# THE ADIPOSE CELL: A MODEL FOR INTEGRATION FOR HORMONE SIGNALLING IN THE REGULATION OF CELLULAR FUNCTION

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January 18-24, 1991

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# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

*Keynote Address (joint)*

**CA 001** A MOLECULAR DISSECTION OF VESICULAR TRANSPORT, James E. Rothman, Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, New Jersey 08544-1014.

Protein transport between successive cisternae of the Golgi stack is reconstituted when isolated Golgi membranes are incubated with cytosol and ATP. This transport is due to rounds of budding and fusion of coated vesicles. When transport is blocked by adding the nonhydrolyzable analogue of GTP, GTP$\gamma$S coated vesicles massively accumulate. The coated vesicles can be purified by density gradient centrifugation following extraction with salt, and contain a number of characteristic polypeptide chains including several GTP-binding proteins, but not clathrin. Following transfer of budded coated vesicles between cisternae, the coats are removed, and fusion proceeds in an ATP dependent process. Fusion requires an NEM-sensitive protein (NSF), a series of soluble NSF attachment proteins (SNAPs), palmityl-CoA as well as other cytosolic factors. The same pathway appears to account for transport from the endoplasmic reticulum to the Golgi, as well as transport within the Golgi, in both animals and in yeast.

*Adipose Cell Growth and Development*

**CA 002** MULTIPLE SIGNALLING PATHWAYS AND ADIPOSE CELL DIFFERENTIATION, Gérard Ailhaud, Ez-Zoubir Amri, Christian Dani, Danielle Gaillard, Paul Grimaldi, and Raymond Négrel, Centre de Biochimie du CNRS, U.F.R. Sciences, Parc Valrose, 06034 Nice, France

The differentiation of preadipocyte Ob17 cells involves sequential events. Emergence of early markers [lipoprotein lipase (LPL), pOb24] is coupled to growth arrest whereas that of late markers such as glycerophosphate dehydrogenase (GPDH) (defining terminal differentiation) are coupled to hormone supplementation and a limited growth resumption of early marker-expressing cells. Among positive factors regulating differentiation, arachidonic acid (ARA) has been identified as the main mitogenic-adipogenic factor controlling growth resumption and terminal differentiation. The effects of ARA are i) blocked by cyclooxygenase inhibitors, ii) mimicked by a stable analogue of prostacyclin (carbaprostacyclin) and iii) potentiated by $PGF_{2\alpha}$. Since these prostanoids are known to be synthesized and secreted by preadipocytes, we have proposed that both prostacyclin and $PGF_{2\alpha}$ play a critical role by means of an autocrine mechanism (1,2). In agreement with this hypothesis, antibodies directed against $PGF_{2\alpha}$ and prostacyclin were found to counteract the effects of ARA. Under these conditions, glucocorticoids appeared to play a permissive role for terminal differentiation, whereas sex steroids were inactive. Glucocorticoids behaved as mitogenic-adipogenic stimuli able to trigger terminal differentiation of growth-arrested, early marker-expressing cells. Corticosterone was found to exert a promoting effect on the metabolism of ARA, leading in turn to an increase in the production of prostacyclin, suggesting a pathway by which glucocorticoids are active. Similar observations could be extended to preadipocyte 3T3-F442A cells and to rat and human adipose precursor cells. The role of growth hormone which acts, at least in part, by means of a phospholipase C-catalyzed hydrolysis of phosphatidylcholine will be discussed. Among negative factors regulating differentiation, TGF-$\beta$ and TNF-$\alpha$ have been reported to induce the phenotypic "dedifferentiation" of differentiated cells. In contrast to the expression of numerous differentiation-specific genes (including that of LPL), the expression of pOb24 gene -a unique indicator of adipose cell committment *in vivo*-, appeared not to be regulated in late post-confuent cells. It is proposed that the developmental stage of differentiating cells is critical for the control by positive or negative effectors.

1. D. Gaillard *et al.*, *Biochem.J.*, 1989, 257:389-397 ; 2. R. Négrel *et al.* , *Biochem.J.*, 1989, 257:399-405.

## The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 003** BROWN ADIPOSE TISSUE GROWTH AND FUNCTION: MECHANISMS LINKING INSULIN RESISTANCE TO DEFECTIVE THERMOGENESIS IN OBESITY. Ludwik J. Bukowiecki, Dept. of Physiology, Fac. of Medicine, Laval University, Quebec, (Que), Canada, G1K 7P4.

The possible cause-effect relationships between insulin resistance and defective thermogenesis in obesity are still poorly understood. Considering that brown adipose tissue (BAT) is the principal anatomical site of facultative thermogenesis in rats, we investigated the metabolic effects of norepinephrine and insulin in brown adipocytes isolated from: (1) obese, nondiabetic LA/N-*cp* rats, (2) obese, nondiabetic Zucker (*fa/fa*) rats and (3) obese, diabetic SHR/N-*cp* rats. The interest in comparing these animals is that they are all hyperinsulinemic, but develop various degrees of resistance to insulin and diabetes. Of these models, only the SHR/N-*cp* rats become overtly diabetic and may therefore be considered as a model of type II diabetes. It was found that the responsiveness and sensitivity of isolated brown adipocytes to the calorigenic effects of norepinephrine (10-100 nM) were markedly reduced in SHR/N-*cp* rats as compared to their lean controls (3-4-fold decrease in Vmax and 2-fold increase in $EC_{50}$, $P<0.01$). In the same cells, there was a similar decrease in the respiratory effects of dibutyryl cAMP, revealing the presence of a major post-receptor defect. Remarkably, total cytochrome oxidase activity (an index of cell mitochondrial content) was also decreased by 3-4 times in SHR/N-*cp* rats, suggesting that a reduced BAT mitochondrial content is responsible for the defective thermogenesis. Adipocytes isolated from SHR/N-*cp* rats were markedly resistant to to insulin (stimulation of glucose transport and antithermogenesis). On the other hand, adipocytes from obese Zucker rats were also desensitized to the metabolic effects of norepinephrine and insulin, but their maximal thermogenic capacity was not reduced. In contrast, all the above parameters were normal in obese-nondiabetic LA/N-*cp* rats. Considering that insulin replacement in streptozotocin-induced diabetic rats restores both BAT thermogenic capacity and cytochrome oxidase activity to normal, it is suggested that insulin resistance and/or diabetes in SHR/N-*cp* rats leads to a decreased mitochondriogenesis resulting in a diminished BAT thermogenesis. This major thermogenic defect may contribute to the development of obesity in SHR/N-*cp*rats. Insulin appears to play a permissive role in modulating the capacity of BAT for responding calorigenically to catecholamines. (Supported by the MRC and CDA).

**CA 004** DIFFERENTIATION-INDUCED GENE TRANSCRIPTION IN 3T3-L1 PREADIPOCYTES: ROLE OF THE CCAAT/ENHANCER BINDING PROTEIN, M. Daniel Lane, Robert J. Christy, Klaus H. Kaestner, Vincent W. Yang and James M. Ntambi, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

During differentiation in cell culture 3T3-L1 preadipocytes acquire the morphological and biochemical characteristics of adipocytes. This process involves the coordinated transcriptional activation of a family of genes whose expression characterize the adipocyte phenotype. We have cloned and determined the structures of three mouse genes, i.e. the genes encoding 422(aP2) protein, stearoyl-CoA desaturase (SCD1) and the insulin-responsive glucose transporter (GLUT4), whose expression is activated during differentiation of 3T3-L1 preadipocytes into adipocytes.

It was determined that a factor, present in nuclear extracts of differentiated but not undifferentiated 3T3-L1 cells, binds tightly to a "homologous" nucleotide sequence element within the promoters of the 422(aP2) protein, SCD1 and GLUT4 genes. We also discovered that the transcription factor, i.e. the CCAAT/enhancer binding protein (C/EBP), binds specifically to the same sites in these promoters to which the nuclear factor from differentiated 3T3-L1 adipocytes binds. Consistent with a role for C/EBP in transcriptional activation during "adipose conversion", expression of C/EBP mRNA and protein is activated during differentiation. Co-transfection into 3T3-L1 preadipocytes of a C/EBP expression vector along with chimeric promoter-reporter genes (containing promoter sequences from the 422(aP2), SCD1 and GLUT4 genes) resulted in trans-activation of the reporter gene. Mutations altering the C/EBP binding sites of the 422(aP2), SCD1 and GLUT4 gene promoters blocked trans-activation. Taken together these findings suggest that the programmed expression of C/EBP during preadipocyte differentiation is involved in the coordinate activation of expression of a family of adipocyte genes.

To investigate regulation of C/EBP expression during differentiation, the mouse C/EBP gene was cloned and its structure determined. The gene is devoid of introns and preliminary studies indicate that its promoter possesses binding sites for transcription factors Zif and C/EBP. Possible autoregulation of the C/EBP gene by its transcription/translation product is now under investigation.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

*Substrates and Signals of the Insulin Receptor (joint)*

**CA 005** INSULIN-STIMULATED ACTIVATION OF THE RAF-1 PROTEIN KINASE, R.-M. Lee and P.J. Blackshear, Howard Hughes Medical Institute Laboratories and Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, NC 27710

One of the most pressing questions in the field of insulin action is how the activation of the insulin receptor protein tyrosine kinase leads to phosphorylation of cellular proteins on serine and threonine residues. Among the serine/threonine protein kinases rapidly activated by insulin in sensitive cells is the Raf-1 proto-oncogene kinase. This kinase is also rapidly phosphorylated on serine residues in response to insulin; however, the question remains as to whether this is the result of activation of an insulin-stimulated Raf-1 kinase kinase or insulin-stimulted Raf-1 kinase autophosphorylation. We have addressed this issue by searching for an insulin-activated Raf-1 protein kinase kinase. Using recombinant Raf-1 protein as a substrate, we have identified a kinase in sensitive cells that can be activated by insulin to phosphorylate the Raf-1 protein within 2-5 min of insulin exposure. Insulin-stimulated phosphorylation of the Raf-1 protein occurs on multiple sites, some of which comigrate with sites phosphorylated in response to insulin in intact cells. Further characterization of this kinase is in progress.

**CA 006** INSULIN STIMULATION OF TYROSINE PHOSPHORYLATION AND MEMBRANE ASSOCIATED PHOSPHATIDYLINOSITOL 3-KINASE ACTIVITY IN CELLS EXPRESSING WILD-TYPE AND MUTANT INSULIN RECEPTORS, Richard A. Roth, Kazuyoshi Yonezawa, Bei Zhang, Janice E. Chin and Kristina S. Kovacina, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5332.

After binding to its receptor, insulin elicits a diverse array of biological responses. Various mutant receptors have been described which differ in their ability to mediate distinct biological responses. For example, a mutant receptor which lacks two critical autophosphorylation sites has been reported to be capable of mediating insulin's ability to stimulate DNA synthesis but not glucose uptake. This mutant receptor was previously found to exhibit no insulin-stimulated kinase activity in vitro. This mutant was now studied to assess its in vivo tyrosine kinase activity by determining whether it was capable of mediating the tyrosine phosphorylation of a putative endogenous substrate of tyrosine kinases, the type I phosphatidylinositol (PtdIns) 3-kinase. This mutant receptor was found to be ~1/3 as potent as the wild-type receptor to stimulate this response. This level of tyrosine phosphorylation of the PtdIns Kinase was clearly elevated in comparison to the parental cells. Western blotting with anti-phosphotyrosine antibodies also documented that this mutant receptor was capable of insulin-stimulated in vivo tyrosine phosphorylation of various substrates. Moreover, in vitro assays demonstrated that this receptor exhibited approximately 10-times higher basal kinase activity than the wild-type receptor. Finally, insulin was found to stimulate an increase in the membrane associated PtdIns 3-kinase activity in cells expressing both the wild-type receptor as well as this mutant receptor, although the mutant was approximately 1/2 as potent as the wild-type receptor.

These results indicate that: 1) In vitro assessments of the tyrosine kinase activity of various mutant insulin receptors may not accurately reflect their in vivo activity; 2) Tyrosine phosphorylation of PtdIns 3-kinase parallels an increase in membrane associated PtdIns 3-kinase activity; and 3) The ability of the mutant receptor lacking two autophosphorylation sites to mediate insulin-stimulated tyrosine phosphorylation of various endogenous substrates may account for the reported ability of this receptor to mediate some biological responses.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

## *Phospholipid/Phospholipase-Based Signalling Systems*

**CA 007** **ACTIVATION OF THE INSULIN RECEPTOR IN RAT EPIDIDYMAL ADIPOCYTES LEADS TO INCREASED PROTEIN KINASE C ACTIVITY AND RELEASE OF $IP_3(1,4,5)$.** John J. Egan, Mary G. Wetzel, Uh-Hyun Kim, Ian A. Simpson, and Constantine Londos. NIDDK/NICHD, National Institutes of Health, Bethesda, MD 20892.

Exposure of isolated rat epididymal adipocytes to physiological concentrations of insulin and vasopressin results in increased protein kinase C (PKC) activity in isolated plasma membranes. The insulin effect is moderately rapid ($t_{\frac{1}{2}} \cong 1$ min) and is explained, at least in part, by translocation of PKC to the membrane. Similarly, in [$^3$H]myo-inositol-loaded, $Li^+$-blocked adipocytes, both insulin and vasopressin rapidly produce a 2- to 3-fold increase in $(1,4,5)IP_3$; peak values are seen within 5 sec, following which they decline to the baseline by 30-60 sec. In contrast to insulin, which does not stimulate formation of 3-phospho-inositol metabolites, vasopressin stimulates the formation of both $(1,3,4)IP_3$ and $(1,4,5)IP_3$, indicating that these hormones act via different pathways. Unlike other tyrosine kinase receptors, the insulin receptor stimulates the phosphorylation of the ß-isoform of phospholipase C, but not the $\gamma$-isoform. Both the time-course and insulin concentration-dependency for phosphorylation parallel those of $(1,4,5)IP_3$ formation. The above data indicate that in the rat adipocyte: 1) insulin signalling proceeds, at least in part, through phospholipid hydrolysis, 2) insulin probably increases intracellular calcium, and 3) PKC should be numbered among the insulin-stimulated ser/thr kinases.

**CA 008** REGULATION OF PHOSPHOINOSITIDE AND PHOSPHATIDYLCHOLINE PHOSPHOLIPASE BY G-PROTEINS, John H. Exton,Stephen J. Taylor,Stephen B. Bocckino,Karen Shaw,Rafat Siddiqui, and Jonathan S. Blank, Howard Hughes Medical Institute and Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232 The G-protein that regulates phosphoinositide phospholipase C in liver plasma membranes has been purified to homogeneity in both the heterotrimeric and dissociated forms. Both forms have been purified on the basis of their ability to stimulate partially purified phospholipase from the same source in a GTPγS-dependent manner. The heterotrimer contains a 42kDa α-subunit and a 35kDa β-subunit. The α-subunit is not ADP-ribosylated by pertussis toxin and differs immunologically from other presently identified G-protein α-subunits. It binds purine nucleotides with the same specificity and affinity as other α-subunits and crossreacts with certain antisera raised to peptide sequences in other α-subunits. The specific G-protein-responsive phosphoinositide phospholipase is being purified from liver plasma membranes and its physiological and immunological characteristics determined.

GTP analogues stimulate phosphatidylcholine hydrolysis in rat liver plasma membranes. The nucleotide specificity of the response indicates that it is mediate by a G-protein. Phosphatidic acid, diacylglycerol, choline and P-choline are the products, indicating that both phospholipase D and C activities are involved. Activation of phospholipase D is also indicated by the enhanced production of phosphatidylethanol in the presence of chanol. No phospholipase D activity can be detected in the presence or absence of GTPγS in other subcellular functions on the basis of this transphosphatidylation reaction. Choline is incorporated into phosphatidycholine in the absence of CTP and ATP in all subcellular functions assayed, presumably via a base exchange activity. This activity is stimulated by millimolar $Ca^{2+}$, whereas transphosphatidylation is unaffected by this ion. On the basis of these and other findings, it is concluded that transphosphatidylation and base exchange are catalyzed by different enzymes. Interestingly, the base exchange activity of liver plasma membranes is stimulated by submicromolar concentrations of GTP analogues, but not other nucleotides. The possibility that this effect also involves a G-protein is being explored.

**CA 009** EFFECTS OF INSULIN ON PHOSPHOLIPID METABOLISM, DIACYLGLYCEROL-PROTEIN KINASE C SIGNALLING AND ITS RELATIONSHIP TO GLUCOSE TRANSPORT IN RAT ADIPOCYTES, Robert V. Farese, Tatsuo Ishizuka, Mary Standaert, Eric Wickstrom, Denise R. Cooper, J.A. Haley Veterans' Hospital and Departments of Internal Medicine, Biochemistry, and Molecular Biology and Chemistry, University of South Florida, Tampa, FL 33612

We presently evaluated whether insulin activates diacylglycerol (DAG)-protein kinase C (PKC) signalling and its potential relationship to stimulated glucose transport in rat adipocytes. After prelabeling adipocytes with [$^3$H]glycerol for 15 minutes to prelabel precursor pools, insulin provoked 25-30% increases in [$^3$H]glycerol incorporation into DAG within 1-2 minutes. The latter was followed by a transient return to the basal level at 5 minutes, and a secondary increase at 10-20 minutes. Increases in DAG mass were not observed consistently, perhaps reflecting the large size of a metabolically inert pool. Increases in [$^3$H]glycerol-labeled DAG at least partly reflected activation of the *de novo* phosphatidic acid (PA) synthetic pathway, as there were associated rapid increases in the activity of glycerol-3-phosphate acyltransferase (G3PAT). The insulin-induced increase in G3PAT was observed both in intact adipocytes and membrane preparations thereof, and this effect of insulin was mimicked by phosphatidylinositol (PI)-specific phospholipase C, suggesting that headgroups released from the PI-glycan through phospholipase C action may account for the insulin-induced increase in G3PAT activity. In addition to activation of the *de novo* pathway, insulin stimulated the hydrolysis of phosphatidylcholine (PC), as indicated by acute (30-second) decreases in [$^3$H]choline-labeled PC in adipocytes labeled overnight to isotopic equilibrium. Insulin-induced decreases in PC were followed by rapid resynthesis, possibly via the *de novo* pathway. These changes in phospholipid metabolism were associated with PKC activation, as evidenced by translocation of PKC from cytosol to membranes, which was evident both with the Mono Q-purified PKC enzymatic assay (histone phosphorylation) and by evaluation of immunoreactive PKC-β. The importance of PKC during insulin-stimulated glucose transport was assessed in several ways: (1) downregulation (DR) of PKC after overnight treatment of adipocytes with phorbol esters (TPA) (as well as other PKC activating agents, including insulin and glucose) was associated with decreases in insulin-stimulated 2-deoxyglucose (2-DOG) uptake; (2) DR of PKC and insulin-stimulated 2-DOG uptake could also be effected by using antisense DNA which was targeted against PKC-β and PKC-α; (3) following TPA-DR of PKC, effects of insulin on 2-DOG uptake were restored by introduction of PKC into adipocytes by electroporation. These findings suggest that: (1) insulin increases DAG production and activates PKC in rat adipocytes; (2) PKC is required during insulin-stimulated glucose transport.

**CA 010** ACTIVATION OF PHOSPHOLIPASE C ISOZYMES BY PROTEIN TYROSINE KINASES AND G-PROTEIN, Sue Goo Rhee, Ha Kun Kim, Jae Won Kim, Hee Sook Kim, and Do Joon Park, Laboratory of Biochemistry, NHLBI, NIH, Bethesda, MD 20892

Three immunologically distinct phospholipase C (PLC) isozymes, PLC-ß, PLC-γ, and PLC-δ, were overexpressed in NIH3T3 cells and CCL39 cells, and PI hydrolysis in the overproducing cells in response to PDGF or bombesin was compared to that in normal cells. PLC-γ-overproducing cells, but not PLC-ß- and PLC-δ-overproducing cells, exhibited enhanced response to PDGF, whereas only PLC-ß-overproducing cells showed enhanced response to bombesin which is known to bind a receptor coupled to G-protein. This result suggests that there are two types of PI-specific phospholipase C (PLC), one activated via a G-protein and the other through phosphorylation by protein tyrosine kinase. PLC-ß belongs to the former type and PLC-γ to the latter. The binding of PDGF, EGF and FGF to the corresponding receptors promotes the association of PLC-γ with the growth factor receptor, stimulates the phosphorylation of PLC-γ at tyr and ser residues, and activates PLC. To investigate the role of tyr phosphorylation in PLC activation, tyr residues 771, 783, and 1254 of PLC-γ, which have been shown to be phosphorylated by purified EGF receptor kinase, were individually changed to phe and the mutant enzymes were expressed in NIH3T3 cells. Phe substitution at tyr 783 completely blocked the activation of PLC by PDGF, whereas mutation at tyr 1254 inhibited the response by 40% and mutation at tyr 771 enhanced the response by 50%. These results suggest that PLC-γ is the isozyme of PLC-γ that mediates PDGF-induced PI hydrolysis. Furthermore, they show that neither the association of PLC-γ with the PDGF receptor nor its phosphorylation on ser residues is sufficient to account for PDGF-induced activation, but that phosphorylation of tyr 783 is essential.

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*Lipolysis*

**CA 011** HORMONE-SENSITIVE LIPASE: PROPERTIES, FUNCTION, HORMONAL CONTROL,
P. Belfrage*, C. Holm*, D. Langin*, A. Hidalgo*, G. Fredrikson*, T.G. Kirchgessner+
R.C. Davies+ and M.C. Schotz+, University of Lund, Sweden* and UCLA, U.S.A.+

The 757 amino acid ($M_r$ 82,820) rat adipose tissue hormone-sensitive lipase (**HSL**) shows no homology with other lipases or proteins, except for a -GXSXG- catalytic site motif. HSL has hydrophobic properties as integral membrane proteins but no membrane-spanning hydrophobic sequence. The human, single HSL gene (20-30 kb) is located at chromosome 19 cent-q13.3. mRNA varies tissue-specifically between 3.3 and 3.9 kb. Human HSL shows > 80% homology with the rat enzyme. Active and phosphorylatable/activatable rat HSL has been expressed in COS and also in CHO cells. The HSL promotor and long-term regulation of HSL is being studied. HSL hydrolyzes all acylglycerol classes and cholesterol esters. The cholesterol ester hydrolase activity is probably important for release of cholesterol for steroid hormone production in steroid-producing tissues, which express HSL at 10% the level of adipose tissue. Rat HSL is phosphorylated on Ser 563 (the *regulatory* phosphorylation site) by cAMP-dependent protein kinase (cAMP-PrK) and thereby activated or on Ser 565 (the *basal* site) probably mainly by AMP-activated protein (AMP-PrK) kinase, with no direct effect on enzyme activity. However, phosphorylation of any of the two sites is mutually exclusive. Protein phosphatase type 2A is mainly responsible for dephosphorylation of both phosphorylation sites in fat cells. Adenylate cyclase stimulators activate HSL in fat cells through cAMP-mediated Ser 563 phosphorylation. Insulin causes its antilipolytic effect through net dephosphorylation of the same phosphorylation site. Under physiological conditions this reflects mainly, or exclusively, diminished cAMP-PrK mediated Ser 563 phosphorylation, secondary to cyclic AMP reduction, which in turn appears to mainly result from insulin induced increase of cyclic AMP degradation.

**CA 012** CONTROL OF ISOLATED ADIPOCYTES, ENZYME TRAFFICKING, AND THE SURFACE OF THE LIPID STORAGE DROPLET. Constantine Londos, John. J. Egan, Andrew S. Greenberg, and Sheree A. Wek. NIDDK, National Institutes of Health, Bethesda, MD 20892.

Three topics will be discussed. 1) The behavior of isolated adipocytes is uncontrolled and unpredictable unless one carefully manages the medium adenosine concentration. The cells contain high affinity A1 adenosine receptors linked, via G-proteins, to adenylyl cyclase and to cAMP-independent processes, such as the glucose transporter. Control is achieved by removing the variable and unpredictable endogenous adenosine from the medium with adenosine deaminase, and supplementing with deaminase-resistant A1 receptor agonists. 2) Western blotting of adipocyte subcellular fractions with antibodies against hormone-sensitive lipase (HSL) shows that the enzyme is in the cytosolic/supernate fraction of lipolytically quiet cells, but in the "fat cake" fraction of lipolytically stimulated cells. Immunocytochemical studies in 3T3-L1 adipocytes confirms that HSL is translocated upon stimulation. These data explain the discrepancy between the great increases in lipolysis in vivo upon HSL phosphorylation and the meager effects of in vitro phosphorylation. Since HSL phosphorylated in vitro exhibits no propensity bind to lipid, we speculate that the surface of the lipid droplet contains a "docking" moiety for the enzyme. 3) Perilipin, a previously undescribed protein, has been isolated from detergent extracts of fat cakes. The protein is: a) adipocyte-specific, as determined by Western and Northern analysis; b) the major cAMP-dependent protein kinase substrate of adipocytes; and c) localized to the lipid droplet surface by immunocytochemistry. It is speculated that perilipin plays a role in lipid metabolism, perhaps as a docking moiety for HSL.

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**CA 013** MOLECULAR MECHANISMS IMPORTANT IN THE ANTILIPOLYTIC ACTION OF INSULIN: PHOSPHORYLATION/ACTIVATION OF ADIPOCYTE PARTICULATE cGMP-INHIBITED cAMP PHOSPHODIESTERASE. V.C. Manganiello, C.J. Smith, E. Degerman, H. Tornqvist, H. Eriksson, A. Rascon and P. Belfrage, NIH, Bethesda, MD and U. Lund, Sweden.

In rat adipocytes lipolysis is promoted by agents that increase intracellular cAMP and antagonized by agents that decrease synthesis of or increase degradation of cAMP. Insulin is a physiologically important and potent inhibitor of lipolysis. Although understanding of the molecular mechanisms involved in the antilipolytic actions of insulin is incomplete, several studies have suggested that insulin-induced activation of the hormone-sensitive, particulate, cilostamide- and cGMP-inhibited cAMP phosphodiesterase (cGI PDE) is an important component.

The particulate cGI PDE in rat adipocytes is subject to dual regulation by insulin and agents that increase cAMP. In intact adipocytes there is a close concentration-dependent and temporal relationship between activation of adenylyl cyclase, cAMP-dependent protein kinase, cGI PDE and the hormone-sensitive lipase. This functional coupling between activation of adenylate cyclase and cGI PDE may be important in regulation of turnover/steady state concentrations of cAMP, the activation state of A-kinase and perhaps in hormonal control of adenosine accumulation in adipocyte preparations. Incubation of adipocytes with insulin also increases cGI PDE activity without changing cAMP-kinase. In the presence of insulin and isoproterenol, there is rapid, synergistic activation of cGI PDE which temporally correlates with insulin-induced reduction in isoproterenol-stimulated cAMP-kinase and lipolysis. In addition, the antilipolytic action of insulin was blocked in adipocytes incubated with OPC-3911, a specific inhibitor of the particulate cGI PDE.

In $^{32}P_i$ labelled adipocytes incubated with isoproterenol or insulin, a 135 kDa particulate protein identified as native cGI PDE was phosphorylated on serine sites. The time courses and concentration dependencies for phosphorylation and activation of cGI PDE were similar for isoproterenol, adenosine deaminase (ADA) and insulin; maximal phosphorylation was similar for the three agents. Phosphorylation and activation by ADA was prevented and/or reversed by phenylisopropyladenosine. Incubation of adipocytes with insulin and isoproterenol resulted in synergistic phosphorylation and activation of cGI PDE. These and other studies suggest that insulin and cAMP promote phosphorylation (and activation) of cGI PDE via distinct serine kinases and that synergistic phosphorylation/activation of cGI PDE may be important in the antilipolytic action of insulin.

**CA 014** THE ADIPOSE CELL IN STATES OF METABOLIC DYSFUNCTION, Ulf Smith, Dept. of Medicine, Sahlgren's Hospital, Gothenburg, Sweden.

The main function of the adipose cell is to store lipids under periods of affluence and to release the stored FFA and glycerol when needed. This function is under precise control by hormonal and other humoral factors. The adipocyte may be used as a model cell for elucidating the mechanisms of action of hormones and other regulators. However, fat cells are also useful to elucidate pathophysiological alterations in man such as the mechanisms for insulin insensitivity in states of insulin resistance, the adrenergic regulation in cells from individuals with hypo- or hyperthyroidism or phaeochromocytoma.

Increased FFA release may also be of pathogenetic importance for the insulin resistance in obesity and NIDDM as well as an important cause of hypertriglyceridemia.

Fat cell metabolism is usually studied by incubating the cells under appropriate conditions in vitro. However, the recently developed microdialysis technique allows experimental studies in vivo. With this technique, a small dialysing probe is implanted in the subcutaneous tissue and perfused with saline at a slow flow rate. The dialysate is collected and can be analysed for the appropriate metabolites or hormones. Using the microdialysis technique, we have verified the presence of regional differences in fat cell lipolysis in situ. Furthermore, fat cells release significant amounts of lactate during an oral glucose tolerance test.

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*Substrate Generation and Transport*

**CA 015** AN INCREASE IN MEMBRANE TRANSPORT OF LONG CHAIN FATTY ACIDS IS AN EARLY EVENT DURING ADIPOSE CONVERSION OF BFC-1 CELLS. Abumrad N.A., Forest C. and Sanders S. Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232.

Fatty acid (FA) permeation across the plasma membrane of adipocytes is carrier mediated (1,2). The physiological importance of the transport system and its role in FA metabolism remain undetermined. Adipose cells in culture offer an ideal system where transport and metabolism of FA can be related as the cell differentiates and acquires the adipose phenotype. We determined uptake of [$^3$H]oleate in BFC-1 cells at various stages of their conversion into adipocytes. BFC-1 cells, established from mouse brown fat tissue, express many of the characteristics of other adipose cell lines like 3T3 L1 and 3T3 F442A. They show a high rate of adipose conversion with temporally well defined stages. Large increases are observed in the activity of the enzyme lipoprotein lipase (LPL), at day 4 after cell confluence, and of glycerol phosphate dehydrogenase and fatty-acyl CoA ligase at 6-8 days following cell confluence. A three fold increase in early rates of oleate uptake, which reflected FA entry into the cells, was apparent three days following cell confluence. This increase was measured under conditions where greater than 95% of intracellular radioactivity was recovered in the form of free unesterified oleate. Uptake of retinoic acid, a molecule which shares structural similarities with long chain fatty acids (FA), remained unaltered throughout the adipose conversion process. The increase in oleate transport was related to an increase in the transport Vmax while the Km remained unchanged ($2 \times 10^{-7}$ M). Oleate transport reached its maximal value at about day 6 following cell confluence as metabolism of the FA (incorporation into lipids) began a gradual increase. The increase in transport preceded by at least three days induction of mRNA for the cytosolic fatty acid binding protein and for the enzyme fatty acid synthetase. The data indicate that increases in activities of FA transport and of LPL, early during the adipose conversion process would favor increased availability of exogenous FA at a stage where endogenous synthesis of FA is limited. This would promote FA esterification and lipid deposition and might contribute to the induction of proteins, which function in FA metabolism. Preliminary data indicate that oleate addition to the culture medium of BFC-1 adipocytes can induce mRNA for FABP and can potentiate its induction by dexamethasone.

References: 1) J. Biol. Chem. (1984) 259, 9183-9191
2) J. Biol. Chem. (1988) 263, 14678-14683
3) Exp. Cell Res. (1987) 168, 218-232

**CA 016** TISSUE-SPECIFIC REGULATION OF LIPOPROTEIN LIPASE IN ADIPOSE TISSUE AND MUSCLE, Eckel Robert H., Daniel H. Bessesen and Mary V. Raynolds, Division of Endocrinology, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262.

Lipoprotein lipase (LPL) is a hydrolytic enzyme present in many tissues including adipose tissue and muscle. The regulation of LPL by hormones and nutrition is tissue-specific. In particular, insulin increases LPL activity and mRNA in isolated adipocytes, but decreases or fails to increase LPL activity in skeletal and cardiac muscle. Insulin-mediated increases in LPL mRNA and rat adipocytes is not due to increases in LPL gene transcription but likely due to increased LPL mRNA stability. Unlike insulin, isoproterenol and cyclic AMP decrease LPL activity, mRNA and gene transcription in isolated rat adipocytes. In cardiac muscle, isoproterenol increases both LPL activity and mRNA. Studies are in progress to determine if this stimulatory effect of isoproterenol on LPL mRNA in muscle is due to increased LPL gene transcription. Tissue-specific alterations in the regulation of LPL have important pathophysiologic implications, particularly in obesity. Specifically, in obese Zucker rats, increases in LPL activity in adipose tissue and decreases in LPL activity in muscle likely contribute to the maintenance of the obese state. The further increase in the ratio of adipose tissue LPL/muscle LPL after maintenance of the reduced-obese state is additional evidence to support this role of the lipase. These tissue-specific changes in LPL are also paralleled by tissue-specific differences in the incorporation and utilization of dietary triglyceride fatty acids. Overall, tissue-specific changes in LPL regulation by hormones (and nutrition) provide mechanisms by which triglyceride-rich lipoprotein triglyceride fatty acids can be partitioned to meet the metabolic needs of the tissue.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 017** LIPOPROTEIN TRANSPORT AND METABOLISM, Silvia Santamarina-Fojo. Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. 20892.

The transport of the major plasma lipoprotein particles from their site of synthesis in the intestine and the liver to peripheral tissues, and their metabolism within the circulation are complex process that involves multiple plasma proteins and receptors. These include lipoprotein lipase (LPL), hepatic lipase (HL), cholesterol ester transfer protein (CETP), lecithin cholesterol acyl transferase (LCAT) and the plasma apolipoproteins. The uptake of chylomicrons and VLDL remnants into peripheral tissues is mediated by the low density lipoprotein (LDL) and putative remnant receptors, an area of active investigation. The important role that LPL and its cofactor, apoC-II, as well as that of HL and CETP play in the regulation of normal cholesterol and triglyceride metabolism has been established by the identification of patients with a deficiency of these proteins. Individuals with a deficiency of LPL or apoC-II have marked derangements in normal triglyceride metabolism and pancreatitis, and patients with a deficiency of hepatic lipase develop both hypertriglyceridemia and hypercholesterolemia. CETP deficiency results in hyperalphalipoprotenemia while patients with a deficiency of LCAT have low HDL levels. Characterization of the molecular defects at the gene level that leads to a deficiency of these proteins have provided new insights into their structure and function.

HDL has been proposed to play a central role in preventing the development of atherosclerosis by removing excess cholesterol from cells by the process of reverse cholesterol transport. As essential components of HDL, the plasma apolipoproteins A-I and A-II, may have an important function in reverse cholesterol transport. Recently, studies in the mouse adipocyte cell line, OB1771, have suggested a differential role of apoA-I and apoA-IV versus apoA-II in the translocation of cholesterol from these cells for transport to the liver. HDL subfractions containing apoA-I, but not apoA-I and apoA-II, translocate cholesterol to the cell membrane in OB1771 cells. The study of these plasma apolipoproteins and their function in the remodeling of lipoprotein particles, as well as the characterization of the genetic defects in dyslipoproteinemic patients have greatly enhanced our understanding of the processes regulating the transport and metabolism of lipoproteins.

**CA 018 REGULATION OF LIPOPROTEIN LIPASE IN HUMAN ADIPOSE TISSUE MAINTAINED IN ORGAN CULTURE**, Susan K. Fried, Dept. of Nutritional Sciences, Rutgers University, New Brunswick, NJ 08903-0231. The cellular and molecular mechanisms underlying nutritionally induced variations in human adipose tissue (hAT) lipoprotein lipase (LPL) are unresolved. Understanding has been limited by lack of appropriate *in vitro* model systems. Smith et al.[1] demonstrated that the long-term hormonal regulation of hAT metabolism can be studied using organ culture. We confirmed that hAT fragments cultured in M199, with or without insulin (INS), remain acutely responsive to stimulatory effect on glucose transport and metabolism, and isoproterenol-stimulation of lipolysis. Expected supressive effects of dexamethasone (DEX), added in the absence or presence of insulin (INS), on glucose metabolism were observed. Thus, organ culture seemed suitable to study hAT LPL regulation. After 1 week of culture, INS (7nM) increased LPL activity ~5-fold over basal, DEX had no effect, and INS+DEX increased it ~20-fold. A physiological concentration of INS (0.7 nM) elicited half maximal stimulation of LPL activity. INS stimulation of LPL activity was explained by increased levels of LPL mRNA and specific rates of LPL synthesis determined by biosynthetic labelling and immunoprecipitation [0.012% (basal) vs 0.063% (INS) of total tissue protein synthesis(PS)]. The combination of INS+DEX increased LPL activity over INS alone (0.59 vs. 4.1 umol/h/gm, $p<.001$), without altering rates of LPL synthesis [0.063 vs 0.070% of total PS]. Pulse-chase studies showed that newly-synthesized LPL was degraded rapidly in the presence of INS alone, but more slowly in the presence of INS+DEX (-66+13% (INS) vs. -29+17% (INS+DEX) in 90 minutes, n=4). Levels of LPL mRNA were approximately 2-fold greater in the presence of DEX+INS than INS alone; LPL mRNA was detectable in tissue cultured with DEX alone, but not in the absence of hormones. Potential down-regulation of LPL were also examined. Isoproterenol (0.1-10 $\mu$M), a beta adrenergic agonist, added for 24 h to tissue cultured for 1 week in the presence of INS alone or INS+DEX, decreased LPL activity only 27% ($p<.01$), and did not consistantly alter rates of LPL synthesis, degradation, or mRNA levels. We have previously shown that LPL mRNA levels, rates of synthesis, and activity are markedly decreased by tumor necrosis factor[2]. In contrast, interleukin-1$\alpha$ decreased LPL synthesis and activity, but not mRNA levels.

In summary, INS is essential for maintenance of LPL gene expression and synthesis in hAT. Glucocorticoids also increase LPL gene expression, but their primary mechanism of action is a post-translational inhibition of LPL degradation.

[1]Cigolini & Smith, Metabolism 28:502, 1979.
[2]Fried & Zechner, JLR 30:1917, 1989.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

*Lipogenesis*

**CA 019** REGULATION OF LIPID SYNTHESIS AND STORAGE BY THE AMP-ACTIVATED PROTEIN KINASE, D. Grahame Hardie, Department of Biochemistry, The University, Dundee, DD1 4HN, Scotland, U.K.

The AMP-activated protein kinase (AMP-PK, formerly called HMG-CoA reductase kinase) is now recognized as a multisubstrate enzyme which regulates several key enzymes of lipid metabolism. AMP-PK phosphorylates and inactivates both acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGR), which are the key regulatory enzymes of fatty acid and cholesterol synthesis respectively. We have shown that all of the sites phosphorylated on these two enzymes *in vitro* (ser-79, -1200 and -1215 on ACC, ser-871 on HMGR) are phosphorylated significantly in rat liver *in vivo*. AMP-PK forms part of a protein kinase cascade, being converted from an inactive to an active form by phosphorylation catalysed by a kinase kinase, with this being reversed by protein phosphatase-2C. AMP-PK is activated allosterically about 5-fold by physiological concentrations of AMP, and the nucleotide is also absolutely required for phosphorylation and activation by the kinase kinase. Thus the system would be activated dramatically by any rises in AMP, which are known to occur in intact cells whenever ATP is in short supply. The kinase kinase is also activated by nanomolar concentrations of long chain acyl-CoA. We have developed a simple and specific assay for AMP-PK involving phosphorylation of a synthetic peptide based on the sequence around ser-79 in ACC, which appears to be phosphorylated uniquely by the kinase. Using this assay we have shown that the kinase is present in tissues other than liver, including the rat adipocyte. We have shown that ACC is partially phosphorylated at ser-79 in isolated rat adipocytes, indicating that the kinase has an important physiological role in this tissue. Phosphorylation at ser-79 (and hence inactivation) is increased in adipocytes by agents which elevate cyclic AMP. The mechanism for this is not clear but it may involve inhibition of protein phosphatases by cyclic AMP-dependent protein kinase. Another target for AMP-PK is hormone-sensitive lipase, which is phosphorylated at ser-563 by cyclic AMP-dependent protein kinase and ser-565 by AMP-PK. Phosphorylation at ser-563 activates the enzyme, while phosphorylation of ser-565 does not directly affect activity, but completely prevents phosphorylation and activation at ser-563. Thus activation of AMP-PK would inhibit lipolysis by antagonizing the effects of cyclic AMP-dependent protein kinase. There is now good evidence that ser-565 is phosphorylated both *in vivo* and in isolated rat adipocytes. AMP-PK may therefore be playing a feedback role in adipose tissue. Elevated concentrations of long chain acyl-CoA would activate the kinase cascade, causing phosphorylation of both ACC and hormone-sensitive lipase, and inhibiting the synthesis of fatty acids, and their release from triglyceride stores. Although hormones may interact with this system, we believe it is primarily controlled directly by metabolites. It may represent a protective mechanism to inhibit synthesis and release of lipids when fatty acid levels rise or ATP is depleted.

**CA 020** MECHANISMS FOR REGULATION OF LIPOPROTEIN LIPASE, Thomas Olivecrona, Department of Medical Biochemistry and Biophysics, University of Umeå, S-901 87 Umeå, Sweden

The amount of lipoprotein lipase at the vascular endothelium in a given tissue is determined by the rate at which the enzyme is delivered to endothelial binding sites, and the rate at which it is lost from these sites. Both of these rates are subject to regulation by physiological and pharmacological agents. In addipocytes, LPL mRNA decreases markedly in response to a variety of agents, exemplified here by tumor necrosis factor and dioxins. It also decreases during fasting. This change is, however, slower and of lesser magnitude than the change in LPL activity, implying that additional mechanisms operate. In isolated adipocytes a large fraction of newly synthesized LPL is rapidly degraded unless agents which promote release are present, e.g. heparin. This suggests regulation of tissue LPL activity by partitioning of the enzyme between transfer to endothelial sites and intracellular degradation. There is continuous dissociation-reassociation of LPL between endothelial binding sites and the circulating blood. This results in some spreading of the enzyme to sites with little or no local synthesis, but the major direction of transport is to the liver where the lipase is taken up and degraded. Accumulation of fatty acids at endothelial sites of LPL action inhibits lipase activity and causes enhanced dissociation of the lipase into blood. This feed-back regulation creates a direct link between tissue energy metabolism and lipoprotein degradation.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 021** Effector Control of Mammalian Pyruvate Dehydrogenase Phosphatase and Kinase and Insights into Insulin Regulation. Thomas E. Roche, Kansas State University, Manhattan, KS.

In energy metabolism, the mammalian pyruvate dehydrogenase complex (PDC) has a critical role in maintaining the reciprocal relationship between metabolism of glucose and fatty acids or ketone bodies. In specialized tissues, PDC has a controlling role in the conversion of glucose to fat reserves. Insulin enhances PDC activity in white adipose tissue, brown adipose tissue, lactating mammary tissue, and to a limited extent in isolated liver cells. The phosphorylation-dephosphorylation cycle that regulates mammalian PDC is tightly regulated responding to changes in the intramitochondrial NADH/$NAD^+$, acetyl-CoA/CoA, ATP/ADP ratios and concentrations of pyruvate, $Mg^{2+}$ (mM), $Ca^{2+}$ ($\mu$M). Polyamines enhance dephosphorylation but the significance in vivo has not been established. My laboratory has contributed insights into the mechanism of each of these effects through studies with purified complexes, over a dozen preparations of subcomplexes, components, and functional domains. These will be succinctly reviewed. Richard Denton's laboratory has established that insulin activates the pyruvate dehydrogenase (PDH) phosphatase by a mechanism that results in a persistent activation that can be still observed in isolated and toluene permeabilized mitochondria. Their work has further demonstrated that the PDH phosphatase in toluene-permeabilized mitochondria prepared from insulin-treated adipose tissue has a reduced $K_m$ for $Mg^{2+}$ similar to that elicited by spermine. However spermine does not reduce the Km for $Mg^{2+}$ when added to toluene permeabilized mitochondria. Thus the mechanism for the effect of insulin remains unknown. We are using polyclonal and monoclonal antibodies to the PDH phosphatase subunits $P_c$ (a 50-kDa catalytic subunit) and $P_f$ (a 90-kDa, FAD-containing subunit) to evaluate whether a PDH phosphatase subunit undergoes a covalent modification. In previous work we have evaluated a large number of "insulin mediator" preparations for their effects on the purified PDH phosphatase and in various assays using mitochondria and mitoblasts. We have been unable to detect any direct effects of these fractions in the PDH phosphatase and we have found that the conditions standardly used to evaluate "insulin mediator" effects are subject to detecting artificial enhancements in PDC activity.

## *The Glucose Transporter: Structure and Function (joint)*

**CA 022**THE GLUCOSE TRANSPORTER FAMILY, Graeme Bell, Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637 The oxidation of glucose represents a major source of metabolic energy for mammalian cells. However, because the plasma membrane is impermeable to polar molecules such as glucose, the cellular uptake of this important nutrient is accomplished by plasma membrane-associated carrier proteins that bind and transfer it across the lipid bilayer. Two classes of glucose carriers have been described in mammalian cells (Table 1): $Na^+$-dependent glucose transporters and facilitative glucose transporters. Sodium- dependent glucose transporters are expressed in the small intestine and kidney where they are responsible for the dietary absorption of glucose and its reabsorption from forming urine, respectively. By contrast, facilitative glucose transporters are widely distributed in mammalian tissues and present on the surface of most if not all cells. A family of five structurally-related and functional facilitative glucose transporters have been described (Table 1) as well as a glucose transporter pseudogene. Each facilitative glucose transporter isoform has a distinct pattern of expression. The amino acid sequences of the facilitative and $Na^+$-dependent glucose transporters are structurally unrelated. The diversity of glucose transporters expressed in mammalian tissues contributes to the precise regulation of glucose uptake under varying physiological conditions.

Table 1. Human glucose transporters

| Isoform | Major sites of expression |
|---|---|
| A. Facilitative glucose transporters | |
| 1. GLUT1/Erythrocyte-HepG2 | Ubiquitous; abundant in fetal tissues, placenta and adult brain, kidney and colon |
| 2. GLUT2/Liver | Liver, pancreatic ß cell, kidney and small intestine |
| 3. GLUT3/Brain | Ubiquitous; most abundant in brain |
| 4. GLUT4/Muscle–fat | Muscle (skeletal and cardiac) and adipose (brown and white) tissue |
| 5. GLUT5/Small intestine | Small intestine (jejunum) |
| 6. GLUT3P1 (formerly GLUT6) | Pseudogene-like sequence related to GLUT3 |
| B. Sodium-dependent glucose transporters | |
| 1. SGLT1 ($Na^+$/glucose cotransporter) | Absorptive epithelial cells of small intestine and kidney |

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 023** OVERVIEW OF ALTERATIONS IN INSULIN ACTION IN HUMAN DIABETES. José F. Caro, Madhur K. Sinha, Walter J. Pories, Hisham A. Barakat, Prabhaker G. Khazanie, G. Lynis Dohm, Departments of Medicine, Surgery, Clinical Pathology, and Biochemistry, School of Medicine, East Carolina University, Greenville, NC 27858.

Insulin resistance and diminished insulin secretion are present in all patients with type II diabetes. One of the metabolic consequences of these alterations is hyperglycemia due to increased glucose production by the liver and decreased glucose utilization by skeletal muscle and adipose tissue.

In order to understand the mechanism(s) of insulin resistance at the cellular level, we have developed methods to isolate human hepatocytes, adipocytes and muscle fibers from morbidly obese patients with and without type II diabetes undergoing gastric bypass surgery.

It is clear that an abnormality in the coupling between insulin and insulin receptor or in the abundance of insulin receptor protein are not responsible for insulin resistance in type II diabetes. However, the ability of insulin to stimulate insulin receptor tyrosine kinase is markedly impaired in the liver, muscle and adipose tissue from these patients resulting in decreased tyrosine phosphorylated endogenous substrates. Furthermore, a novel pathway of "cross-talk" between the insulin receptor and Gi proteins, which seems to be independent of tyrosine kinase activation, is impaired in type II diabetes albeit $Gi_{1-2\alpha}$ immunodetection is normal and $Gi_{3\alpha}$ is only slightly decreased. Therefore, these two separate pathways of insulin signalling are abnormal in type II diabetes and likely responsible for insulin resistance.

Additionally, abnormalities in the expression of the facilitative glucose transporters family are present in type II diabetes. In adipose tissue GLUT-4 protein is decreased by ~ 20% in obesity and by over 50% in diabetes qualitatively consistent with the alterations in insulin stimulated glucose transport. In skeletal muscle the decrease in GLUT-4 is ~ 20% and identical in obese patients with or without type II diabetes suggesting that additional defects in translocation and/or activation must be present in this tissue.

Liver GLUT-2 protein and mRNA are similar in obese patients with or without diabetes. However, obese patients with impaired glucose tolerance who presumably represent a pre-diabetic state have dramatic decrease in GLUT-2 protein and mRNA. If in the liver GLUT-2 serves as a glucose sensor, a decrease in GLUT-2 may result in an erroneous homeostatic increase in glucose production initiating a cascade of events that lead to the end stage hyperglycemic syndrome recognized today as type II diabetes. Once the full-blown diabetic syndrome is established, it is impossible to determine which of the target tissues and beta cell defects, if any, are primary. Since a genetic marker for type II diabetes is not available yet, detailed clinical investigation of subjects with impaired glucose tolerance may allow understanding type II diabetes before the complex end stage hyperglycemic syndrome becomes established.

**CA 024** MECHANISMS OF GLUCOSE TRANSPORT REGULATION: ROLE OF PROTEIN KINASE C I.A. Simpson, S.W. Cushman, J.J. Egan, W. Heath, C. Londos, H. Nishimura, J. Saltis. Eli Lilly Co., Indianapolis, In., and NIDDK, NIH, Bethesda, Md.

Activation of protein kinase C is associated with an increase in glucose transport activity in a variety of different cell types. Insulin, vasopressin, epinephrine and bombesin elicit a comparable stimulation of protein kinase C activity in rat adipose cells although these C-kinase activities may be associated with different isoenzymes. Glucose transport activity is stimulated 3-4-fold by phorbol 12-myristate 13-acetate (PMA) as well as all of these hormones, except for insulin which induces a 40-fold stimulation. In addition to magnitude, several other distinctions can be made between the transport responses to insulin and the other hormones. The time course for activation by insulin is much more rapid than for the other hormones ($t_{1/2} \approx 2$ min. cf $t_{1/2} \approx 8$ min.). Glucose transporter concentration in plasma membranes, as measured by cytochalasin B binding, is increased 6-fold by insulin as compared to a 3-fold increase in response to PMA and other hormones. In addition, the relative levels of GLUT1 and GLUT4 isoforms that are translocated to the plasma membrane are markedly different. Whereas, the translocation of GLUT1 is similar in both cases insulin induces a 2.5-fold greater increase in GLUT4 translocation. These observations confirm that GLUT4 is the primary transporter in rat adipose cells and also suggest that GLUT4 is innately more active than GLUT1. The likelihood that different mechanisms are responsible for these transport responses is supported by the distinct effects induced by catecholamines ($R_s$) and adenosine ($R_i$) on the responses. Whereas these agents modulate the intrinsic activity of the insulin mediated response they alter the translocation response to the other hormones. Although the mechanisms are clearly distinct they are also interactive, as preincubation with PMA both accelerates and augments the actions of insulin. Whether these distinctions reflect the involvement of different isoenzymes of protein kinase C or are due to completely unrelated mechanisms remains to be determined.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

*New Signalling Systems of the Adipose Cell*

**CA 025** **CELLULAR BASIS FOR PROSTAGLANDIN PRODUCTION BY ADIPOSE TISSUE,** Lloyd Axelrod, Diabetes Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114. Disturbances of prostacyclin ($PGI_2$) production by adipose tissue contribute to the pathogenesis of diabetic ketoacidosis and may contribute to the pathogenesis of hypertension and vascular disease. Disturbances of prostaglandin $E_2$ ($PGE_2$) production by adipose tissue may result in altered lipolysis because $PGE_2$ is a potent antilipolytic substance. We studied the cellular basis of $PGI_2$ and $PGE_2$ production in adipose tissue, measured as release of 6-keto-$PGF_{1\alpha}$ and $PGE_2$ respectively, in response to epinephrine. Adipocytes did not produce $PGI_2$ or $PGE_2$ when the nonadipocyte cellular constituents of adipose tissue (nonfat cells) were removed by repeated washing. Nonfat cells did not produce $PGI_2$ or $PGE_2$ in the absence of adipocytes. Both adipocytes and nonfat cells were required for $PGI_2$ and $PGE_2$ production in response to epinephrine. Adipocytes pretreated with 0.2 mM aspirin to inhibit PGH synthase promoted $PGI_2$ production when mixed with nonfat cells. Nonfat cells preincubated with aspirin did not produce $PGI_2$ when mixed with adipocytes. Nonfat cells converted arachidonic acid to $PGI_2$ and $PGE_2$ but adipocytes did not. Epinephrine stimulated lipolysis and $PGI_2$ production in a dose-dependent parallel manner, but the responses were distinct above $10^{-6}$ M. Fractionation of nonfat cells on a Percoll density gradient followed by measurement of angiotensin-converting enzyme activity and 6-keto-$PGF_{1\alpha}$ production indicated that nonfat cells were mostly vascular endothelial cells.

Conclusions. Catecholamine-stimulated $PGI_2$ and $PGE_2$ production in adipose tissue results from cooperation of adipocytes and vascular endothelial cells. Adipocytes provide arachidonic acid, which is converted to $PGI_2$ and $PGE_2$ by vascular endothelial cells. Because adipose tissue is located near blood vessels throughout the body, adipocytes may be an important source of arachidonic acid for vascular endothelial cells in health and disease. Adipocytes may, under some circumstances, release arachidonic acid into the systemic circulation where it is used by vascular endothelial cells to produce eicosanoids.

**CA 026** GROWTH HORMONE AND THE ADIPOCYTE: TOO MANY ACTIONS, TOO FEW MECHANISMS, H. Maurice Goodman, G Peter Frick, Thomas W Honeyman, Yael Schwartz and Lih-Ruey Tai Department of Physiology, University of Massachusetts Medical School, Worcester, MA, 01655

GH produces at least 3 classes of responses in mature rat adipocytes. The initial response is pleiotropic and insulin-like: membrane transport of glucose and synthesis of glycogen and fatty acids are increased, while cyclic AMP-dependent lipolysis is inhibited. The intramitochondrial enzymes, pyruvate and branched-chain $\alpha$-ketoacid dehydrogenases, are activated, as is an actinomycin-sensitive event that terminates the insulin-like response within about 2 h. The insulin-like response is not preceded by a change in cytosolic calcium, and the means of signalling in the various cellular compartments is not known. Upon termination of the insulin-like response, adipocytes are refractory to further insulin-like stimulation by GH for several hours, but GH receptors are not down-regulated. Induction of refractoriness is a separate response to GH that is initiated well after the events that terminate the insulin-like response are underway, and can be produced without a preceding insulin-like response. Conversely, immunoneutralization of GH at 60 min prevents the appearance of refractoriness without diminishing the insulin-like response. Induction of refractoriness is actinomycin-sensitive, associated with increased cytosolic calcium concentration, and may result from an autocrine product secreted in response to GH. A third category of GH responses is characterized by delayed onset of lipolysis and decreased glucose metabolism, and is also at least partly sensitive to actinomycin. Lipolysis is likely due to increased cAMP, but the mechanism for increasing cAMP is unknown. Production of the insulin-like response requires concentrations of GH at the upper end of the physiologic range (100-300 ng/ml), virtually saturating GH receptors, while lipolytic and refractory responses are maximal at or below the $K_d$ (10 ng/ml). Scatchard analysis suggests the presence of only a single class of surface binding sites, but modifications to GH or the carbohydrate component of the GH receptor selectively impairs either the insulin-like or the lipolytic response, suggesting different interactions may mediate different responses. Crosslinking studies reveal at least 3 GH-receptor complexes with $M_r$s of ~250, 130, and 55 kDa, with the ~250kDa diminishing to ~130 kDa upon disulfide reduction. Northern analysis of polyA-selected adipocyte RNA using a cDNA probe corresponding to the extracellular domain of the rat GH receptor reveals 2 major mRNA transcripts of ~1.2 and ~4 kbases, and at least 2 minor transcripts of ~1.6 and ~2.5 kbases. We do not know if different receptor transcripts are related to different responses, how they signal, or if the "secreted" form that lacks a transmembrane domain is involved in signalling.

## The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 027** THE GROWTH FACTOR REPRESSIBLE AP27 GENE REGULATES ADIPOCYTE DIFFERENTIATION, Gordon M. Ringold, Hans-Michael Wenz, Lindsay Hinck, Paul Cannon, Ulrich Danesch and Marc Navre, Institute of Cancer and Developmental Biology, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304

Environmental cues that dictate the ability of adipogenic cells to undergo differentiation must ultimately be detected as intracellular signals that impinge upon nuclear processes required for transcription of adipocyte-specific genes. We have focused substantial effort on understanding the mechanisms by which steroid and polypeptide hormones alter the ability of adipogenic cells to differentiate. Recently, we described the glucocorticoid-inducible and growth factor-repressible clone 5/AP27 gene that appears to play a critical role in "triggering" differentiation. A strict correlation exists between the ability of various agents to inhibit AP27 gene expression and 3T3-L1 or TA1 adipocyte differentiation. Using anti-sense RNA vectors we now show that cells expressing reduced AP27 are markedly compromised in their ability to differentiate. The function of AP27 remains obscure however the protein is clearly related by sequence to a family of NAD-dependent dehydrogenases.

In order to further delineate the signalling pathways by which hormonal cues act upon adipocyte genes, we have recently identified a cis-acting element (FSE) responsible for the differentiation-dependent transcription of the FSP27 gene. Moreover nuclear extracts from adipocytes but not pre-adipocytes contain at least one factor that binds specifically to a palindromic sequence present in the FSE. The FSP27 promoter and the transcription factors that regulate its expression therefore serve as useful tools to elucidate the molecular details associated with differentiation specific gene expression.

### *Altered Adipose Cell Function*

**CA 028** STRUCTURE AND FUNCTION OF AN ADIPOCYTE-SPECIFIC ENHANCER, Reed A. Graves, Peter Tontonoz, Amy Greenstein*, Kenneth Platt*, Susan R. Ross* and Bruce M. Spiegelman, Dana-Farber Cancer Institute and the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston MA 02115 and *Department of Biochemistry, University of Illinois Medical School, Chicago, IL 60612

The molecular basis for adipocyte-specific gene expression is not currently understood. We have recently discovered the first functional enhancer with specificity for adipose cells. This 500 base pair enhancer is found at -5.4 kilobases from the transcription start of the adipocyte P2 gene ands functions to stimulate heterologous gene expression specifically in cultured adipose cells or in the adipose tissue of trangenic mice. This enhancer functions with promoters from the aP2 gene or the SV40 early region. Deletion analysis of the 5' flank of the aP2 gene in transgenic mice demonstrates that this enhancer and not the binding site for C/EBP is the primary determinant of tissue specific gene expression. The molecular dissection of this enhancer reveals that it consists of at least 3 separable activities: (1) a binding site for an NF-1-like factor that functions as a positive element primarily in adipose cells, (2) a positive factor that functions in many cell types and (3) a negative element that suppresses gene expression in non-adipose cells.

Current experiments seek to clone and characterize the key trans-acting factors. We are also attempting to use the adipocyte enhancer to express a number of proteins with regulatory potential (adrenergenic receptors, adipsin) in fat cells in order to analyze their functions and possibly create new models of altered energy balance in vivo.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

*Late Abstracts*

## ROLES OF G PROTEINS AND G PROTEIN SUBUNITS IN SIGNAL TRANSDUCTION

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**Complexity**: G proteins are a complex family of heterotrimers of composition $\alpha\beta\gamma$. Some the native G proteins have been purified ($G_s$, 3 $G_i$s, 2 $G_o$s, $G_z$, and 1 $G_t$). Each of the subunits constitutes a family of homologous gene products of which the most complex is that of the $\alpha$'s for which there are at least 16 genes ($\alpha_s$, $\alpha_{i1}$, $\alpha_{i2}$, $\alpha_{i3}$, $\alpha_o$, $\alpha_t$-r, $\alpha_t$-c, $\alpha_{olf}$, $\alpha_z$,$\alpha_q$, $\alpha_{11}$ through $\alpha_{16}$). Some of these give rise to more than one peptide due to alternative RNA splicing ($\alpha_s$ has 4 forms, $\alpha_o$ has 2 forms). There are also several $\beta$'s ($\beta_1$ through $\beta_4$) and $\gamma$'s ($\gamma_1$(also T$\gamma$) through $\gamma_3$) genes. Not all genes and/or their splice variants are expressed in all cells and some, like $\alpha_{olf}$ and $\alpha_t$'s, and one of the $\gamma$'s, appear to be expressed only in single cell types. Thus it is clear that most G proteins have yet to be purified and also that it is unlikely that any of the purified G proteins can be accepted as homogeneous. Effects of "pure" G proteins need to be interpreted with caution. **Roles of Subunits**: $\alpha$'s have been shown by us and others to regulate adenylyl cyclase (AC), phosphodiesterase, ion channels and, indirectly, also phospholipase C. One hypothesis ascribes all signal transducing roles of animal cell G proteins to $\alpha$'s, but a second hypothesis gives such role also to the $\beta\gamma$'s formed upon G protein activation. Intact membrane systems were used to explore the effects of the latter. One study showed $\beta\gamma$'s to inhibit rather than stimulation G protein-gated $K^+$ channel activity, as shown previously for AC. In so doing, $\beta\gamma$'s were much more potent in inhibiting $K^+$ channel activity under basal (no agonist present) than under agonist stimulated conditions. This effect of agonist stimulation on the response to $\beta\gamma$'s was also found in AC assays using $GH_4C_1$ and wt S49 membranes, where stimulation, respectively, by VIP or isoproterenol essentially abolished any effect of $\beta\gamma$'s. In comparison, AC inhibition by somatostatin, typical PTX sensitive $G_1$ effect, is not inhibited by VIP ($GH_4C_1$) or isoproterenol (wt S49). Thus, we failed to obtain any evidence supporting the hypothesis that ascribes a signal transducing role to $\beta\gamma$ in activation of $K^+$ channels or inhibition of AC by receptor ligands. **Lipidation**: Several co- and postranslational reactions that may modify G protein subunits as obtained upon in vitro translation with reticulocyte lysates, were studied. We confirmed the findings by others that $\alpha$'s of the three $G_i$'s and of the two $G_o$ proteins, but not of the various forms of $G_s$, are co-translationally myristoylated. In addition we found that upon translation of mRNA's in the presence of labeled mevalonic acid or of labeled farnesylpyrophosphate plus unlabeled mevalonic acid, $\gamma$'s, but not the $\beta$ subunit or any one of the $\alpha$ subunits, are quantitatively polyisoprenylated. After cleavage from $\gamma$, the polyisoprenol thus formed co-migrated with 20-carbon geranylgeraniol. We infer that lipid modification is important for membrane localization.

MECHANISMS OF ALTERED GLUCOSE TRANSPORT RESPONSE TO INSULIN IN DIABETES AND OBESITY. S. W. Cushman and O. M. Gonzalez-Mulero, NIDDK, NIH, Bethesda, MD and B. B. Kahn, Department of Medicine, Harvard Medical School, Boston, MA USA.

Insulin stimulates glucose transport in rat adipose cells through the translocation of glucose transporters from an intracellular pool to the plasma membrane. Two glucose transporter isoforms have recently been identified in this cell: GLUT1 comprises <5% of total glucose transporters and is distributed equally between the plasma membranes and intracellular pool in the basal state; intracellular GLUT1 is extensively translocated to the cell surface in response to insulin. GLUT4 comprises >95% of the total, is almost totally sequestered in the intracellular pool in the basal state, and increases ≈20-fold on the cell surface through translocation of ≈50% of the intracellular pool with insulin. The relative roles of these glucose transporter isoforms in the altered glucose transport response to insulin in adipose cells have now been evaluated in the streptozotocin rat model of diabetes before and after insulin treatment and in the aged male rat model of obesity. Diabetes is accompanied by a depletion of basal GLUT4 and a corresponding reduction in GLUT4 translocation. These defects are reversed with in vivo insulin treatment. GLUT1 number and subcellular distribution are minimally changed. The alterations in glucose transport correlate with GLUT4 concentration in the plasma membranes. In the aged, obese rat, cellular enlargement is associated with insulin-resistant glucose transport which also correlates with a marked depletion of GLUT4 and reduction in its translocation. However, the markedly increased basal glucose transport per cell in obesity correlates closely with an expanding plasma membrane surface area containing a slightly increased concentration of GLUT1. These results suggest respective roles of GLUT1 in basal glucose transport and GLUT4 in the response to insulin, and alterations in the relative contributions of GLUT1 and GLUT4 to cellular glucose uptake in insulin-resistant metabolic states where the insulin response is diminished compared to basal activity.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

THE ADIPOSE CELL IN STATES OF METABOLIC DYSFUNCTION, Ulf Smith, Dept. of Medicine, Sahlgren's Hospital, Gothenburg, Sweden.

The main function of the adipose cell is to store lipids under periods of affluence and to release the stored FFA and glycerol when needed. This function is under precise control by hormonal and other humoral factors. The adipocyte may be used as a model cell for elucidating the mechanisms of action of hormones and other regulators. However, fat cells are also useful to elucidate pathophysiological alterations in man such as the mechanisms for insulin insensitivity in states of insulin resistance, the adrenergic regulation in cells from individuals with hypo- or hyperthyroidism or phaeochromocytoma.

Increased FFA release may also be of pathogenetic importance for the insulin resistance in obesity and NIDDM as well as an important cause of hypertriglyceridemia.

Fat cell metabolism is usually studied by incubating the cells under appropriate conditions in vitro. However, the recently developed microdialysis technique allows experimental studies in vivo. With this technique, a small dialysing probe is implanted in the subcutaneous tissue and perfused with saline at a slow flow rate. The dialysate is collected and can be analysed for the appropriate metabolites or hormones. Using the microdialysis technique, we have verified the presence of regional differences in fat cell lipolysis in situ. Furthermore, fat cells release significant amounts of lactate during an oral glucose tolerance test.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

## *Adipocyte Differentiation*

**CA 100** IDENTIFICATION OF LINEAGE-SPECIFIC REGULATORY GENES IN THE COMMITTED ADIPOBLAST
I. E. Akerblom, B. Brunk and C. P. Emerson, Jr. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Multipotential embryonic cells exhibit choices during development that progressively restrict their genetic potential. These decisions are translated into patterns of gene expression unique to each differentiated cell. The 10T1/2 cell line, which converts to stable muscle, adipocyte and chondrocyte lineages after treatment with demethylating agents, has been used extensively as a model system in which to examine genetic changes occurring when a cell is determined to a specific differentiation pathway. The specific aim of the work presented is to identify genes activated when these multipotential cells convert into cells programmed for the adipocyte lineage. A cDNA library from proliferating 10T1/2-derived adipoblasts has been constructed in a novel vector containing a eukaryotic promoter for transfection analysis. This primary library will be selected for adipoblast-specific cDNAs through subtraction of parental 10T1/2 sequences and positive selection with cDNAs from another adipoblast cell line. The final library will be used as a resource for 1) identifying genes capable of conferring the adipocyte potential through transfection studies, 2) characterizing adipoblast-specific genes individually and as a population, thereby developing markers for the adipoblast state, and 3) screening for sequences containing structural domains conserved in a number of developmentally regulated transcription factors. We expect that this approach wil result in the isolation of novel genes specific to determined adipoblasts which can be used to investigate the molecular mechanisms responsible for lineage determination and transduction of the lineage choice during terminal differentiation.

**CA 101** INDUCTION OF ADIPOCYTE DIFFERENTIATION IN CULTURE BY HYPOLIPIDEMIC ARYLOXY-ALKANOIC ACIDS, Jacob Bar-Tana and Ruth Brandes, Department of Biochemistry, Hebrew University-Hadassah Medical School, P.O.Box 1172, Jerusalem 91010, Israel

Hypolipidemic aryloxyalkanoic acids of varying hydrophobic backbones (i.e. clofibrate, bezafibrate, nafenopin) induce adipose conversion in cultured 3T3-L1 as well as in primary rat epididymal preadipocytes. Adipose conversion results in increase in fat globules, glycerophosphate dehydrogenase and hormone sensitive lipase activities as well as glutamine synthase and glycerophosphate dehydrogenase mRNA's. Adipose conversion induced by bezafibrate is synergistic with $bu_2$-cAMP or forskolin. The synergistic effect requires the simultaneous presence of the fibrate inducer and $bu_2$cAMP in the culture medium during the induction phase, while both may be removed during the conversion phase. A single 60K protein appears to be phosphorylated as early as 15 min following the incubation of non-differentiated 3T3-L1 cultures in the presence of both bezafibrate and $bu_2$-cAMP, or in the presence of IBMX. Hence, induction of adipose conversion by aryloxyalkanoic acids or IBMX appears to involve an early cAMP-dependent phosphorylation of a 60K protein. The synergistic action of bezafibrate and cAMP in initating commitment of adipose conversion may indicate that the inductive capacity of IBMX may be accounted for by two independent functions - acting as an inducer as well as a generator of cAMP.

**CA 102** DIFFERENTIATION OF 3T3-L1 PREADIPOCYTES WITH 3-ISOBUTYL-1-METHYL XANTHINE AND DEXAMETHASONE STIMULATES CELL-ASSOCIATED AND SOLUBLE CHONDROITIN-4-SULFATE PROTEOGLYCANS, Juan C. Calvo, David Rodbard and Masaki Yanagishita*, Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development and *Bone Research Branch, National Institute of Dental Research, Bethesda, MD 20892

We analyzed the composition of the proteoglycans in 3T3-L1 preadipocytes cultured in DMEM containing 10% fetal calf serum, before and after differentiation into adipocytes induced by treatment with 0.5 mM 3-isobutyl-1-methyl xanthine (IBMX) and 0.1 $\mu$M dexamethasone for 48 h. Cells were metabolically labeled with [$^{35}$S]sulfate and [$^{3}$H]glucosamine in fresh medium containing insulin (10 $\mu$g/ml) for 20 h. After labeling, the culture medium and the cells were extracted and processed for proteoglycan analysis. There was a 1.67 ± 0.18 fold increase in macromolecular $^{35}$S in the medium during differentiation. The cell extract showed no increase in labeling when expressed per mg DNA. Analyses of radiolabeled molecules using ion exchange chromatography, gel filtration and HPLC in combination with specific enzymes (chondroitinase ABC and heparitinase) and alkaline treatment indicated that virtually all $^{35}$S was incorporated into two major species of chondroitin-4-sulfate proteoglycans (CSPG-I and CSPG-II). CSPG-I has a mass of 970 kDa with multiple CS chains (average 50 kDa each) and a core protein of 370 kDa including the oligosaccharides. CSPG-II has a mass of 140 kDa with one or two CS chains (average 61 kDa each) and a core protein of 41 kDa including oligosaccharides. Differentiation was associated with a specific increase of CSPG-I (4 fold increase of secretion into medium, and 2.5 fold increase of the cell-associated form). Analysis of the time course of the appearance of the two PG peaks revealed that CSPG-I preceded triglyceride accumulation, whereas CSPG-II remained almost constant. IBMX was the main stimulus for CSPG-I, but dexamethasone and insulin were synergistic, with a major effect when the three stimuli were present simultaneously. A part (13%) of CSPG-I is able to bind to hyaluronic acid. CONCLUSIONS: 1) Based on molecular composition, CSPG-I is similar to versican; 2) CSPG-II is similar to either decorin or biglycan; 3) 3T3-L1 preadipocytes provide an ideal model system for study of cell-matrix interaction during growth and differentiation.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 103** DIFFERENTIAL SENSITIVITY OF MOUSE PREADIPOCYTE LINES TO DEVELOPMENTAL BLOCK BY SV40 LARGE T ANTIGEN MUTANTS

Cherington, V.1,2, Chatterjee, R.1, Krieser, R.2, and Higgins, C.1. Departments of Physiology1 and Pathology2, Tufts University School of Medicine, Boston, MA 02111.

Constitutive expression of SV40 large T antigen (SVLT) blocks differentiation of confluent monolayers of murine preadipocyte lines (3T3-F442A and 3T3-L1) prior to induction of differentiation-dependent genes in fetal calf serum-supplemented or serum-free media (containing epidermal growth factor, growth hormone, insulin). We are studying the mechanism of SVLT in preadipocytes in order to determine the functional relationship between oncogene transformation/tumorigenesis and the differentiation block. Certain SVLT point or deletion mutants capable of blocking 3T3-F442A, do not block 3T3-L1 differentiation. SVLT mutants studied include transformation defective mutants (no longer associate with the cellular RB tumor suppressor gene product), and C-terminal deletion mutants (no longer associate with the cellular p53 protein). Because induction of differentiation of 3T3-L1, but not of 3T3-F442A, requires a 1-2 day exposure to glucocorticoid (GC) and isobutylmethylxanthine (MIX), we are investigating the role of GC/MIX in altering SVLT function. The SVLT block is less efficient when differentiation is induced in suspension cultures; lines transformed by wild-type SVLT induce differentiation-dependent genes in suspension indicating at least a partial reversal of the SVLT block. Although SVLT is still present in non-adherent cells, our data indicate that the partial reversal of the SVLT block results from either the elimination of a functional subset of SVLT in these cells, or a reduction of the total SVLT below a functional threshold.

**CA 104** EXPRESSION OF ß1- and ß2- AND ATYPICAL ß-ADRENERGIC RECEPTORS DURING 3T3-F442A ADIPOSE DIFFERENTIATION.

Bruno Fève*, Laurent J. Emorine¶, Françoise Lasnier*, Donny A. Strosberg¶ and Jacques Pairault*, *U282 INSERM-CNRS, Hôpital Henri Mondor, 94010, Créteil, FRANCE and ¶ CNRS, SD1-6231, ICGM, Hôpital Cochin, 75014 Paris, FRANCE

Expression of ß1-, ß2- and atypical β-adrenergic receptors (ß-ARs) during the adipose differentiation of murine 3T3-F442A cells was investigated at receptor protein and mRNA levels. Adenylate cyclase activity and lipolysis of adipocytes was activable by the selective agonist BRL37344 and the ß1/ß2 antagonist CGP12177. Intact adipocytes and adipocyte plasma membranes exhibited both high and low affinity binding sites for (-)-[$^{3}$H]CGP12177 and [$^{125}$I]CYP, respectively. The low-affinity ß-AR component represented ~80 % of total ß-ARs (~60,000 sites/cell) in adipocytes. Competition of [$^{125}$I]CYP binding by agonists or antagonists of various subtype selectivity indicated that the high affinity ß-AR component was related to ß1- and ß2-AR subtypes (15-20% and 2-5% of total ß-ARs, respectively) while the low affinity one corresponded to the atypical ß-AR. Preadipocytes, poorly responsive to ß-AR agonists, only expressed few high affinity ß-ARs (~3000 sites/cell) solely of ß1 subtype. Northern analysis of poly(A)$^{+}$RNA from preadipocytes and adipocytes was carried out using several human ß-AR subtype-specific DNA probes. The ß1-AR mRNA (3.2 kb) was the unique species present in preadipocytes. In adipocytes there was a 3-4.7 fold induction of the ß1-AR mRNA associated to the emergence of strong ß3-AR signals (2.2 and 2.65 kb). The ß2-AR mRNA (1.8 kb) was expressed at low levels in adipocytes.

Based on the close parallelism between ligand binding, functional studies and mRNA analyses, our work provides evidence for the presence of a prominent "β3-AR-like" population, so far known as the atypical ß-AR, mediating the lipolytic effect of catecholamines.

**CA 105** HUMAN RELAXIN INHIBITS CELL DIVISION IN 3T3-L1 FIBROBLASTS,

Susan C. Frost, Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610.

For the first time, we have been able to uncouple cell proliferation from differentiation in the adipogenic cell line, 3T3-L1 fibroblasts. When confluent fibroblasts are induced to differentiate they typically undergo two rounds of cell division followed by accumulation of lipid droplets and expression of insulin-stimulated glucose transport as these cells obtain the adipocyte phenotype. Human relaxin added during induction had no effect on the development of the adipocyte phenotype or insulin-stimulated glucose transport. However, it blocked cell division at a half-maximal concentration of 1.25nM well within physiological range. This could be reversed by the addition of antibodies specific for human relaxin. Thus, relaxin joins a select number of hormones with growth inhibitory properties such as transforming growth factor-ß (TGFß) and mammostatin. Unlike other inhibitory peptides, like TGFß, relaxin does not prevent differentiation but rather uncouples it from cell division. Further studies suggest that relaxin functions as a $G_2M$ inhibitor.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 106 MITOCHONDRIAL UNCOUPLING PROTEIN IN BROWN ADIPOCYTES DIFFERENTIATED IN CELL CULTURE,** Jan Kopecky, Marie Baudysová, Stanislav Pavelka, Franco Zanotti, Dagmar Janíková and Josef Houstek, Institute of Physiology, Czechoslovak Academy of Sciences. 142 20 Prague, Czechoslovakia.

In order to characterize the biogenesis of unique thermogenic mitochondria of brown adipose tissue (BAT), the differentiation of precursor cells isolated from mouse BAT was studied in cell culture. Cells were cultured in the presence of serum , or in chemically defined serum-free medium. Synthesis of mitochondrial uncoupling protein (UCP), $F_1$-ATPase, and cytochrome oxidase was examined by L-[$^{35}$S]methionine labeling and immunoblotting. For the first time, synthesis of physiological amounts of the UCP - a key and tissue-specific component of thermogenic mitochondria, was observed in cultures at about confluence (day 6), indicating that a complete differentiation of brown adipocytes was achieved *in vitro*. In postconfluent cells (day 8) the content of UCP decreased rapidly, in contrast to the other mitochondrial enzymes, $F_1$-ATPase and cytochrome oxidase. In these cells, it was possible, by using norepinephrine, to specifically induce the synthesis of the UCP, but not $F_1$-ATPase or cytochrome oxidase. The maximal response was observed at 0.1 μM norepinephrine and the synthesis of UCP remained activated for at least 24 h. Activation of UCP synthesis was accompanied by a transient stimulation of Type II iodothyronine 5'-deiodinase activity. Stimulation of the β-adrenergic receptors by catecholamine and elevation of intracellular concentration of cAMP played major roles in both, the stimulation of UCP synthesis and the increase of deiodinase activity. Stimulation of $\alpha_1$-adrenergic receptors enhanced the regulatory effect. A quantitative recovery of the newly synthesized UCP in the mitochondrial fraction indicated completed biogenesis of functionally competent thermogenic mitochondria.

**CA 107** CATECHOLAMINE STIMULATED LIPOLYSIS IN HUMAN ADIPOCYTES GROWN FROM PRECURSORS IN SERUM-FREE PRIMARY CULTURE. Derek Litthauer, Maryna van de Venter and Willem Oelofsen. Biochemistry Department, University of Port Elizabeth, P O Box 1600 Port Elizabeth 6000, South Africa.

Catecholamines are the major lipolytic agents in human adipocytes. Stromal-vascular cells from human subcutaneous abdominal or mammary adipose tissue were grown to confluence in DMEM:F12 (1:1) medium containing insulin (10 μg/ml), transferrin (10 μg/ml), dexamethazone ($10^{-8}$M), $T_3$ ($10^{-9}$M), fetuin (100 μg/ml) and human HDL (75 μg protein/ml) when 0.1 mM isobutyl-methyl-xanthine was added for seven days. Under these condition a differentiation rate of about 75% was achieved with glycerol-3-phosphate dehydrogenase activity reaching 1100 to 1500 mU/mg protein. Fully differentiated cells (14-18 days post confluence) were maintained in the above medium, containing 3% BSA for 2 days to minimize breakage before the lipolysis experiments, which were performed in Krebs-Ringer bicarbonate buffer containing 4% BSA. The adipocytes displayed catecholamine induced lipolysis (isoproterenol and norepinephrine) using glycerol release into the buffer as a measure of lipolysis.

**CA 108** REGULATION OF THE INSULIN RECEPTOR GENE DURING ADIPOCYTE DIFFERENTIATION Catherine McKeon and Thang Pham. Diabetes Branch, NIDDK, NIH, Bethesda, MD 20892

Although the insulin receptor gene is expressed in most tissues, the level of expression is regulated in a tissue specific manner. In 3T3-L1 cells, a tissue culture model for adipocyte differentiation, the insulin receptor gene has been shown to be induced 10-fold during differentiation. In order to study the regulation of the insulin receptor gene, we have cloned 40 Kb of genomic DNA containing the 5' end of the human insulin receptor gene. The first 575 bp upstream of the translation initiation site contains promoter activity when inserted in a eukaryotic expression vector fused to the reporter gene, chloramphenicol acetyltransferase (CAT). However, when this vector was stably integrated into 3T3-L1 cells, this region alone could not mediate the induction during adipocyte differentiation. The addition of a 2 Kb region from the insulin receptor gene to this expression vector confers the ability to induce CAT expression in stable transfectants of 3T3-L1 cells during adipocyte differentiation. These studies are the first to demonstrate tissue-specific regulation of the insulin receptor gene. Supported by JDF Grant #188739.

**CA 109** ADRENERGIC REGULATION OF *DIFFERENTIATION* (EXPRESSION OF THE UNCOUPLING PROTEIN THERMOGENIN AND OF LIPOPROTEIN LIPASE) AND OF *PROLIFERATION* (DNA SYNTHESIS) IN BROWN ADIPOCYTE CULTURES

Jan Nedergaard, Stefan Rehnmark, David Herron, Myriam Néchad, Anders Jacobsson, Josef Houstek, Gennady Bronnikov, Michael C. Schotz, Todd G. Kirchgessner and Barbara Cannon; The Wenner-Gren Institute, Stockholm University, S-106 91 Stockholm Sweden; *and* Université P & M Curie, Paris; Czech Academy of Sciences, Prague; USSR Academy of Sciences, Pushchino; Veterans Administration Research, Los Angeles

In order to understand the differentiation and proliferation processes occurring in brown adipose tissue during the recruitment process, the ability of adrenergic stimulation to influence these processes in brown adipocytes proliferating and differentiating in culture from isolated precursor cells was studied. The expression of the uncoupling protein *thermogenin* could be observed both as a norepinephrine-evoked induction of thermogenin mRNA and as the occurrence of thermogenin antigen. Immunoelectron micrographs showed that the induced synthesized thermogenin molecules were transported to the mitochondria and incorporated in the mitochondrial membrane.– *Lipoprotein lipase* gene expression was also influenced by adrenergic stimulation.– *Cell proliferation* was followed as the rate of DNA synthesis and it was found that it was possible to accelerate the rate of DNA synthesis in the exponential growth phase but not in confluent cells. The adrenergic *subtype classification* and the possible intracellular messengers involved was investigated for the above processes. Both $\alpha_1$- and ß-adrenergic responses could affect thermogenin gene expression but cell proliferation was fully under ß-adrenergic control. Concerning the ß-adrenergic responses, the possibility is being investigated that different subtypes are involved in the differentiation and the proliferation response. *It was concluded* that adrenergic pathways are involved in the control of both proliferation and differentiation processes in these cells. (For details see Herron et al. (1990) FEBS Lett. 268, 296-300; and Rehnmark et al. (1990) J. Biol. Chem. 265, 16464-16471)

**CA 110** A MUTATION IN BOVINE GROWTH HORMONE ALTERS ITS ABILITY TO INDUCE ADIPOCYTE DIFFERENTIATION, Shigeru Okada, Wen Y. Chen, Bruce Kelder, Paul E.C. Wiehl and John J. Kopchick, Department of Zoological and Biomedical Sciences, Molecular and Cellular Biology Program and Edison Animal Biotechnology Center, 201 Wilson Hall, Ohio University, Athens, Ohio, 45701.

To determine the importance of the third α-helix of bovine growth hormone (bGH) on its biological activities, we have introduced a series of mutations into bGH. In one mutation (pbGH10Δ6-M8), the amphiphilic α-helix was idealized by the following changes: glutamate-117 to leucine, glycine-119 to arginine, and alanine-122 to aspartate. Transgenic mice expressing the mutated bGH (bGH-M8) were shown to have a significant growth supressed phenotype, although bGH-M8 posessed the same binding affinity to mouse liver membrane preparations as compared to wild-type bGH. Culture fluids from stably transformed mouse L cells secreting either wild-type bGH or bGH-M8 were used to induce differentiation of 3T3-F442A fibroblasts into mature adipocytes. Addition of bGH-M8 resulted in significantly reduced induction of 3T3-F442A cell differentiation compared to wild-type bGH as measured by glycerol-3-phosphate dehydrogenase activity.

**CA 111** THE IDENTIFICATION AND CHARACTERIZATION OF PROTEINS WHOSE REGULATION IS ASSOCIATED WITH COMMITMENT TO 3T3-L1 ADIPOCYTE DIFFERENTIATION. Henry B. Sadowski, Thomas T. Wheeler and Donald A. Young, Environmental Health Science Center, and Endocrine-Metabolism Unit, Department of Medicine, University of Rochester Medical Center, Rochester, NY 14642.

The mouse 3T3-L1 fibroblastic cell line can be induced to differentiate rapidly (4-6 days) and efficiently (80% conversion) to adipocytes by a 48 h treatment of post-confluent cells with the combination of dexamethasone (D), methylisobutyl-xanthine (M) and insulin (I). Biological studies of the effects of these agents on differentiation determined that (i) D acts synergistically with MI to induce differentiation, (ii) maximal commitment to adipocyte differentiation requires the presence of D during the period 30-48 h after the addition of MI, and (iii) the phorbol ester, TPA, completely inhibits adipocyte differentiation unless it is added 36 h or more after initiating DMI treatment. These results define a window in time during which the cells become committed to differentiation. To study gene expression during commitment to, and subsequent expression of the adipocyte phenotype, we used ultra-high resolution two-dimensional electrophoretic analysis of metabolically labeled proteins and *in vitro* translation products of mRNA. This allowed the simultaneous analysis of the regulation of over 4000 synthesized proteins and 2000 mRNA species. We identified and characterized 8 mRNA species: (i) that were synergistically induced by treatments that lead to differentiation, (ii) were first expressed at elevated levels before the appearance of known adipocyte phenotypic markers, (iii) remained elevated in fully differentiated adipocytes, and (iv) whose elevated expression was inhibited by TPA. These novel differentiation-associated proteins may be important in the commitment to and expression of the adipocyte differentiation program. [Supported by grants from NIH; ES07026,DK1677 and CA47650]

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

CA 112 BIOCHEMICAL CHARACTERIZATION OF SERUM-DERIVED ADIPOGENIC FACTORS, Ginette Serrero, Hirokazu Zaitsu, Koichiro Tanaka and Weiguo Zhao, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY 12946

Fetal bovine serum has been shown to contain adipogenic activity for preadipocyte cell lines (1). Subsequently it was shown that serum could be replaced by a fraction called Fetuin (2) which is derived from fetal bovine serum by ammonium sulfate precipitation (3). Recently, fetuin has been widely used also to support the growth and differentiation of adipogenic cell lines and of preadipocytes in primary culture. In the work presented here characterization of adipogenic activity in fetuin was undertaken by following the differentiation of the teratoma-derived adipogenic cell line 1246 and of the 3T3-L1 cell line. Partial purification and biochemical characterization using commercially available crude pedersen fetuin as starting material was performed. The results show the existence of several adipogenic factors with distinct biochemical properties (4). The two factors which have the highest adipogenic specific activity were shown to be different from the two major contaminants found in fetuin: $\alpha 2$ macroglobulin and the 68 kDa acidic glycoprotein fetuin. The details of the experiments described here will be presented.

Supported by grant DK38639 from the National Institute of Health.

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# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

*Regulation of Metabolism and Gene Expression in Adipocytes*

**CA 200** PHOSPHORYLATION OF ADIPOCYTE LIPID BINDING PROTEIN (p 15) BY THE INSULIN RECEPTOR, David A. Bernlohr, Melissa K. Buelt, Ronald S. Sha and Laurie L. Shekels, Dept. of Biochemistry, Univ. of Minnesota, St. Paul, MN 55108

The adipocyte lipid binding protein (ALBP) has been phosphorylated by the insulin receptor (IR) in vitro. following insulin stimulated autophosphorylation of the wheat germ agglutinin purified 3T3-L1 adipocyte IR ALBP was phosphorylated exclusively on tyrosine $^{19}$ in the sequence ENFDDY, analogous to the substrate phosphorylation consensus sequence observed for several tyrosyl kinases. The concentration of insulin necessary for half-maximal IR autophosphorylation ($K_{0.5}^{IR}$) was identical to that necessary for half-maximal ALBP phosphorylation ($K_{0.5}^{ALBP}$), 10 nM. Kinetic analysis indicated that stimulation of ALBP phosphorylation by insulin was attributable to a 5-fold increase in the $V_{max}$ (to 0.27 fmol/minute/fmol insulin binding sites) while the $K_m$ for ALBP (50 μM) was unaffected. Utilizing the soluble kinase domain of the human IR β-subunit, the presence of a fatty acid bound to ALBP reduced the $K_m$ to 10 μM. Unbound fatty acids inhibited soluble IR kinase autophosphorylation in a concentration dependent fashion with a $K_i$ of 4 μM. The inhibitory action of the unbound fatty acids on the soluble kinase was relieved when ALBP was present during autophosphorylation. Therefore, in vitro murine ALBP is phosphorylated on tyrosine$^{19}$ in an insulin stimulated fashion by the IR and the presence of a bound fatty acid on ALBP increases the affinity of IR for ALBP. The effect of fatty acids on IR autophosphorylation in situ in CHO cells transfected with the human IR has also been evaluated. These results suggest that the end products of the lipogenic pathway may feedback inhibit the tyrosyl kinase and that fatty acid binding proteins have the potential to modulate such interaction.

**CA 201** **DIABETES REDUCES THE HEPARIN-, VANADATE-, AND PHOSPHOLIPASE C-RELEASE OF LIPOPROTEIN LIPASE FROM CARDIOMYOCYTES**, J. Braun and D. Severson, Department of Pharmacology and Therapeutics, The University of Calgary, Calgary, Alberta, Canada T2N 4N1

Lipoprotein lipase (LPL) catalyzes the hydrolysis of the triacylglycerol component of lipoproteins at the endothelial luminal surface. LPL is synthesized within parenchymal cells and then is secreted and translocated to its functional site at the endothelial cell surface. We have examined the secretion of LPL from myocytes isolated from hearts of control and acutely diabetic rats (100mg/kg streptozotocin; 3-4 days). LPL was released into the medium after incubation of cardiac myocytes with heparin, phosphatidylinositol-specific phospholipase C (PI-PLC), and decavanadate (DV). Monomeric vanadate, alone or in combination with $H_2O_2$ or phorbol dibutyrate did not release LPL. Heparin pretreatment of myocytes reduced the subsequent release of LPL due to PI-PLC and DV, suggesting that LPL may be bound ionically to heparan sulfate proteoglycans that are covalently linked to the cell surface of cardiac myocytes by a phosphatidylinositol-glycan membrane anchor. Myocytes from diabetic rat hearts secrete less LPL activity in response to heparin, DV and PI-PLC than do myocytes from control rat hearts. *In vivo* treatment of diabetic rats, with insulin, but not *in vitro* insulin treatment of myocytes from diabetic rats rapidly (1 h) reverses the effects of diabetes.

**CA 202** REGIONAL AND OBESITY-RELATED DIFFERENCES IN ADENYLYL CYCLASE-LINKED RECEPTOR SIGNALLING IN HUMAN ADIPOCYTES, Patricia J.Coon, Andrew S.Greenberg, Andrew P.Goldberg, Constantine Londos, Department of Medicine, University of Maryland, Baltimore, Md 21218 and NIDDK, NIH, Bethesda, Md 20892.

The effects of obesity on the regulation of lipolysis in regional fat depots was determined by evaluating the signalling properties of stimulatory and inhibitory adenylyl cyclase-linked receptors subcutaneous abdominal and gluteal adipocytes from lean (19±1% body fat) and obese (31±2%), healthy, middle-aged (60±2 yr) men. In plasma membranes purified from adipocytes isolated by collagenase digestion, we measured adenylyl cyclase activity stimulated by the beta-adrenergic agonist, isoproterenol (Iso), or inhibited by adenosine, nicotinic acid, prostaglandin, and alpha-adrenergic receptor agonists. Results, standardized against maximal forskolin-stimulated adenylyl cyclase activity, showed that in lean men, all receptor stimulators and inhibitors were more efficacious in abdominal than gluteal plasma membranes. In contrast, in obese men, Iso-stimulated adenylyl cyclase activity was blunted in membranes from both sites, but a more pronounced attenuation of activity in abdominal membranes led to a loss of regional differences. Inhibition of cyclase activity by adenosine and prostaglandin receptor agonists also was blunted in abdominal membranes from obese men, thereby reducing the regional differences seen in the lean men. Inhibition mediated by alpha-adrenergic agonists or nicotinic acid did not appear to be affected by obesity. Thus, these data show that there are regional differences in the signalling properties of both stimulatory and inhibitory adenylyl cyclase-linked receptors. The attenuation of the action of some receptors by obesity is region-specific.

**CA 203** SEASONAL CHANGES IN HORMONE SENSITIVE AND LIPOPROTEIN LIPASE mRNA EXPRESSION IN OBESE MARMOTS. Gregory L. Florant, Bruce E. WIlson, D. Scott Weigle, and Samir Deeb. Dept. of Biology, Temple Univ., Philadelphia, PA 19122 and Dept. of Medicine, Univ.of Washington, Seattle, WA 98195.

The mechanisms underlying the circannual rhythms of body fat in mammals that hibernate are poorly understood. We investigated the yearly expression of genes encoding two enzymes involved in fatty acid metabolism, hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) in the adipose tissue of five marmots (Marmota flaviventris). We hypothesized that expression of mRNA for HSL would be high during the winter fasting period and LPL mRNA expression low. The converse relationship would be true during the summer and early fall period of weight gain. Total RNA was isolated from gonadal fat obtained from marmots at four times during their circannual cycle: spring, summer, fall, winter. RNA from each season was analyzed for expression of mRNA for HSL and LPL by Northern blotting. RNA blots were probed with labeled cDNA for HSL and LPL genes. Quantitation of mRNA levels in the different seasons was determined by densitometry. We found that although the pattern of expression was similar for the two genes, HSL mRNA expression was significantly ($P<.05$) elevated during the winter and early spring when compared to LPL m RNA expression. In summer and fall, the HSL/LPL mRNA ratio was low indicating predominance of LPL mRNA expression. We conclude that gene expression for LPL is associated with summer and fall feeding and weight gain whereas expression of HSL mRNA is associated with fasting and hibernation.

**CA 204** A MULTIRECEPTOR-LINKED NADPH-OXIDASE PRESENT IN HUMAN FAT CELL PLASMA MEMBRANES DEFINES A NOVEL PATHWAY OF SIGNAL TRANSDUCTION, Heidemarie I. Krieger-Brauer and Horst Kather, Klinisches Institut für Herzinfarktforschung an der Medizinischen Universitätsklinik, Berheimerstraße 58, D-6900 Heidelberg, W.-Germany.

It has been proposed that hydrogen peroxide ($H_2O_2$) may act as a second messenger for insulin, and evidence has been presented to suggest that adipocytes possess a plasma membrane-bound NADPH-oxidase, producing $H_2O_2$, that is activated on exposure of intact adipocytes to insulin. We have confirmed that human fat cells contain a stimulus-sensitive NADPH-oxidase resembling the respiratory burst oxidase of professional phagocytes with respect to substrate specificity, cofactor requirements, and stbility of activation. The human fat cell oxidase is activated by insulin and cytokines acting through phosphotyrosine kinase receptors (IGF I, EGF, PDGF), or via binding sites that are devoid of enzymic activity, but may associate with non-receptor tyrosine kinases (TNF, IL-1$\alpha$, Interferon$\gamma$). In contrast to all other hormone-, or cytokine-sensitive NADPH-oxidases that have been characterized, the human fat cell oxidase retains its hormone responsiveness after cell disruption.Intriguingly, no ATP is required for a ligand-induced activation in crude plasma membranes, indicating that insulin and various cytokines utilize phosphorylation-independent pathways for receptor signalling.

Overall, the findings demonstrate that human fat cells contain a stimulus-sensitive NADPH-oxidase that is unique in that all components necessary for a ligand-induced activation are plasma membrane-bound, and provide direct biochemical evidence indicating that activation of this multireceptor-linked system defines a novel pathway of signal transduction.

**CA 205** ROLE OF THE FAT CELL ADENYLATE CYCLASE SYSTEM IN REGIONAL- AND SEX-RELATED ADIPOSE TISSUE LIPOLYTIC SPECIFITIES, Danièle Lacasa, Brigitte Agli and Yves Giudicelli, Department of Biochemistry, Faculty of Medicine Paris-Ouest CHI, Poissy 78303, France.

Triglycerides mobilization, one of the two processes controlling the fat mass, depends on the fat cell lipolytic activity. In Humans and rats, this activity varies with both the fat cell anatomical localization and the estrogenic status. To determine the mechanism underlying these sex hormone- and site-related differences, we have compared the effects of long-term ovariectomy ( 3 weeks) on some of the membranous components controlling the lipolytic response in rat subcutaneous ( SC) and parametrial ( PM) fat cells. Our experiments show that:

1. Regardless of the estrogenic status, SC adipocytes are less lipolytic, produce less cyclic AMP and possess lower B-adrenoceptor number than PM adipocytes;
2. Ovariectomy induces a decrease in the lipolytic activity, cyclic AMP production and in both the B-agonist-stimulatory response and the catalytic subunit activity of adenylate cyclase in PM but not in SC adipocytes.

These results showing a rather insensitivity of the SC fat cells to the estrogenic status may contribute to explain some of the sex-related regionalspecifities of adipose tissue metabolism and distribution.

**CA 206** PHOTOAFFINITY LABELLING AND SEQUENCING OF THE ATP-BINDING SITE OF PYRUVATE DEHYDROGENASE KINASE, M. Tony Leesnitzer, William Burkhart and Joseph Espinal, Division of Endocrinology, Glaxo, Inc., Research Triangle Park, NC 27709

One possible mechanism of reducing hyperglycemia in Type II diabetes is to increase peripheral glucose utilization. A key step in glucose disposal is the irreversible oxidation mediated by pyruvate dehydrogenase. PDH activity is regulated in part by reversible phosphorylation of the E1-alpha subunit of the complex. Phosphorylation, which inactivates PDH, is carried out by an intrinsic kinase that co-purifies with PDH complex. In the present study, we have photoaffinity labelled PDH complex in order to sequence the ATP-binding site of the kinase. Photolysis of 8-azido ATP in a purified PDH incubation resulted in inhibition of PDH kinase. Analysis of TCA precipitates of $^{32}$P-azido ATP incubations of PDH by SDS-PAGE and autoradiography indicated labelling of a 44,000 $M_r$ subunit peptide. The peptide was excised from the gel and digested with V8 protease. A single $^{32}$P-labeled peptide was isolated by C18 reverse-phase HPLC, and the amino acid sequence was determined. The sequence of the ATP-binding site will be used to develop inhibitors of PDH kinase.

**CA 207** INVOLVEMENT OF THE RECEPTOR-COUPLED ADENYLATE CYCLASE SYSTEM IN THE ANATOMICAL SITE-RELATED CHARACTERISTICS OF LIPOLYSIS IN FAT CELLS, René Pecquery, Marie-Noêlle Dieudonné and Yves Giudicelli, Department of Biochemistry Faculty of Medicine Paris-Ouest, CHI, Poissy 78303, France.
Various studies have shown that the lipolytic response of white adipocytes to catecholamines was dependent on the anatomical origin of these cells. To explain this phenomenon, we have presently compared hamster white adipocytes from the subcutaneous and epididymal fat for their lipolytic activity, cyclic AMP response and adrenoceptor-coupled adenylate cyclase ( AC ) system. Basal and maximal lipolytic responses to β- ( isoproterenol ) and mixed α2/β ( epinephrine ) adrenergic agonists were lower in subcutaneous than in epididymal fat cells, but the α2- adrenergic antilipolytic response to UK-14304 was higher in subcutaneous than in epididymal fat cells. Identical results were observed for cyclic AMP production. These differences appear to imply several steps of the AC system since 1) the density of inhibitory α2-adrenoceptors ( 3H-RX 821002 binding sites) was higher in subcutaneous than in epididymal fat cells, and, 2) the AC catalytic activity was lower in subcutaneous than in epididymal fat. In conclusion, besides an increased α2-inhibitory pathway in white subcutaneous adipocytes, their lower AC catalytic activity appears to be the major limiting step when considering the lipolytic response to cat echolamines.

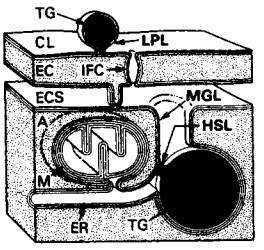
**CA 208** A MECHANISM OF INSULIN RESISTANCE IN CHRONIC DISEASE: COORDINATE SUPPRESSION OF C\EBP, GLUT4 AND INSULIN RECEPTOR mRNA BY TUMOR NECROSIS FACTOR-α, Phillip H. Pekala and Jacqueline Stephens, Department of Biochemistry, School of Medicine, East Carolina University , Greenville NC 27834
Fully differentiated 3T3-L1 adipocytes (7 days post induction) were subjected to continuous exposure to 5 nM TNF for the next 13 days. After 6 days of treatment, mRNA content for both GLUT4 (insulin responsive glucose transporter) and C\EBP (a transcription factor proposed to control expression of GLUT4) decreased by 80% relative to age matched controls, while insulin receptor mRNA levels decreased by at least 50%. These mRNA levels remained suppressed for the duration of the experiment (7 additional days). Messenger RNA content for other differentiation specific genes, including lipoprotein lipase and pAL422 (aP2) remained unchanged. In addition, β-actin mRNA content was not affected by the treatment, while mRNA for GLUT1, a second isoform of the transporter expressed in both fibroblasts and adipocytes exhibited a minor decrease. The observed changes appear to be controlled, at least in part, at the level of transcription based on nuclear transcription run-on assays. Results from hexose transport assays suggest that the TNF-induced depletion of insulin receptor and GLUT4 mRNA corresponds to a loss in the ability of insulin to stimulate hexose uptake. These data suggest two distinct mechanisms for the control of differentiation specific genes in the 3T3-L1 adipocytes: one that can be regulated by TNF and appears to control insulin sensitivity, and another that is TNF-insensitive. Thus, even during chronic exposure to TNF the 3T3-L1 adipocytes, although insulin insensitive, are able to partially preserve their adipose cell phenotype.

**CA 209** INCREASED INSULIN ACTION IN WHITE ADIPOSE TISSUE AT THE ONSET OF EXPERIMENTAL OBESITY : ACTIVATION OF MULTIPLE INSULIN EFFECTORS, Luc Pénicaud, Béatrice Cousin, Anne-Françoise Burnol*, Dominique Perdereau*, Armelle Leturque*, Luc Picon, Jean Girard*, Pascal Ferré*.Centre de Recherches sur la Nutrition, UPR1511*, Meudon, URA 307, Paris, CNRS, France.

We have shown *in vivo* and *in vitro* in the rat that, early after lesion of the ventromedian hypothalamus (VMH), white adipose tissue is hyper-responsive to insulin. We have studied the insulin effectors involved in this increase in white adipose tissue of VMH rats one week after the lesion. The number and affinity of insulin receptors were unchanged whereas autophosphorylation and tyrosine kinase activity were 2.5-fold higher in response to insulin. The increase in basal and insulin-stimulated glucose transport was concomitant with a 4-fold increase in Glut 4 protein and specific mRNA concentrations. The marked increase in basal and insulin-stimulated lipogenesis was related to a higher activity and mRNA concentrations of two key lipogenic enzymes : fatty acid synthase and acetyl-CoA carboxylase. Thus the hyper-responsiveness of glucose metabolism to insulin in white adipose tissue early during the course of obesity is related to both receptors and post-receptors changes. Since an increased insulin secretion is one of the earliest detectable modifications, it is speculated that insulin could play a crucial role in these phenomena.

**CA 210** **TRANSPORT OF FATTY ACIDS AND MONOACYLGLYCEROLS IN WHITE AND BROWN ADIPOSE TISSUES**
**Robert O. Scow and E. Joan Blanchette-Mackie, Endocrinology Section, Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD 20892**

**Fatty acids (FA) and monoacylglycerols (MG) are produced by lipoprotein lipase (LPL) from plasma triacylglycerols (TG) in capillaries of adipose tissue and transported to adipocytes (A) for TG synthesis. Long chain FA and MG are insoluble in aqueous media at pH 7.4. It is widely proposed FA may be transported in cells by FA-binding protein. Mode of transport of MG has received little attention. Our findings in tissues and model membranes (*Prog. Lipid Res.* 24, 197-241, 1985) indicate FA (as 1:1 acid-soaps) and MG can be transported in vivo by lateral movement in an interfacial continuum (IFC) of plasma and intracellular membranes. We postulate FA and MG produced by LPL enter IFC in capillaries and flow in IFC across endothelial cells (EC) and extracellular space (ECS) to sites in adipocytes where MG are hydrolyzed by MG-lipase (MGL) to FA and glycerol, and FA are esterified and stored as TG between leaflets of endoplasmic reticulum (ER), or transferred to inner mitochondrial membrane (M). FA and MG produced by hormone-sensitive lipase from cellular TG also enter IFC. These MG flow in IFC to sites of MGL activity, and the FA flow in IFC to capillaries for transport to other tissues by plasma albumin, or to mitochondria for heat production.**




**CA 211** PROTEIN KINASE C (PKC) ANTISENSE DNA AND PKC(19-31) PSEUDOSUBSTRATE INHIBIT INSULIN-STIMULATED 2-DEOXYGLUCOSE UPTAKE IN RAT ADIPOCYTES.
Mary Standaert, Eric Wickstrom, Bing Zhi Yu, Joachim Sasse, Herman Hernandez, Denise Cooper and Robert Farese. VA Hospital and University of South Florida, Tampa, FL 33612.

Insulin activates PKC in isolated rat adipocytes promoting PKC translocation to the particulate fraction (Ishizuka, *et al*, 1989, *FEBS Lett.* 257:337; Eagan, *et al*, 1990, *PNAS* 87:1052). Here, we have shown that 20 h treatment of isolated adipocytes with decapentadeoxynucleotides, antisense to the mRNA initiation codon regions of PKCα and/or PKCβ, reduces PKC activity, immunoreactive PKC levels, and insulin-, as well as PMA-, stimulated 2-DOG uptake by 50-70%. Treatment with sense or nonsense oligodeoxynucleotides had no effect. Furthermore, acute treatment with the autoregulatory, PKC pseudosubstrate, PKC(19-31), described by House and Kemp (*Science*,1987, 238:1726) effectively blocked insulin-stimulated hexose uptake in electroporated and intact adipocytes with respective IC50 values of 30 μM and 600 μM. PKC(19-31) did not inhibit basal hexose uptake and treatment with an irrelevant peptide failed to inhibit the effect of insulin. PMA-stimulated hexose transport in intact adipocytes was also inhibited by PKC(19-31) but was approximately 2-fold more sensitive to PKC(19-31) inhibition, i.e., IC50 of 300 μM, than was the effect of insulin. Similar relative differences for PMA and insulin were also also noted using the PKC inhibitors staurosporine and sangivamycin, suggesting either both PKC-dependent and -independent effects in insulin-stimulated hexose uptake, or differential inhibitor sensitivities for PMA- and DAG (insulin)-activated PKC. These studies, in which PKCα or PKCβ antisense DNA and treatment with PKC(19-31) each inhibited insulin-stimulated hexose uptake, indicate that PKC plays a positive role in signalling insulin-stimulated hexose uptake in isolated adipocytes.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 212** ALTERED EXPRESSION OF CARBONIC ANHYDRASE III IN OBESE MICE, L. Stanton, R. Coleman, P. Ponte and M. Snyder, California Biotechnology Inc., and Metabolic Biosystems, 2450 Bayshore Pkwy, Mountain View CA 94043

Carbonic anhydrase III (CAIII) is one of a family of closely related enzymes which catalyze the reversible hydration of $CO_2$. The function of this isozyme in metabolism is unknown; however, it has been suggested that it may play a role in fatty acid biosynthesis. We have found that the expression of CAIII is altered in genetically and chemically-induced obese mice. There is a substantial reduction in the level of CAIII in adipose tissue of obese mice relative to lean control animals. Northern analyses on CAIII levels have been performed on samples from several obesity models with respect to fat depot, sex and age. Levels of CAIII mRNA were found to parallel the differences in protein levels. Changes in CA levels are not a general feature of obesity since comparable levels of CAIII mRNA were observed in lean and obese liver, muscle, and kidney. Additionally, the levels of two other CA isozymes, CAI and CAII, were not demonstrably different in tissues from lean and obese mice.

**CA 213** PROGESTERONE REGULATES GLUCOSE METABOLISM IN FEMALE RAT ADIPOCYTES. M.-Th. Sutter-Dub, P. Cordoba, K.T. Ta. Lab. Endocrinologie, Univ.BordeauxI, Avenue des Facultés, F-33405 Talence Cedex, France.
Progesterone (P) inhibit glucose metabolism by a dose-related effect occuring after 20-min incubation. This effect is similar to that observed during pregnancy and after treatment with P. - Mechanisms of action of P may differ from the classical action of steroids. However, as shown by autoradiography, L-leucine is incorporated into 5 proteins and P increases this incorporation. By increasing glucose in the medium (0.56-5.6 mM), P effect on glucose oxidation is decreased and cAMP, increased. This may indicate a relation with glucose transport, although P has no action on it at 0.56 mM glucose. The involvement of purinergic receptors was also investigated. Adipocytes incubated in the presence of P ($10^{-10}$ to $10^{-3}$ M; 0,56 mM) responded biphasically :[1-$^{14}$C]glucose oxidation was increased and then decreased. With adenosine (ado) deaminase P decreased glucose oxidation at all concentrations. Caffeine (non-selective ado antagonist), theophylline ($A_1$< $A_2$ receptor antagonist) and dipyridamole (ado uptake inhibitor) decreased glucose oxidation with the following order of potency: dipyridamole > theophylline > caffeine. These potencies were decreased by P, whereas that of inhibitors of phosphodiesterase activity (papaverine, imipramine) remained unchanged.- In conclusion, these results suggest that adenosine modulates the effect of progesterone on glucose transport and metabolism in adipocytes.

**CA 214** CHRONIC CONTROL OF ADRENERGIC SYSTEM OF ADIPOSE TISSUE BY GROWTH HORMONE AND INSULIN, Richard G. Vernon, Eric Finley, Paul W. Watt, Hannah Research Institute Ayr, Scotland, U.K.
Sheep adipose tissue retains its responsiveness to adrenergic agents and adenosine during tissue culture for 48h; basal lipolysis and sensitivity to catecholamines are enhanced. Tissue culture with growth hormone increases maximum response and sensitivity to the β-agonist isoproterenol. In addition culture with growth hormone increases ligand binding to the β-adrenergic receptor. In contrast, culture with insulin increases basal lipolysis and decreases response to $\alpha_2$-adrenergic agonists and the adenosine analog PIA, but has no effect on response to β-agonists. During culture ligand binding to the $\alpha_2$-adrenergic receptor is increased; this is prevented by insulin which could partly account for the effect of the hormone on $\alpha_2$-adrenergic activity. However, culture with insulin has no effect on the ligand binding to the adenosine receptor indicating a chronic effect of the hormone on some other component of the signal transduction system. Also, whereas culture with insulin virtually eliminated the ability of PIA to inhibit isoproterenol-stimulated lipolysis, PIA still decreased the basal rate of lipolysis. These data suggest a shift in the operating range of cyclic AMP concentrations following culture with insulin, perhaps due to diminished phosphodiesterase or $G_i$ activity. Thus both growth hormone and insulin have chronic effects on the adrenergic/adenosine signal transduction system which regulates lipolysis. The effects of growth hormone are to promote response to β-agonists whereas insulin, paradoxically in view of its well known acute effect on lipolysis, diminishes signal transduction through the $\alpha_2$-adrenergic/adenosine system.

## The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 300** GROWTH HORMONE REGULATES GENE EXPRESSION BY SEVERAL DISTINCT MECHANISMS IN PREADIPOCYTE Ob17 CELL LINE, Sylvie Barcellini-Couget, Anne Pradines-Figuères, Alain Doglio, Paul Grimaldi, Gérard Ailhaud and Christian Dani, Centre de Biochimie du CNRS, U.F.R. Sciences, Parc Valrose, 06034 Nice, France

To gain some insights in the mechanisms by which growth hormone regulates gene expression, we have studied three genes (*c-fos*, LPL, IGF-I) that are positively regulated by GH in Ob1771 preadipose cells. These genes were induced rapidly at the transcriptional level, with distinct relative time course of mRNA accumulation : 1) Transcription of *c-fos* gene was detected within 5 min after GH addition, peaked within 15 min and declined to basal level within 30 min ; 2) mRNA transcripts for LPL and IGF-I accumulated within 4 hours, peaked within 24 hours and declined rapidly only after GH removal. Induction of LPL gene expression by GH was prevented by cycloheximide whereas induction of *c-fos* and IGF-I genes was independent of protein synthesis. The signalling pathways were also different for these genes. Cyclic nucleotide analogs and calcium ionophores cannot mimic any of the effect of GH. In contrast, PMA mimics the effect of GH on *c-fos* and LPL gene expression. The protein kinase C inhibitor H7 prevented completely *c-fos* induction and partially LPL induction whereas IGF-I gene expression appeared independent of protein kinase C activation.

These results indicate that GH may regulate gene expression via different cellular pathways in the same cell.

**CA 301** INDUCTION OF aP2 BY AN INSULIN SENSITIZING AGENT, Steven D. Clarke, Peter K.W. Harris, Lisa A. Foellmi, Beatrice M. Wyse, Jerry R. Colca and Rolf F. Kletzien, Metabolic Diseases Research, Laboratory of Diabetes, Upjohn Company, Kalamazoo, MI, 49001

Pioglitazone is anti-diabetic agent that increases sensitivity of target tissues to insulin through an undefined mechanism. aP2 is a fatty acid binding protein which may mediate some actions of insulin in adipcoytes. The aP2 gene is transcriptionally regulated during differentiation of preadipocytes to mature adipocytes. Pioglitazone accelerates the insulin or IGF induced differentiation of 3T3-L1 cells. Analysis of the kinetics of drug-related changes in the cellular phenotype indicate that pioglitazone is inducing changes in gene expression during the first few hours of treatment. We have studied the effect of the drug on specific mRNA species and have found that the aP2 mRNA is elevated 10-fold within four hours of treatment of appropriately staged 3T3-L1 cells. Several other insulin-regulated mRNA species (c-fos, Glut-4, LPL) were not induced by treatment with pioglitazone alone. Thus, the aP2 gene is candidate for direct action of the drug. Induction of aP2 by pioglitazone was also observed in animal models. Treatment of ob/ob mice with various levels of the drug for six weeks caused a dose-dependent increase in aP2 mRNA in epididymal fat. The increase in epididymal fat pad aP2 mRNA correlated positively with the changes in the physiological parameters effected by the drug. Pioglitazone treatment of $KKA^y$ mice resulted in a 2 to 3-fold increase in aP2 mRNA. A possible direct effect of pioglitazone on the aP2 gene is being evaluated by analysis of aP2 promoter/CAT plasmids transfected into 3T3-L1 cells.

**CA 302** COORDINATED OVERTRANSCRIPTION OF LIPOGENIC ENZYME GENES IN ADIPOSE TISSUE OF GENETICALLY OBESE RATS. I. Dugail, A. Quignard-Boulangé, X. Le Liepvre, C. Guichard, M. Lavau. INSERM U.177, 15 rue de l'Ecole de Médecine, Paris, France.

We have previously shown that the overcapacity for lipid storage in adipose tissue of young obese Zucker rats can be ascribed to a higher content of mRNA encoding for fatty acid synthetase (FAS), malic enzyme (ME) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the increases of which are proportional to the increases in enzyme activities. We have examined here whether the higher levels of these mRNAs resulted from increased transcription rates. Nuclei were isolated from adipose tissue of lean (Fa/fa) and obese (fa/fa) Zucker rats aged either 16 or 30 days. Nuclear run-on assay was performed by incubating nuclei with 100 uCi of $^{32}P$ CTP, and labelled transcripts were hybridized to recombinant plasmids. In 30 day-old hyperinsulinemic obese rats, both mRNA and transcription rate were markedly stimulated : ME x8, FAS x15, GPDH x10, GAPDH x6, over control values. At 16 days of age, when mutant pups are still suckling and normoinsulinemic, the higher level of mRNAs in adipose tissue from obese rats could be explained by a parallel increase in transcription rates : ME x2, GAPDH x2 and FAS x3, suggesting a genotype effect independent of hyperinsulinemia. At this age, GPDH mRNA levels and transcription rate were unaffected in obese rats. We conclude that the expression of a subset of genes involved in fat storage is transcriptionally stimulated in adipose tissue of genetically obese rats. This suggests that a common trans-acting factor could be responsible for the over-transcription of these genes in the mutant rat.

**CA 303** GENE EXPRESSION OF LIPOGENIC ENZYME AND GLUT 4 IN RAT ADIPOSE TISSUE, Pascal Ferré, Dominique Perdereau, Luc Pénicaud*, Françoise Assimacopoulos°, Armelle Leturque, Tarik Issad and Jean Girard. Centre de Recherches sur la Nutrition, UPR1511, Meudon, URA 307*, Paris, CNRS, France et Laboratoire de Recherches Métaboliques°, Genève, Switzerland.

At weaning, the rat switches from a high-fat low-carbohydrate diet, the milk, to the high-carbohydrate, low-fat adult diet. This is is concomitant with a marked increase in plasma insulin and in the glucose transport and lipogenic capacities of white adipose tissue. We have used this model in order to study the regulation of Glut 4, Acetyl-CoA carboxylase (ACC) and Fatty acid synthase (FAS) gene expression. At weaning, the marked increase in the expression of these genes depends upon the meal-induced excursions of plasma glucose and insulin concentrations since it is markedly reduced (1) when acarbose, an inhibitor of intestinal α-glucosidase is included in the weaning high-carbohydrate diet or (2) when the amount of carbohydrate of the diet is reduced. Conversely, in the adipose tissue of Zucker obese (fa/fa) rats which are hyperinsulinemic even during the suckling period, Glut 4, ACC and FAS are overexpressed before and after weaning. In *in vitro* experiments on 24h-incubated white adipose tissue explants of suckling rats, we have shown that insulin is a major factor in the regulation of the FAS and ACC genes, whereas glucose or T3 alone or in combination are ineffective.

**CA 304** CLONING AND SEQUENCING OF THE 5' FLANKING REGION OF THE MURINE LIPOPROTEIN LIPASE GENE, Jeff Gimble, Xianxin Hua, Kellee Youkhana and Jane Hudson, Immunobiology and Cancer Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104.

The lipoprotein lipase (LPL) gene expression in murine bone marrow stromal cells is sensitive to the effects of adipogenic agonists and antagonist. Consequently, it provides a useful model for the study of stromal cell gene regulation. To facilitate these studies, we have cloned the 5' flanking region of the murine LPL gene by screening a genomic library with an oligonucleotide probe derived from the published cDNA sequence (Kirchgessner et al, JBC 267:8463). The original bacteriophage clone consists of a 12 kb fragment of murine genomic DNA which includes approximately 10 kb of the 5' flanking region as well a the first exon. The sequence of over 3.9 kb of the murine gene surrounding the first exon has been determined. Sequence comparison of the murine to the human LPL 5' flanking region reveals an overall 74% identity (Deeb & Peng, Biochem. 28:4131). While the putative octamer binding site (NF-A) is completely conserved in the murine genome, the GAGAGGA motif located upstream of the human LPL gene and common to a number of adipocyte specific genes is not conserved in the murine genome. Experiments have begun to examine the DNase I hypersensitivity of the murine LPL gene in pre-adipocytes and adipocytes. Preliminary results suggest that an adipocyte specific DNase I hypersensitive site exists within the first exon but additional studies will be necessary to confirm this finding.

**CA 305** EXPRESSION OF HUMAN GLUCOSE TRANSPORTERS IN XENOPUS OOCYTES: KINETIC CHARACTERISATION AND SUBSTRATE SPECIFICITIES OF THE LIVER-TYPE (GLUT 2) AND BRAIN-TYPE (GLUT 3) ISOFORMS. Gwyn W. Gould*, Thomas J. Jess* and Graeme I. Bell[X]
*Molecular Pharmacology Group, Department of Biochemistry, The University of Glasgow, GLASGOW G12 8QQ Scotland. [X]Howard Hughes Medical Institute and Departments of Biochemistry and Molecular Biology and of Medicine, The University of Chicago, CHICAGO, IL 60637.

We describe the functional expression of five members of the family of human facilitative glucose transporters, the erythrocyte-type transporter (GLUT 1), the liver-type transporter (GLUT 2), the brain-type transporter (GLUT 3), the insulin-responsive transporter (GLUT 4) and the small-intestine type transporter (GLUT 5) by microinjection of their corresponding mRNAs into Xenopus oocytes. We have measured the $K_m$ for 3-O-methyl-D-glucose of three of these transporter species, and the results are discussed in the light of the possible roles for these different transporters in the regulation of blood glucose. The substrate specificity of these transporter isoforms has also been examined. We show that for all transporters, the transport of 2-deoxy-D-glucose is inhibited by D- but not by L-glucose. In addition, both D-galactose and D-mannose are transported by GLUT's 1,2 and 3 at significant rates; furthermore GLUT 2 is capable of transporting D-fructose. The nature of the glucose binding sites of GLUT's 1,2 and 3 was investigated using hexose inhibition of 2-deoxy-D-glucose uptake. We show that the characteristics of this inhibition are different for each transporter isoform.

**CA 306** PERILIPIN, A MAJOR, HORMONALLY-REGULATED, ADIPOCYTE-SPECIFIC PHOSPHOPROTEIN ASSOCIATED WITH THE PERIPHERY OF LIPID STORAGE DROPLETS,

Andrew S. Greenberg, John J. Egan, Sheree A. Wek, Nira B. Garty, Malcolm C. Moos, E. Joan Blanchette-Mackie, and Constantine Londos, NIDDK, National Institutes of Health, Bethesda, MD 20892.

The lipid fraction ("fat cake") derived from homogenates of rat epididymal adipocytes contains a prominent phosphoprotein (62 kDa by SDS-PAGE) that is multiply phosphorylated by cAMP-dependent protein kinase in vivo (65/67 kDa doublet by SDS-PAGE), at which point it is by far the most heavily phosphorylated protein in the cell. Identity among the 62/65/67 kDa species is established by peptide mapping and by Western blotting with antibodies immunopurified from antiserum raised against the 62 kDa species. Western blot analysis of various tissues with immunopurified antibodies suggests that the protein is specific for adipocytes. Moreover, amino acid sequences of 8 different peptide fragments reveal no homology with known proteins. This protein, which we term perilipin, is found in cultured 3T3-L1 adipocytes, but not in their precursor 3T3-L1 fibroblasts. Immunocytochemical studies indicates that perilipin is closely associated with the periphery of lipid storage droplets in cultured adipocytes. Given its adipocyte specificity, acute regulation by hormones, and subcellular location, we speculate that perilipin plays a role in the specialized lipid storage function of adipocytes.

**CA 307** LONG-TERM REGULATION OF TWO GLUCOSE TRANSPORTER SUB-TYPES IN PRIMARY CULTURED RAT ADIPOCYTES.

M.GUERRE-MILLO, I.HAINAULT, C.GUICHARD and M.LAVAU. U 177 INSERM, 15 rue de l'Ecole de Médecine, PARIS 75006, FRANCE.

We have previously shown that the long-term stimulatory effect of insulin on glucose transport activity in 3T3-F442A adipocytes is mediated through a specific increase in the expression of the erythroïd/brain (GLUT 1) type of glucose transporter, the adipose cell/muscle type (GLUT 4) being unchanged. We have addressed here, the question of the long-term regulation of these two glucose transporter isoforms in primary cultured rat adipocytes. Epididymal fat cells were isolated by collagenase and cultured in DMEM supplemented with BSA 1%, SVF 1%, glutamine 4 mM, glucose 10 mM and ± insulin (10 nM). GLUT 1 and GLUT 4 were assessed in total cellular membranes by Western blotting, using specific antibodies, against the C-terminal peptide of GLUT 1 and the monoclonal antibody 1F8, respectively. GLUT 1 concentration per mg of membrane proteins markedly increased (by more than 200 %) during the culture time (4 days). In sharp contrast, GLUT 4 membrane content decreased gradually to less than 20 % of the initial content. The presence of insulin in the medium did not modify these patterns. These data indicate that culture regulates oppositely the expression of GLUT 1 and GLUT 4 in rat adipocytes.

**CA 308** ISOLATION OF 'FAT' GENES IN DROSOPHILA,

**Deborah K. Hoshizaki, Wade M. Johnson and Rayna L. Lunz, Department of Biochemistry, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612**

We are developing the *Drosophila* fat body as a model system to identify events during differentiation which are necessary to activate genes involved in intermediary metabolism. The fat body is the principle tissues involved in storage and intermediary metabolism in insects and is analogous to the liver and adipose tissue in mammals. Using P-element based 'enhancer trap' mutagenesis, we have detected several enhancers which drive expression of a *lac*Z reporter gene in fat body cells.

To identify and characterize the fat enhancers and their associated genes, we have begun to clone the genomic regions flanking the sites of insertion and to screen for deficiency mutations.

In one adult fat specific enhancer line, we have mobilized the inserted P-element to isolate deficiency mutations due to imprecise excision of the P-element. A pupal lethal mutant was recovered which exhibits incomplete differentiation of adult structure during metamorphosis. A molecular and genetic characterization of this mutant and the other fat enhancer lines will be presented.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 309** **cDNA CLONING AND EXPRESSION OF PERILIPIN, A LIPID DROPLET-ASSOCIATED, ADIPOCYTE-SPECIFIC PROTEIN,** Alan R. Kimmel, Andrew S. Greenberg, Sheree A. Wek, John J. Egan, Malcolm C. Moos and Constantine Londos, Laboratory of Cellular and Developmental Biology, NIDDK/NIH, Bethesda, MD 20892.

Perilipin is an hormonally regulated, phosphoprotein associated with the periphery of the lipid storage droplet in adipocytes. The protein is the major cellular A-kinase substrate in adipocytes and, depending on the extent of phosphorylation, migrates at 62-67 kDa in SDS-PAGE (Greenberg et al., submitted). A lambda gt11 cDNA expression library, derived from rat epididymal adipocyte mRNA, was screened with polyclonal antisera raised against purified perilipin. Nucleic acid sequence analysis was performed on three overlapping cDNA clones which reacted with both polyclonal antisera and affinity-purified anti-perilipin antibodies. Their deduced protein sequence included identities with three separate peptide sequences derived from purified perilipin, thus, confirming the isolation of perilipin cDNA clones. Hybridization of perilipin cDNA probes to blots of rat adipocyte RNA revealed two moderately abundant messages of 3.0 and 3.6 kb. No hybridization was detected to RNA isolated from 8 additional rat tissues, nor was the protein detected in a variety of non-adipocyte tissues by immunoblotting. In addition, we observe no perilipin mRNA in preadipocytes, but significant accumulation occurred after differentiation in culture. These data suggest that perilipin may be specifically expressed in adipocytes. Aspects of perilipin to be discussed include protein structure and function and the hormonal and developmental regulation of gene expression.

**CA 310** REGULATION OF GENE EXPRESSION IN A BROWN FAT CELL CULTURE DERIVED FROM A SV40 - INDUCED TUMOR, Leslie P. Kozak and Ulrike C. Kozak, The Jackson Laboratory, Bar Harbor, ME. 04609

Insulin is essential for cold and dietary induced thermogenesis. However, it is unknown whether the effects of insulin are determined by a direct action on brown fat cells or indirectly through the hypothalamus. The appearance of a SV40 T-antigen induced brown fat tumor has enabled us to establish cultures of brown fat cells in which we analyzed the hormonal induction of the mitochondrial uncoupling protein (UCP) and glycerol-3-phosphate dehydrogenase (GPDH) mRNA. The induction of UCP mRNA was dependent on norepinephrine (10 μM) and did not require insulin or triiodothyronine in short term cultures (3 days in culture). Added insulin (1 nM) and triiodothyronine (50 nM) enhanced induction by norepinephrine in long term cultures (9 days in culture), but were ineffective alone. In contrast, GPDH mRNA, which is also induced in brown fat by cold exposure, was unaffected by norepinephrine, but induced by insulin and triiodothyronine. The results indicate that norepinephrine-dependent and -independent signal transduction pathways regulate the expression of genes during thermogenesis and that insulin is capable of inducing gene expression with kinetics similar to norepinephrine. A tissue culture system as described here has not been previously available and should provide a valuable tool for in vitro analysis of gene expression in brown fat.

**CA 311** PURIFICATION AND INITIAL CHARACTERIZATION OF A 116,000 Da MEMBRANE PROTEIN WHICH IS OVER-EXPRESSED IN GENETIC OBESITY. Christopher J. Lynch, Kendrick M. McCall, Judith A. Williams, Louis F. Martin and Susan J. Vannucci. Departments of Cellular and Molecular Physiology and Surgery, Pennsylvania State University College of Medicine, Hershey, PA 17033.

Protein mapping studies in our laboratory have pinpointed aberrant expression of many adipocyte proteins in obese Zucker rats, a model of genetic obesity. Here we report on our efforts to purify and identify one of these which we term p116. P116 is a particulate protein which is localized in high-density microsomes containing plasma membrane vesicles, mitochondria and nuclei. In this fraction from obese animals which over-express p116, it represents approximately 5% of the microsomal proteins by staining. Early attempts to purify detergent-solubilized p116 by FPLC were unsuccessful because of aggregates it formed with other lower molecular weight proteins. Despite this, we have successfully purified p116 to homogeneity using preparative 2-dimensional IEF/SDS polyacrylamide gel electrophoresis. Polyclonal antibodies directed against purified p116 were produced in rabbits and immunoblottting studies with these show that p116 levels are 2-5 fold higher in young obese Zucker rats compared to their lean littermates. In addition, two dimensional gel analysis and immunoblotting suggest that p116 is present in human adipose tissue. N-terminal sequence analysis of p116 indicated that p116 may be chemically *blocked* at the N-terminus. However, several internal peptides resulting from *Staphylococcus aureus* V8 protease digestion have been sequenced. Analysis of these sequences using the NBRF protein identification resource database suggest that p116 may not have been previously sequenced or translated from an isolated cDNA. We hope that these studies will identify obesity specific proteins which can be used as genetically determined markers of the pre-obese state and determinants of childhood obesity.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

**CA 312** TISSUE-SPECIFIC REGULATION OF LIPOPROTEIN LIPASE GENE EXPRESSION BY cAMP, Mary V. Raynolds and Robert H. Eckel, Division of Endocrinology, University of Colorado Health Sciences Center, Denver, CO 80262

The enzyme lipoprotein lipase (LPL) is synthesized primarily by adipocytes and myocytes, and mediates plasma triglyceride-derived fatty acid uptake for storage (adipocytes) or fuel (myocytes). LPL enzyme activity and mRNA levels are regulated by cAMP in a tissue-specific manner. Adipocytes treated with $10^{-3}$M-$10^{-6}$M of the non-metabolized cAMP analog 8(4-chlorophenylthio)cAMP (8-CPTcAMP) showed a dose- and time-dependent decrease in LPL enzyme activity and steady-state LPL mRNA levels. Decreased LPL gene expression in adipocytes after treatment with 8-CPTcAMP was due in large part to decreased transcription of the LPL gene. Conversely, treatment of rats with 200$\mu$g/kg isoproterenol, a $\beta$-adrenergic agonist which increases intracellular cAMP, caused a 3-fold increase in cardiac muscle LPL mRNA by 30 min. In addition, activity of the reporter gene firefly luciferase, driven by LPL-promoter-luciferase constructs introduced into C2 murine myoblasts, was increased 200% by addition of 8CPT-cAMP to the cultured myoblasts, indicating that 8CPT-cAMP also has an effect on LPL gene transcription in muscle. In both adipocytes and myocytes the effects of increasing intracellular cAMP appear to be predominately on transcription of the LPL gene: curtailing transcription in the adipocyte and stimulating transcription in the myocyte. Either of two putative cAMP response elements in the LPL gene promoter could mediate cAMP responsiveness.

**CA 313** CLONING AND EXPRESSION OF AN ADIPOCYTE SPECIFIC GENE: IDENTIFICATION OF A NOVEL GENE REGULATORY SEQUENCE ELEMENT, Gordon M. Ringold and Ulrich Danesch, Institute of Cancer and Developmental Biology, Syntex Research, Palo Alto, CA 94304

Our laboratory has cloned several cDNAs whose mRNAs are induced during differentiation of TAI adipocytes. One of these cDNA clones, referred to as FSP27, encodes a 1.8 kb mRNA which is detectable only in differentiated adipocytes. This tissue specific expression can be turned off by dedifferentiating TAI adipocytes with TNF or TPA together with a Ca-ionophore. In the mouse, FSP27 expression is restricted to fat tissue and can not be detected in any other organ. The sequence of the FSP27 cDNA, coding for a protein of relative molecular weight of 27 kd, shows no homology to any existing protein as revealed by a computer aided search of current protein data banks. In addition to the cDNA, we cloned the FSP27 gene from TAI cells. A fusion gene containing only 176 bp of 5' upstream flanking sequences of the FSP27 gene and a CAT reporter plasmid is expressed and regulated in a tissue specific manner during adipocyte differentiation of the adipogenic cell lines TAI and 3T3-LI. No expression can be found in JZ hepatoma or L fibroblast cells. Deleting sequences up to position -135 no longer allows expression in adipocytes, thus revealing a fat cell specific element (FSE) between -176 and -135. This region contains the palindromic sequence TTTCGAAA which is footprinted by nuclear extracts from adipocytes but not from adipoblasts. Similarly, an oligonucleotide containing this palindrome leads to an adipocyte specific gel-shift. In addition to the FSE, the FSP27 5'flanking sequences contain a non-fat cell specific enhancer element further upstream that increases the expression from a heterologous promoter in a differentiation independent fashion.

**CA 314** AUTOREGULATION OF MUSCLE HEXOSE TRANSPORT BY GLUCOSE, Shlomo SASSON, Department of Pharmacology, Hebrew University Faculty of Medicine, Jerusalem, 91010, Israel.

Glucose regulates the rate of hexose transport in adipose and skeletal muscle cells. Low glucose increases, while high glucose decreases the Vmax of hexose transport without affecting the Km. [$^3$H]cytochalasin B binding and Western blot analyses with anti-GLUT-1 antibodies of the plasma and microsomal membrane fractions of L8 myocytes indicate that the autoregulation of the transport is accompanied with similar reversible changes in the concentration of transporters in the two fractions with no modification in their intrinsic activity. 2-Deoxyglucoses promotes downregulation of hexose transport and reduces the GLUT-1 signal in the plasma membrane concomitantly with an increased signal in the microsomal membranes. A short exposure to 2-deoxyglucose was sufficient to generate a stable signal to initiate downregulation of hexose transport that lasted for at least 5h. Several controls with uncouplers indicate that this effect of 2-deoxy-glucose did not result from the mild decrease in ATP content. Our data suggest that permanently high glucose concentration are necessary to accumulate hexose-6-phosphate, and low doses of 2-deoxyglucose generate a long-standing 2-deoxyglucose-6-phosphate signal. These signals initiate the redistribution of glucose transporters between the plasma and microsomal membranes, leading to the observed reduced glucose uptake and utilization. These results may explain the reduced peripheral glucose utilization under hyperglycemic conditions.

**CA 315** HORMONAL AND METABOLIC REGULATION OF ADIPOCYTE P2 GENE EXPRESSION IN TRANSGENIC MICE. Hai-Lun Shyu, Reed A. Graves*, Bruce M. Spiegelman* and Susan R. Ross, Department of Biochemistry, University of Illinois, Chicago, IL 60612 and *Dana-Farber Cancer Institute and the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

The mouse adipocyte P2 (aP2) gene encodes an adipocyte-specific member of the intracellular lipid binding protein family. The region immediately upstream from the start of transcription has been shown to contain binding sites for the c-jun/c-fos and C/EBP transcription factors and at least one glucocorticoid regulatory element (GRE). We have been mapping the sequences present in the aP2 gene that are required for both its tissue-specific and hormone-regulated expression in transgenic mice. The adipose tissue-specific expression of aP2 transgenes is dependent on an enhancer element mapping 5kb upstream from the transcription start site and is independent of the promoter proximal elements; however, the promoter proximal elements are required for the 5-fold induction of transgene expression by glucocorticoids. Currently, we are examining the role of the promoter proximal sequences in the regulation of aP2 expression by catabolic and anabolic states and in response to various effector molecules, such as adrenergic receptor agonists. The results of these experiments will allow us to better understand the complex regulation of gene expression in the adipose cell.

**CA 316** INSULIN TREATMENT OF RAT ADIPOCYTES INCREASES THE PLASMA MEMBRANE CONCENTRATION OF GLUT1 AND GLUT4 GLUCOSE TRANSPORTERS DETECTED BY IMMUNOELECTRON MICROSCOPY. R.M. Smith[1], M.J. Charron[2], N. Shah[1], H.F. Lodish[2], and L. Jarett[1]. [1]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and [2]Whitehead Institute for Biomedical Research, Cambridge, MA 02142.

Colloidal-gold immunoelectron microscopy was used to localize brain/erythrocyte (GLUT1) and adipose/muscle (GLUT4) glucose transporters in intact isolated adipocytes (ADIP) which had been incubated in the absence or presence of 4 ng insulin/ml for 15 min at 37°C or, after insulin incubation, were washed to remove insulin and incubated 30 min. ADIP were fixed, dehydrated and embedded in LR White resin. Thin sections were incubated with antibodies against the COOH terminus of GLUT1 or GLUT4 for 72 hr at 4°C. Specifically bound antibodies were detected with protein A labeled colloidal-gold. Controls demonstrated labeling specificity. Sections of untreated adipocytes incubated with GLUT1 or GLUT4 revealed small numbers of gold particles associated almost exclusively with the plasma membrane; little specific labeling was detected in cytoplasmic vesicles. We observed at least a 10 fold increase in the plasma membrane labeling of both transporters in insulin-treated ADIP. The number of transporters in the plasma membrane of insulin-treated and washed ADIP was only slightly greater than control and significantly lower than insulin-treated ADIP. No differences were detected in the number of intracellular transporters under any of the incubation conditions. These observations suggest that insulin causes a conformational change in the glucose transporter, native in or translocated to the plasma membrane, which resulted in increased labeling of the transporters by the COOH terminal antibodies. This change in conformation, in addition to translocation to the plasma membrane, may be the mechanism by which insulin stimulates glucose transport in ADIP.

**CA 317** EVIDENCE THAT TYROSINE PHOSPHORYLATION OF A PUTATIVE GH-RECEPTOR IS INVOLVED IN THE INSULIN-LIKE ANTILIPOLYTIC EFFECT OF GROWTH HORMONE(GH) IN ISOLATED RAT ADIPOCYTES, Hans Tornqvist*, Eva Degerman, Per Belfrage and Hans Eriksson, Depart of Pediatrics*, University Hospital, and Medical and Physiological Chemistry, University of Lund, Lund, Sweden.

Growth hormone(GH) elicits acute insulin-like metabolic effects such as antilipolysis and lipogenesis in isolated rat adipocytes preincubated for 3 hr in the absence of this hormone or in freshly prepared adipocytes from hypophysectomised rats. Phosphotyrosine-containing membrane proteins from these adipocytes, labelled with $^{32}$P-orthophosphate, were isolated by wheat germ agglutinin purification and immunoprecipitation with a polyclonal antiphosphotyrosine antibody(anti-Ptyr) followed by SDS-PAGE and autoradiography. hGH induces within minutes a dose-dependent phosphorylation ($EC_{50}$≈0.7nM) of a 114 kDa membrane glycoprotein(p114) immunoreacting with anti-Ptyr, in rat adipocytes preincubated without hormones for 3 hours . In cells stimulated with $^{125}$I-hGH, this hormone copurified with p114 indicating hormone binding to this protein. The phosphorylation of p114 exhibited a close correlation with phosphorylation of particulate cGMP-inhibited cAMP-phosphodiesterase(cGI-PDE), net dephosphorylation of hormone-sensitive lipase and antilipolysis in a hGH dose-dependent manner. The appearance of $^{32}$P-p114 also correlated with the hGH-induced increase in lipogenesis. The amount of hGH-induced $^{32}$P-p114 is substantially reduced in freshly prepared adipocytes refractory to hGH-stimulation as compared to non-refractory cells. A brief preincubation of non-refractory adipocytes with a low concentration of hGH substantially reduces the antilipolytic effect of hGH concomitant with disappearance of $^{32}$P-p114. No hGH-induced tyrosine phosphorylation of either the insulin or IGF1 receptor was detected. These results suggest that agonist-induced tyrosine phosphorylation of a 114 kD GH receptor is involved in acute insulin-like effects of GH in rat adipocytes. These results are also consistent with the hypothesis that the signal transduction chain for the antilipolytic effect of GH converges with that for insulin at or above the level of cGI-PDE activation.

**CA 318** IDENTIFICATION OF AN OVERFEEDING-INDUCED ADIPOCYTE 5 kB mRNA LACKING SEQUENCE HOMOLOGY TO KNOWN ADIPOCYTE GENES. Bruce E. Wilson, Grant E. Meyer, David S. Weigle, Department of Medicine, University of Washington, Seattle, WA 98195.

We have utilized subtraction cloning to identify adipocyte cDNA preferentially expressed in the overfed state in order to isolate genes with a potential role in the regulation of adipose mass.(Clin Res 38:483A). One overfeeding-induced 1.8 kB cDNA isolated from the omental adipose tissue of an overfed macaque (M nemestrina) identifies an mRNA of 5kB in rodent, subhuman primate and human adipose tissue by Northern analysis. This cDNA also identifies complementary clones in a mouse brain cDNA library and a 5 kB mRNA expressed in subhuman primate brain tissue. Similarities between the 1.8 kB cDNA and 40,127,752 residues of GenBank release 63 were assessed by a double-stranded multiple alignment algorithm using a window size of 30 (International Biotechnologies, Inc. MacVector, version 3.04). No significant homology was noted with the nucleic acid sequence or its predicted translation product. There is an open reading frame extending to base pair number 1238 of the cDNA, the presumed start of the 3' untranslated region of the native message. Further evaluation of the gene for this novel overfeeding-induced adipocyte mRNA will be required to determine whether the encoded peptide has a role in the regulation of adipose mass.

**CA 319** DYSREGULATED GLUCOSE-TRANSPORTER (GT) mRNA ABUNDANCE IN MUSCLE AND FAT DURING THE HYPOGLYCEMIC PHASE OF ENDOTOXIC SHOCK. W. Patrick Zeller, Sian Min The, Masakatsu Goto, Michelle Sweet, R. Morrison Hurley, James P. Filkins, and Cecilia Hofmann, Depts. of Pediatr., Physio., Surg., and Biochem., Loyola Univ. Shock and Trauma Inst., Stritch School of Med., Maywood, IL 60153 and Hines VA Hospital, Hines, IL 60141.

Glucose transport in fat and muscle tissue is altered in endotoxic shock. GT proteins are responsible for cell-specific uptake of glucose and may therefore be altered in endotoxic shock. We investigated the abundance of mRNA for GLUT4 and GLUT1 in epididymal fat and soleus muscle of saline- and endotoxin-treated animals. Male Sprague-Dawley rats were treated with S. enteritidis lipopolysaccharide (LPS, 40mg/kg IP) or saline only. Tissues (5-13 samples/group) were harvested when the animals became hypoglycemic, i.e. 4-6 hours post-injection. When the tissues were harvested, LPS- and saline-treated animals revealed plasma glucose (44+6mg/dl vs 111+4, p<.001), insulin (50+12 vs 40+9uU/ml), and lactic acid (5.9+0.5 vs 1.3+0.1mM/L, p< 0.001) concentrations, respectively.

| Treatment | GLUT1 Abundance | | GLUT4 Abundance (density units) | | |
|---|---|---|---|---|---|
| | Muscle | Fat | Muscle | Fat | |
| Saline | 101±19 | 99±8 | 99±11 | 100±13 | ($\bar{x}$+S.E.M.) |
| LPS | 324±31 | 679±94 | 133±8 | 142±33 | |

At the time of hypoglycemia and lactacidemia, GLUT1 mRNA levels were significantly increased 6-fold in fat and 3-fold in muscle, but GLUT4 mMRNA levels were not significantly changed. This observation of altered GLUT1 expression in muscle and fat may be important for understanding the hypoglycemic-phase of endotoxic shock.

## *Late Abstracts*

ADIPSIN AND AN ENDOGENOUS COMPLEMENT CYCLE FROM ADIPOSE CELLS, Lisa Choy, Barry S. Rosen and Bruce M. Spiegelman, Department of Biological Chemistry and Molecular Pharmacology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

We have previously demonstrated that there is a marked deficiency in the levels of the serine protease adipsin in several forms of genetic and acquired obesity in rodents. Furthermore, purified adipsin was demonstrated to have the catalytic activity of factor D, the initial and rate limiting enzyme of the alternative pathway of complement activation. We have now asked whether adipose cells also produce the other 2 key proteins involved in triggering the alternative pathway of complement: factors C3 and B. Factor C3 mRNA is produced by 3T3-F442A when the cells are in the predipose or adipose states. Factor B is produced by adipocytes but only when they are treated with low levels of TNF. The production of factor B by adipose tissue was demonstrated by radiolabelling tissue slices and immunoprecipitating with anti-B antisera. The endogenous catalytic activation of the proximal steps of the alternative pathway by adipose cells was indicated by the formation of cleavage products characteristic of this pathway (Bb, C3$\alpha$'). Since several soluble ligands generated by this pathway, such as C3a, are known to have receptor mediated pharmacological effects on a variety of cells, the role of these ligands in energy balance is now under investigation.

INTERACTION BETWEEN GLUCOSE AND FREE FATTY ACID AVAILABILITY IN DETERMINING RATES OF LIPID SYNTHESIS IN HUMAN ADIPOCYTES *IN VITRO*, Edens, N.K., Rockefeller University, New York, NY 10021

It is important to determine whether physiological variations in substrate availability can affect the rate of acylglyceride synthesis (AS) in human adipose tissue. Both 0.5 and >10 mM glucose have been reported to support maximal rates of AS in human adipocytes *in vitro*. These inconsistent results might be due to variations in free fatty acid (FFA) availability in incubations. Isolated adipocytes obtained from six human volunteers were incubated with adenosine deaminase (0.8 U/ml) and $N^6$-phenylisopropyl adenosine ($10^{-7}$ M) to minimize lipolysis. Either 0 or 0.5 mM palmitic acid (PA) (PA:albumin ratio ~0.68) and a range of concentrations of glucose ([Glu]) were added to the incubation medium. The rate of AS was measured by incorporation of $^{14}$C-glucose into $^{14}$C-acylglyceride. Without exogenous PA, increasing [Glu] from 0.1 to 10 mM increased AS ~2-fold, from (Mean ± SEM) 0.029 ± 0.007 to 0.066 ± 0.015 $\mu$mol/$10^6$ cells-2hr. With 0.5 mM PA, the same increment in medium [Glu] increased AS ~8-fold, from 0.055 ± 0.018 to 0.424 ± 0.169 $\mu$mol/$10^6$ cells-2hr ($p < .05$ for interaction). At 1 mM glucose, insulin (500 $\mu$U/ml) increased AS by 0.010 ± 0.002 $\mu$mol/$10^6$ cells-2hr without PA, but by 0.043 ± 0.014 $\mu$mol/$10^6$ cells-2hr with 0.5 mM PA (n=3). Medium FFA availability, which can vary with the rate of lipolysis and adipocyte concentration in the incubation, may be rate-limiting for AS and can affect the response to increasing glucose availability and insulin.

DIFFERENTIAL EXPRESSION OF GLUCOSE TRANSPORTER ISOFORMS IN THE RAT ADIPOSE CELL IN OBESITY. O.M. Gonzalez-Mulero, I.A. Simpson, and S.W. Cushman, NIDDK, NIH, Bethesda, MD USA.

Adipose cellular enlargement in the aging rat model of obesity is accompanied by increased basal glucose transport activity per cell, but no change in the maximally insulin-stimulated rate. Per unit cellular surface area, the basal activity is unchanged and the stimulation by insulin(INS) is markedly decreased. These alterations are explained by a constant basal concentration of glucose transporters(GLUT) in the plasma membrane and a relative depletion of GLUT from the intracellular pool, as assessed by cytochalasin B binding. We have now reevaluated the changes in GLUT to determine the relative roles of the two isoforms normally present in the rat adipose cell, GLUT1 and GLUT4. Large and small cells(=0.8 and ≈0.08 µg lipid/cell, respectively) were prepared from ≈900- and ≈200-g rats, respectively, and incubated ± INS. 3-0-methylglucose transport was assessed and aggregate GLUT were measured by cytochalasin B binding in the plasma membranes(PM) and low-density microsomes(LDM). The relative concentrations of GLUT1 and GLUT4 were then determined by Western blotting. With increasing cell size, the basal concentration of GLUT4/mg protein in LDM decreases markedly(≈85%) and its relative concentration in LDM compared to PM decreases from ≈10- to ≈4-fold. The appearence of GLUT4 in PM in response to INS also markedly decreases from ≈3- to ≈1.25-fold. While the basal concentration of GLUT1 in LDM and its relative concentration in LDM compared to PM also decrease, these decreases are much smaller than for GLUT4. In addition, the presence of GLUT1 in PM does not change in either the basal or INS-stimulated state. The glucose transport response itself to INS decreases from ≈21- to ≈2-fold. These results suggest that the INS resistance associated with cellular enlargement in obesity is mediated by a selective depletion of GLUT4 from the intracellular pool and that the increased basal glucose transport activity per cell is mediated by an expanding PM containing a constant concentration of GLUT1. However, we must presume that GLUT4 is intrinsically more active than GLUT1 to explain the residual INS response in the largest cells studied.

DEVELOPMENTAL CHANGES IN ADENYLYL CYCLASE AND GTP-BINDING PROTEINS IN BROWN ADIPOSE TISSUE, J.G.Granneman and A. Chaudhry, Center for Cell Biology, Sinai Hospital, Detroit MI 48235

During the perinatal period, norepinephrine (NE)-stimulated adenylyl cyclase (AC) activity increased in brown adipose tissue membranes and then declined to adult levels by 23 days postpartum. The postnatal patterns of NE- and NaF-stimulated activities were identical, indicating that the increase in NE-stimulated activity resulted from the increased interaction of $G_s$ with the catalytic subunit (C). This increased $G_s$-C interaction was the result of an increase in $G_s$ specific activity, as assessed in cyc- reconstitution assays, as well as an increase in C activity, as assessed by forskolin/$Mn^2$+-stimulated AC activity. Total $G_{s\alpha}$ levels significantly declined during the perinatal period due to the loss of the the small molecular weight form of $G_{s\alpha}$. Thus, the ratio of large to small form of $G_s$ increased three-fold, and might have contributed to the peak in activity observed after birth. $G_i$-like proteins, as assessed by pertussis toxin-catalyzed [$^{32}$P]ADP-ribosylation, declined after birth and remained low. However, loss of $G_i$ did not appear to contribute to the perinatal changes in AC activity because pertussis toxin treatment failed to alter NE-stimulated activity in IBAT. In contrast to $G_\alpha$ subunits, membrane levels of $G_\beta$ subunits did not change.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

IDENTIFICATION AND ANALYSIS OF A POTENT ADIPOCYTE-SPECIFIC ENHANCER, Reed A. Graves, Peter Tontonoz, Susan Ross* and Bruce M. Spiegelman, Dana-Farber Cancer Institute, and the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA and *Department of Biochemistry, University of Illinois Medical School, Chicago, IL

We have identified an enhancer with specificity for adipose cells at -5.4Kb of the adipocyte P2 gene. This 500 bp enhancer directs CAT gene expression specifically to cultured fat cells but not preadipocytes when linked to its own minimal promoter or an enhancerless promoter from SV40 virus. In transgenic mice this enhancer directs CAT expression specifically to white and brown adipose tissues. Molecular analysis of the enhancer DNA demonstrates that it contains a binding site for a nuclear protein in the NF-1 transcription factor family. Mutational analysis indicates that this enhancer plays an important role in enhancer action. Further analysis indicates that other cis- and trans-acting elements may function in silencing expression from non-adipose cells. We will also describe experiments that utilize this adipocyte enhancer to investigate the potential regulatory roles of certain fat cell proteins, such as adipsin, by overexpressing them in the fat tissue of transgenic mice.

Phenylarsine oxide (PAO) induces an insulin-dependent, Glut 4-specific lyzosomal degradation in adipocytes. Chan Y. Jung and Ben H. Jhun, State University of New York, and VA Medical Center, Buffalo, NY 14215

An incubation of rat adipocytes with PAO caused an inhibition of 3-O-methyl glucose equilibrium exchange flux upon the addition of insulin, and a parallel reduction in adipocyte Glut-4 content in Western blots. Both the transport inhibition and the Glut-4 reduction were saturable with an increasing concentration of PAO showing essentially an identical Ki value of 35 uM. Both effects were not observed without the addition of insulin or if cells were incubated with insulin first. SDS-PAGE analysis of iodine-125 forscolin-labelled adipocytes revealed a disappearance of lable from 55KDa band region, demonstrating that the reduction in Glut-4 immunoreactivity is due to the chemical degradation of Glut-4 protein. The degradation (1) was specific to Glut-4, as neither Glut-1 nor clathrin light chain was affected in immunoblots, (2) requires intact cells, as it was not observed with homogenate or fractionated membranes, and (3) was due to a lysosomal pathway as it was effectively arrested by chloroquine. These results demonstrate that there is a physiologically suppressed, insulin-dependent, Glut-4-specific, lyzosomal degradation pathway in adipocytes that can be activated by PAO.

REGULATION OF GLUCOSE TRANSPORT IN RAT ADIPOSE CELLS: EFFECTS OF POTASSIUM DEPLETION. Haruo Nishimura, Samuel W. Cushman, and Ian A. Simpson, NIDDK, NIH, Bethesda, MD USA.

Several early studies reported that potassium depletion results in an increase in the transport and metabolism of glucose in isolated rat adipose cells. Here we have reinvestigated this phenomenon specifically to ascertain by which mechanism this enhanced transport activity is achieved. $K^+$ depletion results in a slow ($t_{1/2}$ ≈30 min) 4-fold increase in basal 3-O-methylglucose transport (3-OMGT) compared to $K^+$-replete (4mM) conditions but is without effect on the 35-fold insulin-stimulated 3-OMGT. The effects of $K^+$ depletion are reversed on addition of $K^+$ with a $t_{1\backslash 2}$ ≈15 min. The apparent $EC_{50}$ for $K^+$ is ≈1.2mM. Comparable stimulations of 3-OMGT are achieved in the presence of $K^+$ when cells are incubated with ouabain, a $Na^+/K^+$ ATPase inhibitor. The distribution of glucose transporters GLUT1 and GLUT4 were monitored by Western blotting; $K^+$ depletion induces a 1.4-fold increase in plasma membrane GLUT1 (compared to 1.7-fold by insulin) and a 2.2-fold increase in GLUT4 (compared to 5.0-fold by insulin). The translocations induced by insulin are independent of $K^+$. Thus, modulations of cellular $K^+$ appears to stimulate 3-OMGT by translocation mechanisms analogous to those of insulin and may underlie the mechanism of insulin action.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

THE IDENTIFICATION AND CHARACTERIZATION OF PROTEINS WHOSE REGULATION IS ASSOCIATED WITH COMMITMENT TO 3T3-L1 ADIPOCYTE DIFFERENTIATION. Henry B. Sadowski, Thomas T. Wheeler and Donald A. Young, Environmental Health Science Center, and Endocrine-Metabolism Unit, Department of Medicine, University of Rochester Medical Center, Rochester, NY 14642.

The mouse 3T3-L1 fibroblastic cell line can be induced to differentiate rapidly (4-6 days) and efficiently (80% conversion) to adipocytes by a 48 h treatment of post-confluent cells with the combination of dexamethasone (D), methylisobutyl-xanthine (M) and insulin (I). Biological studies of the effects of these agents on differentiation determined that (i) D acts synergistically with MI to induce differentiation, (ii) maximal commitment to adipocyte differentiation requires the presence of D during the period 30-48 h after the addition of MI, and (iii) the phorbol ester, TPA, completely inhibits adipocyte differentiation unless it is added 36 h or more after initiating DMI treatment. These results define a window in time during which the cells become committed to differentiation. To study gene expression during commitment to, and subsequent expression of the adipocyte phenotype, we used ultra-high resolution two-dimensional electrophoretic analysis of metabolically labeled proteins and *in vitro* translation products of mRNA. This allowed the simultaneous analysis of the regulation of over 4000 synthesized proteins and 2000 mRNA species. We identified and characterized 8 mRNA species: (i) that were synergistically induced by treatments that lead to differentiation, (ii) were first expressed at elevated levels before the appearance of known adipocyte phenotypic markers, (iii) remained elevated in fully differentiated adipocytes, and (iv) whose elevated expression was inhibited by TPA. These novel differentiation-associated proteins may be important in the commitment to and expression of the adipocyte differentiation program. [Supported by grants from NIH; ES07026,DK1677 and CA47650]

RECYCLING OF CELL SURFACE GLUT4 GLUCOSE TRANSPORTERS IN RAT ADIPOSE CELLS PHOTOLABELED IN THE INSULIN-STIMULATED STATE. S. Satoh, O. M. Gonzalez-Mulero, A. E. Clark, I. J. Kozka, G. D. Holman, and S. W. Cushman, Department of Biochemistry, Bath, UK and NIDDK, NIH, Bethesda, MD USA.

Insulin stimulates glucose transport in rat adipose cells through the translocation of glucose transporters, primarily the GLUT4 isoform, from a large intracellular pool to the plasma membrane. Here we examine directly the recycling of GLUT4 transporters initially present on the cell surface in the insulin-stimulated state by photolabeling them with a novel impermeant [$^3$H]-bis-mannose derivative. When insulin-stimulated cells are treated with 1 mg/ml crude collagenase as described by T. Kono, 3-0-methylglucose transport rapidly decreases from its maximal level towards the basal level with a $t_{\frac{1}{2}}$ of ≈15 min. Unlabeled GLUT4 transporters detected by Western blotting decrease in the plasma membranes and increase in the low-density microsomes with the same time course. Over a period of 60 min with colla-genase, ≈55% of the GLUT4 transporters labeled on the cell surface in the insulin-stimulated state leave the plasma membrane and appear in the low-density microsomes. However, ≈30% of the cell surface-labeled GLUT4 transporters redistribute from the plasma membranes to the low-density microsomes even in the continuous presence of insulin. Thus, the specific recycling pathways of GLUT4 transporters can now be directly followed in response to hormonal stimulation through the use of this novel cell surface photolabeling technique, although small differences in trafficking kinetics between labeled and unlabeled GLUT4 transporters remain to be resolved.

REGULATION OF THE PHOSPHORYLATION/ACTIVATION OF LOW $K_m$ cAMP PHOSPHODIESTERASE (PDE) IN RAT ADIPOCYTES BY INSULIN AND CALCIUM MOBILIZING AGENTS, Carolyn J. Smith, *Valeria Vasta,**Eva Degerman, **Per Belfrage and Vincent Manganiello, Lab. of Cellular Metabolism, NHLBI/NIH, Bethesda, MD 20892, *Dept. of Biochemistry, Univ. of Florence, Italy, and **Dept. of Medical and Physiological Chemistry 4, Univ. of Lund, Lund, Sweden S221 00. Incubation of $^{32}$P-labeled rat adipocytes with insulin (INS) or isoproterenol (ISO) induced serine phosphorylation of a 135 kDa particulate protein identified as low $K_m$ cAMP PDE (1). To determine whether C-kinase or other $Ca^{+2}$-sensitive kinases contribute to regulation of PDE, the effects of phorbol ester (PMA) and $Ca^{+2}$-mobilizing hormones were compared to INS and ISO for changes in the phosphorylation and activation of PDE in intact adipocytes. INS or ISO evoked ~50% or ~100% respective increases in PDE activity; either agent promoted ~10-fold increases in PDE phosphorylation. Compared to INS and ISO, the corresponding effects of PMA, vasopressin and angiotensin II on PDE were smaller (i.e., increases of 30% for activation and 2- to 3-fold for $^{32}$P-labeling). In combination, ISO and INS produced additive or synergistic effects on the phosphorylation/ activation of PDE. However, the effects of PMA together with insulin (or with ISO) were less than additive. The $^{32}$P-135 kDa from INS-, ISO- or PMA-treated cells was proteolyzed and analyzed by autoradiography for $^{32}$P-peptides. Limited digestion of PDE with V8 protease generated common $^{32}$P-peptides of 42, 28/31 and 14/18 kDa for either INS, ISO or PMA; a ~24 kDa was seen only with PMA. Digestion of PDE from INS- or ISO-treated cells with chymotrypsin plus trypsin produced at least 3 to 4 $^{32}$P-peptides (on thin layer chromatography), one of which was also observed in PMA-treated cells. These results suggest that the adipocyte hormone-sensitive particulate PDE is regulated by insulin and cAMP, as well as by PMA and $Ca^{+2}$-mobilizing hormones (in analogy with the hepatocyte); $Ca^{+2}$-dependent phosphorylation of PDE may be associated with activation, partially mimicking the effects of cAMP or INS; and that various agonists effect similar <u>in situ</u> phosphorylations of PDE.

1) Degerman, E., Smith, C. J., Tornqvist, H., Vasta, V., Belfrage, P. and Manganiello, V. C. (1990) **Proc.Nat.Acad.Sci.(USA)** <u>87</u>:533-537.

# The Adipose Cell: A Model for Integration of Hormone Signalling in the Regulation of Cellular Function

TGF-β BLOCKS EARLY BUT NOT LATE DIFFERENTIATION-SPECIFIC GENE EXPRESSION AND MORPHOLOGIC DIFFERENTIATION IN 3T3 T PROADIPOCYTES. Rodney L. Sparks, Ethan E. Strauss, and Blake J. Allen, Department of Cell Biology and Anatomy, School of Medicine, Oregon Health Sciences University, Portland, OR 97201.

Transforming growth factor-β (TGF-β) inhibits differentiation of BALB/c 3T3 T cells as well as various other proadipocyte models. TGF-β has recently been shown to induce dedifferentiation of 10T1/2 TA1 proadipocytes. Our prior studies did not suggest such an action on 3T3 T cells. Therefore, in the current study we tested the effects of exogenous TGF-β (0.1-5.0 ng/ml) on morphologic differentiation and on differentiation-dependent gene expression (northern and slot blot analyses) at various times during differentiation of 3T3 T proadipocytes. When induced to differentiate, 3T3 T cells first undergo predifferentiation growth arrest at a specific state ($G_D$) of the $G_1$ phase of the cell cycle. From this state molecular, biochemical, and morphological differentiation proceeds. When added prior to the onset of differentiation, TGF-β was a potent inhibitor of morphologic differentiation as well as of the expression of differentiation-specific genes such as lipoprotein lipase (LPL) and insulin-regulated glucose transporter (IRGT). TGF-β also blocked differentiation induced by the antidiabetic AD4743, a derivative of ciglitazone. However, once differentiation began, TGF-β was ineffective in blocking differentiation. In addition, fully differentiated cells exposed to TGF-β for up to 7 days did not undergo significant morphologic dedifferentiation or down-regulation of differentiation-specific gene expression (LPL, IRGT, etc.). Tumor necrosis factor also had no effect on fully differentiated cells. These data suggest that TGF-β may not inhibit 3T3 T adipocyte differentiation by directly blocking the expression of known differentiation-specific genes, but rather by blocking an as yet unidentified master regulatory gene(s) [analogous to myoD] and that this inhibition occurs in a cell cycle and differentiation-dependent manner. (Funded by NCI, Fraternal Order of Eagles, and The Smokeless Tobacco Research Council).

ADIPOCYTE REGULATION OF ANGIOGENESIS: DIFFERENTIATION-DEPENDENT BIOSYNTHESIS OF MONOBUTYRIN, A NOVEL ANGIOGENIC FACTOR. William Wilkison, Lisa Choy, and Bruce Spiegelman. Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School and Dana-Farber Cancer Institute, Boston, MA 02115.

Adipocyte differentiation is accompanied by the directed invasion of new capillaries. We have previously shown that differentiating adipocytes secrete activities that stimulate angiogenesis in vivo and vascular endothelial cell growth and chemotaxis in vitro. We have purified the major differentiation-dependent chemotactic and angiogenic activity and have determined the structure of the active molecule to be 1-butyryl-glycerol (monobutyrin). To determine the biosynthetic pathway of this novel signalling lipid, we have developed an in vitro assay for the formation of monobutyrin. Of many substrates tested, [C-14] butyryl-CoA and [C-14] glycerol were substrates for the formation of monobutyrin in fat cell total homogenates. This enzymatic activity was only observed in adipocytes and was localized to particulate fractions. The enzyme has a high affinity for butyryl-CoA ($K_{m\,app}$ = 5 uM). In addition to characterizing the biosynthetic activity, the differentiation-dependent secretion of monobutyrin into culture media was quantitated using a novel enzymatic assay. The effect of different lipolytic and lipogenic hormones on monobutyrin secretion by adipocytes is examined.