG or cholesteryl esters via a lipase similar, if not identical, to hormone sensitive lipase. While these results point to a role for perlipin in lipid hydrolysis, they do not preclude participation of the protein in structural or lipogenic functions.

PHOSPHATIDYLINOSITOL 3-KINASE ACTIVATION IS REQUIRED FOR INSULIN-STIMULATED DNA SYNTHESIS BUT NOT FOR INSULIN-STIMULATED GLUCOSE TRANSPORT OR ANTI-LIPOLYSIS, Joseph L. Evans and Christian M. Honer, Diabetes Department, Sandoz Research Institute, East Hanover, NJ 07936

The significance of phosphoinositide second messengers as mediators of eukaryotic signal transduction is universally accepted. The hydrolysis of phosphatidyl(Ptd)inositol(Ins) (4,5) bisphosphate(P) by phospholipase C generates diacylglycerol, which activates protein kinase C, and PtdIns (1,4,5)P_3, which binds to its intracellular receptor and mobilizes calcium. The physiological function of another group of inositol lipids including PtdIns-3-P, PtdIns (3,4)P_2, and PtdIns (3,4,5)P_3 is presently unknown despite being the focus of intensive investigation. These molecules are produced following the activation of PtdIns 3-kinase (PI-3-kinase), which selectively phosphorylates the 3-position of the inositol ring. PI-3-kinase is rapidly activated following stimulation by insulin and other growth factors, an effect which is mediated by SH2 interaction of PI-3-kinase with receptor tyrosine kinases or their substrates. In this study, we have begun to investigate the role of PI-3-kinase activation in mediating selected metabolic and mitogenic effects of insulin employing a recently discovered (by others) inhibitor of PI-3-kinase activity. In isolated rat adipocytes, the inhibitor was inactive (at concentrations up to 10 μM) on either basal or insulin-stimulated glucose transport and lipolysis. In contrast, the inhibitor (100 nM) caused a substantial reduction (> 60%) in insulin-stimulated DNA synthesis in Fao hepatoma cells. It is concluded that 1) the metabolic and mitogenic effects of insulin are mediated by distinct signalling pathways, 2) PI-3-kinase activation is necessary for maximum insulin-stimulated DNA synthesis, and 3) targeting PI-3-kinase for activation (via SH2 agonists) would not significantly enhance glucose transport or glucose disposal.

PROTEIN AND LIPID INTERACTIONS IN THE ADIPOCYTE LIPID BINDING PROTEIN, Christopher D. Kane*, Judith M. LaLonde#, Leonard J. Banaszak#, and David A. Bernlohr*||, the *Department of Biochemistry, College of Biological Sciences, #Department of Biochemistry, Medical School, || Institute of Human Genetics, University of Minnesota, St. Paul, MN 55108

The Adipocyte Lipid Binding Protein (ALBP) is a low molecular weight, soluble protein expressed specifically in adipocytes and which displays the ability to bind hydrophobic ligands such as fatty acids and retinoic acid. Previous work by our laboratory has shown that these ligands are bound through a combination of both enthalpic and entropic factors. Given the heterogeneous character of these binding forces, we hypothesize that ALBP may be capable of binding alternative ligands. An analysis of hexadecanesulfonic acid (HDSA) via sulfhydryl protection assay and X-ray crystallography shows that this C:16 dianionic fatty acid analog is capable of being bound by ALBP in a manner homologous with other fatty acid molecules. The conformation of bound HDSA as well as the distance of its dianionic head group from the calyx of the binding pocket remain highly conserved with the conformation of monoanionic palmitic acid. The ability to bind hydrophobic ligands other than fatty acids alludes to the possibility that ALBP may have alternative roles within the adipocyte that have yet to be addressed. Grant support provided by NSFDMB 9118658 and a Arnold H. Johnson Fellowship.

GLUCOSE TRANSPORT, GLUCOSE TRANSPORTER AND GLUCOSE TRANSPORT GENE IN LONG-TERM PRIMARY CULTURE ADIPOCYTES, Shinobu Satoh, Stephan Krief, Andrew S. Greenberg, Takehiko Takeda, Constantine Londos, Alan R. Kimmel and Samuel W. Cushman, Yokohama City University, Yokohama, Japan and NIDDK/NIH, Bethesda, MD 20892

We have placed adipose cells isolated from rat epididymal fat pads in culture with serum-free DMEM and at various times analyzed glucose transport activity (GTA), glucose transporter (GLUT1 and GLUT4) and the expression levels of mRNA for glucose transporters. Basal GTA increased gradually and acutely insulin-stimulated GTA decreased markedly, then more gradually. After 7 days of culture, insulin no longer stimulates GTA over basal level. The total number of GLUT1 per rat adipose cell increases almost 2-fold over 10 days. GLUT1 spontaneously redistributes from low density microsomes (LDM) to plasma membranes (PM) with time in the basal state until no intracellular pool is left after 3-4 days. The total number of GLUT4 per rat adipose cell decreases markedly over the first 2 days, then more gradually until almost no GLUT4 are detectable after 10 days. A small spontaneous redistribution of GLUT4 from LDM to PM occurs over 3-4 days in the basal state but then the overall disappearance of GLUT4 becomes the predominant feature. GLUT 1 mRNA levels increase slightly during the initial hours of culture, but remain constant during the next 7 days. In sharp contrast, the levels of mRNA for glucose transporter GLUT4 decline precipitously within 6 h, but begin to rise again after 24h. A plateau is reached by 72 h which is maintained over the next 4 days at a level lower than that observed in fresh cells. In addition, the pattern of change in GLUT4 mRNA levels generally parallels that of several fat-specific genes such as hormone-sensitive lipase. Thus, while the functional consequences of these changes remain to be established, stable glucose transporter gene expression is achieved in 72 h. The insulin resistance observed when isolated rat adipose cells are placed in primary culture is the consequence of 1) a switch from the GLUT4 to GLUT1 glucose transporter isoform and 2) loss of the mechanism which maintains a sequestered intracellular pool of glucose transporters for translocation in response to insulin.

BIOLOGICAL EFFECTS OF HUMAN GROWTH HORMONE ON RAT ADIPOCYTE PRECURSOR CELLS IN PRIMARY CULTURE, Martin Wabitsch[1,2#], Mapoko M. Ilondo[1], Eberhard Heinze[2], Ronald M. Shymko[1], Walter M. Teller[2], and Pierre De Meyts[1], Hagedorn Research Institute[1], DK-2820 Gentofte, Denmark, and Department of Pediatrics 1, University of Ulm[2], D-87075 Ulm, Germany

The effects of growth hormone (GH) on the differentiation and the proliferation of primary adipocyte precursor cells isolated from rat epididymal fat pads were studied under chemically-defined conditions using a serum-free culture medium. In the presence of both triiodothyronine and insulin, up to 90% of the initially fibroblast-like cells differentiated within 8 days into mature fat cells. ^{125}I-hGH binding to the cells increased significantly during adipose conversion. In newly differentiated cells (day 6), the binding kinetics reached steady-state after 180 min at 22° C. Scatchard analysis yielded linear plots with an apparent K_d of 0.16 nM and 8400 sites/cell. Human GH (hGH) at 0.5-50 nM dose-dependently inhibited the differentiation and decreased glycerophosphate dehydrogenase activity. In cultures treated with 5nM hGH, insulin-stimulated glucose uptake and lipogenesis were significantly reduced (61 ± 3 and 89 ± 4 % of control, respectively, $p<0.01$), whereas a 5-fold stimulation of lipolysis was observed. All these hGH effects were not affected by an anti-IGF I monoclonal antibody (IGF I-MAb), suggesting a direct GH action, and were observed even in the presence of corticosterone which by itself stimulated adipocyte differentiation, glucose uptake and lipogenesis. In undifferentiated cells, 5 nM hGH markedly stimulated IGF I production (12-fold) and slightly increased both cell number and ^{3}H-thymidine incorporation (1.3 and 2.6-fold, respectively). At variance with the direct GH action on cell differentiation, the cell proliferation effects were suppressed by the IGF I-MAb. Taken together, these data show that, in contrast to established preadipocyte cell lines, hGH directly inhibits the adipose conversion in primary cultures of rat adipocyte precursor cells while indirectly promoting cell proliferation through an increase in IGF I production. #was recipient of an ESPE Research Fellowship.