

**DIFFERENCES IN METABOLITES IN PAIN PROCESSING BRAIN
REGIONS IN PATIENTS WITH DIABETES AND PAINFUL
NEUROPATHY**

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Running Title: MRS of Brain in Painful Diabetic Neuropathy

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ABSTRACT

Aim: Magnetic Resonance Spectroscopy (^1H -MRS) has been used to show changes in the brain following peripheral nerve injury in subjects without diabetes. This study uses ^1H -MRS to examine the brain in subjects with or without painful diabetic neuropathy.

Methods: Twenty six diabetic subjects (12 with and 14 without chronic neuropathic pain) were compared with 18 subjects without diabetes and pain. The left thalamus, anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) were assessed using ^1H -MRS.

Results: In the DLPFC, diabetic subjects had a decrease in NAA and creatine relative to the control group. In the thalamus, there was a reduction of NAA in the diabetic group with pain compared to those with diabetes and no pain.

Conclusion: Subjects with diabetes have metabolite differences in the brain compared to controls. Subjects with painful neuropathy showed reduced NAA in the thalamus which may explain the genesis of pain in some cases.

Pain due to diabetic neuropathy can be severe and disabling. Despite numerous studies of the peripheral nervous system, the genesis of this type of neuropathic pain remains poorly understood. Studies of the central nervous system after peripheral nerve injury have demonstrated biochemical and structural changes including abnormal firing of thalamic neurons [1] and changes in metabolite concentrations in the thalamus [2]. Thus, the central nervous system may be important in the genesis of pain in diabetic neuropathy.

Magnetic resonance spectroscopy (MRS) provides an excellent tool to study the central nervous system. However, MRS studies in diabetes are limited [3,4] and none have focused directly on painful neuropathy. It is therefore the purpose of the current study to use MRS to provide further information on diabetes, particularly in the context of painful neuropathy.

RESEARCH DESIGN AND METHODS

Twelve diabetic subjects with chronic neuropathic pain of more than six months duration, were matched for age, sex, type of diabetes and glycaemic control with 14 patients without pain. They were compared with 18 subjects without diabetes and pain. Subjects were excluded from the study if they had acute or chronic pain not associated with diabetic neuropathy. All subjects had a blood glucose level ≥ 4.0 mmol/L prior to scanning. Vibration perception threshold (VPT) was measured using a biothesiometer (Bio-medical Instrument, Newbury, Ohio). Pain was measured on a 10cm Visual Analogue Scale (VAS).

The left thalamus, anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) were assessed using ^1H -MRS. Magnetic resonance examinations were performed using a 1.5 T MRI scanner and standard quadrature head coil. Following image guided voxel placement, water suppressed and unsuppressed ^1H -

MRS data were collected using a stimulated echo acquisition mode (STEAM) sequence [5] with chemical shift selected water suppression. Acquisition parameters were TE/TR 25/1500 ms, voxel size 8 cm³, 256 signal acquisitions, spectral width 2500 Hz, and 2K data points.

Interpretation of spectra was performed using the java-based magnetic resonance user interface (jMRUI version 2.0) [6] and a non-linear least squares algorithm (AMARES). Metabolites are expressed relative to the water resonance of the water un-suppressed spectra. Differences between groups were calculated using ANOVA with a Levenes test for equality of variance with post hoc independent t-test. Data are presented as median and interquartile range or mean with standard deviation.

RESULTS

Clinical profiles of the 18 control, 26 diabetic subjects and their pain scores, are shown in the table. VPT scores were higher in the pain group and this was the only difference between diabetes groups ($t = -2.2$; $P = 0.04$). In the DLPFC, diabetes independent of pain was associated with a significant decrease in both NAA and creatine relative to the control group. In the thalamus, there was a significant reduction of NAA in the diabetic group with pain compared to those with diabetes and no pain. The diabetes no pain group also showed an elevation in creatine relative to the control group in the thalamus. There were no differences found in the anterior cingulate cortex.

CONCLUSIONS

Our study demonstrates that there are significant changes in brain metabolites in pain processing regions of the brain associated with diabetes and with neuropathic pain. People with diabetes demonstrated a marked reduction in NAA and creatine in the DLPFC, independent of pain. This observation demonstrates that diabetes is associated with altered cerebral

metabolites, although by themselves they could not explain the presence of pain. Within the diabetic cohort, subjects with pain had significantly reduced NAA in the thalamus compared with the no pain group. This novel finding of a reduction in thalamic NAA in subjects with pain due to diabetes, is consistent with findings from studies on other types of neuropathic pain using MRS in people without diabetes [2,7]. The reduction in thalamic NAA is likely to represent abnormal neuronal activity and oxidative energy transmission. With relevance to neuropathic pain, a reduction in NAA in the thalamus may be responsible for altered amplification and perception of the pain signal in people with diabetes.

It should be noted that usual pain medications were not withdrawn prior to scanning. However, not all of the subjects with pain used medication and the reduction in NAA in the diabetes group with pain occurred irrespective of the use (or not) of pain medication. The control group were also younger than the diabetic group, however there was no significant age difference between the two diabetic cohorts to explain the association of pain with reduced NAA. Those with painful

neuropathy had a higher VPT than those without pain. However, many patients amongst the no pain group have both high VPT and NAA. Thus it is more likely that the presence of pain, rather than sensory loss, accounts for the difference in NAA in the two diabetic groups.

In summary, MRS has identified metabolite differences in the brain in subjects with diabetes compared to controls. We also demonstrated reduced NAA in the thalamus of diabetic subjects with painful neuropathy. Further studies are required to determine whether this helps to explain the genesis of pain in some, but not all, patients with diabetic neuropathy.

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TABLE1. Clinical Profile of Subjects and Concentrations of Metabolites in the Dorsolateral Prefrontal Cortex, Anterior Cingulate Cortex and Thalamus Brain Regions

	Controls N=18	Diabetes No Pain N=14	Diabetes Pain N=12
Age (years)	48 [†] [33-60]	57 [53-63]	61 [56-68]
Duration Diabetes (years)	N/A	13.5 [8.8-27]	15 [10.3-22.8]
Gender (No. of Males)	9	13	15
No. with Type 2 diabetes	N/A	9	10
HbA1c %	N/A	7.7 [6.7-8.9]	7.5 [6.8-8.6]
Vibration Perception Threshold (volts)	N/A	26.6 [16-41]	37.5 [#] [26-50]
Pain Score 10cm VAS	N/A	0	6 ± 2.45
Dorsolateral Prefrontal Cortex	Choline 218 ± 36	Choline 204 ± 31	Choline 207 ± 35
	Creatine 172 ± 22	Creatine 152 ± 26*	Creatine 148 ± 16*
	NAA 128 ± 10	NAA 108 ± 23*	NAA 113 ± 8*
Anterior Cingulate Cortex	Choline 247 ± 58	Choline 237 ± 41	Choline 249 ± 46
	Creatine 182 ± 30	Creatine 189 ± 38	Creatine 182 ± 17
	NAA 127 ± 28	NAA 127 ± 17	NAA 132 ± 15
Thalamus	Choline 195 ± 26	Choline 201 ± 36	Choline 194 ± 29
	Creatine 169 ± 13	Creatine 184 ± 16*	Creatine 172 ± 15
	NAA 108 ± 12	NAA 117 ± 19*	NAA 98 ± 13 [#]

Results are in median [interquartile range] or mean ± standard deviation; NAA: N-acetyl aspartate; † Different to diabetic group p<0.001; * Different to control group (p=0.04), # Diabetes pain different to no pain group (p<0.01).