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Regular Insulin Secretory Oscillations Despite Impaired ATP Synthesis in Friedreich Ataxia Patients

Abstract

Friedreich Ataxia is an inherited disorder caused by decreased expression of a mitochondrial protein called frataxin. Deficiency of this protein causes reduced biogenesis of iron-sulfur clusters, and subsequently impaired synthesis and replenishment of ATP *in vivo*. Basal secretion of insulin occurs in an oscillating manner presumably triggered by ATP-dependent feedback inhibition of glycolytic flux. Hence, individuals with reduced ATP synthesis rates should possibly exhibit impaired insulin secretory oscillations if these were solely dependent on ATP. In the present study Friedreich Ataxia patients with a presumptive impairment of ATP

synthesis in pancreatic beta-cells were evaluated for regularity of basal secretory oscillations of insulin. Healthy siblings were employed as controls. In conflict with the initial hypothesis, no differences in regards to oscillation patterns were observed between patients and controls. Supported by *ex vivo* evidence, these findings tentatively suggest that pulsatile insulin secretion might not be exclusively dependent on ATP feedback inhibition in humans.

Key words

Frataxin · pulsatile insulin secretion · oxidative phosphorylation · ATP · type 2 diabetes

Introduction

Friedreich's Ataxia (FA) is an autosomal-recessively inherited disease, leading to neurodegeneration [1] as well as cardiomyopathy causing premature death at an average age of 37 years [2]. In most cases, FA is caused by an intronic GAA triplet repeat expansion [3] impairing expression levels of a fully functional protein named frataxin [4]. Frataxin in its mature state is an 18 kD protein encoded in the nucleus and located at the mitochondrial matrix [2]. It has been previously demonstrated that the yeast homologue of this protein primarily directs iron-sulfur

cluster assembly [5,6]. Despite their severely reduced life expectancy, a subset of FA patients develops diabetes mellitus of unknown origin [7]. Non-diabetic FA patients exhibit normal glucose-stimulated insulin secretion [7] but show some degree of insulin resistance [8], which is also observed in heterozygous siblings [9]. While studies on a possible association between the common type 2 diabetes and the *frataxin* GAA triplet repeat expansions in humans are inconclusive [10–16], linkage of type 2 diabetes with the locus harboring the human *frataxin* gene at 9q13 was found in at least four different populations worldwide up to date [17–20].

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Oscillations of insulin secretion were first described decades ago [21–24]. Its relevance to the pathogenesis of type 2 diabetes was repeatedly shown in later years, as impaired oscillations were proposed to be an early step in the development of type 2 diabetes [25,26]. Thus, reduced pulsatility may be considered one important factor contributing to the impaired beta-cell function observed in type 2 diabetes. Studies by Tornheim and others have demonstrated that pulsatile insulin secretion may be linked to glycolytic oscillations in the pancreatic beta-cell [27]. In detail it was proposed that basal glycolysis induces oxidative phosphorylation (OXPHOS) and ATP synthesis which then in turn down-regulates a rate limiting step of glycolysis, the enzyme phosphofructo-1-kinase (PFK1) (EC 2.7.1.11) via allosteric inhibition. Subsequently ATP levels would drop and in turn up-regulate PFK1-activity, leading to an oscillation of ATP-synthesis and – presumably – insulin secretion via the K_{ATP} channel. We have previously tested this hypothesis in humans, deficient in the muscle subtype of PFK1 (PFK1-M) which is predominantly expressed in pancreatic beta-cells. We have previously found a significant impairment in the pulsatility of insulin secretion in such individuals suggesting a link between PFK1-M activity and insulin oscillations [28]. These findings supported the view that synchronized oscillations of both glycolysis and ATP-synthesis might indeed govern pulsatile insulin secretion. Based on these findings we now asked whether a disease with a specific impairment of ATP-synthesis might further support the initial hypothesis. Of all diseases known in humans, FA probably comes closest to a selective impairment of OXPHOS and hence ATP synthesis. We have previously shown that overexpression of the FA-associated protein frataxin in fibroblasts was able to increase respiration and OXPHOS cumulating in elevated ATP levels in such cells [29]. In turn fibroblasts derived from FA patients show decreased levels of both frataxin as well as ATP (M. Ristow et al., unpublished). Employing nuclear magnetic resonance spectroscopy we and others have demonstrated a severe impairment of post-exercise ATP replenishment in skeletal muscle from FA patients *in vivo* [30,31]. This deficit was unambiguously observed in every FA patient investigated [31], and correlated with disease duration and the shorter repeat expansion [30,31]. Hence we decided to study non-diabetic FA patients as a model for ATP deficiency regarding the regularity of pulsatile insulin secretion in humans.

Subjects, Material and Methods

We studied two male individuals suffering from FA. All available siblings of these index patients were employed as controls ($n=3$). The study protocol was approved by the local ethics committee and included minutely sampling of whole blood over a time period of 2 hours starting at 9 AM. All other details concerning the sampling procedure were described previously [28]. Explicitly, study patients and control subjects were in a fasting state. To determine a possible pre-existence of diabetes or impaired fasting glucose (iFG) repeated determinations of fasting blood glucose were performed. All determinations and calculations were performed as previously described [28]; briefly, insulin was determined in quadruplicate by radioimmunoassay, pulse analysis was performed employing the pulse-detection software Ultra™. The times series of insulin data was normalized

to have mean 0 and variant 1. Then spectral and autocorrelation analysis were performed using the statistical software STATISTICA™. As an additional complementary method, sample entropy [32,33] was calculated for the different pulses. The sample entropy was calculated with a Matlab™ script developed by authors. The input factors for the sample entropy was set to $m=3$ and $r=0.3$ as suggested [33]. The sample entropy method is based on the method of approximate entropy [34] but is assumed to be more consistent. The sample entropy of a signal is a measure of the regularity of the signal. A lower sample entropy means a more regular pulsatility. For comparison, surrogate pulses, where the imaginary part of the fast Fourier transform of the pulses were shuffled and then inverse transformed, leading to a pulse with same spectral properties as the original pulses but complete random (please see [35] for a more detailed explanation) were created. For each of the five pulses 100 surrogate pulses were created. If the sample entropy value was lower than 95 % of the sample entropy within the series of the surrogate data, then there were assumed to be some regularity within the pulses. Genotype analysis was performed as previously described [36] briefly by PCR amplification using primers flanking the GAA repeat of the first intron of the *frataxin* gene and subsequent electrophoresis and hybridization with a $(TTC)_6$ -probe.

Results

Five individuals in total were evaluated, two of them suffering from FA. Both index patients revealed a phenotype typical for FA including mandatory use of wheelchairs. All three unaffected siblings were unapparent regarding symptoms of FA and otherwise healthy. Neither diabetes nor iFG were detected in all five study subjects as fasting blood glucose was repeatedly found to be lower than 5 mmol/l (data not shown). Relevant additional information on the history of the disease is given in Table 1. Genotype analysis revealed the expected allele structure regarding intron 1 of the *frataxin* gene: both index patients (subjects #1 and 4) were found to be homozygous for the GAA repeat expansion, while all siblings were either homozygously normal (subject #2) or were carrying a heterozygous expansion of the repeat (subjects #3 and 5) (Table 1). As previously observed [9,36], GAA repeats exhibit a high degree of transmission instability as reflected by the occurrence and distribution of allelic expansions in both families (Table 1). Subsequent analysis of putative pulsatility of insulin secretion in all five individuals revealed possible oscillations as can be estimated from the insulin levels derived from minutely sampled serum (Fig. 1). The pattern observed suggests that both frataxin-deficient FA individuals [subjects #1 (Fig. 1A) and #4 (Fig. 1B)] as well as all controls [subjects #2 (not shown), #3 (not shown) and #5 (Fig. 1C)] exhibit oscillations of insulin secretion. Since these raw data (Fig. 1) are difficult to interpret, spectral (Fig. 2) and autocorrelation (Fig. 3) analyses were applied. To determine the time period of these apparent oscillations, spectral analyses were performed. The analyses for both FA patients shows a period of about 10 and 12 minutes, respectively (Fig. 2A, B), while control subjects showed period times between 11 and 15 minutes (example: Fig. 2C), altogether consistent with previous published data in pulsatility in humans [37], suggesting a normal time period of oscillations in ATP-

Table 1 Data on the history of FA disease investigated

Family #	Subject #	Depicted in Panel	Age (years)	Sex	Origin	GAA Repeats Frataxin Intron 1	FRDA Symptoms	Age of Onset	Wheelchair Usage since
1	1	A	21	male	Germany	660/895	yes	8	16 years of age
1	2	none	24	male	Germany	5/7	no	n.a.	n.a.
1	3	none	20	male	Germany	8/1030	no	n.a.	n.a.
2	4	B	23	male	Turkey	1000/1000	yes	6	13 years of age
2	5	C	25	male	Turkey	6/850	no	n.a.	n.a.

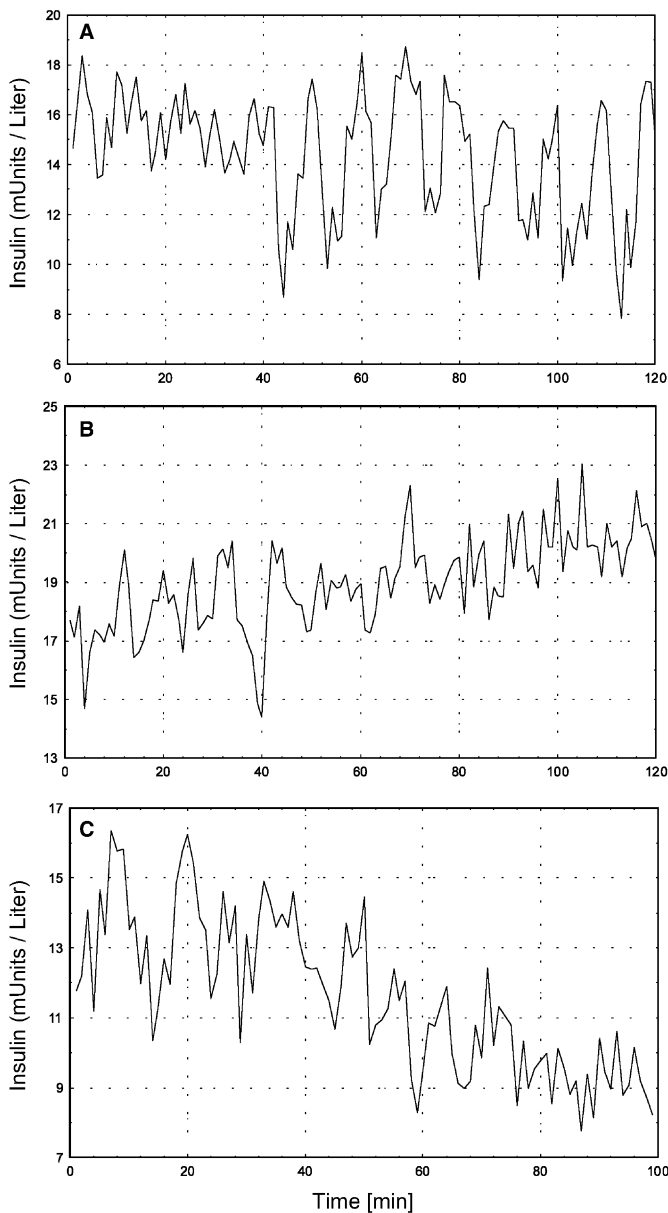


Fig. 1 Basal serum insulin concentrations. Panel **A** and Panel **B** depict basal serum insulin concentrations at different time points obtained from two unrelated Friedreich Ataxia patients. Panel **C** depicts basal serum insulin concentrations at different time points obtained from a representative control subject (sibling of the patient depicted in Panel **B**).

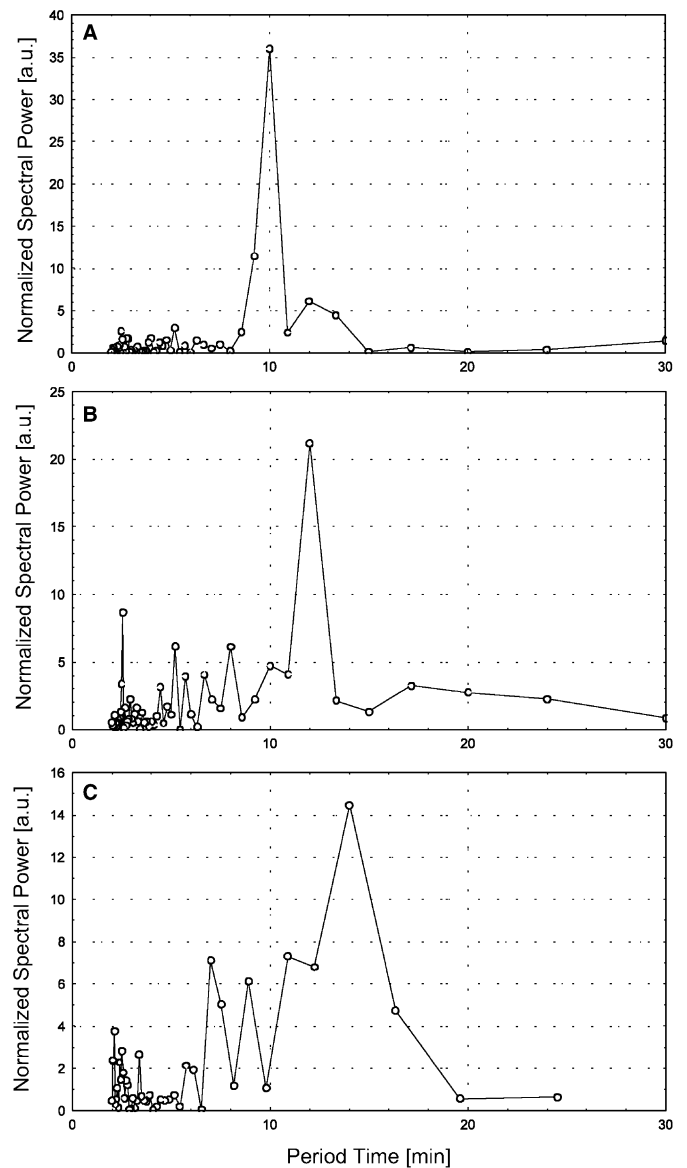


Fig. 2 Spectral analysis of insulin secretion. Panel **A** and Panel **B** depict spectral analyses obtained from insulin secretion patterns (Fig. 1) of two unrelated Friedreich Ataxia patients. Panel **C** depicts spectral analysis obtained from insulin secretion pattern (Fig. 1) of a representative control subject (sibling of the patient depicted in Panel **B**).

deficient FA patients. Subsequently, to evaluate these oscillation period times generated by spectral analysis for significance, autocorrelation analyses were employed. Individual significance level for the autocorrelation was 0.1 which was achieved in all

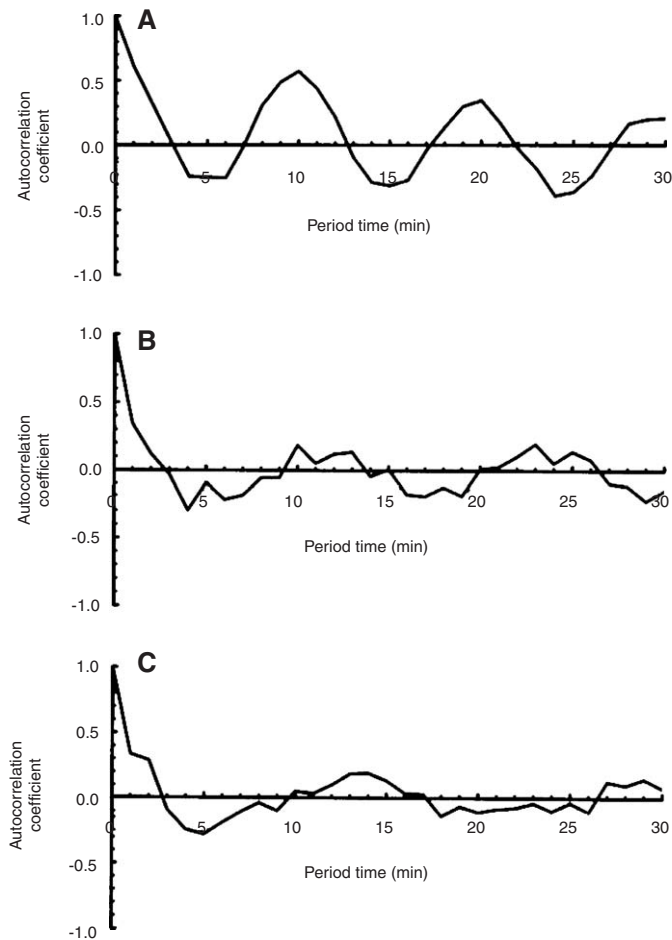


Fig. 3 Autocorrelation analysis of time periods. Panel **A** and Panel **B** depict autocorrelation analyses of time periods derived from spectral analyses (Fig. 2) obtained from two unrelated Friedreich Ataxia patients. Panel **C** depicts autocorrelation analyses of time periods derived from spectral analyses (Fig. 2) obtained from a representative control subject (sibling of the patient depicted in Panel **B**). Maximal individual significance level P per calculation was 0.1.

pulses. The sample entropy data were for the five pulses 1.72, 1.81, 1.59 (for the three controls), 1.66 for first subject and 1.75 for the second subject. The 5% lowest value for the surrogate data were for the three controls 1.95, 1.84, and 1.77, and for the two subjects 1.91 and 2.03, respectively. All pulses show some regularity but none of them differ significantly in regularity. In summary, non-diabetic FA is not associated with impaired insulin oscillations in humans.

Discussion

Normal oscillatory secretion of insulin is known to be impaired in individuals with type 2 diabetes [26,38] as well as their immediate relatives [25] and subjects with impaired glucose tolerance [38]. Therefore, this early defect may be involved in the pathogenesis of type 2 diabetes [25,26,38]. Furthermore, pulsatile administration of insulin has been shown to exert a greater hypoglycemic effect than continuous delivery [39]. Oscillating insulin secretion has been suggested to contribute to a cyclic ATP-controlled feed-back inhibition of glycolysis both *in*

vitro [27,40] as well as *in vivo* [28]. In contrast, it was suggested that pulsatility might be controlled by oscillations of cytosolic Ca^{2+} alone, thus possibly being independent of metabolic oscillations [41]. Bergsten's group has concluded that both mechanisms, i.e. oscillations of either metabolism or Ca^{2+} , may promote pulsatile insulin secretion independently, but can act synergistically if attributed to pancreatic islets in parallel [42]. To specifically support the findings of the present study it should be mentioned that a diazoxide-derived constitutive opening of the K_{ATP} -channel in pancreatic islets was unable to abolish pulsatile secretion *ex vivo* [43]. While our previous data suggest that glycolytic oscillations are essential for pulsatile insulin secretion in humans [28], the findings of the present study indicate that an impairment of ATP-synthesis is insufficient to abolish insulin secretory oscillations in humans. Clearly we are unable to quantify ATP levels in humans islets of Langerhans *in vivo* for obvious reasons. Hence it should be mentioned that evidence for impaired OXPHOS in islets due to FA is merely presumptive, though very likely [29–31,44,45]. Furthermore, the study was performed in the fasting state, hence further studies are required to determine whether the observations on FRDA and normal insulin oscillation also exists at states of hyperglycemia. Lastly, isolated islets from the recently published beta-cell specific frataxin knock-out mouse [46] will hopefully enable further studies to elucidate this problem as well as the underlying mechanism. In summary, the current data suggest that insulin is secreted in a pulsatile manner despite an impairment of oxidative phosphorylation in humans with Friedreich ataxia.

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