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IMPAIRED INSULIN SECRETION AND UPTAKE IN PATIENTS WITH DIFFUSE IDIOPATHIC SKELETAL HYPEROSTOSIS

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Objective. So far, high prevalence of metabolic symptoms accompanying diffuse idiopathic skeletal hyperostosis (DISH) appears not definitely elucidated because of their possible origin from other disorders such as diabetes and/or body mass differences. From such reasons this study was aimed to compare non-diabetic DISH patients to a group of age and BMI matched controls in order to distinguish the influence of DISH proper on metabolic parameters free of additional metabolic effects caused by diabetes and/or body weight differences.

Methods. Both groups of patients were subjected to oral glucose tolerance test (OGTT) and fasting serum levels of glucose, insulin, C-peptide, growth hormone, insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGF-BP3) were assayed. Fasting serum total cholesterol, HDL cholesterol, triglycerides, non-esterified fatty acids (NEFA) and uric acid were determined as well. The indices of insulin sensitivity and insulin secretion were calculated.

Results. With the exception of decreased NEFA serum level and decreased insulinogenic index and insulin/C-peptide ratio in DISH patients any other significant differences in serum parameters and indices of insulin sensitivity were not found.

Conclusions. The data obtained suggest impaired ß-cell pancreatic stimulation and increased insulin hepatic extraction in DISH. It is assumed that the above mentioned conditions, if persisting for a long time, might lead to decreased ability of insulin to maintain normal serum glucose level and consequently to insulin resistance which is highly prevalent in symptomatic DISH patients.

Key words: Diffuse idiopathic skeletal hyperostosis – Glucose metabolism – Insulin secretion – Insulin uptake

Diffuse idiopathic skeletal hyperostosis, or Forestier's disease, is a condition characterized by calcification and ossification of ligaments. This condition was described by Forestier and Rotes-Querol (1950) more than 50 years ago and was termed senile ankylosing hyperostosis. This disease is characterized by the ossification of anterior longitudinal ligament and by the production of flowing osteophytes particularly involved the right side of the spine. Although the etiologic factors of DISH are not clearly definitely elucidated, several metabolic, endocrine, genetic, and environmental factors have been considered, but none has been definitely proved so far (Mata et al. 1997).

Several factors have been implicated in the disease based on frequent associations with various metabolic conditions (El Miedany et al. 2000). These factors include hyperinsulinemia, high growth hormone levels (Weinfeld et al. 1997), diabetes mellitus (Littlejohn and Smythe 1981), obesity (Pavelkova and Pavelka 2005), gout (Littlejohn and Hall 1982), dyslipidemia (Vezyroglu et al. 1996), and prolonged use of isoretinol (Kanauchi et al. 2003). Since the association of DISH

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with obesity and diabetes mellitus was most frequently noticed, the metabolic studies of DISH patients are significantly affected by this fact. The presence of hyperinsulinemia in DISH was described by several authors (e.g. by Littlejohn and Smithe 1981; Littlejohn 1985; Denko et al. 1994). In addition to insulin, the growth hormone (GH) is implicated in pathogenesis of DISH since elevated GH serum levels were found in patients with hyperostosis (Moskowitz et al. 1991; Denko et al. 1994; Denko et al. 2002). However, the above mentioned studies used either low number of patients or the DISH and control groups differed significantly in terms of body weight. Anyway, the majority of DISH related studies is in accordance with the fact that the patients with hyperostosis have higher prevalence of obesity than controls (Smythe and Littlejohn 1994; Denko et al. 1994; Kiss et al. 2002; Mader et al. 2005).

Considering the above circumstances, in our study we used age and BMI matched groups of non-diabetic subjects in order to compare DISH patients and controls without any influence of metabolic disturbances caused by obesity and diabetes.

Methods

Patients. The study group consisted of 20 men (mean age ± SEM., 61.2 ± 2.3 yr.) and 9 women (62.4 ± 1.2 yr.) identified as having DISH. All subjects were recruited from the National Institute of Rheumatic Diseases in Piestany, Slovakia. Criteria for DISH were based on Resnick criteria included new bone flowing over, decreased range of motion in the spine, symptoms of pain and stiffness in the spine with radiological changes in the involved areas (Resnick et al. 1975). The criteria as outlined by Resnick differentiate DISH from degenerative disk disease and ankylosing spondylitis. The control population included 8 men (60.2 ± 2.1 yr.) and 5 women (58 ± 4.1 yr.). All DISH patients were on treatment with non-steroidal anti-inflammatory drugs (NSAID). In order to minimize the acute effect of therapy on insulin sensitivity, the last dose of the medicaments was administered two weeks prior to the investigation. All subjects gave informed written consent before the study. The study was approved by the Ethics Committee of the National Institute of Rheumatic Diseases, Piestany, Slovakia.

The subjects were asked to fast and restrain from the use of strong physical activity, tobacco and ingesting alcohol and caffeine for 12 hours prior to the examination. At 8.00 a.m., an indwelling catheter was inserted into an antecubital vein and the subjects were asked to rest in a comfortable armchair. Basal blood samples were drawn at least 30 min after the catheter insertion. Fasting samples were used for measurement of serum lipids and NEFA concentrations and for measurement of plasma GH, IGF1, IGF-BP3 and uric acid concentrations. After obtaining the fasting samples, the subjects underwent the oral glucose tolerance tests after ingesting 75 gram of anhydrous glucose diluted in 250 ml water within a time period of 1-3 minutes. Blood samples were obtained 30, 60, 90 and 120 min after the complete glucose solution had been swallowed.

Biochemical assays. Serum glucose and uric acid were determined by using auto-analyzer Hitachi 911 (Hitachi, Tokyo, Japan). Serum insulin, C-peptide and GH were measured by RIA (Immunotech, France). Serum IGF-1 and IGF-BP3 were determined with the aid of commercially available RIA kits (Immunotech, France). Serum total cholesterol, HDL cholesterol and triglycerides levels were measured with enzymatic kits from Roche Diagnostics using an auto-analyzer Hitachi 911. Serum non-esterified fatty acids levels were measured by using commercial kits (Randox, UK).

Calculation of indices of insulin sensitivity and insulin secretion. Indices of insulin sensitivity were calculated essentially as described by Radikova et al. (2006). Fasting concentrations of glucose and insulin were used to estimate the percentage of beta-cell secretion (%B) and percentage of insulin sensitivity (%S) by homeostasis-model assessment 2 (HOMA2, available online at www.OCDEM.ox.ac.uk). Glucose and insulin concentrations from the oral glucose tolerance tests were used to calculate following indices of insulin sensitivity and secretion: insulin sensitivity index (ISI_{Ced}) by Cederholm and Wibell (1990), composite whole body insulin sensitivity index (ISI_{Mat}) by Matsuda and DeFronzo (1999) and index of insulin sensitivity (ISI_{Gutt}) proposed by Gutt (2000). Insulinogenic index (IRG) according to Seltzer et al. (1967) was calculated as the ratio of the incremental area under the insulin curve above the fasting level ($I_{30} - I_0$, $mU \cdot min \cdot l^{-1}$), to the incremental area under the glucose curve above the fasting level ($G_{30} – G_0$, $mmol \cdot min \cdot l^{-1}$) during the first 30 min of the OGTT. Areas under the curve (AUC) of glucose, insulin and C-peptide during OGTT were calculated using the trapezoidal method.

Statistical evaluation. The results were expressed as means±SEM, the differences between groups being evaluated by t-test. Difference between groups were considered statistically significant at $p<0.05$.

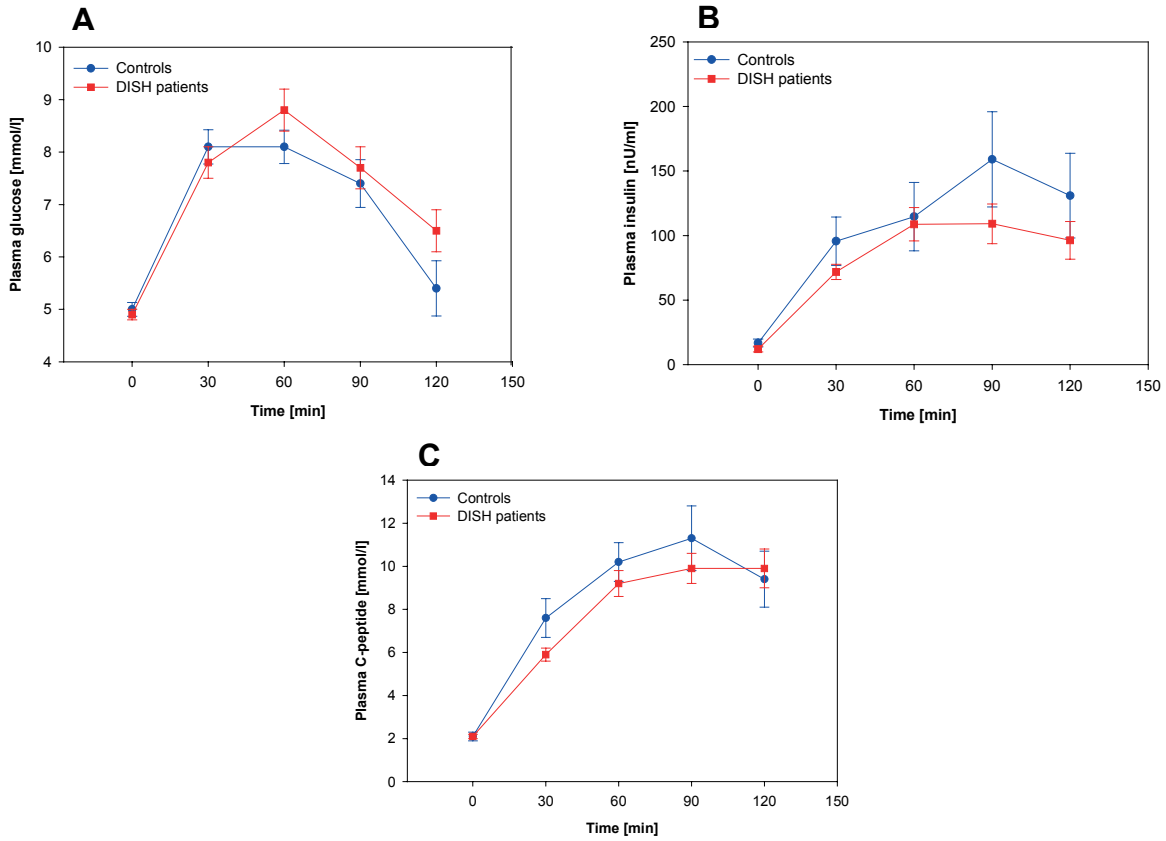

Fig 1 Serum glucose (A), insulin (B) and C-peptide (C) levels in DISH patients and controls during OGTT. DISH patients: n = 27; controls: n = 13. Values are the mean ± SEM.

Results

Clinical characteristics of the studied subjects are shown in Table 1. As shown, two groups of age- and BMI-matched non-diabetic subjects were compared. Those with diffuse idiopathic skeletal hyperostosis had a tendency to higher levels of uric acid and TG when compared with non-DISH subjects (Tab. 1). According to the diagnostic criteria of the American Diabetes Association (2004), 17 patients (58.6%) of the DISH group had normal glucose tolerance (NGT) and 12 patients (41.4%) had impaired glucose tolerance (IGT). In control group, 12 (92.3%) subjects had NGT and only one (7.8%) had IGT. Thus, the group of DISH patients had significantly higher abundance of subjects with impaired of glucose metabolism as compared to controls ($p<0.05$).

Any statistical differences were not found in time course of serum glucose, insulin and C-peptide level during OGTT (Fig. 1A, B, C), although, in patients with hyperostosis, there appeared a tendency to elevated levels of glucose (Figure 1A), and decreased levels of

Table 1

Comparison of DISH patients with controls

	Controls	DISH patients
Age (years)	59.3 ± 2.0	61.5 ± 1.6
BMI (kg/m^2)	27.3 ± 1.1	29.4 ± 0.6
Fasting serum glucose (mmol/l)	5 ± 0.1	4.9 ± 0.1
Fasting serum insulin (μU/ml)	16.7 ± 3.1	12 ± 2.2
Fasting serum C-peptide (ng/ml)	2.1 ± 0.2	2.1 ± 0.1
IGF-1 (ng/ml)	112.3 ± 9.3	109.4 ± 7.8
IGF-BP 3 (ng/ml)	2955.5 ± 153.9	2795.2 ± 165.8
NEFA (mmol/l)	0.4 ± 0.1	0.3 ± 0.0*
Cholesterol (mmol/l)	5.6 ± 0.3	6 ± 0.3
HDL cholesterol (mmol/l)	0.7 ± 0.1	0.9 ± 0.1
Uric acid (mmol/l)	295.9 ± 23.6	342.3 ± 14.9
GH (ng/ml)	1.2 ± 0.5	0.6 ± 0.2
Triglycerides (mmol/l)	1.5 ± 0.2	2.1 ± 0.3

DISH patients: n = 27; controls: n = 13. Values are the mean ± SEM.
* Significantly different at $p<0.05$ when compared to controls.

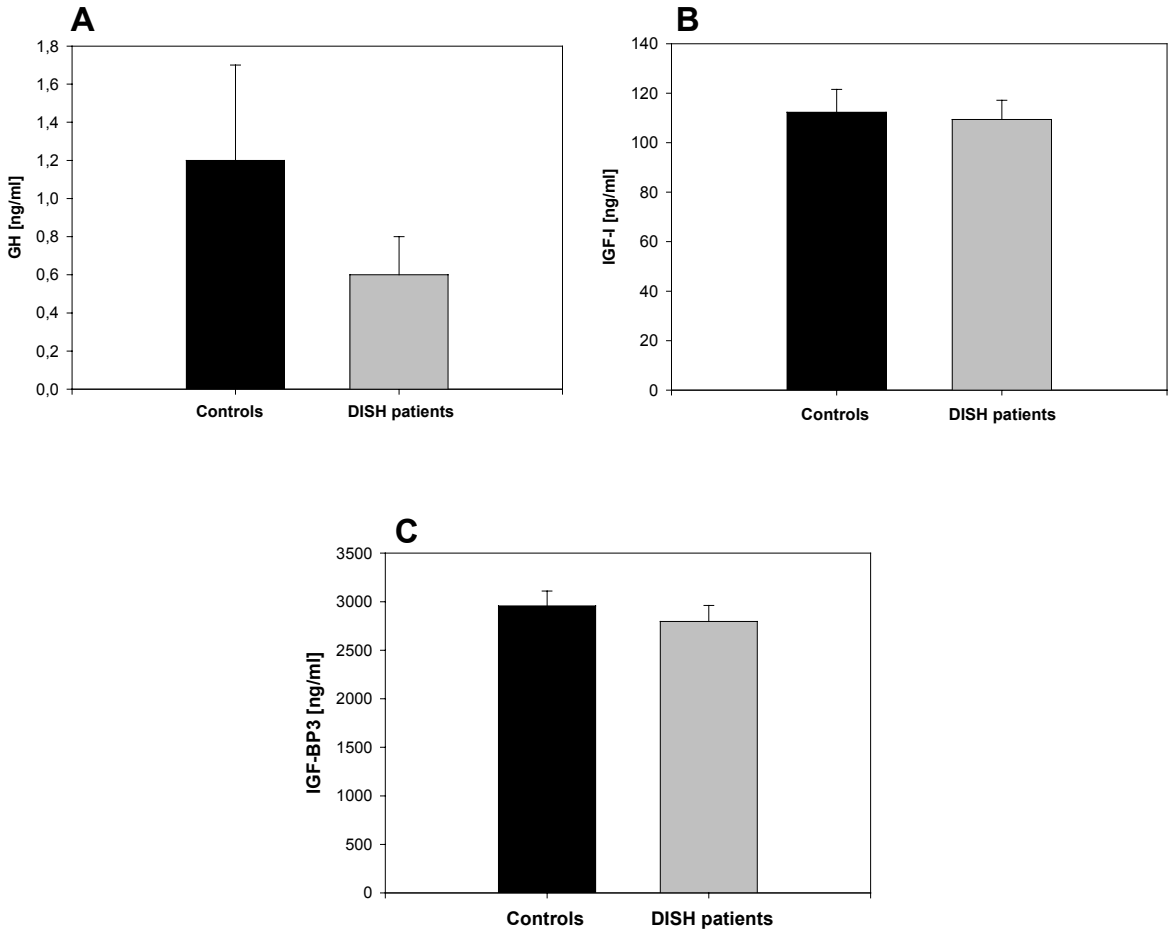

Fig 2 Serum growth hormone (A), IGF-1 (B) and IGF-BP3 (C) levels in DISH patients and control. DISH patients: n = 27; controls: n = 13. Values are the mean ± SEM.

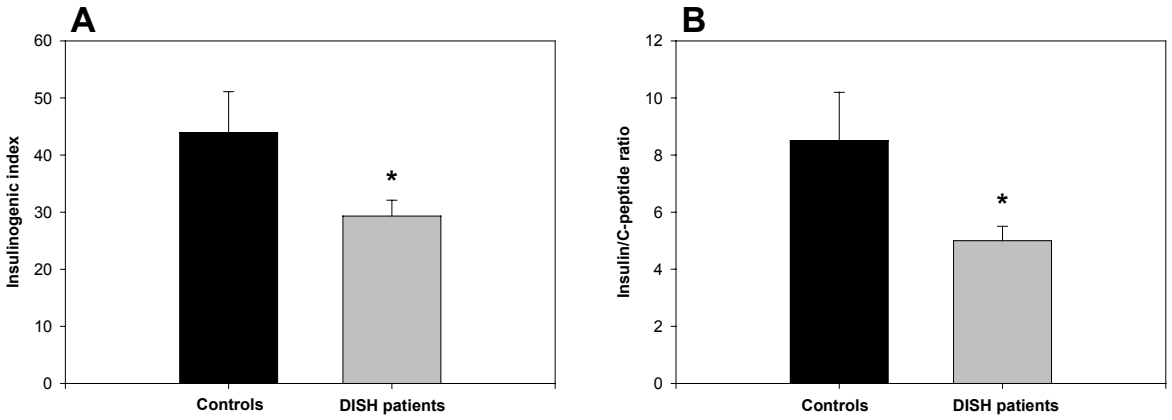

Fig 3 Insulinogenic index (A) and insulin/C-peptide ratio (B) in DISH patients and controls.
DISH patients: n = 27; controls: n = 13. Values are the mean ± SEM. * Significantly different at $p<0.05$ when compared to controls.

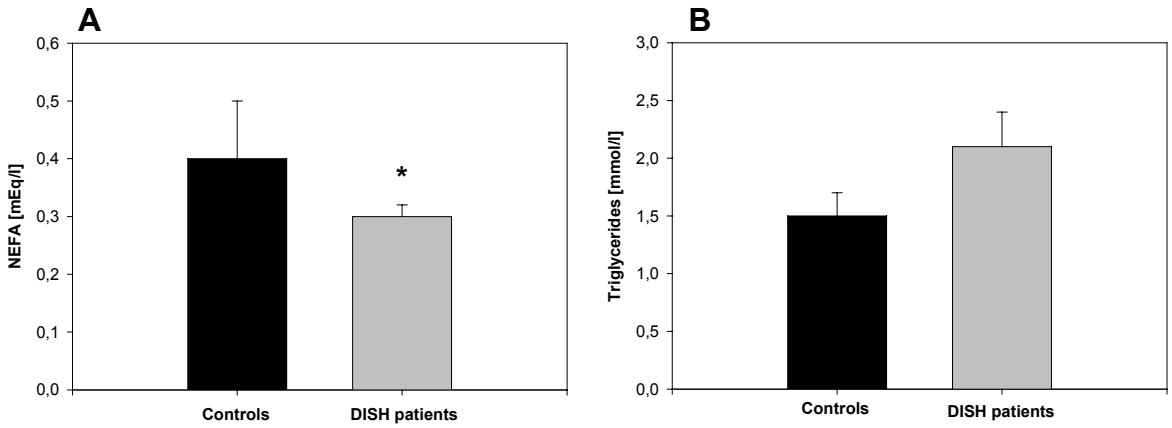

Fig 4 Concentrations of non-esterified fatty acids (A) and triglycerides (B) in DISH patients and controls. DISH patients: n = 27; controls: n = 13. Values are the mean ± SEM. * Significantly different at p<0.05 when compared to controls.

insulin (Figure 1B) at 120 min. The area under the curve (AUC) of glucose, insulin and C-peptide concentrations and also the suprabasal areas (ΔAUC) of glucose, insulin and C-peptide under the OGTT curves (ΔAUC) were not significantly different in DISH patients as compared to controls. Using homeostasis model assessment (HOMA) we have simultaneously assessed insulin sensitivity (HOMA-%S) and β-cell function (HOMA-%B) by using HOMA2 method in a group of DISH patients and controls. No significant differences were found in HOMA-%S, HOMA-%B as well as in HOMA-IR and indexes of insulin sensitivity and secretion ($ISI_{(Ced)}$, $ISI_{(Mat)}$, $ISI_{(Gutt)}$), between patients and controls. In addition, we did not find any significant differences between the levels of GH, IGF-1 and IGF-BP3 in DISH patients and controls (Fig. 2A, B, C).

The group of patients with hyperostosis had significantly lower insulinogenic index ($p<0.05$) (Fig. 3A). Significant difference was also found in insulin/C-peptide ratio with lower value in DISH patients ($p<0.05$) (Fig. 3B). Surprisingly, the subjects with DISH showed decreased levels of NEFA in contrast to controls ($p<0.05$) (Fig. 4A). Finally, we did not find any significant difference between levels of triglycerides in DISH patients and controls (Fig. 4B).

Discussion

Diffuse idiopathic skeletal hyperostosis is a disease of unknown etiology characterized by calcification and ossification of soft tissues, mainly ligaments and enthesis (Mader and Lavi 2009).

Generation of ossification in DISH is, in principle, a new formation of bones in atypical localizations, and therefore bone by itself and its metabolism merits intentness in this problem. A metabolism of bone depends on the activity of bone's cells. Important role in maintenance of bone's mass has growth hormone, which effect to bone is realized through IGF-1. It stimulates differentiation of osteoblasts (Brixen et al. 1990). Literature, however, brings controversial data on the role of GH and IGF-1 in pathogenesis of DISH. On the one hand there are studies showing elevated basal GH levels in DISH (Moskowitz et al. 1991; Denko et al. 1994; Denko et al. 2002), on the other hand some studies do not prove these findings (Littlejohn and Smythe 1981; Zlnay et al. 1988; Altomonte et al. 1992). Moskowitz et al. (1991) reported that plasma insulin and growth hormone levels being increased in DISH but IGF-1 levels were normal. Altomonte et al. (1992) found similar GH and insulin in patients with DISH in comparison to the controls. Denko et al. (1994) found insulin and growth hormone levels to be higher in DISH patients than in a control group, whereas IGF-1 levels were normal in both these groups. In another study, the same authors stated that plasma growth hormone levels were significantly lower in asymptomatic patients with DISH and there was no change in levels of IGF-1, whilst in symptomatic patients elevated levels of GH and IGF-1 were found (Denko et al. 2002). In our study there was no difference between patients with DISH and controls regarding plasma insulin, GH, IGF-1 and IGF-BP 3 except a tendency to decreased GH. We conclude that differences in GH serum levels

reported between DISH patients and controls might be due to non-equal BMI of the studied groups. Since GH deficiency leads to obesity (Ukropec et al. 2008) one would expect rather a lower GH levels in DISH disease typically highly associated with obesity. Anyway, the role of growth hormone in pathogenesis of DISH is still waiting for elucidation.

The association of DISH with excess of body weight is well known since the early descriptions by Forestier and Lagier (1971). Obesity is associated with elevated serum insulin concentrations and indeed the presence of hyperinsulinemia in DISH was described by several authors e.g. by Littlejohn and Smythe (1981), Littlejohn (1985) and Denko et al. (1994). On the other hand, several authors did not find elevated basal serum insulin as well as insulin levels during OGTT (Zlnay et al. 1988; Altomonte et al. 1992; Troilett and Gerster 1993) in DISH patients. Moreover, a number of studies did not notice any impaired lipid parameters such as total cholesterol, HDL, TG (Troilet and Gerster 1993), total cholesterol, TG (Kiss et al. 2002), total cholesterol, TG, HDL and LDL (Mader et al. 2004, 2008) in hyperostosis. The present study confirms the absence of elevated serum glucose, insulin and lipid parameters in DISH when subjects with equal age and BMI are using for comparison.

An empirical "insulinogenic index", is the ratio relating enhancement of circulating insulin to magnitude of corresponding glycemic stimulus (Seltzer et al. 1967). Based on decreased insulinogenic index we anticipate decreased secretion of insulin in DISH patients in comparison to controls. This assumption is in accordance with our finding of decreased NEFA concentrations in DISH since NEFA are potent stimulators of glucose-stimulated insulin secretion (Doshi et al. 2009). We also determined insulin/C-peptide ratio, which was also significantly reduced in patients with DISH. This ratio is showing the extent of clearance and degradation of insulin by the liver. Decreased insulin/C-peptide ratio means increased clearance and degradation of insulin in DISH.

In summary, our study shows that DISH patients display inadequate - decreased insulin secretion in response to glucose stimuli. In addition, insulin levels and perhaps action is reduced by elevated hormone clearance. We assume that both above conditions, if persisting for a long time, could lead to the development of insulin resistance and diabetes. This finding might be an explanation for high prevalence of obesity and diabetes in DISH patients.

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