the influence of physical activity on glucoregulation depends on intensity of exercise.

253. Activation of insulin-receptor-kinase in intact HIRc and hepatoma G2-cells: Effect of dexamethasone

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We have studied the effect of a pretreatment with dexamethasone (D) on insulin (I)-binding and I-activation of insulin receptor kinase (IRK) in intact HIRc-cells (Rat1-fibroblasts expressing human I-receptor, provided by Dr. A. Ullrich, Genentech) and hepatoma G2-cells (HG2-cells). Cells were preincubated with (+D) or without (−D) 100 nmol/l D for 48 h. Fresh medium with 0, 10, or 500 ng/ml I was then added to the cells for 30 min to activate cellular IRK. I-receptors were isolated and immunoprecipitated in the presence of kinase and phosphatase inhibitors. IRK-activity towards histone that was then measured at 0.5 μmol/l ^{32}P-ATP reflected the activity state of the receptors while the cells were intact. In HIRc-cells +D the number of I-receptors was reduced by 58 ± 5% ($p < 0.01$). In HG2-cells, no D-effect on binding was found. D increased the I-effect on IRK-activity (expressed as femtomol P into histone/pmol I-binding activity/min ± SEM) in HIRc-cells at a submaximal (10 ng/ml; −D: 19.4 ± 1.6, +D: 37.8 ± 2.4; $p < 0.01$) and at a maximally effective I-concentration (500 ng/ml; −D: 61.0 ± 7.4, +D: 83.3 ± 8.7; $p < 0.01$). A comparable increase was observed in HG2-cells. Conclusions: (1) D reduces the number of I-receptors in HIRc, but not in HG2-cells, (2) D increases I-activation of IRK in both HIRc and HG2 cells. (3) Our data indicate that D could lead to an amplification of the I-signalling into the cell by increasing IRK-activity. This may explain previously described permissive effects of D on biological actions of I.

254. The effect of omega-3 fatty acids on insulin binding and action in normal and hereditary hypertriglyceridaemic rats fed a high sucrose diet

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To clarify mechanisms of beneficial effects of omega-3 fatty acids (w-3 FA) on glycide and lipid metabolism we investigated the outcome of 24 days administration of w-3 FA rich fish oil (FIO) on serum triglycerides (TG), glucose (G) and insulin (I). I binding (B) and action (A) in liver and fat tissue in normal and hypertriglyceridaemic (HTG) rats fed sucrose (SR) diet (70 cal%). In HIG rats, SR feeding further increased fasting TG levels (1.4 ± 0.1 vs 3.5 ± 0.7 mmol · l^{-1}, $p < 0.01$) abolished by FIO treatment. I levels (34.7 ± 4.9 μU · ml^{-1}) increased further on SR (61.2 ± 8.7, $p < 0.01$) along with G (6.10 ± 0.04 vs 7.72 ± 0.29 mmol · l^{-1}, $p < 0.001$) suggesting worsening of IA. FIO kept I and G within their baselines. FIO raised w-3 FA content in liver (14.34 ± 1.32 vs 18.58 ± 1.8 weight%) and adipocyte membranes accompanied by increased hepatocyte membrane fluidity (0.550 ± 0.002 vs 0.508 ± 0.007, $p < 0.005$). FIO increased IB in adipocytes (0.43 ± 0.008 vs 0.83 ± 0.04 femtomol · 10^{-6} cells, $p < 0.02$) with no effect in liver. In fat tissue FIO enhanced IA (incorporation of 14-C-glucose into lipids). Comparable data were generated in normal rats. FIO treatment inhibited SR-induced HTG in both animal models and prevented the suggested worsening of IA. This probably concerns fat tissue as FIO did not affect IB or IA in liver regardless of the change in hepatocyte fluidity.

255. A mutation in the insulin receptor causes insulin resistance and leprechaunism

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We have recently described a leprechaun patient with severe insulin resistance. Cultured fibroblasts of the patient showed 2-deoxyglucose-uptake stimulation by insulin of 1.2-fold compared to 1.7-fold in control fibroblasts. The resistance was related to a malfunction of the insulin receptor as concluded from the absence of significant insulin binding to the patient's cells and an impaired insulin stimulation of β subunit autophosphorylation. Molecular cloning and sequencing of insulin-receptor cDNA of the patient showed a homozygous leucine-to-proline mutation in the α subunit of the receptor. This mutation is located outside the regions known to be involved in insulin binding and tyrosine kinase. However, it seems to inhibit the transduction of the insulin-binding signal, generated on the insulin-binding site of the α subunit, to the tyrosine-kinase domain on the β subunit. By means of DNA amplification techniques we found that the family members are heterozygous for this mutation. These individuals have only one allele coding for a functional insulin receptor. As a result the insulin-binding levels are only 30% of the control levels. Oral glucose tolerance tests show increased insulin levels but low normal glucose levels. These findings indicate that an insulin-binding defect is not sufficient to induce Type 2 (non-insulin-dependent) diabetes.

256. T cell-dependent class II MHC antigen expression induced by streptozotocin

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T lymphocytes can be sensitised specifically by streptozotocin (STZ). We aimed at analysing the effects of STZ on class II MHC expression in vivo. Subcutaneous injection of STZ led to prominent induction of class II antigens in multiple organs of BALB/c mice. By an immunoperoxidase method on cryo-cut sections Ia antigens were evaluated with monoclonal antibodies detecting I-A^d, antibodies with specificity for Ia^k were used as controls. Treatment with STZ resulted in definite, yet transient increases of class II-positive cells within days in the pancreas, liver, heart and kidney, but not the brain. Pancreatic B cells, however, were devoid of class II antigen positivity. These immune phenomena were T cell-dependent as assessed in tissues from BALB/cJ nu/nu recipients and their +/nu counterparts. The upward regulation of cellular class II antigen expression by STZ is presumed to ensue local release of lymphokines. The intriguing concept of T cell activation by class II-positive beta cells still remains to be proven.

257. Platelet alpha granule release of growth factors in diabetic patient and non-diabetic subjects with non-healing wounds

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Platelet alpha-granules contain several growth factors (GF): platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), platelet-derived epidermal growth factor (PDEGF), platelet factor 4 (PF4), and transforming growth factor-B (TGFB). Beta thromboglobulin (B-TG) is also found in the alpha granule. Patients with non-healing wounds had blood sampled for preparation of platelet GF according to published techniques. B-TG was measured by radioimmunoassay, PDGF by ELISA, mitogenic index by H^3 thymidine incorporation into Balb 3T3 cells, and capillary endothelial chemotaxis by modified Boyden chamber technique. Patient diagnosis included diabetes mellitus, peripheral vascular disease (PVD), decubitus ulcer (DU), surgical wound dehiscence (SURG), radiation burn (RB), and venous stasis (VS). Seven patients with diabetes mellitus had B-TG = 167 ± 0.17 ng/ml, mitogenic index (MI) = 2.5 ± 0.17, PDGF = 42.7 ± 4.9 ng/ml, and capillary endothelial chemotaxis (CEC) of 2.8 ± 0.54 × control. All 20 control wound patients had lower values. B-TG was significantly higher than all groups ($p < 0.05$), MI was greater than PVD and DU ($p < 0.05$) and PDGF values were uniformly higher ($p < 0.05$). CEC was not statistically different. Platelets from diabetic wound patients have high levels of alpha granule GF necessary for wound repair. A local block in GF release or enhanced degradation may be present at the wound site.

258. Autoimmune and metabolic risk markers in siblings of Type 1 (insulin-dependent) diabetic probands

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To evaluate the relationship between autoimmune and metabolic risk markers for Type 1 (insulin-dependent) diabetes we analysed the occurrence of conventional (IF-ICA) and complement-fixing cytoplasmic islet cell antibodies (CF-ICA) and insulin autoantibodies (IAA) in siblings between 3–19 years of age of all newly diagnosed diabetic children younger than 15 years participating in a nationwide study. Twenty-three of 514 (4.5%) were positive for IF-ICA, 19 (3.7%) for CF-ICA and 5/589 (0.9%) for IAA at the presentation of diabetes in