OP 1
Diabetic Foot

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High prevalence of foot ulceration in the Balkan region - a multicenter study from the BALKANDIAB network.

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Background and Aims: The epidemiology of foot ulceration (FU) is still not well understood because of the absence of reliable estimates of the true prevalence from the non-industrialized regions worldwide. Since much of our understanding of the pathophysiology of FU is derived from cross-sectional surveys this multicenter survey was undertaken to identify the prevalence of FU and potential risk factors related to peripheral neuropathy (DPN) in patients attending diabetic clinics in all the countries of the Balkan Region as a initiative of the former established Balkandiab Network
Materials and Methods: Data were obtained from 1491 diabetic subjects (mean age: 56.4±13.7 years and mean duration of diabetes 10±8.1 years, males 774 (52%), type 2 1160 (78%). The diagnosis of DPN was based on a standardized clinical examination applicable in every center according to the Neurodiab criteria. Motor and sensory deficits were assessed in both legs and scored (tendon reflexes - maximum 8 and reduced sensation of pain, touch, cold and vibration- maximum 8- respectively) using a modified scoring system of this proposed of P.Dyck. NDS≥3 established the diagnosis of DPN
Results:1) The prevalence of FU was 7.5% (SE= 1.4) and of motor deficits (MS≥1) 35.7% (SE=1.24) and of sensory deficits (NS≥2) 43.2% (SE=1.2). 2) Mean age and mean duration of diabetes were significantly higher in ulcerated patients (60.5 ±11.8 vs. 55.7±13.8 and 11.9±1.3 vs. 9.1±2.4 yrs respectively, ($p<0.05$ in both cases). FU was more prevalent in male population than in female (7.8% vs. 6.8%, $p<0.05$). 3) Furthermore FU was more common in patients with even small motor deficits (MS≥1 – reduced reflexes) (15% vs. 5% $p<0.05$) and sensory deficits (NS≥2) (22.96% vs. 8.9% $p<0.05$). The odds ratio for the patients with reduced reflexes or reduced sensations to get an ulcer are 3.5 and 2.36 respectively. The prevalence of FU did not differ between smokers and non smokers.
Conclusion: The present study showed that intrinsic factors related to neuropathy such as small abnormalities in motor or sensory function detectable in daily practice are important risk factors for FU. Further hypothesis is that extrinsic risk factors could also contribute to this high prevalence of FU. Preventive strategies should be addressed to both of the above groups of predisposing factors to FU.

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Reduction in diabetes related lower extremity amputations in the Netherlands: 1991 - 2000.

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Background and Aim: Lower extremity amputations are a prevalent complication among patients with diabetes worldwide. However, there is little data on the actual impact of the increased attention towards diabetic foot ulcers and lower extremity amputations in particular. The aim was to determine the incidence of lower extremity amputations among diabetic patients from 1991 till 2000 in the Netherlands.
Materials and Methods: A secondary database containing information regarding all hospital admissions in which a lower extremity amputation occurred for the years 1991 till 2000 was obtained from the Dutch national medical register. As a patient unique identifier was included multiple amputations and hospitalizations for a single individual could be identified. Furthermore, constant age and sex specific diabetes prevalence rates were used to calculate the diabetic population at risk for every year.
Results: In the year 1991 a total of 1687 patients with diabetes was admitted 1865 times for 2409 amputations. In the year 2000 these numbers were 1673, 1932 and 2448 respectively. The overall incidence rates of the number of patients that underwent a lower extremity amputation decreased over the years from 51.0 to 44.5 per 10,000 patients with diabetes ($p<0.05$). For male patients with diabetes the rates remained stable (62.6 versus 64.2) whereas the female population showed a significant decrease (43.4 versus 31.2, $p<0.05$). When the different levels of amputations were considered the rates showed similar trends as for the total population. In the total population the average hospitalization duration was 45.0 days in 1991 and decreased to 36.2 days in 2000. This trend continued to be true for both male patients (42.4 versus 33.8 days) and female patients with diabetes (47.3 versus 39.7 days).
Conclusions: Over the years observed in this study the incidence rates of diabetes related lower extremity amputation in the Netherlands was found to decrease in both the total and female diabetic population. This decrease is supposed to be an underestimation, because an expected increase in the occurrence of diabetes has not been taken into account. Furthermore, the duration of hospitalization decreased in time. Possible explanations may be the growing attention towards the diabetic foot problem by specialized foot clinics and the preventive actions taken by podiatrists.

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Validity of dorsal foot surface monofilament testing for neuropathy in 15,692 diabetic patients.

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Background and Aims: Screening methods for peripheral neuropathy in UK diabetes clinics include assessment of plantar insensitivity to the 10g monofilament (MF) and/or neuropathy disability score (NDS) ≥6/10. We aimed to determine the validity of MF testing on the dorsal site of the diabetic foot compared to these established neuropathy assessments.
Materials and Methods: Over 4 years we assessed 15,692 diabetic patients for peripheral neuropathy in a mass screening programme using: (a) Semmes Weinstein 1g-MF, 10g-MF and 75g-MF at 3 plantar and 1 dorsal site(s) on both feet; (b) NDS.
Results: Neuropathy prevalence, defined by NDS≥6, plantar insensitivity to 10g-MF and dorsal insensitivity to 10g-MF, was 21.3%, 17.1% and 3.8%, respectively. Exact MF score agreement between right and left feet was very high at 93.6% (kappa=0.83, $p<0.0001$) for the dorsal site and 85.7% (kappa=0.77, $p<0.0001$) for plantar sites. However, exact MF score agreement fell to 46.9% (kappa=0.17, $p<0.0001$) comparing dorsal with plantar sites, although this improved to 86.3% (kappa=0.30, $p<0.0001$) using insensitivity to 10g-MF as cut-off. Logistic regression analysis confirmed the strong relationship between NDS and dorsal insensitivity to 10g-MF (odds ratio (OR) = 10.6 [8.8-12.8 95%CI, $p<0.0001$]) and NDS and plantar insensitivity to 10g-MF (OR = 9.1 [8.3-10.0, $p<0.0001$]). The positive predictive value (PPV) of dorsal insensitivity to 10g-MF compared to the NDS≥6 was 71.5%, whereas the PPV of plantar insensitivity was 58.5%. All three neuropathy tests were strongly associated with foot ulcer history: dorsal insensitivity OR=6.05 [4.9-7.5]; NDS≥6 = 4.5 [3.9-6.2]; plantar insensitivity = 4.4 [3.7-5.1] ($p<0.0001$).
Conclusion: Although Semmes Weinstein monofilaments are highly reproducible instruments, the predictive value of the 10g-MF monofilament for defining neuropathy during mass screening is very different between the plantar and dorsal surfaces. Testing of both plantar and dorsal surfaces are recommended, however, in light of close associations with foot ulceration.

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The North Catalonia Diabetes Study (NCDS): diabetic polyneuropathy and foot risk.

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Background and Aims: The diabetic Polyneuropathy (DPN) is the most important predictor of foot ulcer and amputation in type 2 diabetes mellitus (T2DM). It is fundamental to identify the foot risk for an effective treatment

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Atypical mechanisms of Ca^{2+} release from the endoplasmic reticulum contribute to the cytosolic Ca^{2+} rise induced by depolarization in mouse ß-cells.

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Background and Aims: In pancreatic ß-cells, Ca^{2+} influx through voltage-dependent Ca^{2+} channels plays a prominent role in the depolarization-induced rise in free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). The contribution of Ca^{2+} release from the endoplasmic reticulum (ER) to this $[Ca^{2+}]_c$ elevation is largely debated and was investigated here.
Materials and Methods: $[Ca^{2+}]_c$ was measured by the fura-2 technique and the voltage-dependent Ca^{2+}current was recorded in the perforated mode of the patch-clamp technique. The expression of ryanodine receptors was studied by RT-PCR.
Results: Depolarization of single ß-cells by 45 mmol/l K^+ (in 10 mmol/l glucose + 0.1 mmol/l diazoxide) evoked two types of $[Ca^{2+}]_c$ responses: a monotonic and sustained elevation (~60% of cells) or a sustained elevation superimposed by a single large and transient $[Ca^{2+}]_c$ peak (TCP) ~60s after the onset of depolarization (~40%). TCP occurred only once during continuous depolarization, but was repeatedly activated by cycles of repolarization/depolarization. Simultaneous measurements of $[Ca^{2+}]_c$ and voltage-dependent Ca^{2+} current established that TCP did not result from a larger Ca^{2+} current. As the abolition of TCP by thapsigargin pretreatement indicated that it is caused by Ca^{2+} mobilization from the ER, we investigated the mechanisms of this mobilization. In other cell types, the ER releases Ca^{2+} upon activation of ryanodine receptors (RYR1 activated by depolarization and/or Ca^{2+}, RYR2-3 activated by Ca^{2+}) or IP_3 receptors (activated by IP_3 and/or Ca^{2+}). Whereas expression of the 3 RYR isoforms was prominent in control tissues (skeletal muscles for RYR1, cardiomyocytes for RYR2 and spleen for RYR3), mRNA of RYR1-2 was not found and mRNA of RYR3 was detected at low levels only in purified ß-cells. In a Ca^{2+}-free medium, attempts to activate RYR1 by 100 mmol/l K^+ and all RYRs by 10 mmol/l caffeine did not affect $[Ca^{2+}]_c$ in ß-cells, although similar protocols mobilized Ca^{2+} in skeletal and cardiac myocytes. 0.1 mmol/l ryanodine, a concentration that blocks all RYRs and efficiently suppressed the high K^+- or caffeine-induced Ca^{2+} mobilization in muscle cells, did not prevent the TCP elicited in ß-cells by 45 mmol/l K^+ in a Ca^{2+}-containing medium. Microinjection of ß-cells with heparin (a blocker of IP_3 receptors) suppressed acetylcholine-induced Ca^{2+} mobilization but did not prevent the TCP triggered by high K^+.
Conclusion: The TCP observed in 40% of β-cells in response to a depolarization-induced Ca^{2+} influx corresponds to a Ca^{2+}-induced Ca^{2+} release from the ER. Its mechanism is unusual as it does not involve ryanodine or IP_3 receptors.

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The timing for [Ca2+]i response to various glucose-concentrations show cell-specific profiles in the individual ß-cell.

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Background and Aims: That there are variations in the pattern of cytosolic free Ca2+ concentration ([Ca2+]i) among pancreatic ß-cells is well-known. Likewise varies the length of lag-time before onset of the [Ca2+]i response among the ß-cell population. The lag-time for [Ca2+]i/insulin response is of interest to characterise in view of the fact that early stages of type II diabetes mellitus are associated with an impairment of the first phase insulin release. We have previously shown that the [Ca2+]i response enclose a cell-specific lag-time for the single ß-cell when maximally stimulated. We have now studied whether the timing for the [Ca2+]i response is reproducible also when the cells are stimulated at various glucose-concentrations: 5.5, 8.3, 11.1 and 16.7 mM.
Materials and Methods: The early [Ca2+]i response was recorded from ob/ob mouse single ß-cells, by comparing the response from two consecutive exposures (10 min) of the same cell at the different glucose-concentrations (above). A rest period of 30 min at 3 mM glucose was included between the stimulations. Microfluorimetry with the fura 2/AM as probe was used.
Results: Correlation coefficient for onset of initial [Ca2+]i lowering, defined as the first value under an extrapolated base-line was, for 5.5 mM glucose; r=0.64, P<0.001 (86±23 vs. 64±10 s) for 8.3 mM glucose; r=0.44, P<0.05 (46±8 vs.43±10 s), for 11.1 mM glucose; r=0.69, P<0.01 (33±8 vs.47±16 s) and for 16.7 mM glucose; r=0.02 (18±4 vs. 19±4 s). Lag-time for [Ca2+]i rise, defined as the first value over the extrapolated baseline was, for 5.5 mM glucose; r=0.81, P<0.001(292±42 vs.303±30 s), for 8.3 mM glucose; r=0.81, P<0.001 (211±20 vs.257±29 s), for 11.1 mM glucose; r=0.77, P<0.001 (190±18 vs. 231±33 s) and for 16.7 mM glucose; r=0.70, P<0.001 (131±11 vs.159±15 s). Lag-times for nadir of the initial lowering and for maximum of the first peak were also compared within the pairs of stimulation, for each glucose-concentration tested. The timing of these events showed similar high degrees of correlations as the lag-times for initial [Ca2+]i lowering and lag-times for [Ca2+]i rise.
Conclusion: The results indicate that each single ß-cell is pre-programmed to react with a specific [Ca2+]i response within a certain time to a certain glucose-concentration. ß-Cells showing either a fast or a late response during the first glucose-stimulation reproduced their time pattern during the second stimulation. An individual lag-time may correspond to different function for ß-cells and may have importance for the impairment of the first phase insulin release.

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Acetylcholine restores glucose-induced insulin exocytosis in the diabetic GK islet by affecting post-cytosolic calcium signalling.

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Background and Aims: A markedly impaired insulin release in response to glucose (G) while responsiveness to others secretagogues is maintained, is present in the Goto-Kakizaki (GK) rat, a lean spontaneous model of type 2 diabetes. We and others have previously reported that besides its K^+channel-dependent effect, the glucose action via the K^+-independent pathway was also impaired in the GK β-cell. In the normal β-cell acetylcholine (Ach) potentiates glucose-stimulated insulin release by actions predominantly at a site distal to the elevation of $[Ca^{2+}]_c$. Accordingly we have now studied the location of the action of Ach and its interaction with the glucose pathway in GK islets.
Materials and Methods: Insulin secretion was determined from perifused freshly isolated islets. $[Ca^{2+}]_c$ was measured in parallel using the same fura-2 loaded islets. Double-stranded cDNA was synthesized starting from RNA extracted from 16 wk-old male W and GK islets and related cRNA was hybridized to Affymetrix RG-U34A rat array. Expression values for the genes were determined using Affymetrix Microarray Suite 5.0 and Affymetrix Data Mining Tool 2.0.
Results: We have first verified that the addition of Ach (1 mM) to perifused GK islets amplified (by ten times) their insulin response to 16.7 mM G with a clear return of the biphasic pattern of insulin release. In comparison the non-diabetic Wistar (W) islets exhibited only a two fold increase. Then we have demonstrated that: 1/ Ach elicited a first phase insulin release at 0 or 2.8 mM glucose in GK islet, while it has no significant effect in W; 2/ basal activity of total phospholipase C was not impaired in GK islets; 3/ Ach-induced inositol-phosphates production was normally enhanced in GK islets; 4/ SERCA-3 gene expression (Affymetrix microarray) was decreased in GK islets as compared to W, while IP3-receptor subtype3 gene expression was increased; 5/ Ach in the presence of 2.8 mM G, triggered a transient peak of $[Ca^{2+}]_c$ in the W islets which reflected mostly mobilization of Ca^{2+} from intracellular Ca^{2+} stores since it was suppressed by thapsigargin. In the GK islets, the Ca^{2+} response to Ach was not enhanced; 6/ inhibition of PKCs (Bim) did not affect the $[Ca^{2+}]_c$ nor the insulin release responses to Ach in GK islets; 7/ inhibition of PKAs (Rp-cAMP or H89) or adenylate cyclases (2-5 dideoxyadenosine), while not affecting the $[Ca^{2+}]_c$ response, significantly lowered the insulinotropic response to Ach (at low G) in GK islets.
Conclusion: At variance with W islets, Ach is a triggering signal for exocytosis at low G in GK islets and an hyperactive amplifying signal at high G. This is by a mechanism largely involving direct interaction with the insulin exocytotic machinery, since it reflects an enhanced efficacy of Ca^{2+} on exocytosis. Such a sensitization to Ach is independent of PKC pathway and is related to cAMP generation and the PKA pathway.