ABSTRACT SERVICE*

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EPIDEMIOLOGY

Association between Advanced Microangiopathy and Clinically Significant Macroangiopathy in Patients with Type 2 Diabetes: a Cross-Selection Study.

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The purpose of this research was to assess the association between advanced Microangiopathy and clinically significant macroangiopathy in patients with type 2 diabetes. The study included 2493 patients with type 2 diabetes (mean age 60.0 yr; mean age at diabetes onset 48.7 yr; mean duration of diabetes 11.3 yr; 59% female patients) who were treated between 1994 and 1997 in the Karlsburg Diabetes Clinic. Advanced microangiopathy (proliferative retinopathy and/or macroalbuminuria > 200 g/min) was present in 573 patients. Clinically significant macroangiopathy in 515 patients (history of myocardial infarction or CABG or PTCA in 193 patients; claudication, pain or necrosis as symptoms of peripheral vascular disease in 158 patients; history of TIA or stroke in 97 patients; a combination of two or three of these forms of macroangiopathy in 67 patients). Multiple logistic regression analysis revealed a significantly increased risk of cerebral (OR adjusted for duration of diabetes, age at onset of diabetes and for sex 2.7; $p < 0.001$), peripheral (OR 2.3; $p < 0.001$) or combined (OR 2.8; $p < 0.001$) macroangiopathy in patients with advanced microangiopathy versus those without advanced microangiopathy. In contrast, the risk of severe coronary heart disease (OR 1.1) was not higher in patients with advanced microangiopathy. Risk factors for macroangiopathy were significantly ($p<0.01$) higher in patients with advanced microangiopathy than in those without advanced microangiopathy (triglycerides 4.1 vs 3.1 mM, total cholesterol 6.8 vs. 6.2 mM, HbA_{1c} 9.5 vs. 9.2%, presence of hypertension 89 vs 72%). In conclusion: Patients with type 2 diabetes and advanced microangiopathy have a higher prevalence of cerebral and peripheral arterial disease than patients without advanced microangiopathy. This association is not explained by age, duration of diabetes or by sex. Excess prevalence of macroangiopathy in these patients might be related to the more atherogenic risk profile. The lack of association between advanced microangiopathy and severe coronary heart disease is an unexpected, but possibly interesting result. However, this finding must be interpreted with caution because of the possibility of sample distortion bias; i.e. patients with advanced microangiopathy and severe coronary heart disease might have died from acute myocardial infarction and might therefore be underrepresented in the sample studied.

Low BMI Predicts Mortality in Indian Patients with Fibrocalculous Pancreatic Diabetes (FCPD).

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We followed 77 consecutive FCPD patients (49 men, 28 women) during the period 1984 to 1997 who were referred to us at the King Edward Memorial (K.E.M.) Hospital, a medium-sized referral hospital in Pune, Maharashtra, India. At presentation the median age was 31y in men and 21y in women, median BMI was 17.8 and 16.2 kg/m^2, respectively. Fifty five of these were known diabetic (median duration 15 mths), others were newly diagnosed. Thirty one (40%) patients resided in rural areas. They were thinner [BMI 15.5 (3.5), mean (SD) vs 18.3 (3.0), $p<0.001$], younger (median age 23 vs 30 y) and more hyperglycemic (mean glycated Hb 13.1 vs 11%) as compared to urban patients. During the follow up period (median duration 6y, range 0-14y) 23 patients (30% 13men) died, 7 were lost to follow up. Causes of death included chronic renal failure (n=8), malnutrition and neglect of health (6), post-operative portal vein thrombosis (2), septicaemia, hypoglycaemia, old age and road accident (1 each). Cause of death could not be confirmed in 3 patients. Median survival of those who died was 4 y (range 0-40 y) from diagnosis of diabetes. Fourteen out of 31 (45%) rural patients died compared to 9/46 (20%) urban patients ($p<0.001$). Rural as well as urban patients who died had a lower BMI compared to those who are alive [12.9 (2.1) vs 18.2 (3.0), for rural and 15.7 (3.1) vs 19.1 (2.6) for urban patients, $p<0.001$ and 0.015 respectively]. Kaplan Meier survival curve revealed that survival was significantly lower in rural compared to urban patients. Multiple logistic regression analysis showed that mortality was predicted by lower BMI ($p = 0.00035$) and higher age ($p = 0.056$) while the place of residence did not relate significantly. It is possible that poor nutrition related to the pancreatic disorder as well as socioeconomic status is responsible for the high mortality in these FCPD patients.

GESTATIONAL DIABETES

High Frequency of Congenital Malformations in Type 1 Diabetes despite Optimal Glycemic Control?

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It is well known that adequate glycemic control early in pregnancy in women with type 1 diabetes (IDDM) is associated with an increased frequency of congenital malformations (CM). Adequate periconceptional glycemic control decreases the occurrence of CM. We have conducted a retrospective study in three large centers in the Netherlands to access how often good glycemic control had been achieved periconceptionally and if the frequency of CM in well regulated women approaches that of non-diabetic pregnancies. A total of 220 pregnancies in 172 women occurring between 1/1/86-1/1/97 were studied. Mean duration of disease was 12.6± 7.6 years and mean age of onset of disease was 17.6± 7.6 years; mean HbA_{1c} at 10 weeks gestation (HbA_{1c}) 6.3± 1.3%, at 20 weeks 5.9± 1.0% and at 30 weeks 6.0± 1.0% (normal non-diabetic range: 4.0-6.0%, mean 5.0%, SD 0.5%). Sixty percent of the women had a HbA_{1c} <3SD (<6.5%) above the non-pregnant mean. CM were seen in 19 children (8.3%; normal 2.0%)' 15 children had a single defect and 4 children had two or more defects (14 cardiovascular, 4 urogenital, 1 spina bifida, 1

club-foot, 1cleft palate, 1 lung hypoplasia). We found 4.8% CM when the HbA_{1c} value was 4.0-6.0%, 9.1% when HbA_{1c} was between 6.0-7.0% and 11.6% when HbA_{1c} was above 7%. Perinatal mortality was 3.5% (normal 0.9%) (4 stillbirth, 4 neonatal death). In conclusion, these retrospective data suggest that in IDDM-pregnancies with a HbA_{1c} within the normal range the frequency of major CM is still increased.

Gestational Age at Screening, Diagnosis and Management for Gestational Diabetes in a Canadian Community.

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A retrospective chart audit was performed for women delivering at the Kingston General from 1994-1998 to determine at what gestational age women with an eventual diagnosis of gestational diabetes (GDM) received glucose screening and testing. Fifty women were identified with an average maternal age of 31 years (SD 4.6). Thirty-two percent received initial prenatal care from an obstetrician, 66% from a family physician, and 2% from a midwife. A 50 g glucose screen was performed on 48. This was done between 24-28 weeks in 39.6%, at 22 weeks in 4.2% and 29 weeks in 25%; and not done in 31.2%. Of the 33 women seen by a family physician 42.4 % received the 50 g screen between 24-28 weeks. For the 16 seen by an obstetrician, 31.3% were screened between 24-28 weeks. Thirty-three women had risk factors for GDM: family history of diabetes (38%), obesity (30%), previous GDM (18%), previous LGA infant (10%), Native Canadian (4%), and unexplained stillbirth (3%). In the high risk women, a screen was done between 24-28 weeks in 36%, at 22 weeks in 6.1%, after 29 weeks in 21.2%; and not done in 33.3%. One woman received a 75g screen at 28 weeks. Twenty-two high risk women were seen by a family physician and 8 were screened between 24-28 weeks. Ten were seen by an obstetrician and 4 were screened between 24-28 weeks. A 100g OGTT was performed on 49. This was done before 30 weeks in 49%, at 30 weeks in 6.1%, at 31 weeks in 14.3%, at 32 weeks in 4.1% and after 32 weeks in 8.2%. Formal testing was not done in 18.4%. There was a mean delay of 1.5 weeks between a positive screen and formal testing. There was a mean delay of 2 weeks between positive testing and specialist consultation. This study demonstrated that the majority of women diagnosed with GDM are not being screened between the recommended 24-28 weeks. Women with risk factors for GDM and women receiving care from an obstetrician fared no better than the entire population. In many women there was a significant delay between screen, diagnosis and initiation of management.

MONITORING AND NON INVASIVE GLUCOSE TESTING

Non-Invasive Continuous Glucose Monitoring During Physiological Blood Glucose Changes in volunteers and Diabetic Patients.

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Continuous glucose monitoring by means of optical glucose sensors would allow diabetic patients to check their metabolic control at their convenience. In experimental glucose clamp studies with patients with type 1 diabetes mellitus we have demonstrated that non-invasive glucose monitoring is possible by registration of the scattering coefficient of human skin. In order to evaluate if physiological changes in glycemia can also be detected, we monitored blood glucose in 5 healthy volunteers (age 26± 1 y, BMI 22.3± 2.4 kg/m^2) consuming a large breakfast and in 13 patients with type 2 diabetes mellitus (age 57± 8 y, BMI 29.2± 2.0 kg/m^2) receiving an oral glucose tolerance test (75 g) for 4 h. Two simultaneous measurements of the skin tissue scattering coefficient were carried out in each volunteer / patient by means of a portable system in order to evaluate reproducibility. An optic sensor head was attached directly to the skin and light was applied for registration of reflected light intensity. Additionally measurements of the interstitial fluid glucose concentration were performed simultaneously by means of the microdialysis technique (CMA catheter and analyzer.) Blood glucose increased from baseline levels of 3.9± 0.4 mmol/l to maximal values of 12.3± 0.7 mmol/l after 122± 12 min in healthy subjects (with low-dose somatostatin infusion) and from 8.8± 0.8 to 17.1± 2.2 mmol/l after 114± 17 min in diabetic patients. In 8 of the 10 measurements with the volunteers the observed changes in scattering coefficient correlated well with changes in glycemia (linear regression coefficient r=0.75). No correlation was observed in 2 measurements. Reproducibility was good in 3 of the 5 volunteers. In the diabetics, 16 of the 26 measurements showed a good correlation (r=0.77). Two measurements showed a moderate correlation and 9 measurements no correlation. Reproducibility was good in 11 patients. The interstitial glucose concentration showed a good correlation with the intravasal measurements in the patients (r=0.84). This study shows that in principle physiological changes in blood glucose can be monitored by registration of scattering coefficient changes in volunteers and diabetic patients in most, but not all experiments.

Influence of Glucose Test Strip Design on the Accuracy of Test Results.

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Biosensor glucose test strips can be designed to minimize error from testing with insufficient blood or specimens that contain interfering substances. We studied the influence of test strip design on the accuracy of two biosensor systems. The Bayer Glucometer Elite test strip, introduced in 1991, has 2 electrodes (a reference electrode surrounded by an active electrode). The Precision QID test strip, introduced in 1997, contains 3 electrodes (the third electrode is designed to correct for interference from medications and endogenous substances in the blood). In studying the effect of sample sizes (20 replicates at 10, 5 and 1 m L), we found that a small drop of blood (1 m L) incompletely covered the active electrode, where reaction takes place, and lowered the Elite results by an average of 49%; the mean value dropped from 244 mg/dL (13.6 mmol/L) to 125 mg/dL (6.9 mmol/L) when the sample size was decreased from 10 m L to 1 m L. The Precision QID was not attached because the tests did not start with insufficient sample. In that situation, a second blood drop may be applied to the same test strip to complete the test and obtain an accurate reading. We attribute the performance of the Precision QID to the test strip design: the

reference electrode is located further back on the test strip blood is drawn to completely cover the active electrode before it can reach the reference electrode to trigger the test to start. In the interference study, acetaminophen at 55 m L/mL and uric acid at 23 mg/dL falsely elevated Elite results by 20%, and 40%, respectively. Both substances showed a concentration-dependent elevation of the Elite results. When the two substance were combined, the effects were additive. The Precision QID test strip, containing and interference correction electrode, maintained accuracy in all condition tested.

Development of a Rapid, Non Invasive Test for Diabetes Using Fluorescence Spectroscopy.

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We will be describing a new tabletop instrument designed to non-invasively measure optic lens fluorescence. The instrument is used to detect elevated advanced glycosylated end products (AGEs) in the crystalline lens of the patient. Elevated AGEs are a significant symptom of diabetes. The crystalline lens of the eye is excited with blue laser (473 nm), and the fluorescence and scattering intensities are measured. These measurements require only 20 seconds and are used as inputs to a mathematical screening mode, which generates an output. The output is used to determine whether or not the measurement subject has a low or high probability of having diabetes. The instrument uses low-intensity, eye-safe light to measure changes in the fluorescence and scattering properties of the lens related to the glycosylation of the proteins in the lens of the eye. These glycosylation end products build up at faster rates in patients with elevated glucose levels and can be used to distinguish patients with diabetes. The results of multi-site clinical studies using preproduction instrumentation on a population of 800 patients will be presented. Screening for diabetes is difficult since it typically requires a lengthy fasting period and an invasive blood collection. By evaluation the fluorescence products that accumulate in the lens of the eye, one can rapidly screen a patient for diabetes with similar sensitivity and specificity as existing screening approaches. Since no invasive blood collection is required, many of the infection control issues are reduced. As with any screening method, follow-up confirmatory tests will be required to make a final diagnosis.

PATHOGENESIS

Impaired Survival of Differentiating B Cells but no Insulin Secretory Defects Result from Ablation of IRS-2.

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IRS-2 null mice (KO) have been shown to have severe insulin resistance and markedly reduced beta and non beta cell mass that result in rapid progression to diabetes. At both E18 or 2 d of age even with a reduced islet mass evident, KO and multiple ;(5+) apoptotic bodies in beta cells and peri-islet ducts while WT had few to none (0-2) on propidium iodide/insulin immunostained pancreatic sections; mitotic figures were equally frequent (7+) in both. At age 4 wks, the % beta cells incorporating BrdU over 6 hrs did not differ between KO and WT (IRS-2: 1.04± .22 % VS wt: 0.061± . 18 %, N=4 FOR BOTH). Thus, no replication defect was seen. Isolated KO islets were smaller than Wt with a normal ratio of beta/non beta cells and a lower insulin content/DNA (1.77± .15 ng/n vs 2.65± .16), probably due to degranulation from the mild hyperglycemia. So, in 5 independent experiments, islets isolated from 6-8 wk WT and slightly hyperglycemic female KO mice were cultured for 24 hrs. in RPMI 1640, and aliquots of size-matched islets then incubated at 3, 6, 10, 15, 20 or 30 mM glucose in buffered bicarbonate solution. Ko islets showed no impairment of secretion in response to glucose, whether secretion was expressed as ng insulin/ islet per hr or as ng insulin/ng DNA per hr. In summary, beta cells of IRS-2 KO mice have no intrinsic insulin secretory no replication defects but have impaired survival as they differentiate.

Involvement of Smad Proteins in the Differentiation of Pancratic AR42J Cells Induced by Activin A.

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Pancreatic Ar42J cells are derived from a chemically-induced pancreatic tumor and express both exocrine and neruoendocrine properties. Upon treatment with Activin A, ArA2J cells stop growing and their morphology changes significantly. Activin-treated cells express mRNA for GLUT2, ATP-sensitive potassium channel and pancreatic polypeptide. Collectively, Activin A converts AR42J cells into endocrine cells. Many of the Activin-treated cells eventually die by apoptosis unless an additional factor is added. In the presence of either betacellulin or hepatocyte growth factor (HGF), Activin-treated AR42J cells survive and further convert to insulin secreting cells. Smad proteins are now thought to be involved in the action of TGF-B and related ligands. The present study was conducted to investigate the involvement of Smad proteins in the differentiation-induced activity of Activin A in AR42J cells. In these cells, mRNAs for Smad2 and Smad4 were abundantly expressed whereas the expression of mRNA for Smad1 and Smad3 was very low. Activin A induced serine-phosphorylation and the subsequent accumulation of Smad2 in nuclei. Transfection of the N-domain of Smad2 (Smad2-N), which acts as a dominantly negative mutant, blocked the morphological changes induced by Activin A whereas the C-domain of Smad2 (Smad2-C), which acts as a constitutively active mutant, reproduced the Activin-induced morphological changes. Similarly, Smad2-N blocked apoptosis induced by Activin A whereas Smad2-C induced apoptosis. In the presence of HGF, Activin A converted Ar42J into insulin-secreting cells and Transfection of Smad2-N inhibited the differentiation. Smad2-C, however, did not induce differentiation in the presence of HGF, These results suggest that activation of the Smad2 pathway is necessary and sufficient to induce apoptosis and morphological changes, while differentiation into endocrine cells requires the activation of Smad2 and possibly another intracellular signal.

Regulation of the Uncoupling Protein-3 Gene Expression by Thiazolidinediones.

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Uncoupling protein-3 (UCP3) is a recently identified mitochondrial carrier protein expressed highly in the skeletal muscle and brown adipose tissue (BAT). It was reported that the UCP3 gene expression was reduced in the skeletal muscle of NIDDM patient. A subject heterozygous for a nonsence mutation of UCP3 gene was morbidly obese and diabetic. To elucidate pathophysiologic significance of UCP3 in the effect of Thiazolidinediones (TZDs) on glucose metabolism and energy expenditure, we investigated expression of UCP3 gene in Wister fatty rats with 2 weeks oral administration of 3 mg/lg/day pioglitazone, a TZD derivative. In pioglitazone-treated Wistar fatty rats, UCP3 mRNA levels were significantly increased by 2.1-fold, 2.0-fold and 1.6-fold in the epididymal white adipose tissue (WAT), retroperitoneal WAT and BAT as compared with those in nontreated fatty rats, respectively. No significant change of UCP2 mRNA levels was observed. In addition, we examined the regulation of UCP3 gene expression using primary culture of mature adipocytes from SD rats. In cultured adipocytes, UCP3 mRNA levels were increased in a dose-responsive manner by 10^{-5} M^{-4} M pioglitazone, while there was no significant change of UCP2 mRNA levels. These results clearly demonstrate that the UCP3 gene expression is up regulated by TZDs in adipocytes both in Wistar fatty rats and in vitro. The present study suggests the involvement of UCP3 in the effects of TZDs on energy and glucose metabolism. The elucidation of the mechanism of the regulation by TZDs using 5' flanking region of the UCP3 gene is on going.

Leptin Regulates Gene Expression In Brown And White Adipose Tissue Through The Sympathetic Nervous System.

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Exogenous leptin enhances energy utilization in ob/ob mice by binding to its hypothalamic receptor and selectively increasing peripheral fat tissue oxidation. Leptin also increases uncoupling protein 1 (UCP1) expression in brown adipose tissue (BAT) but it has not been established whether this effect is mediated by central or peripheral leptin receptors. The present experiments sought to determine whether leptin regulates UCP1 expression in BAT and its own expression in white adipose tissue (WAT) through modulation of sympathetic tone. Mice lacking the enzyme responsible for synthesizing norepinephrine and epinephrine $dbh^{+/-}$ from dopamine were treated with leptin (20m g/g body weight/day) for 3 days prior to sacrifice. UCP1 mRNA and protein expression were 5-fold higher in BAT from control $dbh^{+/-}$ compared to $dbh^{+/-}$ mice, and leptin produced a 4-fold increase in UCP1 mRNA levels in $dbh^{+/-}$ mice. Leptin had no effect on UCP1 expression in $dbh^{+/-}$, but the b 3-adrenergic agonist, CL316, 243 increased UCP1 expression and established the b -adrenergic responsiveness of BAT from these mice. Similarly, exogenous leptin reduced leptin mRNA in WAT from $dbh^{+/-}$ but not $dbh^{+/-}$ mice. CL316,243 reduced leptin mRNA in WAT from both genotypes. Lastly, db/db mice lacking the long form of the leptin receptor failed to increase UCP1 mRNA in response to exogenous leptin, but increased UCP1 mRNA in response to CL316,243. These studies establish that leptin regulates its own expression in WAT and UCP1 expression in BAT through hypothalamic leptin receptors and modulation of sympathetic tone.

Genome-wide Search for Type 2 Diabetes Susceptibility Genes in Caucasians, Mexican Americans African Americans, and Japanese Americans.

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We have completed a genome-wide screen to identify genes that contribute to type 2 diabetes in samples of 497 Caucasian, 365 Mexican American, 229 African American, and 128 Japanese American individuals collected as part of the ADA's GENNID study. Family and medical histories, anthropometric measurements, and physiological parameters were collected by the individual GENNID investigators. Based on these data, we assigned the phenotypes diabetes and impaired glucose homeostasis according to the ADA criteria rather than the classification developed by the National Diabetes Data Group. The genome-wide screen consisted of approximately 390 markers in each sample. Multipoint nonparametric linkage analyses were performed with diabetes and diabetes or impaired glucose homeostasis (IH). The phenotype diabetes or IH was linked at the p=0.005 level (LOD >1.44) to markers near D5S1404 (LOD =2.66), D9S178 (LOD=1.67), D12S853 (LOD=2.74), and GATA172D05 (on chromosome X; LOD=1.81) in Caucasians, to markers near D2S439 (LOD=1.85) and D3S2432 (LOD=4.17) in Mexican Americans, and to markers near GATA129H04 (ON CHROMOSOME 1;lod=1.84) in Japanese Americans. Further analyses showed a correlation in Caucasians between the extent of allele sharing at the chromosome 5 region indicated above and a region on chromosome 12 containing the MODY 3 gene (HNF-1[alpha]), suggesting the genes in these two regions interact to produce type 2 diabetes. Fine mapping of the chromosome 5 and 12 regions is in progress.

Hexosamine Excise in Liver results in Obesity, Hypertriglceridemia, and Glucose Intolerance.

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Hexosamines are mediatos of cellular glucose sensing and have been implicated in the pathogenesis of type 2 diabetes. We previously reported that transgenic (TG) mice over expressing the rate limiting enzyme in Hexosamine biosynthesis, glutamine: fructose-6-phosphate amidotransferase (GFA) in liver show increased glycogen storage at an early age. We have now followed glucose homeostasis in these animals as they age. Intraperitoneal glucose tolerance testing revealed that by 12-14 months the TG mice had a significant increase in the glucose area under the curve (TG 416± 21 mg[cdot]h; control [C] 334± 43 mg[cdot]h p<0.005). The TH mice were also significantly heavier months (TG 27.8± 6.2 g, 24.5± 3.9 g at 12-14). Free (nonesterified) fatty acid (FFA) and triglyceride levels were studied in the random fed state or after 6, 12, or 24^{th} of fasting in age and weight matched 2-4 month male mice (n=4/group). FFA and triglyceride levels were 4.9-fold and 3.5-fold elevated in random-fed TG mice (FFA: 0.67 vs. 0.14 Mm IN c, P<0.05; triglyceride: 1.34 vs. 0.38 mM in C, p<0.05). With fasting, C animals showed the expected increase in FFA (0.87 mM at 6h, 3.33 mM at 6h, 3.33 mM at

12h, and 1.55 mM at 24h). In contrast, the TG animals showed no change in FFA levels during the fast (0.46 mM at 6h, 0.41 mM at 12h, and 0.64 mM at 24h). The data demonstrate that the Hexosamine pathway not only regulates carbohydrate metabolism as has been previously demonstrated by fat metabolism as well. The data are consistent with a role for the Hexosamine pathway as a general "satiety sensor", shifting fuel utilization towards storage when Hexosamine flux is increased. The GFA transgenic model is therefore similar to the "thrifty" phenotype that has been hypothesized to underlie the syndrome of obesity, insulin resistance, hyperlipidemia, and type 2 diabetes.

Birth Weight And The Insulin Resistance Syndrome: Thrifty Phenotype Or Thrifty Genotype?

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A number of studies have established that low birth weight is associated with the insulin resistance syndrome (IRS) in adult life. Since birth weight is thought to be determined principally by environmental factors, its long-term consequences on the IRS have also been thought to be principally environmentally determined. Recently two genes that affect insulin metabolism, glucokinase and the insulin gene have been shown to influence birth weight, raising the possibility that the inverse association between birth weight and the IRS could have a genetic basis. We examined; these issues in two large family studies of low-income Mexican Americans. The first study involved 1431 individuals in 41 randomly ascertained families and the second study involved 579 individuals in 32 families ascertained on a type 2 diabetic proband. Birth weights were available from birth certificates on 418 individuals from the first study and on 184 individuals from the second. Using maximum likelihood variance decomposition techniques, we partitioned the phenotypic correlations between birth weight and the following IRS-related variables into their genetic and environmental components: anthropometric variables reflecting obesity and body fat distribution; serum leptin; fasting and 2-hour glucose and insulin; total and HDL cholesterol, and triglyceride; and systolic and diastolic blood pressure and heart rate. Intact and split proinsulin were also available, but from the second study only. Except for HDL cholesterol and 2-hour glucose and insulin, all of the genetic correlations were positive. The genetic correlations with blood pressure were low, but the other ranged from .19 to .58 and was statistically significant for percent fat by bioimpedance, serum leptin, fasting insulin, HOMA model for insulin resistance and b -cell function, and heart rate ($p = 0.002$ to 0.040). With the same exceptions, the environmental correlations were all inverse, ranging from -.06 to -.36, and statistically significant for subscapular to triceps skin fold ratio and fasting insulin ($p = 0.041$ and 0.050). We conclude that genes that tend to increase birth weight tend also to increase IRS-related variables, whereas environmental factors act in the opposite direction. The reverse pattern, i.e., an inverse genetic ($p = 0.018$) and a positive environmental correlation, for HDL cholesterol and a weak pattern for blood pressure and total cholesterol are all consistent with this conclusion.

Cow Milk Insulin as Trigger of Cellular and Humoral Immunity to Insulin in Infants with genetic Risk for IDDM

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We have reported that CM (cow milk) formula contains bovine insulin (BI) which induces insulin-binding antibodies in children. We studied the emergence of T-cell responses to BI and human insulin (HI) by peripheral blood proliferation test and insulin-binding antibodies by EIA in children who participated in a dietary prevention study (2^{nd} pilot of TRIGR). All children had a first degree relative with IDDM and carried HLA-risk alleles for IDDM (DQB1*0302 and/or *02 without protecting alleles). They were randomized to receive after exclusive breast feeding either ordinary CM formula or casein hydrolysate (CH) for the first 6-8 months of life. During intervention CM containing foods and beef were avoided. lgG-antibodies to BI and T-cell reactivity to BI were higher in the CM formula group at 3 months of age when compared to the CH group or to infants exclusively breast l-fed (EBF) (table). Antibodies to Bi and Hi correlated at 3 months of age in the CM formula group ($r=0.69$, $p<0.0001$). At 3 months of age none (0 of 14) and at 9 months of age 7 of 21 infants in the CM formula group showed T-Cell responses to HI ($Si>2.0$, $p<0.03$) At 9 months T Cell responses to BI and HI correlated in the CM-group ($r=0.662$; $p<0.001$). The first insulin-specific T-cell response is induced by CM exposure in infants with a genetic risk for IDDM. This immune response later spreads to include reactivity to HI, too.

	IgG (median)	SI (median)	SI>2 (number)
CM	0.210	2.2	9/14
CH	0.113 ($p<0.01$)	1.8 (NS)	3/9 (NS)
EBF	0.151 ($p<0.02$)	1.6 ($p<0.02$)	2/17 ($p<0.007$)

Lack of Suppression of Glucagon After Meal Causes Higher Postprandial Plasma Glucose in Presence of (type-2) Diabetic but not Nondiabetic Insulin Profile.

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People with type 2 diabetes have defects in both [alpha] and b cell function. To determine whether lack of suppression of glucagon causes hyperglycemia when insulin secretion is impaired but not when insulin secretion is intact, twenty nondiabetic subjects were studied on two occasions. On both occasions, a "prandial" glucose infusion was given over 5 hrs while endogenous hormone secretion was inhibited. Insulin was infused so as to mimic either a nondiabetic (n=10) or diabetic (n=10) Postprandial profile. Glucagon was infused at a rate of 1.25 ng/Kg/min beginning at time zero to prevent fall in glucagon (nonsuppressed study day) or beginning at two hours so as to create a transient fall in glucagon (suppressed study day). During "diabetic" insulin profile, lack of suppression of glucagon resulted in a marked increase ($p<0.002$) in both the peak glucose concentration (11.9± 0.4 vs. 8.9 ± 0.4 mM) and the area above basal glucose (927 ± 77 vs. 546 ± 112 mM for 5 hr) due to impaired ($p<0.001$) inhibition of endogenous glucose production (0.19 ± 0.12 vs. –0.90 ± 0.11 mmol/kg) for the first 2 hours. In contrast, during the "nondiabetic" insulin profile, lack of suppression of glucagon concentration (9.1 ±

0.4 vs. 8.4 ± 0.3 mM) and the area above basal of glucose (654 ± 146 vs. 488 ± 118 mM over 5 hours) due to a less impaired (p<0.01) inhibition of endogenous glucose production (-0.57 ± 0.15 vs. –1.08 + 0.06 mmol/kg) for the first 2 hours. Of interest, when glucagon was suppressed, glucose concentrations differed only minimally during the "nondiabetic" and "diabetic" insulin profiles. These data indicate that lack of suppression of glucagon can ;cause substantial hyperglycemia when insulin availability is limited, therefore implying that inhibitors of glucagon secretion and/or glucagon action are likely to be useful therapeutic agents in such individuals.

Manifestations of Syndrome X I Premenopausal Women with or at Risk for Type 2 DM.

Louise Deriso' Darinka Sipula, Mary T Korytkowski, Pittsburgh, PA University of Pittsburgh.*

Androgen excess (AE) is a component of Syndrome X in Premenopausal women with polycystic ovary syndrome (PCOS) and postmenopausal women with type 2 DM. To investigate the presence of AE in eumenorrheic premenopausal women with at risk for or with type 2 DM. SHBG, bioavailable testosterone (BAT), and lipid profiles were obtained before insulin-modified FSIGT in 10 at risk (based on family history or history of gestational DM) and 7 with DM. Ten women without DM or risk factors served as controls ©. All studies took place during the early follicular phase of the menstrual cycle. At risk and DM had higher diastolic (DBP) and mean arterial pressure (MAP) THAN c (77± 3 vs 86± 1 vs 68± 4; 90± 3 vs 100± 2 vs 82± 4, p<0.05). Fasting glucose, insulin (I) and C-peptide levels were higher in DM than in at risk and C (p<0.05). Fasting I correlated with TG (r=0.42, p+0.03) and BAT (r=0.39, p+0.045), but not with HDL, TC: HDL or SHBG. In conclusion, at risk premenopausal women have higher DBP and MAP than C, while those with type 2 DM have even higher DBP and MAP with low SHBG and higher BAT. This suggests that AE is a component of Syndrome X in premenopausal women with but not at risk for type 2 DM.

	Controls	At risk	DM
Age	37 ± 3	41 ± 2	45 ± 2 *
BMI (kg/m^2)	26.9 ± 1.9	28.7 ± 1.9	34.3 ± 2.0 *
WHR	0.79 ± 0.02	0.78 ± 0.02	0.89 ± 0.02 *
Cholesterol (TC)	165 ± 10	154 ± 10	170 ± 6
Triglycerides (TG)	114 ± 35	101 ± 22	185 ± 16 *
HDL	44 ± 4	44 ± 4	32 ± 4 *
LDL	95 ± 7	90 ± 9	103 ± 8
TC:HDL	3.8 ± 0.5	3.8 ± 0.4	5.4 ± 0.4 *
SHBG (nmol)	45 ± 9	53 ± 16	17 ± 19 *
BAT	3.8 ± 0.6	4.7 ± 1.4	7.8 ± 0.7 *

(*p<0.05)

COMPLICATIONS

Methicillin Resistant Staphylococcus aureus: an Increasing Problem in a Diabetic Foot Clinic.

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Diabetic foot ulceration is an important cause of lower extremity amputation, and infection can result in delayed wound healing. We aimed to define the microbiology of foot ulcer infections and study the prevalence and outcome of methicillin-resistant Staphylococcus aureus (MRSA) colonized or infected ulcers in a cohort of diabetic patients in a diabetic foot clinic. Seventy-nine ulcers had swab cultures during the period 1996-1998. Gram-positive aerobic bacteria predominated (56.7%) while Gram-negative aerobic bacteria and anaerobes were less frequent (29.8% and 13.5% respectively). Among Gram-positive aerobes, Staphylococcus aureus predominated and 40% of the strains were MRSA. MRSA isolation from ulcers was significantly more common in patients treated vs non-treated with antibiotics before the swab was taken (p=0.01). The mean duration (range) of MRAS colonization was 16.6 (4-30) weeks, Patients whose foot ulcers were colonized / infected by MRSA had significantly longer time to healing than patients with ulcers infected by methicillin-sensitive Staphylococcus aureus [mean time (range): 35.4 (19-64) weeks and 17.8 (8-24) weeks respectively, p = 0.03]. in conclusion, Gram-positive aerobic bacteria predominated in the swab cultures from diabetic patients with mild and moderately sever foot ulcer infections. We found an alarmingly high prevalence of MRSA, which was related to previous exposure to antibiotics and resulted in prolonged dime to ulcer healing.

High Levels Of Angiostatin In The Vitreous Of Patients With Proliferative Diabetic Retinopathy After Previous Laser Coagulation.

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Proliferative diabetic retinopathy is a major debilitating disease causing most cases of blindness in the western population. It is characterized by abnormal growth of new retinal blood vessels. Growth factors such as insulin like growth factor I have been proposed to initiate retinal angiogenesis. Photocoagulation is the well established therapy of PDR often resulting in regression of retinal neovascularization. However, the molecular mechanism is still unknown. Recently angiostatin, a fragment encompassing the kringle region of plasminogen, has been characterized as a potent inhibitor of neovascularization and therefore is a potential mediator of the positive effect of retinal photocoagultion. Aim: To investigate whether ocular vitreous levels of the angiogenesis inhibitor angiostatin are elevated in patients with PDR after photocoagulation. Methods: Vitreous was obtained from 19 control patients without retinal neovascularization and 32 patients with PDR on occasion of pars plana vitrectomy. 26 out of these patients with diabetes mellitus has had retinal photocoagulation previously. Angiostatin was detected by western blotting using specific plasminogen antibodies. Results: A protein sized 43 kDs, corresponding to the predicted molecular mass of the angiostatin kringle fragment. 1-4 was detected. The K1-4 ban did not appear after preincubation of the antibody with a previously isolated angiostatin K1-4 fragment. Angiostatin could be detected in 2 out of 19 control patients and 29 out of 32 diabetes patients (p<0.00001). However, production of angiostatin in human vitreous correlates also with previous retinal photocoagulation. Conclusions: Angiostatin is not a physiologically expressed angiogenesis inhibitor in human

vitreous. Proliferative diabetic retinopathy and previous retinal photocoagulation are significantly associated with production of angiostatin, suggesting that angiostatin may be the mediator of photocoagulation effects in proliferative diabetic retinopathy.

Retinal Levels Of Occluding are Increased in Background Diabetic Retinopathy and are Associated With Overexpression of Glial Fibrillar Acidic Protein.

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We have recently shown that abnormalities of retinal Muller cells – as detected by increased levels of glial fibrillar acidic protein (GFAP) – occur early in the development of diabetic retinopathy. Because Muller cells contribute to the induction of blood-barrier properties in the retinal vasuclature, dysfunction of these cells could play a role in the increased vascular permeability of background diabetic retinopathy. To test this hypothesis we studied the expression of the tight junction protein occluding and its relationship to that of GFAP in the retina of 10 diabetic patients (age 65± 5 y, diabetes duration 7± 5 y) and 10 age and sex-matched non diabetic donors. We also evaluated the occurrence of vascular leakage by immunohistochemical localization of serum albumin in radial sections of formalin-fixed retina of the same donors. Western blot analysis of retina homogenates, prepared within 36 hours of death, showed a 4-fold increase in occluding levels in the diabetic retinas compared to control retinas (8.5± 2.9 vs 2.3± 1.6 unit/m g of protein, $p < 0.0001$), whereas no significant changes were observed in the levels of another endothelial cell protein (eNOS). When levels of occluding and GRAP were compared in individual retinas, a positive, significant correlation was observed ($r=0.6$, $p=0.006$). Extravasation of serum albumin, as indicated by intense staining of the vessel walls, was present in diabetic retinas only. These data indicate that increased vascular permeability in diabetic retinopathy is associated with altered regulation of tight junction proteins. The concordant increase of occludin and GFPA suggests a possible link between dysfunction of endothelial and Muller cells in the diabetic retina. Experiments are in progress to define the mechanism(s) leading to increased occludin and the involvement of Muller cells in this phenomenon.

Efficacy of the 5.07 Semmes Weinstein Monofilament (SWM) for the Quantitative Assessment of Diabetic Polyneuropathy

Helana Schmid, Ana L Gleisner, Anthero S Ferreira, Cristina Neumann, Porto Alegre, Brzil UFRGS.*

In the previous study the cutaneous perception threshold (CPT) measured as the number or errors out of 54 responses when the 5.07 SWM was applied at the foot showed a high sensitivity and specificity for detecting Diabetic Polyneuropathy (DPN) defined as 2 or more abnormalities in nerve conduction studies. Now the clinical characteristics and CPT results of 30 diabetic patients (10 type 1; 20 type 2) and 26 healthy subjects are presented, according to the presence of: 1) less than 2 nerve conduction abnormalities (NCA), 2) 2 to 5 NCA; 3) 6 NCA and absence of Diabetic Autonomic Neuropathy (DAN); 4) 6 NCA plus DAN. CPT, vibration perception threshold (Biothesiometer – Bio Medical Instruments Co. Ohio), answers to the questionnaire of the Michigan Neuropathy Score, cardiovascular autonomic function tests (Ewing), NC velocity and action potential amplitude of the peroneal, tibial and sural nerve were evaluated. Results are showed in the table. The 5.07 SWM is a sensitive, specific and quantitative tool for detection and staging of early as well as advanced DPN.

Group	CPT(errors)	VPT (volts)	No with Ulcers	No symptoms
1	1.2 ± 2.9	8.1 ± 9.5	0	1.3 ± 1.2
2	3.3 ± 5.0	8.6 ± 10.8	0	1.3 ± 1.2
3	8.7 ± 4.3	18.7 ± 8.3	25%	2.2 ± 1.2
4	31.6 ± 17.6 * †	37.8 ± 13.6 *	67% * †	5.7 ± 2.3

$p<0.05$* 1 vs 4; † 1 vs 3; 2 vs 4.

CLINICAL

Eating Disorders in Adolescent Females with Type 1 diabetes (DM).

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Eating disturbances in adolescent females with DM are common and associated with poor metabolic control and increased risk of diabetic retinopathy. There is ongoing controversy as to whether DM is associated with an increased frequency of eating disorders. Previous studies have lacked the statistical power to detect if differences exist. We conducted a 3 site (Toronto, Ottawa, Hamilton, ON) cross-sectional study to determine the prevalence of clinical (DSM-IV) and sub-threshold eating disorders in 361 adolescent females with DM and 1123 age matched non-DM controls. Subjects with DM represented 85% of those eligible. Controls were drawn from local schools in which 75% of eligible subjects participated. Eating disorder status was assessed using self report measures and a standardized interview (Eating Disorder Examination). Metabolic control was measured in subjects with DM using HbA{1c}. The mean age of the sample was 15± 2 yrs., and mean HbA{1c} for the DM cohort was 8.9± 1.7%. DSM-IV eating disorders were significantly more prevalent among subjects with DM (10.2%) than in the controls (4.8%, P=0.0001). Sub-threshold eating disorders were also more common in adolescents with DM (12.4%) compared to controls (6.5%, P=0.0001.) Those with DM had higher BMI values (22.7± 3.8) than those in the control group (20.6± 3.8) than those in the control group (20.6± 3.3, P=0.0001). DM subjects with DSM-IV and subthreshold eating disorders were older at screening (P=0.003), and older at diabetes onset (p=0.002) compared to non-disordered DM subjects. Mean HbA_{1c} was higher in subjects with DM and DSM-IV eating disorders (9.5± 2.1%) than in the non-disordered DM group (8.8± 1.7%, P=0.05). Omission and/or underdosing of insulin for weight loss was reported 11.4% of adolescents with DM, which is similar to previous finding. Clinical and sub-threshold eating disorders are more prevalent in young females with DM than in their non-diabetic peers, and are associated with impaired metabolic control. These finding support the role of DM and its treatment in the onset or

maintenance of eating disturbances in susceptible adolescent females.

Acanthosis Nigricans and Acrochordons in Insulin Resistant Subjects With and Without Glucose Intolerance: A Study in Three Ethnic Groups.

Paul Y Casanova-Ramero, Hermes J Florez, Ronald B Goldberg, Miami, Fl University of Miami School of Medicine.

Paul Y Casanova-Ramero, Hermes J Florez, Ronald B Goldberg*, Miami, FL Acanthosis nigricans (AN) and acrochordons [or skin tags (SKT)] are skin lesions that have been associated with type 2 diabetes, insulin resistance (IR) and obesity. We have explored the relationship between glucose tolerance, the presence and extent of AN and SKT, (using both site-specific and global scoring systems), and components of the IR syndrome. The study population consisted of 94 White non-Hispanic (WHN), Hispanic (H), and African American (AA) adults with a positive family history of diabetes and/or obesity who had entered a screening program to detect glucose intolerance. AN was present in 72% of subjects (cervical 68%; axillary 49%), while SKT were detected in 54% (cervical 22%; axillary 43%). Although there were no differences in prevalence or extent of AN and SKT between those with normal (n=54), impaired (n=19) or diabetic (n=19) OGTTs, global AN and SKT scores each correlated with fasting glucose levels (AN; r=0.22, p=0.036: SKT; r=0.29, p=0.005). There was a greater prevalence of axillary and cervical AN in AA and H compared to WNH chi^2=39.1, p<0.001 and chi^2=20.2, p=0.01, respectively). Conversely, axillary SKT were more frequently in H and WNH compared to AA chi^2=7.0, p=0.03), while cervical SKT did not differ significantly among ethnic groups chi^2=4.9, p=0.08). IR (measured by the homeostasis model assessment [HOMAIR]), ethnicity (AA and H) and body mass index (BMI), were associated with the global AN score (model R^2 = 0.52, p=0.001), after controlling for age, sex, family history of diabetes, and presence/absence of glucose intolerance. The global SKT score was also associated with HOMA IR and BMI (model R^2 = 0.27, p < 0.01), after adjusting for the same factors. These results suggest that while AN and SKT may be useful screening tools in identifying glucose intolerance, they are strong indicators of IR independent of glucose tolerance categories, and are influenced in their location and severity by ethnicity and BMI.

Finding from the Global LEA Study.

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Objective: To compare the incidence of lower extremity amputations (LEA) in different centers around the world. Setting: 11 participating centers, all with populations greater than 250,000, in Western Europe, Japan, Taiwan and North America. Main Outcome Measures: Incidence of first ever and all major and minor amputations and the proportion associated with diabetes. Methods: A standard data collection form was developed and data were collected using multiple sources with checks on ascertainment levels using capture-recapture. Denominator population data were collected from each center and both crude and age-adjusted rates were calculated. Results: Marked differences in amputation incidence rates were found between different centers around the world, most notably between the Navajo Indian population and the populations of Tochigj, Japan and Madrid, Spain. The Navajo center also had the higher proportion of patients undergoing amputation where diabetes and/or infection were underlying causal factors. Conclusion: The study has enabled meaningful and realistic comparisons to be made of the incidence of lower extremity amputations in different centres and over time. Marked differences in rates have been found; even between centres such differences require further investigation.

Skin Manifestations of Diabetes Mellitus.

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The skin is a frequently overlooked source of pathology in diabetes mellitus except in cases where this is the reason for the diagnosis of the disease or there are serious manifestations of diabetes mellitus. This is because most diabetics do not frequently complain of skin disease unless it is a source of significant discomfort. In this study the skin disorders of 134 (72 females and 62 males) consecutively admitted cases of diabetes at our center were noted. Ages ranged from 11-76 years, 63 (47.0%) cases were type 2 and 71(53.0%) cases type 1. In this group, 27(20.1) patients had no significant skin pathology while, of the remaining patients, the commonest disorders were dry soles found in 35(26.1%) cases, hyperkeratosis plantaris in 15 (11.9%) cases, tinea pedis 25(18.7%) of cases while total number of fungal infections was 52 (38.8%). Other disorders included Necrobiosis lipoidica 2(1.4%), diabetic shin spots 4 (2.9%). There was a high incidence of cherry nevi present in these cases 21 (15.7%) which may also be related to the higher incidence of liver disease in diabetic patients. The commonest disorders associated with diabetes in this group of patients comprised fungal infections 38.8% and dry soles related to peripheral neuropathy 26.1%. these disorders, though not frequently cause for severe discomfort in our patients are high risk factors for the development of the diabetic foot and thus always require treatment. Other disorders like necrobiosis lipoidica though generally rare are a source cosmetic concern and my thus affect the quality of life of these patients.

Thyroid Disease Prevalence in Diabetic Patients.

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The association between thyroid disease and type 1 diabetes mellitus (DM1) is well known, but is not clear in the case of type 2 diabetes mellitus (DM2). The aim of this study was to assess the prevalence of thyroid dysfunction (TD) and autoimmune thyroiditis (AT) in patients with DM1 and DM2. We included 266 diabetic patients without known thyroid disease: 128 with DM1(69, 59) and 138 with DM2 (58, 80). TSH, FT_{4} and antibodies to thyroid microsomal (TMA) and thyroglobulin antigen (TTA) were measured. AT was defined on the basis of positive results of TMA and/or TTA tests: and TD when TSH values were out of the normal range. Statistical analysis was made by Chi-Square and t-tests. The descriptive data (mean ± SD) and prevalence results were: No association was found between AT and type of diabetes, however TD was significantly higher in DM2 (p=0.039). In the whole diabetic group, TD and AT

prevalances were higher in females (p = 0.02 and p = 0.018 respectively). Moreover, TD was positively associated with AT (p = 0.001) and age (p = 0.026). In conclusion, these data show a high prevalence of AT in both groups of diabetes and a greater prevalence of TD in the DM2 one, probably due to the higher age of the patients in this group.

TREATEMENT

Adjunctive Therapy with Inhaled Human Insulin in Type 2 Diabetic Patients Falling Oral Agents: A Multicenter Phase II Trial.

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Patients with type 2 diabetes mellitus failing oral agent therapy often resist initiating insulin therapy, at least partially due to the need for injections. A reproducible dry powder aerosol delivery system has been developed to dose human insulin without injection. We asked whether type 2 diabetics falling oral agent (OA) therapy can improve glycemic control by adding pre-meal inhaled insulin (INH) to their usual OA. Study entry required HbA_{1c} > 8.0% despite therapeutic doses of a sulfonylurea (e.g., > = 5mg/d glyburide) and/or metformin (> = 1.5 g/d). After a 1-mo run-in, 69 subjects from 9 sites were randomized to a 3-mo treatment period of either continued usual OA alone or usual OA plus INH,1-2 puffs TID before meals. INH doses were titrated based on QID glucose testing. For the 66 subjects with complete data, baseline HbA_{1c} was comparable in the 2 groups: 9.92± 1.32% for OA alone (n=35) and 9.78± 1.28% for OA plus INH (n=31). While patients on OA alone showed little change in HbA_{1c} after 12 wk (-0.13± 1.15%), those receiving INH plus OA exhibited a marked improvement in HbA_{1c} (-2.28± 1.17%, p < .0001 vs. OA alone).INH has been very well tolerated. There was only one report of severe hypoglycemia (home BG=54mg/dl). Pulmonary function testing showed no change over the 3 mo study. We conclude that in patients with type 2 diabetes failing oral agents, a no-injection regimen with adjunctive INH therapy markedly improves glycemic control with low risk of hypoglycemia.

Use of Insulin Pump Therapy At Night Only for Pre-Teen Children with Type 1 Diabetes.

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Although continuous subcutaneous insulin infusion via an insulin pump has gained wide-spread acceptance in adolescents & adults with type 1 diabetes, it may have limited usefulness in pre-teen children because they may not be developmentally capable of managing the pump without parental supervision. As a result of this, we evaluated the efficacy of pump therapy (MiniMed 507C) only at night in 10 children, ages 7-10 years. Children were randomly selected based on inclusion criteria; mean duration of diabetes was 3.4 ± 1.6 years, mean HbA1C of 7.6 ± 0.9% and patients took 0.9 ± 0.3 units of insulin/kg/day. After 4 wks of 3 insulin injections/d plus supplemental correction doses per standardized algorithm, a cross-over design with random assignment to either 3 insulin injections/d or pump at night (pump used for dinner insulin & throughout the night with a.m. insulin injection of lispro and NPH; total daily dose distributed as 60% injectable and 40% pump) was implemented. Study time on pump and injections was 4 wks with a 2 wk stabilization period for pump initiation. Data collection included fructosamine levels & 5 BG levels/d to determine changes in diabetes control as outlined in Table 1. Scores of fear of hypoglycemia were pre 27 ± 15 and post 12 ± 9 (p=0.02) & for adherence were pre 77 ± 11 & post 86 ± 6 (p=0.04). These data suggest that the use of the insulin pump at night only in pre-teen children improves overall glycemia & BG levels at breakfast, bed & in the night without causing an increase in hypoglycemia. Insulin pump therapy at night only may be a viable option for pre-adolescents who need to improve glucose control but who are not yet ready to mange the pump during times when there is no parental supervision.

Table 1

	Fruc	Mean BG	AVI	Lunch	Din	Bed	0300	%Tar	%	%
Pre Pump	391 ± 58	186 ± 32	196 ± 31	137 ± 39	191 ± 54	141 ± 31	173 ± 49	36 ± 8	21 ± 10	43 ± 13
Post Pump	345* ± 37	160* ± 20	125* ± 31	157 ± 39	183 ± 42	118* ± 14	132* ± 25	44* ± 7	15 ± 6	41 ± 11
Pre Inject	354 ± 36	172 ± 38	167 ± 50	133 ± 30	186 ± 47	128 ± 35	170 ± 53	40 ± 8	18 ± 9	43 ± 15
Post Inject	390** ± 46	181 ± 34	185* ± 35	146 ± 39	191 ±	156** ± 28	178** ± 46	37** ± 7	20 ±	43 ±

* p < 0.05 pre vs post pump
** p < 0.05 post pump vs. post injection

Improved Glycemic Control Induced by Dietary Monounsaturated Fatty Acids is Mediated by Enhanced Secretion of Glucagon-Like Peptide-1

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Diets enriched in monounsaturated fatty acids (MUFAs) have been shown to benefit glycemic control. Furthermore, MUFAs specifically stimulate secretion of the antidiabetogenic hormone, Glucagon-Like Peptide-1 (GLP-1) in vitro. To determine whether the MUFA-induced benefit in glycemic control was due to increased GLP-1 release, lean Zucker rats were fed a synthetic diet containing 5% fat derived from either Olive Oil (OO; 74% MUFA) or Coconut Oil (CO; 6% MUFA) for 2 wk. Food Intake and body weight gain were similar for both groups over the feeding period. The OO group had improved glycemic tolerance as compared to the CO group in both oral and duodenal glucose tolerance tests (AUC 121 ± 61 vs 290 ± 24 mM[cdot]120min, P < = 0.05; 112 ± 28 vs 266 ± 65 mM[cdot]120min, P < = 0.05, respectively). This was accompanied by increased secretion of gut Glucagon-Like Immunoreactivity (gGLI; an index of GLP-1) in the OO rats as compared to the CO rats (402 ± 96 vs 229 ± 33 pg/ml at t = 10 min, P < = 0.05). Tissue levels of GLP-1 and plasma glucagon levels were not different between the two groups. To determine the total contribution of GLP-1 to the enhanced glycemic control, the GLP-1 antagonist exendin^{9-39} (Ex^{9}) was infused 3 min prior to a duodenal glucose tolerance test. This abolished the benefit in glycemic control conferred by OO feeding (OO+Ex^{9} vs

CO+Ex^{9}, P=NS), and resulted in a deterioration of glycemic control in the OO+Ex^{9} group when compared to the OO controls (AUC 331± 21 vs 112 ± 28) mM[cdot]120min, P < = 0.05). This was partially due to increased release of glucagon with Ex^{9}. To probe the mechanism by which the MUFA diet improved GLP-1 secretion, an in vitro model of the GLP-1 secreting L cell was incubated for 24 h in the presence of either 100m M palmitic acid (Saturated FA) and subsequently challenged with Glucose-dependent Insulinotropic Peptide (GIP), a known stimulator of the L cell. Pre-exposure to oleic acid significantly increased GIP-induced GLP-1 secretion when compared to controls (178 ± 14 vs 132 ± 9 pg/ml, P < = 0.01). However, palmitic acid pre-exposure abolished GLP-1 secretion induced by GIP. In conclusion, these results demonstrate that the benefit in glycemic control obtained with MUFA diets is the result of increased GLP-1 secretion through a mechanism of enhanced L cell sensitivity. These results suggest that diet therapy with MUFAs may benefit the treatment of impaired glucose tolerance through increased GLP-1 secretion.

Potent Enterogastrone Actions of the Insulinotropic Hormone, Glucagon-Like Peptide-1 (GLP-1): Mechanisms of Action.

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The insulinotropic gut hormone, GLP-1, has turned out to be one of the most potent entergastrones (inhibitors of gastric emptying and secretion) in man, an effect that contributes greatly to its glucose-lowering effects. As a result, near-normalization of blood glucose may be brought about by iv infusions of GLP-1 in patients with type II diabetes. The hormone is metabolized with a half-life of 1-1.5 min and its clearance exceeds cardiac output by a factor of 2, indicating that only a fraction of the secreted hormone reaches its targets by way of the circulation. Experiments involving isolated prefused small intestine have shown that 50-70% of the peptide is metabolized already before it leaves the intestine, presumably catalyzed by the enzyme, dipeptidyl peptidase IV, located in capillaries draining the intestinal mucosa, harbouring the GLP-1 secreting cells. How is the compatible with the hormonal function of GLP-1? We studied the effects of GLP-1 on gastric motility and secretion stimulated by insulin hypoglycemia in anesthetized pigs (n = 8) equipped with transducers sewn onto the antral serosa. GLP-1 at 2 pmol/kg x min nearly abolished secretion and motility. The effect was abolished by vagotomy. Near-brain arterial infusion (carotid and vertebral arteries) required similar infusion rates to be effective, indicating that the effect was not exerted centrally. In isolated prefused porcine antrum (n=7) GLP-1 (0.01 – 10 nmmol/l) had no effect on motility stimulated by electrical stimulation of the vagus nerves, indicating that GLP-1 does not inhibit peripheral, efferent vagal effects. Finally, in anesthetized pigs prepared as above, we isolated completely and perfused the stomach in situ, so that it was attached to the pig only by its intact vagus nerves. Insulin hypoglycemia in the pig greatly stimulated antral motility. IV infusion in the pig of GLP-1 in doses up to 20 pmol/kg x min had no effect, whereas infusion into the gastric arterial perfusate (1 nmol/L, N = 6) strongly inhibited antral motility. We conclude that the enterogastrone activity of GLP-1 involves inhibition of ventral parasymthetic outflow, transmitted to the brain via vagal afferents. Presumably, the local degradation occurs after this interaction has been taken place.

Cellular Engineering Approaches to the treatment of IDDM.

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We have discovered that intermediate lobe (IL) pituitary cells can be engineered to produce insulin (ins) at sufficient levels to cure diabetes in NOD mice but, in contrast to transplanted islets, Ilins cells are resistant to immune-mediated attack. In an attempt to introduce glucose sensing properties into Ilins cells, we constructed recombinant adenoviruses containingGLUT-2 (Ad-GLUT-2) and the islet isoform of glucokinase (Ad-GK). While GK activity was not detectable in primary cultures of Ilins cells, transduction with ad-GK resulted in high levels of functional GK activity, comprising > 90% of the total glucose phosphorylating activity. Likewise, treatment of Ilins cells with Ad-GLU-2 resulted in glucose transport kinetics similar to ß cells. To determine whether either of these manipulations, alone or in combination, could confer glucose sensing capabilities to Ilins cells, the conversion of 5-[^{3}H] glucose to ^{3}H_{2}) was measured at 0.3, 3, and 20 mM glucose. Although the effects of treatment of Ilins cells with either virus alone was minimal, the co-transduction of Ad-GLUT-2- and – GK resulted in a marked stepup in metabolism over the physiologic range of 3 to 20 mM glucose, with glucose usage increasing from 1.61 ± 0.27 to 3.20 ± 0.60 nmol/h/m g protein, respectively. In contrast, in control AdLacZ transduced cells glucose usage from 3 mM to 20 mM glucose remained essentially unchanged (0.84 ± 0.13 to 0.97 ± 0.23 nmol/h/m g protein). These findings demonstrate that the co-expression of GLUT-2 and GK can confer glucose-sensing capabilities in the physiologic range to Ilins cells. Using transgenic mouse techniques, we have recently created NOD Ilins cells that stably express GLUT-2 and GK. These studies represent the first important steps towards the creation of immunoresistant b -cell surrogates for the treatment of diabetes.

Low-Dose Rosiglitazone (RSG) provides Additional Glycemic Control When Combined with Sulfonylureas in Type 2 Diabetes (T2D).

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RSG improves insulin sensitivity and reduces hyperglycemia at doses up to 8mg/d as monotherapy in T2D. Efficacy of low doses of RSG (2 & 4mg, as 2 divided doses) and PBO, added to existing SU therapy, were assessed after 6 mos in 574 T2D patients in this randomized, double-blind study. Patients had been raking gliclazide, glibenclamide, or glipizide for >=6 mos before the study. RSG produced clinically and statistically significant reductions in HbA1c and FPG, with greatest effects at 4mg/d. The improvements in HbA1c were the same, regardless of the SU used. Decreases in FFA (c.15%) and increases in HDL (C.10%) & LDL (c.5%) were seen with RSG4mg/d. The overall incidence of AEs was similar in all 3 groups, with no significant hypoglycemia or hepatotoxicity. RSG 4mg/d is

safe, well tolerated, and effective when added to SU therapy. Its benign safety profile at 4mg/d supports the investigation of higher doses of RSG in combination with Sus.

	SU Alone	SU+RSG 2mg/day	SU+RSG 4mg/day
HbA1c (%)	(n=192)	(n=199)	(n=183)
Mean baseline	9.2	9.2	9.2
Mean D from Baseline ± SD	+0.2±1.11	-0.5*±1.05	-0.9*±1.10
Difference from SU alone	NA	-0.6*	-1.0*
% with reduction 30.7%	19%	39%†	60%†
FPG (mg/dl)			
Mean baseline	207.3	203.7	205.4
Mean D from baseline ± SD	+5.8±49.4	-17.1*±48.5	-37.7*±47.2
Difference from SU alone	NA	-24.3*	-43.9*
% with reduction 30mg/dl	21%	38%†	56%†

* P < 0.0001; † P < 0.0001 for comparison with SU alone

BAY 27-9955, A novel, Non-Peptide Antagonist of Glucagon Binding to the Glucagon Receptor.

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BAY 27-9955 (BAY), a small molecular weight compound, blocks binding of glucagon to the glucagon receptor. BAY is species-selective, having the highest affinity for human and dog receptors (IC-50 values of 110 nM and 140 nM, respectively), followed by the mouse and rabbit receptors (400 nM and 700 nM), and lowest affinity for the rat receptor (8000 nM). With the human receptor, BAY inhibited glucagon-stimulated camp generation with an IC-50 value of 46 nM. Schild plots indicate that the inhibition was competitive with glucagon, being characterized by a pA_{2} value of 7.8. The inhibitory effect of BAY on glucagon action was fully reversible. Chimeric receptors comprised of human and rat amino acid sequences that retain full glucagon binding activity were used to map the BAY site on the receptor. a rat chimera with human 6^{th} transmembrane amino acid sequences was sensitive to BAY whereas rat sequence in these domains were insensitive to the compound. Site directed mutagenesis showed that lys-388 in the human receptor was required for full inhibitory activity. BAY has little effect on the GLP-1 receptor or on a variety of other receptors ;(- 23 tested). In conclusion, BAY is a highly specific, species-selective antagonist of glucagon binding and action. Its ability to lower plasma glucose in patients with type 2 diabetes is under investigation.

An Amylin Antagonist has opposite effects to Amylin on Basal Lipids and Counteracts Insulin Resistance in high Fat Fed Rats.

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Lipid accumulation in muscle and liver is associated with insulin resistance. Although amylin has been implicated in insulin resistance, its role in lipid metabolism is not clear. We investigate the effect of amylin on lipid metabolism and insulin resistance in rats. In normal chow-fed rats, rat-amylin infusion (5nM/h, iv) elevated plasma tree fatty acids (FFA), glycerol, triglycerides, glucose and insulin over a period of 4 hrs. (all p<0.001, repeated measures ANQVA) with increments of 83%, 82%, 45% 13% and 46%, respectively. In contrast, rat-amylin-(8-37) (125nM/h), a specific but relatively low affinity amylin antagonist, lowered FFA (-32%), glycerol (-30%), glucose (7%) and insulin (-35%) (all p<0.05) by itself and blocked elevations of these parameters induced by amylin (all p<0.01). In insulin resistant high fat fed (3 wks) rats, infusion of amylin-(8.37) (5.5h) markedly increased the glucose infusion rate (19.0 ± 1.7 vs saline control 11.4± 0.5 mg/kg.min, p<0.01) during euglycemic-hyperinsulinemic clamp (insulin 0.25U/kg.h). This was associated with both enhanced insulin-induced suppression of hepatic glucose production (1.5± 0.5 vs 5.1± 0.5 mg/kg.min, p<0.01) and increased skeletal muscle glucose metabolic index [24.3± 3.4 vs 12.8± 0.7 mM/100g.min), p=0.01]. During the clamp, amylin-(8-37)-infused rats had significantly lower plasma triglycerides (0.59± 0.03 vs 0.94± 0.l7 mM, p<0.05), FFA (0.31± 0.05 vs 0.68± 0.12 mM, p<0.02) and glycerol (0.33± 0.03 vs 0.62± 0.11 mM, p<0.02). We conclude that in the basal state amylin and the amylin antagonist amylin-(8-37) have reciprocal effects to increase and decrease circulating lipids, respectively. In high fat fed rats, amylin-(8-37) substantially ameliorates muscle and liver insulin resistance. This study supports a role of amylin in insulin resistance, possibly via modulating lipid availability.

Losartan Improves Insulin Sensitivity and Modifies Glomerular Hyperfiltration In Type 1 Diabetes.

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The effect of the angiotensin II receptor antagonist, losartan on insulin mediated glucose disposal and renal hemodynamics was examined in normotensive, normoalbuminuric Type 1 diabetic patients using a double-blind, placebo controlled, cross-over design. Diurnal blood pressure, GFR (125, iothalamate), RPF (131, hippuran), UAE were measured and a hyperinsulinaemic, euglycaemic clamp with indirect calorimetry was performed in 9 patients (age 30± 7 years (mean ± SD), HbA_{1c} 8.1 ± 1.1%) following 6 week losartan 50 mg/day and 6 weeks placebo. Diurnal blood pressure was significantly reduced after losartan compared with placebo (122/70± 11/8 vs 130/76± 12/6 mmHg, p<0.05). Isotopically determined glucose disposal rates were similar after losartan and placebo in the basal (2.61± 0.53 vs 2.98± 0.93 mg/kg/min) and insulin stimulated states (6.84± 2.52 vs 6.97± 3.11 mg/kg/min). However, glucose oxidation rate increased significantly after losartan vs placebo in the basal state (1.72± 0.34 vs 1.33± 0.18., mg/kg/min, p<0.01) and during insulin stimulation (2.89± 0.75 vs 2.40± 0.62 mg/kg/min, p<0.03). Basal and insulin stimulated non-oxidative glucose disposal tended to decrease, however not significantly, after losartan. Endogenous glucose production and lipid oxidation were unchanged after treatment and similarly suppressed during hyperinsulinaemia. Glycaemic control, FFA total cholesterol, HDL-cholesterol, triglycerides, and metabolites were stable during losartan and placebo. A significant decline in GFR (133± 23 vs 140± 22 ml/min, <0.05) and

filtration traction (GFR/RPF) (24.6± 3.5 VS 26.2± 3.6%, P<0.05) was observed during losartan vs placebo. RPF and UAE did not change. In conclusion, losartan reduces blood pressure, glomerular hyper filtration and filtration fraction and improves glucose oxidation in normotensive, normoalbuminuric Type 1 diabetic patients.

Rosiglitazone (RSG) does not Increase the Risk of Alcohol Induced Hypoglycemia in Diet-Treated Type 2 Diabetics.

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RSG, a potent thizolidinedione insulin sensitizer, is an effective glucose (Glc) – lowering agent that is not expected to induced hypoglycemia. To determine if RSG increased the Hypoglycemic risk of moderate alcohol consumption, type 2 diabetics previously treated with diet and exercise were randomized to receive 8mg RSG (n=11) or placebo (n=12) once daily for 8 wks. On 2 separate occasions during the final week, subjects randomly ingested either ethanol (E) 0.6g/kg body weight or a non-ethanol control (NE) with a standard evening meal. Plasma Glc was measured over the 14-hr interval following the start of the E/NE challenge. Glc concentrations were analyzed by ANOVA and point estimates (Pt Est) and 90% confidence intervals (Cl) derived for the ratio of E:NE values. Lack of effect was prospectively defined if the 90% Cl was contained within a 30% equivalence range. There were no episodes of hypoglycemia. There were no clinically meaningful ethanol-associated reductions in plasma Glc at either the 4-hr or 14-hr time point. Plasma Glc concentration-time profiles over the 14-hr interval were similar between regimens. No clinically meaningful changes in overnight urinary cortisol: creatinine ratios, another index of hypoglycemia, were observed prior to or after ethanol vs. non-ethanol challenges. These results demonstrate that RSG does not increase the risk of hypoglycemia associated with occasional moderate ethanol consumption with a meal.

Time (hours)	Treatment	E:NE Pt Est	90% CI
0 (Prechallenge)	RSG	0.90	(0.73, 1.08)
	Placebo	0.90	(0.76, 1.04)
4	RSG	1.12	(1.00, 1.24)
	Placebo	1.04	(0.95, 1.13)
14	RSG	1.01	(0.92, 1.10)
	Placebo	0.96	(0.89, 1.03)

Synergistic Anti-hyperglycemic Activities of Zinc, Cyclo(His-Pro) and Arachidonic acid.

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We have previously shown that oral administration of animal prostrate extract improved oral glucose tolerance, and lowered blood Hba_{1c}, insulin and glucose levels in both animal and human subjects with diabetes. Since animal prostate contains the highest amount of zinc, prostaglandins, cyclo (his-pro) (CHP) of all other organ tissues, we have examined the effects of oral administration of chemical mixtures of zinc, arachidonic acid (AA), CHP and L-histidine (L-His) on the blood glucose levels of streptozotocin-induced diabetic rats. When the diabetic rates were given 11 mg AA (the optimal level), 0.5 mg L-His and 5 mg zinc/kg body weight in drinking water, blood glucose levels decreased from 393 to 184 mg/dL in 5 days. When 0.3 mg CHP/kg (the optimal level) was given with the same amounts of L-His and zinc, blood glucose levels decreased from 422 to 192 mg/dL in one day. Water consumption of those rats given distilled water was 1,24 L/kg/day, while those given water containing zinc, AA and L-His was 0.61 L/kg/day and 0.45 L/kg/day when zinc, CHP and L-His were added to the drinking water. These results suggest that oral intake of a capsule containing proper amounts of zinc, AA, CHP and L-His can be a novel anti-hyperglycemic agent for the treatment of patients with diabetes.

Fluid Silicone Prevention of Calluses and Diabetic Ulcers: A 35-Year Report.

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Loss of plantar fat is a common finding with calluses and neuropathic ulcers. From 1964 though 1998, fluid silicone (dimethylpolysiloxane 350 cs viscosity) was independently studied as an injectable soft tissue prosthesis for weightbearing press reduction. Each patient was fully informed that injecting fluid silicone was experimental and not federally approved. Among 1450 patients treated for corns, calluses or atrophic fat pads, 38 had diabetic neuropathic ulceration, which in 37 was preceded by painless callus. Four had Charcot joint. After healing, each site received serial subdermal doses of 0.10-0.25 ml and total amounts of 0.65-5.0 ml, mean 1.25 ml. Only Charcot foot patients wore special shoes postimplant. All others wore standard shoes. In 1-25 yrs follow-up, mean 7 yrs, 27 of 38 ulcers (71%) did not recur. Including all patients, rare fluid migration requiring surgical removal was the only adverse reaction of significance. Infection, fluid rejection, inflammation or allergic reaction, was not seen. Host response was analyzed by light and electron microscopy from 33 surgical biopsies and 134 skin specimens gathered from 35 patients postmortem. Tissue samples studied 1-29 yrs postimplant, mean 13.9 yrs, found an essentially noninflammatory fibrosis and histiocytosis. The efficacy of injected silicone to increase skin thickness and reduce plantar pressure has recently been confirmed by a randomized placebo controlled double-blind trial. The authors conclude that silicone fluid safely and effectively decreases callus and neuropathic ulcer formation, and that fluid drift is a nonsignificant risk compared to the potential of preventing ulceration and amputation.

LIPIDS AND OBESITY

Fasting and Postprandial Abnormalities in Triglyceride Metabolism are Greater in Diabetic Women with Coronary Artery Disease.

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Type 2 diabetes mellitus (DM) is associated with greater increases in risk of premature coronary artery disease (CAD) in women than in men. Although some previous studies have suggested that DM has a greater impact on fasting lipid

levels in women, little is known about gender-specific effects of DM on Postprandial lipemia (PPL). Since PPL has been shown to predict the presence of CAD Sin nondiabetic subjects, we studied PPL in 28 men and 12 women with CAD and DM and 18 men and 9 women with CAD but without DM. The groups were matched by age and BMI. The patients received a high-fat liquid meal containing 105 gms fat and 100,000 units vitamin A per $2m^2$ body surface. Fasting and Postprandial lipids and lipoproteins were measured by conventional methods. Total cholesterol (C) and high density lipoprotein (HDL) C levels were significantly lower in the DM group but low density lipoprotein C levels were similar in the DM and nonDM groups. When patients were divided by gender, fasting triglyceride (TG) levels (mg/dl± %SE) were 170.4± 14% in DM women vs 117.5± 15% in non DM women (p=0.08) and 147± 11% in DM men vs 162.5± 12% in nonDM men (p=0.54). Fasting HDL C levels were lower in DM women (37.7± 2.8 vs 51.9± 4.7, p=0.02 and in DM men (29.5± 1.2 vs 35.7± 2.1, p=0.01). The area under the postprandial TG curve (AUC) was greater in DM women (1292.3± 19%) vs nonDM women (630.6± 24%) but was similar in DM and nonDM men (1189.1± 9% vs 1159.8± 16%). Triglyceride-rich lipoprotein (TGRL)-TG AUC (isolated at d<1.006) was increased in both DM women (1032.8± 21% vs 429.7± 22%) and DM men (1023.1± 10% vs 682.4± 17%) compared with nonDM subjects. Retinyl palmitate AUC, used as a measure of chylomicron remnant clearence, did not differ between DM and nonDM subjects of either gender. These studies, conducted in patients with CAD, indicate that DM has a greater impact on fasting levels of TG and HDL C in women than in men. Additionally, these results indicate that although DM was associated with increased PPL in both genders, the effect of DM was more pronounced in women with DM, affecting both TGRL and total TG AUCs. Together with luck of a gender difference in remnant clearence, our result suggests that the gender-specific effects of DM are related to defects in lipolysis of TG in all lipoproteins postprandially. This findings may, in part, explain the greater impact of DM on CAD risk in women compared to men.

Effects of Dietary Fish Oil Supplementation in Type 2 Diabetes: A Cochrane Systematic Review.

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Hypertriglceridemia is the commonest lipid disturbance in type 2 diabetes mellitus (DM2). Dietary fish oil (FO) supplementation is known to lower triglycerides (Tg) in non-diabetic individuals but may lead to deterioration in glycemic control in DM2. the randomized controlled trial (RCT) is the optimal way to assess an intervention for efficacy and safety. We undertook a systematic review of RCTs assessing the effects of FO in DM2. Using a combination of search strategies (MEDLINE, EMBASE, the Cochrane Clinical Trials Registry, handsearching and expert input) updated through September 1998 we identified 158 articles of which 63 were considered relevant based on title; 18 RCTs were included in the final analysis (11 crossover and 7 parallel group design). Of these, 4 recruited patients with baseline hypertriglyceridemia (> 200 mg/dl). Using methods developed by the Cochrane Collaboration we independently assessed study quality, abstracted and combined data from the 18 RCTs. The pooled data set included 823 subjects (range 8 to 418) followed for a mean of 12 weeks (range 2 to 52). Ten studies were of high quality Doses of FO ranged from 1.7 to 10 g/d. Weighted mean differences derived from meta-analysis of the pooled data demonstrated a statistically significant effect of FO on lowering Tg (-49.6 mg/dL 95% Cl –36 to –63) and raising LDL cholesterol (+8.2 mg/dL 95% Cl 0.6 to 15.8). no statistically significant effect was observed for fasting plasma glucose (4.72 mg/dL 95% Cl-1.5 to 10.9), HbA1c (0.14% 95% Cl –0.08 to 0.37), total cholesterol (0.28 mg/dL 95% Cl –5.23 to 5.8) or HDL cholesterol (0.76 mg/dL 95% Cl –0.59 to 2.13). The Tg lowering effect was most marked (-64.8 mg/dL) in the RCTs, which recruited hpertriglyceridemic subjects. Heterogeneity was observed and could be accounted for by the presence of a single large RCT and selection of study participants with and without baseline elevations in Tg. Trail design, dose of FO, duration, of RCT, participant selection and quality of the trials did not account for heterogeneity we conclude that FO supplementation in DM2 decreases triglycerides without a statistically significant effect on glycemic control. RCTs assessing the long term effects of FO supplementation on morbidity and mortality in DM2 are needed.

Intracerebroventricular (Icv) Infusion of Alpha-MSH lowers food intake and body weight via central pathways associated with both Energy Balance and Satiety.

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Hypothalamic melanocortins are proposed to reduce food intake by acting upon key forebrain nuclei such as the paraventricular nucleus (PVN) that are involved in energy homeostasis. To test the hypothesis that chronic central melanocortin signaling causes sustained anorexia and weight loss, male Wistar rats received chronic infusion into the third cerebral ventricle of either vehicle (n = 7) or alpha-melanocyte stimulating hormone ([alpha]-MSH, 24 m g/day, n = 9). Food intake was reduced by 39.9% on Day 1 ($p < 0.05$) in [alpha]-MSH-infused animals relative to vehicle-infused controls. By day 6, cumulative food intake in animals receiving [alpha]-MSH remained decreased by 10.7% ($p < 0.05$) and was accompanied by a 4.3% decrease in body weight ($p < 0.05$) and a comparable 20.1% nonsignificant reduction in leptin levels with no change in glucose. Since similar responses to [alpha]-MSH were detected in group of icv vehicle-treated animals that were pairfed to the [alpha]-MSH group (n = 9), these metabolic effects of [alpha]-MSH appeared to result primarily from reduced food intake. To identify brain pathways that respond to [alpha]-MSH, a separate group of rats received a single icv injection of saline (n = 5) or [alpha]-MSH (20 m g, n = 10). After 2 hours, brains were removed for histochemical assay of cFos-like immunoreactivity (cFLI), a measure of neuronal activation. Compared to vehicle-treated controls, we detected an increase of 250% in cFLI-positive cell nuclei in the PVN ($p < 0.01$), and increase of 200-1000% in the supraoptic nucleus ($p < 0.05$), the central nucleus of the amygdala ($p < 0.01$), and the parabrachial nucleus of the brainstem ($p < 0.001$). We conclude that chronic central administration of [alpha]-MSH reduces food intake and body weight by not only activating PVN neurons, but also pathways in the hindbrain associated with satiety. These findings strengthen the hypothesis that the melanocortin

system is involved in the regulation of food intake and body weight, and support a role for agonists targeting central melanocortin receptors in the pharmacological treatment of obesity.

Lipoic Acid Protects Against Oxidation –Induced Insulin Resistance in 3T3-L1 Adipocytes.

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Insulin stimulation of GLUT4 translocation and glucose transport activity has been recently shown to be impaired following exposure of 3T3-L1 adipocytes to oxidative stress. This study explores the potential of lipoic acid (LA), a unique antioxidant, to protect the cells against the induction of insulin resistance when administered prior to oxidative stress. Two hours exposure to a stable 25 m M H2O2 concentration produced by adding glucose oxidase to the culture medium, resulted in a 50-70% reduction in insulin stimulated glucose transport activity. This was associated with a decrease in reduced glutahione context from 37.4± 3.1 to 26.4± 4.9 nmol/mg prot., (P<0.005). Sixteen hours pre-treatment with 200 m M racemic LA provided a significant protection against both the reduction in insulin stimulated glucose transport following oxidative stress, reaching 84.8± 4.4% of control, as well as full protection against the reduction in GSH. Oxidative stress impaired the 4.89± 0.36 –fold insulin stimulated increase in GLUT4 context in plasma membrane lawns of control cells. However, LA pre-treatment was associated with preserved insulin induced GLIT4 translocation in cells exposed to oxidation, yielding 80% the GLUT4 content observed in non-oxidized cells. Insulin stimulated tyrosine phosphorylation of bands corresponding to IRS and the b subunit of the insulin receptor, was not affected by oxidation nor by LA pre-treatment as assessed in total cell lysates. In contrast, insulin stimulated PKB serine 473 phosphorylation and activity were markedly impaired by oxidation, but were protected by LA pre-treatment. Protection against the effect of oxidation on insulin stimulated glucose transport activity could not be observed with neither troglitazone, its isolated vitamin E moiety, nor with vitamin C. In conclusion, this study demonstrates the unique ability of LA to protect against the impaired insulin stimulated GLUT4 translocation and PKB activation induced by oxidative stress, potentially by its capacity to maintain intracellular redox state.