6th Milano International Meeting on Diabetes

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Medical School

Milano, 21–23 March 1996
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Scientific Programme

Thursday, 21 March 1996

Morning

Opening Ceremony

Biology of Islets
Chairman: G. C. Weir
Invited Speakers:
W. J. Malaisse, F. Purrello, G. C. Weir

Islets Transplantation and Bioengineering
Chairman: C. Ricordi
Invited Speakers:
M. Lipes, C. Newgard, C. Ricordi

Afternoon

Free Communications

Type I Diabetes
Chairman: U. Di Mario
Invited Speakers:
E. Bonifacio, G. F. Bottazzo, P. Pozzilli

Free Communications

Friday, 22 March 1996

Morning

Free Communications

Molecular Basis of Insulin Action
Chairman: J. Roth
Invited Speakers:
F. Barbetti, J. Roth, S. Shoelson

Afternoon

Free Communications

Poster Session

Saturday, 23 March 1996

Morning

Lipid Metabolism
Chairman: G. Crepaldi
Invited Speaker:
B. Howard

Free Communications

Final Lecture
Chairman: G. Pozza
Invited Speaker:
D. Mintz

Invited Speakers

Studies on the glycogenin gene and on the insulin receptor gene promoter and their implications on insulin action.

Fabrizio Barbetti M.D., Ph.D. H San Raffaele Scientific Institute, Milan.

Genetic factors play a major role in the pathogenesis of non-insulin dependent diabetes mellitus (NIDDM) and the search for new potential candidate genes is influenced by the current understanding of the pathophysiology of disease. The insulin resistance found in subjects with NIDDM originates in part from an impairment of the glycogen synthesis in tire skeletal muscle. Thus, genes encoding enzymes of the glycogen synthesis can be considered reasonable candidates in the pathogenesis of NIDDM. Recently, the mechanisms of glycogen biogenesis in muscle have been further elucidated. Glycogen synthase alone is unable to promote the *de novo* synthesis of glycogen and requires the priming activity of a protein named glycogenin. This self-glucosylating enzyme generates an oligosaccharide primer suited to initiate glycogen synthesis reactions. Glycogenin is complexed to the 86 kDa catalytic subunit of glycogen synthase and an impaired glycogenin activity may have negative effects upon glycogen biosynthesis. We have cloned the glycogenin cDNA from human skeletal muscle mRNA: human glycogenin is a 333 amino acid protein exhibiting 93% identity with rabbit glycogenin. A single transcript of about 2.4 kb, prominent in skeletal muscle, was detected by Northern blot analysis. *In situ* hybridization unequivocally located the human glycogenin gene to chromosome 3q25.1. We also mapped two intronless glycogenin-related sequences to human chromosomes 12 and 13. The effects of different glycemic and insulin levels on glycogenin mRNA in normal and diabetic animals are now under investigation.
The maintenance of glucose homeostasis is a complex process in which the insulin receptor (HIR) plays an important role: major changes in the number of receptors in insulin-dependent tissues may have pathophysiological consequences. Insulin levels affect the receptor cellular trafficking and density on the cell surface, but in the steady state the absolute number of receptors is function of the abundance of cellular HIR mRNA. Genetic mutations altering the insulin receptor protein are found in genetic syndromes of severe insulin resistance. We have been studying four patients in which a reduction of HIR mRNA accounts for a decreased number of receptors on the cell surface. Pulsed-field gel electrophoresis (PFGE) was applied to perform long-range restriction analysis: blots were hybridized with HIR cDNA. A large rearrangement of the HIR gene has been found in one patient. We have amplified by polymerase chain reaction (PCR) the promoter region of the HIR gene in two overlapping fragments. High resolution agarose gel of PCR products did not reveal microdeletions. Denaturing gradient gel electrophoresis (DGGE) of HIR promoter region spanning -1676 to -684 from the ATG did not show sequence variations. The entire promoter is now subjected to sequence analysis. We have preliminarly concluded that the impaired HIR gene transcription in these patients is due to range of molecular defects.

The IDDM-Sardinia Project: from the lab to the field.

G.F. Bottazzo[1], A. Loviselli[1], S. Mariotti[2], E. Cossu[3], R Cirillo[2], V Sepe[1], M Songini[1] and the Sardinian School Children-IDDM (SSI) & Newborn-IDDM (SNI) Study Groups.
[1]Department of Immunology, St Bartholomew's and the Royal London School of Medicine and Dentistry, London, UK; [2]Institute of Internal Medicine, Medical Clinic I "Mario Aresu", Cagliari, Italy; [3]Center for Metabolic Diseases & Atherosclerosis, H San Michele, Cagliari, Italy

The incidence of IDDM is growing world wide. The data from Europe has disclosed unexpected insights. Apart from the confirmation of the known North to South gradient, with Finland and the other Scandinavian countries leading the unfortunate race and the Mediterranean area with the lowest incidence, unexpectedly it has been shown that Sardinia is an exception to this rule. In fact, the Island has a similar incidence of IDDM as that reported in Finland, the highest in the world. Interestingly, in both countries the occurrence of the disease began to rise around the mid 60's and the trend does not seem to have stopped. From the migration, linguistic and genetic view points, there are very few similarities between the inhabitants of the two areas, thus indicating that, if an environmental factor (?new) has hit these locations, it acted with a similar mode of action, but on a distinct background.
In order to understand what has caused this incredible increase of IDDM in Sardinia (200 new cases per year only in the age group 0-29), we have organised and carried out a series of projects, which, among others, include: 1) the "Sardinian School Children-IDDM (SSI) Study", where over 10,000 young individuals aged 6-14 have been registered and bled for the measurement of ICA and other islet-related autoantibodies. We have identified 439 ICA positive individuals and during an initial follow up of 1071 of them (median 4.8 years of follow up), 5 developed diabetes. They were all ICA positive, 4 had '37 antigen' antibodies, 2 had also GAD antibodies, and all had a family history of DM; 2) the "Sardinian Newborn-IDDM (SNI) Study", where 30,000 (the cohort of children born in 2 years in the Island) cord blood will be collected and the children regularly followed every year for 5 years. So far, the prevalence of ICA in 9174 mothers tested at the time of delivery was 2,5%, and of the 1101 children tested at year 1, only 1 (0.1%) had ICA. GADA was measured in 303 of these children and 5 (1.7%) were positive. The only one with both autoantibodies developed IDDM at the age of 1 year and 7 months.
Other projects include: 1) continuation of the collection of incidence data; 2) a close collaboration with the veterinary colleagues, in order to identify common or rare diseases, occurring in animals, which might be related to IDDM in humans; 3) comparing data provided by similar protocols carried out in Finland; and 4) the study of the Sardinian emigrants in various locations in the world. This latter project should quantify differences between the number of cases with IDDM in the Island compared with those developing the disease abroad.
The ultimate aims of the entire "IDDM Sardinia" project is: 1) to design maps of incidence of overt IDDM and pre-IDDM in the Island (Hot and Cold spots), in order to see if environmental factor(s) can be pinpointed; 2) to design models of prediction in familial versus sporadic cases; and 3) to prepare the ground for safe preventive trials to hold the progression of the disease in the identical individuals at high risk to develop IDDM.

Islet antibodies and insulin dependent diabetes: fact, fiction and prediction.

Ezio Bonifacio Ph.D. Department of Medicine I, Istituto Scientifico San Raffaele, Milan, Italy.

Islet cell antibodies (ICA) are the traditional marker of preclinical insulin dependent diabetes (IDDM). Numerous islet antigens have been proposed as autoantibody targets. Not all of these have been confirmed. Nevertheless, several islet antigen specific markers are now available in addition to ICA. We have demonstrated that glutamic acid decarboxylase (GAD) and the protein tyrosine phosphatase-like antigen IA-2 are components of the traditional ICA reactivity, and that combined measurement of these two antibody markers can identify 90% of IDDM cases. The use of IA-2 and GAD antibodies together with ICA markedly improve the ability to identify preclinical IDDM subjects. Our studies in almost 3,000 school children show that while elevated levels of these islet antibodies can be detected in a significant proportion of the background population, only few have elevated levels of more than one marker. This is in marked contrast to IDDM and preclinical IDDM cases where the over 80% have elevated levels of at least two markers. These studies show that through measurement of these three markers around 70% of future IDDM cases can be predicted with a positive predictive value above 50%. We will present strategies for accurate IDDM risk assessment in the general population using islet autoantibody markers.

Risk Factors for Cardiovascular Disease in Individuals with Diabetes - The Strong Heart Study.

Barbara V. Howard Ph.D. Medlantic Research Institute, Washington D.C., U.S.A.

Coronary heart disease (CHD) is the leading cause of death among individuals with diabetes. However information on CHD and its association with known risk factors in populations with high rates of diabetes is limited. The purpose of the Strong Heart Study is to quantify CHD and its risk factors among three geographically diverse groups of American Indians who have high prevalence of diabetes. The study group consists of 4549 adults between 45 and 74 years of age in 13 Indian communities in Arizona, Oklahoma, and South and North Dakota.

Rates of diabetes ranged from 33% to 72% in men and women in the three centers. Prevalence rates of definite myocardial infarction (MI) and definite CHD were higher in men than in women in all three centers (P<.0001) and higher in those with diabetes (P=.002 and P=.0003 in women and men, respectively). Diabetes was associated with a relatively greater increase in prevalence of MI (PR = 3.8 vs. 1.9) and CHD (PR = 4.6 vs 1.8) in women than in men. Logistic regression analysis indicated that prevalent CHD was significantly related to age, diabetes, hypertension, albuminuria, percent body fat, smoking, high concentrations of plasma insulin and low concentrations of HDL cholesterol. These findings from the baseline Strong Heart Study examination emphasize the relative importance of diabetes associated variables as risk factors for CHD among populations with high rates of diabetes.

Engineering Insulin Secretion in Non-Islet Cells for β-Cell Replacement in IDDM.

Myra A. Lipes, Alberto M. Davalli, Gordon C. Weir, Robert H. Skelly. Joslin Diabetes Center, Boston, MA.

We have recently created transgenic nonobese, diabetic (NOD) mice in whom insulin expression was targeted to the pituitary via the proopiomelanocortin (POMC) promoter. POMC-expressing intermediate lobe pituitary cells may be potentially useful cells for insulin gene delivery in IDDM since they efficiently secrete fully processed, mature insulin via a regulated secretary pathway, similar to islet β cells. However, in striking contrast to islet β cells, the insulin-producing intermediate lobe cells are not immunologically attacked in diabetic NOD mice. To assess whether the transgenic tissues could reverse hyperglycemia, diabetic NOD mice were grafted under the kidney capsule with transgenic intermediate lobe tissue. This resulted in the return to near-normoglycemia, with mean blood glucose levels decreasing from 484 ± 21 mg/dl before transplantation to 150 ± 43 mg/dl after transplantation (n=6). In addition, mice receiving transgenic intermediate lobe grafts had a significant gain in body weight and complete remission from diabetic symptoms. Random insulin levels increased from 4 ± 0.2 μU/ml pre-transplantation to 42 ± 9 μU/ml post-transplantation, similar to nondiabetic control mice (39 ± 9 μU/ml, n=6). As expected, diabetic NOD mice receiving control (nontransgenic) intermediate lobe pituitaries had no reduction in their blood glucose levels and had increasingly severe diabetic symptoms which resulted in their demise within 3 weeks after transplantation. The absence of autoimmunity in intermediate lobe pituitary cells engineered to secrete *bone fide* insulin provides encouraging *in vivo* evidence of the potential of these cell types for β cell replacement therapy for IDDM.

IS GLYCEMIC CONTROL IMPORTANT TO PREVENT COMPLICATIONS IN NIDDM?

Daniel H. Mintz, M.D.
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Since the DCCT involved patients with Type 1 diabetes mellitus, a question exists as to the extent that these results apply to patients with Type 2 diabetes. In Type 2 diabetes there are yet no comparable intervention studies, although the large multicenter trial in the United Kingdom, the UK Prospective Diabetes Study (UKPDS), scheduled to be completed in 1998, has already reported a relationship between hyperglycemia and albumin excretion rate. Two other intervention studies of patients with Type 2 diabetes have been undertaken. One, the U.S. Veterans Administration Cooperative Study on Glycemic Control and Complications, was aborted because of lack of funds. The second, the Japan Kumamoto trial in thin Type 2 patients studied over six years has shown that improved glycemic control lowered risk for two-step progression of diabetic retinopathy, similar to the results in the older patients studied in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). In the Kumamoto study, glycemic control also lowered the risk for progression of nephropathy, improved nerve conduction velocities and substantially reduced the incidence of major macrovascular, cardiac, cerebral vascular and peripheral vascular events.

Thus, although multicenter, large-scale study results (UKPDS) are not yet available, the data that is available places hyperglycemia as the defining issue in micro and potentially macrovascular disease in Type 2 diabetes, as it is in Type 1.

However, in Type 2 diabetic patients the choice of treatment targets and treatment interventions may be different from Type 1 diabetes. The risks of hypoglycemia in the elderly, for example, may outweigh the benefits of intensive therapy aimed at normoglycemia. Treatment targets need yet to be defined; in particular, we await information whether the threshold for progression of micro and macrovascular diseases is different. This information will allow us to rationally individualize therapies for patients with Type 2 diabetes.

As trials for prevention of progression of Type 1 diabetes from a seemingly indolent autoimmune diathesis to frank diabetes have been initiated, so have multicentered trials for prevention of Type 2 diabetes been undertaken. The design of these studies provides important clues to direct clinicians to consider the therapeutic options that are available to them today to care for the largest number of patients with diabetes mellitus.

Regulation, perturbation and correlation of metabolic events in pancreatic islets.

Willy J. Malaisse Ph.D. Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium

The regulation of metabolic events in pancreatic islet cells plays a key role in their physiological response to circulating nutrients, especially D-glucose. Recent findings reinforce the view that the B-cell glucose-sensing system should not be considered as being restricted to the participation of glucokinase to the metabolism of hexoses in this cell. Likewise, further evidence is accumulating to indicate that the coupling of metabolic to more distal events in the secretory sequence is not restricted to the role of ATP in causing the closing of K^+ channels. Site-specific defects of nutrient metabolism may participate in the perturbation of insulin release in non-insulin-dependent diabetes. For instance, glycogen accumulation in conditions of sustained hyperglycemia may account, at least in part, for the secondary phenomenon of glucose incompetence, that should not be ascribed to a process of either glucotoxicity or desensitization. New tools to correct for such metabolic defects include non-sulfonylurea hypoglycemic agents of the meglitinide family, adenosine analogue such as formycin A, and esters of carboxylic metabolites that are intermediates of the Krebs cycle or their precursors.

Engineering of Cell Lines for Insulin Replacement in Diabetes.

Christopher B. Newgard, Hans Hohmeier, Karl Normington, Anice Thigpen, Christian Quaade, and Samuel Clark. University of Texas Southwestern Medical Center and BetaGene, Inc., Dallas, TX, USA.

While islet transplantation has shown promise for treatment of a small number of IDDM patients, wide-scale application of this approach may be hindered by the inherent expense and difficulty in procurement of transplantable tissue. We have therefore been engaged in studies in which molecular tools are used for creating insulin secreting cell lines that simulate the performance of the normal islet β-cell. Our strategy involves iterative engineering, or the step-wise stable introduction of genes designed to enhance the performance of neuroendocrine cell lines. Rodent insulinoma (RIN 1046-38) cell lines engineered to contain multiple copies of the human insulin gene exhibit an increase in insulin content of more than 10-fold relative to untransfected parental cells (2.5 $\mu g/10^6$ cells versus 0.2 $\mu g/10^6$ cells, respectively). HPLC analysis reveals that the human proinsulin is efficiently processed to mature insulin, both in the intracellular and secreted pools, indicating that the RIN cell complement of PC2 and PC3 can accommodate human insulin overexpression. Further iterative engineering has allowed us to introduce the GLUT-2 glucokinase genes into RIN cell lines previously engineered for insulin overexpression. Such cells exhibit strong secretory responses to glucose (6-14 fold in different clonal lines) that are potentiated in a fashion similar to that seen in β-cells by agents that raise cAMP and by amino acids. Overexpression of glucokinase to high levels (8.8-fold increase in activity relative to parental cells) induces a shift in the glucose concentration required for maximal insulin secretion from approximately 0.05 mM to 1 mM. Further adjustment of the glucose dose-response to the physiologic range (4-9 mM) is likely to be achievable by stable suppression of low Km hexokinase activity, based on studies in which transient inhibition of this activity has been achieved with chemical approaches (exposure of cells to 2-deoxyglucose or 5-thioglucose) or by expression of hexokinase in antisense configuration via recombinant adenovirus. In order to affect permanent suppression of hexokinase activity in RIN cells, we have recently knocked out 1 allele of the hexokinase I gene by homologous recombination and are in the process of characterizing resulting cell lines. Transplantation of our cell lines into nude rats reveals that stably integrated transgenes (GLUT-2, glucokinase, human insulin) are expressed at constant levels in the *in vivo* environment over the full duration of experiments performed to date (48 days). These results provide encouragement for upcoming studies on insulin replacement with engineered cells in animal models with chemically induced or spontaneous autoimmune diabetes.

Non HLA genes and their significance in the pathogenesis of type 1 diabetes.

Paolo Pozzilli, Lorenza Nisticò, Raffaella Buzzetti Istituto di Clinica Medica II, universita' degli studi di Roma "La Sapienza", Policlinico Umberto I, Roma, Italy

Insulin dependent diabetes (IDDM) is an autoimmune disease with a strong genetic background. Major genes involved in this disease are the HLA gene (IDDM1) on chromosome 6 and a gene in the insulin gene region(IDDM2) on chromosome 11. However, these genes account for not more than 50% of the genetic susceptibility depending on the population of patients studied. By using the candidate gene approach we have investigated non HLA genes in the susceptibility to IDDM. We analyzed linkage of IDDM to chromosome 2q in 48 Italian families to test if the human homologue of Idd5 in non-obese diabetic (NOD) mouse is linked to disease. Tightest linkage was obtained with the CTLA-4 microsatellite, at the 3' untranslated region of the CTLA-4 gene. This gene codes for the CTLA-4 T cell receptor which possesses inhibitory function on T cells via interaction with the B7 ligand. Interestingly, the frequency of the same allele (the second most frequent allele of the CTLA-4 microsatellite locus in Caucasian populations) was significantly increased in Caucasian patients with Graves' disease in a previous study. Given these data, we analysed a point mutation in exon 1 of CTLA-4 (an A-G transition at position 49, encoding an Ala/Thr substitution in the leader peptide). The A allele was in strong linkage disequilibrium with the 104 bp microsatellite allele in the Italian families (χ 2 = 58; 2 degrees of freedom) and it was preferentially transmitted to diabetic offspring; transmission disequilibrium data was highly significant (p=0.0001). We replicated these observations in a data set of Spanish IDDM families (n=47) with still high significant p value (p<0.004) (in collaboration with MT Martinez Larrad and M Serrano-Rios).
In conclusion, we have demonstrated a linkage and association of CTLA-4 gene with IDDM. Not since the discovery of the association of HLA and autoimmune diseases in 1974 has a locus been associated and linked to more than one autoimmune disease (IDDM and Graves' disease).

Human islet transplantation.

Camillo Ricordi, M. D. Cell Transplant Center, Diabetes Research Institute, University of Miami, Miami, Florida, USA.

Few areas of biomedical research have raised such hope and, over time, led to such frustration as islet cell transplantation. Nevertheless, even the most skeptical observers will recognize that substantial and definitive progress has occurred in the field. These advances continue to fuel enthusiasm amongst scientists that a cure for diabetes by cellular replacement therapy isachievable. a goal worthy of the intense pursuit.
Since 1990, the availability of techniques and reagents for high yield recovery of islets from human cadaveric pancreata has resulted in a renewed interest in testing the clinical applicability of the approach, with several centers resuming clinical trials of islet cell transplantation in patients with insulin-requiring diabetes mellitus. Some patients became completely free from exogenous insulin therapy, and several patients have sustained islet function for years. The majority of recipients of islet allografts, however, either did not become insulin independent or eventually had to return to insulin therapy. Causes of islet graft failure have included rejection, insufficient beta cell mass and/or problems related to islet engraftment (i.e., cytokines and radicals released at the transplant site before the islets are revascularized during the peri-transplant period). Despite the requirement for some exogenous insulin, glycemic control improved in recipients of functioning islet grafts, compared to the degree of metabolic control preceding the islet allografts. Long-term metabolic control was in fact similar or even better than that observed in patients undergoing intensive insulin therapy as reported by the DCCT. Reports from several centers on patients with long term partial islet graft function clearly indicated that the levels of glycosylated hemoglobin could be maintained in the normal or near normal range for several years. This was a critical finding, since it was unknown whether intrahepatic islet allografts could function long term in an ectopic site. Moreover, the metabolic control achieved in some islet transplant recipients was not associated with an increased risk of severe hypoglycemic episodes, although this is still a relative advantage that needs to be weighed by the fact that intensive insulin treatments do not require immunosuppression.
It is indeed the continuous requirement for immunosuppressive drugs that still limits the applicability of islet transplantation to diabetic patients undergoing organ allotransplantation. Besides the need for clinical strategies to eliminate the requirement for continuous immunosuppression, critical issues remain and need to be resolved before the procedure can be proposed for wide clinical application as a treatment for patients with insulin requiring diabetes mellitus. These include the need for improved reagents and techniques to reduce islet cell loss following purification and the problem of primary non-function, that results from the non-specific inflammatory response to allogeneic islets at the transplant site.

Effects of prolonged glucose stimulation on beta cells: from supersensitivity to desensitization.

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The prolonged exposure of pancreatic islets and isolated beta cells to elevated glucose concentrations induces a state of unresponsiveness to glucose (desensitization). However, an increased sensitivity to glucose (detected by a shift to the left of the dose-response curve of glucose-induced insulin release) has been also reported after the chronic exposure to glucose, making the overall response less comprehensible. In vitro models have many theoretical and practical advantages to better understand the effects of the prolonged glucose stimulation; moreover, they are also suitable for studying the mechanisms responsible of the observed alterations. We have performed a time-course of the effect of the exposure to high glucose on the secretory behaviour of beta cells. Rat pancreatic islets exposed for 30 min at high glucose showed an increased basal insulin secretion (79±27 vs. 28±5 pg/islet/30', n=5, p<0.05) as the only difference in respect to control islets (exposed to 5.5 mM). After 3 h exposure to high glucose, also an increased sensitivity to glucose was observed, as indicated by a shift to the left of the glucose dose-response curve (EC_{50} 6.0±0.9 and 11.5±1.2, respectively; n=5, p<0.05). After 6 h exposure to high glucose, besides the two alterations already described, also a decrease in glucose-induced insulin release was observed (426±52 vs. 988±65 pg/islet/30', n=5 p<0.01). We studied the mechanism responsible for these alterations and we found that the "supersensitivity" to glucose may be related to alterations of the "glucose sensing" mechanism of beta cells, in particular of glucose phosphorylation. In contrast, in islets desensitized to glucose our data suggest that ion flux and consequent membrane potential changes play a role in determining the secretory defect. Since a normal response to glyburide was observed, a proximal signal defect for closure of K-channels rather than an intrinsic defect in the channel is more likely. In conclusion, the prolonged stimulation of beta cells with high glucose induces a series of distinct secretory abnormalities, with a pattern of response that lead first to an increased sensitivity and then to a decreased responsiveness to glucose.

MECHANISMS OF INSULIN ACTION AND INACTION

Jesse Roth, M.D., Raymond and Anna Lublin Professor of Medicine, and Director, Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A.

Over the last fifty years there has been an enormous interest in understanding how insulin carries out its many biological functions. Progress has been especially rapid in the last several years. One of the most important foci of this research is to try to understand why patients with Type II diabetes have a diminution in their sensitivity to insulin and how these defects can be prevented or overcome.

Insulin itself, while stimulating biological actions, also brings with it inhibitory responses of multiple types. Indeed, insulin is one of the "insulin antagonists" for which investigators searched in earlier decades. Our studies with transgenic mice (that have multiple copies of the human insulin gene) that hypersecrete insulin show that hyperinsulinemia alone as the primary defect is capable of generating insulin resistance and glucose intolerance. These data are consistent with studies of Jeanrenaud et al. which have shown that hyperinsulinemia may be the initiating pathophysiological event with lesions of the brain that produce insulin resistance and glucose intolerance.

The insulin receptor, with its kinase that activates the receptor, has been well studied. Recently attention has been turned to the receptor phosphatase which inactivates the receptor. Studies from our group (Bernier, Kole, and Liotta) as well as the group of Goldstein et al. have shown that the phosphatase can be regulated experimentally in such a way as to maintain the activated form of the receptor longer, thereby enhancing insulin action. Other studies of regulators of target cell sensitivity to insulin have revealed that TNF-alpha, produced by fat cells, especially in response to insulin, may be a major agent for insulin resistance in both fat and muscle cells through its actions on IRS-1 (Speigelman et al.). On the other hand, studies from our group (Montrose, Egan, and Wang) have shown that GLP-1, known primarily for its ability to enhance glucose-induced insulin secretion, may also be active in promoting insulin actions in the periphery, both in concert with insulin and as an independent agent.

By delineating the signalling molecules and their receptors that regulate cell sensitivity to insulin, new therapeutic agents and regimens will emerge that will be broadly useful in patients with NIDDM.

Abstract. Cellular Mechanisms of Insulin Action.

Steven Shoelson, MD, PhD

In insulin-responsive tissues like muscle and fat, activated insulin receptors directly phosphorylate substrate proteins like IRS-1, IRS-2 and Shc. The sites of phosphorylation on the substrate proteins serve to dock various SH2 (Src homology 2) domain proteins, including phosphatidyinositol (PI) 3-kinase, the phosphatase SHPTP2, and a linker protein Grb2 that helps regulate the activation state of Ras. Stimulation of Shc and IRS protein pathways by the insulin receptor leads to gene expression and cellular proliferation, and IRS proteins may regulate glucose transport, as well. Each of the insulin receptor substrates contains a PTB (phosphotyrosine binding) domain, while downstream enzymes either contain SH2 domains or associate with SH2 proteins. Our laboratory has studied how these modular proteins function in insulin action. As one strategy, the modular proteins were divided into isolated domains, and these have been characterized in detail. For example, we have determined binding characteristics of all SH2 and PTB domains of IRS-1, Shc, PI 3-kinase, SHPTP2 and Grb2 to understand how they interact with one another. In addition, we have determined crystal structures of many of the corresponding complexes, including those for IRS-1 with the insulin receptor and IRS-1 with PI 3-kinase. Once biochemical and physiological functions and high resolution three-dimensional structures have been determined, the isolated modules can be reassembled to learn how the modules influence one another and, ultimately how the intact proteins and complexes work. As an example of this approach, both isolated SH2 domains of the tandem SH2 phosphatase, SHPTP2, were analyzed. Subsequently, it was found that the domains work together to regulate catalysis, so a crystal structure of the tandem domains was solved. This showed that two domains can function together in phosphoprotein recognition. The cellular signaling pathways of insulin will be discussed along with selected detailed examples of how the molecules work in propagating signals into the cell.

Beta cell adaptation to the diabetic state.

Gordon C. Weir M.D. Joslin Diabetes Center, Harvard Medical School, Boston MA, USA

Beta cell function is profoundly altered in all hyperglycemic states. Potential contributors to the secretory abnormalities have included reduced GLUT2, glycogen accumulation, glucose recycling, changes in glucokinase, reduction of mitochondrial glycerol phosphate dehydrogenase (mGPDH), and reduction of the ATP-sensitive potassium channel activity, but none of these alone seem sufficient to account for the insulin secretory dysfunction found in diabetes.

We have recently found comparable reductions in GLUT2 and insulin mRNA in the islets of the rats after partial pancreatectomy (Px). In addition, we found a striking reduction in protein levels of the transcription factor idx-1. Idx-1 (also called ipf-1, pdx-1, luf-1 and stf-1) may be particularly important for pancreas development, presumably by influencing the expression of multiple genes. We have developed a hypothesis about pdx-1 being of major importance in maintaining the unique differentiation of beta cells. The machinery that allows optimal insulin secretion includes the enhanced expression of genes responsible for insulin, GLUT2, glucokinase, mitochondrial glycerol phosphate dehydrogenase, pyruvate carboxylase and, no doubt, a variety of others. On the other hand, the expression of other genes must be suppressed, some of these presumably being glucose-6-phosphatase, lactate dehydrogenase, and PEP carboxykinase. We suspect that beta cells exposed to the diabetic milieu lose their unique differentiation and become more like other cell types. Such changes in the machinery, upon which glucose induced insulin secretion depends, would have an especially crippling effect upon the glucose component of insulin secretion.

Free Communications and Posters

COMPARATIVE DERMATOGLYPHICAL ANALYSIS OF TYPE 1 AND TYPE 2 DIABETES

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The groups of Azerbaijans with clinically diagnosed diabetes mellitus were examined using a comprehensive dermatoglyphic program. Examined were men of 14-45 age suffering from Type 1 (n=20) and Type 2 (n= 13) diabetes mellitus.
As a control, a group of healthy men (n= 84) of the same nationality were examined too. To identify the dermatoglyphics the method by Cummins and Midlo (1967) was used. The comparative analysis of dermatoglyphics patterns has demonstrated that, in some cases, there are statistically significant differences between the groups of diabetics and the control one. The data optained testify to prospects of dermatoglyphic analysis in working out the genetical aspects of diabetes mellitus.

FLORID DIABETIC RETINOPATHY: A LONG-TERM FOLLOW-UP STUDY

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Sometimes proliferative retinopathy in young diabetics shows peculiar clinical features and evolution. These forms have a rapid course which leads to blindness in a short time and were for this reason defined as "florid" proliferative retinopathy. We present our experience on 118 eyes of 66 patients followed for a mean time of 46.4 months ± 36.3.
The clinical characteristics of the patients were: 46 females (69.7%); mean age 26.7 yrs ± 5.0; mean duration of diabetes 16.2 yrs ± 5.0; coexisting HBP in 17 patients (25.7%). All of them were insulin-dependent (100%). Mean initial visual acuity was 0.64 ± 0.27. In all the eyes a confluent panretinal photocoagulation extended to all areas of retinal ischemia was performed. At the end of follow-up visual acuity was 0.47 ± 0.33. Eyes with a final visual acuity lower than 0.1 were 25 (21.2%). The causes of blindness, in the majority of cases (90.4%), were retinal detachment and vitreous hemorrhages. Twenty-eight eyes (23.7%) were submitted to pars plana vitrectomy; the mean post-surgical visual acuity was 0.18 ± 0.24. Considering the total number of eyes, diabetic retinopathy improved in 81 out of 118 eyes (68.6%); on the contrary in 31.4% of the eyes retinopathy appeared worsened. Neovascular glaucoma occurred in 7 eyes (5.9% of cases).
Although florid retinopathy remains a major cause of blindness in diabetics, early laser treatment may reduce this risk.

GLYCATED AND OXIDIZED LDL IN NIDD DIABETIC PATIENTS: CORRELATION WITH STANDARD PARAMETERS

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We have evaluated LDL glycation in 45 patients aged between 18 and 86 years and in 17 normal subjects of comparable age, through a monoclonal antibody directed against glycated Apo B in a competitive ELISA test; as sustained by some authors, due to the higher susceptibility of LDL glycated during oxidation, we have compared the values detected with oxidized anti-lipoprotein antibodies (ELISA test, using copper oxide-treated LDL as antigen) and with standard parameters such as glycemia and glycated haemoglobin (determined with glucose-oxidase and HPLC method, respectively).
Results are reported in Table 1

	Glycemia (mg%)	Glycated Hb (%)	Glycated LDL (mg% apoB gl)	AbOxLDL (mU/ml)
NIDD	144.18+59.75	7.88+2.48	0.10+0.15	158.43+154.64
Normal	94.24+7.58	5.65+0.0046	0.04+0.03	105.82+40.29

High levels of glycated LDL (expressed as glycated Apo B) have been observed in sera of patients with non-insulin dependent diabetes (NIDD) which do not correlate with glycated Hb levels (r=0.144), while they correlate with oxidized anti-LDL antibodies (r=0.716).
Therefore, glycated lipoproteins are likely to be one of the sources of in vivo formation of oxidized LDL in diabetic patients.

DIABETIC PAPILLOPATHY: A 4-YEAR FOLLOW-UP STUDY OF 54 EYES

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Diabetic papillopathy (DP) is characterized by a temporary edema of the optic disc, with minimal visual impairment. Our caselist consists of 69 eyes (50 pts), evaluated in our Department during the last 13 yrs. The clinical characteristics of the patients were: 34 females (68%); mean age 49.5 yrs ± 16.8; mean duration of diabetes 13.2 yrs ± 7.8; 58% of the patients were insulin-dependent; HBP coexisted in 29 pts (58%). The lesion was bilateral in 19 pts (38%); the optic disc involvement was sectorial in 41 eyes (59.4%) and total in the remaining 40.6% of cases. In 10 out of 46 eyes (21.7%) in which a visual field examination was performed, only a slight enlargement of the blind spot was observed. With regard to the coexisting diabetic retinopathy (DR), it was absent in 4.3% of cases, non-proliferative in 55% of cases, pre-proliferative in 34.8%, proliferative in 5.9%.
We followed 38 out of the 50 pts (54 eyes) for an average period of 52.7 mos. ± 34.1. The initial visual acuity was 0.67 ± 0.30 and 0.58 ± 0.32 at the end of follow-up. The average time between diagnosis and regression of DP was 6.2 mos. ± 3.4. In 16/54 eyes (29.6%) DP reappeared during the follow-up: in 18.5% in the same sector and in 11.1% in different sectors of the optic disc. With regard to the DR evolution, 46.1% of the eyes showed a progression of retinal lesions during the follow-up and in 10.7% of cases a proliferative stage occurred.
In conclusion in our caselist DP turned out to be associated with pre-proliferative DR in a high percentage of cases. In order to explain this association, it's possible to speculate that DP is a consequence of lesions localized at the level of the third retinal capillary layer; the characteristics of this network (i.e., long and tortuous vessels, few anastomoses, high resistance) could justify a specific involvement in cases of impaired blood circulation as typically present in the pre-proliferative stage of retinopathy.

POSSIBLE INVOLVEMENT OF TUMOR NECROSIS FACTOR (TNF) IN SERUM LIPID ALTERATIONS IN IDDM PATIENTS. B.Batetta, P.P. Contini*, M.Manai*, F.Sanna, P.Pani, S.Dessì.

Alterations of lipid metabolism are frequently observed in patients with IDDM. These alterations include high triglyceride and low HDL cholesterol (HDL-C) levels in the plasma. It has been suggested that hypertriglyceridemia could be related to a decrease of lipoprotein lipase (LPL) activity and or to an increase of VLDL production as a consequence of insulin deficiency. Since precursor particles of HDL are thought to derive from lipolysis of triglyceride rich lipoproteins, the possibility that low HDL-C observed in IDDM patients may be secondary to the decreased triglyceride clearance from plasma, must be considered. To better understand the physiologic basis for the altered lipid metabolism in IDDM patients, in the present study, the levels of triglycerides and HDL-C in the plasma were correlated with the expression, in monuclear cells, of some genes known to be involved in the regulation of lipid metabolism such as HMGCoA reductase and LDL receptors. In addition the expression of TNF was also investigated since this cytokine has been frequently reported as being responsible for changes in lipid metabolism which occur in association with other wasting diseases such as infections and cancer. The study was conducted on six IDDM patients, at diagnosis, two days after insulin treatment and after reaching the glycaemic control as evidenced by hemoglobin A1c measurement. At the time of diagnosis hypertriglyceridemia and low HDL-C levels were associated with low expression of HMGCoA reductase and LDL receptors and high expression of TNF as compared to controls. Two days after therapy the triglyceride levels strongly decreased in association with reduction of TNF gene expression and the increase of HMGCoA reductase and LDL gene expression while the HDL-C levels were still low. Recovery of the normal pattern of HDL-C was seen when glycaemic control was obtained. Overall, these results suggest that the decreased LPL activity observed in IDDM patients may be mediated in part by TNF other than by insulin deficiency. In addition they suggest that hypertriglyceridemia and low HDL-C levels in IDDM patients are regulated, at least in part, by independent mechanisms.

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Proinsulin-like molecules and insulin release in human islets: effect of chronic exposure to high glucose concentrations.
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Besides being a consequence of inadequate insulin release or insulin action, hyperglycemia might itself impair islet insulin response to glucose. Mimicking the effects of a poor metabolic control on transplanted islets, we studied in an in vitro system the effect of hyperglycemia on islet function, by evaluating the release of insulin and proinsulin-like molecules of human islets after 48 hours of culture in high glucose concentrations.
Islets were isolated from human pancreata; after a 12-h overnight culture at 37° C in CMRL 1066 (10% FCS, 1% L-glutamine, 1% antibiotics), islets were divided in two groups and cultured for 48-h in standard CMRL 1066 (control islets), and CMRL 1066 plus glucose 16.7 mM (test islets). After the 48 h experimental culture, islets were perifused and acutely stimulated for 20 minutes with glucose 16.7 mM, followed by glucose 3.3 mM for 20 min. As expected, islets cultured in high glucose released more insulin under basal conditions than control islets; in addition these islets lost the ability to release insulin in response to an acute glucose stimulation, and showed a paradoxical response to low glucose levels. Moreover, the proinsulin-like materials / insulin ratio was higher in islets cultured in high glucose than in control islets, after high glucose (0.23$\pm$ .04 in control islets vs 0.47 $\pm$ 0.10 in islets cultured in high glucose; n=12; $p<0.05$) and low glucose (0.24$\pm$ .04 in control islets vs 0.92 $\pm$ 0.24 in islets cultured in high glucose; n=8; $p<0.05$).
The evidence that culture conditions can affect the release of proinsulin-like molecules of isolated human islets suggests that a chronic glucose stimulation can imbalance the rates of synthesis, intracellular degradation, and release of insulin from ß cells, so that insulin is released before its maturation is complete.

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Hypoglycemia after pancreas and islet transplantation. A. Battezzati, D. Bonfatti, G. Calori, A. Secchi, R. Caldara, L. Luzi, G. Pozza. Department of Medicine, Istituto Scientifico San Raffaele, Milan, Italy.
Hypoglycemia (hypo) is a major iatrogenic complication in IDDM. Can pancreas or islet transplantation (PTx,ITx) cure IDDM avoiding hypo? The prevalence of hypo after Ptx or ITx is unknown, although persisting counterregulatory abnormalities and occurrence of hypo were reported after Ptx. Aim of this study was to assess the prevalence of hypo in functioning PTx and ITx (function defined by ≥ 3 of: HbA1c<6.5%, fasting plasma glucose(PG) <7.8mM, >4wks insulin independence and fasting c-peptide>2ng/ml). 43 diabetic-uremic subjects were studied at 2mos and yearly up to 3yr after kidney and PTx. Patients received a isocaloric diet fractionated in 3 meals and their PG sampled at 2h intervals for 1 day. Prevalence of hypo:

PG	2 mos (n=37)	1 yr (n=38)	2 yr (n=26)	3 yr (n=19)
<3.6 mM	4	3	4	3
<3.0 mM	1	1	1	1

No subjects reported symptoms of hypo. The lowest PG was 2.6 mM. 6 (32%) of 19 PTx functioning at 3yr had at least 1 PG <3.6mM in one study, and 3 (16%) had at least 1 PG <3.0mM. 7 (50%) of all PG <3.6mM recorded occurred > 4h from last meal, including 3 of the 4 PG <3.0mM. 3 subjects had hypo after 10h overnight fasting, 2 reaching PG <3.3mM. Prevalence of hypo was not different between sexes. 4 ITx recipients were studied with the same protocol, 2 of them complying with inclusion criteria at 6 mos, 1 at 1yr and 1 at 2yr follow-up. The lowest PG was 3.6mM. SUMMARY: 10-15% of PTx have hypo during their yearly 24h PG profile. One third of PTx have at least 1 hypo over 3yr follow-up. Hypo is spread through the day, the lowest values being post-absorptive. No hypo occurred in functioning ITx studied up to now, although the sample size is too small to draw significant conclusions. CONCLUSION: functioning PTx can cure diabetes at the expense of mild hypo. Since our results reflect a 1day/year sample, prevalence of hypo after PTx may be greater than estimated.

PREVALENCE OF SEVERE HYPOGLYCAEMIA IN A COHORT OF DIABETIC CHILDREN. E. Bognetti, A. Brunelli, F. Meschi, R. Bonfanti, M. Viscardi, A. Zoja, A. Cogliardi, G. Chiumello. *Department of Pediatrics, Scientific Institute H San Raffaele, University of Milan, Italy.*
Severe hypoglycaemia is one of the worst experience for IDDM patients. We have retrospectively evaluated the prevalence of severe hypoglycaemia (hypoglycaemia with seizure or impairment of consciousness) during the years 1992-94. Data have been obtained by interview from 187 (105m/82f) patients randomly selected in our out-patient clinic. Mean age at interview was 14.2±5.3 years and duration of diabetes 6.4±4.2 years. Prevalence of hypoglycaemia as episodes/100 patients/year was: during 1992: 12%; during 1993: 11% and during 1994: 20%. Frequency of hypoglycaemia was: 39% during the night; 51% during the morning until lunch; 10% after lunch until midnight. Hypoglycaemia was explained in the 27% of episodes by errors in diet; in 11% by insulin mistakes but in about 42% of episodes was impossible to identify a cause. Hypoglycaemia was treated by oral glucose or glucagon respectively by 73% and 15%. Hospital admission was necessary in 11% of episodes. Transient palsy was reported in 1 patients. Mean glycaemia before treatment was 29±11.5 mg% but it was performed before treatment only in 23 patients. Insulin requirement and metabolic control before and after hypo were not different (insulin: 0.87±0.2 Vs 0.88±0.2 U/Kg/day; delta HbA1c: 32.8±25.7 Vs 37.4±26.1%). Relative body weight (RBW) was modestly higher after hypoglycaemia (105.8±14.7 Vs 108.8±15.8, $p<0.01$). We conclude that severe hypoglycaemia is a frequent acute complication of insulin therapy in children and adolescent. Hypoglycaemia is particularly frequent during night-time and errors in diet explain many episodes.

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ROLES OF TRANSMEMBRANE GLUCOSE TRANSPORT AND INTRACELLULAR GLUCOSE PHOSPHORYLATION IN MUSCLE INSULIN RESISTANCE OF NON-INSULIN-DEPENDENT DIABETES MELLITUS. R.C. Bonadonna, S. Del Prato, E. Bonora, E. Ferrannini, M.P. Saccomani, C. Cobelli, R.A. DeFronzo.

The quantitative role of the cellular effectors of glucose metabolism in determining muscle insulin resistance in non-insulin dependent diabetes mellitus (NIDDM) is still imperfectly known. We assessed transmembrane glucose transport and intracellular glucose phosphorylation *in vivo* in skeletal muscle in non-obese NIDDM patients. We performed euglycemic insulin clamp studies in combination with the forearm balance technique (brachial artery and deep forearm vein catheterization) in 5 non-obese NIDDM patients and 7 age- and weight-matched controls (study 1). D-mannitol (a non-transportable molecule), 3-O-[^{14}C]-methyl-D-glucose (transportable, but not metabolizable) and [3-^{3}H-]-D-glucose (transportable and metabolizable) were simultaneously injected into the brachial artery, and the washout curves were measured in the deep venous effluent blood. In vivo rates of transmembrane transport and intracellular phosphorylation of D-glucose in forearm muscle were determined by analyzing the washout curves with the aid of a multi-compartmental model of glucose kinetics in forearm tissues. At similar steady state concentrations of plasma insulin (≈500 pmol/l) and glucose (≈5.0 mmol/l), the rates of transmembrane influx (34.3±9.1 vs 58.5±6.5 μmol·min^{-1}·kg^{-1}, p<0.05) and intracellular phosphorylation (5.4±1.6 vs 38.8±5.1 μmol·min^{-1}·kg^{-1}, p<0.01) in skeletal muscle were lower in the NIDDM patients than in the controls. In the NIDDM patients (study 2) the insulin clamp was repeated at hyperglycemia (≈13 mmol/l) trying to match the rates of transmembrane glucose influx measured during the clamp in the controls. The rate of transmembrane glucose influx (62±15 μmol·min^{-1}·kg^{-1}) in the NIDDM patients was similar to the controls, but the rate of intracellular glucose phosphorylation (16.6±7.5 μmol·min^{-1}·kg^{-1}), although 3-fold higher than in the patients during study 1 (p<0.05), was still ~60% lower than in the controls (p<0.05). We conclude that, when assessed in vivo, both transmembrane transport and intracellular phosphorylation of glucose are refractory to insulin action and add to each other in determining insulin resistance in skeletal muscle of NIDDM patients.

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INTRACELLULAR PARTITIONING OF GLUCOSE DISPOSAL IN DIABETIC SUBJECTS WITH OR WITHOUT HYPERTENSION. E. Bonora, M. Alberiche, G. Targher, F. Saggiani, M. Zenere, R.C. Bonadonna and M. Muggeo.

In this study we quantitated rates of glucose disposal throughout the different intracellular biochemical pathways (glycogen synthesis, total glycolysis, non-oxidative glycolysis, glucose oxidation), as well as hepatic glucose production, Cori cycle and lipid oxidation by using a 4-h euglycemic (~5 mM) insulin clamp (~300 pM) in combination with dual tracer infusion ([^{3}H]-3-glucose and [^{14}C]-U-glucose) and indirect calorimetry in 20 normotensive diabetic (D) and 20 sex- age- and BMI-matched hypertensive diabetic (HD) nonobese (BMI<30) patients. No differences were found between the two groups in the basal rates of glucose metabolism, while significantly lower rates (μmol/min·kg FFM) of total glucose disposal (20.85 ± 2.33 vs 28.54 ± 3.01, p<0.05) and glycogen synthesis (3.69 ± 1.50 vs 12.43 ± 3.29, p<0.025) were found in HD vs D subjects during insulin infusion. In this condition, total (17.13 ± 3.13 vs 16.11 ± 1.61) and non-oxidative glycolysis (7.50 ± 2.70 vs 3.86 ± 1.46) were not significantly different in HD and D, while glucose oxidation was substantially lower in HD vs D (9.60 ± 0.66 vs 12.22 ± 1.26, p<0.07). Hepatic glucose production (5.02 ± 0.88 vs 4.15 ± 1.43) and Cori cycle (1.09 ± 0.73 vs 0.37 ± 0.19) were not different in the two groups whereas lipid oxidation was significantly higher in HD than in D subjects (1.34 ± 0.13 vs 0.91 ± 0.15, p<0.05). These results suggest that in nonobese diabetic subjects the presence of hypertension is associated with a more severe degree of insulin resistance and that this defect is mainly due to a more pronounced defect in glycogen synthesis.

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PREVALENCE OF CELIAC DISEASE IN A COHORT OF CHILDREN AT ONSET OF INSULIN DEPENDENT DIABETES. R.Bonfanti, E.Bazzigaluppi*, M.Viscardi, E.Bognetti, F.Meschi, C.Riva, P.Macellaro, G.Barera, C.Bianchi, G.Chiumello.
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The prevalence of celiac disease in Insulin Dependent Diabetes Mellitus (IDDM) patients has been reported between 1 to 4 %. Antiendomysial antibodies (EMA) detected by indirect immunofluorescence on section of monkey's distal oesophagus, are a sensitive and specific marker of celiac disease. The aim of the study was to screen a cohort of newly diagnosed IDDM patients for celiac disease with EMA.

172 patients, referred to our Department for IDDM onset, have been consecutively studied since 1/93 to 12/95 (71 F, 101 M, mean age 8.7 ± 4.7 yrs). All of them were tested for EMA and total IgA (to exclude selective IgA deficit); patients with positive EMA were indicated for the intestinal biopsy. We also evaluated: metabolic data (HbA1c, Fructosamine, venous blood gas analysis), and auxologic parameters (Height standard deviation score, relative body weight).

All patients studied had normal IgA values; 9/161 (5.6%, 3F/6M) had positive EMA antibodies. The jejunal biopsy was performed in 5 of them (4 EMA positive patients refused) and a subtotal villar atrophy was found in 4 patients (confirmed celiac disease prevalence =2.3%). All patients with celiac disease at biopsy were HLA DR3/ DQ2. There were no significative differences for all the parameters studied at onset of IDDM between EMA positive and negative patients, but EMA positive patients were younger than EMA negatives : 4.86 ± 2.3 yrs vs 8.83 ± .7 yrs (p=0.01 Mann Whitney U test).

In conclusion these results show that the increased prevalence of celiac disease, according to antiendomisyal antibodies screening, is already present at clinical onset of IDDM. Clinical parameters, apart from age, are not different between patients with diabetes at onset and with or without markers for celiac disease. We can speculate that a common immune-mediated mechanism is involved in both diseases: a longitudinal study of EMA in this patients could add further information on this hypothesis.

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NA^{+}/Li^{+} COUNTERTRANSPORT (SLC) AND CARDIOVASCULAR RISK IN HYPERTENSIVE NON NEPHROPATIC NIDDM PATIENTS.
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High SLC have been proposed as a marker of hypertension (HT). Individuals with HT and metabolic abnormalities had significantly higher SLC than those without these alterations. Increased SLC has been found in insulin-dependent diabetes (IDDM) with diabetic nephropathy (DN) or poor cardiovascular risk profile [HT, low HDL, high triglycerides (TG)]. In non insulin-dependent diabetes (NIDDM), high SLC has been found when HT and/or DN are present. **Objective of study**: To assess the relationship between SLC, metabolic abnormalities and diabetic complications in NIDDM HT without DN. **Methods**: 150 NIDDM normoalbuminuric patients in therapy with antidiabetic drugs and sodium-free diet were visited. Patients with systolic blood pressure (SBP) ≥ 140 and/or diastolic (DBP) ≥ 90 (WHO criteria) were enrolled (n=43, 16M/27F, BMI 28.±3.8 Kg/m^{2}; duration 8.4±5.9 yrs; age 61.6 ±7.5 yrs (Mean ± SD). Blood glucose, HbAlc, C-Peptide, cholesterol (CHOL), HDL, TG, uric acid, creatinine, urinary albumin concentration (UAC), erithrocyte SLC (Canessa method), fundus examination for the presence of diabetic retinopathy (DR), US Doppler for carotide plaques (CP), electroneurography for somatic neuropathy (SN) and Ewing tests for the cardiovascular autonomic neuropathy (CAN) were evaluated. Patients were divided in 2 groups based on their SLC: Group I (G1), SLC <0.4, n= 24; Group 2 (G2), SLC ≥0.4 mmol/L cell x h, n =19. **Statistics**: Unpaired Student T, Chi^{2} and Pearson R; p level <0.05. **Results**: Groups were comparable regarding BMI, sex, age, duration, glucose, HbAlc, CHOL, uric acid, UAC, SBP and the prevalence of DR, CP and SN. Significative differences were found for HDL (G1: 1.28±0.31, G2: 1.05±0.24 mmol /L), TG (G1: 1.79 ±0.92, G2: 2.58±1.32 mmol/L), CHOL/HDL ratio (G1: 4.8± 1.5, G2: 5.9±2.1), C-Peptide (G1: 0.95±0.39, G2: 1.42±0.70 nmol/L), DBP (Gl: 91.6±11.3, G2: 97.8±6.7 mmHg) and CAN score (G1: 0.6±0.9, G2: 2.1±1.2). In all patients SLC was correlated with: C-Peptide (R=0.38), TG (R=0.29), CAN (R=0.45) and HDL (R=0.37). **Conclusions**: Our data confirm that, as reported in general population and IDDM, higher SLC levels are found in NIDDM HT normoalbuminuric patients with associated metabolic abnormalities. Moreover, irrespective of other diabetic complications, Group 2, with higher DBP levels and a cardiac autonomic involvement, has a poor cardiovascular risk profile.

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A NEW PROTOTYPE OF COHERENT MICROCAPSULES (CM) FOR PANCREATIC ISLET GRAFT (TX) IMMUNOISOLATION R. Calafiore, G. Basta, G. Luca, C. Tortoioli, G. Selvaggi*, C. Ricordi*, P. Brunetti

To minimize the size of standard alginate/polyaminoacidic (AG/PA) immunoprotective microcapsules, measuring 700-800μm in equatorial diameter, we developed CM, which based on a new two-phase, aqueous multi-reagent emulsification process, would allow formation of a highly biocompatible, permselective, and ultra-thin hydrogel cast, tightly enveloping each individual islet, so as to eliminate any idle, dead space. CM morphologic integrity was documented by either light (staining with Hematoxilin-Eosin) or scanning/transmission electron, or confocal laser, microscopy examination. In vitro CM immunobarrier competence was demonstrated, *in vitro*, by both membrane's impermeability to Ig, and splenocyte/lymphocyte's proliferation inhibition in allo-/xenogeneic mixed islet/splenocyte or lymphocyte co-cultures. Rat, canine or human islets in CM showed physiologic in vitro glucose-stimulated insulin release (ANOVA, $p<0.001$ vs. baseline). Moreover, CM did not provoke any inflammatory cell reaction, after 30 days of TX beneath the renal capsule of normal CD-1 mice. In either CD-1 mice or Lewis rats with streptozotocin-induced diabetes, intraperitoneal (the former) and/or renal subcapsular TX of xeno- or allogeneic Wistar/Furth rat islet containing CM, respectively, resulted in full remission of hyperglycemia in all recipients which was sustained for a maximum of 140 days of graft. Intrahepatic TX of canine islet containing CM in 6 dogs with total pancreatectomy-induced diabetes, two of these animals being treated with Cyclosporin, hesitated in induction of hyperglycemia remission, in all but one recipients, which, however, lasted to a maximum of 8-10 days. Rapid graft failure was associated with liver histologic findings of dense inflammatory cell infiltration, whose immunocytochemical characterization is in actual progress, surrounding the islet containing, but not empty, CM. In summary, CM seem to retain standard microcapsule's functional properties, both *in vitro* and in diabetic rodents, while occupying neglectible volume. Relative to the preliminary dog study, whether implant site-related effects may adversely affect TX survival, and alternative sites should be instead considered, will require further investigation.

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KIDNEY AND KIDNEY-PANCREAS TRANSPLANTATION FOR UREMIC IDDM PATIENTS:A TWO YEARS FOLLOW-UP

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Aim of our study was to evaluate the two years follow-up period of three groups of uremic-diabetic patients who received a single kidney or a simultaneous kidney and pancreas transplantation: group SPK(25 pts-26 segmental neoprene injected pancreas transplantation); group WPK (35 pts-whole pancreas transplantation with bladder diversion); group K(17 pts-19 single kidney transplantation). Immunosuppression was based on ALG or ATG globulins (Merieux), azathioprine and cyclosporine. 33 patients [12 (48%) in the SPK group, 15 (41.6%) in the group WPK and 6 (31.5%) in the K group] had postoperative surgical complications. 15 patients underwent reintervention [6 (24%) in the SPK group, 8 (22.8%) in the group WPK and 1 (5.2%) in the group K]. 54 patients experienced an acute kidney rejection episode [15 (60%) in the SPK group, 27 (77.1%) in the group WPK and 10 (52.6%) in the group K). Two years HbA1c (SPK 5.6±0.2, WPK 5.8±0.5, K 8.1±0.2) and plasma creatinine levels (SPK 1.3±1.1, WPK1.3±0.1, K 1.7±0.2) were higher in K patients than in simultaneous kidney and pancreas recipients ($p<0.01$ and $p<0.05$). Survival rate for patients was similar in the three groups (SPK 88.0%, WPK 88.4%, K 88.3%) and higher than patients who were in waiting list for transplantation in our centre (67%). Two years kidney survival rate was lower in K recipents than in SPK and WPK recipents (SPK 93.1%, WPK 88.4%, K 79.4%). In 7 patients [5 (20%) in the SPK group and 2 (5.7%) in the group WPK] vascular thrombosis led to pancreas failure. A pancreatic fistula was diagnosed in 7 patients [6 (24%) in the SPK group and 1 (2%) in the group WPK]. Haemorragic complications occured in 4 SPK patients (16%) and in 1 WPK patient (2%). A duodenum bladder anastomosis leakage was confirmed in 3 WPK patient (8.5%) and a urether bladder anastomosis leakage was diagnosed in 3 K subjetcs (15.7%).

FUNCTIONAL HUMAN ISLET GRAFTS IN THE SCID MOUSE: A NOVEL MODEL FOR THE STUDY OF HUMAN TYPE I DIABETES. F. Calcinaro, G. Gambelunghe, G. Murdolo, G. Pelosi, K.J. Lafferty and P. Brunetti.

Previous studies have provided evidence that IDDM recurrence in islet tissue transplanted to diabetic NOD mice is a CD4+ T-cell dependent process, non-restricted by the MHC antigens carried on the islet tissue. That is, IDDM in this animal model results from non-specific inflammatory islet tissue damage. If that is true also for the human disease it would be possible for unfractionated peripheral blood mononuclear cells (PBCs) from diabetic patients to damage non-syngeneic islets. This report proposes a novel animal system for the study of human islet-specific CMI. Two batches of 200-400 freshly isolated hand-picked human islets were transplanted to the kidney capsule of SCID mice. Graft function was determined by assaying human C-peptide production following i.p. glucose challenge (2 gr/Kg B.W.), 3-4 weeks after transplantation (Tx). Animals showing significant C-peptide production (>0.6 pmol/l) served as the recipients of unfractionated PBCs. 10^7 fresh PBCs from either new onset diabetic patients (n=12) or age-matched control subjects (n=5) were transplanted, in a blood cloth, adjacent to the islet grafts. Groups of 1-4 mice were transplanted with PBCs isolated from the same donor. Four weeks after Tx of PBCs, human C-peptide production was assayed again and the animals sacrified for histology. One half of the islet graft was fixed and stained (H&E, insulin and glucagon). The reminder was snap-frozen and stained for the presence of CD3+ human T-cells using a specific biotinilated monoclonal antibody and two secondary revealing antibodies. Preliminary experiments served to determine that purified human islets do not trigger an allograft reaction when co-transplanted with HLA-mismatched control PBCs. RESULTS. 13 of 23 SCID mice transplanted with PBCs from diabetic patients showed various degrees of islet graft infiltration and the presence of CD3+ human T-cells at the islet site. In the control group (n=12) only two mice, both transplanted with PBCs from the same donor, showed infiltration and the presence of human T-cells at the islet site (P=0.034; Fisher's exact test). The analysis of C-peptide production before and after PBC Tx provided evidence for islet damage in the test group (-Test group- Before PBCs: 1.9±0.16 pmol/l, mean±SEM; after PBCs: 1.04±0.6; P=0.0004. -Control group- Before PBCs: 1.66±0.2 pmol/l; after PBCs: 1.36±0.31; P=0.42; Mann-Whitney U test). CONCLUSIONS. This system provides a way to study human islet-specific CMI outside the diabetic patient. We propose it for: 1) determining cellular involvement in the pathogenetic process; 2) establishing human islet-specific T-cell clones and 3) defining pathogenicity of already established human islet-specific clones.

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REG GENE EXPRESSION IN A PSEUDOISLET REGENERATING MODEL
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In 1988 Okamoto and coworkers identified a new gene named Reg (i.e. RF-gencrating Gene). This gene was expressed in a model of surgical diabetes during the adaptive repair/regeneration process.

Many authors have investigated the role of the Reg Protein encoded by this gene in different sperimental model.

In this work Reg gene expression was studied in a pseudoislet model in which endocrine β-cells are induced to restore their functional structure by re-aggregation. Islets of Langerhans were obtained from adult Sprague Dawley rats using a modification of the method proposed by Sutton et al. islets were divided into two groups. Islets of the first group were dissociated after 24 h of culture using a chemical, mechanical and enzymatical method. The cells obtained were divided in 4 sub-groups and were cultivated for seven days in RPMI 1640 medium supplemented with FCS 2% and antibiotcs rispectively in presence of 2.8 mM glucose, 16.7 mM glucose, 10 mM Nicotinamide and 10% amino acid solution. During the culture period the cells spontaneously reaggragate in pseudoislets. Islets of the second group were not dissociated and cultured at the same conditions. Rna was extracted from each group of islets and was subjected to RT-PCR using 32P endlabelled primers for a specific region of Reg cDna. At the same time a second PCR was performed with endiabelled primers specific for preproinsulin as a marker for pancreatic endocrine cells. PCR products were analysed with 12 % polyacriamide gel electrophoresis and autoradiography.

In pseudoisiets preproinsulin gene expression showed the expected modulation in the different conditions; Reg gene expression showed a 218 % increase at 16.7 mM glucose, a 183% increase with Nicotinamide and a 114 % increase with amino acids when compared with 2.8 mM glucose. In islets preproinsulin gene was regularly expressed, while Reg gene was not expressed at all.

In conclusion, these data show that in the pseudoislets model, i.e. a model where endocrine, pancreatic cells undergo to repairing/regenerating processes, Reg gene is expressed and glucose and Nicotinamiide increase this expression, while Reg gene is not expressed in islets at the same conditions. These results suggest a possible organizing role for Reg protein in islet formation.

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Renal sub-capsular islet grafts fully correct the impaired skeletal muscle glucose transport system of streptozotocin diabetic rats.

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Chronic insulin therapy improves, but does not restore insulin-mediated muscle glucose uptake in human diabetes or muscle glucose uptake, transport, and transporter translocation in streptozotocin diabetic rats. To determine wether this inability is due to inadequate insulin replacement, we studied fasted streptozotocin-induced diabetic Lewis rats either untreated or after islet transplantation under the kidney capsule. Plasma glucose was increased in untreated diabetics and normalized by the islet transplantation (110±5, 452±9, and 102±3 mg/dl in controls, untreated diabetics and transplanted diabetics, respectively. Plasma membranes and intracellular microsomal membrane vesicles were prepared from hindlimb skeletal muscle of basal and maximally insulin stimulated rats. Islet transplantation normalized plasma membrane carrier mediated glucose transport V_{max}, plasma membrane glucose transporter content and insulin-induced transporter translocation. There were no difference in transporter intrinsic activity (V_{max}/R_o) among the three groups. Microsomal membrane GLUT4 content was reduced by 30% in untreated diabetics rats and normal in transplanted diabetics, while the insulin-induced changes in microsomal membrane GLUT4 content were quantitatively similar in the three groups. There were no differences in plasma membrane GLUT1 among the groups and between basal and insulin stimulated states. Microsomal membrane GLUT1 content was increased 60% in untreated diabetics and normalized by the transplantation.

In conclusion, our data show that islet transplantation under the kidney capsule completely restores the impaired skeletal muscle glucose transport system of streptozotocin diabetic rats, supporting the hypothesis that the route of insulin delivery is not a significant variable.

COGNITIVE FUNCTIONS IN PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS
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Objective of study .The goal of this study was to assess the cognitive functions of subjects with insulin-dependent diabetes mellitus (IDDM) that is not associated with manifestations of cerebral pathology.
Subjects . IDDM patients were selected based on the following inclusion criteria:- insulin therapy started before the age of 35; - age between 20 and 50; - schooling ≥5 years; absence of any concurrent pathologies that could justify cognitive deficits (e.g. thyroid dysfunction, uremic or hepatic encephalopathy, alcohol abuse); - EEG and cerebral CT within normal range.
Methods . Cognitive function was investigated using a battery of neuropsychological tests aimed towards examining intellectual functions, memory, attention, visuospatial analysis, language, praxia, perceptual recognition, "frontal" functions, visuomotor coordination (Raven's CPM 47, WAIS digit span, Corsi Cubes, prose memory, Rey's Figure A, WAIS digit symbol, Wisconsin card-sorting test, Buschke-Fuld verbal learning, Benton Judgment of Line Orientation, Grooved Pegboard test, Trail Making test A and B, Stroop test, phonological fluency, semantic fluency). Performance was compared with the normative data from control subjects equivalent in age and schooling. The clinical and laboratory parameters characterizing the patients with cognitive deficits were also analyzed, with particolar reference to the following: glycemia at the beginning and end of the neuropsychological examination; same-day HbA_{1c}; onset and duration of illness; therapy; history of severe hypoglycemia/coma; presence of hypertension; heart disease dyslipidemia, smoking; presence of peripheral polyneuropathy, dysautonomia, retinopathy, nephropathy.
Results. The preliminary results of the study have shown that, cognitive functions may be altered in the population examined. In fact, three subgroups were identified. Group A was characterized by difficulty in most neuropsychological tests, regardless of which cognitive area was being investigated, while group B revealed selective difficulty in specific cognitive areas, especially as far as memory is concerned. Lastly, the neuropsychological performance of group C was within normal range. Based on a preliminary analysis of this data, a relationship could be observed between high HbA_{1c} values, indicating irregular control of blood sugar, and cognitive abnormalities.

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EFFECT OF EXERCISE ON THE URINARY EXCRETION OF ALBUMIN AND ALPHA 1 MICROGLOBULIN IN PATIENTS WITH DIABETES MELLITUS AND INCIPIENT NEPHROPATHY P. Desenzani, P. Perini, A. Burattin, C. Mascadri, A. Giustina.
Nephropathy is the most frequent and serious microangiopathic complication in the diabetic patient. The early marker of renal damage is represented by microalbuminuria at rest (urinary albumin excretion, UAE between 20 and 200 μg/min). Physical exercise is thought to be able to unmask earlier stages of glomerular damage in patients without microalbuminuria at rest. We have studied 20 diabetic patients, 11 insulin-dependent and 9 non insulin-dependent, all normotensives: 13 were normoalbuminuric at rest, while 7 had baseline microalbuminuria. The aim of our study was to ascertain if the determination of a tubular protein (urinary alpha 1-microglobulin) (Immunonephelometric Method, BNA 100, Behring, Italy) after exercise (submaximal; 90% of theoretical heart rate) was able to give additional informations with respect to microalbuminuria in diabetics patients in the early stages of diabetic nephropathy. Our data show that: a) in normoalbuminuric diabetic patients at rest there is a significant ($p<0.05$) increase in urinary excretion of alpha 1-microglobulin after exercise; b) in the majority of the normoalbuminuric patients (67%), after exercise there is an increase in the urinary excretion of alpha 1-microglobulin but not of albumin; c) urinary alpha 1-microglobulin does not increase after exercise in diabetic patients with microalbuminuria at rest; d) there are no significant correlations between urinary alpha 1-microglobulin and urinary albumin excretion after exercise in both normoalbuminuric and microalbuminuric diabetic patients. We conclude that tubular proteinuria after exercise (detected by the determination of urinary alpha 1-microglobulin) may represent on useful tool in addition to microalbuminuria for the diagnosis and follow-up of early diabetic nephropathy.

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INFLUENCE OF ANTIDIABETIC THERAPY ON PHAGOCYTIC ACTIVITY OF HUMAN DIABETIC BLOOD LEUCOCYTES

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Diabetes mellitus (DM) is defined as chronical hyperglycaemia caused by absolute or/and relative insulin deficiency. Increased infection as one of the major problems of patients with DM. The aim of study was to ascertain whether different antidiabetic therapy can modulate the phagocytic activity of peripheral blood cells from patients with DM. 62 NIDDM patients were classified into three groups homogenous in age, sex and quality of their glycoregulation (FBG, MBG and HbA1): A (17) - treated with oral antidiabetics or were on dietary regimen; B (15) - received insulin and oral antidiabetics; C - (15) received insulin only, in two doses. Control group include 15 healthy volunters. Polymorphonuclear (PMN) and mononuclear (MO) were obteined from peripheral blood by centrifugation on density gradient. Percentage of phagocytes (PP), phagocytic index (PI) and absolute index of phagocytosis (API), as parameters of phagocytic activity, have been determined by method of ingestion yeast particles labeled by neutral red. Results showed no difference between groups in phagocytic activity of MO, but patients who recived insulin (Group B and C) showed positive correlation between dose of insuline (DI) and API of MO: $API=9.306+1.911*DI$. All diabetic patients show raise parameters of phagocytic activity of PMN in comparation with control group: PP: 73.6 vs 63.3, $p=0.001$; API: 283.4 vs 217.8, $p=0.001$. It was noticed statistcly significant difference between groups in respect of PP of PMN: Group A (71.35), B (72.5); Group C (77.39) vs Control (63.29), $p=0.008$, 0.004 and 0.009 respectivly; Group A vs B, $p=0.04$. AIP: Group A (281.86), B (284.95), C (276.92) vs Control (217.83) p= 0.01, 0.004 and 0.02 respectivly. In conclusion, diabetic patients who received insulin show enchance of PP and API of PMN; we have observed and characterized positive correlation between dosis of insulin and API of MO.

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NOD-MHC IS NECESSARY BUT NOT SUFFICIENT FOR THE OCCURENCE OF ANTI-GM2-1 AUTOANTIBODIES IN MICE: CONTRIBUTION OF *IDD9* ON CHROMOSOME 4

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The GM2-1 ganglioside is an islet specific molecule which has been shown to be target of autoantibodies at and before development of autoimmune diabetes both in man and the NOD mouse. In order to determine the immunogenetics of this autoantibody response in the NOD mouse, we have analyzed the occurence of anti-GM2-1 autoantibodies in NOD mice and in NOD-derived congenic lines carrying one or more diabetes-resistance genes from the C57/BL10 (B10) strain. Furthermore, B10 derived congenic lines carrying one or more diabetes-susceptibility genes (Idd) from the NOD mouse were also analyzed. These lines display various degrees of islet autoimmunity, ranging from absence of insulitis and diabetes to overt diabetes. Anti-GM2-1 autoantibodies were determined employing an indirect immunoperoxidase technique on TLC plates. The following mice were tested: NOD (n=47), NOD having B10-MHC (NOD $H2^b$, n=10), B10 having the NOD-MHC (B10 $H2^{g7}$, n=8), NOD having a diabetes resistance gene (*Idd9*) on a portion of chromosome 4 derived from B10 (NOD B10*Idd9*, n=21) and B10 $H2^{g7}$ having an additional diabetes-susceptibility gene at *Idd9* derived from NOD (B10 $H2^{g7}$ *Idd9*, n=6). Anti-GM2-1 autoantibodies were found in 44/47 NOD and only in 6/21 NOD B10*Idd9*. In contrast anti-GM2-1 antibodies were absent in sera of NOD $H2^b$ and of B10 $H2^{g7}$ mice. Furthermore 3/6 B10 $H2^{g7}$ Idd9 expressed these autoantibodies. These data indicate that expression of NOD-MHC is necessary but not sufficient for the production of a humoral autoimmune response to GM2-1. Finally, the data from the chromosome 4 congenic lines indicate that, together with NOD MHC, *Idd9* contributes to the presence of such autoantibody response.

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DIAGNOSTIC SENSITIVITY OF IMMUNODOMINANT EPITOPES OF GLUTAMIC ACID DECARBOXYLASE (GAD65) AUTOANTIBODIES IN CHILDHOOD IDDM

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The prevalence and titre of epitope-specific autoantibodies to glutamic acid decarboxylase (GAD65) in 155 IDDM and 9 GAD65Ab-positive healthy children were determined using 4 GAD65/67 chimaeric molecules which discriminate among the N-terminal (N), middle (M) and C-terminal (C) epitopes of GAD65. Radioligand binding assays for IgG antibodies (Ab) used immunoprecipitation of in vitro translated ^{35}S-GAD. We found autoantibodies to GAD65 in 116/155 (75%), to GAD67 in 19/155 (12%) ($p<0.0001$) and to the GAD65-N-67 chimaera in 25/155 (16%) ($p<0.0001$) IDDM sera. GAD67Ab were found almost exclusively (17/19,89%) in GAD65Ab-positive sera and the levels of GAD67Ab correlated with those of GAD65Ab ($r^2=0.5913$; $p=0.009$). GAD6.5Ab directed to GAD65-M were found in 104/155 (67%), to GAD65-C in 104/155 (67%) and to GAD65-M+C in 116/155 (75%) of IDDM sera, and indicated reactivity to two distinct epitopes. Additional antibody epitope(s) on GAD65 are cross-reacting with GAD67. Among the 9 GAD65Ab-positive healthy children, 2,/9 were also positive with GAD67, 9/9 (100%) with GAD65-M+C, 7/9 (78%,) with GAD65-M, 8/9 (89%) with GAD65-C and 2/9 (22%) with GAD65-N-67. Titres of GAD65-Ab ($P=0.007$), GAD65-C-Ab ($p=0.002$) and GAD65-C+M-Ab ($p=0.003$), but not of GAD65-M-Ab ($p=0.101$) were significantly higher in IDDM than in healthy children. We conclude that GAD65Ab in IDDM and healthy children are highly conserved and are directed to middle and C-terminal epitopes, and propose that levels of antibodies specifically directed to the carboxy-terminal end of GAD65 may distinguish IDDM from healthy children.

HISTOLOGICAL AND IMMUNOCHEMICAL STUDIES IN A CASE OF A NEONATE WITH CONGENITAL AUTOIMMUNE DIABETES DIED SOON AFTER DIAGNOSIS.

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Congenital diabetes is an extremely rare form of the disease and very little is known about the pathogenetic mechanisms involved in such a condition. In the present work we report a case of a neonate who developed insulin-dependent diabetes (IDDM) in the first day of life, followed by the development of diarrhea and of eczematous diffuse skin lesions. He died at 29 days of age because of necrotizing enterocholitis. He had no family history for type 1 or type 2 diabetes, while 6 other male neonates in his mother's family died within the first year of life (at least one with diarrhea and skin lesions). At day 4 of life he and his mother were studied for the presence of anti-islet (ICA), anti-insulin (IAA), anti-GAD65 and anti-ICA512 autoantibodies; circulating levels of C peptide and T lymphocyte subsets were also determined. We could also perform immunohistochemical and morphological studies on the pancreas and on several other organs including heart, kidney, liver, thyroid, thymus, spleen and brain obtained at autopsy in the neonate.
Plasma C peptide was undetectable in the neonate and was normal in the mother; T cell subsets were normal in both subjects,while the baby, but not the mother, was positive at high titer both for IAA and for anti-GAD65 autoantibodies indicating an autoimmune basis of the diabetic syndrome in this case. Both subjects were negative for ICA and anti-ICA512 autoantibodies. Histological studies showed the presence of a massive mononuclear cell infiltrate destroying the endocrine pancreas and leaving the exocrine portion of the gland intact. Such infiltrate contained CD4, CD8 and NK cells. By immunohistochemistry, glucagon-positive but not insulin-positive cells were found among infiltrating mononuclear cells, indicating the ß cell specificity of the destructive process within the pancreas. Interestingly, a similar infiltrate was found in the heart, while other organs examined were normal.
In conclusion, we have demonstrated that ß cell autoimmunity can be involved in the development of congenital IDDM and, therefore, that loss of immune tolerance to islet components can already start in intrauterine life. The presence of myocarditis together with insulitis may suggest that a viral infection may be the cause or at least the precipitating event leading to ß cell destruction in this case.

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GENE THERAPY FOR IDDM: DEVELOPMENT OF MATURE INSULIN-SECRETING CELL LINES BY RETROVIRAL VECTORS.

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Primary autologous cells, engineered for self-regulated insulin production, are the long-distant target of IDDM gene therapy. By retroviral vector technology, we transduced the human proinsulin cDNA into murine fibroblasts (3T3-HPI) and human hepatoma cells (HepG2-HPI), under the viral LTR transcriptional control, and obtained an *in vitro* constitutive production of proinsulin (detected by RIA, ng/24h/1x10^6 cells) of 0.5-1 and 2.5-4.9, respectively. When transplanted intraperitoneally into streptozotocin-induced diabetic athymic mice (BG>400 mg/dl), HepG2-HPI cells reduced blood glucose levels in 6/8 mice, and 4/6 experienced hypoglycemia (BG<40 mg/dl), with plasma proinsulin (IRI) levels of 2.5-8.6 ng/ml. To obtain the production of mature insulin in non-endocrine cells, the human proinsulin cDNA was modified by PCR, with the insertion of two mutations, so as to be translated into a proinsulin molecule mutated at the positions 51 (Lys -> Arg) and 84 (Leu -> Arg). Such a modified protein should be cleavable by the ubiquitous protease furin, with the production of mature insulin. The vector carrying the double mutated human proinsulin cDNA (HPIdm) was then transduced into 3T3 and HepG2 cells, and an insulin production of 3.8 ng/24h/1x10^6 cells developed (detected as IRI by BI-insulin IRMA, Pasteur, cross reactivity with proinsulin <0.0001%). Both cell lines have been i.p. transplanted into diabetic athymic mice. Moreover, primary cultured rat hepatocytes are transfected with these vectors and tested for in vitro and in vivo insulin production. The development of insulin secreting cells represents a first step towards the engineering of an artificial β-cell, with the HPIdm gene under the control of a glucose-regulated promoter.

CROSS-TALK BETWEEN INSULIN AND ANGIOTENSIN SIGNALING: ANGIOTENSIN II STIMULATES THE PHOSPHORYLATION OF IRS-1 AND IRS-2 BUT INHIBITS PHOSPHATYLINOSITOL 3-KINASE

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Angiotensin II (AII) acting via G-protein linked receptor, is an important element of mechanisms that regulate hemodynamics and water and electrolyte balance. Following *in vivo* injection of 10^{-6} to 10 M AII in rats, rapid tyrosyl phosphorylation of the major insuln receptor substrates IRS-1 and IRS-2 (2. 6 and 3.8 fold, respectively) as well as association of IRS-1 and IRS-2 with the p85 subunit of PI 3-kinase (PI 3-K) (3.2 and 4.4 fold) in the heart is observed. Phosphorylation of IRS-1 and IRS-2 appears to involve tyrosyn phosporylation of JAK2, which is complexed with IRS-1 and IRS-2 both after AII as well as insulin stimulation. In heart, there is a 7.3 and 28-fold stimulation of IRS-1 and IRS associated PI 3-K activity following insulin injection. Surprisingly, however, following AII treatment term was a dose dependent inhibition of IRS-1 and IRS-2-associated PI 3-K activity, with maximal decreases of 55% and 60% folling injection of 200 pmole of AII. The effect of AII to inhibit PI 3-K was also observed during insulin stimulation, with AII treatment one minute before insulin infusion reducing the level of IRS-1 and IRS-2 associated PI 3-K activity by 74% and 86% respectively. The ability of AII to inhibit insulin-stimulated PI 3-K activity occurred without any effect of AII to alter insulin stimulation of insulin receptor or IRS-1/2 tyrosyl phosphoryiation and with no significant reduction of in the binding of p85 to IRS-1/2 or p110 catalytic subunit to p85 regulatory subunit of PI 3-K. Infusion of a peptide blocker (QGVYVHPV) of the AII receptor completely prevented the inhibitory effect of AII on insulin stimulated PI 3-kinase activity, with no effect on model for Aii action, AII is able to inhibits basal as well as insulin and IGF-1 stimulated IRS-1 associated PI 3-K activity. Phorbol esters, possibly via stimulation of PKC, mimic AII inhibition of PI 3-K. Conclusions: 1) AII and insulin/IGF-1 signalling pathways meet at the IRS/PI3-kinase level, where AII acting through the AT1 receptor is able to inhibit PI 3-kinase while stmulating efficient IRS-1/2 phosphorylation and PI 3-K association to them. 2) This phenomenon might involve tyrosyl-phosphorylation on alternative sites in the IRSs or PKC-dependent serine phosphorilation of the PI 3-K complex. 3) It seems of physiological relevance, also given the hypoglicemic effects of some ACE inhibitors. 4) This intracellular interaction may play an important role in the insulin resistance of hypertension and the association of these conditions with cardiovascular diseases.

SELECTIVE REGULATION OF PHOSPHATIDYLINOSITOL 3-KINASE SUBUNITS BY GLUCOCORTICOIDS REVEALS A NOVEL MECHANISM MODULATING ENZYME ACTIVITY. F. Giorgino, M. T. Pedrini, L. Matera, and R. J. Smith.

Activation of phosphatidylinositol (PI) 3-kinase represents a key signaling event for insulin and IGF-I regulation of cellular metabolism. Hormone stimulation induces the phosphorylation of specific tyrosine residues in the receptor substrate IRS-1 which mediate the association of the 85-kDa regulatory subunit (p85) of PI 3-kinase and the activation of the 110-kDa catalytic subunit (p110) of the enzyme. The objective of this study was to investigate the effects of the glucocorticoid dexamethasone on expression of components of the PI 3-kinase complex and on their association with IRS-1 in rat skeletal muscle cells. Two highly homologous isoforms of p85 have been cloned, p85α and p85β, which are encoded by distinct genes and form each a complex with p110. Dexamethasone treatment induced a 4-fold increase in p85α protein content, but had no effect on p85β. The increase in p85α protein was associated with a coordinate increase in p85α mRNA, indicating that dexamethasone enhances p85α gene expression. Stimulation of cells with insulin or IGF-I induced the association of both p85 isoforms and of p110 with IRS-1 and markedly increased PI 3-kinase activity in IRS-1 immunoprecipitates. In cells treated with dexamethasone, greater amounts of p85α and lower amounts of p85β, respectively, were found in IRS-1 immunoprecipitates in response to hormone stimulation so that the α/β ratio was markedly higher than in control cells. Despite the increase in both total and IRS-1-associated p85α, IRS-1-associated PI 3-kinase activity was decreased by 45%, which was explained by lower levels of the catalytic subunit p110 associated with IRS-1. In conclusion, dexamethasone specifically increases the expression of p85α but has no effects on p85β. The cellular excess of "free" p85α induced by dexamethasone may compete with both p85α/p110 and p85β/p110 complexes for the association with IRS-1 leading to increased p85α and decreased p85β and p110 in IRS-1 immunoprecipitates. These results suggest a novel PI 3-kinase regulatory mechanism which may inhibit cell signaling through this protein.

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TRANSPLANTATION OF BOVINE ISLETS INTO THE THYMUS OF NON-IMMUNOSUPPRESSED PIGS. R. Giannarelli, P. Marchetti, M. Carmellini, A. Coppelli, R. Lupi, C. Tellini, S, Del Guerra, F. Mosca, R. Navalesi.

Intrathymic islet allo-transplantation allows prolonged islet function in temporarily immunosuppressed rodents, dogs and pigs. In addition, the thymus is a suitable site for islet survival in concordant xeno-transplantation experimental rodent models. No information is currently available on the survival of pancreatic islets transplanted into the thymus in discordant xenograft models. In this study we prepared pure bovine islets, of proven in-vitro and in-vivo viability, and cultured them at 37 °C in medium M199, with 10% bovine serum and antibiotics for 15 to 21 days. Then, approximately 10,000 islets were transplantanted into the thymus of eight 3-mo old, non-immunosuppressed pigs, under general anesthesia. The surgical procedure was simple, safe and effective. The glands were retrieved after 3 (2 animals), 7 (3 animals) and 14 (3 animals) days from implantation, and processed for light microscopy (hematoxylin-eosin staining, immunocytochemistry). In 2 pigs of the 14 day study, intravenous glucose was administered at 3, 7 and 14 days after islet transplantation, and bovine insulin measured by RIA following HPLC separation from porcine insulin. Well preserved islets were shown both at 3 and 7 days from transplantation, and insulin containing cells were evidentiated by immunocytochemical staining. No apparent modification of the islet surrounding thymus was observed. Bovine insulin was found in the plasma of transplanted pigs, which increased upon glucose stimulation. These results demonstrate that bovine islets can successfully engraft in the thymus of non-immunosuppressed pigs, with avoidance of hyperacute rejection. Further studies are ongoing to evaluate how long the islets can function in this discordant xenograft model.

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INSULIN SIGNALING ABNORMALITIES IN SKELETAL AND CARDIAC MUSCLES OF DIABETIC RATS: CORRECTION BY TRANSPLANTATION OF PANCREATIC ISLETS. F. Giorgino, F. Logoluso, A. Davalli, R. Napoli, R. J. Smith, G. C. Weir, and R. Giorgino.

Insulin-deficiency and associated hyperglycemia produce metabolic derangements and changes in gene expression in several insulin target tissues including skeletal muscle and heart. This results in an altered capacity of insulin to properly signal and function in these tissues that cannot be normalized by the administration of exogenous insulin. We analyzed the alterations of insulin signaling that occur in the skeletal and cardiac muscles of streptozocin (STZ) diabetic rats and investigated the effects of diabetes treatment by islet transplantation. Male Lewis rats were studied: controls (C, n=8), untreated 6-week diabetics (D, n=8), and islet transplanted diabetics (T, n=8). T were transplanted with syngeneic islets under the kidney capsule 2-3 weeks after STZ administration and studied 8 weeks later. Fasting plasma glucose was increased in D and normalized in T (106±5, 456±10, and 97±3 mg/dl in C, D, and T, respectively). *In vivo* protein tyrosine phosphorylation was studied by i.p. injection of 20 U insulin, removal and homogenization of tissues 30 min later, and analysis of tyrosine phosphoproteins with phosphotyrosine antibodies. In the skeletal muscle of D, insulin-stimulated tyrosine phosphorylation of the IR β-subunit and IRS-1 was increased 1.6- and 2.3-fold, respectively, compared to C, and IRS-1 tyrosine phosphorylation was also 2.3-fold higher in the absence of insulin stimulation. D had a 50% decrease in IRS-1 protein content and a 2-fold increase in the amounts of IR and p85, respectively. Although with lower magnitude compared to skeletal muscle, similar changes in IR and IRS-1 tyrosine phosphorylation and IR, IRS-1 and p85 protein expression were also found in the heart of D. Islet transplantation fully corrected the diabetes-induced changes in tyrosine phosphorylation and expression of insulin signaling proteins in both skeletal and cardiac muscles. Thus, the adequate insulin replacement obtained with islet transplantation is capable of maintaining normal insulin signaling reactions in peripheral tissues of diabetic animals.

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GLUTAMATE DECARBOXYLASE AUTOIMMUNITY AND GROWTH HORMONE (GH) RESPONSE TO GH-RELEASING HORMONE IN TYPE I DIABETES MELLITUS A. Giustina, P. Perini, P. Desenzani, S. Bossoni, C. Poiesi, E. Bazzigaluppi, E. Bosi.

Insulin-dependent (type I) diabetic patients are known to have exaggerated growth hormone (GH) responses to GH-releasing hormone (GHRH) which are hypothesized to be due to decreased somatostatin tone. Aim of the study was to ascertain the influence of the presence and the of the activity of the autoimmune process involving a key enzyme (glutamic acid decarboxylase, GAD) in the synthetic pathway of a neurotransmitter regulating somatostatin secretion, i.e. gamma aminobutiric acid (GABA), on the GH response to GHRH in patients with type I diabetes mellitus. Twenty non-obese type I diabetic patients and seventeen normal subjects underwent an iv injection of 100 μg GHRH(1-29)NH_2. The GH peaks after GHRH were significantly (r_s= 0.46, $p<0.05$) correlated with the level of GAD antibodies (GADA) in the whole population of type I diabetic subjects studied. Diabetic subjects with serum GADA levels > 3 Units (n. 10) showed significantly higher serum GH levels after GHRH injection as compared both to diabetic patients with GADA < 3 Units (n. 10) and to normal controls both when expressed as absolute and peak values. Our findings suggest that autoimmunity may play a key role in determining the exaggerated GH response to GHRH observed in type I diabetes mellitus. The mechanism underlying this effect is hypothesized to be the production of antibodies to GAD, a key enzyme in the synthesis of GABA, and in turn a reduced GABAergic inhibitory tone on somatostatin production at the hypothalamic level.

Dr. Andrea Giustina: Endocrine Section, c/o 2^ Medicina, Spedali Civili, 25123 Brescia, Italy.

EFFECT OF SHORT AND LONG-TERM ADMINISTRATION OF PICOTAMIDE ON BASELINE AND EXERCISE-INDUCED URINARY ALBUMIN EXCRETION IN TYPE II PATIENTS (NIDDM) WITH INCIPIENT NEPHROPATHY. A. Giustina, P. Desenzani, P. Perini, S. Bossoni, A. Burattin, C. Mascadri, F. Manelli, M. Milani, G. Romanelli.

The aim of our study was to investigate the short- and the long-term effect of picotamide, a dual inhibitor of tromboxane synthesis and action, on baseline and exercise-induced urinary albumin excretion (UAE) in normotensive patients with NIDDM. In the short-term investigation, 15 NIDDM patients microalbuminuric at rest (12 men and 3 women, age 56±2, BMI 28±1 Kg/m^2) performed 5 submaximal exercise tests (90% of theoretical heart rate) on a cycloergometer: the first two under basal conditions; the third and fifth exercise after subjects had received picotamide (300 mg TID) or placebo (3 tablets/day) for 10 days; the fourth exercise was performed after 10 days of wash-out. When diabetic patients were untreated, a significantly ($p<0.05$) higher UAE with respect to baseline levels was observed immediately after and 1h after the exercise test. After picotamide administration, UAE significantly decreased ($p<0.05$) immediately after and 1h after exercise, as compared to diabetic patients given a placebo. In the long-term investigation, 6 patients microalbuminuric at rest with NIDDM were studied: 4 patients (2 men and 2 women, age 52±11) were treated for 9 months with picotamide (300 mg TID) and 2 patients who did not receive the drug served as controls. Three of the picotamide treated-patients were also given a cycloergometric exercise test at baseline and after 3 and 6 months of therapy. Microalbuminuria at rest was measured in all patients at baseline and after 3, 6 and 9 months. At the end af the study, all the picotamide-treated patients demonstrated a significant decrease in microalbuminuria at rest (from 41.7±12.7 μg/min at baseline to 11.8±3 μg/min after 9 months) and after exercise (peak at baseline 103±36 μg/min vs 65.8±11 μg/min after 6 months). Conversely, in the two controls, microalbuminuria at rest increased from 45.1±0.9 μg/min at baseline to 151±59 μg/min at the end of the 9-months study period. Our results show that in normotensive NIDDM patients with incipient nephropathy: a) short-term administration of picotamide is associated with a reduction in UAE after exercise; b) long-term treatment with picotamide reduces both baseline and exercise-induced microalbuminuria.

Progetto Finalizzato Microalbuminuria (Legge Regione Lombardia 31/08/91 n. 53).
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ALTERED REGULATION OF LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE TISSUE AND MUSCLE OF TRANSGENIC MICE OVEREXPRESSING GLUT4 SELECTIVELY IN FAT. L. Gnudi, B.B. Kahn, and A. Tiengo.

Transgenic mice overexpressing GLUT4 selectively in adipose tissue using the aP2 promoter/enhancer develop obesity, enhanced glucose tolerance, increased insulin sensitivity, and elevated glucose transport in isolated adipocytes. To understand the obese phenotype, we measured adipose tissue and muscle lipoprotein lipase (LPL) activity, the rate limiting step for supply of triglycerides fatty acid to tissues. Fasting LPL activity tended to be lower in transgenic skeletal muscle and was reduced 50% in transgenic heart compared to nontransgenic, whereas fasting LPL in transgenic adipose tissue was unaffected. Stimulation of LPL activity by feeding is blunted in parametrial fat from 22-fold in nontransgenic mice to 3-fold in transgenic and in perirenal fat from 15-fold to 7-fold, respectively. LPL activity in the fed state in transgenic mice is reduced 60-75% in parametrial and perirenal fat. In the combined muscle mass (heart, gastrocnemius and soleus muscle), LPL activity in the fasted state is 55-65% lower than in nontransgenic and feeding induces an unexpected rise in LPL activity. In sum, increased glucose transport into fat in transgenic blunts the meal induced stimulation of LPL in fat and reverses the inhibition in muscle. Whereas stimulation of adipose LPL may be blunted by lower plasma insulin levels in transgenics, fasting muscle LPL may be suppressed by elevated plasma lipids. Thus, altering the partitioning of glucose between adipose tissue and muscle by adipose-specific overexpression of GLUT4 alters the rate limiting step for the partitioning of lipoprotein fatty acids between these tissues.

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THE INTERRELATION BETWEEN INFANT FEEDING PATTERNS AND DIABETES MELLITUS.

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Purpose: To determine the prevalence of infant fee ding patterns (breast or artificial feeding) in first 4 months of life among patients with IDDM and NIDDM in Azerbaijan.

Patients and desing: The prospective study was performed on 177 patients with IDDM (85 males and 92 females, mean age 13,4 ± 3,6) and 147 patients with NIDDM (65 males and 82 females, mean age 56± ± 4,8). The data were obtained by randomised revie wing of 160 patients card and by questioning of 164 NIDDM and IDDM patients.

Results: Our data revealed that 60,5% patients with Type 1 diabetes mellitus had had the artificial feeding in first 4 month of life and only 39,5% patients were breast fed. Among patients with NIDDM 95,2% had had breast feeding.

Conclusion: Our results were evidence of the presence a positive link between early cow milk exposure, breast feeding and IDDM in Azerbaijan population and confirms the conception of a role of bovine serum albumin on development of IDDM.

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IMPROVEMENT OF HYPERTENSION AFTER COMBINED KIDNEY PANCREAS TRANSPLANTATION.

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Hypertension after kidney transplantation (**TX**) has particularly damaging consequences for renal graft function and for the vascular system of patients (**PTs**). Hyperinsulinemia, insulin resistance and hyperglycemia are considered the major causes of hypertension in diabetic patients. We compared, through a 1 year follow up study, the posttransplant hypertension (**H**) in **40** hypertensive diabetic recipients who received combined kidney and pancreas transplant (**KP**) with **20** who received a kidney transplant alone (**K**). In the former group, **13** recipients had segmental pancreas (**KPS**) while **27** a whole pancreas (**KPW**). All **PTs** were treated with the same immunosuppressive treatment including: steroids, azathioprine and cyclosporine (**CSA**). Before and after **TX** glycosylated hemoglobin (**HbA1c%**), fasting free insulin (**F IRI uU/ml**), creatinine (**creat mg/dl**), and the prevalence of **H** was evaluated. The prevalence of **H** was **88% , 48 %** and **53 %** in **K, KPW** and **KPS** group 1 year after **TX** respectively. Considering both **KP** groups ,1 year after **TX** the rate of **H** was statistically lower than in **K** groups (**p=.04**). Before **TX** the 3 groups of **PTs** were comparable for clinical charactheristic of cadaveric donors and recipients, creatinine, CSA, **HbA1c** and **F IRI.** After **TX** a good renal function was observed in all **PTs (KPW vs KPS vs K creat. mg/dl 1.3 vs 1.2 vs 1.5**). **F IRI** as well as **HbA1c** levels were statitically lower in both **KP** group than in **K** group (**KPW* vs KPS** vs K°. F IRI: 16.4* vs 15.1** vs 26.0°; *vs° p=.004; **vs°p=.008. HbA1c: 5.5*vs 6.7** vs 7.4°; *, ** vs ° p=.0001. * vs ** p=.01**). In conclusion, we observed a clear improvement of **H** in **KP** transplanted **PTs** more than in diabetic **Pts** recipients of renal transplant alone. The improvement of results observed is associated to lower fasting **F IRI** levels and to a better metabolic control reached in **KP** recipients.

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF THE LABIAL SALIVARY GLANDS IN CHILDREN WITH TYPE I DIABETES.

A.MARKOPOULOS, M. BELAZI, A.GALLI- TSINOPOULOU

The aim of this study was to investigate the morphological alterations of labial salivary glands in children with type I diabetes. For this purpose labial salivary glands under local anesthesia were excised from the lower lip of ten diabetic children at the onset of diabetes (mean age : 10 years). Additionally ten similar biopsies were obtained from ten healthy children (mean age : 9 years). The tissues were conventionally processed and embedded in paraffin wax. The first section was stained with hematoxylin- eosin, while two additional sections were stained immunohistochemically using the indirect immunoperoxidase avidin - biotin method in order to detect T and B cells.

The results of this study showed that a mononuclear infiltration was apparent in all diabetic children. Lymphocyte aggregations were randomly seen throughout the salivary gland section. Lymphocytes were particularly seen around various ducts. In contrast the salivary glands of healthy controls were free of infiltrates and were characterized normal. The immunohistochemical analysis for the characterization of the infiltrates showed that the majority of these cells were T cells. B cells were also observed to a lesser extend and were predominantly found on the periphery of infiltrates.

These findings indicate that a destruction of labial salivary glands takes place in children with type I diabetes. This phenomenon is mainly T cell mediated. The similarity of this condition with the well - known destructive process of the pancreas of diabetics (insulitis) suggests that the labial salivary glands and pancreas may share a common antigen that might be the target of the autoimmune process in typeI diabetes.

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INCREASED URINARY EXCRETION OF GLUCAGON-LIKE PEPTIDE 1 (GLP1) 7-36 AMIDE IN EARLY PHASE OF DIABETIC RENAL INVOLVEMENT.

R.Lugari, L.Sarti, S.Coppi C.Dell'Anna, P.Sbordone, A.Gnudi, R.Zandomeneghi.

The metabolism of human GLP1, considered the most important physiologic insulinotropic factor, is still unknown. In uremic subjects plasma GLP1 is reported to be significantly increased, suggesting that the kidneys play a role in the removal of the circulating peptide. Studies performed in the rat demonstrate that the renal clearance of GLP1 involves its glomerular filtration, subsequent tubular uptake and intracellular catabolism or intraluminal brush border-associated peptidases degradation. In condition of maintained glomerular filtration, thus, the negligible urinary excretion of the peptide would confirm the integrity of tubular function.Aim of this study was to investigate the urinary excretion of GLP1 in non insulin-dependent diabetic patients, evaluating different stages of renal function. 29 type 2 diabetics, mean age 58.4±7.8 yr (M±SD) and 7 healthy volunteers were studied. Urinary albumin excretion rate (UAE) was assessed by RIA on 3 consecutive overnight collections. Glomerular filtration rate (GFR) was evaluated as creatinine clearance.Urinary excretion of GLP1 was determined by RIA on a single overnight collection and expressed as pg/min. Four groups of subjects were individuated: group1=controls (UAE=3.4±2.6 µg/min),group 2=normoalbuminuric diabetic patients (n=8; UAE=5.2±3.1), group 3=microalbuminuric patients (n=11; UAE=81.7±14.6),group4=macroprotenuric patients (n=10;UAE=448.9±94.8) Mean values of urinary GLP1 resulted significantly different between the groups ($p<0.03$). With respect to group 1(275.5±132.1 pg/min.),the urinary peptide excretion significantly increased in group 2 (490.4±311.5;$p<0.05$) and further in group 3 (648.6±305;$p<0.01$),whereas no significant difference resulted between macroproteinuric patients (317.9±183.3) and controls. Mean values of GFR was lower in group 3 (57.4±28 ml/min.) with respect to both group 1 and group 2 (105.6±6.1,91.2±17:$p<0.01$).Considering all subjects examined, a significant relationship emerged between urinary GLP1 and GFR ($p=0.004$).In conclusion, the significant increase of the peptide excretion observed in normoalbuminuric diabetic patients could indicate an early tubular dysfunction which would occur in condition of still maintained glomerular integrity, becoming then more evident with the onset of glomerular diabetic impairment. In condition of overt diabetic nephropathy the tubular defect, in terms of urinary peptide excretion, would be masked by the concomitant serious damage of glomerular function.

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WHICH RELATIONSHIP BETWEEN Na-H ANTIPORT ACTIVITY AND PLASMA LIPIDS?

E. Matteucci, C. Bertoni, E. Boldrini, F. Piazza, F. Ruberti, M. Casini and O. Giampietro.

Na-H antiport is closely related to cytoskeleton and involved in cell physiopathology. Membrane lipid composition and fluidity may influence ionic transport. We checked the relation between exchange activity and serum lipids in 33 healthy controls (35±9 y), 18 non-diabetic pregnant women (32±4 y), 42 patients (33±11y) with type 1 insulin-dependent diabetes mellitus (IDDM), 26 non diabetic normotensive siblings (30±7 y) and parents (56±8 y) of type 1 diabetics, 30 hypertensives (55±9 y), and 20 uremic subjects (43±9 y) on regular hemodialysis. In healthy people, serum glucose ($p<0.05$), total cholesterol ($p<0.001$) and triglycerides ($p<0.05$) increased with age, while the exchange rate was unmodified. In pregnants, at 14, 24, 33 weeks, 2-hour OGTT glycaemia ($p<0.01$), serum total cholesterol and triglycerides ($p<0.001$) increased, while antiport was unaffected. IDDM patients had higher glycaemia ($p<0.001$) and normal plasma lipids, but higher Na-H antiport ($p<0.05$). Parents and siblings of IDDM people had raised antiport activity ($p<0.05$), but only relatives of microalbuminuric probands showed concurrent dyslipidemia: siblings had elevated serum triglycerides ($p=0.01$), parents higher serum cholesterol ($p<0.05$). Compared with matched controls, uremics had elevated systolic blood pressure ($p<0.01$), glycaemia ($p<0.001$), triglycerides ($p<0.001$) and Na-H exchange rate ($p<0.05$). Hypertensives had higher systolic and diastolic blood pressure ($p<0.001$), serum triglycerides ($p<0.05$), the lowest HDL cholesterol concentration ($p<0.001$) with the highest antiport activity ($p<0.001$). While in healthy people by alone Na-H antiport activity correlated with no parameter, in the whole study population (grouped by age), Na-H exchange rate correlated with blood pressure (diastolic or mean) only, both in young ($p<0.05$) and in aged ($p<0.001$) subjects. Hence, Na-H antiport activity seems unaffected by circulating lipids both in healthy and disease conditions.

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EFFECT OF THE CALCIUM ANTAGONIST AMLODIPINE ON INSULIN SECRETION AND SENSIVITY IN NIDDM.

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Amlodipine is a calcium channel blocker with a long-lasting antihypertensive effect, without known major effects on glucose and insulin levels in normotensive or hypertensive patients.
The purpose of this study was to evaluate the effect of treatment with amlodipine on the glucose and insulin response to intravenous glucose tolerance test (IVGTT) in NIDDM patients with essential hypertension.
We studied 15 patients (8 men, 7 women) with NIDDM and mild hypertension, receiving treatment with oral hypoglycaemic agents and/or diet. Their age was 62±4 years, the known duration of diabetes was 9±6 years, glycaemic control was stable.
The protocol was designed as a randomized, double-blind, placebo-controlled cross-over study, with each treatment phase lasting for 6 weeks. A 3-week washout was undertaken before each treatment phase.
To evaluate insulin secretion and sensitivity, an IVGTT was perfomed before and at the end of each treatment period. Calculated Conard constants, reflecting insulin sensitivity, remained unchanged after amlodipine (-0.00175±0.00012 vs placebo -0.00185±0.00020, mean±SEM, p=ns). Insulin secretion, as evaluated by the calculation of areas under curve was also unaffected by amlodipine (3452±711 vs placebo 3481±605, p=ns). Delta areas were consistently unchanged, and no significant differences in blood glucose or insulin levels were found at any single point during IVGTT.
Major blood lipids, glycaemic profiles and HbA1c levels also remained unchanged, whereas blood pressure was reduced by amlodipine treatment as expected (systolic: 132.2 ± 19.7 vs 140.2 ± 15.6 mmHg, diastolic: 76.4 ± 7.8 vs 85.4 ± 7.7 mmHg, p<0.05 for both).
We conclude that amlodipine represents an effective antihypertensive treatment also in NIDDM patients. The notion that amlodipine may not adversely affect insulin secretion and sensitivity is extended to hypertensive NIDDM patients. This may be a perspectively useful characteristic in clinical practice.

INSULIN, PROINSULIN AND C-PEPTIDE RELEASE AFTER INTRAVENOUS GLUCOSE CHALLENGE IN SUBJECTS WITH INSULINOMA.

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Subjects with insulinoma show hypoglycemic symptoms due to glucose overutilization combined with high insulin (I), proinsulin (PI) and C-peptide (C-pep) levels during the fasting period, but little is known about the dynamics of I, PI and C-pep release after a glucose challenge. The aim of this study was to evaluate the effects of an acute intravenous glucose tolerance test (IVGTT: 0.3 g/kg) on (I), (PI) and (C-pep) release in ten subjects with insulinoma, confirmed histologically after surgery, in comparison with eight normal subjects matched for age, sex and body weight. After the IVGTT, 32 blood samples were withdrawn in 240 min. In subjects with insulinoma, fasting G levels were significantly lower and I , PI and C-pep levels were significantly higher than in normal subjects, as expected. Two min after IVGTT, G concentrations peaked at about 16 mM in both groups declining thereafter to basal levels in 160±26 min in insulinoma and in 76±8 min in normal subjetcs ($p<0.02$). The time to peak I, PI and C-pep release after IVGTT was longer in insulinoma than in control subjects (6.1±0.8 *vs* 3.8±0.3, 6.7±1.2 *vs* 3.5±0.3 and 7.1±1.1 *vs* 3.8±0.5 min, respectively, $p<0.03$) while the return to baseline values was similar in the two groups. First-phase (from basal to peak) I and PI integrated release (Δ-AUC) was similar while first-phase C-pep Δ-AUC was significantly higher in insulinoma than in normal subjects (6.0±1.6 vs 1.9±0.4 nM/min; $p<0.05$). Second-phase (from peak to basal levels) I and C-pep Δ-AUC was significantly higher in insulinoma than in normal subjects ($p<0.05$) while second-phase PI Δ-AUC was similar in the two groups. In conclusion, I, PI and C-pep response to an acute glucose challenge is delayed in subjects with insulinoma with an oversecretion of I and C-pep while PI secretion seems not different. These findings could be useful in the early diagnosis of insulinoma.

CRF (CORTICOTROPIN-RELEASING-FACTOR) STIMULATION TEST IN DIABETIC PATIENTS WITH OR WITHOUT DIABETIC NEUROPATHY.

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We evaluated HPCA axis function by high-dose CRF test (100 ug e.v.) in 12 normal subjects (mean age+SE: 44,75 + 4,01) group 1), 8 diabetic patients without diabetic neuropathy (mean age + SE: 54,5 + 3,57; group 2), and 7 diabetic patients with established symtomatic polyneuropathy (mean age + SE: 58,86 + 3,84; group 3). All diabetic patients presented a good control of metabolic status. Experiments started at 7:30 am; CRF was injected after 2 basal blood samples (-30'; 0); further blood sampleswere taken at + 15' +30' +60' +90' minutes after CRF administration. Mean basal ACTH levels were higher in the subject of group 3 than in those groups 1 and 2, but these differences did not achieved the statistical significance. Administration of CRF induced a significantly increases in ACTH levels in all subjects; however, the ACTH response was lower in diabetic patients (groups 2 and 3) than in controls (group 1) and in particular between diabetic patients without neuropathy and normal subjects these differences achieved the statistical differences ($p<0.05$). Concerning the cortisol pattern, basal levels of the hormone were higher in the group 3, while CRF-induced response in this group was lower than in the others (these differences did not achieved the statistical significance).

Groups	basal ACTH (pg/ml)§	basal Cortisol (ug/ml)	ACTH Δ%§	Cortisol Δ%§	ACTH Δ area (pg/ml)§	Cortisol Δ area (ug/100ml)§
Group 1	22.4 ±4.1	10.7±0.7	5.1±1.6	1.8±0.2	2527.6±572.0*	430.5±108.7
Group 2	20.9±3.4	10.8±0.9	2.5±0.2	1.7±0.1	974.1±150.5*	285.7±90.0
Group 3	31.1±5.2	12.6±1.0	2.7±0.6	1.4±0.1	1374.3±476.7	89.1±128.2

§ Values are expressed as mean ± SE; * p<0.05.

These data suggest that in diabetic neuropathy there is a tonic hyperactivity of the HPAC axis in basal conditions, in accordance with the data in literature. However, the hyporesponsiveness of ACTH and cortisol to CRF test suggests the presence of a reduce ability of HPAC axis in responding to acute pituitary stimulation, probably related either to a decrease pituitary ACTH reserve or to a down-regulation interesting the whole HPAC axis consequent to the basal hyperactivity. The hormonal pattern in diabetic patients without neuropathy showes under stimulus, a significant HPAC hyporesponsiveness when compared to the normal subjects, while the cortisol response to CRF did not differ significantly neither from neuropathic patients nor from control subjects.

THE INCIDENCE OF DIABETES MELLITUS IN OLD PEOPLE AND ITS INFLUENCE ON SURVIVAL.

M. Motta, D. Rosso, G. Carnazzo.

In recent years the improvement in enviromental conditions, the increase in average life span, the improvement in diagnostic and therapeutic possibilities have determined a noteable increase of diabetes in middle age and in senility. The epidemiological data of the past can no longer be considered valid, and even the most recent research must be considered provisional as data increases ever more rapidly. The incidence of diabetes in the Italian population aged between 65 and 84, according to the latest data from the CNR project ILSA, is around 14%. This data is less than that which we met in Catania where the incidence is 22%. This confirms our previous research which noted a high incidence of diabetes in our population; in fact, in a study of the population of Militello (CT) undertaken in 1988 there was an incidence of 18%. In the "Multiscope " Survey of the Family between 1987-91 carried out by ISTAT in questionnaire form, in the volume dedicated to old people, the percentage of diabetes in the over-seventies, sub-divided into five-years groups, is as follows: 10.5% in those between 70-74, 11.6% in those between 75-79 and 13.3% from 80 upwards. A noteable decrease emerges in the number of cases of diabetes in the 70-74 age range compared to the preceeding five-year band. In the successive five years the number of new cases increases on average by 1.4%. The percentage of diabetes onsets after 70 (senile diabetes) which we noticed in the Militello study, and in another survey by L.Motta and co-workers in 1976, is equal respectively to 8% and 6.5%. The low percentage of diabetes in centenarians is important, and has been studied by the Italian Multicentric Study on Centenarians. In this survey the percentage is 3.7%. In only one case was the diabetes present for more than 50 years, while in all the other cases it appeared at an average age of 91.2±13.3 (median=96 years). Analysing the prevalence of diabetes we can put forward the hypotesis that long-term diabetes causes an increase in mortality in old people. Senile diabetes is largely responsible for the high percentage of diabetes in old people. The particular pathogenesis of senile diabetes on the base of arteriosclerosis, together with factors concerning age, should be considered likely to be responsible for the mortality in senile diabetes progressing more rapidly. Summing this two events causes the incidence of diabetes in ultra centenarians to go down by 3.7%. These data allow us to state that long-term diabetes constitutes an element of risk for longevity, and that diabetes rarely allows the patients to achieve a century.

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ISLET AUTOANTIBODIES AS SCREENING MARKERS IN IDDM FIRST DEGREE RELATIVES FROM NORTHERN ITALY

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Islet cell antibodies (ICA) are the established marker for IDDM risk assessment. Antibodies to glutamate decarboxylase (GADA) have been recently proposed as an alternative to ICA measurement for screening purposes. The aim of this study was to determine the effectivness of GADA measurement as a primary screening test in first degree relatives of IDDM patients. GADA, measured by immunoprecipitation of ^{35}S-labelled in vitro translated antigen (positivity at the upper 1st percentile of controls), ICA, detected by immunofluorescence and quantified in JDF-units (positivity above 5 JDF-units), and insulin autoantibodies (IAA), measured by a radiobinding assay (positivity above mean + 3 SD of controls) were determined in 821 first degree relatives (465 parents, 356 siblings) participating in the prospective San Raffaele IDDM Family Study within the Lombardy region. GADA were detected in 43 (5.2%) relatives, and were more frequent in siblings than parents (8.1% vs 3.0%; $p<0.01$). In comparison, 30 (3.7%) relatives had ICA, 30 (3.7%) had IAA, and both were more frequent in siblings. At least one antibody was detected in 82 (10%) relatives, including 13.2% of siblings ($p<0.01$ vs parents). GADA alone were present in 28 relatives, GADA plus ICA in 13 ($p<0.01$ vs GADA-neg), and GADA plus IAA in 8 relatives ($p<0.01$ vs GADA-neg); 6 had all three antibodies. Over a maximum follow-up of 6 years, 4 relatives developed IDDM: all were siblings; all had GADA, 3 had ICA, and 2 had IAA. This study demonstrates a high prevalence of humoral islet autoimmunity in siblings of IDDM patients, and in particular a GADA frequency much higher than the expected number of future IDDM cases. The detection of GADA in all cases progressing to IDDM, however, indicates that GADA is likely to be a sensitive marker for identifying future IDDM cases in first degree relatives. The addition of other islet antibody markers (e.g. IA-2 antibodies) will be required to increase specificity.

MULTICENTER CLINICAL TRIAL OF THE PROMEDOS 3 IMPLANTABLE INSULIN PUMP IN IDDM.

Giovanna Petrella for The Point Study Group II.

Safety, feasibility and efficacy of the Promedos 3 (Siemens-Elema AB) implantable programmable pump for long-term intraperitoneal delivery of HOE 21 PH insulin (Hoechst AG) were evaluated in a multicenter, non-randomised clinical trial on 47 IDDM patients followed at 6 European centers for 94.5 pt yr.

HbA1c was on average 8.1±1.4% during the 3 month run-in period of subcutaneous intensive insulin therapy and 7.5±0.9% after one year of pump treatment. Severe hypoglycaemic events decreased from 34.0 % pt yr during intensive insulin therapy to 2.0 % pt yr during pump insulin treatment ($p<0.01$). Ketoacidosis events were 8.0 and 1.0 % pt yr during subcutaneous and pump insulin treatment respectively. Pump pocket complications were 3.2 % pt yr and resulted in explantation of 3 devices. Twenty-three events of irreversible catheter occlusions (incidence 24.3 % pt yr) were managed by catheter replacement. Two devices were explanted because of pump occlusion failure (no flow). Programmer problems were 103.7 % pt yr, and were managed by programmer reconfiguration or replacement.

In conclusion, the Promedos 3 device is safe, feasible and effective for long-term intraperitoneal insulin treatment of IDDM patients.

GROWTH HORMONE AND MICROALBUMINURIA IN TYPE I DIABETIC PATIENTS WITH NORMAL RENAL FUNCTION P. Perini, P. Desenzani, S. Bossoni, A. Burattin, C. Poiesi, A. Giustina.

Type I diabetic patients (IDDM) are known to have abnormalities in growth hormone (GH) secretion. In normal subjects hyperglicemia inhibits the pituitary GH response to provocative stimuli. In contrast, IDDM patients, despite elevated blood glucose levels, show higher mean 24-h GH levels than normal subjects, exaggerated GH responses to exercise (via somatostatin) and pharmacological provocative tests, and paradoxical GH secretion after TRH. Several experimental studies suggest the existence of a possible relationship between GH and development of diabetic microvascular disease such as nephropathy. Aim of our study was to evaluate the correlation between baseline GH level and microalbuminuria (urinary albumin excretion, UAE, between 20-200 μg/min) (Immunonephelometer method, BNA100, Behring, Scoppito, Italy) at rest and after submaximal exercise in 9 males normotensive IDDM patients normoalbuminuric at rest (age 37.3±2.9 yrs, duration of disease 14.9±3.7 yrs, HbA1c 7.6±0.2%). Five of the patients had microalbuminuria after exercise (stage II diabetic nephropathy); three of these patients were also affected by background retinopathy. Our results demonstrate significantly ($p < 0.042$) higher baseline GH levels (1.54±0.52 ng/ml) in the 5 patients at stage II of diabetic nephropathy as compared to patients without microalbuminuria after exercise (baseline GH levels 0.08±0.05 ng/ml). No other significant differences between the two groups considered were found with respect either to glycometabolic control, age, BMI or duration of diabetes. Our findings show that in IDDM patients with very early stage diabetic nephropathy, higher basal GH levels may be found as compared to patients with no evidence of nephropathy. Therefore, these data seem to lend support to the hypothesis linking GH hypersecretion (probably caused by low somatostatin) and microangiopathic complications in type I diabetes mellitus.

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CAN AN ACUTE OR A CHRONIC HYPERINSULINEMIA AND HYPERTRIGLYCERIDEMIA INCREASE ENDOTHELIN-1 RELEASE IN MAN?

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Several studies have shown the association amongst insulin resistance, hyperinsulinemia, hypertriglyceridemia, hypertension and cardiovascular disease in subjects affected by plurimetabolic syndrome (syndrome X). However, it is difficult to characterize the contribution of each metabolic variable in the induction of hypertension and cardiovascular disease. Considerable attention has been recently attributed to the potential role of endothelin-1 (ET-1) in vascular disease since ET-1 is a potent vasocostrictor peptide synthetized and secreted by endothelial cells. We tested the hypothesis whether subjects with syndrome X show high ET-1 levels and we evaluated the single contribution of insulin and triglyceride levels in the induction of arterial ET-1 release.

Fasting arterial ET-1 levels were measured in normal subjects (n=31), in subjects with syndrome X (n=16) and in subjects with insulinoma (n=11). Arterial ET-1 levels were evaluated in normal subjects during intralipid (test 1) or saline (test 2) infusion lasting 360 min (study A). Moreover, in study A a euglycemic two-step hyperinsulinemic (25 and 125 mU/kg/h) clamp was started at 120 min.

Subjects with syndrome X showed higher fasting ET-1 levels than normal subjects and subjects with insulinoma (7.22±0.89 *vs* 2.61±0.38 and 2.35±0.22 pg/ml; $p<0.01$). A significant relationship was found between fasting ET-1 levels and fasting triglyceride levels ($r=0.53$, $p<0.0001$) and between fasting ET-1 levels and baseline systolic ($r=0.61$, $p<0.0001$) and diastolic blood pressure ($r=0.40$, $p<0.001$). Study A: in test 1, arterial ET-1 levels increased significantly in response to hypertriglyceridemia and furthermore, in a dose-dependent manner, in response to insulin. In test 2, arterial ET-1 levels increased significantly only in response to high insulin levels, but remained signifcantly lower than in test 1.

In conclusion, subjects with syndrome X showed higher fasting ET-1 levels than normal subjects and subjects with insulinoma. These levels were reproduced in normal subjects by the simultaneous increase of insulin and triglyceride levels. Therefore, an increased secretion of ET-1 may be one of the causes of cardiovascular disease in patients with hyperinsulinemia and hypertriglyceridemia.

CULTURE OF HUMAN ENDOTHELIAL CELLS ON HIGH CONCENTRATIONS OF FIBRONECTIN DECREASES Na^+/H^+ EXCHANGER ACTIVITY.

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Human vascular endothelial cells cultured in high glucose concentrations decrease their proliferation and exhibit decreased Na^+/H^+ antiporter activity which is known to regulate intracellular pH and modulate cell proliferation. It has been shown that also insoluble fibronectin decreases endothelial cell proliferation and that Na^+/H^+ antiporter activity is sensitive to signal deriving from fibronectin. The aim of this study was to investigate whether the activity of the Na^+/H^+ antiporter was altered in human endothelial cells exposed to different amounts of an extracellular matrix component such as fibronectin. Human endothelial cell cultures obtained from twelve individual umbelical cord vein were studied. Cells were plated in 35 mm tissue culture dishes coated with two different concentrations of fibronectin (1 and 50 µg/ml) and compared with cells grown on uncoated dishes. Cultures were studied 72 hours after seeding. Na^+/H^+ exchange was measured by unidirectional ^{22}Na influx stimulated by cellular acidification in the presence or absence of dimethylamiloride. In 12 experiments, Na^+/H^+ exchange activity was lower in cells cultured on 50 µg/ml of fibronectin (FN_{50}) when compared with the activity of cells plated on uncoated dishes (P) and 1 µg/ml of fibronectin (FN_1). The mean ± SE of the Na^+/H^+ exchange activity were for P 8.36±1.07 nmol/mg protein x min, for FN_1 7.18±0.69, and for FN_{50} 6.24±1.11 ($p<0.02$).
In endothelial cells cultured on high concentrations of fibronectin the activity of the Na^+/H^+ exchange is decreased. This phenomenon may parallel and perhaps contribute to the decreased replication observed under these experimental conditions.

EXOCYTOSIS AND ITS MODULATION IN SINGLE RAT INSULINOMA CELLS ASSAYED USING MEMBRANE CAPACITANCE MEASUREMENTS AND SEROTONIN RELEASE

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Electrophysiological measurements of cell capacitance (C_m) and biochemical assays of [^{3}H]-serotonin ([^{3}H]-5HT) release were combined to study the control of secretion in rat insulinoma RINm5F cells.
Depolarizing pulses produced C_m changes (DC_m), indicative of exocytosis, with the same voltage- and Ca^{2+} dependency as the inward Ca^{2+} currents (ICa). Ba^{2+} was able to substitute for Ca^{2+} in stimulating exocytosis, but not endocytosis. However, both the relative potency and kinetics of Ca^{2+} versus Ba^{2+} triggered exocytosis differed significantly.
Serotonin synthesis and uptake were demonstrated in RINm5F cells. This allowed the use of [^{3}H]-5HT to study hormone release from cells populations.
[^{3}H]-5HT was released in a depolarization, Ca^{2+} and time-dependent manner.
Ba^{2+} also substitued for Ca^{2+} in depolarization-induced [^{3}H]-5HT release.
Thapsigargin used to deplete Ca^{2+} stores had no effetcs on Ca^{2+} triggered C_m increases, but Ca^{2+} triggered [^{3}H]-5HT release was strongly reduced. Ba^{2+}-triggered [^{3}H]-5HT release, however, was not affected by Ca^{2+} store depletion.
Finally, noradrenaline was found to potently block both [^{3}H]-5HT release and C_m increases. However, its ability block secretion was not correlated with its ability to inhibit the calcium channels, suggesting that noradrenaline acts directly on the secretory apparatus.

INSULIN RESISTANCE IS A MAJOR RISK FACTOR FOR MORBIDITY AND MORTALITY IN PATIENTS WITH LONG STANDING NON-INSULIN-DEPENDENT DIABETES (NIDDM) WITH SECONDARY FAILURE OF ORAL HYPOGLYCAEMIC AGENTS

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Secondary failure of oral hypoglycaemic agents (SF) is a frequent clinical entity, commonly understood as the inability of oral agents, after a proven period of efficacy, to control blood glucose levels in NIDDM patients. SF has a different etiology in lean (progressive reduction of insulin release) and in obese (progressive increase in insulin resistance) NIDDM patients, and population studies have indicated a greater prevalence of coronary heart disease (CHD) and of mortality in insulin-treated patients or in patients with high endogenous insulin levels. Therefore we planned a cross-sectional and a 5-year follow-up study in NIDDM patients with SF. In 112 SF patients, CHD was associated with higher C-peptide release and diastolic blood pressure at univariate analysis and at multiple logistic regression, and with higher uric acid and creatinine levels at univariate analysis only. Of 93 patients retraced 5 years later, 25 had died; alive patients differed at baseline from dead patients for lower age, creatinine levels, prevalence of CHD, and dose of insulin both at univariate analysis and at multiple logistic regression, and for lower C-peptide release at univariate analysis only. Obese patients had higher C-peptide release and more markers of insulin resistance than lean patients, and died more frequently (29.3% vs 16.7%, $p = 0.05$). Alive patients, in spite of a rather poor metabolic control, did not show a significant increase in the prevalence of complications. These data indicate the need for reduction of insulin resistance, more than the achievement of strict metabolic control, in order to prevent morbidity and mortality in long standing NIDDM with SF of oral agents.

PREVALENCE OF HCV ANTIBODIES IN EGYPTIAN PATIENTS WITH DIABETES MELLITUS. *Hassan Rizk, Ossama Saad, Ahmed Rassem, Magdy Hamed.*

It is commonly believed that diabetics are prone to many varieties of complications including infections, requiring surgical interference, blood transfusion and parenteral drug adminstration (Joslin, 1994).
Epidemics of blood borne hepatitis were described in patients attending diabetes clinics where they received injection treatment during the early 20 th century (Vannafalt et al., 1944). Recently, hepatitis C virus has been identified and considered a major cause of post transfusion and community acquired hepatitis (Koziel et al., 1993). The higher prevalence of HCV carriers among Turkish diabetics was described by Ozyilkan et al., (1994).
The aim of this study is to determine the prevalence of HCV among Egyptian diabetics.
One hundered patients (37 males and 63 females) with diabetes mellitus and 35 non diabetic healthy subjects were investigated for anti HCV antibodies. HCV antibodies was detected by second - generation ELISA (Abbott Laboratories). The results were compared using the chi-squared test with Yate's correction.
Whilst the prevalence of HCV antibodies was 31.4% among the control group, 51% of the diabetic group were positive for HCV antibodies with significant statistical increase ($P = 0.04553$).
In the present study, there was no significant statistical difference between HCV diabetics and non HCV diabetics as regards duration of diabetes, micro-angiopathic complications of diabetes, type of diabetes, insulin therapy, history of surgical operations or blood transfusion ($P>0.05$).
We conclude that diabetes mellitus is a risk factor for HCV infection.

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LATE COMPLICATIONS IN NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM): A MULTIVARIATE LOGISTIC ANALYSIS OF RISK FACTORS IN 2500 PATIENTS.

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Objective: To determine frequency and risk factors of chronic complications in a large cohort of NIDDM patients.
Methods: Prevalence of late complications and their association with potential risk factors, including age, sex, duration of diabetes and hypertension, glycosylated hemoglobin A1C (HBA1C) and mean arterial pressure (MAP) were cross sectionally studied in 2500 NIDDM patients referred to nine diabetes outpatient clinics of the Bergamo area. Nephropathy was defined as albumin concentration in spot morning urines >300 mg/L (multistix +, Bayer Diagnostics S.p.A.) without signs of urinary tract infection. Retinopathy was diagnosed by funduscopy, neuropathy by electromyography, peripheral artery disease (PAD) and ischemic heart disease (IHD) by history and clinical examination.
Results: Prevalence of complications and results of multivariate logistic analyses are in the table.

Prevalence	NEPHROPATHY 11%	RETINOPATHY 23%	NEUROPATHY 11%	I.H.D. 23%	P.A.D. 13%
Age	N.S.	N.S.	N.S.	0.0001	0.0001
Sex	0.02	0.009*	N.S.	0.047	0.003
Diab. Duration	0.0001	0.0001	0.0001	0.007	0.004
Hypert. Duration	0.02	N.S.	N.S.	0.0001	0.003
HbA1C	0.0001	N.S.	N.S.	N.S.	N.S.
MAP	0.0004	0.0002	N.S.	N.S.	N.S.

*Female predominance

Duration of diabetes increased the risk of all complications. Age, male sex, and duration of hypertension were main factors associated with macroangiopathy. Female sex and high MAP were strongly associated with increased risk of retinopathy. High HBA1C and MAP with nephropathy.
Conclusions: A great percent of NIDDM patients require medical assistance for chronic complications. Efforts aimed at identifying potentially treatable risk factors and at optimizing diagnosis and therapy of complications should help minimize the burdens of long-term morbidity and mortality of NIDDM.

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LONG-TERM RESULTS OF THE MULTICENTER CLINICAL TRIAL WITH THE INFUSAID MODEL 1000 IMPLANTABLE PROGRAMMABLE INSULIN PUMP.

Marina Scavini for The Implantable Insulin Pump Trial Study Group.

Long-term metabolic outcome and adverse event rates of insulin therapy using the Infusaid Model 1000 implantable programmable pump were examined in 76 IDDM patients followed for 24 pt yr of intensive SC insulin therapy, and for 213 (IP) and 38 (IV) pt yr of pump treatment.

HbA1c was 7.9±1.5% at study entry, 7.3±1.3% at the end of the run-in period, remaining between 6.9±1.0% and 7.5±1.2% during 3 yr of pump therapy. Mean %IBW was 106.3±12.1 at the end of the run-in period and 110.4±14.2 after 3 yr of pump therapy (obesity rate 2.7% pt yr). HbA1c and %IBW were not different between IP and IV treatment. Rate of severe hypoglycemia was 33.0% pt yr during the run-in period and significantly decreased to 13.0 (IV) and 2.0 (IP) % pt yr during pump therapy.

Pump slowdown occurred every 9 months, and were managed with alkaline solution flush. Catheter occlusion rates were 12.0 (IP) and 20.0 (IV) % pt yr. Rate of pump pocket complication and of programmer problems were 5.3 and 19.9% pt yr.

Pump therapy achieves similar metabolic control to SC intensive insulin treatment, lowering the incidence of severe hypoglycemia. Catheter occlusions and pump slowdown are the commonest complications.

METABOLISM OF POSTPRANDIAL LIPOPROTEINS IN PATIENTS WITH BENIGN INSULINOMA. G. Ruotolo, M. Mori, F. Ragogna, C. Tettamanti, G. Derosa, and G. Pozza.

Insulin certainly plays an important role in the regulation of lipoprotein metabolism in the postprandial state. Benign insulinoma represents an interesting model of chronic hyperinsulinemia, but data on the lipoprotein metabolism in this condition are still lacking.

The aim of this study has been to analyze many aspects of fasting and postprandial lipoprotein metabolism in 6 patients with benign insulinoma, and in 8 healthy control subjects. The two study groups were comparable for age, sex, body mass index, and apoE phenotype. Postprandial values have been expressed as area under the concentrations from time 0 (fasting 8:00 am) to the end of the test (sampling at 0, 1, 2, 3, 4, 5, 6, 8, and 10 hours).

Fasting and postprandial levels of glycemia (basal: 49±7 vs 79±7 mg/dl; postprandial: 498±58 vs 813±38 mg/dl), and free fatty acids (basal: 0.39±0.16 vs 0.62±0.28 mEq/l; postprandial: 5.2±1.3 vs 7.7±1.6 mEq/l) were significantly lower, whereas those of insulin were significantly higher (basal: 17.0±6.3 vs 8.3±5.5 μU/ml; postprandial: 324±81 vs 123±39 μU/ml) in the insulinoma than in the control group. Fasting total cholesterol (150±11 vs 172±21 mg/dl), and LDL cholesterol levels (91±12 vs 112±20 mg/dl) of insulinoma patients were significantly lower than controls, whereas no statistical differences between the two study groups have been detected concerning postprandial levels of triglycerides and retinyl esters in plasma and triglyceride-rich lipoproteins. Plasma postheparin lipoprotein lipase (158±53 vs 198±43 mU/ml) and hepatic lipase (163±60 vs 210±50 mU/ml) activities in benign insulinoma patients were lower than those of controls (p=0.10).

In conclusion, patients with benign insulinoma show a fast catabolism of lipoproteins of intestinal origin in the presence of a rather low postheparin hepatic lipase activity, and elevated fasting and postprandial plasma insulin levels.

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REAL TIME SPECTRAL ANALYSIS. A QUICK METHOD FOR CARDIOVASCULAR AUTONOMIC NEUROPATHY SCREENING IN DIABETIC SUBJECTS.

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We developed an instrument able to measure R-R interval and to estimate and display in real time the spectrum of heart rate variability (HRV). Both the reliability of the instrument and accuracy of the results has been checked by suitable test signals. Aim of this work was to evaluate the sensitivity of this method in 18 Control subjects (9M/9F, age range 25-36 yrs, BMI range 20-26 Kg/m^2) and 9 IDDM subjects (5M/4F, age range 22-39 yrs, BMI 22-26 Kg/m^2, with severe autonomic cardiovascular neuropathy, Score >4 as evaluated according to Ewing: deep breathing, lying to standing, Valsava maneouvre and postural hypotension). Subjects were tested at 8.00 a.m., with serum glucose levels below 11 mmol/l, after an overnight fast, resting in bed and in a controlled temperature room (21°C). Under low frequency (LF), peak area was: Control group 3.66±0.4; IDDM group 0.11±0.06; t test 26.3, p<0.001. Under high frequency (HF), peak area was: Control group 1.7±0.26; IDDM group 0.11±0.05; t test 17.9, p<0.001 (values expressed as mean ± SD, to the sixth power in arbitrary units). In this preliminary validation study, this method was able to detect a significative difference of the areas under the peak of both LF and HF. This instrument can therefore be considered a reliable tool both to quantitate in real time spectrum analysis of HRV and to accurately detect autonomic neuropathy.

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VOLTAGE OPERATED CALCIUM CHANNELS CONTROLLING INSULIN SECRETION FROM HUMAN PANCREATIC BETA CELLS.

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Calcium ion entry through voltage-operated calcium channels is a crucial step in the coupling of β cell depolarization with insulin secretion. Using the patch-clamp technique, we investigated the biophysical and pharmacological properties of calcium channels in freshly dispersed human pancreatic β cells.

Both low and high voltage activated currents were expressed, the two current types being easily distinguishable on the basis of biophysical criteria. The high voltage activated currents were not homogeneous: one component was affected by the dihydropyridine antagonist nitrendipine and the agonist Bay-K-8644; the other was insensitive to both dihydropyridines and ω-conotoxin. Noradrenalin, a potent inhibitor of insulin release, specifically inhibited the dihydropyridine-sensitive channels, in a voltage- independent manner.

In line with this pharmacology, nitrendipine reduced and Bay-K-8644 increased glucose-induced insulin secretion from perifused human islets, whereas ω-conotoxin had no effect. However, about 20% of the glucose-induced insulin release was found to be resistant to high nitrendipine concentrations.

These data show that human pancreatic β cells express heterogeneous voltage-operated calcium channels, only one of which is dihyciropyridine-sensitive (L-type). The L-type channels are clearly involved in the control of insulin secretion, but our data suggest that dihydropyridine and ω-conotoxin-insensitive channels (P/Q-like) may also play a role in the stimulus-secretion coupling of human β cells.

THE CENTRAL DIABETIC NEUROPATHY: A NEUROPHYSIOLOGICAL STUDY IN INSULIN-DEPENDENT DIABETIC PATIENTS. M. Sterlicchio*, M. Croci, N. Akhevan, M. Ferrari, A. Tufano, T. Hassan **, F. Rognone***, F. Caviezel.

The so-called diabetic encephalopathy (i.e. central diabetic neuropathy, CDN) is still matter of debate, due to several difficulties of investigation and to frequent coexistence of vascular damage. In this study 9 type 1 (insulin - dependent) diabetic patients (7 M, 2 F, aged 21-49 y, duration of diabetes 0.5-24 y, mean HbA1 12.9%) underwent electromyography (EMG), somatosensorial evoked potentials (SEP) by median and tibial nerve stimulation, magnetic resonance imaging (MR) of the brain, and the usual test for autonomic neuropathy (AN). Only 2 patients had mild abnormalities at the bedside neurological examination. Almost all patients (8/9) showed peripheral neuropathy (by EMG), 2 of them were found as having undetectable SEP and 4 an increased latency time (>N20) at cerebral level. The two patients without SEP at cortical level (aged 47 and 48 y) showed diffuse T1-weighted low signal images at MR, possibly due to (micro)vascular damage. 6 out of 9 subjects had AN. Only one patients (aged 23 y) showed no pathological findings at the central (CNS) and peripheral nervous system. No clear correlation has been found between the altered cerebral conduction velocity and metabolic control, duration of diabetes, degree of peripheral neuropathy, frequency of hypoglycaemic episodes. In conclusion, abnormalities of SEP at cerebral level could be interpreted as signs of CDN in diabetic subjects. Moreover, SEP may represent a useful tool in monitoring the evolution of diabetic neuropathy, including that at the CNS level.

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Higher incidence of severe hypoglycemias with glibenclamide / glyburide than with glibornuride

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Little is known about the incidence of severe hypoglycemias (i.e. requiring hospital admission) related to different sulfonylureas, i.e. glibenclamide / glyburide and glibornuride. In Switzerland, 85-90% of all NIDDM patients treated with sulfonylureas take either of these 2 drugs. - During 3 years, we looked at all NIDDM patients admitted to our hospital due to a severe hypoglycemia. Basel has a population of 199'000, and about 95% of all emergencies are sent to our emergency room. The total number of NIDDM patients treated with sulfonylureas was estimated by the number of tablets sold by all pharmacies and by the defined daily dose, i.e. 7.5 mg for glibenclamide / glyburide and 37.5 mg for glibornuride, respectively.

Sulfonylurea	Number of patients treated	Number of severe hypoglycemias		
		total	per year	per 1000 patient-years
Glibenclamide	447	7	2.33	**5.2**
Glibornuride	1100	2	0.7	**0.64**

The initial blood-sugars were all around 2 mmol/l, and no other reason for the hypoglycemia could be found. - These results indicate that the incidence of severe hypoglycemias is significantly higher with glibenclamide / glyburide than with glibornuride ($p < 0.01$). Therefore we do not recommend glibenclamide / glyburide as a first choice drug in NIDDM.

Metabolic efficacy of pig islet transplanted in different sites of diabetic nude mice.

Taglietti MV, Socci C, Bertuzzi F, Caumo A, Monti L, Freschi M, Magistretti P,Di Carlo V, Pozza G.

Aim of this study was to compare the efficiency of pig islets in reversing diabetes according to different sites of implantation. Pig islets were isolated using the digestion-filtration method and purified by centrifugation on discontinous EuroFicoll gradients. Aliquots of 1000 hand picked islets were transplanted into the portal vein (n= 7), kidney capsule (n=9) and spleen (n=10) of STZ diabetic nude mice. Graft function over the time was studied by an IVGTT (0,5 mg/kg) performed at 15, 30, 60, 90 days on the transplanted recipients and in two control groups: normal mice (n=4) and STZ diabetic mice (n=7). Glycemia was assessed at 0, 1, 3, 5, 15, 30 minutes after glucose bolus. Results were expressed by index Kc, i.e the slope of the regression line of the glucose concentrations logarithm, measured between 3 and 30 minutes after bolus. Pig C-peptide serum concentration (ng/ml) was assessed 5 minutes after glucose bolus in some subjects of each group.

Portal vein mortality was 71% (n=5) while kidney and spleen mortality were 11% (n=1) and 40% (n=4),respectively. Pancreas **histologic examination** of the dead transplanted animals showed no residual mice islets. **C-peptide serum concentration** (ng/ml) at. 15, 30 days was 0.8 ± 0.8 and 1.54 ± 0.5 for the kidney site; 0.63 ± 0.73 and 0.08 ± 0.01 for the spleen site, 0.15 ± 0.03 and 0,09 for the portal site.

At 15 days **fasting glycemia** was 132±123, 190±184, 56±37 mg/dl in the kidney,.spleen and portal group. At 90 days kidney and spleen group.in creased to 172±86 and 253 ± 249 respectively. **Kc** in normal and diabetic groups was 0.02 and 0.006 ($p<0.001$). Kc was 0.027, 0.033, 0.031, 0.021 at 15, 30, 60, 90 days in the kidney group; 0.036, 0.035, 0.037, 0.015 at 15, 30, 60, 90 days in the spleen group; 0.021, 0.026 at 15, 30 days in the portal groups. (Each group vs diabetics < 0.05 ; vs normal mice = ns).

In conclusion, these findings showed that pig islets are able to restore a normal metabolic control after transplantation in diabetic mice. Site of transplantation does not seem to influence islet engrafment while the glycemic control over the time worsened both in kidney and soleen grafted mice.

ARE HLA-DR AND HLA-DQ PHENOTYPES ASSOCIATED WITH ICA, GAD65 AND IA2/ICA512 AUTOANTIBODIES?

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Type 1 diabetes is due to a progressive autoimmune destruction of beta cells. The autoimmune response is directed against a multiplicity of autoantigens. Insulin, glutamate decarboxylase (GAD) and 37K antigen, now identified as the protein tyrosin phosphatase IA2/ICA512, are the main autoantigens. HLA alleles confer susceptibility to type 1 diabetes. The aim of our study was to determine whether ICA, GAD65 and IA2/ICA512 autoantibodies were associated with different HLA-DR and HLA-DQ phenotypes. We examined 39 patients with type 1 diabetes aged from 3 to 14 years at diagnosis of disease. The patients' sera were tested for ICA, GAD65 and IA2/ICA512 antibodies. All patients were also typed for HLA-DR and HLA-DQ. ICA, detected by indirect immunofluorescence, were present in 76.9% of patients, GAD65 and IA2/ICA512, evaluated by immunoprecipitation of in vitro translated ^{35}S-methionine labelled recombinant proteins, were found in 71.8% and 69.2% of patients respectively. All the three autoantibodies were present in 51.3% of patients, one or two Abs in 3 8.5% of pts, while no Ab was detected in 10. 2% of pts. No correlation was observed between GAD and IA2/ICA512 levels, while we found an association between ICA and IA2/ICA512 titres ($p<0.001$). We didn't find significant differences in ICA, GAD65 and IA2/ICA512 Ab frequency and levels between HLA-DR3, DR4 and DR3/4 patients. Moreover, we didn't observe significant differences in Ab frequency between patients homozygotes or heterozygotes for HLA-DQA1*0301/0501 and HLA-DQB1* 0201/0302. Our preliminary results don't suggest that production of Ab specifities are influenced by HLA-DR and HLA-DQ phenotypes.

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SOLUBLE CD23 RELEASE BY PBMC FROM YOUNG DIABETIC PATIENTS.

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It has been suggested that serum levels of soluble CD23 (sCD23) provide an indirect means of assessing the overall balance of Th1 and Th2 activity. In a previous study we observed significant enhancement of serum sCD23 levels in newly diagnosed and long-standing diabetic patients compared to controls (Diabetes 44-S1: 138A, 1995). The aim of present study was to evaluate sCD23 production by PBMC activated by rIL-4 from 17 diabetic patients, aged from 3 to 25 years (8 at diagnosis and 9 long-standing) and 7 age and sex matched healthy subjects. Serum IL-4 levels were also determined. Soluble CD23 concentrations in supernatants and serum IL-4 levels were assayed using immunoenzymetric methods. Soluble CD23 release was similar in supernatants of non activated PBMC from patients (newly diagnosed pts. median 1.73 U/ml, range 0-2.7; long-standing pts: median 2.6 U/ml, range 0.4-3.3) and controls (median 1.64 U/ml, range 0-2.57).. Moreover the addition of rIL-4 to the medium culture similarly increased sCD23 concentrations either in newly diagnosed (median 8.26 U/ml, range 5.6-10.3) and long-standing pts (median 7.8 U/ml, range 5.2-13.6) or healthy subjects (median 6.18 U/ml, range 3.05-6.4). Our diabetic patients also showed serum IL-4 levels comparable to controls. The factors responsible for elevated serum sCD23 levels, previously observed in our diabetic patients, remained to be determined.

NEW PORTABLE GLUCOSE METERS - A COMPARISON WITH THE REFERENCE GLUCOSE OXIDASE METHOD.

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Patients as well as medical personnel are increasingly confronted with new devices for blood glucose measurement. Devices smaller in size, faster in action and easier to handle are developed by various companies. We questioned whether precision and accuracy of recently marketed blood glucose meters would be efficient and safe enough for clinical use. We compared the meters Companion 2 plus (C2), Glucometer Elite (GE), Hypocount Supreme (HS) and Omnican Control (OC) with our reference glucose oxidase method. In average 190 pairs of blood glucose values from capillary blood samples of type1 and 2 diabetic patients were determined using two meters of each brand. The measurements were performed by one and the same experienced technician, using blood from the same sample for the meter and the Beckman Analyzer 2. For evaluation a linear regression analysis [1], the percentage of values with a max. deviation of less than 10% from the reference value [2], Koschinsky's Acceptance Analysis (TDF= Total Deviation Factor) [3] and the Error-Grid Analysis [4] were used:

	Companion 2 plus	Glucometer Elite	Hypocount Supreme	Omnican Control
[1]	r = 0.975	r = 0,977	r = 0,920	r = 0,907
[2]	56%	72%	40%	32%
[3]	TDF = 1,25	TDF = 1,25	TDF = 1,38	TDF = 1,72
[4]	Zone A: 97%	97%	86%	71,5%
	B: 3%	3%	14%	28%
	C: 0	0	0	0
	D: 0	0	0	0,5%
	E: 0	0	0	0

Combining all analyses, C2 and GE showed the least deviations in all blood glucose ranges. According to Koschinsky's clinical acceptance scale C2, GE and HS can be classified as good and OC as acceptable. The criteria of the American Diabetes Association (max. deviation of +/- 5% in 100% of measurements) were not met by any device, but after training and with proper handling all devices work efficiently enough and are safe for clinical use.

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THE BENEFICIAL EFFECTS OF DIFFERENT TYPE THERAPY ON BETA CELLS FUNCTION IN RECENT-ONSET INSULIN DEPENDENT DIABETES(IDDM) MELLITUS(IDDM)

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It has been previously shown that a different treatment in recent onset IDDM patients might have a beneficial effect on beta cells function and early clinical remission, defined as optimal metabolic control, HbA1c <7% more than 30 days.The aim of this study is to compare residual beta cells function (C-peptide before and 6 min after 1 mg Glucagone iv) in IDDM patients at time of onset and at time of clinical remission, regarding the type of therapy. In group A(MC insulin-two doses) 5 out of 25 patients had remission (20%) with duration 32+/- 8days and C-peptide at time of onset were 0,27+/- 0,02nmol/l vs 0,38+/- 0,03nmol/l.In group B (MC insulin-two doses + Cyclosporine A) 9 out of 11(81,2%) had remission with duration 323+/-43days and C-peptide were 0,24+/- 0,02nmol/l vs 0,35+/-0,03nmol/l.In group C (HM insulin 4 doses by PEN) 19 out of 38 (50%) had remission with duration 145+/-37 days and C-peptide at onset time were 0,24+/-0,02nmol/l vs 0,40+/-0,06nmol/l.In group D (HM insulin pump therapy) 20 out of 28 (71,4%) had remission with duration 139+/-22 days and C-peptide were at time of diagnosis 0,28+/-0,02 nmol/l vs 0,44+/-0,03 nmol/l. In group E(HM insulin 4 doses by PEN + 250 mg/day Nicotinamide) 6 out of 10 (60%) had remission with duration 150+/-25 days and C-peptide at time of onset were 0,30+/-0,01 nmol/l vs 0,48+/-0,02nmol/l.In group F(HM insulin pump therapy + 250 mg/day Nictotinamide) 7 out of 10(70%) had remission with duration 150+/-22days and C-peptide at time of diagnosis were 0,33+/-0,01 nmol/l vs 0,61+/-0,06 nmol/l.At time of remission C-peptide before and after Glucagon stimulation were in group A 0,35+/-0,01nmol/l vs 0,45+/-0,02nmol/l;In group B 0,29+/-0,01nmol/l vs 0,54+/-0,03nmol/l;In group C 0,49+/-0,02nmol/l vs 0,74+/-0,05nmol/l;In group D 0,47+/-0,03nmol/l vs 0,79+/-0,05 nmol/l;In group E 0,39+/-0,02 nmol/l vs 0,92+/-0,06 nmol/l;In group F 0,42+/-0,03nmol.l vs 0,89+/-0,05 nmol/l.Our results suggest that the best influence on preservation beta cells function had combined therapy insulin pump with Nicotinamide, but the longest remission had patients on Cyclosporine A therapy.

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EFFECTS OF HYPERINSULINEMIA AND HYPERAMINOACIDEMIA ON INTRACELLULAR PROTEIN TURNOVER IN THE HUMAN FOREARM. **M. Zanetti, R. Barazzoni, M. Vettore, P. Tessari.**

The effects of insulin and of amino acids on protein turnover in human muscle are controversial. In this study, we have employed an *intracellular* model of forearm leucine kinetics in the investigation of the effects of systemic hyperinsulinemia, without or with hyperaminoacidemia, on leucine exchange between plasma and cell, release from proteolysis, utilization for protein synthesis, oxidation and interconversion with KIC. Hyperinsulinemia ($\approx$80-100 μU/ml) with resulting hypoleucinemia decreased forearm leucine inflow (-22$\pm$8%, $p<0.05$) and outflow (-32$\pm$8%, $p<0.02$) into/from cell, but it did not significantly modify proteolysis (-20%, $p=0.138$), protein synthesis (-4%, NS), leucine oxidation and leucine-KIC interconversions. Hyperaminoacidemia and hyperinsulinemia increased sharply leucine inflow from plasma to forearm cell (+148$\pm$44%, $p<0.01$), protein synthesis (+79$\pm$22%, $p<0.01$), and net leucine balance (from -6$\pm$5 to +58$\pm$12 nmol/100 ml of forearm·min, $p<0.01$). Leucine release from endogenous protein breakdown, oxidation, outflow from cell, and interconversions with KIC did not change. Fractional contributions by estimated total skeletal muscle to whole body leucine kinetics were not modified by insulin alone, while following hyperaminoacidemia with hyperinsulinemia the percent contribution by muscle to body leucine oxidation decreased ($p<0.01$), from 21$\pm$6% to 6$\pm$1%, while that to protein synthesis increased ($p<0.01$), from 27$\pm$7% to 41$\pm$13%. Thus, in the forearm, hyperaminoacidemia (with hyperinsulinemia) is required to stimulate leucine inflow from plasma to cell and protein synthesis. Insulin alone decreases leucine exchange between plasma and cell, it may decrease proteolysis, but it does not stimulate protein synthesis. Finally, hyperaminoacidemia results in a preferential stimulation of protein synthesis in muscle tissues, while the infused leucine is largely oxidized at extramuscular sites.

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ACE-INHIBITORS IMPROVE ENDOTHELIAL FUNCTION IN PATIENTS WITH TYPE I DIABETES (IDDM) AND MICROALBUMINURIA. M. Zenere, G. Arcaro, F. Saggiani, M.G. Zenti, T. Monauni, A. Lechi, M. Muggeo, R.C. Bonadonna.

In human large conduit arteries flow(shear stress)-induced dilation (FID), obtained through distal ischemia-induced vasodilatory response, is endothelium (E)- and nitric oxide (NO)-dependent. We have previously shown that both FID and E-independent (elicited by glyceryl trinitrate administration) vasodilation (GTN-ID) are severely impaired in the femoral artery of normotensive microalbuminuric IDDM patients. ACE-inhibitors may reduce albumin excretion rate and exert a cardiovascular protective action. We performed a double-blind placebo-controlled crossover study, in which 9 (3 males, 6 females) normotensive microalbuminuric IDDM patients underwent a one-week trial of either placebo (P) or captopril (C) (25 mg t.i.d.) or enalapril (E) (10 mg/die) in randomized order, to ascertain whether short time ACE inhibition obtained with (C) or without (E) a sulphydryl donor molecule can improve vessel wall function in these patients. FID and GTN-ID were evaluated in the right common femoral artery by high resolution echo-doppler. Both C and E treatment improved endothelium-dependent vasodilation (+1.55±0.54 and +1.45±0.42 vs -0.93±0.35 mm per 8 min, $p<0.05$ for both C and E vs P, respectively), with minimal changes in arterial pressure and microalbuminuria. C, but not E, significantly enhanced maximal GTN-ID (+0.74±0.09 mm for C, +0.50±0.08 mm for E and +0.44±0.09 mm for P; $p<0.05$ for C vs both E and P). Thus, a short term course of ACE inhibition improves E-(NO)-dependent vasodilation in normotensive microalbuminuric IDDM patients. Furthermore, C causes a drug-specific improvement in E-independent femoral artery vasodilation, possibly through its sulphydryl donor properties.

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AGE AND DIABETES DURATION AS RISK FACTORS OF COMPLICATIONS IN THE FRAME OF VERONA DIABETES STUDY. G.Zoppini, G.Verlato, M.De Marco, M.Muggeo.

The Verona Diabetes Study is a population-based study including a cross-sectional study of prevalence of known diabetes in the city of Verona on 31 December, 1986 and of a longitudinal study of mortality over the following 5 years in the prevalent cohort. In the prevalence study, patients were identified by using three indipendent sources: family physicians, diabetes clinics, and drug prescriptions for diabetes. The Verona Diabetes Study led to the identification of 7488 diabetic patients whose main clinical features were: age 65.5 $\pm$ 12.8 yr (mean $\pm$ SD), age at diagnosis 55.0 $\pm$ 13.3 yr, diabetes duration 10.1 $\pm$ 7.4 yr, BMI of 27.3 $\pm$ 4.6 kg/sm. 2.7 % of patients were type 1 diabetes, 95,5% type 2 diabetes and 1,8% were unclassified. In these patients the prevalence of micro and macrovascular complications were: retinopathy 31,4%, hypertension 49,2%, coronary artery disease 9,2%, stroke 8,5%, peripheral vascular disease 12,5 and polivascular disease 16,2%. The influence of sex, age and diabetes duration on the complication prevalence was evaluated by a logistic regression model. The prevalence of retinopathy and vascular disease was significantly increased by diabetes duration and age ($p< 0.001$). On the other hand, the prevalence of hypertension was influenced mainly by age ($p<0.001$) and less by diabetes duration ($p=0.085$). In interpreting these data, it must be considered that the majority of patients were elderly type 2 diabetics and, often, hypertension was already present at the time of the diagnosis of diabetes. The risk of developing retinopathy and vascular disease is increased both by diabetes duration and age.

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