Univerzita Palackého v Olomouci
Lékařská fakulta

Využití analýzy variability srdeční frekvence v hodnocení autonomní dysfunkce a nefarmakologických intervencí při metabolickém syndromu: 20 let zkušeností

Disertační práce

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Prohlášení

Prohlašuji, že jsem tuto disertační práci zpracoval samostatně, pod vedením školitelky Prof. MUDr. Elišky Sovové, PhD MBA, opírajíc se o výsledky vlastních a týmových výzkumů, probíhajících ve spolupráci s Institutem biomedicínské techniky a fyziky Lékařské univerzity Vídeň, Klinikou tělovýchovného lékařství a kardiovaskulární rehabilitace a 1. interní klinikou FN a LF Univerzity Palackého v Olomouci, jakož I ve spolupráci s vývojovými partnery společností Advanced Medical Diagnostics Group UK, MIE Medical Research, PreventaMed s.r.o. a Sima Media Olomouc s.r.o.

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Význam zkratek

ABPM	Ambulatory blood pressure monitoring / 24h ambulantní měření TK
ACE	Angiotensin-converting enzyme
ADA	American Diabetes Association
AR	Autoregresivní model
BMI	Body Mass Index [kg/m^2]
BP	Blood pressure/Krevní tlak
CAD	Coronary artery disease
CAN	Cardiovascular autonomic neuropathy/Kardiovaskulární autonomní neuropatie
CCV	Coefficient of component variance
Chol	Cholesterol
CRP	C-reaktivní protein
CSII	Continuous subcutaneous insulin infusion / Kontinuální subkutánní infuze inzulinu, obvykle inzulinovou pumpou
DALY	Disability-adjusted life years
DCCT	Diabetes Control and Complications Trial
DM	Diabetes mellitus
EASD	European Association for Study of Diabetes
EBM	Evidence-based medicine
ECG	Elektrokardiografie
EEG	Electroencefalografie
ESC	European Society of Cardiology
FFT	Fast Fourier transform / rychlá Fourierova transformace
FIT	Functional insulin treatment / Funkční podávání inzulinu
HbA1c	Glykovaný hemoglobin A1c (použito jednotek DCCT)
HbA1c (Rel)	(Relativní) hemoglobin A1c (100% = průměr referenční hodnoty)
HDL	High density lipoprotein
HF	High frequency HRV / Vysokofrekvenční pásmo HRV
HRV	Heart rate variability / Variabilita srdeční frekvence
IDDM	Diabetes mellitus 1.typu (Inzulindependentní diabetes)
IDF	International Diabetes Federation

ICHS	Ischemická choroba srdeční
IGT	Impaired glucose tolerance
LDL	Low density lipoprotein
LF	Low frequency HRV / Nízkofrekvenční pásmo HRV
LN	Natural logarithm/Přirozený logaritmus
MI/IM	Myocardial infarction/Infarkt myokardu
MSSD / RMSSD	Root of the mean square of difference of normal successive R–R Intervals / druhá odmocnina průměru čtverců odchylek po sobě následujících normálních R-R intervalů
NIDDM	Diabetes mellitus 2.typu (Non-inzulindependentní diabetes)
PP	Pulse pressure / Pulsní tlak
PPP	Public Private Partnerships / Partnerství veřejného a soukromého sektoru
R–R interval	Interval mezi dvěma R vlnami po sobě následujících normálních srdečních stahů
RCT	Randomized controlled trial/Randomizovaná kontrolovaná studie
SDNN	Standard deviation of all normal R–R intervals / Směrodatná odchylka po sobě následujících normálních R-R intervalů
TAG	Triacylglyceroly
TK	Krevní tlak
UK-HEART	United Kingdom Heart Failure Evaluation and Assessment of Risk Trial
UKPDS	United Kingdom Prospective Diabetes Study
ULF	Ultra-low frequency HRV / Pásmo ultranízkých frekvencí HRV
VLCD	Very-low calory diet
VLDL	Very-low density lipoprotein
VLF	Very-low frequency HRV / Pásmo velmi nízkých frekvencí HRV
WHR	Waist-hip ratio / Poměr pas-boky
WHO	World Health Organisation

Seznam komentovaných prací autora

(Úvodní číslo označuje kapitolu, v níž se práce nachází)

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Obsah

Seznam obrázků a tabulek:

SOUHRN

Úvod

Ač redukovaná variabilita srdeční frekvence (heart rate variability, HRV) je prokázaná jako významný prognostický marker v kardiologii a diabetologii, lze nalézt málo výzkumných prací na téma nefarmakologických intervencí s cílem úpravy/zlepšení této autonomní dysfunkce. Během posledních dvou dekád jsme úspěšně klinicky ověřili diagnostický postup ke kvantifikaci možných pozitivních vlivů léčebných intervencí u pacientů s autonomní dysfunkcí v rámci diabetu a/nebo metabolického syndromu.

Cíle

(1) charakterizovat klinický vývoj diagnostické metody určené ke kvantifikaci autonomní dysfunkce

(2) ověřit tyto diagnostické metody krátkodobé časové i frekvenční analýzy HRV v rutinní praxi

(3) zhodnotit přínos analýzy HRV při sledování terapeutických účinků nefarmakologických léčebných opatření u pacientů s metabolickým syndromem.

Metody

K realizaci cílů práce byla použita metody časové a frekvenční analýzy krátkodobého záznamu HRV během modifikovaného ortostatického pokusu *(Opavský 1995)* a/nebo baterie reflexních kardiovaskulárních testů dle Ewinga *(Ewing 1985)*.

Dále byly dle cílů v jednotlivých studiích použity následující diagnostické nebo terapeutické metody: diagnostické metody -- analýza 24h záznamu krevního tlaku (ABPM), echokardiografie a další sonografická vyšetření, EKG, zátěžová spiroergometrie, EEG, odběry krve. Použité terapeutické metody zahrnovaly kromě standardní medikamentozní léčby také nefarmakologická opatření, jako např. 12-týdenní vytrvalostní trénink na rotopedu, spinální stimulaci u pacientů s refrakterní anginou pectoris při chronické ICHS, řízené zpomalení dýchání pomocí bio-feedbacku nebo bezkontaktní radiofrekvenční redukci subkutánního břišního tuku. Jednotlivé metody jsou podrobně popsány v konkrétních publikacích.

Výsledky

Získali jsme následující výsledky:

(1) U skupin pacientů s diabetem s různým stupněm autonomního postižení jsme za použití „zlatého standardu“ Ewingovy baterie testů stanovili normy pro spektrální analýzu krátkodobé HRV během modifikovaného ortostatického pokusu *(Publikace č. 3.2.1 dle Seznamu komentovaných prací)*

(2) U skupin zdravých dobrovolníků a pacientů s diabetem jsme během výše uvedeného standardizovaného testu zjistili časné vagální postižení u pacientů, které původně nebylo diferencovatelné robustnější Ewingovou baterií testů, a potvrdili tak zvýšení senzitivity vyšetření ortostatickou manipulací *(Publikace č. 3.4.1 dle Seznamu komentovaných prací)*

(3) U skupiny pacientů s diabetem s různým stupněm autonomnho postižení jsme ověřili dostatečnou reprodukovatelnosti/opakovatelnost vyšetření HRV v časové i frekvenční doméně v průběhu dvou po sobě jdoucích měření provedených během dvou dnů *(Publikace č. 3.1.1 dle Seznamu komentovaných prací)*

(4) U pacientů s diabetem s různým stupněm autonomní dysfunkce jsme zjistili po 12-týdenní tréninkové intervenci na rotopedu statisticky významné zlepšení HRV v obou hlavních frekvenčních pásmech (bez nebo s pouze časnou dysfunkcí), čímž jsme prokázali reverzibilitu postižení. Žádné změny nebyly zaznamenány u pacientů s těžkou CAN. *(Publikace č. 4.1.1 dle Seznamu komentovaných prací)*

(5) V kontrastu k těmto nálezům, 13-hodinová restrikce stravy vedla k nárůstu HRV u pacientů s diabetem se všemi stupni CAN, včetně i těch s pokročilou autonomní dysfunkcí (p=0.02) *(Publikace č. 4.2.1 dle Seznamu komentovaných prací)*

(6) U pacientů s diabetem a hypertenzí jsme použili spektrální analýzu HRV ke sledování efektu nefarmakologické redukce krevního tlaku pomocí zpomalení dechové frekvence biofeedback-systémem RespeRate. Při aplikaci 4xtýdně vedla tato metoda ke statisticky významnému poklesu TK i u uspokojivě korigovaných parametrů krevního tlaku, jakož i významnému nárůstu spektrálního výkonu v LF pásmu HRV. *(Publikace č. 4.4.1 dle Seznamu komentovaných prací)*

(7) Při retrospektivní bezpečnostní analýze vlivu série čtyř 30-minutových radiofrekvenčních ošetření subkutánního tuku u pacientů s metabolickým

syndromem jsme sledovali dopad léčby na kardiovaskulární autonomní regulace: Prokázali jsme, že ač 20±14 minut ihned po ukončení ošetření lze detekovat významnou sympatoadrenergní reakci (vzestup spektrální energie LF a pokles HF pásma), 39±18 dnů po posledním ošetření nejsou detekovatelné změny v autonomních regulacích. *(Publikace č. 4.5.1 dle Seznamu komentovaných prací)*

(8) Při využití analýzy HRV během spinální stimulace u pacientů s chronickou ICHS a refrakterní anginou pectoris jsme prokázali signifikantní vliv této intervence na autonomní funkci — zvýšení spektrálního výkonu v oblasti parasympatiku. Tento nález podporuje hypotézu vysvětlující klinický přínos spinální stimulace pozitivním ovlivněním autonomní balance ve prospěch ochranného vlivu vagu. *(Publikace č. 4.3.1 dle Seznamu komentovaných prací)*

Závěr

Stanovených cílů bylo dosaženo a výsledky byly publikovány v recenzovaných časopisech, většinou s impact faktorem. Podrobnosti viz kapitola 8 této disertační práce, „Publikační a vědeckovýzkumná činnost autora“. Náš výzkum poskytl dostatečně podložené argumenty k podpoře využití krátkodobé analýzy HRV v rutinní ambulantní praxi při diagnostice a sledování účinku léčebných opatření u pacientů s metabolickým syndromem a diabetem:

(1) Při charakteristice diagnostické metody byly definovány teoretické základy HRV, nezbytné technické vybavení, průběh, časová náročnost a standardní podmínky pro měření. Spektrální analýza HRV má oproti Ewingově baterii reflexních testů výhodu v kratší době a menší náročnosti na kooperaci pacienta při vyšetření, vyšší senzitivitě zvláště při detekci časné dysfunkce, a umožňuje kvantifikovat vliv obou regulačních podsystémů

(2) V rutinní praxi byla frekvenční analýza HRV v rámci předložené práce ověřena v 9 studiích u celkově 590 pacientů. Na podkladě našich prací byla metodika rozšířena a zpřesněna, jak uvedeno v publikacích. Tato metoda byla zavedena na cca 30 pracovištích v CZ a zahraničí.

(3) Analýza HRV byla přínosná při sledování vlivu vytrvalostního tréninku, periodického lačnění, řízeného poklesu TK zpomalením dechové frekvence, efektu radiofrekvenční léčby podkožního tuku, a efektu spinální stimulace, kde přispěla k rozpoznání přínosu i potenciálních rizik intervence. Zvlášť výhodná se metoda jeví při kvantifikaci vlivu parasympatiku a při dlouhodobém intraindividuálním sledování efektů léčebných opatření.

SUMMARY

Introduction

Despite the fact that reduced heart rate variability (HRV) is a proven prognostic marker in cardiology and diabetology, there is only a few scientific papers available dealing with non-pharmacological interventions aiming for change/improvement of this autonomic dysfunction.During the last two decades we successfully validated a diagnostic approach for quantification of possibly positive impact of therapeutic interventions in patients with autonomic dysfunction within diabetes and/or metabolic syndrome.

Aims

(1) To characterize clinical development of diagnostic method used for quantification of autonomic dysfunction
(2) To validate these diagnostic tools for short-term time- and frequency-domain HRV analysis in a routine practice
(3) To evaluate benefits of HRV analysis during observation of effects caused by non-pharmacological therapeutic measures in patients with metabolic syndrome

Methods

Methods of short-term time- and frequency analyses of HRV as obtained during the modified orthostatic load *(Opavsky 1995)* and/or battery of cardiovascular reflex tests by Ewing *(Ewing 1985)* have been explored/used to reach these aims.

Further on, the following diagnostic or therapeutic methods have been used in individual studies: diagnostic methods – ambulatory 24h blood pressure monitoring (ABPM), echocardiography and other sonographical examinations, exercise spiroergometry, ECG, EEG, blood sampling. The applied therapeutic methods included – in addition to standardized pharmacological treatment – non-pharmacological measures, such as 12-weeks bicycle ergometer training, spinal stimulation in CAD patients with refractory angina pectoris, 8-weeks´ guided breathing sessions using biofeedback device, or 4 treatments for non-contact radiofrequency reduction of subcutaneous abdominal fat layers. Individual methods are described in detail in respective papers.

Results

We obtained following results:

(1) In patients with diabetes and various degrees of autonomic impairment, while using the Ewing battery of reflex tests as a gold-standard, we established normal values for spectral analysis of short-term HRV as obtained during the modified orthostatic load. *(Publication No 3.2.1. as of Annotated bibliography)*

(2) In healthy volunteers and patients with diabetes, using the above mentioned orthostatic load we discovered an early vagal impairment in patients, which originally was not detectable by the more robust Ewing test battery. This finding confirms an improved sensitivity of the test due to orthostatic manipulation. *(Publication No 3.4.1 as of Annotated bibliography)*

(3) In patients with diabetes and various degrees of autonomic impairment, we verified a satisfactory reproducibility/repeatability of HRV tests in time- and frequency-domains, as examined with two successive measurements on two different days. *(Publication No 3.1.1 as of Annotated bibliography)*

(4) In patients with diabetes and various degrees of autonomic impairment, we observed statistically significant improvement of HRV parameters in both main frequency bands (no or early CAN patients) after 12-weeks bicycle ergometer training, which fact demonstrated reversibility of the impairment. No change has been recorded in patients with severe CAN *(Publication No 4.1.1. as of Annotated bibliography)*

(5) In contrast to these findings, a 13-hour food restriction lead to increase of HRV in patients with diabetes and all degrees of CAN, including those with developed autonomic dysfunction (p=0.02). *(Publication No 4.2.1 as of Annotated bibliography)*

(6) In patients with diabetes and hypertension we used the spectral analysis of HRV to observe effects of non-pharmacological lowering of blood pressure via reduction of respiration rate by means of biofeedback system RespeRate. When used 4x weekly, this method lead to a statistically significant reduction of blood pressure even in satisfactorily controlled patients, as well as to a significant increase of HRV spectral power in LF frequency band. *(Publication No 4.4.1. as of Annotated bibliography)*

(7) In a retrospective safety analysis of a series of four 30-min radiofrequency treatments of subcutaneous fat in metabolic syndrome patients, we measured impact of the

treatment on autonomic control: we have demonstrated that – despite the sympathoadrenergic response (elevation of spectral energy of LF and reduction of HF frequency bands) 20±14 minutes immediately after the treatment -- no changes in autonomic control could be detected 39+18 days after the last treatment. *(Publication No 4.5.1 as of Annotated bibliography)*

(8) By means of HRV analysis during the spinal stimulation in CAD patients with refractory angina pectoris we demonstrated a significant influence of this intervention on the autonomic function – the increase of spectral power in parasympathetic domain. This finding supports the hypothesis explaining the clinical benefit of spinal stimulation by positively influenced autonomic balance towards augmented vagal protection. *(Publication No 4.3.1 as of Annotated bibliography).*

Conclusion

We reached the predefined goals and the results have been published in peer-reviewed journals, mostly with impact factor. For more details, the chapter 8 of this dissertation ("Publications and research activities of the author") can be consulted. Our research delivered satisfactorily supported arguments favouring usability of short-term HRV analysis in routine outpatient practice for diagnostics and observation of therapeutic effects in patients with metabolic syndrome and diabetes:

(1) While exploring this diagnostics method, theoretical fundaments, necessary technical equipment, test protocol, time efforts as well as standardized test conditions have been defined.

(2) The frequency analysis of HRV has been verified in a routine practice in 9 trials with the total of 590 patients. Based on our results, the method has been extended and improved, as described in the papers attached. This diagnostic method has been introduced in 30 centres in CZ and abroad.

(3) The HRV analysis was useful for assessment of benefits or potential risk of endurance training effects, effects of periodic food restriction, BP reduction via device-guided breathing, effects of radiofrequency treatment of subcutaneous abdominal fat layers and of effects of spinal stimulation. In particular, this HRV method is beneficial in quantification of parasympathetic influence, as well as in long-term intra-individual observations of effects of therapeutic measures.

1. ÚVOD

1.1. Krátký (ale důležitý) pohled do historie

Tato disertační práce shrnuje výsledky mého více než **20-letého zájmu a odborných aktivit** v oblasti ambulantní analýzy variability srdeční frekvence (HRV) při metabolickém syndromu.

Problematika kardiovaskulární autonomní dysfunkce mne zaujala již **v roce 1991**, kdy jsem se při pobytové stáži na Lékařské univerzitě ve Vídni u Prof. Dr. Ing. Herwiga Thomy a Prof. Dr. med. Kingy Howorky začal věnovat zavádění klinické rutinní diagnostiky autonomní neuropatie. Na tyto vstupní kroky pak navázala spolupráce s Prof. MUDr. Jaroslavem Opavským a Doc. Ing. Jiřím Salingerem CSc, která vedla od **roku 1993** k rozvoji technologie původně vyvinuté pod vedením obou jmenovaných autorů na Fakultě tělesné kultury Univerzity Palackého v Olomouci. Podíl na tomto rozvoji mělo rovněž i založení společnosti Sima Media Olomouc s.r.o. v roce 1993, která zajistila ekonomické podmínky, a ve spolupráci s oběma univerzitními pracovišti dále rozvíjela klinicky využitelný systém, později známý pod názvy VariaPulse a VariaCardio. Úspěšná součinnost mezi uvedenými subjekty pak vedla k rychlému rozšíření ambulantní diagnostiky kardiovaskulární autonomní dysfunkce: v České a Slovenské republice jsme provedli několik desítek instalací na předních odborných pracovištích a spolupracovali na řadě studií. Na mezinárodní úrovni se nám tuto původem českou technologii podařilo rozšířit do mnoha špičkových univerzitních center v Evropě, především díky dlouhodobé spolupráci s Prof. Howorkou z uvedeného vídeňského pracoviště, a díky řadě odborných publikací a prezentací (viz seznam). Kolem **roku 2000** pak byla navázána intenzivní spolupráce s partnery ve Velké Británii, z níž pak vzešel rozvoj nové generace technologie HRV, dva podané evropské patenty, na nichž jsem se spoluautorsky podílel, i první aplikace v USA a Jižní Africe, a intenzivní spolupráce s vybranými univerzitními i farmaceutickými partnery v Německu. Ačkoliv v dalším běhu času mi díky osobním, pracovním i zdravotním záležitostem již nebylo možné věnovat se pouze rozvoji HRV, zůstává pro mne tato oblast medicíny celoživotním profesním koníčkem a stále zajímavým tématem, s jehož souvislostmi se setkávám i v každodenním ambulantním životě naší interní ambulance i při různých klinických studiích.

V předloženém komentovaném souhrnu zmiňuji některé milníky mé odborné činnosti v oblasti HRV, zejména se zaměřením na aspekty reverzibility autonomní dysfunkce a nefarmakologická opatření při léčbě metabolického syndromu, jimž jsem se věnoval **od r. 2006** po dobu mého doktorského studia na Lékařské fakultě Univerzity Palackého v Olomouci.

1.2. K teorii variability srdeční frekvence

Jednou z nejzajímavějších metod, která si v posledních dvou desetiletích úspěšně razí cestu do rutinní praxe interní neinvazivní diagnostiky, je analýza variací srdeční frekvence (v anglosaské literatuře nejčastěji označována jako „heart rate variability“, zkratkou HRV). Již v roce 1872 publikoval Darwin svou teorii o vzájemné interakci mezi srdcem a mozkem cestou pneumo-gastrické (vagální) inervace *(Thayer 2009)*. Roku 1884 konstatoval Jackson, že srdeční činnost je tonicky inhibována mozkovými vzruchy, přičemž odstranění této inhibice „umožní“ -- a nikoliv „vyvolává“ -- zvýšení fyziologické aktivity srdce *(Thayer 2009)*. Tyto dva postuláty jsou dodnes platnými tezemi teorie variability srdeční frekvence.

Kardiovaskulární systém vykazuje prvky sebeorganizovanosti směřující k udržení své dynamické stability. Ta se udržuje přizpůsobováním srdeční frekvence, krevního tlaku a dalšími mechanismy, které reagují na řadu vnitřních a zevních vlivů jako jsou např. vznik ischemie, metabolická dysbalance či významná fyzická a/nebo psychická zátěž *(Mathias 2013)*. V odpovědi na uvedenou situaci se srdeční frekvence zrychluje či zpomaluje, mění se tedy délka R-R intervalu. Tato fyziologická adaptace srdeční frekvence patří k typickým znakům autonomních, integrativních funkcí živých organizmů. Mnozí autoři popisují HRV jako fenomén, který časně a velmi citlivě reaguje na přechod mezi zdravím a nemocí *(Thayer 2012)*. Vysoká variabilita srdeční frekvence je indikátorem dobré adaptability systému, tedy „zdravých“ regulací srdečních funkcí a potažmo „zdravého“ organismu. Naopak, snížená variabilita bývá známkou porušení adaptability systému a měla by vést k detailnější, cílené diagnostice její příčiny *(Dekker 2000).* Z klinického pohledu je snížená HRV je vnímána jako indikátor rizika spojeného s rozvojem řady chorob především kardiovaskulární a/nebo metabolické etiologie *(Task Force 1996).*

1.3. Klinický význam vyšetření/nálezu autonomní dysfunkce

Autonomní dysfunkce je nedílnou součástí kardiovaskulárních komplikací řady chronických onemocnění. Excesivní sympatikotonie spolu s chybějícím ochranným vlivem vagu je jedním ze spouštěcích faktorů maligních arytmií a příčin náhlé smrti nejen při akutním koronárním syndromu ale i řadě jiných, metabolických onemocnění s kardiálním postižením *(Metelka 2014)*. Kromě kardiovaskulárního efektu má sympatikotonie významnou korelaci

s rozvojem inzulinorezistence a komponent metabolického syndromu včetně diabetu 2. typu *(Pumprla 2014)*.

Odhaduje se, že tato kardiovaskulární autonomní neuropatie (CAN) se spolupodílí na až 70% všech úmrtí pacientů s diabetem, vzhledem k tomu, že jak při "tiché" formě infarktu myokardu tak při náhlé smrti arytmogenní etiologie se rozhodující vliv přisuzuje právě CAN *(Vinik 2003)*. Navzdory dřívějším předpokladům, že se autonomní dysfunkce manifestuje teprve v pozdních fázích diabetu a metabolického syndromu, existuje dnes řada publikací, které nachází časnou formu CAN již v prvních letech existence onemocnění *(Ziegler 2001, Vinik 2003, Viggiano 2009).*

Jeden z hlavních důvodů pozdní diagnostiky této závažné dysfunkce u metabolického syndromu a diabetu byla nedostupnost účinné metodiky a technologie k odhalení tohoto stavu v předchozích letech. Díky rozvoji výpočetních technologií je dnes již možno i v běžné rutinní praxi diagnostikovat (a následně zahájit léčbu) poměrně jednoduše, rychle a neinvazivně, za využití počítačové analýzy variability srdeční frekvence. Tato metoda se dnes rozšířila hlavně v kardiologii a diabetologii, přičemž stovky odborných publikací dokazují její využití i v dalších oborech medicíny *(Task Force 1996, Pumprla 2014).*

Spolu s ostatními rizikovými faktory, sledovanými při rozvoji metabolického syndromu, je dlouhodobá neuspokojivá metabolická/glykemická kontrola zásadním faktorem přispívajícím k progresi postižení autonomních regulací a k rozvoji neuropatie *(DCCT 1993, UKPDS 2000)*. Nicméně, recentní studie poukazují na to, že inzulinová rezistence (IR) je pravděpodobně ještě významnějším rizikovým faktorem než samotná hyperglykemie, přičemž oxidativní stres je kandidátním mechanismem propojujícím neurohumorální stimulaci projevující se excesivní sympatikotonií s IR *(Vinik 2013)*.

Při diabetu -- dle definice Americké diabetologické společnosti a podvýboru Toronto Consensus Panelu o diabetické neuropatii -- je kardiovaskulární autonomní neuropatie definována jako postižení autonomní kontroly kardiovaskulárního systému, po vyloučení ostatních příčin *(Tesfaye 2010)*. Prevalence CAN se pohybuje mezi 2.5 až 50%, dle metodiky vyšetření, věku (až 38% u pacientů s IDDM a až 44% u NIDDM), i trvání diabetu (až 35% u IDDM a až 65% u NIDDM). Incipientní formu autonomní dysfunkce lze nalézt až u 7% nově diagnostikovaných pacientů s diabetem *(Vinik 2003)*. Vyjma uvedených faktorů je CAN významně častěji sdružena s polyneuropatií, retinopatií, mikroalbuminurií resp. diabetickou

nefropatií a renálním selháním *(Vinik 2003, Valensi 2003, Low 2004, Boulton 2005).* Symptomatická kardiovaskulární autonomní dysfunkce zahrnuje sinusovou tachykardii, intoleranci zátěže a ortostatickou hypotenzi (incidence 6-32% dle metodiky a studované populace) *(Spallone 2011)*. V souvislosti s CAN bylo zjištěno mnoho dalších kardiovaskulárních abnormit, např. postprandiální hypotenze, poškozená baroreflexní senzitivita, peroperační instabilita, a řada dalších *(Ziegler 1993, Boulton 2005).*

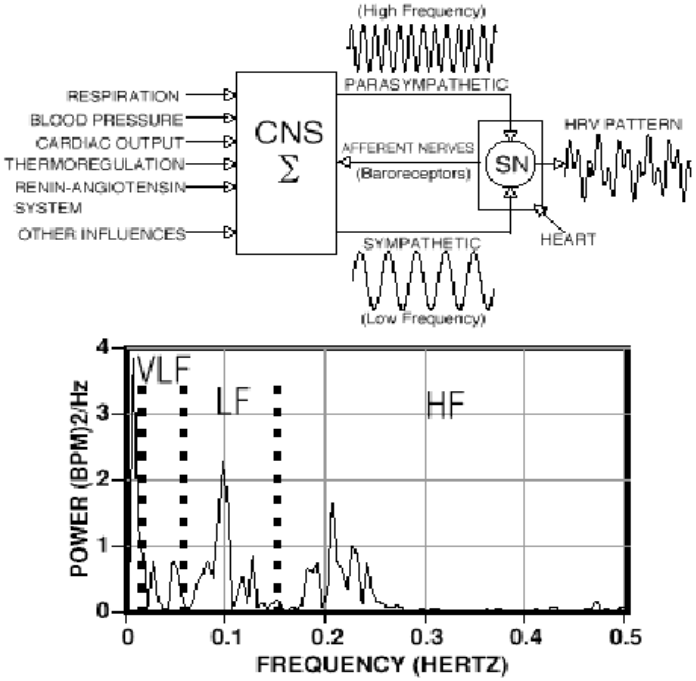
CAN je rovněž významně asociována s mortalitou. Meta-analýza z 15 longitudinálních studií zahrnujících 2900 pacientů sledovaných 1-16 let demonstrovala, že diagnoza CAN založená na aspoň 2 patologických reflexních testech zvyšovala relativní riziko mortality na 3.65 (2.66-4.47, *Maser 2003)*. Tyto studie prokázaly, že CAN je nezávislý prediktor mortaility. Obdobně, na základě metaanalýzy *Wheelera et al* s 12 zařazenými studiemi je kromě HRV také stanovení QTc intervalu vhodným nezávislým prediktorem mortality a kardiovaskulárního úmrtí *(Wheeler 2002, Ziegler 2008).* Obecně platí, že ke stanovení stratifikačního rizika arytmií a náhlé smrti lze využít řady dalších elektrofyziologických aspektů srdeční stability, s různou výpovědní schopností a senzitivitou/specificitou, např. pozdních potenciálů, turbulence srdečního rytmu, senzitivity baroreflexu, alternace vlny T (T-wave alternans), délky intervalu QT, variability intervalu QT, disperze intervalu QT a invazivního elektrofyziologického vyšetření *(Heinc 2006)*. Nicméně, s ohledem na multifaktoriální a časově závislou etiologii letálních arytmií je nutno dodat, že selekce jednoho rizikového faktoru a jeho samostatné užití k predikci arytmogenních komplikací či náhlé smrti vždy představuje jen část problému. To také vysvětluje, proč v prospektivních studiích při různých patologických stavech nebyl žádný samostatný rizikový faktor dostatečně senzitivní, a s dostatečnou pozitivní prediktivní přesností nebyl schopen identifikovat rizikovou skupinu nemocných *(Heinc, 2006).*

Ve srovnání s komplexností některých výše uvedených diagnostických postupů je námi používané krátkodobé stanovení HRV na základě variací R-R intervalů poměrně jednoduché rutinní vyšetření, které dostatečně senzitivně (ač nespecificky) a během krátkého, standardizovaného a nezatěžujícího testu identifikuje a kvantifikuje i časnou dysfunkci. To umožňuje zahájit časná léčebná opatření, která v iniciální fázi postižení poskytují ještě dostatečnou pravděpodobnost reverzibility stavu, jak jsme prokázali v několika studiích analyzovaných v této práci *(Howorka 1997, 1998).*

1.4. Záznam a analýza variability srdeční frekvence

Analýza HRV je založena na vyhodnocení fluktuace intervalů mezi normálními, po sobě následujícími srdečními stahy, nejčastěji mezi vlnami R. Díky výpočetním technologiím je dnes rutinní analýza HRV jednou z mála metod umožňujících neinvazivně a rychle kvantifikovat kardiovaskulární autonomní regulace, a v případě využití tzv. spektrální dekompozice i posoudit podíl obou hlavních složek, sympatiku a parasympatiku, viz **Obr.1**.

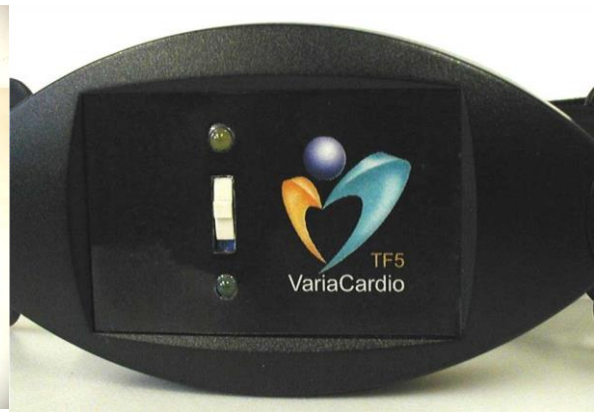
Obr.: 1: Schematické znázornění „filtrační" a kontrolní funkce centrálního nervového systému *ve vztahu ke kardiovaskulárním autonomním regulacím – externí i interní vlivy jsou „převáděny" do typických oscilačních funkcí viz dvourozměrný obrázek výsledného frekvenčního spektra s jeho typickými oblastmi VLF (very-low-frequency), LF (low-frequency) a HF (high-frequency) znázorněnými v dolní části obrázku (zdroj www.heartmath.com)*



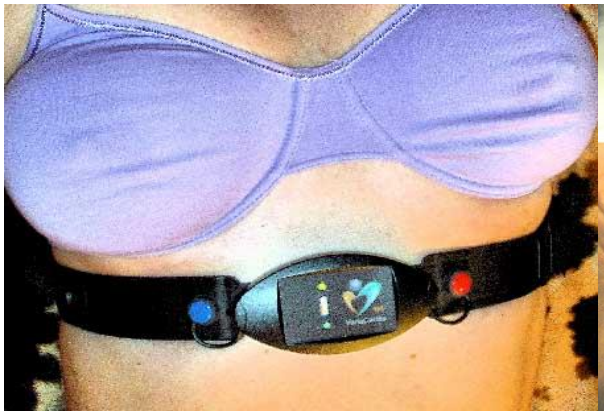
U nás se v posledních dvou desetiletích do klinické praxe rozšířily systémy VariaPulse a VariaCardio, viz **Obr.2**, vývojově pocházející z olomoucké Univerzity Palackého *(Opavský 1995, Salinger et al, 1995)* a klinicky rozvíjené ve spolupráci s řadou univerzitních center u nás i v zahraničí, mj. s Lékařskou univerzitou ve Vídni, pražským IKEMem nebo Jesseniovou Univerzitou v Martině *(Pumprla 1995, Howorka 1997, Jirkovská 1999, Javorka 1999).* Tento měřící systém sestává z hrudního snímače s elektrodami a vyhodnocovací/vysílací jednotkou, přijímače a software na dedikovaném počítači. Průběh měření je řízen přístrojovým software a je minimálně náročný na spolupráci pacienta. Sledované parametry jsou online znázorněny během vyšetření na obrazovce a lze tak sledovat okamžité změny autonomních regulací v souvislosti s např. ortostatickou manipulací nebo některým z testů Ewingovy baterie.

Obr.2: Systém VariaCardio TF5 *určený k ambulantnímu vyšetření kardiovaskulární autonomní neuropatie: (a) celkový pohled snímač EKG/vysílač ve formě hrudního pásu s dvěma elektrodami a vyhodnocovací a vysílací jednotkou uprostřed, a přijímač s dvěma antenami zajišťujícími kvalitnější příjem signálu, (b) pohled na přední stěnu snímače/vysílače srdeční frekvence a EKG,*

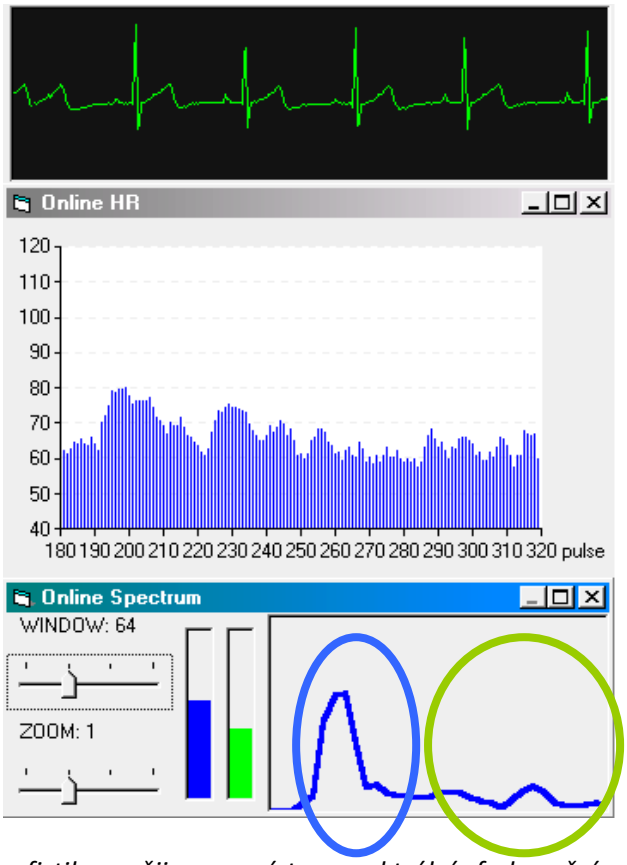
(a) *(b)*



(c) umístění snímače/vysílače na hrudníku při měření HRV, (d) dobíjení akumulátoru systému (Autor: J Pumprla)



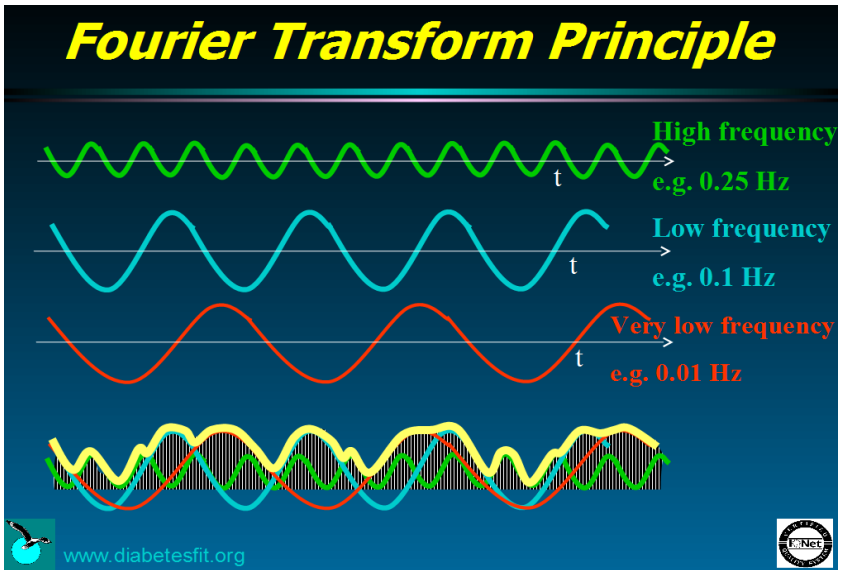
Podstatou frekvenční/spektrální analýzy je rozložení nepravidelného průběhu HRV na pravidelné cykly=frekvence, reprezentující procesy ovlivňující její kolísání, viz **Obr.3**. Protože oba recipročně řídící subsystémy, sympatikus a parasympatikus, „pracují“ s odlišnými frekvencemi (parasympatikus „reaguje“ rychleji, sympatikus pomaleji, zřejmě v souvislosti s odlišnými charakteristikami jejich neurotransmitterů), je možné je matematicko-statisticky rozlišit a následně kvantifikovat jejich tzv. „spektrální výkon“ (spectral power). K tomuto rozlišení se obecně používají dvě procedury – rychlá Fourierova transformace (FFT), umož-



Obr.3: Praktické provedení měření a online analýzy variability srdeční frekvence: typické nálezy. *Horní a prostřední obrázek: povrchové jednosvodové EKG s vysokou vzorkovací frekvencí 500Hz a individuálně snímané R-R intervaly snímané se vzorkovací frekvencí 1000Hz jsou telemetricky odesílány do přijímače, kde se převádí do vizuální podoby sloupcového grafu, viz prostřední obrázek, kde jeden sloupek vždy reprezentuje jeden srdeční stah. Křivka proložená vrcholy jednotlivých sloupků průběhu srdeční frekvence -- tedy její variabilita – může být dále analyzována jednak v tzv. časové doméně – a jednak sofistikovaněji, pomocí tzv. spektrální, frekvenční analýzy, v predefinovaných časových analytických „oknech“ (v tomto případě 300 sekund). Výsledné spektrum je znázorněno v dolní části obrázku: zde jsou zřejmé typické dva peaky obou regulačních větví autonomního nervového systému: vlevo peak nízkofrekvenčního spektra s centroidní frekvencí kolem 0.1Hz s dominantním vlivem sympatiku, a vpravo peak vysokofrekvenčního spektra kolem 0.25Hz přisuzovaný výlučně parasympatickému vlivu. (Autor: J.Pumprla)*

ňující vyhodnotit průběh HRV online *(Salinger 1995)*, nebo autoregresní (AR) analýza *(Hartikainen 1998)*, kterou lze využít pouze off-line po ukončení záznamu a je subjektivně ovlivnitelná výběrem odpovídajícího analytického modelu. Ačkoliv obě tyto analýzy jsou principiálně ve svém differenciálně-diagnostickém přínosu srovnatelné *(Hartikainen 1998)*, vlivem vyšší nejistoty použitého AR modelu (fázová závislost, „tail“ efekt, model order) v analýze biologických časových řad variability srdeční frekvence se doporučuje preferenčně využívat rychlou Fourierovu transformaci *(Chemla 2005, viz* **Obr.4***).*

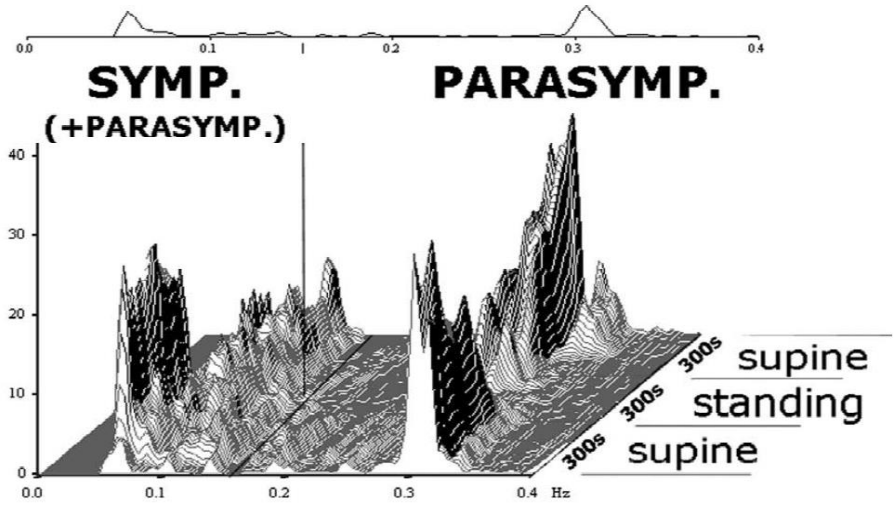
Obr.4: Princip matematicko-statistického procesu tzv. rychlé Fourierovy transformace *využitého ve spektrální analýze HRV: dekompozice nepravidelného průběhu variací srdeční frekvence (na obrázku dole) na pravidelné oscilace s typickými frekvencemi viz jednotlivé průběhy 0.25 Hz, 0.10 Hz a 0.01 Hz, typicky reprezentující high-, low- a very-low frekvenční pásma (Autor J.Pumprla)*



Při pohledu na získané frekvenční spektrum HRV se zobrazuje vliv sympatiku /s částečným podílem parasympatiku/ v rozsahu frekvencí 0,04-0,15 Hz (tzv. nízkofrekvenční pásmo, LF), a zastoupení parasympatiku v rozsahu 0,15 až 0,40 Hz (tzv. vysokofrekvenční pásmo, HF). Pásmo velmi nízkých frekvencí 0,01-0,04 (VLF) Hz se považuje za indikátor aktivity termo- či chemoreceptorů a systému renin-angiotenzin *(Task Force 1996)*. Nicméně, v rámci krátkodobých záznamů je jejich podíl na spektru – zvláště při frekvenci pod 0.01 Hz – diskutabilní, protože tyto cykly mají svou vlnovou délku při např. 0.01 Hz až 100 sekund, tj. při neúměrně krátkých záznamech pod 100 sekund může dojít k falešné redukci dat potřebných k frekvenční analýze *(Task Force 1996)*. Kromě absolutních hodnot spektrálního výkonu v jednotlivých pásmech se posuzují i poměry, relativní zastoupení jednotlivých komponent, a klinicky přínosnější se jeví kumulované parametry sčítající výkon jednotlivých frekvenčních pásem v průběhu celého vyšetření, např. provokace sympatiku i parasympatiku během ortostatické manipulace *(Howorka 1998)*. Celkovým výsledkem vyšetření je typicky dvojrozměrný graf zobrazující spektrální energii obsaženou v predefinovaném časovém intervalu, nebo trojrozměrný spektrogram zobrazující spektrum variací srdeční frekvence v

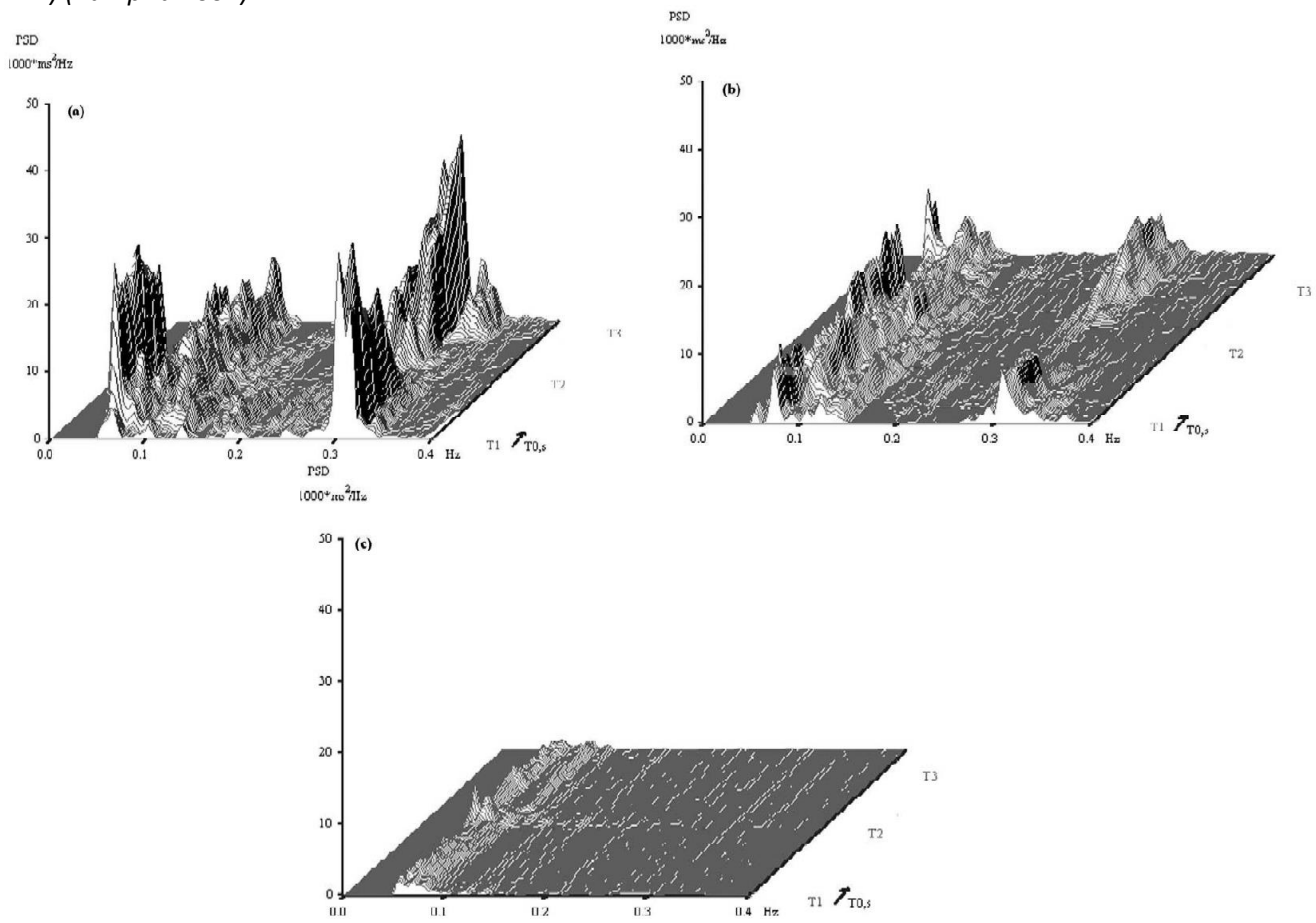
průběhu vyšetření, viz **Obr.5**. Při vyšetření systémy VariaPulse a VariaCardio se k provokaci obou větví autonomních regulací využívá standardizovaný ortostatický pokus, kdy vyšetřovaná osoba 5 minut leží, 5 minut stojí a poté opět 5 minut leží *(Opavský 1995)*. Cílem této kombinace je provokace obou regulačních větví -- sympatiku i parasympatiku – přirozeným, fyziologickým podnětem, a dále také standardizace testu. Na přiloženém obrázku č.5 je znázorněn průběh HRV během vyšetření modifikovaným ortostatickým pokusem: leh s převahou parasympatiku (kolem 0.3 Hz), poté recipročí vzestup aktivity sympatiku (kolem 0.1 Hz) vestoje, a v poslední fázi pokles sympatikotonu a opětný vzestup parasympatikotonu po opětné supinaci.

Obr. 5: Schematické znázornění výsledku krátkodobého vyšetření HRV *za použití modifikovaného ortostatického pokusu: standardizované vyšetření leh-stoj- leh, každá pozice 5 minut (Pumprla 2002)*



Rozvoj případné poruchy kardiovaskulárních autonomních regulací lze pak posoudit vizuálně i kvantitativně. V časné fázi autonomní regulační dysfunkce dochází především k redukci v oblasti parasympatiku, zatímco při rozvinutém postižení dochází k poklesu už i v oblasti nízkofrekvenční, tedy převážného vlivu sympatiku (*Ziegler 2001),* viz následující **Obr.6** s různými fázemi autonomní dysfunkce.

Obr. 6: Typické nálezy krátkodobé HRV *registrované během modifikovaného ortostatického pokusu: (a) normální regulace bez kardiovaskulární autonomní dysfunkce, se zachovalými dominancemi parasympatiku vleže a sympatiku vestoje, (b) počínající dysfunkce (se sníženým parasympatikotonem, oblast 0.3 Hz), a (c) u pokročilá dysfunkce (redukce sympatiko- i parasympatikotonu, oblasti 0.1 i 0.3 Hz) (Pumprla 2002)*



Průběh srdeční frekvence není izolovanou veličinou, ale úzce souvisí s regulacemi krevního tlaku, dýchání a dalšími faktory. Proto je vždy nutno interpretovat výsledky HRV v souvislosti s celkovým klinickým stavem či léčbou. Průběh vyšetření je třeba striktně standardizovat a vyloučit externí stimuly, přičemž zřejmou roli může hrát frekvence a hloubka dýchání *(Pumprla et al 2001).* Při longitudinálním, intraindividuálním sledování pacienta se však tento fenomen uplatňuje minimálně. Navíc snaha o řízenou dechovou frekvenci během vyšetření HRV je de facto externí, organismu nepřirozený stimul, který v konečném nálezu může významně znehodnotit přirozený nález HRV u pacienta *(Task Force 1996, Aysin 2007).*

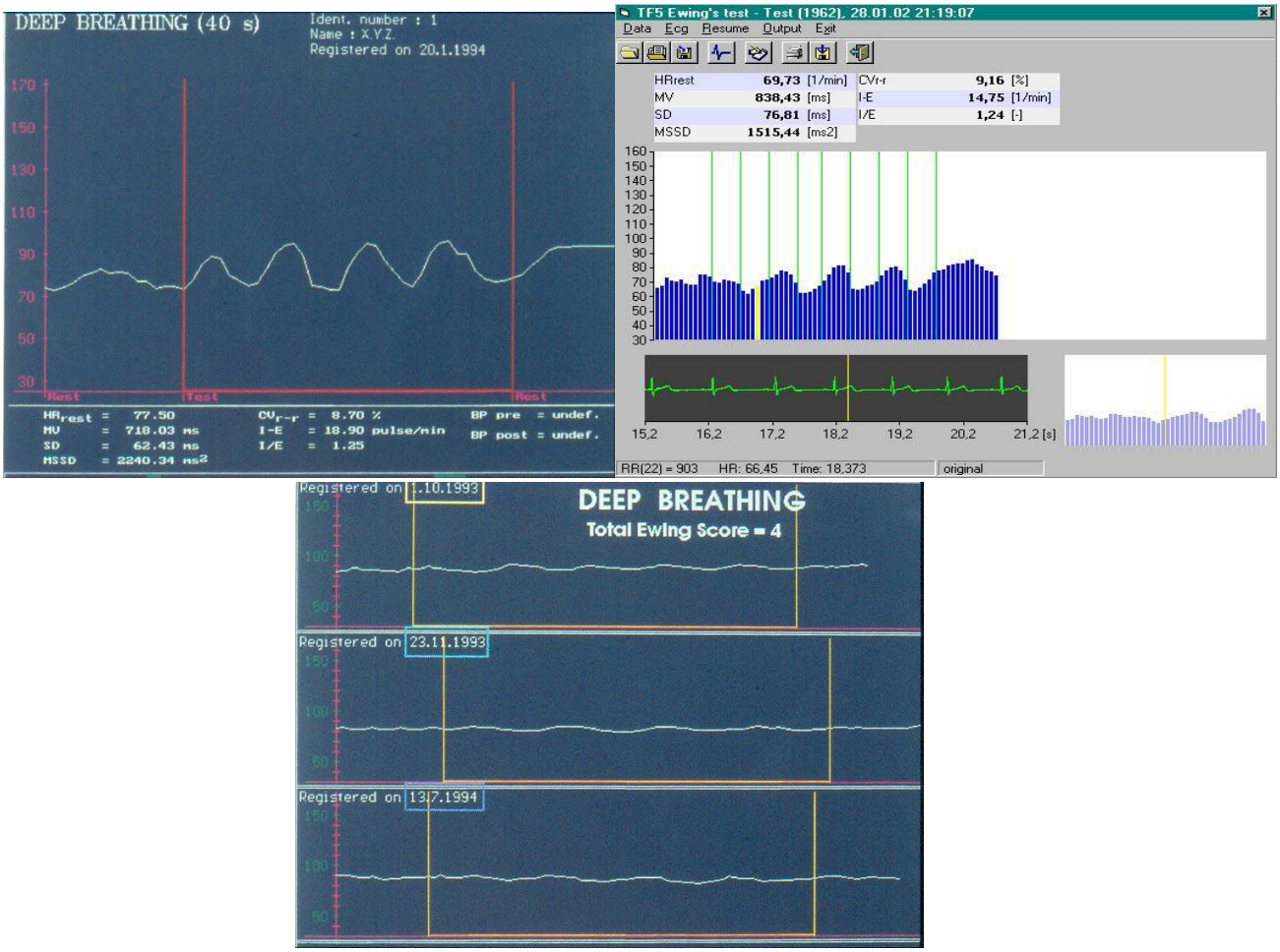
Jako u většiny biologických funkcí, je i zde vysledovatelný vliv řady faktorů včetně věku: s jeho nárůstem přirozeně klesá spektrální výkon. Z hlediska případné terapeutické intervence

je možné nález časného postižení -- tedy nižší spektrální výkon jen v oblasti parasympatiku — efektivně ovlivnit jak nemedikamentozně, tak medikamentozně. Při aplikaci vytrvalostního tréninku, krátkodobého lačnění nebo tréninku v řízeném dýchání jsme v našich studiích našli statisticky významné zlepšení HRV zvláště v oblasti vlivu parasympatiku *(Howorka 1997, 2013)*. Medikamentozně lze HRV ovlivnit typicky pomocí betablokátorů, ACE, v metabolické oblasti např. pioglitazonem *(Kobayashi 2010)*. Problematika nefarmakologických intervencí je podrobně diskutována později.

Pro frekvenční analýzu časové řady je bezpodmínečně nutná equidistantnost dat a proto je třeba odstranit/sofistikovaně nahradit případné artefakty získané během záznamu. Tento fakt také vylučuje z analýzy pacienty např. s fibrilací síní nebo frekventní komorovou extrasystolií *(Task Force 1996)*.

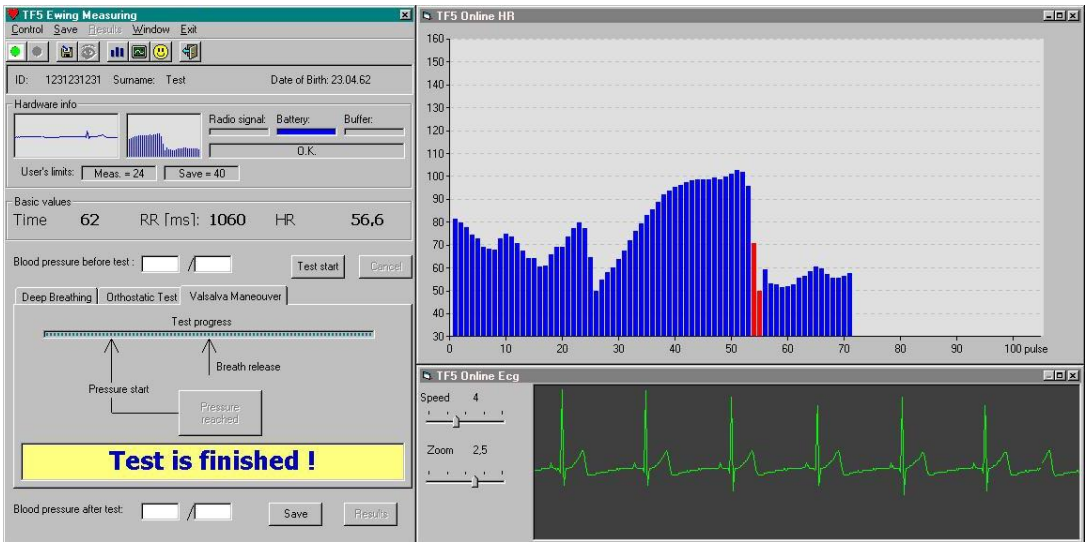
Časově a na vyšetřovací instrumentárium náročnější jsou kardiovaskulární reflexní testy analyzované v tzv. **časové doméně** (obvykle matematické průměry, rozdíly parametrů před-po a statistické kalkulace např. disperze dat kolem průměru, geometrické metody apod.). Jednou z nejčastěji užívaných je skupina čtyř (resp. pěti) kardiovaskulárních reflexních testů navržených Ewingem *(Ewing 1980 a 1985),* která zahrnuje test hlubokého dýchání, Valsalvův manévr, ortostatický test a hand-grip test. Typickými sledovanými parametry jsou zde rozdíl max-min srdeční frekvence, či poměry hodnot srdeční frekvence v různých predefinovaných okamžicích vyšetření. Ačkoliv jsou tyto testy v praxi principiálně proveditelné, jejich nevýhodou je jejich robustnost a nemožnost odhalit incipientní jemné změny regulací, resp. kvantifikovat podíl obou regulačních subsystémů. Nicméně, Ewing byl jedním z prvních autorů, prokazujících významnou prediktivní hodnotu nálezu autonomního postižení ve vztahu ke zvýšené mortalitě *(Ewing 1985)*. Na následujících **obrázcích 7, 8 a 9** je znázorněn průběh praktického vyšetření pomocí baterie čtyř reflexních testů dle Ewinga.

Obr. 7: Test hlubokého dýchání *– průběh variací srdeční frekvence v průběhu čtyř hlubokých vdechů a výdechů, s 1 cyklem trvajícím 10 sek., tj celkově 40 sek. Srovnání normálního nálezu (horní řádek, starší a novější systém) s patologickým (dolní řádek, zde celkově zobrazeny 3 záznamy, nález stacionární při 3 různých měřeních). Hlavním sledovaným parametrem je průměrný rozdíl maximální a minimální srdeční frekvence dosažené během testu, označováno rovněž jako „I-E" (inspiration – exspiration) diference, případně poměr I/E.*

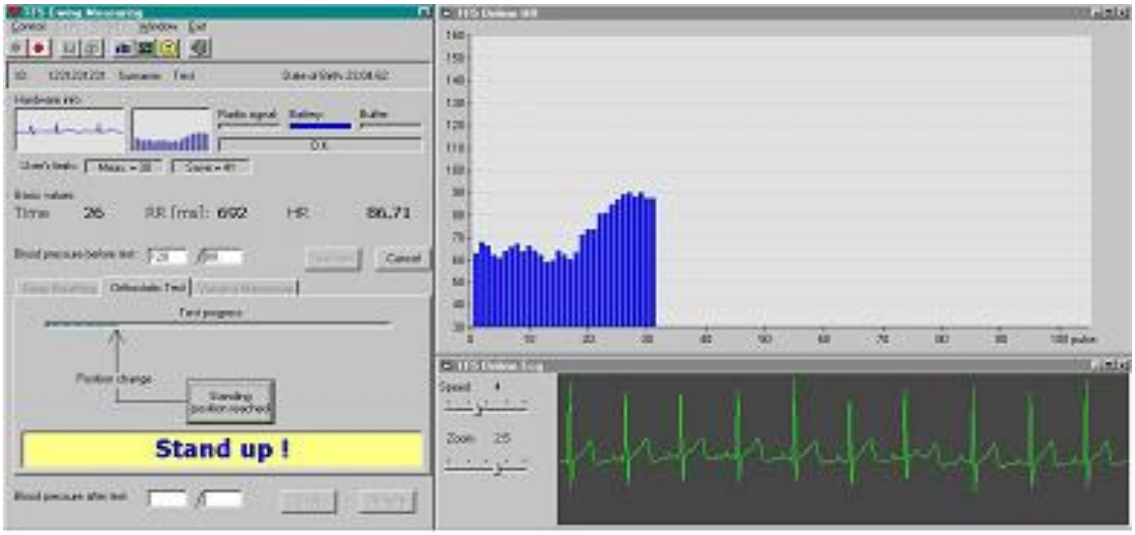


Prodloužení doby vyšetření na 24 hod. v rámci holterovského monitorování může přinést další informace zvláště z pásem tzv. velmi nízkých (0.01-0.04 Hz) či ultranízkých (pod 0.01 Hz) frekvencí, které dle některých autorů mohou dále zvýšit výtěžnost testu vzhledem k tomu, že se na celkové spektrální energii podílí až z 90%. Nicméně, při těchto „dlouhodobých" testech obvykle nelze – na rozdíl od výše referovaného krátkodobého 15-minutového ortostatického testu – standardizovat podmínky vyšetření, což může značně

Obr. 8: Valsalvův test 15-sekundového usilovného *výdechu proti odporu 40 mmHg. Hlavním sledovaným parametrem je tzv. Valsalva ratio, poměr srdeční frekvence na konci a začátku testu (fáze IV a II).*



Obr. 9: Ortostatický test: *zde se sledují dvě hlavní veličiny – průběh srdeční frekvence a krevního tlaku – v průběhu ortostatické manipulace. Hlavními sledovanými parametry jsou Index 30:15 (délka nejdelšího R-R interval kolem 30. srdečního úderu a nejkratšího kolem 15. úderu v průběhu testu), resp. R-R max/min, tedy poměr nejdelšího a nejkratšího R-R intervalu v průběhu celé manipulace. Krom srdeční frekvence se jestě vyhodnocuje pokles krevního tlaku po aktivním postavení, s patologickými hodnotami více než 20 mmHg rozdílu.*



Tab. 1: Normální hodnoty testů Ewingovy baterie *ve vztahu k věku: Je nutno zdůraznit, že výsledky a případnou patologii všech testů je třeba posuzovat striktně ve vztahu k věku pacienta a věkově-relevantním normám (Ewing 1985 a Metelka 2014)*

Index	I-E	I/E	RR_{max} / RR_{min}	ratio 30:15
Věk 20-29	20 min^{-1}	>1.36	>1.28	>1.07
30-39	15 min^{-1}	>1.23	>1.20	>1.07
40-49	15 min^{-1}	>1.14	>1.20	>1.03
50-59	12 min^{-1}	>1.10	>1.13	>1.03

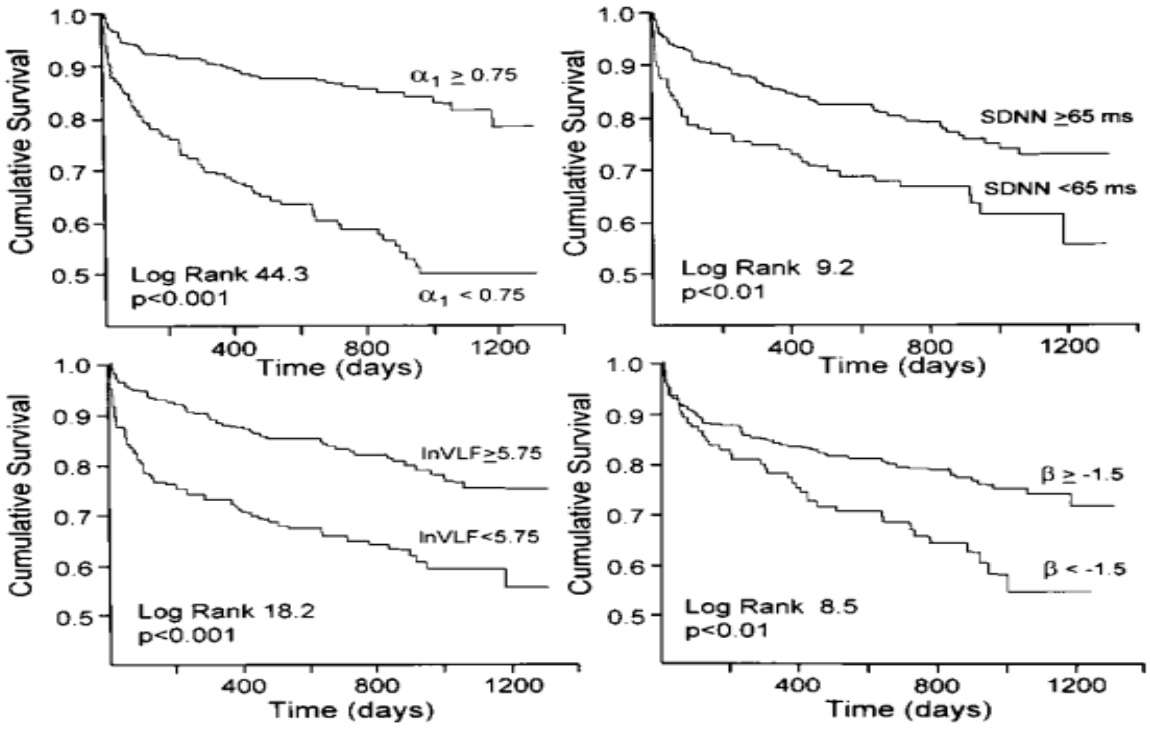
ztížit jeho výpovědní hodnotu. Například *Fujita et al* zjistili při holterovském 24h monitorování svých pacientů signifikantně rozdílné výsledky srdeční frekvence a spektrálních výkonů ve vazbě na polohu těla ve spánku: poloha na pravém boku byla preferována pacienty se srdečním selháním více než vlevo či na zádech *(Fujita 2000)*. Navíc se zdá, že výsledky HRV analýzy pečlivě standardizovaných krátkodobých vyšetření se zásadně neliší od analýz provedených na základě holterovského monitorování *(Sloan 1994).* V populační studii zjistil Sinnreich et al, že pětiminutové záznamy po dvouměsíčním opakování nevykazovaly signifikantní odchylku pro časovou ani frekvenční analýzu *(Sinnreich 1998)*. *Migliaro et al* při srovnání časové i frekvenční analýzy 24h záznamů a 10-minutových standardizovaných vyšetření nalezl u diabetiků statisticky významnou korelaci (r=0.70-0.85, p=0.003) obou veličin.

Vedle výše uvedené lineární analýzy se v literatuře uvádí také zajímavé výsledky výzkumu tzv. **nelineárních** komponent HRV, kdy se posuzuje „chaos“ v regulacích srdeční frekvence *(Wu 2009)* a řada dalších parametrů *(Tan 2009)*. Např. *Huikuri et al* nalezl u skupiny pacientů po akutním infarktu myokardu se sníženou ejekční frakcí významnější predikci all-cause úmrtí pomocí fraktální analýzy než „klasickou“ analýzou HRV v časové nebo frekvenční doméně (*Huikuri 2000)*.

Kromě dnes již standardní možnosti zjednodušené analýzy HRV u většiny systémů k holterovskému monitorování EKG, existuje řada komerčně dostupných, dedikovaných systémů určených k diagnostice autonomní dysfunkce v diabetologii a kardiologii, využívajících výše

zmíněnou lineární časovou a frekvenční krátkodobou analýzu. Jistou limitací je pak fakt, že ne vždy jsou výsledky měření získané jednotlivými systémy zaměnitelné, obvykle v důsledku použití odlišných zpracování dat či kalkulačních procedur. Typickým příkladem aplikace **časové analýzy** s cílem komerčního využití ve smyslu telemedicínské podpory analýzy HRV byl systém ANSCORE *(Risk 2001)*. Systém umožňoval provedení třech testů z původní Ewingovy baterie a komunikaci výsledků se vzdáleným (remote) centrem. Ač smysluplně verifikován tricentrickou studií u více než 200 pacientů *(Risk 2001)*, nedosáhl efektivního rozšíření a byl z trhu stáhnut. **Frekvenční/spektrální analýzu** dnes nabízí řada vendorů a není předmětem této práce se jednotlivostem věnovat. Obecně lze říci, že navzdory pokročilosti technologií a znalostí v oblasti autonomních dysfuknací, nadále existují signifikantní rozdíly ve výsledcích a transferabilitě metodiky mezi jednotlivými výrobci. Proto jsme věnovali pozornost řádné validaci námi využívaného systému, viz dále.

Obr. 10: Kaplan-Mayerovy křivky přežívání *pacientů po akutním IM se sníženou ejekční frakcí, pro parametry nelineární/fraktální (vlevo nahořea a vpravo dole) a lineární analýzy HRV (Huikuri 2000). Nicméně, tyto metody však obvykle zůstávají díky své komplexnosti a svízelné reprodukovatelnosti analytické metodiky omezeny na výzkumná pracoviště a v rutinní praxi se rozšířily minimálně.*



1.5. Přehled aplikací HRV v interní medicíně

Analýza HRV se využívá v praxi od 60.let minulého století, přičemž první zjednodušené sledování variací srdeční frekvence bylo využito v kardiotokografii *(Hon 1965)*. Zanedlouho na to se začala HRV uplatňovat v kardiologii *(Wolf 1978)* a následně rychle rostl počet aplikací v řadě dalších oborů. K listopadu 2014 bylo publikováno a indexováno v databázi PubMed americké národní lékařské knihovny (www.pubmed.gov) celkem 11 772 prací na klíčová slova „heart rate variability".

V roce 2002 jsme byli vyzváni skupinou kolem Dr Jima Nolana, která publikovala významnou studii UK-Heart prokazující prognostickou hodnotu analýzy HRV u pacientů s chronickým kardiálním selháním *(Nolan 1998)*, k sestavení přehledového článku k tématu funkčního, praktického využití analýzy variability srdeční frekvence. Review jsme publikovali v časopise Int J Cardiology a k dnešnímu dni článek zaznamenal 160 citací dle citační databáze Scopus/Elsevier (viz 8.kapitola Publikační a vědeckovýzkumná činnost autora). Podle této citační databáze (Scopus/Elsevier) bylo 30 našich prací k 1. 11. 2014 citováno celkem ve 450 publikacích. Celkový počet citací je 502.

V další části této disertační práce jsou k jednotlivým tématickým celkům („Přehledové články", „Metodologické cíle: Vývoj a validace metodiky, stanovení norem" a „Klinické/aplikační cíle: Výzkum reverzibility autonomní dysfunkce. Kvantifikace účinků terapeutických opatření") uvedeny in extenso nejvýznamnější publikace -- viz Seznam komentovaných prací autora. Součástí každé publikace je podrobná diskuse.

1.5.1. Pumprla J, Howorka K, Groves D, Chester M, Nolan J: Functional assessment of heart rate variability: Physiological basis and practical applications. International Journal of Cardiology, 2002, 84 (1): 1-14.
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Review article

Functional assessment of heart rate variability: physiological basis and practical applications

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Abstract

The autonomic nervous system dynamically controls the response of the body to a range of external and internal stimuli, providing physiological stability in the individual. With the progress of information technology, it is now possible to explore the functioning of this system reliably and non-invasively using comprehensive and functional analysis of heart rate variability. This method is already an established tool in cardiology research, and is increasingly being used for a range of clinical applications. This review describes the theoretical basis and practical applications for this emerging technique.

Keywords: Heart rate variability; Autonomic system; Sympathetic; Parasympathetic; R–R interval; Spectral analysis

1. Introduction

One of the most interesting non-invasive diagnostic methods increasingly used in medicine is analysis of heart rate variability (HRV). Detailed and sophisticated analysis of fluctuation in heart rate can be used to indirectly assess autonomic control of the heart [1–5]. Change in the HRV pattern provides an early and sensitive indicator of compromised health [6–10]. A high variability in heart rate is a sign of good adaptability, implying a healthy individual with well functioning autonomic control mechanisms. Conversely, lower variability is often an indicator of abnormal and insufficient adaptability of the autonomic nervous system, implying the presence of a physiological malfunction in the individual for which further investigations are required to yield a specific diagnosis. Simple analysis of variation in heart rate has been used in clinical practice since the early 1960s, with reduced foetal HRV indicating that clinically significant hypoxia may be developing [11]. In the late 1970s, a reduction in HRV was first correlated with increased mortality and arrhythmic events in survivors of myocardial infarction [12]. More recently, reduced HRV has emerged as a strong indicator of risk related to adverse events in normal subjects [7,13] and patients with a wide range of diseases [14–19], reflecting the vital role the autonomic nervous system plays in maintaining health. The purpose of this review is to discuss physiological and technical aspects of HRV analysis, along with an

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overview of the research and clinical applications of the techniques.

2. Physiological background of HRV analysis

The cardiovascular system displays features typical of self-organising systems designed to achieve dynamical stability [20]. In the case of the cardiovascular system, stability is achieved by autonomically mediated control of heart rate, blood pressure and other factors which react rapidly to a range of internal and external stimuli such as acute ischaemia, metabolic imbalance and changes in physical or mental activity. In particular, heart rate varies in a complex reactive manner to these stimuli (which occur even in resting individuals). The sinus node is densely innervated by both autonomic divisions, and heart rate will reflect their modulating effect on the intrinsic firing rate of its pacemaker cells. Parasympathetic activation slows heart rate. This effect is mediated by synaptic release of acetylcholine, which possesses a very short latency period and high turnover rate. The rapid response of this biological mechanism enables the parasympathetic nervous system to regulate cardiac function on a beat to beat basis. Sympathetic activation results in an increase in heart rate and conduction system velocity, together with an increase in contractility. This is mediated by synaptic release of noradrenaline which is reabsorbed and metabolised relatively slowly. Changes in cardiovascular function mediated by alterations in sympathetic activity therefore have a slower time course. Because of these differences in neurotransmitter function the two subsystems of the autonomic nervous system tend to operate at different frequencies and variation in heart rate related predominantly to changes in sympathetic or parasympathetic activity can be identified and quantified [21–23]. Although the interpretation of this type of data requires some caution, it does provide the basis for non-invasive semi-quantitative assessment of autonomic activity [5,24].

In normal individuals cyclical changes in heart rate occur in association with respiration [25]. This respiratory related variation occurs at a high frequency (typically around 0.25 Hz or 15 times per minute at rest) and can be abolished by vagal blockade [26]. These two factors suggest that this particular type of high frequency cyclical HRV is parasympathetically mediated. Cyclical variation occurring in association with changes in baroreceptor activity (due to fluctuation in blood pressure) can also be easily identified [27]. This baroreceptor mediated variation occurs at a lower frequency (typically 0.10 Hz or six times per minute) and can be significantly modified by sympathetic blockade [26,28]. There is also a close correlation between this low frequency variation in heart rate and direct measures of muscle sympathetic nerve activity [29]. These factors suggest that sympathetic activity is an important mediator of this low frequency cyclical HRV. Recently, studies have demonstrated that vagal blockade also produces some modification of this low frequency HRV, suggesting that there is also a parasympathetic component to this cyclical activity. Because of this dual modulation, measurement of low frequency cyclical HRV does not provide a direct quantitative index of sympathetic activity [26]. Simultaneous measurement of high and low frequency HRV, however, can be used to investigate changes in sympathovagal balance. Since the two arms of the autonomic nervous system operate in a co-ordinated fashion, relative changes in the amount of high and low frequency HRV provide a semiquantitative index of the direction and magnitude of reciprocal alteration in sympathovagal balance (with a high frequency bias suggesting parasympathetic dominance, and a low frequency bias suggesting sympathetic dominance) [30,31].

Superimposed on this sympathetic and parasympathetic mediated activity are very slow cyclical variations occurring at a rate of one per minute or less (equal to or less than 0.01 Hz). These have not been well characterised, but are thought to be related to changes in autonomic activity associated with thermoregulatory mechanisms [32], changes in peripheral chemoreceptor activity [33,34] and/or fluctuations in activity of the renin-angiotensin and parasympathetic systems [35,36].

In addition to these cyclical variations, there are frequent, sudden large beat to beat changes in R–R intervals that are superimposed on the cyclical changes, and occur throughout the day and night [1]. Sudden beat to beat changes in R–R interval, such as those that occur in association with changes in posture or muscular exercise are abolished by atropine but unaffected by beta-blockers. In animals,

beat to beat variation in R–R interval is abolished by vagal section, but returns with direct stimulation of the sectioned vagus. Beat to beat variation in R–R interval is reduced or absent in subjects with parasympathetic neuropathy [37]. Thus the non-cyclical beat to beat variation in R–R interval that occurs constantly in man is thought to be parasympathetically mediated. The spontaneous variation in efferent cardiac parasympathetic activity that generates these constant beat to beat changes is a normal physiological response to afferent inputs from the periphery, higher centres, and other neuroendocrine systems.

Heart rate variability is quantified by analysis of variations of the intervals between consecutive normal heart beats. The usual definition of a heart beat interval is the time between consecutive R wave peaks. Advances in computer technology have allowed sequential R–R intervals to be measured accurately and recorded in real time. After passing through automated ectopic beat and artefact handling procedures [38], sophisticated and fast methods of analysis can then be applied to the data to determine HRV measurements which reflect autonomic nervous system activity. Typically, HRV can be measured using add-on software in some standard ECG machines or by dedicated HRV analysers. Instantaneous heart rate is displayed on a computer screen in the form of a bar graph (known as a tachogram), where each bar represents the heart rate associated with an individual sequential R–R interval (Fig. 1). A curve passing through the peaks of these bars represents the variation in heart rate. Useful parameters which relate to autonomic processes are determined by further quantitative analysis of this curve.

3. Assessment of HRV by cardiovascular reflex testing

Early techniques for analysis of autonomic activity were based on evaluating heart rate changes evoked by stimulation of cardiovascular reflexes. One of the most widely used early sets for investigation of cardiovascular reflexes was proposed by Ewing et al. The Ewing 'battery' of tests includes measurement of heart rate changes induced by deep breathing, Valsalva-manoeuvre, orthostatic load and a hand-grip test [39]. The total Ewing score based on the results of the above tests provides a semi-quantitative measure of the presence and magnitude of autonomic dysfunction in an individual. More complex reflex tests use tilt tables [31], suction chambers [40], or pharmacological manipulation of blood pressure [41] to stimulate reflex changes in heart rate. All of these reflex tests suffer from similar problems [1]. It is difficult to standardise the stimulus used to evoke the reflexes, with a resultant high degree of variability in the range of results produced by the tests. Measurements of reflex responses are made over a short time period, and may produce atypical results solely due to natural variation. The stimulus used to evoke the reflex may be responsible for temporarily changing cardiovascular function (for example by inducing myocardial ischaemia) and this may significantly influence the result of the test. Autonomic reflexes studied in a physiology laboratory may not relate directly to autonomic function in an ambulant individual. The problems associated with the use of reflex testing stimulated investigators to develop techniques for investigating autonomic function based on more detailed analysis of HRV.

4. Time domain analysis of HRV

These methods use mathematically simple techniques to measure the amount of variability present in a pre-specified time period in a continuous electrocardiogram [1,21–23,42]. After editing to remove non-sinus beats and artefact, the remaining normal to normal R–R intervals are measured and subjected to simple statistical analysis. The most commonly used technique is to plot a histogram of R–R interval duration against the number of R–R intervals in a 24-h period and then to calculate the standard deviation of the frequency distribution (SDNN index). An alternative approach is to use a technique based on geometric analysis of the 24-h R–R interval histogram (St George's index or HRV index [16]). These methods are closely related to the SDNN index, but have the advantage of being less dependent on accurate classification of individual beats, and therefore reduce the need for extensive editing of ambulatory electrocardiograms. All of these indices quantify

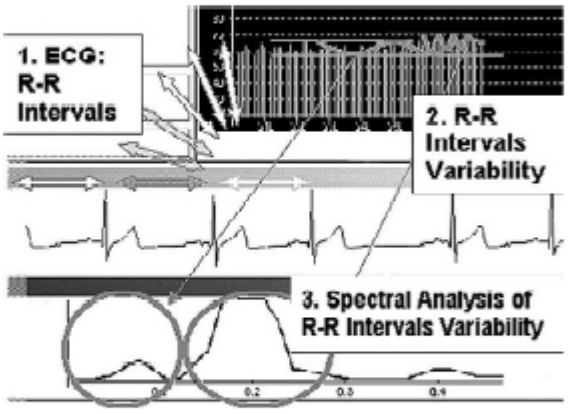

Fig. 1. Tachogram of heart rate (R–R intervals) variability as obtained during evaluation in a supine position. Each bar in the upper part of the figure represents one heart beat. The main principle of spectral analysis of HRV is a detection and quantification of harmonic, regularly occurring components within the irregular course of heart rate. The HRV displayed here shows predominant vagal influence as it physiologically appears during quiet, prone position (fast, regular changes of heart rate).

the total amount of variability present in a 24-h recording, and are influenced by changes in both sympathetic and parasympathetic activity, making them non-specific measures of sympathovagal balance. They are useful clinical tools for detecting abnormalities of autonomic activity (Fig. 2), but cannot be used to quantify specific changes in sympathetic or parasympathetic activity.

There are a number of techniques available to measure beat to beat R–R interval variability, providing interchangeable measurements of parasympathetic activity. One approach is to measure successive beat to beat R–R interval differences, and calculate an index that expresses the distribution of these differences such as the rMSSD index, based on the standard deviation of successive differences. An alternative technique is to count the number of large beat to beat changes that exceed a pre-set threshold in a recording. If only beat to beat changes in excess of 50 ms are counted (sNN50 index), an index that clearly separates normal individuals from those with parasympathetic dysfunction is obtained (Fig. 3). These indices provide sensitive and specific interchangeable time domain measurements of parasympathetic activity, which are easy to measure in clinical quality ambulatory electrocardiograms [42–44].

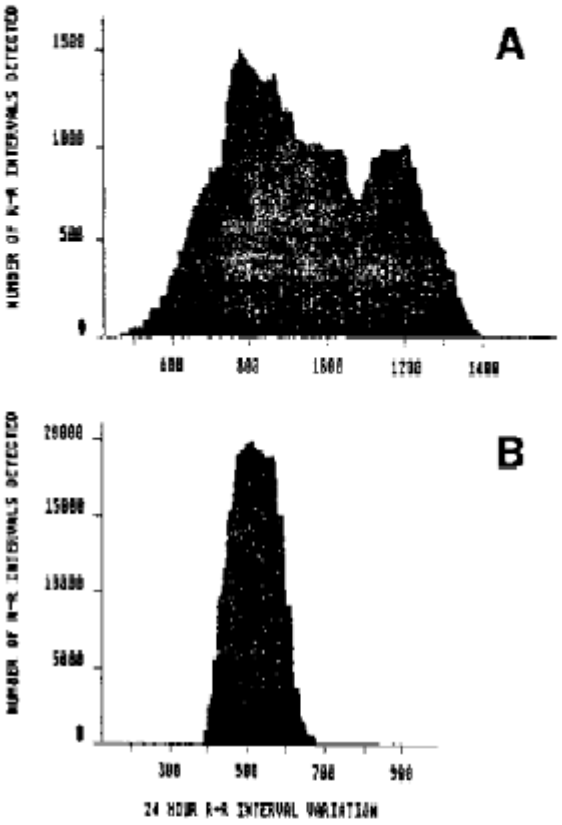

Fig. 2. A 24-h R–R histogram in a normal subject (A) and a patient with chronic heart failure (B). The standard deviation obtained from plot A will be considerably greater than in plot B, providing a numerical index that signifies the presence of autonomic dysfunction in the heart failure patient.

5. Frequency domain analysis of HRV

It is difficult to obtain precise physiological data about changes in autonomic function using relatively unsophisticated time domain analysis of HRV. Because of this, investigators have invested considerable time and effort in developing alternative techniques to investigate cyclical changes in HRV. Before this type of analysis can be performed, extensive editing and review of the electrocardiogram by an experienced operator is required to remove/edit non-sinus ectopic beats, pauses, tape artefact and non-periodic R–R interval changes. Gaps in the R–R interval series associated with data deletion are replaced by interpolating beats using a variety of

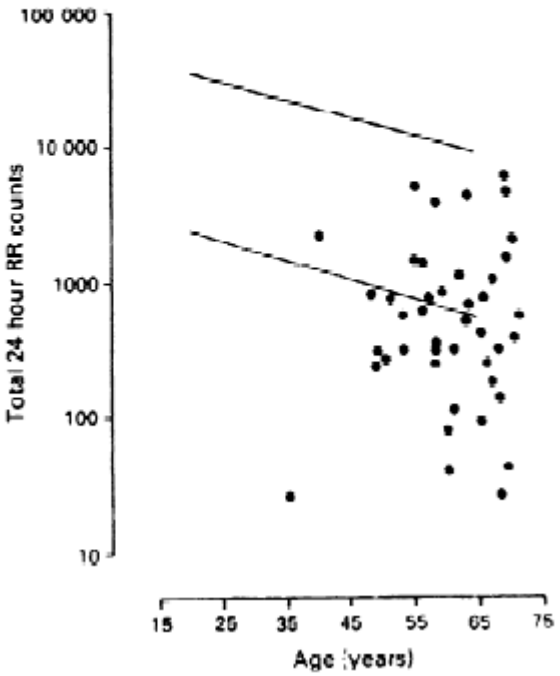

Fig. 3. Measurement of sNN50 index in patients with chronic heart failure. Solid lines represent 95% confidence intervals for counts in normal subjects. Measurements in more than half of the heart failure patients lie below the lower 95% confidence interval and thus have detectable parasympathetic impairment.

different algorithms [38]. The edited normal to normal R–R interval series is then analysed using the mathematical techniques of fast Fourier transformation or autoregression analysis [45], and the amount of cyclical variation present at different frequencies can be detected and quantified [4]. This information is usually presented graphically by plotting the amount of variation present in a recording on the vertical axis against the frequency at which it occurs on the horizontal axis. By measuring the area under the curve at different frequencies (expressed as spectral power) a numerical measure of the amount of high and low frequency cyclical variability present in the recording is obtained.

Early studies used strictly controlled laboratory conditions to obtain short high quality R–R interval recordings from which accurate frequency domain measurements were used to explore autonomic activity in animal studies [4]. Frequency domain analysis has also been applied to human 24-h ambulatory electrocardiograms. Although the information obtained has value in risk stratification, the large amount of artefact, ectopy and non-stationary heart rate behaviour that is present in these long-term recordings renders analysis difficult and poorly reproducible [46]. More recently, advances in technology have facilitated rapid on-line frequency domain analysis of HRV during a modified orthostatic test where the individual lies supine for 5 min, stands for 5 min and lies supine again for another 5 min [47–49]. The autonomic system responds to changes in posture via blood pressure receptors in both the lungs and arterial system (as well as to barometric receptors in the lungs). Hence this postural test provides a method of evaluating the individual's autonomic response to standardised changes in position and the associated changes in blood pressure. These short recordings, obtained during controlled conditions, are relatively free of noise and artefact, simplifying the analytical process. The result of this examination protocol is displayed in the form of a three-dimensional graph of frequency (x-axis) plotted against time and spectral power. This type of analysis generates an easily understood visual representation of heart rate variations occurring during the examination. Fig. 4 shows a clear predominance of parasympathetic (high frequency) activity during supine positions and its reduction during standing where mostly 'sympathetic' (low frequency) autonomic activity predominates. In the early stages of autonomic dysfunction parasympathetic activity is reduced. In severe autonomic dysfunction, activity in both subsystems (high and low frequency bands) reduces by a number of magnitudes (Fig. 5a–c). In addition to the visual representation, normal ranges for high and low frequency activity (spectral power) during the three periods of the orthostatic test have been established to allow the rapid identification of autonomic dysfunction [47]. This type of rapid on-line analysis facilitates the clinical application of frequency domain techniques while meeting technical and clinical prerequisites for meaningful power spectral analysis of heart rate variability (sufficient length of signal (at least ten times the wavelength of the lowest frequency component), stationarity of data in each position due to controlled examination conditions, relative ease of test performance including

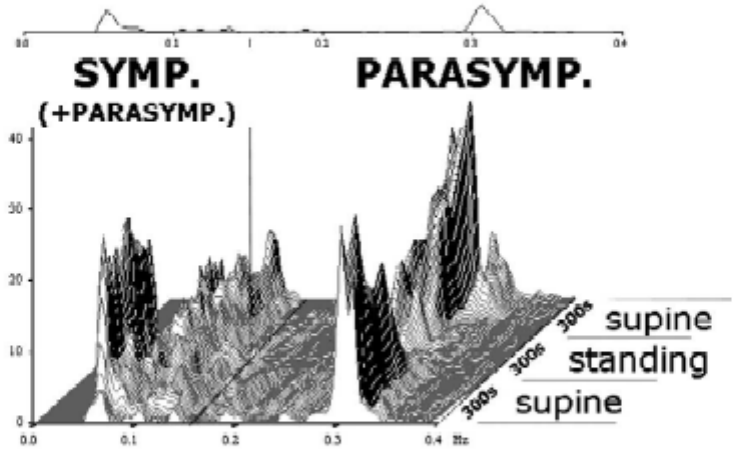

Fig. 4. Typical pattern of short-term spectral analysis of HRV record as obtained during a modified orthostatic load (standardised assessment by VariaCardio TF4 in supine-standing-supine, each position 5 min). Both supine positions show a clear predominance of vagal tone (high frequency band, above 0.15 Hz), whereas after orthostatic manipulation an activation in low frequency band (below 0.15 Hz) and reduction in vagal tone is shown.

artefact processing due to restricted duration of recordings and minimum dependence on the co-operation of the subject) [5,26,45].

6. Comparison of time and frequency domain techniques for analysis of HRV

Some time and frequency domain HRV measurements are closely related [42]. Indices that measure beat to beat parasympathetically mediated HRV (rMSSD, sNN50 and high frequency power) and measures of the total amount of variability present in a long-term recording, such as SDNN and total spectral power, are strongly correlated. These time and frequency domain indices can therefore be used interchangeably. The HRV technique chosen for a particular study will depend on a variety of different factors. Frequency domain techniques facilitate a more precise evaluation of the direction and magnitude of changes in sympathovagal balance than is possible with time domain analysis. Accurate assessment of autonomic activity by frequency domain techniques require that heart rate data are free of artifact and obey strict mathematical criteria, conditions that can only be reliably obtained when subjects are studied under controlled conditions [50]. Since time domain techniques do not have such strict requirements, they are easier to apply to the study of clinical quality ambulatory electrocardiograms. A potential limitation of tests based on 24-h recordings is that it is not possible to standardise the conditions for the examination. This may be of particular importance, since recent studies indicate that physical activity is a major contributor to the lowest frequency components of HRV [45,46,51]. Despite this, these techniques are highly reproducible in a variety of different clinical situations [52]. Although 24-h ambulatory electrocardiograms are commonly used for HRV studies, the optimal time period required to obtain useful data has not been determined, with some investigators reporting that clinically useful information can be obtained from recordings as short as 5 min [53,54]. Moreover, short, 2–15-min samples are excellent predictors of mortality and are correlated with prognostically important data from sustained recording periods [55]. This suggests that frequency domain measurements obtained from short recordings (particularly when conditions are standardised by using the modified orthostatic protocol), may prove to be useful without requiring a long-term electrocardiogram [47,56].

7. New analytical techniques

In the last decade a series of complex techniques have been developed to provide additional infor-

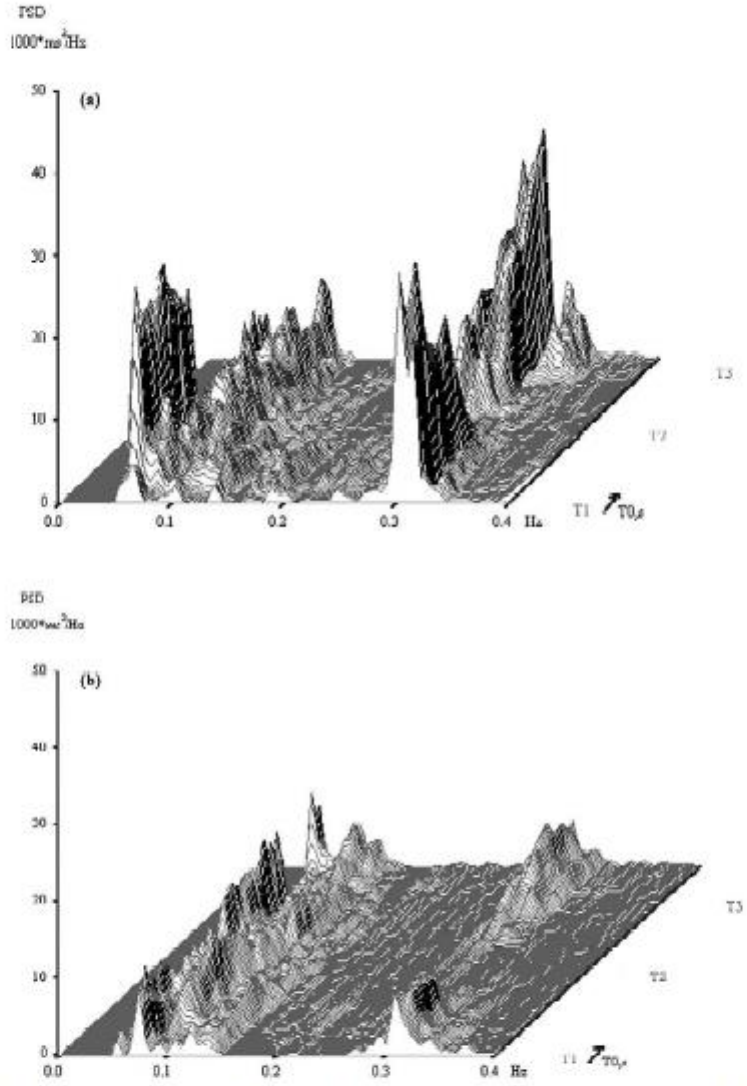

Fig. 5. Typical findings of short-term spectral analysis of HRV as obtained during a modified orthostatic load (compare with Fig. 4) in patients (a) *without* cardiovascular autonomic dysfunction, (b) with *early* stage and (c) with *severe* stage of cardiovascular autonomic dysfunction. Note a typically depressed HRV within the high frequency band, preserved autonomic control in low frequency band in the *early* dysfunction, and reduction of HRV throughout the whole spectrum with only a minimum reaction to orthostatic manipulation in *severe* dysfunction.

mation over and above that available from standard time and frequency domain analysis of HRV. Investigators have recently reported on new time domain techniques that provide some information on sympathetic activity [57–59]. The techniques of peak — through analysis, complex demodulation and acceleration–deceleration oscillation analysis, are closely related and based on similar principles. Post ectopic turbulence analysis may prove useful as a non-invasive index of cardiovascular reflex integrity [60]. Analysis of beat to beat QT interval variability may emerge as a useful quantitative index of sympathetic activity if reliable methods of correcting for changes in heart rate can be developed [61]. The use of

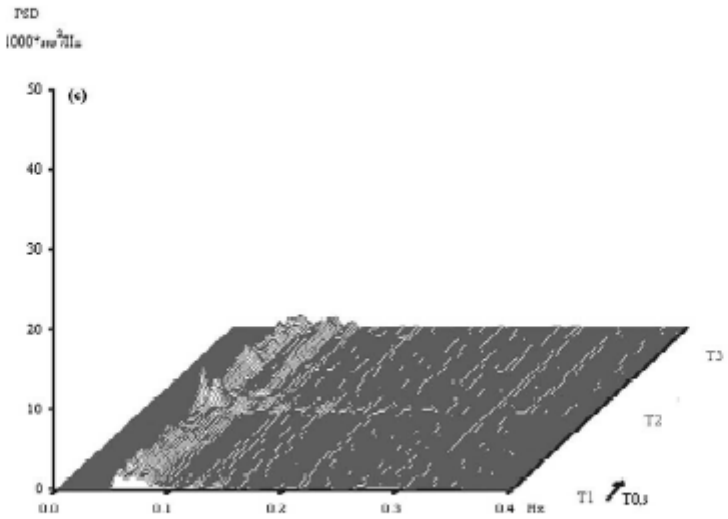

Fig. 5. (*continued*)

non-linear dynamics or chaos analysis for evaluation of autonomic control processes has also been investigated in normal subjects and patients with cardiovascular disease [62–64]. The techniques can be applied to clinical quality ambulatory electrocardiograms, but they are mathematically complicated and have yet to be extensively validated or applied to clinical investigations. Further research and more consistent evidence based on larger studies is needed before these techniques can be incorporated into routine practice.

8. Practical applications of HRV analysis

In the last two decades, analysis of HRV has been extensively applied to the investigation of normal physiology. Prior to the HRV era, investigation of autonomic physiology required the use of complex highly invasive techniques in animal models or imprecise reflex based tests in humans. The use of HRV analysis has provided a simple reproducible method of non-invasive autonomic assessment. This has helped to clarify the role of the autonomic nervous system in regulating the cardiovascular response to changes in posture (parasympathetic dominance when supine, sympathetic dominance when standing), stress (sympathetic dominance) and exertion (sympathetic dominance) [4]. The role of the autonomic nervous system in regulating cardiovascular circadian rhythms has also been evaluated, with HRV studies demonstrating sympathetic dominance in the morning, but parasympathetic dominance at night [65]. This early morning increase in sympathetic tone may be a trigger for the cascade of pathophysiological changes underlying the increase in acute cardiac events during the first few hours of the morning [66] and can be slowed down in angina patients by metoprolol [67]. Interestingly, a rapid spontaneous vagal withdrawal in the morning seems to be a sign of a healthy autonomic control [67]. The physiology of normal ageing can also be explored, with a progressive decline in sympathetic and parasympathetic activity occurring in association with increasing age. Measurement of HRV may be of prognostic value even in individuals who are free of overt clinical disease, with low values identifying those at increased risk of premature cardiac disease [13].

Measurements of HRV have been used to assess autonomic function in a variety of non-cardiac diseases. Diabetes mellitus is commonly associated with autonomic neuropathy, with the degree of dysfunction related to the severity and duration of the disease [39,68,69]. Measurement of HRV is superior to reflex

testing for the detection of autonomic neuropathy [65]. Diabetic patients with detectable autonomic neuropathy have a substantially increased risk of premature death [14,19]. Reduced HRV in a type 1 diabetic subject is an early sign of systemic diabetic complications [70]. In type 2 diabetes with autonomic neuropathy, an additional risk related also to other aspects of metabolic syndrome accounts for up to four-fold risk of cardiovascular death when compared with non-diabetic individuals [71].

More than half of all patients with end stage renal disease have detectable autonomic neuropathy, which may be aetiologically linked to the build-up of metabolic waste products [72,73]. Metabolic derangement in chronic liver disease and/or hypoxia in chronic respiratory disease can also induce autonomic abnormalities leading to reduced HRV [74,75]. It is not surprising that disorders of the central and peripheral nervous system are also associated with autonomic dysfunction leading to abnormalities of HRV [76]. Also, abnormal autonomic function tests are common in some other systemic diseases, such as in HIV infected individuals. It has been shown that it may be attributed to severe global autonomic dysfunction which is not related to heart disease [77]. A progressive reduction in HRV occurs in patients on intensive therapy units who develop brain death. This phenomenon may help to identify candidates for organ donation [78]. In diabetes, renal, hepatic and neurological disease, improvement in metabolic or neurological function is commonly associated with a return to a normal HRV pattern [50,79,80]. A similar effect was seen after physical training in individuals with autonomic dysfunction [81,82]. Analysis of HRV may also have a clinical role in occupational health, for example when exploring elevated cardiovascular risk in shift workers [83] or in evaluation of associations between ambient pollution levels and cardiovascular function [84].

Autonomic dysfunction plays an important role in the pathophysiology of ischaemic heart disease. Although patients with uncomplicated ischaemic heart disease have minimal [85] or undetectable autonomic abnormalities [86], acute myocardial infarction is associated with adverse changes in autonomic activity, which can be easily detected and quantified by measuring different components of HRV [87]. The degree of autonomic dysfunction depends on the site and size of the infarct and can be favourably modified by drugs such as streptokinase [88]. Patients who have marked autonomic dysfunction leading to reduced time or frequency domain measurements of HRV are at increased risk of premature death [15,89]. Decreased HRV was reported to be more sensitive and specific as a predictor of mortality than conventional risk factors, such as a low ventricular ejection fraction, abnormal exercise test, adverse clinical grading score or the presence of ventricular arrhythmias on ambulatory electrocardiograms [42].

Patients with chronic heart failure (CHF) also have evidence of autonomic dysfunction, with the maximal derangement occurring in patients with severe left ventricular dysfunction [90]. The reduced HRV seen in CHF relates to the occurrence of significant compensatory neuroendocrine dysfunction, which will predispose patients to myocardial electrical instability or promote the development of progressive heart failure due to deleterious changes in cardiac loading conditions. Recently published data from the United Kingdom Heart Failure Evaluation and Assessment of Risk Trial (UK HEART) confirms that reduced HRV is a significant independent predictor of death in CHF in a large prospective study powered for mortality [18]. These promising results indicate that reduced HRV may be more sensitive and specific in CHF than in post-MI patients, suggesting a more important clinical role for HRV analysis in the heart failure population. Cardiovascular deconditioning including autonomic dysfunction as found in patients with developed cardiac disease such as CHF can be improved with physiological measures like regular exercise training [91] that can also be effectively performed under home-based conditions [92].

Patients with hypertension have detectable changes in HRV, reflecting adverse autonomic modification that may play an important role in the pathophysiology of the disease [93,94]. Essential hypertension is associated with an increase in sympathetic activity and abnormalities of autonomic circadian rhythm. Control of hypertension is associated with an increase in HRV and an associated reduction in cardiovascular morbidity and mortality [95].

Transplanted hearts show detectable HRV despite iatrogenic denervation, which may be due to indirect effects dependent on the Starling mechanism or variation in circulating catecholamine levels [96].

Additionally, it is apparent that HRV can increase with time in some long-term transplant survivors, indicating that reinervation has occurred [97,98]. Some studies also suggest that episodes of rejection are associated with variation in HRV, which could therefore be useful as a guide to the need for cardiac biopsy [99].

The relationship between reduced HRV and prognosis in patients with cardiovascular disease may in part be related to the role of the autonomic nervous system in regulating myocardial electrical stability. Sympathetic activation favours the onset of life-threatening ventricular tachyarrhythmias, whereas parasympathetic activation exerts a protective and antifibrillatory effect, and abnormalities of HRV reflecting adverse changes in autonomic activity have been demonstrated immediately prior to the onset of ventricular tachyarrhythmias [100]. Patients who survive cardiac arrest are at increased risk of further arrhythmic events if they have reduced HRV, suggesting that autonomic dysfunction plays a clinically important role in the pathogenesis of ventricular tachyarrhythmias. Autonomic changes may play a role in the pathogenesis of atrial fibrillation. Vagal stimulation decreases the atrial refractory period, facilitating the development of re-entrant wavelets [101]. The haemodynamic and structural changes associated with cardiac disease result in adrenergic stimulation, which predisposes to electrical instability by shortening action potential duration, and increasing automaticity and triggered activity. Recently, a number of studies utilised HRV analysis to assess autonomic modulation prior to the onset or early recurrence of paroxysmal atrial fibrillation in patients with and without structural heart disease [102]. An increase in vagal tone was seen in patients with idiopathic paroxysmal atrial fibrillation, whereas in patients with structural heart disease, an increase in sympathetic activity was observed [101,103]. Potentially, these changes in autonomic activity prior to the onset of paroxysmal atrial fibrillation can help in guiding antiarrhythmic therapy. For instance, patients with vagally induced atrial fibrillation may benefit from antiarrhythmic drugs with inherent vagolytic activity (e.g. disopyramide or quinidine), whereas patients with adrenergically induced atrial fibrillation may benefit from agents with beta-blocker activity (e.g. atenolol, sotalol and amiodarone). Reduced quality of life in patients with paroxysmal atrial fibrillation correlates with depressed vagal function, which may be of value for risk assessment or for consideration of treatment strategies in these subjects [104].

The potential value of risk stratification in patients with cardiovascular disease using HRV analysis lies in identifying a subgroup of patients at high risk of premature death, who can then be selectively targeted for intervention. The use of a combination of reduced HRV, QT dispersion, late potential and ventricular arrhythmia analysis may help increase the sensitivity and specificity of non-invasive electrophysiological risk stratification [105–108]. A high-risk subgroup identified in this way could be targeted with interventions aimed at reducing the risk of death, such as cardioverter-defibrillator implantation, or allocation to additional drug therapy. A further unexplored therapeutic avenue is the use of agents that modulate autonomic activity. Drugs that decrease HRV have an adverse effect on prognosis in patients with cardiac disease [109]. Agents that increase heart rate variability, such as angiotensin converting enzyme inhibitors and beta-blockers, reduce death rates in high-risk populations [110,111]. Recently, agents such as scopolamine or related compounds, have been evaluated and shown to improve sympathovagal balance, with a consequent improvement in myocardial electrical stability [112]. As yet, no prospective randomised clinical trials have evaluated these interventional strategies in high-risk populations identified by the techniques discussed above.

9. Conclusions

The autonomic nervous system plays a major role in normal physiological function and in the pathogenesis of many medical disorders. Measurement of HRV provides an easily applied non-invasive method of assessing autonomic function. Time domain techniques are mathematically simple and easy to apply to clinical quality ambulatory electrocardiograms. Frequency domain techniques are more complex and technically demanding, but provide more physiological information. Recent advances in computing hold out the possibility of fully automated signal processing which will facilitate rapid, reliable and reproducible HRV analysis with minimal operator input, increasing clinical accessibility. In 1996 a Task Force

of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology published guidelines for the measurement of HRV which have helped to standardise the application and interpretation of the techniques [5]. Analysis of HRV was first used in clinical practice almost 40 years ago. The last two decades have seen increasing exploration of the potential clinical value of HRV analysis, particularly in patients with cardiac disease and diabetes mellitus. Now that HRV analysis equipment is available in most cardiac departments, the next two decades may see HRV techniques move into routine clinical use, particularly for risk stratification of cardiac and diabetic patients or in the follow-up of patients after therapeutic intervention.

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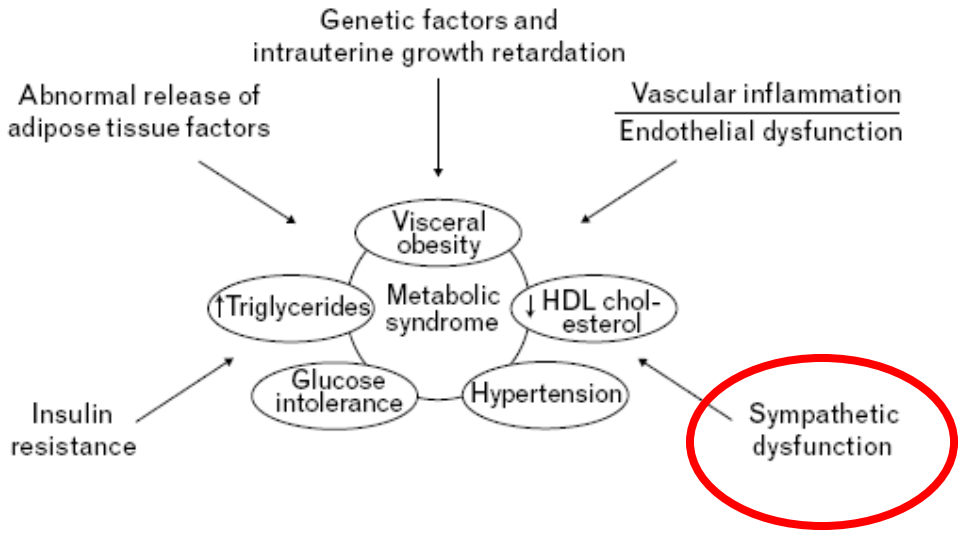
1.6. Metabolický syndrom a autonomní dysfunkce

Metabolický syndrom sdružuje soubor chronických faktorů lipidového a nelipidového charakteru a metabolického původu, které jsou asociovány s inzulinorezistencí, a které ve svém vývoji vedou k rozvoji diabetu 2.typu a kardiovaskulárního postižení *(Reaven 1988, Isomaa 2001, Lakka 2002)*. Za společného jmenovatele se považuje inzulinorezistence, která je definována jako snížená citlivost tkání (játra, svalová tkáň) a schopnosti využívat inzulin ke zpracování glukozy. V dalším rozvoji syndromu dochází k hyperinzulinemii mající za následek progresi dyslipidemie, obezity a rozvoj diabetu 2.typu.

Existuje řada důkazů, že excesivní sympatikotonie hraje významnou patogenetickou roli v rozvoji metabolického syndromu. Neuroadrenergní vlivy reprezentují klíčové regulační mechanismy, mající vliv jak na procesy a funkce kardiovaskulární tak i metabolické (krevní tlak, homeostaza glukozy, koncentrace inzulinu a inzulinorezistence, spotřeba energie). Většina komponentů metabolického syndromu jako je viscerální obezita, hypertenze a inzulinorezistence, je spojována se sympatikotonním vlivem, viz **Obr. 11** *(Reaven 1996, Fliers 2003, Grassi 2006).*

Obr. 11: Potenciální faktory** spojené s patogenezou metabollického syndromu (**Grassi 2006)



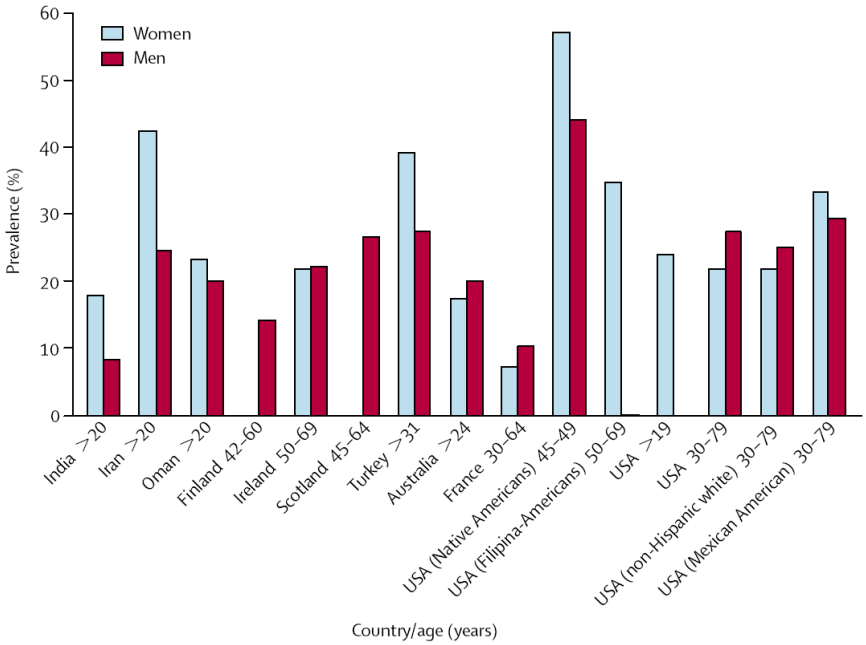
V této souvislosti je potřeba zmínit, že autonomní abnormality lze nalézt i u těch pacientů s metabollickým syndromem, kteří netrpí hypertenzí. Tento fakt poukazuje na to, že

nadměrná sympatikotonie je hlavní charakteristikou metabolického syndromu, nezávisle na výši krevního tlaku, a že je jakousi „vnitřní vlastností“ tohoto syndromu. Navíc, sympatikotonii lze nalézt v celém věkovém spektru populace, včetně starší generace.

Diabetes mellitus 2.typu (DM2) je typickou komponentou metabolického syndromu. Je to chronické, heterogenní, progredující onemocnění, charakterizované hyperglykemií, postihující u nás v současné době více než ¾ milionu obyvatel, ve světě pak na 200 milionů lidí. Dle odhadů např WHO, lze počítat se zdvojnásobením těchto čísel během příštích 25 let. Jak zdravotní následky u každého individuálního pacienta, které zkracují jeho životní vyhlídky a zhoršují kvalitu života, tak dopady následků chronických komplikací diabetu na společnost a ekonomiku, jsou závažné. Diabetes mellitus patří do obrazu metabolického syndromu, společně s dalšími klinicky závažnými odchylkami jako jsou hypertenze, dyslipidemie a centrální obezita, které podstatně přispívají ke zvýšení morbidity a mortality, především na kardiovaskulární komplikace. Náklady na léčbu diabetu u nás přesahují 20 miliard Kč ročně, přičemž převažující hrazenou diagnozou jsou jeho pozdní komplikace. Dle studie CODE2 *(Massi-Benedetti 2002)* je v 8 evropských zemích ročně vydáváno na léčbu pacienta s diabetem bez pozdních komplikací průměrně 1500 €, s pozdními komplikacemi pak 5400 €. Jedním z hlavních důvodů neutěšeného stavu vývoje chronických komplikací diabetu je fakt, že ve chvíli nově diagnostikovaného diabetu typu 2 lze zjistit až ve 40% pacientů již rozvinutou makroangiopatii, až u 40% nefropatii a/nebo neuropatii, u 15% retinopatii, 50% hypertriacylglycerolemii nebo v 50% hypertenzi. Tato skutečnost znamená, že již ve fázi prediabetu, porušené glukozové tolerance, dochází k poškozování tkání a orgánů – včetně rozvoje autonomní neuropatie, jak jsme sami sledovali *(Howorka 2010)* – negativním vlivem ostatních aspektů metabolického syndromu. Právě od tohoto faktu by se také měla odvíjet časná léčebná strategie pacienta s diabetem a metabolickým syndromem. Optimálním a doporučovaným řešením je zahájit léčbu nemedikamentozními opatřeními.

Vysoká prevalence metabolického syndromu (viz **Obr. 12**) a obezity souvisí mj. fyzickou inaktivitou a sedavým způsobem života. V souvislosti s autonomní dysfunkcí sledovanou u **metabolického syndromu** byl v rámci populační studie prokázán vzestup rizika mortality při poklesu fluktuací srdeční frekvence. Zde je příčina zřejmě multifaktoriální, jak v porušeném řízení srdeční činnosti v rámci neuropatie, tak i v poškození cílového orgánu např. vlivem dlouhodobě nekontrolované hypertenze *(Liao 1998).* Jedna z největších studií založená

Obr. 12: Prevalence metabolického syndromu *při použití definice ATPIII (Eckel et al, 2005)*

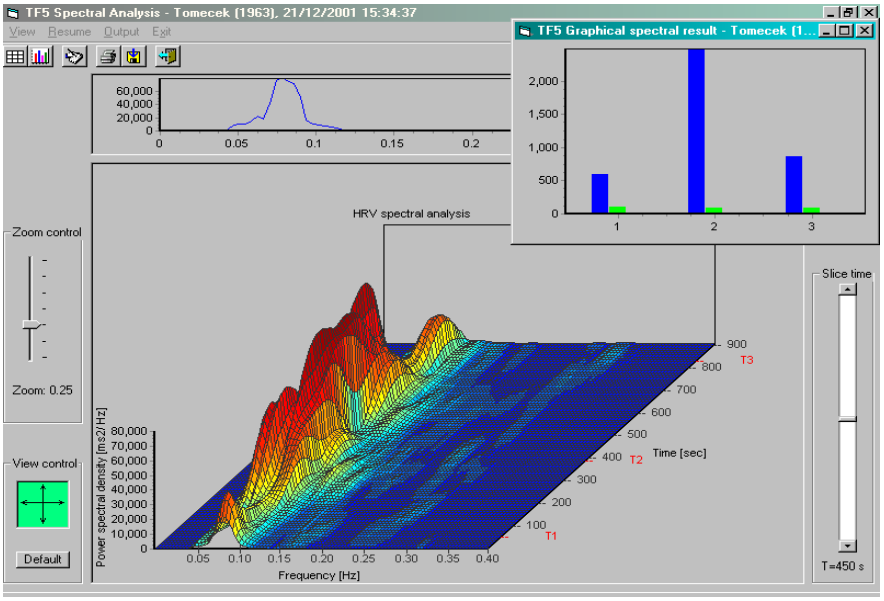


na vstupních datech studie DECODE *(DECODE 1998)*, demonstrovala při prospektivním sledování populace s metabolickým syndromem 1.4x nárůst všeobecné mortality a dokonce více ne 2x nárůst kardiovaskulární mortality, a to pro obě pohlaví. Výsledky sledování jsou nicméně obdobné např. se studii Kuopio ischemic Heart Disease *(Lakka 2002)* nebo ARIC *(McNeill 2005)*, kde byla sledována obdobná incidence chronické ICHS a mrtvic u obou pohlaví. Zajímavým je i nález korelace mezi vzestupem **koncentrace lipidů v krvi** a poklesem HRV *(Christensen 1999)*. Odpovídá tak shora uvedenému pojetí HRV jako jednoho z nezávislých indikátorů kardiovaskulárního rizika.

Dysfunkce v autonomních regulacích s významnou sympatikotonií a poruchou baroreflexu jsou typickým nálezem u **obézních pacientů** s metabolickým syndromem *(Straznicky 2012)*. Významná korelace byla nalezena mezi množstvím viscerálního tuku a zvýšenou aktivitou sympatiku *(Beske 2002)*. Redukce nadváhy koreluje s normalizací autonomního tonu, zvláště pak se zvýšením „ochranného“ vlivu parasympatiku *(Karason 1999).* U neobézních jedinců je porušená HRV s významnou dominancí sympatiku považována za rizikový faktor přírůstku váhy a rozvoje obezity *(Davy 2009)*. Zvýšená sympatikotonie časově předchází rozvoji inzulinorezistence a rozvoji diabetu 2.typu *(Straznicky 2012)*. Mírné přejídání vedlo u zdravých neobezních dobrovolníků k nárůstu váhy spojenému s relativním

nárůstem sympatikotonie *(Gentile 2007)*. U obézních jedinců je sympatikotonní odpověď po požití sacharidové stravy nebo chladové provokaci nižší než u neobézních jedinců *(Matsumoto 2001)*. Ve srovnání skupin žen s rozdílným BMI byl spektrální výkon v pásmu VLF-- jako index termoregulace a vasomotoriky--snížen u skupiny s průměrným BMI=23 ve srovnání s nižším BMI=19. Příklad nálezu významné sympatikotonie v rutinní praxi u pacienta s metabolickým syndromem je prezentován na **Obr. 13**.

Obr.13: Typický příklad dominance nízkofrekvenčního spektra *kolem 0.08 Hz, tedy převahou sympatikotonie /modré-tmavé sloupce/, u pacienta s nedostatečně kontrolovanou hypertenzí při metabolickém syndromu. Standardní modifikovaný ortostatický test, leh-stoj-leh (vlastní sledování J.Pumprla, 2001)*



V rámci populační studie ARIC byl u pacientů s metabolickým syndromem rovněž prokázán vzestup rizika celkové mortality při poklesu HRV. Zde je příčina zřejmě multifaktoriální, jak v porušeném řízení srdeční činnosti v rámci neuropatie, tak i v poškození cílového orgánu např. vlivem dlouhodobě nekontrolované hypertenze *(Liao 1998)*. Rovněž nález snížené HRV u obézních pacientů s esenciální hypertenzí a syndromem spánkové apnoe--zřejmě jako důsledek dlouhodobé excesivní sympatikotonie--může být součástí následující diagnosticko-terapeutické rozvahy *(Salo 2000)*.

Při redukci váhu dochází ke zlepšení odpovědi v VLF a LF komponentech spektra HRV *(deJonge 2010)*, zvláště pak ke zvýšení „ochranného“ vlivu parasympatiku *a* senzitivity kardiovagálního baroreflexu *(Alvarez 2005)*. Obézní subjekty s nízkou vstupní sympatickou aktivitou však dosáhly v 12-týdenním redukčním programu menšího úbytku váhy než ti s vyšším vstupním sympatikotonem *(Straznicky 2012)*.

Všechny tyto nálezy mohou přispívat k definici adekvátních, HRV-podporovaných, diferencovaných terapeutických postupů při moderní léčbě metabolického syndromu a obezity. Jako příklad lze uvést například využití chladem indukované termogeneze s cílem sympatoadrenergní redukce nadváhy stimulací hnědé tukové tkáně *(Howorka 2014)*: nedávno bylo zjištěno, že stimulovaná hnědá tuková tkáň je schopna zvýšit denní energetický výdej těla cca až o 200-400kcal což může vést ke snížení hmotnosti a případného rizika metabolických onemocnění jako diabetes 2.typu *(Zafir 2013)*. U pacientů se syndromem obstrukční spánkové apnoe korelovala při polysomnografických záznamech tíže postižení vyjádřená indexem apnea/hypopnea s progresí relativní sympatikotonie (poměr spektrálních výkonů LF/HF), přičemž ke zlepšení HRV parametrů docházelo při podání kontinuální léčby CPAP, s lepšími výsledky při auto-titrované APAP *(Karasulu 2010)*.

Ačkoliv prognostická relevance autonomní dysfunkce při metabolickém syndromu zůstává předmětem odborných diskusí, v řadě studií bylo demonstrováno, že – obdobně jako u pacientů s chronickým srdečním selháním, po akutním infarktu myokardu nebo mozkové mrtvici – stupeň sympatické aktivity inverzně koreluje s přežitím jedince. Tento nález znovu zdůrazňuje potřebu snižovat případnou excesivní sympatikotonii – jak nefarmakologicky, tak farmakologickou cestou – a v obecnějším pohledu redukovat autonomní dysfunkci, která je jednou z hlavních prognosticky významných charakteristik metabolického syndromu.

1.7. Přehled aplikací HRV v metabolickém syndromu

Vzhledem k zaměření činnosti naší výzkumné skupiny na funkční rehabilitaci a sekundární a terciární prevenci při metabolickém syndromu jsme v roce 2014 vyhodnotili dvacetileté zkušenosti z využití analýzy HRV v rutinní praxi, odborné informace nashromážděné během doktorského studia na UP v Olomouci a publikované state-of-the-art informace v přehledovém článku, vydaném v recenzovaném časopise Interní medicína pro praxi.

1.7.1. Pumprla J, Sovova E, Howorka K: Variabilita srdeční frekvence: Využití v interní praxi se zaměřením na metabolický syndrom. Interni Med, 2014, 16(5): 205-208

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Variabilita srdeční frekvence: Využití v interní praxi se zaměřením na metabolický syndrom

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Schopnost rychlých adaptačních změn je důležitou podmínkou k udržení dlouhodobé homeostázy organizmu. Autonomní nervový systém představuje dynamický řídicí systém, který citlivě reaguje na řadu zevních a vnitřních podnětů, a zajišťuje tak integritu organizmu. Kardiovaskulární reflexy, které jsou základem tohoto systému, se mohou vlivem různých faktorů narušit. Díky výpočetním technologiím lze dnes jejich případnou časnou dysfunkci odhalit poměrně rychle, jednoduše a neinvazivně, pomocí analýzy variací srdeční frekvence. Tato metodika si již našla svou pozici mezi vyšetřovacími postupy v kardiologii a diabetologii, a nabízí své využití u pacientů s metabolickým syndromem i v řadě dalších klinických oborů. Autor článku pojednává o teoretických základech, praktických aplikacích a vlastních cca dvacetiletých zkušenostech s analýzou variability srdeční frekvence se zaměřením na obor vnitřního lékařství.

Klíčová slova: variabilita srdeční frekvence, autonomní nervový systém, sympatikus, parasympatikus, R-R interval, spektrální analýza.

Heart rate variability: applications in medical practice with focus on metabolic syndrome

The capability of quick adaptations is an important condition for preserving a long-term homeostasis of the body. Autonomic nervous system represents a dynamic control system that sensitively manages bodily response to a range of external and internal stimuli and in this way secures integrity of the organism. Cardiovascular reflexes, which represent basic principle of this system, can get impaired due to various factors over the time. Nowadays, due to swift technological developments, it is possible to detect even its early dysfunction by a relatively quick, simple and non-invasive method using analysis of heart rate variability. This method is already well positioned within the clinical armamentarium in cardiology and diabetology, and opens its use for patients with metabolic syndrome, as well as in a range of other clinical disciplines. Author of this review deals with theoretical fundaments, practical applications and own 20-years´ experience in heart rate variability analysis with special focus on internal medicine and metabolic syndrome.

Key words: heart rate variability, autonomic nervous system, sympathetic, parasympathetic, spectral analysis, metabolic syndrome.

Interní Med. 2014; 16(5): ???

K teorii variability srdeční frekvence

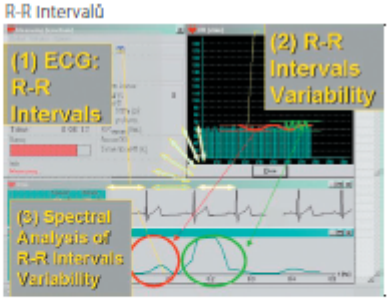
Kardiovaskulární systém vykazuje prvky samoorganizovanosti směřující k udržení dynamické stability. Ta se udržuje přizpůsobováním srdeční frekvence, krevního tlaku a dalšími mechanizmy, které reagují na řadu vnitřních a zevních vlivů, jako jsou např. vznik ischemie, metabolická dysbalance či významná fyzická a/nebo psychická zátěž. V odpovědi na uvedenou situaci se srdeční frekvence zrychluje či zpomaluje, mění se tedy délka R-R intervalu. Tato fyziologická adaptace srdeční frekvence patří k typickým znakům autonomních, integrativních funkcí živých organizmů. Vysoká variabilita srdeční frekvence je znakem dobré adaptability systému, tedy „zdravých" regulací srdečních funkcí a potažmo „zdravého" organizmu. Naopak, snížená variabilita bývá známkou porušení adaptability systému a měla by vést k detailnější, cílené diagnostice její příčiny (1, 2).

Záznam a analýza variability srdeční frekvence

Analýza HRV (heart rate variability, variabilita srdeční frekvence) je založena na posouzení fluktuace intervalů mezi normálními, po sobě následujícími srdečními stahy, nejčastěji mezi vlnami R. Díky výpočetním technologiím je dnes rutinní analýza HRV jednou z mála metod umožňujících neinvazivně a rychle kvantifikovat kardiovaskulární autonomní regulace, a v případě využití tzv. spektrální dekompozice i posoudit podíl obou hlavních složek, sympatiku a parasympatiku. Záznamem vyšetření je časové zobrazení průběhu srdeční frekvence na obrazovce počítače, obvykle ve formě sloupkového grafu, kde jeden sloupek vždy reprezentuje jeden srdeční stah (obrázek 1). Křivka proložená vrcholy jednotlivých sloupků průběhu srdeční frekvence – tedy její variabilita – může být dále analyzována jednak v tzv. ***časové doméně*** (viz dále) a jednak sofistikovaněji, pomocí tzv. spektrální, ***frekvenční analýzy***.

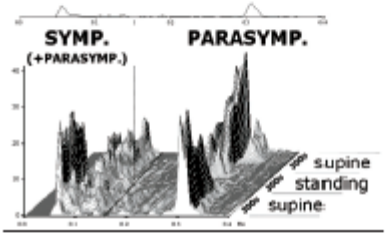
Podstatou spektrální analýzy je rozložení nepravidelného průběhu HRV na pravidelné cykly, reprezentující procesy ovlivňující její kolísání. Protože oba recipročně řídicí subsystémy, sympatikus a parasympatikus, „pracují" s odlišnými frekvencemi (parasympatikus „reaguje" rychleji, sympatikus pomaleji, zřejmě v souvislosti s od-

Obrázek 1. Princip analýzy variability srdeční frekvence (2): (1) záznam EKG a výpočet R-R intervalu, (2) znázornění variací R-R intervalu ve formě sloupkového grafu, (3) spektrální analýza variability R-R intervalů

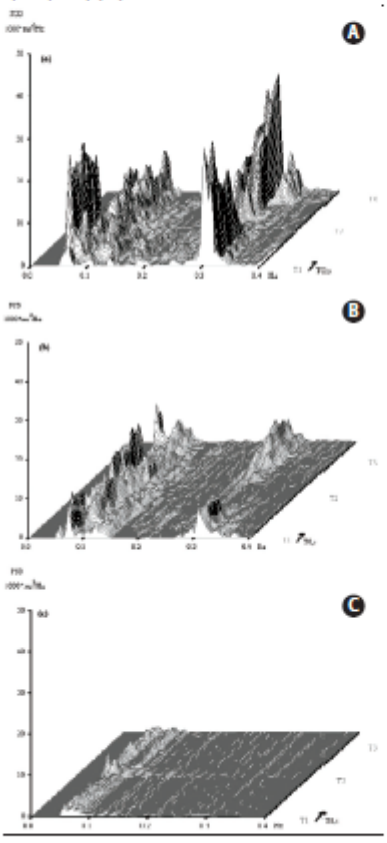


lišnými charakteristikami jejich neurotransmitterů), je možné je matematicko-statisticky rozlišit a následně kvantifikovat jejich tzv. „spektrální výkon" (spectral power). Při pohledu na frekvenční spektrum HRV se předpokládá vliv sympatiku-/s částečným podílem parasympatiku/v rozsahu frekvencí 0,04-0,15 Hz (tzv. nízkofrekvenční pás-

Obrázek 2. Schematické znázornění výsledku krátkodobého vyšetření HRV za použití modifikovaného ortostatického pokusu: standardizované vyšetření leh-stoj- leh, každá pozice 5 minut (4, 12)



Obrázek 3. Typické nálezy krátkodobé HRV registrované během modifikovaného ortostatického pokusu: A) normální regulace bez kardiovaskulární autonomní dysfunkce, se zachovalými dominancemi parasympatiku vleže a sympatiku vestoje, B) počínající dysfunkce (se sníženým parasympatikotonem, oblast 0,3 Hz), a C) u pokročilé dysfunkce (redukce sympatiko- i parasympatikotonu, oblasti 0,1 i 0,3 Hz) (12)



mo, LF), a zastoupení parasympatiku v rozsahu 0,15 až 0,40 Hz (tzv. vysokofrekvenční pásmo, HF). Pásmo velmi nízkých frekvencí 0,01–0,04 (VLF) Hz se obvykle považuje za indikátor aktivity termo- či chemoreceptorů a systému renin-angiotenzin (1, 2). Nicméně, v rámci krátkodobých záznamů je jejich podíl na spektru diskutabilní, protože tyto cykly mají svou vlnovou délku při 0,01Hz až 100 sekund (1). Kromě absolutních hodnot spektrálního výkonu v jednotlivých pásmech je vhodné posuzovat i poměry, relativní zastoupení jednotlivých komponent, a klinicky smysluplné se jeví kumulované parametry sčítající výkon jednotlivých frekvenčních pásem v průběhu celého vyšetření, např. provokace sympatiku i parasympatiku během ortostatické manipulace (3).

Celkovým výsledkem vyšetření je obvykle trojrozměrný graf zobrazující spektrum variací srdeční frekvence v průběhu vyšetření. U nás se v posledních dvou desetiletích do klinické praxe rozšířily systémy VariaPulse a VariaCardio, vývojově pocházející z olomoucké Univerzity Palackého (4, 5) a klinicky rozvíjeny mj. ve spolupráci s Lékařskou univerzitou ve Vídni nebo pražským IKEMem (3, 6, 7). Při vyšetření se k provokaci obou větví autonomních regulací využívá standardizovaný ortostatický pokus, kdy vyšetřovaná osoba 5 minut leží, 5 minut stojí a poté opět 5 minut leží (4). Na přiloženém obrázku (obrázek 2) je znázorněn průběh HRV během vyšetření, s převahou parasympatiku (kolem 0,3 Hz) vleže, poté reciproční vzestup aktivity sympatiku (kolem 0,1 Hz) vestoje, a v poslední fázi pokles sympatikotonu a opětný vzestup parasympatikotonu po opětné supinaci. Rozvoj případné poruchy kardiovaskulárních autonomních regulací lze pak posoudit vizuálně a kvantitativně. V časné fázi dochází především k redukci v oblasti parasympatiku, zatímco při rozvinutém postižení dochází k poklesu už i v oblasti nízkofrekvenční, tedy převážného vlivu sympatiku (obrázek 3).

Jako u většiny biologických funkcí, je i zde vysledovatelný vliv řady faktorů včetně věku: s jeho nárůstem přirozeně klesá spektrální výkon. Z hlediska případné terapeutické intervence je možné nález časného postižení – tedy nižší spektrální výkon jen v oblasti parasympatiku – efektivně ovlivnit jak nemedikamentózně, tak medikamentózně. Při aplikaci vytrvalostního tréninku, krátkodobého lačnění nebo tréninku v řízeném dýchání jsme v našich studiích našli statisticky významné zlepšení HRV zvláště v oblasti vlivu parasympatiku (8, 9, 10). Medikamentózně lze HRV ovlivnit typicky pomocí betablokátorů, ACE, v metabolické oblasti např. pioglitazonem (11).

Průběh srdeční frekvence není izolovanou veličinou, ale úzce souvisí s regulacemi krevního tlaku, dýchání a dalšími faktory. Proto je vždy nutno interpretovat výsledky HRV v souvislosti s celkovým klinickým stavem či léčbou (12). Průběh vyšetření je třeba striktně standardizovat a vyloučit externí stimuly, přičemž zřejmou roli může hrát frekvence a hloubka dýchání (1, 12). Při longitudinálním, intraindividuálním sledování pacienta se však tento fenomén uplatňuje minimálně. Navíc snaha o řízenou dechovou frekvenci během vyšetření HRV je de facto externí stimul, který v konečném nálezu může významně znehodnotit přirozený nález HRV u pacienta.

Časově a na vyšetřovací instrumentárium náročnější jsou kardiovaskulární reflexní testy analyzované v tzv. ***časové doméně*** (obvykle matematické průměry a statistické kalkulace např. disperze dat kolem průměru, geometrické metody apod.). Jednou z nejčastěji užívaných je skupina reflexních testů navržených Ewingem (13), která zahrnuje test hlubokého dýchání, Valsalvův manévr, ortostatický test a hand-grip test. Těmito robustními testy však nelze odlišit či kvantifikovat aktivity obou subsystémů. Nicméně, Ewing byl jedním z prvních autorů, prokazujících významnou prediktivní hodnotu nálezu autonomního postižení ve vztahu ke zvýšené mortalitě (13).

Prodloužení doby vyšetření na 24 hod. v rámci holterovského monitorování může přinést další informace zvláště z pásem tzv. velmi nízkých (0,01–0,04 Hz) či ultranízkých (pod 0,01 Hz) frekvencí, které dle některých autorů mohou dále zvýšit výtěžnost testu vzhledem k tomu, že se na celkové spektrální energii podílí až z 90 %. Nicméně, při těchto „dlouhodobých" testech obvykle nelze standardizovat podmínky vyšetření, což může značně ztížit jeho výpovědní hodnotu. Navíc se zdá, že výsledky HRV analýzy pečlivě standardizovaných krátkodobých vyšetření se zásadně neliší od analýz provedených na základě holterovského monitorování (14).

Vedle výše uvedené lineární analýzy se v literatuře uvádí také zajímavé výsledky výzkumu tzv. ***nelineárních*** komponent HRV, kdy se posuzuje „chaos" v regulacích srdeční frekvence (15) a řada dalších parametrů. Tyto metody však obvykle zůstávají díky své komplexnosti omezeny na výzkumná pracoviště a v praxi se rozšířily minimálně. Naproti tomu existuje na trhu řada komerčně dostupných dedikovaných systémů využívajících výše zmíněnou lineární časovou

Tabulka 1. Příklady asociací nálezů HRV a modifikovatelných rizikových faktorů (volně dle 30)

Riziko	Autor	Sledovaný parametr a populace	Závěr
Hypertenze	Liao	HRV a hypertenze (ARIC studie). n=2061 1/3 hypertonici	1,2–5,2x riziko rozvoje hypertenze u subjektů v nejnižším kvartilu HF pásma
Hypertenze	Singh	HRV a hypertenze (Framingham Study). n=2024, 17 % incidence hypertenze	U mužů 1,38x a u žen 1,12x (NS) riziko rozvoje hypertenze při nižší HRV a relativní sympatikotonii
Hypertenze	Schroeder	HRV, hypertenze, krevní tlak. n=11061, 28 % incidence hypertenze	1,1–1,6x riziko rozvoje hypertenze u subjektů s patologickými hodnotami časové analýzy HRV (SDNN, rMSSD, délka R-R int.)
Hypertenze	Virtanen	HRV, hypertenze, renin. n=191 hyper-toniků a 105 kontrol	Nižší HRV (časová i frekvenční analýza) u nově diagnostikovaných hypertoniků. Zvýšená aktivita reninu jako nezávislý faktor poklesu HF
Cholesterol	Christen-sen	HRV a cholesterol. n=85, 55 % s ICHS	Asociace mezi nižší HRV (časová analýza, SDNN, rMSSD) a vyšším cholesterolem u pacientů s ICHS i u zdravých jedinců
Cholesterol	Kupari	HRV a LDL cholesterol. n=88, bez projevu ICHS	Korelace hladiny LDL cholesterolu s rMSSD r=-0,22 (p=0,08) a s celkovým spektrálním výkonem r=-0,25 (p=0,07)
Diabetes	Liao (ARIC studie)	HRV, diabetes, glyke-mie, inzulinemie. n=1933, 8 % diabetiků	Snížený výkon v HF pásmu u diabetiků ve srovnání s nediabetiky (p=0,01)
Diabetes	Carnethon (ARIC studie)	HRV, typ 2 diabetes. n=8185, 13 % diabetiků	1,2x relativní risk rozvoje diabetu během 8,3 let sledované populace z nejnižšího kvartilu spektrálního výkonu v LF pásmu ve srovnání s nejvyšším
Diabetes	Singh	HRV, typ 2 diabetes. n=1919, 4 % diabetiků	Průměrný LN spektrálního výkonu v LF pásmu u nediabetiků vs. diabetici 6,7 vs. 6,5 (p=0,008). Poměr LF/HF u stejných skupin 1,22 vs. 1,08 (p=0,02)
Obezita	Petretta	HRV, obezita. n=20, u 50 % časný rozvoj obezity	Srovnání kontrolních/neobézních a obézních subjektů – nižší spektrální výkony v ULF (8,67 vs. 8,43 LN ms²) a VLF (7,57 vs 7,37 ms²) pásmech
Pohyb	Sloan	HRV, aerobní aktivita. n=149 zdravých	Zvýšení SDNN (o 0,12 LN ms) a HF výkonu (o 0,39 LN ms²) po 12týdenním aerobním tréninku. Opětný pokles SDNN (o -0,20 LN ms) a HF výkonu (o -0,54 LN ms²) za 4 týdny po ukončení vytrvalostního tréninku
Pohyb	Howorka	HRV, vytrvalostní trénink. n=22 diabetiků	Zvýšení celkového spektrálního vý-konu z 7,5 na 8,0 (LN ms², bez neuro-patie) a z 6,1 na 6,6 (LN ms², s počína-jící neuropatií) po 12týdenním vytrvalostním tréninku. Vymizení efektu do 6 týdnů po ukončení tréninku.
Kouření	Hayano	HRV, krátko- a dlouhodobý efekt kouření. n=81 mužů, 69 % kuřáci	Pokles HF výkonu již po 1 cigaretě (p=0,006). Zvýšení CCV v LF pásmu za 10–17 min po kouření (p=0,0001). Nižší CCV v HF pásmu u těžkých kuřáků ve srovnání s nekuřáky/lehkými kuřáky (p=0,008)

Vysvětlivky:
HRV heart rate variability, variabilita srdeční frekvence
ULF ultra-low frequency/pásmo ultranízkých frekvencí
VLF very-low frequency/pásmo velmi nízkých frekvencí
LF low frequency/nízkofrekvenční pásmo
HF high frequency/vysokofrekvenční pásmo
SDNN směrodatná odchylka po sobě následujících normálních R-R intervalů
rMSSD druhá odmocnina průměru čtverců odchylek po sobě následujících normálních R-R intervalů
R-R int. interval R-R
LN přirozený logaritmus
CCV coefficient of component variance, odvozený parametr frekvenční analýzy přepočítaný na průměrnou délku R-R intervalu

a frekvenční krátkodobou analýzu. Jistou limitací je pak fakt, že ne vždy jsou výsledky měření získané jednotlivými systémy zaměnitelné, obvykle v důsledku použití odlišných zpracování dat či kalkulačních procedur.

Hodnocení variability srdeční frekvence v klinické praxi

V klinické praxi se sledování HRV poprvé objevilo ve zjednodušené podobě v porodnictví v kardiotokografii. Nicméně, hlavním oborem využívajícím výsledky analýzy HRV je samozřejmě ***kardiologie***. Koncem 70. let Wolf poprvé publikoval studie popisující sníženou HRV korelující se zvýšenou mortalitou a/nebo četností klinicky významných arytmických příhod u pacientů po infarktu myokardu (16). Od té doby bylo publikováno několik tisíc prací z mnoha oborů medicíny, přičemž jednou z nejvíce popisovaných problematik je posuzování aktuálního stavu a/nebo prognostického rizika pacienta s chronickým srdečním nebo metabolickým postižením. Příklady aplikace HRV jsou uvedeny v tabulce 1.

Významnou pozici v diagnostice si našla analýza HRV i v ***diabetologii***. Tíže kardiovaskulární autonomní neuropatie (KAN) prokázaná jinými metodami přímo koreluje s poklesem HRV (2). Vyšetření KAN bylo donedávna poněkud opomíjenou součástí v diagnostice klasické triády pozdních komplikací diabetu, ačkoliv je např. známo, že pacienti s její těžkou formou mají několikanásobně vyšší mortalitu než pacienti bez KAN v průběhu pětiletého sledování (17). Příčinou tohoto fenoménu je zřejmě opět dysbalance v autonomních regulacích spolu s poklesem prahu pro vyvolání letální arytmie (17). Obnovení autonomních regulací lze podpořit např. systematickým vytrvalostním tréninkem (8). Příklady aplikace HRV jsou uvedeny v tabulce 1.

Metabolický syndrom a variabilita srdeční frekvence

Dysfunkce v autonomních regulacích s významnou sympatikotonií a poruchou baroreflexu jsou typickým nálezem u obézních pacientů s metabolickým syndromem (18). Významná

korelace byla nalezena mezi množstvím viscerálního tuku a zvýšenou aktivitou sympatiku (19). U neobézních jedinců je porušená HRV s významnou dominancí sympatiku považována za rizikový faktor přírůstku váhy a rozvoje obezity (20). Zvýšená sympatikotonie časově předchází rozvoji inzulinorezistence a rozvoji diabetu mellitu 2. typu (18). Mírné přejídání vedlo u zdravých neobézních dobrovolníků k nárůstu váhy spojenému s relativním nárůstem sympatikotonie (21). U obézních jedinců je sympatikotonní odpověď po požití sacharidové stravy nebo chladové provokaci nižší než u neobézních jedinců (22). Ve srovnání skupin žen s rozdílným BMI byl spektrální výkon v pásmu VLF – jako index termoregulace a vazomotoriky – snížen u skupiny s průměrným BMI=23 ve srovnání s nižším BMI=19.

V rámci populační studie ARIC byl u pacientů s metabolickým syndromem rovněž prokázán vzestup rizika celkové mortality při poklesu HRV. Zde je příčina zřejmě multifaktoriální, jak v porušeném řízení srdeční činnosti v rámci neuropatie, tak i v poškození cílového orgánu např. vlivem dlouhodobě nekontrolované hypertenze (12, 23). Rovněž nález snížené HRV u obézních pacientů s esenciální hypertenzí a syndromem spánkové apnoe – zřejmě jako důsledek dlouhodobé excesivní sympatikotonie – může být součástí následující diagnosticko-terapeutické rozvahy (24).

Při redukci váhy dochází ke zlepšení odpovědi v VLF a LF komponentech spektra HRV (25), zvláště pak ke zvýšení „ochranného" vlivu parasympatiku a senzitivity kardiovagálního baroreflexu (26). Obézní subjekty s nízkou vstupní sympatickou aktivitou však dosáhly v 12týdenním redukčním programu menšího úbytku váhy než ti s vyšším vstupním sympatikotonem (18).

Všechny tyto nálezy mohou přispívat k definici adekvátních, HRV-podporovaných, diferencovaných terapeutických postupů při moderní léčbě metabolického syndromu a obezity. Jako příklad lze uvést například využití chladem indukované termogeneze s cílem sympatoadrenergní redukce nadváhy stimulací hnědé tukové tkáně (27): nedávno bylo zjištěno, že stimulovaná hnědá tuková tkáň je schopna zvýšit energetický výdej těla cca až o 200–400 kcal, což může vést ke snížení hmotnosti a případného rizika metabolických onemocnění jako diabetes mellitus 2. typu (28). U pacientů se syndromem obstrukční spánkové apnoe korelovala při polysomnografických záznamech tíže postižení vyjádřená indexem apnea/hypopnea s progresí relativní sympatikotonie (poměr spektrálních výkonů LF/HF), přičemž ke zlepšení HRV parametrů docházelo při podání kontinuální léčby CPAP, s lepšími výsledky při auto-titrované APAP (29).

Na našem pracovišti jsme v rámci klinického hodnocení účinnosti a bezpečnosti systému Vanquish®, určeného k neinvazivní radiofrekvenční redukci podkožních depozit tuku, zjistili, že významnější redukci obvodu pasu po sérii 4 ošetření selektivním vysokofrekvenčním polem dosáhly subjekty s vyšším spektrálním výkonem v HRV pásmu s velmi nízkou frekvencí (VLF, 0.01–0.04 Hz) na začátku studie. Detaily jsou nad rámec tohoto sdělení, nicméně tento nález je v souladu se studií Matsumota et al, (22) který si povšiml, že autonomní odpověď na termogenní impuls nebo kapsaicinem kořeněnou potravinu, sledovaná v HRV spektru o velmi nízké frekvenci, byla u obézních jedinců nižší než u neobézních. Hodnocení spektrálního výkonu v VLF pásmu by mohlo přinést bližší informace o podílu autonomního systému na regulaci energetické bilance organizmu. Hypoteticky by tedy snížený vstupní výkon ve VLF pásmu mohl implikovat nižší sympatikotermogenní odpověď na terapeutická opatření zaměřená na redukci nadváhy.

Další klinické obory a HRV

Prokázaný prognostický význam má HRV u řady dalších klinicky závažných stavů, od renálního selhání a stavů po cévní mozkové příhodě po peroperační sledování hemodynamického profilu nebo pooperační sledování na jednotkách intenzivní péče a odhad rizika případných komplikací. Další zajímavou oblastí je například posuzování účinku podaných léčiv na kardiovaskulární autonomní regulace a potažmo na stav a/nebo prognózu pacienta, nebo aplikace HRV při řízení kardiovaskulární rehabilitace po infarktu myokardu (31), tréninkových procesů ve sportu a úpravě tréninkové zátěže dle aktuálního stavu organizmu sportovce. To vše je ale nad rámec tohoto sdělení.

Závěr

Vyšetření variability srdeční frekvence poskytuje kvalitativně nový pohled na diagnostiku a léčbu řady chronických onemocnění, s nimiž se setkáváme v denní praxi. Analýza HRV se etablovala především v kardiologii, diabetologii a při vyšetřování metabolického syndromu, kde byla prokázána korelace mezi poklesem variací R-R intervalů a nepříznivou prognózou nemoci, což odpovídá všeobecně vnímané pozici metodiky jako časného a nezávislého indikátoru rizika.

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2. CÍLE DISERTAČNÍ PRÁCE

Cíle této disertační práce jsou:

Metodologické

(1) Definice optimálních parametrů k deskripci autonomní dysfunkce, zahrnující definici věkově vztažených normálních hodnot, a sdružených/kumulativních parametrů spektrálního výkonu, použitých ke zlepšení diskriminační schopnosti testu mezi jednotlivými stupni postižení CAN

(2) Zahrnutí ortostatické manipulace k ověření efektu na diagnostiku časné autonomní poruchy, a k posouzení časného postižení sympatické nebo parasympatické odpovědi při rutnním vyšetření HRV

(3) Ověření reprodukovatelnosti/opakovatelnosti vyšetření krátkodobé HRV v časové i frekvenční doméně v průběhu dvou po sobě jdoucích měření během 2 dnů

Klinické/aplikační

(4) Ověření vlivu vytrvalostního fyzického tréninku u pacientů s diabetem s různým stupněm autonomní dysfunkce

(5) Kvantifikace vlivu minimálně 12-hodinové restrikce stravy u pacientů s různými stupni CAN

(6) Posouzení nefarmakologické redukce krevního tlaku a případně dalších efektů na kardiovaskulární autonomní regulace při aplikaci řízeného zpomalení dechové frekvence biofeedback-systémem RespeRate

(7) Sledování vlivu série radiofrekvenční redukce subkutánního tuku na kardiovaskulární autonomní regulace u pacientů s metabolickým syndromem

(8) Prúkaz vlivu spinální stimulace na autonomní funkci u pacientů s chronickou ICHS a refrakterní anginou pectoris

3. VÝVOJ A VALIDACE METOD ANALÝZY VARIABILITY SRDEČNÍ FREKVENCE

3.1. Vývoj a validace analytických standardů k posouzení HRV:

Reprodukovatelnost krátkodobého vyšetření HRV

Úvod: V rámci validace diagnostického procesu bylo třeba ověřit reprodukovatelnost vyšetření. V literatuře lze nalézt kontroverzní názory na reprodukovatelnost krátkodobého měření HRV. Dostatečnou stabilitu nalezl např. *Sinnreich 1998*, přičemž méně senzitiviní parametry časové analýzy, jako jsou rMSSD nebo SDNN, se zdají být robustnější, a tedy i lépe reprodukovatelné než parametry spektrální analýzy. Zde byla demonstrována lepší stabilita v čase pro celkový spektrální výkon než pro individuální části frekvenčního spektra *(Tarkiainen 2005)*. Na druhou stranu, spolehlivost výsledků krátkodobých záznamů HRV nepotvrdil např. B*ootsma 1996* nebo *Lord 2001*. Nicméně je nutno podotknout, že vysoká, „zdravá" HRV je odrazem řady zcela fyziologických dějů v organismu a tudíž velmi záleží na standardizaci podmínek vyšetření *(Pumprla 2001)*, což ve většině uvedených publikací nebylo dostatečně zajištěno. Náhodná chyba měření představuje pouze menší část celkové interindividuální variability, což představuje dostatečnou relativní relabilitu. Lze tedy předpokládat, že rozdíly mezi zdravými probandy jsou způsobeny především reálnými hodnotami a nikoli náhodnou chybou měření. Efektivním řešením tohoto přirozeného rozptylu normálních dat s ohledem na pozdější analýzy a srovnání s věkově-relevantními normami je jejich logaritmizace, což jsme prokázali i v našich studiích *(Howorka 1998).* Na druhou stranu, nižší HRV má reprodukovatelnost lepší právě v souvislosti s již přítomnou dysfunkcí v regulacích a tudíž je spolehlivost detekce postižení u těchto pacientů vyšší.

Cílem naší práce bylo posouzení reprodukovatelnosti/ opakovatelnosti (repeatibility) výsledků krátkodobé HRV u 55 pacientů s různými stupni diabetické autonomní dysfunkce, získané dle popsaného modifikovaného ortostatického protokolu ve dvou po sobě následujících měřeních v průběhu 2 po sobě následujících (1.47±1.23) dnů, při využití standardů dle Bland-Altmanna *(Bland et Altmann 1986)*.

Výsledky (viz „Výsledky" str.61 a **Obr.14 a 15**): Parametry reprodukovatelnosti pro obě domény HRV jsou uvedeny v tabulce. Vyšetření SA-HRV trvalo v průměru 17±2 min, vyšetření Ewingovou baterií testů trvalo v průměru 38±7 min ($p<0.001$).

Závěr: Vyšetření krátkodobé variability srdeční frekvence v časové a spektrální doméně vykazují srov-natelnou reprodukovatelnost/opakovatelnost v krátkém časovém odstupu. Oproti Ewingově baterii je spektrální analýza signifikantně kratší a méně náročná na compliance pacienta během vyšetření, přičemž umožňuje kvantifikovat stav obou subsystémů autonomního nervového systému.

3.1.1. Pumprla J, Howorka K, Schabmann A et al: Reprodukovatelnost vyšetření diabetické kardiovaskulární autonomní neuropatie pomocí spektrální analýzy variability srdeční frekvence a Ewingovy baterie funkčních testů. Diabetologické dny, Luhačovice, 1999. Sborník abstrakt.

REPRODUKOVATELNOST VYŠETŘENÍ DIABETICKÉ KARDIOVASKULÁRNÍ AUTONOMNÍ NEUROPATIE POMOCÍ SPEKTRÁLNÍ ANALÝZY VARIABILITY SRDEČNÍ FREKVENCE A EWINGOVY BATERIE FUNKČNÍCH TESTŮ

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Úvod

- Reprodukovatelnost/opakovatelnost vyšetření diabetické kardiovaskulární autonomní neuropatie (CAN) pomocí baterie funkčních testů dle Ewinga (1982) byla dostatečně ověřena.
- Principy a normy nové diagnostické metodiky, krátkodobé spektrální analýzy variability srdeční frekvence (SA-HRV), byly rovněž publikovány (1997, 1998).
- Dostatečně však prozatím nebyla prozkoumána reprodukovatelnost/opakovatelnost tohoto vyšetření při jeho opakování v krátkém časovém horizontu.

Cíle

1. Ověření reprodukovatelnosti/opakovatelnosti vyšetření krátkodobé SA-HRV v krátkém časovém odstupu (spektrální analýza HRV)

2. Ověření reprodukovatelností/opakovatelnosti Ewingovy baterie funkčních testů (časová analýza HRV)

3. Posouzení časové náročnosti trvání obou typů vyšetření.

Pacienti a metody

Intraindividuální korelace a koeficienty reproducibility výsledků 2 konsekutivních měření v časovém rozmezí 2 dnů, u 55 diabetiků s různým stupněm postižení (*bez/lehká/těžká* CAN: n=21/22/12) získaných pomocí systému *VariaCardio TF4* pro:

- spektrum variability srdeční frekvence v modifikovaném krátkém ortostatickém testu (polohy leh-stoj-leh, 3x5 minut)
- baterii funkčních testů dle Ewinga

Byly striktně dodrženy standardní podmínky vyšetření (ADA, 1996).

Výsledky

(1) spektrální analýza

Ln kumulativní spektrální výkon [ln(ms^2)]	$x_1 \pm SD_1 / x_2 \pm SD_2$	*r=*	*p=*
• ve vysokofrekvenčním spektru (HF)	5.7±1.2/5.6±1.4	0.95	<0.001
• v celkovém spektru (LF+HF)	7.2±1.2/7.1±1.3	0.94	<0.001
• v nízkofrekvenčním spektru (LF)	6.5±1.4/6.2±1.4	0.90	<0.001
• v hlubokofrekvenčním spektru (VLF)	5.9±1.0/5.8±1.1	0.74	<0.01

Nejvyšší korelace v podskupinách dle tíže CAN:

- *bez a lehká* CAN: vysokofrekvenční spektrum (HF; r=0.91 a r=0.84; oba parametry p<0.001)

- *těžká* CAN: nízkofrekvenční spektrum (LF; r=0.75; p<0.001)

Koeficienty reproducibility/opakovatelnosti dle Bland-Altmanna	*CR=*
• kumulativní spektrální výkon LF+HF	0.9 ms^2
• kumulativní spektrální výkon HF	1.1 ms^2
• kumulativní spektrální výkon LF	1.2 ms^2

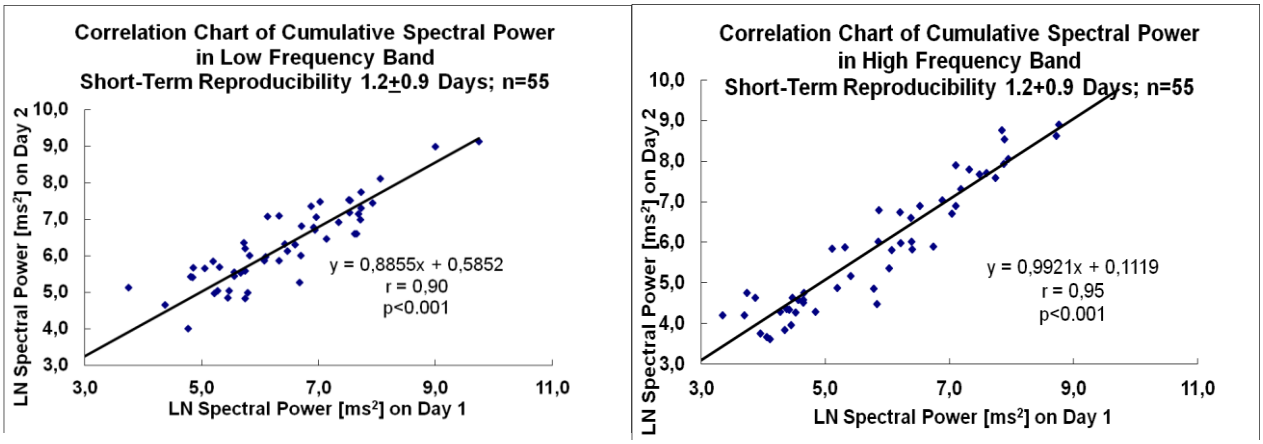
Výsledky

(2) časová analýza

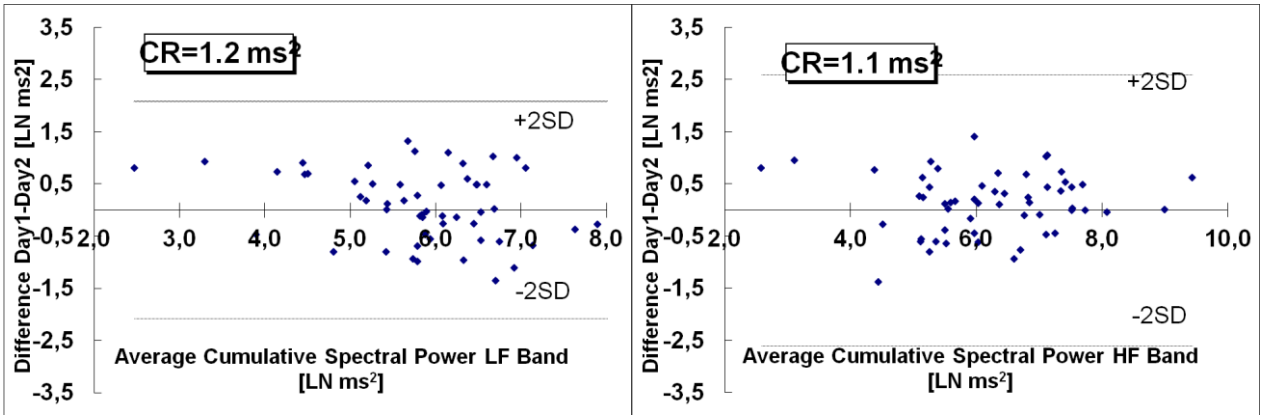
Test	*$x_1 \pm SD_1 / x_2 \pm SD_2$*	*r=*	*p=*
• I-E během testu hlubokého dýchání [min^{-1}]	12.1±3.8/11.7±3.1	0.88	<0.001
• Valsalvův manévr	1.2±0.1/1.1±0.2	0.82	<0.001
• Ortostáza -- rozdíl TK_{sys} [mm Hg]	18.6±4.3/17.3±4.4	0.58	<0.01
-- $HR_{max}/_{min}$	1.3+0.2/1.2+0.2	0.80	<0.001

Koeficienty reproducibility/opakovatelnosti dle Bland-Altmanna	*CR=*
• I-E během testu hlubokého dýchání [min^{-1}]	9.6
• Valsalvův manévr	0.29
• Ortostáza -- rozdíl TK_{sys} [mm Hg]	49.1
-- $HR_{max}/_{min}$	0.34

Obr. 14. Korelace mezi dvěma měřeními HRV *spektrální analýzou v časovém odstupu 1.2±0.9 dnů v nízko- a vysokofrekvenčními pásmu*



Obr. 15: Bland-Altmannův graf pro LF a HF pásmo*, dvě měření HRV spektrální analýzou v časovém odstupu 1.2±0.9 dnů (CR=coefficient of repeatability)*



3.2. Vývoj a validace analytických standardů k posouzení HRV: Srovnání krátkodobé spektrální analýzy HRV s Ewingovou baterií reflexních testů jako referenční metodou a stanovení věkově-korigovaných normálních hodnot

Úvod: Vzhledem ke zvolenému modelu testování HRV (ortostatická manipulace za účelem provokace odpovědi obou větví autonomního systému, *Opavský 1995*), jakož i díky některým specifikám využitých matematicko-statistických kalkulací při výpočtu frekvenčního spektra, využívajícím v rámci Fourierovy transformace metodu tzv. „coarse-graining“ analýzy dle Yamamota, která v principu umožňovala redukovat vliv excesivních neharmonických hodnot kontaminujících zvláště nižší frekvence *(Yamamoto 1991, Salinger 1995)*, a rovněž s ohledem na potřebu jednotné metodiky vyhodnocení a srovnatelnosti výsledků s ostatními pracovišti, bylo potřeba sestavit specifické normy platné pro vyšetřování dedikovanými systémy.

V souvislosti s výše zmíněným neudiví dvojí pohled na problematiku srovnatelnosti metod měření HRV různými procedurami. Např. *Sandercock 2004* nachází při srovnání tří komerčně dostupných systémů deklarovaných pro měření krátkodobé HRV signifikantní rozdílnost ve výsledcích, které přisuzuje odlišným záznamovým a analytickým protokolům jednotlivých zařízení. Naproti tomu *Weipert 2010* nachází při srovnání 3-minutových záznamů z mobilních komerčních aplikací „excelentní“ zaměnitelnost pokud jde o měření R-R intervalů a dostačující srovnatelnost spektrálních parametrů při jejich relativizaci/normalizaci.

Cílem práce bylo vybrat ty parametry spektrální analýzy, které měly obdobnou diskriminační schopnost jako parametry “zlatého standardu” diagnostiky diabetické autonomní neuropatie, baterie reflexních testů dle Ewinga, a zjistit normální hodnoty pro skupiny pacientů bez, s počáteční a pokročilou autonomní dysfunkcí. Druhotným cílem analýzy bylo stanovit věkově diferencované normy.

Výsledky a závěr: Na základě naší analýzy je možné říci, že spektrální analýza krátkodobé HRV dle modifikovaného ortostatického pokusu vykazuje obdobnou diagnostickou hodnotu jako časová analýza, “zlatý standard”, baterie freflexních testů dle Ewinga. Kumulativní spektrální výkon celého frekvenčního rozsahu (v tomto případě 0.06-0.50Hz) lze používat jako celkový deskriptor stupně kardiální denervace při diabetu při využití

popsané metodologie. Z klinického hlediska je důležité také zmínit, že ve skupině konsekutivně vyšetřených 119 probandů s diabetem 1.typu jsme nalezli pouze 50 pacientů bez projevů CAN.

Při použití identické vyšetřovací procedury, *Lacigová 2007 z* plzeňské Fakultní nemocnice nalezla ve skupině 107 asymptomatických probandů s diabetem 1.typu pouze 46% pacientů bez CAN, obdobně jako v naší sledované skupině. *Hosová 2001* z pražského IKEMU vyšetřila identickou metodou 123 nediabetiků, přičemž výsledky kumulativních spektrálních výkonů byly obdobné nálezům dosaženým v naší skupině diabetiků bez projevů CAN.

3.2.1. Howorka K, Pumprla J, Schabmann A: Optimal parameters of short-term heart rate spectrogram for routine evaluation of diabetic cardiovascular autonomic neuropathy. Journal of the autonomic nervous system, 69(2-3), 1998, pp. 164-172. IF 1.4

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Optimal parameters of short-term heart rate spectrogram for routine evaluation of diabetic cardiovascular autonomic neuropathy

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Abstract

Our aim was to select those parameters of heart rate variability (HRV) within its short-term power spectral analysis (PSA), which have a capability similar to that of the standard Ewing battery of cardiovascular function tests in determining different degrees of cardiovascular autonomic neuropathy (CAN) in diabetes and to compare the usefulness of both methods for diagnostic purposes in the everyday routine. Commonly used standard battery of cardiovascular autonomic function tests evaluated as total Ewing score as well as short-term PSA of HRV were used in 119 diabetic patients (age: 52.7 ± 9.8, diabetes duration: 22.2 ± 12.7 years). From this cohort, patients were selected according to the total Ewing score by matching for age, gender, BMI and diabetes type for 3 groups, each of 17 patients, with no CAN (total Ewing score 0–0.5), with early involvement (score 1.0–2.5) and with definite or severe CAN (score 3.0–5.0). Short-term PSA of HRV performed in three positions (supine1–standing–supine2) included frequency-domain and time-domain parameters of HRV. Cumulative spectral power of total frequency band (0.06–0.50 Hz) and spectral power of low-frequency band (0.06–0.15 Hz) during both supine positions proved to be the most selective and discriminating among all patient groups in inter-group comparison and in analysis of discriminance. The correlation between the total Ewing score and the cumulative spectral power of total frequency band was $r = -0.87$ ($P < 0.001$). About 83.2% of cases classified by short-term PSA of HRV using the variables selected by analysis of discriminance was congruent with the classification by the total Ewing score alone. Time expenditure for the performance of each examination was 31 ± 10 min for Ewing test battery vs. 14 ± 2 min for short-term PSA of HRV ($P < 0.001$). In summary, the latter method showed similar diagnostic value concerning the CAN as the classical Ewing standard battery of cardiovascular function tests, although its application proved to be shorter, less stressful and more independent from patient cooperation. Cumulative spectral power of total frequency band (LFHF cumpower) can be used for overall description of the degree of cardiac denervation in diabetes while using short-term PSA of HRV.

Keywords: Cardiovascular autonomic neuropathy; Heart rate variability; Time-domain analysis; Frequency-domain analysis; Power spectral analysis; Diabetic late complications

1. Introduction

Fluctuation of heart rate is used widely as an index of the level of autonomic traffic to the heart (Ewing et al., 1980; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The Ewing battery of cardiovascular function tests (American Diabetes Association, 1996; Ewing et al., 1985) is an established set of standardised stimuli with analysis of effects on heart rate and blood pressure in time-domain. Increased mortality risk is associated with cardiovascular autonomic neuropathy (CAN) in diabetes (Luft et al., 1993; O'Brien et al., 1991) and diminished heart rate variability (HRV) also in coronary artery disease (Bigger et al., 1993; Dreifus et al., 1993; Hayano et al., 1990a; Kleiger et al., 1987). In diabetic patients with microproteinuria, a diminished HRV was predictive for further renal deterioration (Molgaard et al., 1994; Poulsen et al., 1997; Sundkvist and Lilja, 1993). As the impact of CAN on prognosis in diabetes was recognised (Ewing et al., 1980), it became clear that the estimation of cardiac autonomic function should be routinely performed in diabetic patients.

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Analysis of HRV in the frequency/=spectral/-domain allows an assessment of autonomic function with the investigation of the effects of sympathetic and parasympathetic subsystems (Hayano et al., 1991; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Even its short-term version proved a significant predictive value (Bigger et al., 1993). We decided to compare the short-term power spectral analysis (PSA) of HRV to the reference battery of Ewing (American Diabetes Association, 1996; Ewing et al., 1985) on the level of clinical application in diabetes. Our aims were (1) to select those parameters of short-term PSA of HRV, which have the highest diagnostic impact and a capability similar to that of the standard Ewing battery of cardiovascular function tests in determining the degree of cardiovascular autonomic denervation, and (2) to compare the usefulness of short-term PSA of HRV to that of Ewing battery for routine diagnostic purposes of the diabetic outpatient department.

2. Subjects and methods

2.1. Subjects

2.1.1. Study population

Both diagnostic methods were used in a non-selected diabetic cohort of our outpatient department, although the quantification of CAN was preferably used for patients with longer diabetes duration or even with clinical signs of CAN. Patients with arrhythmias, those taking alpha- or beta-blockers or any other medication considerably influencing the cardiovascular system, were excluded (American Diabetes Association, 1996). Clinical characteristics (mean ± SD) of the whole cohort ($n = 119$) were: age 52.7 ± 9.8 years, diabetes duration 22.2 ± 12.7 years, type 1 diabetes $n = 107$, f/m 66/53, BMI 25.5 ± 3.7 kg/m^2, casual blood pressure 128 ± 19/75 ± 9 mmHg, HbA_{1c} 6.8 ± 1.2% (reference range: 4.2–6.2%), casual blood glucose 146 ± 64 mg/dl, total daily insulin consumption 43.5 ± 17.9 U. The distribution of neuropathy subgroups according to the total Ewing score was as follows: no CAN: $n = 50$ (42%), early involvement: $n = 51$ (43%), and definite/severe CAN: $n = 18$ (15%).

2.1.2. Subcohorts for intergroup comparison

In order to compare results (intergroup comparison) of singular tests and parameters of both methods—the reference method (standard battery of Ewing) and short-term spectral analysis—we selected from the whole above described cohort three patient groups with different stages of neuropathy, each of 17 patients. Selection was performed according to the total Ewing score by matching for age, gender, diabetes type, BMI and casual blood pressure (Table 1).

2.1.3. Subcohort for assessment of time expenditure during examination

Another subgroup was selected (first 26 patients as presented for the examination) in order to estimate the time expenditure required for performing the Ewing battery and/or the PSA of HRV (not including the preparatory phase, accounting for 15 min before each type of examination).

2.2. Experimental procedure and clinical setting

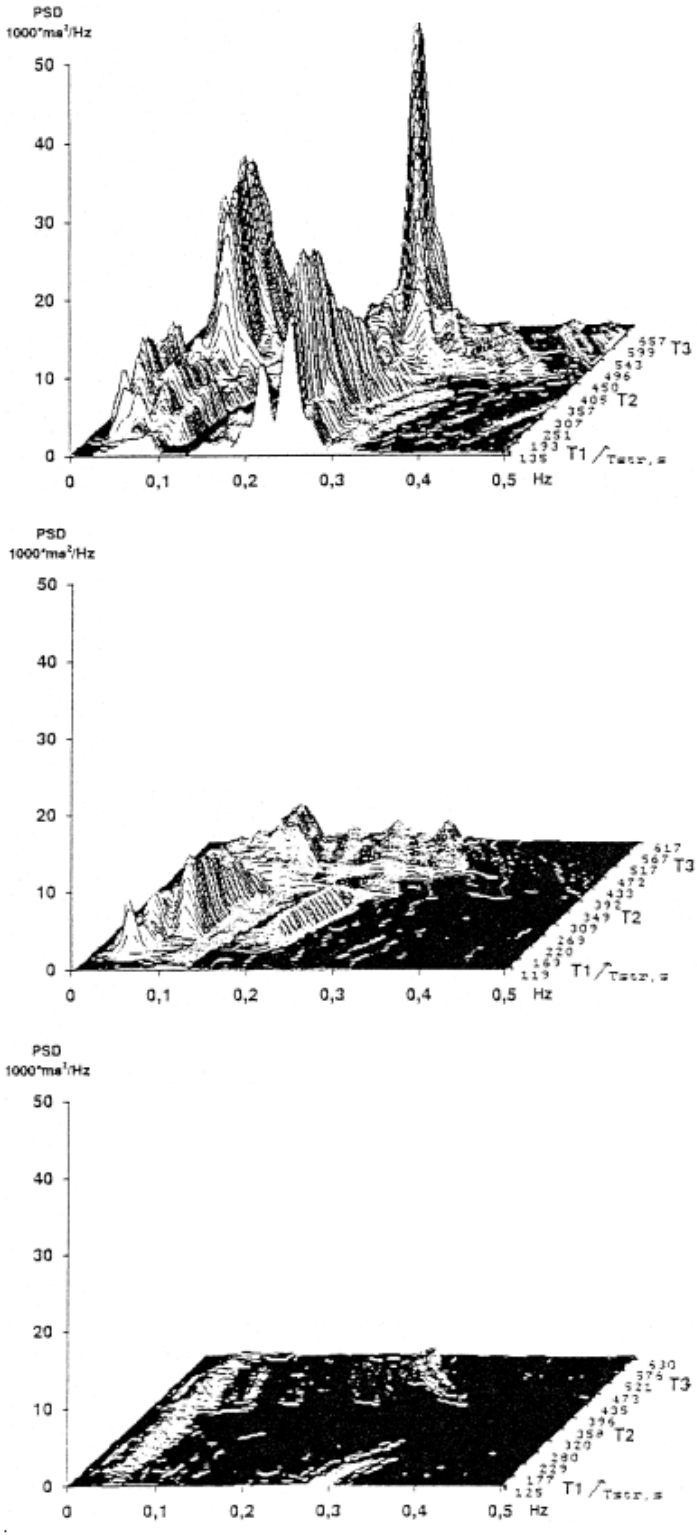
All patients gave their informed consent. The subjects were requested to refrain from smoking and coffee, tea, alcohol and other sympathomimetics administration within the last 12 h, as well as to maintain their usual diabetes-related medication and nutrition. Particularly intensive self-monitoring was requested to avoid casual, subclinical hypoglycaemia. In case of self-monitored values below 70 mg/dl or clinical hypoglycaemia within the last 12 h, the examination was postponed.

The examinations were performed in a quiet, slightly illuminated room with a constant temperature of 23°C, between 8:00 a.m. and 1:00 p.m. Speaking or physical activities not related to the testing procedure were not permitted. Patients were requested to lie down quietly for a preparatory phase of at least 15 min to exclude relevant emotional or external influence on the autonomic system. Only after this resting phase that the data acquisition was

Table 1
Clinical characteristics of patients (x ± SD) grouped according to total Ewing score

Matched variables	*No* CAN	*Early* CAN	*Severe* CAN
n	17	17	17
HbA_{1c} (%)	6.8 ± 0.9	7.1 ± 0.9	6.9 ± 1.6
Age (years)	52.0 ± 9.7	53.4 ± 9.5	52.8 ± 10.7
Gender (f/m)	9/8	9/8	9/8
Type 1/Type 2 diabetes	14/3	14/3	14/3
Diabetes duration (years)	22.3 ± 13.2	23.6 ± 14.8	21.2 ± 10.1
BMI (kg/m^2)	25.7 ± 3.6	25.0 ± 3.4	25.8 ± 4.2
BP sys./diast. (mmHg)	125 ± 19/75 ± 9	126 ± 10/75 ± 6	134 ± 23/74 ± 10

No significant differences between groups.


PSD
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PSD
1000*ms²/Hz
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0,4
0,5 Hz
T1
T2
T3

Table 2
Intergroup comparison for parameters of Ewing battery

	no CAN	A	*early* CAN	B	*severe* CAN	C
I–E difference (beats)	20.8 ± 8.7	**	13.6 ± 7.7	***	5.9 ± 3.5	***
Valsalva ratio	1.5 ± 0.2		1.4 ± 0.2	***	1.2 ± 0.1	***
30:15 ratio	1.1 ± 0.1		1.0 ± 0.1		1.0 ± 0.0	***
ΔsBP (orthostatic load; mmHg)	-7.2 ± 8.0	***	5.9 ± 10.2	***	17.4 ± 7.9	***
ΔdBP (handgrip test; mmHg)	23.6 ± 11.4		16.3 ± 5.8		20.0 ± 17.3	

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
A: comparison of groups *early* CAN vs. *no* CAN; B: *severe* CAN vs. *early* CAN; C: *severe* CAN vs. *no* CAN.

started. Both examinations, the Ewing standard battery and short-term PSA of HRV were performed consecutively in random order in a diabetes outpatient department beyond the usual routine.

2.2.1. Standard battery of cardiovascular autonomic function tests

CAN was assessed by standard battery of cardiovascular reflex tests, i.e. blood pressure and/or heart rate responses to standardised stimuli (American Diabetes Association, 1996; Ewing et al., 1985) including deep breathing, Valsalva manoeuvre, orthostatic load and sustained handgrip. Each borderline test was scored with 0.5, a pathological result as 1.0 score, so that the maximal given score (= total Ewing score) in case of severe autonomic neuropathy could be 5.0. Age-related thresholds for borderline and pathologic values were applied as previously described (O'Brien et al., 1986; Ziegler et al., 1992). Diabetic patients with total Ewing score of 0–0.5 were considered as those without CAN, patients with score of 1.0–2.5 as with early CAN and those with score 3.0–5.0 as with definite/severe CAN (Ewing et al., 1985). In this way, the Ewing standard battery of cardiovascular autonomic function tests was considered as a reference method.

A cuff sphygmomanometer was used to measure blood pressure. A fall in systolic blood pressure of 10 mmHg or less, assuming an erect posture, was defined as normal, a fall of 11–29 mmHg as borderline, and 30 mmHg or more as abnormal (Ewing et al., 1980).

2.2.2. Spectral analysis of heart rate variability

A computer-aided examination and evaluation system *VariaPulse TF3* (Sima Media, Olomouc, Czech Republic) was used for telemetric on-line transfer and analysis of HRV in time- and frequency- domain. A measurement system monitored continuously a surface ECG with a resolution time of 1 ms (Salinger et al., 1995). The data were telemetrically (infrared-light radiation) transferred into a receiver connected to a PC-compatible computer and displayed on-line together with an instantaneous spectral curve on a monitor. Each dataset was filtered automatically by excluding recorded artefacts using a recognition algorithm, and manually as well. Computational method was based on fast Fourier transform modified by algorithm of Coarse graining spectral analysis (Yamamoto and Hughson, 1991). That allowed to discard a broad-band nonharmonic 'noise' contaminating particularly the lower frequencies ($1/f$ component). The final results were immediately displayed on the monitor as three-dimensional running spectra, permitting a general overview on dynamics and on absolute energy contents of the system. We used three consecutive examination positions supine1–standing–supine2 (adapted after Bellavere et al. (1992), Fig. 1) and calculations were made for every position on 256-beat-window basis. Parameters of frequency-domain were observed in every position within the high-frequency band (0.15–0.50 Hz) which has been attributed exclusively to parasympathetic tone (Hayano et al., 1991), and within the low-frequency band (0.05–0.15 Hz) which is said to represent (although still equivocal; Eckberg, 1997) combination of sympathetic and parasympathetic effects on cardiac autonomic tone (Pagani et al., 1993). Main outcome variables were spectral power and power spectral density (peak value) in both frequency bands and coefficient of component variance (CCV) (Hayano et al., 1990b). To increase the reliability of short-term measurements for assessment of global autonomic tone, we considered cumulative indices (spectral power of total frequency band /LFHF cumpower/ with its high /HF cumpower/ and low frequency /LF cumpower/ components over all three positions) representing the total averaged area under all consecutive spectral curves within the short-term recording (Howorka et al., 1997). Standard deviations were calculated for each parameter and findings bearing more than 30% relative standard deviation within any of positions recorded were not considered to be representative. In those

Fig. 1. Typical patterns of short-term spectral analysis of HRV in three consecutive positions, 256 heart beats each (on time axis z: T1 = supine1, T2 = standing, T3 = supine2) in female diabetic subjects: in a patient without CAN (*upper panel*; total Ewing score 0.5, age 43, diabetes duration 14 years), in another one with early involvement (total Ewing score 1.5, age 34, diabetes duration 13 years) and in one with severe CAN (*lowest panel*; total Ewing score 4.0; age 42, diabetes duration 33 years). Vertical axis: power spectral density [ms^2/Hz], horizontal axis: centre frequency [Hz], z-axis: time [s].

cases, the examinations were repeated on another occasion. Calculations of time-domain analysis were made as well for consideration of Mean square of difference of successive $R–R$ intervals (MSSD).

2.3. Statistical analysis

For the analysis, standard statistical packages (SPSS, Statistical Package for the Social Sciences V2.2 SPSS, Chicago, USA and SAS V6.07, SAS Inst., Cary, NC, USA) were used. The examination of normality was performed with Kolmogorov–Smirnov goodness of fit test. For the comparison of multiple groups, a one-way analysis of variance with post hoc comparison was used. Student's *t*-test, with level of significance adapted for multiple comparisons, or Mann–Whitney *U*-test, if appropriate, were applied for estimation of significant differences between groups. For further selection of parameters of HRV with the highest impact, discriminance analysis was applied: the impact of individual parameters is given as percentage of explained variance. Factor analysis (principal component analysis, Varimax rotation) was used to define the functional relationship between variables. For the comparison of time expenditure required for performance of both methods used, a paired *t*-test was applied. Because of the skewness of the frequency-domain data distribution, log transformation was used to produce a normal distribution before the statistical analyses were performed.

3. Results

3.1. Discrimination among diagnostic groups by both methods

The intergroup comparison of the main parameters of the *Ewing test battery* (Table 2) reveals statistical differences for inspiratory/expiratory difference obtained during the deep breathing test and for the decrease of systolic blood pressure in orthostatic load.

In the intergroup comparison of the main parameters of *short-term spectral analysis of HRV* (Table 3a), cumulative spectral power of total frequency band /ln(LFHF cumpower)/ and of low-frequency band /ln(LF cumpower)/ over all three positions, as well as spectral power and power spectral density in low frequency band during both supine positions proved to be sufficiently selective by discriminating among all patient groups. The analysis of HRV in time-domain (Table 3b) while using this method revealed no sufficient selectivity for MSSD and the mean of $R–R$ intervals, lacking the discrimination between the groups with *no* and *early* CAN. In the whole investigated diabetic population, a nearly linear functional relationship between the Ewing total score and logarithmic values of cumulative spectral powers of low and high frequency bands /ln(LFHF cumpower)/ ($n = 119$, $r = -0.87$, $P < 0.001$, Fig. 2) as well as of low ($r = -0.82$, $P < 0.001$) and high frequency bands ($r = -0.75$, $P < 0.001$) was found. A significant correlation of ln(LFHF

Table 3
(a) Intergroup comparison for parameters [ln(ms^2)] of frequency domain analysis of heart rate variability

	no CAN A		*early* CAN B		*severe* CAN C	
ln spectral power — cumulative indices (Supine 1 + Standing + Supine 2)						
Total Cumpower	7.4±0.9	**	6.7±0.6	***	5.2±1.1	***
LF Cumpower	6.7±0.9	**	5.8±0.8	***	4.4±1.5	***
HF Cumpower	6.3±1.2		5.8±1.0	***	4.4±1.2	***
ln spectral power						
Supine 1						
Total Power	6.4±0.7	**	5.7±0.7	***	4.4±1.0	***
LF Band	5.6±0.8	**	4.7±0.8	***	3.3±1.4	***
HF Band	5.5±1.1		4.9±1.0	**	3.7±1.2	***
Standing						
Total Power	5.9±1.1		5.2±1.0	**	4.1±1.1	***
LF Band	5.6±1.1	*	4.8±0.9	**	3.4±1.5	***
HF Band	4.1±1.4		3.6±1.5		2.8±1.2	**
Supine 2						
Total Power	6.6±0.8	**	5.8±0.6	***	4.6±0.9	***
LF Band	5.8±0.7	***	4.7±0.9	**	3.5±1.6	***
HF Band	5.7±1.2		5.1±0.9	***	3.8±1.1	***
ln power spectral density						
Supine 1						
LF Band	8.8±0.9	**	7.8±0.8	***	6.5±1.3	***
HF Band	8.4±1.1		7.6±1.1	**	6.4±1.4	***
Standing						
LF Band	8.7±1.3		7.9±0.9	**	6.6±1.5	***
HF Band	6.9±1.3		6.2±1.4		5.4±1.3	**
Supine 2						
LF Band	8.9±0.7	**	7.9±1.0	**	6.7±1.5	***
HF Band	8.5±1.0		8.0±1.0	**	6.7±1.4	***

(b) Intergroup comparison for parameters of time domain analysis of heart rate variability (One-Way ANOVA, post hoc tests, Alpha = 0.05)

	no CAN A	*early* CAN B		*severe* CAN C	
Supine 1					
$R–R$ interval (s)	0.91±0.18	0.87±0.13		0.83±0.13	
MSSD[a] (ms^2)	997.4±939.5	653.1±734.2	**	142.8±126.1	***
Standing					
$R–R$ interval (s)	0.77±0.15	0.75±0.10		0.74±0.13	
MSSD[a] (ms^2)	526.2±827	424.7±1004.4	*	96.9±119.7	***
Supine 2					
$R–R$ interval (s)	0.92±0.17	0.89±0.12		0.84±0.13	
MSSD[a] (ms^2)	1447.2±1559.6	630.3±551.8	***	76.8±172.7	***

* $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$.
A: comparison of groups *early* CAN vs. *no* CAN; B: *severe* CAN vs. *early* CAN; C: *severe* CAN vs. *no* CAN.
[a]Mean square of successive differences of $R–R$ intervals (Mann–Whitney *U*–Wilcoxon Rank Sum *W* tests).

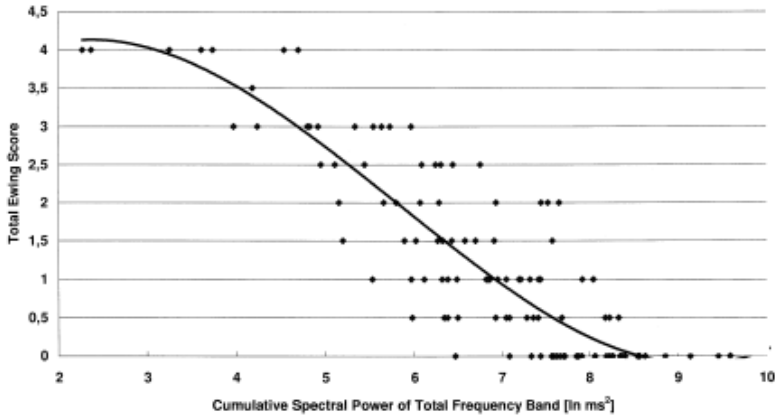

Fig. 2. Relationship between the Total Ewing score (vertical axis) and cumulative spectral power of total frequency band (horizontal axis) in the whole unselected diabetic cohort ($n = 119$).

cumpower) with age was present only in the diabetic subcohort with no CAN ($n = 50$, $r = -0.56$, $P < 0.001$, Fig. 3). The presence of early ($n = 51$, $r = -0.08$, N.S., Fig. 3) or definite CAN ($n = 18$, $r = -0.06$, N.S., Fig. 3) fully abolished this relationship.

3.2. Factor analysis

In short-term PSA of HRV, the variables of frequency-domain were grouped by factor analysis into four independent factors which altogether explain 91.2% of variance. Factor 1 describes the dimension *low frequency* band and explains 70.2% of variance. The second factor describes the dimension *high frequency* band and explains 9.8% of variance. The third factor describes the dimension *standing* and explains 6.5% of variance. The fourth one represents the dimension *coefficient of component variance* (CCV) and explains 4.7% of variance. The partial components of the mentioned factors are given in Table 4.

3.3. Analysis of discriminance

The variables of short-term PSA of HRV allow to discriminate between groups with different degrees of cardiac denervation as categorised after the total Ewing score ($\chi^2 = 192.3$; $df = 44$; $P < 0.001$). The most relevant parameters were those of cumulative spectral power of total frequency band, total spectral powers in supine positions, and spectral powers of low frequency band in both positions (Table 5). About 83.2% of cases classified

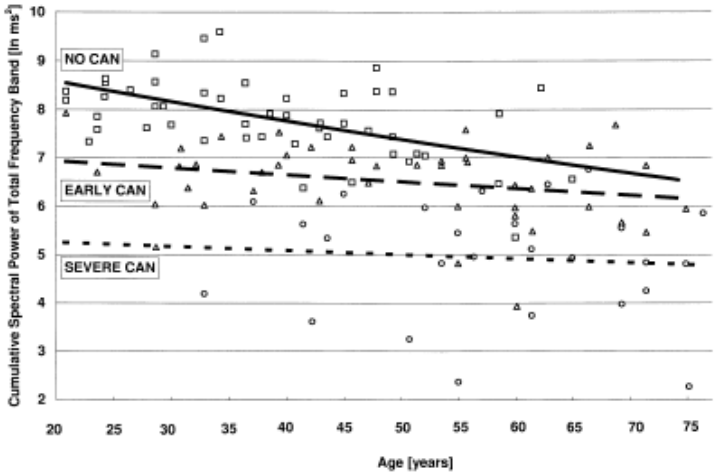

Fig. 3. Relationship between patient age (horizontal axis) and cumulative spectral power of total frequency band (vertical axis) ($r = 0.56$, $P < 0.0001$), in the patients with no CAN ($n = 50$, total Ewing score ≤ 0.5, $r = -0.56$, $P < 0.01$, *continuous line/square symbols*), with early CAN ($n = 51$, total Ewing score ≤ 2.5, $r = -0.08$, $P =$ N.S., *dashed line/triangle symbols*) and in those with definite/severe CAN ($n = 18$, total Ewing score ≥ 3.0, $r = -0.06$, $P =$ N.S., *dotted line/circle symbols*) from the whole described diabetic cohort ($n = 119$).

Table 4
Factor analysis

	Factor 1	Factor 2	Factor 3	Factor 4
LN Density Low Frequency Band Supine2	0.83			
LN Power Low Frequency Band Supine1	0.82			
LN Density Low Frequency Band Supine1	0.81			
LN Power Low Frequency Band Supine2	0.81			
LN Density Low Frequency Band Standing	0.77			
LN Power Low Frequency Band Standing	0.74			
CCV High Frequency Band Supine2		0.84		
LN Power High Frequency Band Supine1		0.83		
LN Density High Frequency Band Supine1		0.83		
CCV High Frequency Band Supine1		0.83		
LN Density High Frequency Band Supine2		0.82		
LN Power High Frequency Band Supine1		0.82		
LN Power High Frequency Band Standing			0.81	
LN Density High Frequency Band Standing			0.80	
CCV High Frequency Band Standing			0.78	
CCV Low Frequency Band Supine1				0.81
CCV Low Frequency Band Supine2				0.75
LN Total Cumpower Sup.1 + 2 + Standing	0.65	0.60		
LN Power Total Frequency Band Supine1	0.62	0.68		
LN Power Total Frequency Band Supine2	0.63	0.66		
LN Power Total Frequency Band Standing	0.60		0.69	

Significant correlations for 117 degrees of freedom ($r > 0.195$, $P < 0.05$; $r > 0.254$, $P < 0.01$; $r > 0.321$, $P < 0.001$), two-tailed test.

Table 5
Analysis of discriminance (matrix of structure)

LN Total Cumpower Supine 1 + 2 + Standing	0.74
LN Power Total Frequency Band Supine2	0.71
LN Power Total Frequency Band Supine1	0.68
LN Power Low Frequency Band Supine2	0.65
LN Power Low Frequency Band Standing	0.64
LN Density Low Frequency Band Supine2	0.63
LN Power Total Frequency Band Standing	0.57
LN Density Low Frequency Band Standing	0.57
LN Power High Frequency Band Supine2	0.57
LN Density Low Frequency Band Supine1	0.55
LN Density High Frequency Band Supine1	0.54
LN Power Low Frequency Band Supine1	0.54
LN Density High Frequency Band Supine2	0.53
LN Power High Frequency Band Supine1	0.52
LN Density High Frequency Band Standing	0.39
Coeff. of Component Variance High Frequency Band Supine2	0.38
LN Power High Frequency Band Standing	0.38
Coeff. of Component Variance Low Frequency Band Standing	0.36
Coeff. of Component Variance Low Frequency Band Supine2	0.36
Coeff. of Component Variance Low Frequency Band Supine1	0.33
Coeff. of Component Variance High Frequency Band Standing	0.27

by short-term PSA of HRV using the variables selected by analysis of discriminance was congruent with the classification by the total Ewing score alone (Table 6). As can be derived from the predicted group membership table (Table 6), the specificity of short-term spectral analysis of HRV for severe neuropathy (positive result of test in existence of severe CAN) is 0.89; whereas, the sensitivity (negative result in nonexistence of severe CAN) is 0.82 if the definition of 'existence' of severe CAN would be based on total Ewing score.

3.4. Practicability of both methods in routine measurements

As in our setting, where the use of both methods was computer-aided, it was possible to estimate exactly the time expenditure required for the performance of each examination. The mean time (excluding preparatory phases and/or pauses needed for explanations) for the performance of Ewing standard battery was 31.4 ± 9.6 and 13.5 ± 2.2 min for the short-term PSA of HRV ($p <$

Table 6
Predicted values as defined by the variables of analysis of discriminance versus the categorization by Ewing score

Group (Ewing score)		Predicted group membership (Spectral analysis of HRV)		
Actual group	No. of cases	*no* CAN	*early* CAN	*severe* CAN
no CAN	50	40 (80%)	9 (18%)	1 (2%)
early CAN	51	5 (9.8%)	43 (84.3%)	3 (5.9%)
severe CAN	18	0	2 (11.1%)	16 (88.9%)

Percentage of 'grouped' cases correctly classified: 83.2%.

0.0001). Moreover, the latter method proved to be more stress-free and almost independent from patient cooperation in comparison to Ewing standard battery.

4. Discussion

Similar to previous investigations (Ewing et al., 1985), our study using Ewing battery also identified two major indices of autonomic failure: (1) reduced inspiratory/expiratory difference, an index of cardiovagal activity, and (2) fall of orthostatic blood pressure during standing, an index of adrenergic function. Our data, however, show that the reliable discrimination between no CAN and early involvement in diabetes requires application of the whole Ewing battery and not only selected tests (Gelber et al., 1997). Moreover, 'classical' time-domain parameters as MSSD and averaged *R–R* interval were also not sufficient to discriminate between the above mentioned patient groups. In contrast, the use of short-term PSA of HRV allowed the reliable discrimination among all subgroups. This diagnostic discrimination has been currently shown to be of extraordinary importance as early stages of cardiovascular denervation in diabetes seem to be reversible (Howorka et al., 1997).

The multiple correlation between variables of PSA and Ewing battery was high and even over 83% of cases was classified in an identical way by both diagnostic methods. The differences in predicted membership might be due to even higher sensitivity of PSA to initial cardiac denervation in comparison to the Ewing battery, confirming previous similar findings (Comi et al., 1990; Yamasaki et al., 1991). Similarity in predicted membership by both methods is particularly relevant for our short-term approach, as sufficient reproducibility of time and frequency-domain analysis of HRV was found mainly for long-term recording periods (Kamalesh et al., 1995). Our study delivers one more proof that short-term spectral measures of HRV can also be considered for reliable diagnostic evaluation of CAN in diabetes in everyday routine. It confirms previous reports where immediate variability of short-term recordings was low (Freed et al., 1994) and short, 2- to 15-min samples were excellent predictors of mortality and correlated well with prognostically important data from sustained recording periods (Bigger et al., 1993; Fei et al., 1996).

The performance of the standard battery of Ewing cardiovascular reflex tests requires appropriate equipment (ECG device, sphygmomanometer, dynamometer for the handgrip test, manometer for Valsalva load) which only at first glance is simpler than the necessary PC-computer for spectral analysis. Our study demonstrates an easy and reliable evaluation of neuropathy by computer-aided calculations.

Under routine everyday conditions in an outpatient department, this method proved to be shorter, almost independent from patient cooperation and delivering immediately a graphic information of the instantaneous autonomic supply to the heart.

Further investigations are required to define/confirm the optimal frequency ranges for extracting relevant information about instantaneous autonomic control of the heart (Jaffe et al., 1993), to improve understanding of the outputs of different frequency bands (Eckberg, 1997; Pagani et al., 1993), reproducibility of the short-term PSA of HRV (Coats, 1995; Freed et al., 1994; Huikuri et al., 1990; Töyry et al., 1995) and to define factors which might reduce the reproducibility such as concomitant medication (Jokkel et al., 1995), nutritional state (e.g. fasting, Pumprla et al., 1996), and the use of common sympathomimetic substances like coffee, tea, alcohol or cigarettes (Hayano et al., 1990b). Further investigations—including those in healthy population—are required to define confidence intervals for the main variables defined by our study.

This investigation showed that short-term PSA of HRV is of similar diagnostic value as the Ewing battery concerning the presence of cardiovascular autonomic neuropathy in diabetes. This instrument, with its indices of cumulative power corresponding to the total Ewing score, proves to be a practical tool for screening purposes in a large population of diabetic patients. The index of overall cumulative power [ln(LFHF cumpower)] was *not* conceived to *replace* Ewing battery but it could be used similarly to the total Ewing score. The proposed cumulative indices are representative for the actual global state of autonomic regulation and reflects the total of all instantaneous sympathetic and parasympathetic effects. Graphic display of short-term spectral analysis illustrates the autonomic balance with its specific functional components even better than the reference method. However, for the assessment of the degree of orthostatic hypotension and further differentiated consideration of autonomic influence, the performance of Ewing standard battery with the application of other approaches (e.g. variations of blood pressure) might be helpful.

Further investigations are required to promote its use in everyday clinical routine for evaluation in diabetes.

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3.3. Vývoj a validace analytických standardů k posouzení HRV: posouzení odlišností v autonomních regulacích při hypoglycemia unawareness syndromu

Úvod: V souvislosti s nálezem významného podílu dosud asymptomatických diabetických pacientů s počínající CAN jsme se zaměřili na problematiku vnímání a reakce na hypoglykemii („hypoglycemia unawareness syndrome“). Tato problematika má řadu závažných klinických a sociálních souvislostí, přičemž postihuje cca 5-10% pacientů především s diabetem mellitus typu I, u nichž jsou výkyvy v průběhu glykemie významně výraznější než u typu II. Zatímco klinicky mírná hypoglykemie 2x týdně je defacto nepřímou známkou dobrého nastavení kontroly diabetu *(Howorka 1996, 2001)*, a vede maximálně k sociálně složitějším situacím při obleněném vědomí pacienta, klinicky závažná hypoglykemie s kvantitativní změnou vědomí může vést k život ohrožujícím situacím, přičemž se odhaduje, že 6-10% pacientů s porušeným vnímáním příznaků hypoglykemie na tento klinický problém zemře *(Reno 2013)*.

Cílem naší práce bylo posoudit, zda existují rozdílné modely autonomních kontrolních regulací u pacientů se zachovalým vnímáním hypoglykemie a s porušenou schopností reakce na podhraniční snížení glykemie (problematika tzv. „hypoglycemia awareness“). Porovnali jsme dvě skupiny pacientů se zachovalou („+“, n=14, z nichž 5 mužů) a porušenou („-“, n=13, z nichž 6 mužů) hypoglycemia awareness, s průměrným věkem 36±10/36±12 let, BMI 24±2/23±2 kg/m2, trvání diabetu 16±9/17±8 let, HbA1c 7.3±1.0/7.6±1.0, denní celková spotřeba inzulinu 49±14/42±13 IU, s celkovým Ewing skorem 0.3±0.1/0.2±0.2.

Výsledky: Bylo provedeno vyšetření krátkodobé HRV dle modifikovaného ortostatického testu a data analyzována ve spektrální doméně s následnými výsledky (skupina (+) se zachovalou vs.skupina (-) s porušenou hypoglycemia awareness):

- LF band cumulative spectral power (x±SEM: ln[ms^2]=7.8±0.3 vs. 7.0±0.3, *p=0.048*),
- LF band cumulative coefficient of component variance (3.5±0.4 vs. 2.5±0.3, *p=0.047)*
- centroid frequency of LF band vestoje (81.8±6.1 vs. 65.7±4.2 mHz, *p=0.036*).

Závěr: Zjistili jsme, že ve srovnání se skupinou bez dysfunkce (+), skupina pacientů s porušenou schopností vnímat hypoglykemii (-) vykazuje snížený spektrální výkon a nižší centroidní frekvenci v oblasti LF band (převážně sympatikonie). Jako možné vysvětlení se nabízí zpomalení a snížení kontraregulační sympatikotonní odpovědi na příchod hypoglykemie, což odpovídá klinickému nálezu u této ohrožené skupiny pacientů. Typický

nález HRV u těchto dvou klinicky odlišných skupin pacientů je znázorněn na **Obr.16**.

3.3.1. Pumprla J, Howorka K, Anderer P et al: Reduction of low-frequency band spectral power and centroid frequency in an analysis of heart rate variability in diabetic patients with hypoglycemia unawareness. Diabetologia, 41, 1998, p. 1184 (Abstrakt). IF: 5.7

REDUCTION OF LOW-FREQUENCY BAND SPECTRAL POWER AND CENTROID FREQUENCY IN ANALYSIS OF HEART RATE VARIABILITY IN DIABETIC PATIENTS WITH HYPOGLYCAEMIA UNAWARENESS

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Aim: To compare patterns of spectral analysis of heart rate variability (HRV) in patients *without* and *with* hypoglycaemia unawareness in diabetes. **Patients and Methods:** Short-term spectral analysis of HRV in positions supine-standing-supine (each 300 seconds, low-frequency /LF/ band 0.05-0.15 Hz, high-frequency /HF/ band 0.15-0.5 Hz) was performed under standardised conditions in two groups of IDDM-patients *without* and *with* hypoglycaemia unawareness (x±SD: n=14/15, age 35±10/36±11, diabetes duration 16±9/18±11 years, HbA_{1c} 7.9±0.4/8.0±0.6%), using the evaluation system *VariaPulse TF3®*. **Results:** When compared to those *without* unawareness, patients *with* hypoglycaemia unawareness demonstrated a statistically significant decrease of LF band cumulative spectral power (x±SEM: ln[ms^2]=7.8±0.3 vs. 7.0±0.3, p=0.048), LF band cumulative coefficient of component variance (3.5±0.4 vs. 2.5±0.3, p=0.047) and centroid frequency of LF band during standing (81.8±6.1 vs. 68.7±4.2 mHz, p=0.036). **Conclusions:** IDDM-patients *with* hypoglycaemia unawareness demonstrate lower spectral power and centroid frequency in LF band when compared to those *without* unawareness. One of possible explanations of this finding could be a slowing of rhythms and/or reduction of heart rate control as depicted in LF band during short-term spectral analysis of HRV. Large-scale study is needed to elucidate this interesting phenomenon.

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Aim

To compare patterns of spectral analysis of heart rate variability (HRV) in patients *without* and *with* hypoglycaemia unawareness in diabetes.

Patients		
Patient group	***Without* history of severe hypoglycaemia**	***With* history of severe hypoglycaemia**
n (m/f)	14 (5/9)	13 (6/7)
Age (years)	36±10	36±12
BMI (kg/m^2)	24.1±2.4	22.5±2.2
Diabetes duration (yrs)	16±9	17±8
Duration of FIT (yrs)	6.8±3.5	5.3±3.8
HbA_{1c} (%)	7.3±1.3	7.6±1.0
Insulin consumption(U)	49±7	42±14
Microalbuminuria (ug/min)	4.5±2.4	7.0±7.5
Total Ewing score	0.3±0.7	0.2±0.4

Methods

Short-term spectral analysis of HRV in positions supine-standing-supine (each 300 seconds, low-frequency /LF/ band 0.05-0.15 Hz, high-frequency /HF/ band 0.15-0.5 Hz, modified fast Fourier transform, noise suppression using Coarse-graining algorithm by Yamamoto, 1991) was performed under standardised conditions, using the commercially avai-lable telemetric evaluation system *VariaPulse TF3*®.

Results

When compared to those *without* unawareness, patients *with* hypoglycaemia unawareness demon-strated a statistically significant decrease of

- LF band **cumulative spectral power** (x±SEM: ln[ms^2]=7.8±0.3 vs. 7.0±0.3, p=0.048),

- LF band cumulative coefficient of component variance (3.5±0.4 vs. 2.5±0.3, p=0.047) and

- centroid **frequency of LF band** during standing (81.8±6.1 vs. 65.7±4.2 mHz, p=0.036).

Conclusions

IDDM-patients *with* hypoglycaemia unawareness demonstrate lower spectral power and centroid frequency in LF band when compared with those *without* unawareness.

One of possible explanations of this finding could be a slowing of rhythms and/or reduction of heart rate control as depicted in LF band during short-term spectral analysis of HRV. Large-scale study is needed to elucidate this interesting phenomenon.

Examination method:

Short-term power spectral analysis of heart rate variability obtained during a modified orthostatic load (system *VariaPulse TF3*; positions supine-standing-supine, each 300 se-conds; low frequency band 0.05-0.15, high frequency band 0.15-0.50 Hz). Computational method based on fast Fourier transform algorithm modified by Coarse graining approach in order to discard a broad band non-harmonic „noise“ *(Yamamoto 1991)*. On-line display of telemetrically (UHF transmission) measured ECG and instantaneous spectral curve with three-dimensional graphics display of results immediately after the examination (s. above).

3-D graphics - description of axes:

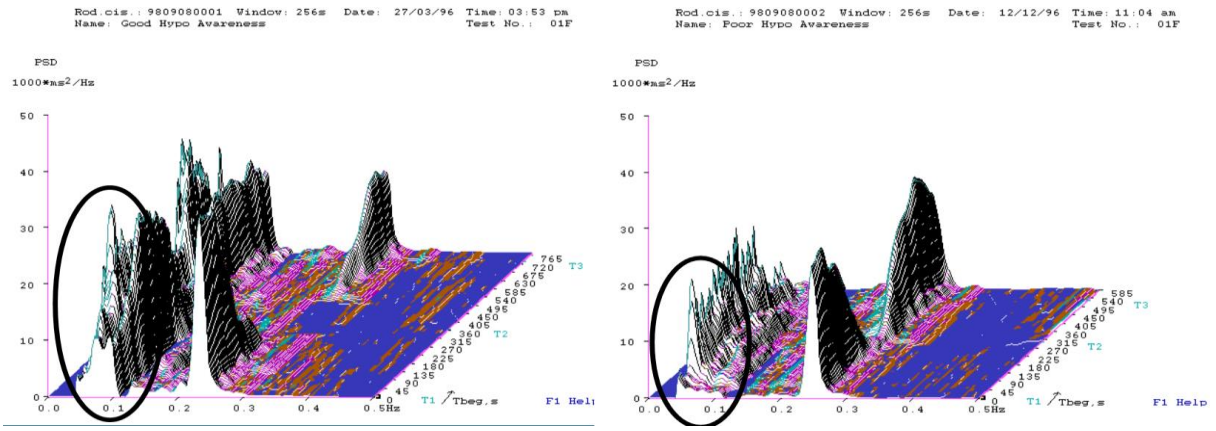
Horizontal axis:	frequency of fluctuations of R-R intervals [Hz]
Vertical axis:	power spectral density [ms^2/Hz]
z-axis:	time relapsed [s]

Main parameters - frequency domain:	
Spectral power	area under the spectral curves
Power spectral density	amplitude of spectral curves
Ratio of spectral power low/high frequency bands	
CCV (coeff. of component variance) relates power to instantaneous heart rate	
Centroid frequency	
Main parameters - time domain:	
mean R-R interval	
MSSD	mean square of differences of successive R-R intervals

Results of spectral analysis of HRV

Patient group	***Without* history of severe hypoglycaemia**		***With* history of severe hypoglycaemia**
	x+SEM	t-test	x+SEM
Cumulative total spectral power (*LF+HF bands*) [ln(ms^2)]	8.4+0.3	0.09	7.8+0.3
Cumulative spectral power (*LF* band only) [ln(ms^2)]	7.8+0.3	**0.05**	7.0+0.3
Cumulative spectral power (*HF* band only) [ln(ms^2)]	7.5+0.4	0.3	7.0+0.3
Centroid frequencies supine1 (LF/HF bands) [mHz]	82+7/249+19	0.7/0.9	79+5/245+14
Centroid frequencies standing (LF/HF bands) [mHz]	82+6/196+13	**0.04**/0.9	66+4/193+15
Centroid frequencies supine2 (LF/HF bands) [mHz]	96+6/252+20	0.2/0.9	84+7/253+15

Obr. 16: Vyobrazení dvou typických nálezů HRV *u pacienta se zachovalou (n=13 pacientů, obrázek vlevo) a s porušenou (n=14 pacientů, obrázek vpravo) schopností vnímání hypoglykemie. Skupina pacientů s porušenou awareness mají významně nižší spektrální energii a posun centroidní frekvence nalevo (k nižším, pomalejším regulačním frekvencím) v LF pásmu*



3.4. Vývoj a validace analytických standardů k posouzení HRV: modifikovaný ortostatický pokus vs. záznam bez ortostatické manipulace

Úvod: Dalším důležitým aspektem při validaci metodiky bylo posouzení přínosu délky vyšetření a ortostatické či jiné manipulace ve snaze po zvýšení výpovědní schopnosti (krátko- či déledobého) vyšetření HRV, za zohlednění nutné balance mezi náročností/zátěží vyšetřovaného probanda a diskriminačním přínosem testovacího protokolu. Řada autorů totiž využívá pro záznamy HRV kratší záznamy než našich 5 minut – např. pouze 1-2 minuty *(Weck 1997, Katz 1999)*, nicméně kombinované s manipulací autonomních regulací např. řízeným hlubokým dýcháním, čímž ovšem vnáší do systému externí parametr systematizované respirační frekvence, který významně ruší přirozený individuální podíl parasympatické regulační vazby na HF frekvenčním pásmu. Jiní autoři pak analyzují nestandardizované dlouhodobé záznamy, přičemž za jeden z podstatných faktorů poukazující na autonomní postižení považují sníženou noční aktivaci parasympatiku, případně redukované cirkadiánní sympatovagální variace *(Spallone 1996)*. Bottini et al nalezl sníženou sympatoadrenergní reakci během fyzické zátěže na ergometru u pacientů s autonomním postižením *(Bottini 1995)*.

Cílem naší studie bylo srovnat nálezy HRV v průběhu modifikovaného ortostatického testu u 80 pacientů (IDDM n=72, věk 38±14 let) s dobře kontrolovaným diabetem (HbA1c 146% průměru normy) a bez průkazu autonomní dysfuknce pomocí zlatého standardu, Ewingovy baterie reflexních testů, se věkově a složením srovnatelnou skupinou 150 probandů bez diabetu nebo jiného klinicky relevantního chronického onemocnění.

Výsledky a závěr: Nalezli jsme statisticky významný rozdíl (p=0.001) mezi hodnotami spektrálního výkonu v LF spektru po ortostatické manipulaci u pacientů s diabetem vs. zdravých probandů, přičemž ve skupině s diabetem byla zpožděna/oslabena redukce sympatikotonní odpovědi po ulehnutí, vyjádřena vyšším LF spektrálním výkonem. Tento nález lze interpretovat – v souladu s dostupnou literaturou – jako příznak oslabené parasympatické kontroly autonomní reaktivity v průběhu ortostatické manipulace, tedy jako příznak incipientní parasympatické dysfunkce u pacientů bez jinak prokazatelného autonomně-regulačního postižení. V tomto smyslu je modifikovaný ortostatický test, aktivující obě autonomní regulační větve, efektivní v detekci již minimálního postižení. Ve srovnání s

parametry individuálních 5-minutových pozic, modifikovaný ortostatický test zvyšuje senzitivitu krátkodobého vyšetření HRV. Navíc je testovací protokol jednoduše replikovatelný a za dodržení obvyklých vyšetřovacích standardů (vyloučení externích vlivů včetně případné hypoglykemie, odstup od posledního jídla, vysazení betablokrů či jiné pro HRV relevantní medikace) i časově (15 minut, leh-stoj-leh, 3x5 minut) uspokojivě realizovatelný v rutinním ambulantním provozu.

3.4.1. Howorka K, Pumprla J, Jirkovska A et al: Modified orthostatic load for spectral analysis of short-term heart rate variability improves the sensitivity of autonomic dysfunction assessment. Journal of Diabetes and its Complications, 2010, 24 (1): 48-54). IF 1.9

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Modified orthostatic load for spectral analysis of short-term heart rate variability improves the sensitivity of autonomic dysfunction assessment

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Abstract

Aim: To evaluate the impact of orthostatic load for sensitivity of short-term spectral analysis of heart rate variability (HRV) assessment of potential early autonomic dysfunction in diabetes mellitus. **Methods:** Comparison of results of short-term time- and frequency-domain analysis of HRV during single positions and during modified orthostatic load (supine 1–standing–supine 2, each position 300 s) in diabetic subjects with good glycemic control (n=80, age 38±14, diabetes duration 16±10 years) and without autonomic neuropathy as assessed by a standard bedside reflex test battery, and in nondiabetic controls (n=150, age 40±13 years). **Results:** None of the short-term frequency-domain parameters [absolute and logarithmic (LN) values of spectral powers in total- (TF), low- (LF), and high-frequency (HF) bands and its centroid frequencies] as obtained in single positions "supine" or "standing" revealed a significant difference between well-controlled patients and healthy controls (P>.3). However, during modified orthostatic load, significant differences in ΔLN $TF_{(supine\ 1-supine\ 2)}$ and in ΔLN $LF_{(supine\ 1-supine\ 2)}$ as well as in ΔLN $LF_{(standing-supine\ 2)}$ values between diabetic and healthy subjects were recorded [−0.2±0.5 vs. −0.1±0.4 LN (ms^2), P=.05; −0.3±0.8 vs. 0.1±0.7 LN (ms^2), P=.001 and 0.2±1.0 vs. 0.4±0.9 LN (ms^2), P=.05, respectively] with insignificant intergroup differences in related centroid frequencies. This finding suggests a delayed recovery of LF spectral power in diabetic subjects after orthostatic challenge. **Conclusions:** When compared with single position measurements, the modified orthostatic load protocol improves the sensitivity of short-term HRV examination. In well-controlled diabetic subjects without cardiovascular autonomic neuropathy (as excluded by standard cardiovascular reflex testing), the delayed recovery of LF band spectral power after orthostatic load with standing up indicates diminished parasympathetic activation.

Keywords: Autonomic neuropathy; Spectral analysis; Heart rate variability; Diabetes mellitus; Orthostatic test

1. Introduction

Cardiovascular autonomic neuropathy (CAN) in diabetes mellitus is a typical late complication that is associated with up to fivefold increase in mortality when compared with diabetic patients without CAN (O'Brien, McFadden, & Corrall, 1991). Analysis of heart rate variability (HRV) allows indirect assessment of cardiac autonomic control. Evaluation of HRV permits the diagnosis of autonomic dysfunction in diabetes (American Diabetes Association: Diabetic Neuropathy, 1996; Maser & Lenhard, 2005; Vinik & Mehrabyan, 2004; Ziegler et al., 1992) and risk stratification in congestive heart failure and/or coronary heart disease (Adamopoulos et al., 1992; American College of Cardiology Cardiovascular Technology Assessment Committee, 1993; Nolan et al., 1998; Task Force of the European Society of Cardiology & North American Society of Pacing and Electrophysiology, 1996). Clinical evidence

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was found for the association between incidence of lethal arrhythmias and signs of an increased sympathetic and reduced vagal activity (Kleiger, Miller, Bigger, & Moss, Multicentre Post Infarction Research Group, 1987; Malliani, Lombardi, Pagani, & Cerutti, 1994; Priori et al., 2001). A reduced HRV is an indicator of compromised health (Dekker et al., 1997, 2000) and/or increased mortality in population studies (Tsuji et al., 1996).

Analysis of HRV can be performed during a standardized short-term examination procedure such as the battery of bedside reflex tests (Ewing, Martyn, Young, & Clarke, 1985; Opavsky, 1988), or using nonstandardized long-term data, typically Holter 24-h ECG monitoring. The immediate variability of short-term spectral measures of HRV was found to be low (Freed, Stein, Gordon, Urban, & Kligfield, 1994). Moreover, short, 2- to 15-min samples were reported to be excellent predictors of mortality and were correlated with prognostically important data from sustained recording periods (Bigger, Fleiss, Rolnitzky, & Steinman, 1993; Fei, Copie, Malik, & Camm, 1996). These studies suggest that easy applicable short-term HRV analysis may have an important clinical role. Early techniques for assessment of HRV were based on simple evaluation of heart rate change during standardized bedside reflex tests as proposed by Ewing et al. (1985) two decades ago. These tests are quite robust but sometimes not sensitive enough for identification of early stages of autonomic dysfunction where the interventions towards restoration of autonomic control are still possible and effective (Howorka, Pumprla, Haber, Koller-Strametz, & Mondrzyk, 1997; Howorka, Pumprla, & Schabmann, 1997). Moreover, strong stimulus used to evoke the single reflex may be responsible for temporarily changing the cardiovascular function that might significantly influence the test result (Batin & Nolan, 1996). A newer method, based on frequency/spectral analysis approach, identifies harmonic, cyclical changes in heart rate course (Task Force of the European Society of Cardiology & North American Society of Pacing and Electrophysiology, 1996). This allows to differentiate and to quantify the energy content of both main branches of autonomic control, the sympathetic and parasympathetic one. Due to high sensitivity, hence vulnerability, of spectral methods to various influences occurring during the recording, it is necessary to carefully standardize the examination protocol (Howorka, Pumprla, & Schabmann, 1998). Usually, HRV obtained from 2- to 10-minute recordings in a supine position is analyzed. However, in contrast to the aforementioned conventional bedside reflex test battery, there is no widely accepted gold-standard examination procedure yet, despite recommendations made by the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology (1996).

The aim of our study was (a) to evaluate the cardiovascular autonomic response as obtained during short-term modified orthostatic load (Opavsky, 1988) in diabetic patients without clinically obvious CAN, as well as (b) to compare these results with nondiabetic controls. Specifically, the effect of implementation of short-term orthostatic load (standing up) is to be investigated.

2. Patients and methods

In our outpatient databases, we identified a group of 80 diabetic subjects (for details see Table 1) with good glycemic control, using functional insulin treatment—discriminating between prandial, basal, and correctional insulin use—to prevent neuropathic dysfunction (Howorka, Pumrla, Haber, et al., 1997; Howorka et al., 1997, 1998). These patients had no clinical signs of any late diabetic complications. Inclusion criteria were (1) age between 15 and 80 years; (2) no cardioactive medication such as beta-blockers; (3) no evidence of heart disease, as assessed by history and physical examination; (4) no evidence of concomitant disease or late complication of diabetes that might interfere with quality of autonomic control; (5) good metabolic control of diabetes, with HbA_{1c} up to 175% of reference mean (=100%); (6) no hypoglycemia during and up to 12 h before the examination; (7) blood pressure under 150/90 mmHg; and (8) body mass index (BMI) <30 kg/m^2. Nondiabetic controls were selected in accordance with the above inclusion criteria.

The presence of CAN in diabetic subjects was assessed by a conventional battery of cardiovascular reflex tests to standardized stimuli (Ewing et al., 1985) including deep breathing, Valsalva maneuver, and orthostatic load. Each single test of Ewing's battery was scored with 0 when normal, 0.5 when borderline, and 1 when out of age-related normal values range (Ewing et al., 1985), summing up the total Ewing score. Diabetic patients with total score of 0–0.5 were free of detectable CAN and therefore eligible for the study.

Short-term spectral analysis of HRV was obtained from recordings consisting of 256 s of artifact-free records each, using a VariaCardio system (Advanced Medical Diagnostics Group, UK) (Salinger et al., 1999). High-resolution one-channel ECG was recorded and identification of R-R intervals with sampling rate of 1000 Hz was performed to be telemetrically transferred into a receiver connected to a PC-compatible computer and displayed on-line together with an

Table 1
Clinical characteristics of study subjects

Diabetic patients
• n=80
• Age 38±14 years (range 18–80 years)
• Type 1 diabetes: n=72
• Diabetes duration: 16±10 years
• Functional insulin treatment: n=76
• HbA_{1c} 146±21% of mean reference range
• Gender (f/m): 46/34
Nondiabetic controls
• n=150
• Age 40±13 years (range 18–72 years)
• Gender (f/m): 81/69

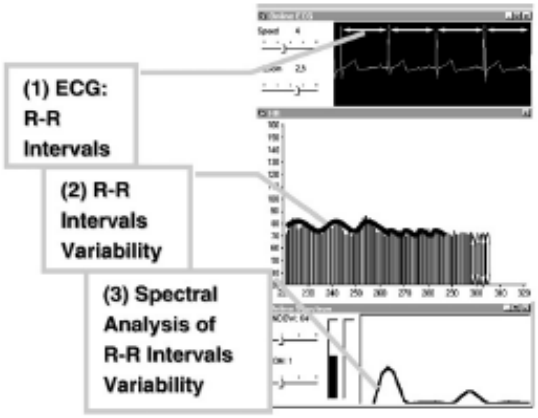

Fig. 1. Principle of measurement of HRV: The main principle of spectral analysis of HRV is a decomposition of physiologically irregular course of heart rate (as identified by R-R intervals in ECG) and quantification of its harmonic components.

instantaneous spectral curve on a monitor (Fig. 1). Computational method was based on fast Fourier transform (FFT). Recordings were obtained in three positions (supine–standing–supine). Each dataset was filtered automatically by identifying artifacts using a recognition algorithm and was overread manually, as well. Artifacts were labeled, and a specific algorithm imputed beat-to-beat intervals throughout an artifact period to preserve the timing relationships of the adjacent, uncorrupted heart rate data (Liao, Barnes, Chambless, & Heiss, 1996). The final results were immediately displayed on the monitor as three-dimensional running spectra (Fig. 2). Parameters of *frequency-domain* HRV were measured in every position within the low-frequency (LF) band (0.05–0.15 Hz) and high-frequency (HF) band (0.15–0.50 Hz), as well as total spectral variability (TF). Main outcome variables were spectral power [unit (ms^2)] in both frequency bands. To assess even small intraindividual changes in global autonomic tone during the trial, we used cumulative indices (spectral power of total frequency band with its low- and high-frequency components over all three positions; Howorka et al., 1998). Standard deviations were calculated for every parameter as this information was necessary for assessment of stationarity during the examination. We excluded the findings demonstrating more then 30% relative deviation in any of positions recorded. In those cases the examinations were repeated. *Time-domain* analysis included the averaged R-R interval (ms) and root of mean square of difference of successive R-R intervals ms. Because of the skewness of the frequency-domain data distribution, log (LN) transformation was performed to produce a normal distribution before the final results were analyzed.

2.1. Statistical analysis

Statistical analysis was performed using standard statistical packages (Statistical Package for the Social Sciences v. 10.0, SPSS, Inc., Chicago, IL, USA). Two-tailed unpaired Student's *t* test was applied to estimate differences between groups. Data are presented as means±S.D., unless otherwise indicated.

3. Results

None of the short-term frequency-domain parameters [absolute and logarithmic (LN) values of spectral powers in total- (TF), low- (LF), and high-frequency (HF) bands and its centroid frequencies] as obtained in single positions "supine"

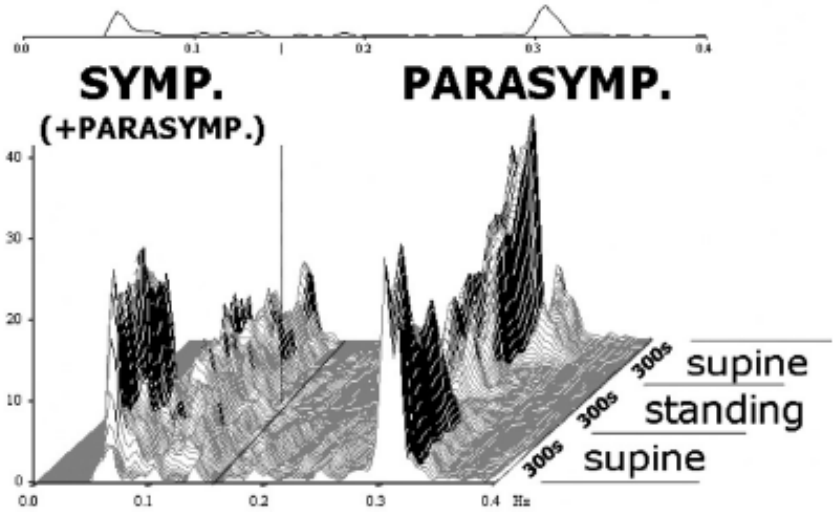

Fig. 2. Three-dimensional display of HRV pattern as obtained during a modified orthostatic load (standardized assessment by VariaCardio TF4 in positions supine–standing–supine, each position 5 min). Horizontal axis: frequency of fluctuations of R-R intervals (Hz); vertical axis: power spectral density (ms^2/Hz); z-axis: time relapsed (s).

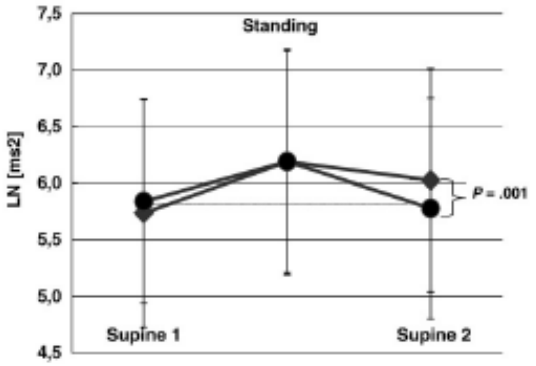

Fig. 3. Dynamic changes of spectral power in low-frequency band (predominantly sympathetic control) during the modified orthostatic load (in positions supine 1–standing–supine 2). Diamonds=diabetic subjects; circles=nondiabetic controls.

or "standing" revealed a significant difference between well-controlled patients and healthy controls ($P>.3$). However, during modified orthostatic load, significant differences in ΔLN $TF_{(supine\ 1–supine\ 2)}$ and in ΔLN $LF_{(supine\ 1–supine\ 2)}$ as well as in ΔLN $LF_{(standing–supine\ 2)}$ values between diabetic and healthy subjects were recorded [−0.2±0.5 vs. −0.1±0.4 LN (ms^2), $P=.05$; −0.3±0.8 vs. 0.1±0.7 LN (ms^2), $P=.001$ and 0.2±1.0 vs. 0.4±0.9 LN (ms^2), $P=.05$, respectively; Figs. 3 and 4], despite no significant intergroup differences in related centroid frequencies. Other nonsignificant results are displayed in Table 2.

4. Discussion

Analysis of HRV is a well-established tool in cardiology and diabetology. Important environmental and physiological factors should be considered in interpretation of HRV

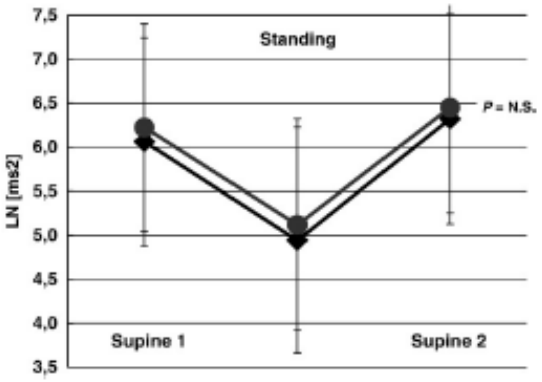

Fig. 4. Dynamic changes of spectral power in high-frequency band (parasympathetic control) during the modified orthostatic load (in positions supine 1–standing–supine 2). Diamonds=diabetic subjects; circles=nondiabetic controls.

Table 2
Differences between single postural positions (parameter ΔLN spectral power) in TF, LF, and HF bands as obtained in groups of diabetic subjects (DM) and nondiabetic controls (non-DM)

	DM (n=80)	Non-DM (n=150)	P
Supine 1 vs. supine 2			
TF	−0.2±0.5	−0.1±0.4	.05
LF	−0.3±0.8	0.1±0.7	.001
HF	−0.3±0.6	−0.2±0.5	NS
Supine 1 vs. standing			
TF	0.3±0.9	0.3±0.8	NS
LF	−0.5±1.1	−0.4±0.8	NS
HF	1.1±1.2	1.1±0.9	NS
Standing vs. supine 2			
TF	−0.5±0.9	−0.4±0.8	NS
LF	0.2±1.0	0.4±0.9	.05
HF	−1.4±1.2	−1.3±1.0	NS

patterns. Typically, the effect of aging on autonomic nervous system cardiac control is progressive and continuous throughout an 18- to 80-year age range (Fluckiger, Boivin, Quilliot, Jeandel, & Yannad, 1999). Interestingly, Goto et al. (1997) reported that while the HF component—as an index of autonomic tone—increased significantly with age from 3 to 6 years, it decreased with age from 6 to 15 years. Additionally, gender and BMI have been reported by some authors (Fagard, Pardaens, & Staessen, 1999; Stein et al., 2008) to have an effect on HRV pattern. Further environmental and physiological factors, such as temperature, position, respiration rate, circadian rhythm and/or postprandial changes, physical exercise, etc. (Bellavere et al., 1996; Bottini et al., 1995; Howorka, Pumprla, Haber, et al., 1997; Howorka et al., 1997), might play a role in autonomic control, as well, and it is therefore preferable to evaluate the autonomic control in response to a standardized manipulation protocol rather than in a single, steady position (Keselbrener & Akselrod, 1998). Furthermore, previous investigations with prolonged measurements of HRV in young insulin-dependent subjects have shown virtually no abnormalities in parasympathetic or sympathetic circadian patterns of heart rate control (Hoffman & Kienzle, 1996). We could reveal such differences vs. healthy controls exclusively while including a standardized orthostatic load for improvement of sensitivity of short-term HRV methodology.

In our previous study, we explored patterns of HRV in a group of diabetic patients without CAN and in nondiabetic controls (Pumprla et al., 2000). Comparison of data obtained in a single position supine or standing did not reveal any significant difference so that well-controlled diabetic patients were assumed to display virtually normal pattern of autonomic control. However, it is known that baroreflex sensitivity is reduced in type 1 diabetes with microalbuminuria but without autonomic neuropathy as assessed by standard bedside tests (Lefrandt et al., 1999). Patients with relatively well-controlled diabetes mellitus with normal reaction to deep breathing test (2-min test; respiration rate, 6/min) did not activate the sympathetic

baroreflex loop during orthostatic challenge (Weck et al., 1997). In diabetic patients with increasing degree of autonomic neuropathy, there was a progressive reduction of day-to-night change in HF/LF ratio of HRV spectrum and reduction of LF during the day. Furthermore, in this study the day LF spectral power was related to postural change, demonstrating that in diabetic patients with increasing autonomic damage, there is a blunted nocturnal increase of vagal activity and lower circadian variation in sympatho-vagal balance (Spallone et al., 1996). Moreover, during exercise, heart rate, systolic blood pressure, norepinephrine, and epinephrine increases were significantly blunted in diabetic patients with autonomic neuropathy with sympathetic and parasympathetic damage (Bottini et al., 1995). Already in 1987, DeBoer, Karemaker, and Strackee (1987) described using their beat-to-beat computer model of the cardiovascular system the 10-s rhythm in heart rate and blood pressure variations as a simple resonance phenomenon due to the delay (prolonged phase shift) in the sympathetic control loop of the baroreflex. Recently, it has been confirmed that the changes in baroreflex delay may be explained by the changes in parasympathetic modulation alone (Simek et al., 2002). All these facts confirm that the choice of natural, orthostatic challenge in the second part of our short-term HRV investigation is the best choice for easy applicable test standardization.

In the present study, we compared groups of diabetic patients without clinical signs of CAN as assessed by standard time-domain bedside reflex tests with nondiabetic controls that did not differ in their main clinical characteristics. In contrast to other authors relying on HRV examination based on a single position protocol or during 24-h nonstandardized ECG-Holter recordings, in order to obtain maximum information on the cardiac autonomic control in our subjects within a relatively short test, we used physiological provocation in form of a modified orthostatic load where subjects are measured during supine, active standing, and supine positions, each for 300 s (Pumprla, Howorka, Groves, Chester, & Nolan, 2002). This test procedure has already been thoroughly evaluated for validity (Howorka et al., 1998). In the present study, however, we focused on dynamics of HRV patterns observed during postural change. This demonstrated significant differences in reactivity of autonomic response between diabetic patients without CAN (assessed by robust albeit less sensitive method) and nondiabetic controls, suspecting a significant role of a prolonged phase-shift phenomenon in an early onset of diabetic CAN.

Normal HRV values have been explored by many researchers. Among the first authors, Ewing et al. (1985) established reference values for the battery of time-domain cardiovascular reflex tests in 1985. While there is no particular discrepancy between results of normative studies in time domain—dependence on age being the only exception (Diehl, Linden, & Berlit, 1997; Ziegler et al., 1992)—reference values based on analysis of frequency-domain HRV data using various devices reveal often significantly different results. This has been confirmed also in comparative studies, using a single R-R time series being analyzed by various HRV analysis devices. Although both main methods [i.e., FFT and autoregressive modeling (AR)] used in frequency-domain analysis at present deliver clinically similar information (Hartikainen, Tahvanainen, & Kuusela, 1998), the commercially available devices differ in data pre- and post-processing methods (Garcia-Gonzalez, M Fernandez-Chimeno, & Ramos-Castro, 2004). According to Chemla et al. (2005), due to higher uncertainty of AR estimates (particularly model order, phase dependency, or tail effect) when compared with FFT, for short-term HRV measurements in patients with diabetes the FFT analysis method should be preferably used. Furthermore, attempts to improve the HRV information by adapting the FFT (e.g., by coarse-graining process that allows extraction of nonlinearities located around 1/f of the spectrum; Yamamoto & Hughson, 1991) produce further—albeit sometimes only slight—differences in the results. These facts, together with the application of a variety of examination protocols—when compared with strictly standardized bedside reflex test battery (Ewing et al., 1985)—produce the conflicting results seen in some studies regarding the usefulness of a spectral analytical approach (Bellavere et al., 1992; Wawryk, Bates, & Couper, 1997). In our project, we studied patients as well as controls using the same method to prevent any protocol-dependent variability in our results.

Similarly to older, more complicated, and device-intensive procedures or more time-consuming methods for assessment of HRV, we identified—using a short (15 min), well-standardized, and easy-to-perform test—a significant difference in autonomic response between diabetic subjects without clinically detectable CAN and healthy controls. The delayed response in reduction of LF spectral power during the orthostatic manipulation from standing to supine position might be interpreted as an early sign of blunted parasympathetic control in diabetes mellitus with no otherwise detectable CAN. Thus, in our study, the modified orthostatic load for short-term spectral analysis of HRV improves the sensitivity of the method which was able to indicate even a slight autonomic impairment in comparison to healthy controls. Clinical relevance of this interesting finding needs, however, further confirmation in prospective observations.

Finally, this study demonstrates the necessity and feasibility of good glycemic control over long time periods with functional insulin treatment (Howorka, in press; Howorka et al., 2000) as an efficient prevention of more advanced autonomic neuropathy. In any case, the assessment of autonomic neuropathy in type 1 diabetes should be performed periodically, preferably in annual intervals (American Diabetes Association: Diabetic Neuropathy, 1996; Vinik & Mehrabyan, 2004). We suggest the convenient use of the described modified orthostatic load with spectral analysis of short-term HRV for this purpose.

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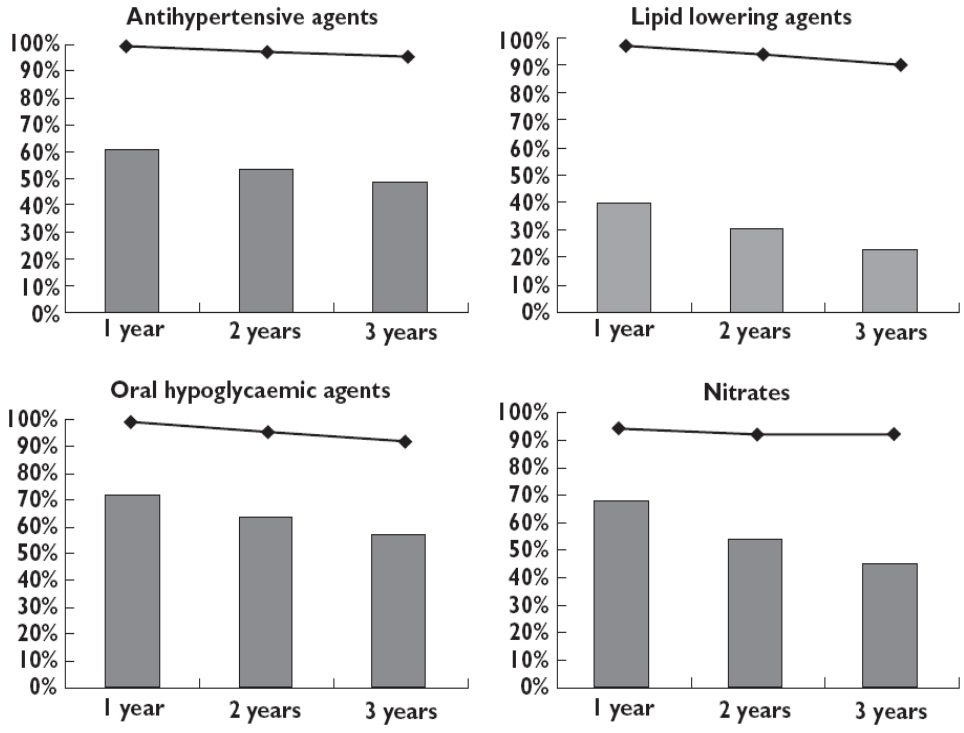
4. APLIKACE METOD ANALÝZY VARIABILITY SRDEČNÍ FREKVENCE V PRAXI

4.1. Praktická aplikace: výzkum reverzibility CAN a kvantifikace efektu nefarmakologických opatření - vytrvalostní trénink

Adherence k předepsané medikaci, resp. přesněji non-adherence, je jedním z hlavním důvodů, proč – přes současný stav a aplikaci medicíny založené na důkazech -- významná část pacientů nedosahuje klinických cílů terapie. Adherence je rozlišovaná jako *úmyslná* (aktivní - rozhodnutí nebrat medikaci), a *neúmyslná* (pasívní - bezstarostnost, zapomnětlivost, cena medikace, stigma nemoci, množství předepsaných tablet, ne/důvěra ke svému lékaři apod., *Gadkari 2012)*. Jak je známo z praktických terapeutických zkušeností s pacienty vyžaujícími chronickou systematickou medikaci, je adherence – navzdory očekáváním – k medikaci typu antihypertenziva, hypolipidemika nebo orální antidiabetika z dlouhodobého hlediska relativně nízká *(Poluzzi 2005)*. Dlouhodobě (3 roky) brává antihypertenziva pouze cca 50-60% pacientů, přičemž ještě méně (20-40%) jich pravidelně medikuje hypolipidemika, viz **Obr.17**.

Obr. 17: Relativní poměr pacientů medikujících *uvedené lékové skupiny po dobu 3 let s alespoň 90% pokrytím (Poluzzi 2005)*



Navíc, ani v případě systematického léčebného dohledu a pravidelného kontaktu mezi pacientem a lékařem, který předepisuje medikaci a kontroluje krevní tlak pacienta minimálně 1x za tři měsíce v ambulanci, nedochází k významnému zlepšení aderence *(McMannus 2005).*

Na základě více než 25-letých zkušeností se skupinovými edukacemi na vídeňském pracovišti *(Howorka 2002)* můžeme konstatovat, že rozhodujícím faktorem pro účinnou kontrolu aspektů metabolického syndromu je edukace a předání spoluodpovědnosti za svou léčbu nemocnému samotnému, přičemž role lékaře ustupuje do pozadí, ve formě supervizora

Obr. 18.: Výsledky klinických studií k dlouhodobé účinnosti edukace *k self-managementu pacientů s metabolickým syndromem včetně diabetu mellitus (Norris 2002)*

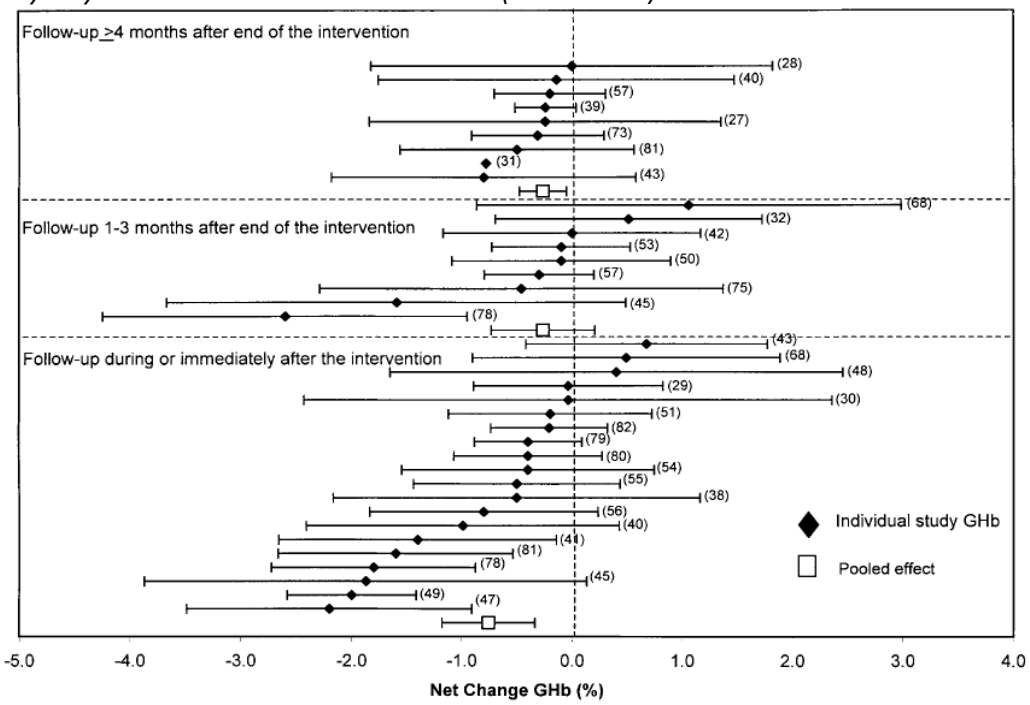


Figure 2—*GHb, stratified by follow-up interval. Net change in GHb is shown for each individual study, with lines extending from the symbols representing 95% CIs. Pooled results are for each follow-up interval, with* r = 0.5.

a poradce *(Norris 2002)*. Tento proces empowermentu (některými autory označován také jako „therapeutic patient education“) se provádí ve formě skupinových edukačních seminářů, obvykle v ambulantních podmínkách, a byl dostatečně popsán i prezentován na odborných fórech *(Howorka 2009).* Úspěšná a efektivní edukace vede k dlouhodobému zlepšení kompenzace diabetu a přidružených komplikací, viz **Obr.18**.

Úvod: Poskytnutí dostatečné informace o nemoci a její léčbě, a edukace chronicky nemocného ve smyslu „therapeutic patient education“ je základním stavebním kamenem úspěšnosti *nefarmakologické léčby* chronicky nemocných v rámci sekundární a terciární prevence komplikací metabolického syndromu *(Howorka 2002)*. Kromě klinických aspektů a dopadů na zdraví jedince, přináší efektivní prevence i snížení ekonomických nákladů na celkovou péči v přepočtu na DALY (disability-adjusted life years) o 15% ročně *(Pumprla 2008)*. S **cílem** redukovat vysokou non-adherenci k chronické medikaci a s ní spojené obavy i strach ze stigmatu nemoci jsme zaměřili naši pozornost v rámci aplikace sekundární a terciární prevence u našich pacientů s metabolickým syndromem na efekt vybraných aspektů životosprávních a dalších nefarmakologických opatření. V další části práce jsou popsány účinky střednědobého vytrvalostního tréninku, lačnění a aplikace řízeného domácího dýchání/ biofeedbacku u pacientů s komponenty metabolického syndromu. V rámci ověření procesů vedoucích k empowermentu pacientů – tedy ve svém důsledku efektivity sekundární a terciárn í prevence při metabolickém syndromu – jakož i k ověření reverzibility autonomního postižení při diabetu, jsme provedli studii s 22 pacienty s inzulin-dependentním diabetem.

Cílem práce bylo analyzovat účinnost pravidelně (minimálně 2x týdně po 30 minutách) prováděného vytrvalostního tréninku (rotoped při 65% individuální maximální srdeční frekvence) u pacientů s různým stupněm postižení autonomní neuropatií.

Výsledkem vytrvalostního tréninku v uvedené formě bylo statisticky významné zlepšení autonomních regulačních funkcí ve skupině pacientů bez a s počínající dysfunkcí a potvrdilo tak sledování jiných skupin např. u pacientů rehabilitujících po infarktu myokardu. Prokázali jsme tak reverzibilitu autonomního postižení, přinejmenším po dobu pravidelné tělesné zátěže. Skupina pacientů s pokročilou CAN nevykázala změnu HRV po této formě intervence.

4.1.1. Howorka K, Pumprla J, Haber P et al. Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy. Cardiovasc Res 1997; 34: 206-214. IF 5.2

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Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy

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Abstract

Objective: To investigate the effects of regularly performed endurance training on heart rate variability in diabetic patients with different degrees of cardiovascular autonomic neuropathy (CAN). **Methods:** Bicycle ergometer training (12 weeks, 2×30 min/week, with 65% of maximal performance) was performed by 22 insulin-requiring diabetic patients (age 49.5 ± 8.7 years; diabetes duration 18.6 ± 10.6 years; BMI 25.1 ± 3.4 kg/m^2): i.e., by 8 subjects with no CAN, 8 with early CAN and by 6 patients with definite/severe CAN. A standard battery of cardiovascular reflex tests was used for grading of CAN, a short-term spectral analysis of heart rate variability for follow-up monitoring of training-induced effects. **Results:** While the training-free interval induced no changes in spectral indices, the 12-week training period increased the cumulative spectral power of the total frequency band ($P = 0.04$) but to a different extent ($P = 0.039$) in different degrees of neuropathy. In patients with no CAN the spectral power in the high-frequency (HF) band (0.15–0.50 Hz) increased from 6.2 ± 0.3 to 6.6 ± 0.4 ln [ms^2]; $P = 0.016$, and in the low-frequency (LF) band (0.06–0.15 Hz) from 7.1 ± 0.1 to 7.6 ± 0.3 ln [ms^2]; $P = 0.08$, which resulted in an increase of total spectral power (0.06–0.50 Hz) from 7.5 ± 0.1 to 8.0 ± 0.3 ln [ms^2] ($P = 0.05$). Patients with the early form of CAN showed an increase of spectral power in HF (5.1 ± 0.2 to 5.8 ± 0.1 ln [ms^2], $P = 0.05$) and LF bands (5.6 ± 0.1 to 6.3 ± 0.1 ln [ms^2], $P = 0.008$), resulting in an increase of total power from 6.1 ± 0.1 to 6.6 ± 0.1 ln [ms^2] ($P = 0.04$), whereas those with definite/severe CAN showed no changes after the training period. Training improved fitness in the whole patient cohort. The increased autonomic tone as assessed by spectral indices disappeared after a training withdrawal period of 6 weeks. **Conclusions:** In diabetic patients with no or early CAN, regularly performed endurance training increased heart rate variability due to improved sympathetic and parasympathetic supply, whereas in subjects with definite/severe CAN no effect on heart rate variability could be demonstrated after this kind of training.

Keywords: Autonomic nervous system; Heart rate, variability; Spectral analysis; Diabetes; Endurance training; Human

1. Introduction

Diabetic autonomic neuropathy is associated with a reduced autonomic supply to the heart [1] which can be estimated by a decrease of heart rate variability (HRV) [2]. Although cardiovascular autonomic neuropathy (CAN) and decreased HRV are associated with an increased mortality [3–6], induced in part by sudden death, no data are available on factors which might improve the autonomic supply to the heart in diabetes. In healthy athletes [7–9], in hypertension [10], in patients with chronic congestive heart failure [11] and/or coronary artery disease [12], and in patients after recent myocardial infarction [13,14] systematic physical training was shown to induce improvement in the autonomic balance with a restoration to normal of the

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reflex activity of the system. Although the mechanisms of training-induced regulatory changes have still not been completely delineated, regularly performed physical training is generally thought to improve cardiovascular performance and to increase HRV [15]. Moreover, in diabetes physical exercise is considered to be essential in reducing insulin resistance [16], overweight [17], and enhancing the change in life style [18].

The prevalence of cardiovascular autonomic dysfunction in diabetes is high [2] and in part responsible for the diminished life expectancy in this chronic disease [4]. The primary goal of our study was therefore to investigate the effects of regularly performed physical training on HRV in insulin-requiring diabetic patients with different degrees of CAN as assessed by variables of short-term analysis of HRV in frequency and time domains. The trial was designed to investigate the potential reversibility of at least the early stages of autonomic neuropathy by those means which are available to every sedentary diabetic subject.

2. Methods

The investigation conforms with the principles outlined in the Declaration of Helsinki [19].

2.1. Study design

Intraindividual comparison of baseline values (mean of two measurements before and after a run-in period of 4–6 weeks with sedentary, unchanged life style) with values after a training intervention of 12 weeks endurance training and with final values after a consecutive period of 4–6 weeks of training withdrawal. Every patient in this design served as his own control.

2.2. Patients

From our clinical survey population of about 300 insulin-requiring diabetic patients we recruited 3 patient groups comparable in age, glycemic control and BMI, but differing in severity of their CAN as assessed by a standard battery of cardiovascular reflex tests [1]. Clinical data of patients who completed the study are summarized in Table 1. Patients with exercise-limiting circumstances, uncontrolled associated diseases such as hypertension, and those with clinical and/or electrocardiographic signs of coronary artery disease were excluded. Pharmacological treatment of concomitant diseases and nutrition were kept constant during all study periods. All patients had been receiving functional insulin treatment for at least 3 months before the study. As previously described [20] this program involves separate substitution of fasting requirements by twice-daily long-acting human insulin independent of prandial and correctional use of regular insulin according to individual algorithms. All patients undergo an extensive, individually tailored patient education program including group and individual sessions.

Table 1
Clinical characteristics of insulin-requiring diabetic patients with various degrees of autonomic neuropathy

Patient groups	All	No CAN	Early CAN	Severe CAN
n (F/M)	22 (13/9)	8 (5/3)	8 (5/3)	6 (3/3)
Age (years)	49.5 ± 8.7	44.1 ± 8.5	51.8 ± 7.2	50.3 ± 11.5
BMI (kg/m^2)	25.1 ± 3.4	24.4 ± 2.8	25.3 ± 3.1	25.8 ± 4.8
Diabetes duration (years)	18.6 ± 10.6	18.9 ± 12.7	15.5 ± 8.5	21.7 ± 8.1
HbA_{1c} (%)	6.9 ± 0.9	6.7 ± 0.7	7.1 ± 1.0	6.9 ± 1.1

2.3. Training procedures

Patients underwent 12 weeks bicycle ergometer training controlled by individual training heart rate twice per week 30 min per training unit with 60–70% of individual maximal capacity. The target training heart rate was estimated during initial spiroergometry and was defined as heart rate at 65% of maximal exercise capacity [training heart rate = resting heart rate + (maximal heart rate − resting heart rate) × 0.65] [21]. This endurance training modality was chosen because it is known from clinical experience [22,23] to improve cardiovascular performance.

2.4. Outcome variables and their assessment

Main outcome variables were those of short-term spectral analysis of HRV.

2.4.1. CAN assessment

CAN was assessed initially by a standard battery of cardiovascular reflex tests to standardized stimuli [1,24] including deep breathing, Valsalva maneuver, orthostatic load and sustained handgrip. Diabetic patients with a Total Ewing Score of 0–0.5 were considered as those without CAN, patients with score of 1–2.5 as with early CAN and those with a score of 3–5 as patients with definite/severe CAN [1,2]. For follow-up monitoring a short-term spectral analysis consisting of 3 time segments (adapted from Bellavere [25], in positions supine–standing–supine, 256 artifact-free heart beats each) was performed. This short-term option delivers well-reproducible results when recordings are performed under similar laboratory conditions as applied in our study [25]. Both CAN examinations were performed with the VariaPulse TF3 system (Sima Media Olomouc, Ltd., Czech Republic) [26]. A surface ECG was continuously monitored with a time resolution of 1 ms. R–R intervals were telemetrically transferred to a receiver connected to a PC-compatible computer and displayed on-line together with an instantaneous spectral curve on a monitor. The computational method was based on fast Fourier transform modified by algorithm of coarse-grain-

ing spectral analysis [27]. This allowed one to extract a broad-band non-harmonic 'noise' contaminating particularly the lower frequencies (1/f component). Each dataset was filtered automatically by excluding recorded artifacts using a recognition algorithm, as well as manually. The final results were immediately displayed on the monitor as 3-dimensional running spectra (Fig. 1), permitting a general overview of the dynamics and of the absolute energy content of the system. Parameters of the frequency domain were observed in every position within the high-frequency

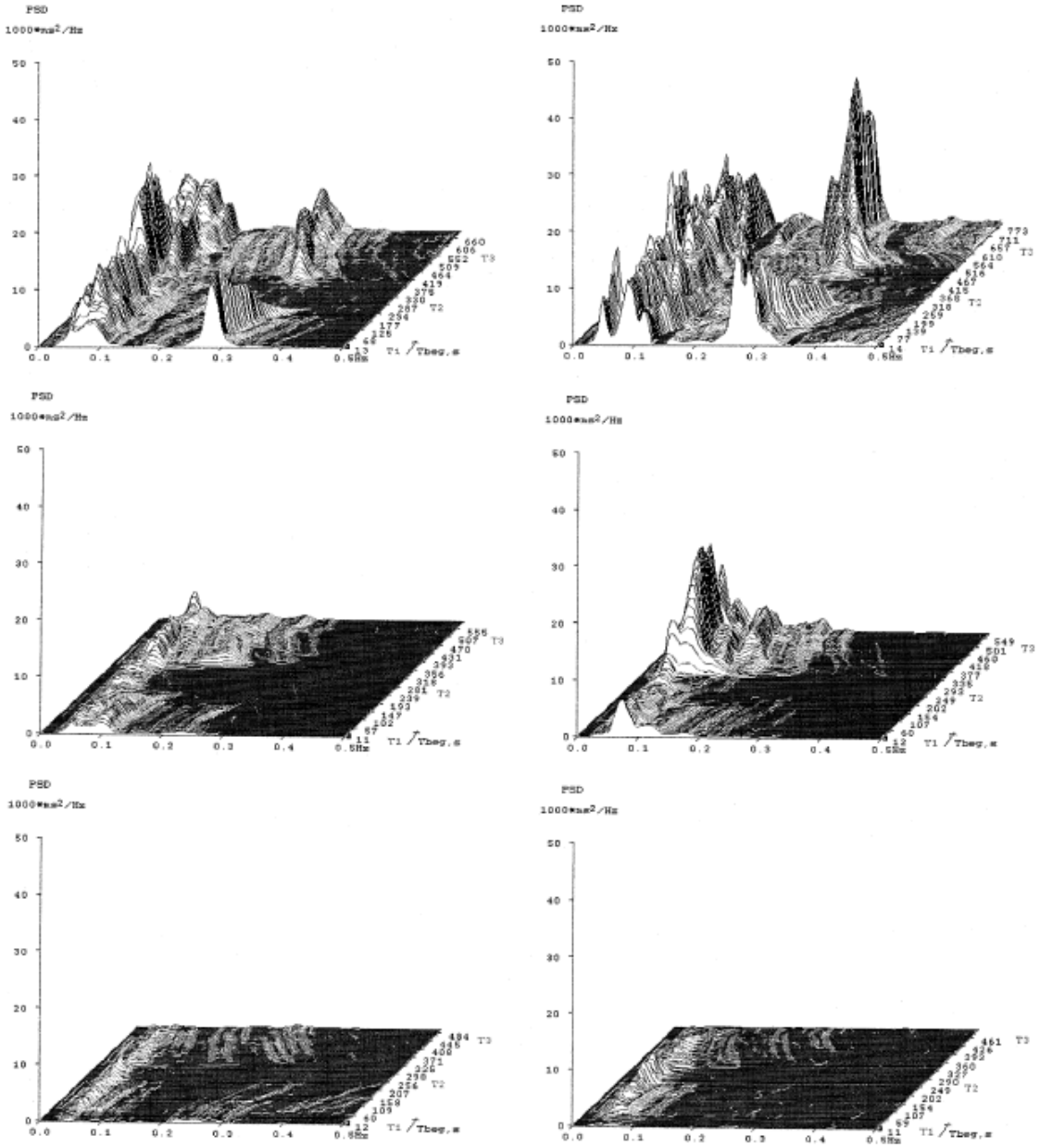

Fig. 1. Examples of typical impact of regularly performed physical training on heart rate variability as assessed by short-term spectral analysis before and after (right panel) training period in patients with various degrees of CAN. Diabetic patient with no CAN (41-year-old male, Ewing score 0, BMI 27.4 kg/m^2, diabetes duration 8 years, top panel), with early involvement (50-year-old male, Ewing score 2, BMI 23.5 kg/m^2, diabetes duration 29 years, middle panel), and one with severe involvement (43-year-old male, Ewing score 4, BMI 21.4 kg/m^2, diabetes duration 25 years, bottom panel).

band (0.15–0.50 Hz), which has been attributed exclusively to parasympathetic tone [28], and within the low-frequency band (0.05–0.15 Hz), said to represent a combination of sympathetic and parasympathetic effects on cardiac autonomic tone [29,30]. Main outcome variables were spectral power (units [ms^2]) in both frequency bands. To increase the reliability of short-term measurements and to assess even small intraindividual improvements in global autonomic tone during the trial, we used cumulative indices (spectral power of the total frequency band with its high- and low-frequency components over all 3 positions) representing the total averaged area under all consecutive spectral curves within the short-term recording. Standard deviations were calculated for each parameter as this information was necessary for assessment of the stationarity of the examination. We excluded any findings having a more than 30% relative deviation in any of the positions recorded. In those cases the examinations were repeated. Time-domain analysis was also performed to calculate the mean square of the difference of successive R–R intervals (MSSD) [ms^2].

2.4.2. Spiroergometry

Symptom-limited incremental bicycle spiroergometry (beginning with 25 W, increments of 25 W every 2 min) was performed (Computer Ergometer Ergoline, Ergometrics 900, Bits, Germany, and SensorMedics Metabolic Measurement Cast 2900, CA, USA) during all 4 main points of the trial before and after the training period to define maximal work capacity and maximal oxygen consumption [31], anaerobic threshold [32], and training heart rate [21].

2.4.3. Ambulatory blood pressure monitoring (ABPM)

ABPM was performed using a SpaceLabs 90207 monitor (SpaceLabs, Redmond, USA, 1993) according to the recommendations of the British Hypertension Society [33]. Additional outcome variables such as mean systolic and diastolic blood pressure, mean heart rate, and night-time dipping were assessed as previously described [34].

2.4.4. Echocardiography

Transthoracic two-dimensional echocardiography was performed to assess the presence and/or changes in left ventricular hypertrophy, end-systolic and end-diastolic left ventricular diameter and wall thickness of the interventricular septum. Continuous waved Doppler sonography was used to assess disturbances of relaxation [35] and left ventricular fractional shortening. All measurements were performed by the same physician (J.K.-S.) experienced in echocardiography, using the VingMed CFM 750 system (Diasonics-Sonotron, Zug, Switzerland).

2.4.5. Blood analysis

HbA_{1c} was assessed by the HPLC method (Variant, Bio-Rad, Richmond, CA, USA; reference range 4.4–6.6%) and blood lipids by standard commercial kits from Boehringer Mannheim (Mannheim, Germany).

2.4.6. Depression scale

Symptoms of depression were assessed during all main investigation points by respective scores of the Beck Depression Inventory [36], a well-established self-reporting measure frequently utilized in empirical evaluation of psychiatric depression.

2.5. Statistical analysis

Statistical analysis was performed using standard statistical packages (SPSS, Statistical Package for the Social Sciences V7.0, SPSS Inc., Chicago, USA). Analysis of variance with repeated measures (General Linear Model) was used to evaluate differences between groups in the course of parameters during and after endurance training. The two-tailed paired Student's *t*-test was applied to estimate differences vs. baseline values. Because of the skewness of the frequency-domain data distribution, log (ln) transformation was performed to produce a normal distribution before the final results were assessed.

Demographic data are presented as means ± s.d., outcome variables as means ± s.e.m., unless otherwise indicated.

3. Results

While the training-free run-in interval induced no changes in spectral indices in all patient groups, the 12-week training period increased the cumulative spectral power of the total frequency band ($P = 0.04$) but to a different extent (two-way interaction, differences in course between groups, $P = 0.039$) in various degrees of neuropathy (Figs. 1 and 2). Whereas patients with severe CAN showed after the training period no measurable changes in cumulative total spectral power (0.06–0.50 Hz) (ln $[ms^2]_{before}$ 4.7 ± 0.3; ln $[ms^2]_{after}$ 4.6 ± 0.5; $P = 0.8$), those with the early form of CAN and those without any detectable neuropathy showed an increase from ln $[ms^2]_{before}$ 6.1 ± 0.1 to ln $[ms^2]_{after}$ 6.6 ± 0.1; $P = 0.04$ and from ln $[ms^2]_{before}$ 7.5 ± 0.1 to ln $[ms^2]_{after}$ 8.0 ± 0.3; $P = 0.05$, respectively (Fig. 2, top panel).

Spectral power in the low-frequency band (0.06–0.15 Hz) indicated significant effects of training ($P = 0.002$), but also to a different degree between groups (two-way interaction, differences in course between groups, $P = 0.01$), showing a highly significant rise in the group with early CAN (from ln $[ms^2]_{before}$ 5.6 ± 0.1 to ln $[ms^2]_{after}$ 6.3 ± 0.1; $P = 0.008$), borderline amplification in the group without CAN (from ln $[ms^2]_{before}$ 7.1 ± 0.1 to ln $[ms^2]_{after}$ 7.6 ± 0.3; $P = 0.08$), but no changes in the group with severe CAN (ln $[ms^2]_{before}$ 4.1 ± 0.3; ln $[ms^2]_{after}$ 4.1 ± 0.4; $P = 0.6$) (Fig. 2, middle panel).

In the high-frequency band (0.15–0.5 Hz) no more statistical differences between the groups in the course of the parameters were revealed (two-way interaction, $P = 0.121$). If compared to baseline values, however, increases were still evident in both groups without severe CAN (early CAN from ln $[ms^2]_{before}$ 5.1 ± 0.2 to ln $[ms^2]_{after}$ 5.8 ± 0.1; $P = 0.05$; no CAN group from ln $[ms^2]_{before}$ 6.2 ± 0.3 to ln $[ms^2]_{after}$ 6.6 ± 0.4, $P = 0.016$), whereas the group with severe CAN showed no statistical changes (ln $[ms^2]_{before}$ 3.7 ± 0.5; ln $[ms^2]_{after} = 3.4 \pm 0.7$, $P = 0.6$) (Fig. 2, bottom panel).

Time-domain analysis of HRV showed the effects of training on the MSSD ($P = 0.03$), but the differences in course between the groups were not significant (two-way interaction, $P = 0.15$). When values after training were compared to baseline values, significant amplification was present only in the group with no CAN (from $[ms^2]_{before}$ 1848 ± 375 to $[ms^2]_{after}$ 3339 ± 797, $P = 0.03$; early CAN from $[ms^2]_{before}$ 636 ± 115 to $[ms^2]_{after}$ 1062 ± 372, $P = 0.3$; severe CAN from $[ms^2]_{before}$ 201 ± 51 to $[ms^2]_{after}$ 227 ± 132, $P = 0.9$).

After training withdrawal of 4–6 weeks, all training-induced changes in HRV could no longer be detected.

The impact of training on spectral and time-domain indices of HRV, if considered in any particular position (supine–standing–supine), as well as on their ratios, was not sufficient to reveal any statistical changes.

As assessed by incremental spiroergometry, maximal performance capacity increased over the training period in the whole patient cohort ($P < 0.001$), although the impact of endurance training was different between the groups (two-way interaction, differences in course between the groups, $P = 0.02$) and was higher in patients with no and early involvement (Fig. 3, top panel). The maximal oxygen consumption increased in the whole cohort ($P = 0.005$) and no differences in its course between the groups could

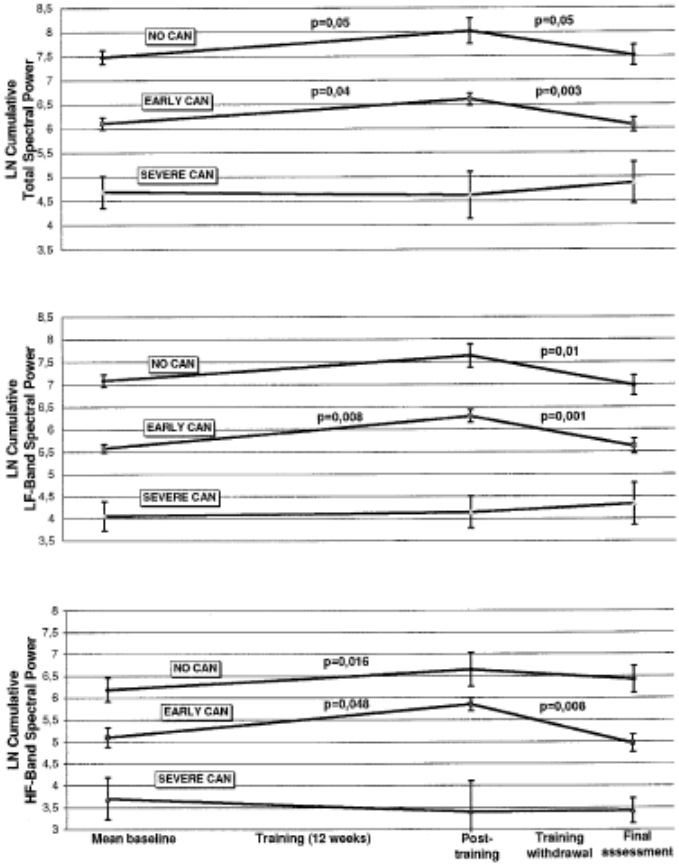

Fig. 2. Effect of regularly performed physical training and training withdrawal period on spectral indices of HRV in groups of diabetic patients with various degrees of CAN: cumulative spectral power of total frequency band (top panel), cumulative spectral power in low-frequency band (middle panel) and in high-frequency band (bottom panel).

be detected (two-way interaction, $P = 0.11$) (Fig. 3, lowest panel). Similarly, a significant increase in anaerobic threshold was observed in all patient groups ($P < 0.001$ Fig. 3, middle panel), and no differences in its course between the groups were assessed ($P = 0.79$). At different load-levels (e.g., 50 and 100 W) slight, but non-significant decreases in heart rate were recorded after endurance training in all groups, indicating a tendency to improvement of cardiac output/beat. With the exception of the group with no CAN, which still showed an increased exercise capacity even after training withdrawal (Fig. 3, top panel, $P = 0.002$ vs. baseline values), after the period without training all initially induced beneficial effects had disappeared.

Ambulatory blood pressure monitoring demonstrated a numerical decrease of a mean of 24 h heart rate in the whole patient cohort ($P = 0.081$) and no differences between the degree of impact were detectable between the groups ($P = 0.58$). Moreover, the heart rate remained slightly lower even after 6 weeks of training withdrawal. Endurance training induced an increase of night-time dipping only in the group with no CAN for systolic (16 ± 3 vs. 21 ± 2 mmHg, $P = 0.03$) as well as for diastolic blood pressure (14 ± 2 vs. 17 ± 2 mmHg, $P = 0.04$).

Echocardiographic evaluation revealed during baseline investigations no signs of left ventricular hypertrophy in patients with no and early involvement. In patients with severe CAN septum thickness (10.9 ± 1.8 mm) indicated left ventricular hypertrophy. Training induced no significant reductions of interventricular wall thickness (no CAN from 9.5 ± 0.6 to 9.0 ± 0.5 mm, $P = 0.48$; early CAN from 9.2 ± 0.8 to 9.2 ± 0.6 mm, $P = 0.85$) and of left ventricular end-diastolic diameter (no CAN from 52.5 ± 1.9 to 51.9 ± 2.5 mm, $P = 0.57$; early CAN from 49.2 ± 1.6 to 47.4 ± 1.1 mm, $P = 0.14$). In contrast, in the group with severe involvement, training had an inverse but non-significant effect on both interventricular wall thickness (10.9 ± 1.8 to 11.4 ± 2.2 mm, $P = 0.52$) and left ventricular end-diastolic diameter (from 50.9 ± 1.6 to 53.3 ± 1.8 mm, $P = 0.43$). Disturbance of relaxation was present only

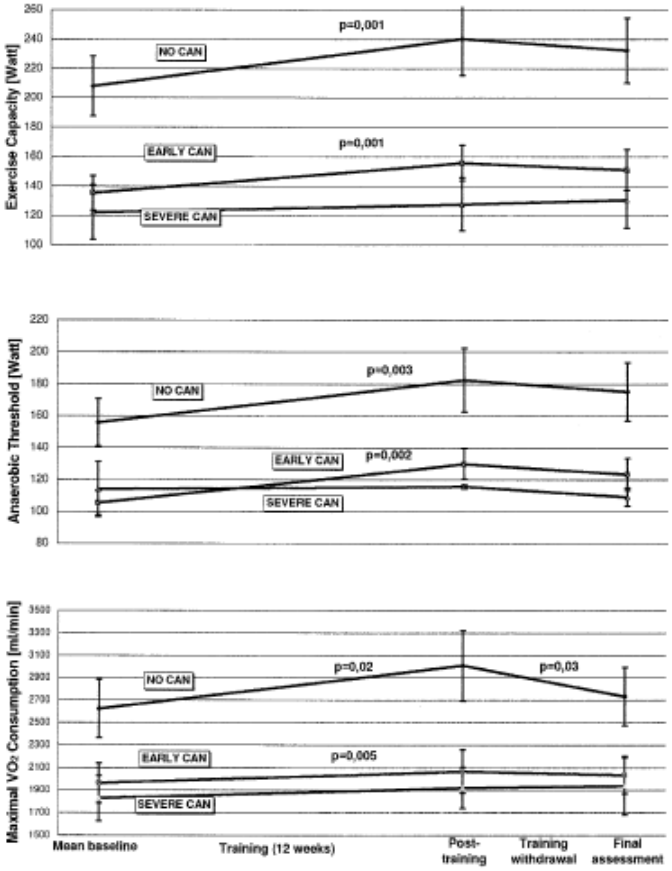

Fig. 3. Effect of regularly performed physical training and training withdrawal period on maximal performance capacity (top panel), anaerobic threshold (middle panel) and maximal V_{O_2} consumption (bottom panel) in groups of diabetic patients with various degrees of CAN.

in patients with early and severe neuropathy. Training induced minor improvements in both groups in this respect.

The training period had no relevant effects on blood lipids or HbA_{1c}.

Depression symptoms as assessed by Beck's Depression Inventory were not affected by the training ($P = 0.66$), but it should be stressed that the depression level revealed was quite low (level of severity 0–26), with the exception of 2 individuals in the group with severe involvement (level of severity 26–40).

4. Discussion

This study documents for the first time the beneficial effects of low-grade endurance training on heart rate variability in the early stages of diabetic cardiovascular autonomic neuropathy. As our trial was designed to investigate the training-induced reversibility of diabetic cardiovascular autonomic neuropathy and data are gathered on the HRV-related impact of endurance training in healthy subjects [7–9], we dispensed with a non-diabetic control group.

The effect of low-grade endurance training was more pronounced in the group with early CAN than in subjects with no CAN. Our study demonstrates the already known phenomenon that pathologic values (including blood lipids, blood pressure values, incipient CAN, low initial fitness levels) are more strongly influenced towards normal by endurance training than normal values. This finding is consistent with our previous observations [22] and reports of others [37]. Additionally, it is possible that the chosen training modality represented a stronger stimulus for patients with early involvement, as they displayed a much lower exercise capacity.

The design included a period of run-in with no training to ensure that the selected methods for assessment of CAN deliver reproducible staging of neuropathy. In order to reduce the standard deviation of particular indices of HRV, mean values of both pre-training investigations were used for baseline. The relevant reconditioning of autonomic balance of the heart supply was expected first after a training period of 12 weeks [22,23]; the described training intensity and length were well accepted and tolerated by the majority of patients, although in 5 initially selected cases (3 with severe and 2 with early involvement) the training could not be completed. These early drop-outs were caused by acute severe pemphigus (hospitalization), severe neuropathic diarrhea, benign lung tumor (surgery), and non-compliance with the necessary supervised heart-rate-controlled training.

This study provided evidence that the used method of short-term spectral analysis of HRV is practicable and easily applicable in ambulatory routine. As it was found that the immediate variability of short-term spectral measures of HRV was low [38] and short, 2- to 15-min samples were excellent predictors of mortality and correlated with prognostically important data from sustained recording periods [39,40], we have chosen to exclusively perform short-term spectral analysis for repetitive assessment of HRV. Due to this simplification, patients could accept 4 consecutive measurement series during the study; the method takes only a short time and is quite independent of the patient's compliance during the examination.

In contrast to the previous, much less sensitive standard score of the cardiovascular reflex battery, the selected cumulative indices over all 3 time periods (supine–standing–supine) proved able to assess even small changes in cardiac autonomic supply. The cumulative indices proposed here should be understood as a method for increasing the reliability of the measurements during short-term recordings in all 3 positions. Although the information given by the cumulative spectral indices corresponds to that given by indices of time-domain analysis (MSSD), it became evident that this index is more representative of the actual global state of autonomic regulation and reflects the total instantaneous sympathetic and parasympathetic effects. A similar investigation using an analogue training program in sedentary middle-aged men [41] showed apparently no effects on HRV, which—to our present knowledge—can be viewed partly as a methodological problem since the cumulative indices designed by us were not considered in this previous investigation.

The current methods most commonly used for analysis of HRV in the frequency domain are based either on the fast Fourier transform or on the autoregressive model. Under several conditions considered also in our study design both analytical approaches deliver similar results [28,42]. The procedure of coarse-graining spectral analysis [27] was additionally chosen to increase the reliability of the measurement process. This method has the advantage that the non-harmonic 'noise' contaminating the spectrum is removed and the high processing speed allows on-line graphic display.

Our study documented a training-induced increase of complex sympathetic and parasympathetic supply as reflected by amplification of the spectral power of the low- and high-frequency bands. A similar finding was reported by Furlan [9], although in healthy subjects the changes induced by heavy training were mainly restricted to the low-frequency band.

The endurance training modality applied was not able to improve cardiac supply in patients with severe CAN. In 3 severely affected subjects we recorded even an on-going loss of total spectral power. Training improved maximal performance capacity and maximal oxygen consumption, and increased the anaerobic threshold in the whole diabetic cohort, but, similar to the effects on HRV, the effects of endurance training on fitness were dependent on the degree of CAN.

It is evident that neither the emotional state as assessed by Beck's Depression Inventory nor other factors such as

current treatment of diabetes or hypertension were responsible for the changes assessed as the impact of training disappeared after training withdrawal.

If the demonstrated reversibility of CAN as induced by systematic endurance training in our study corresponds to the decrease of risk associated with cardiac denervation, endurance training and rehabilitative exercise programs in diabetic patients with early involvement should be recommended. Further studies are necessary to investigate the potential reversibility of nephropathy and other cardiac denervation-associated phenomena, such as increased microalbuminuria [43] and decreased glomerular filtration rate [44]. It should be kept in mind, however, that—as documented also by our investigation—apparently only a systematic, regularly performed physical training is necessary for lasting beneficial effects. Our study provides the first necessary rationale for a long-term study of the effects of endurance training upon HRV indices in diabetic patients with cardiovascular autonomic neuropathy. Although that should still be proven in longer studies, it is already known that the time period needed to inverse the training-induced effects on fitness (including its morphological correlates) is related to the duration and intensity of the training [37].

Recent data suggest that maximal oxygen consumption itself is a good predictor of mortality risk [45]. Further mortality analyses in diabetes should consider the physical state and exercise behavior as important variables which might influence the mortality risk.

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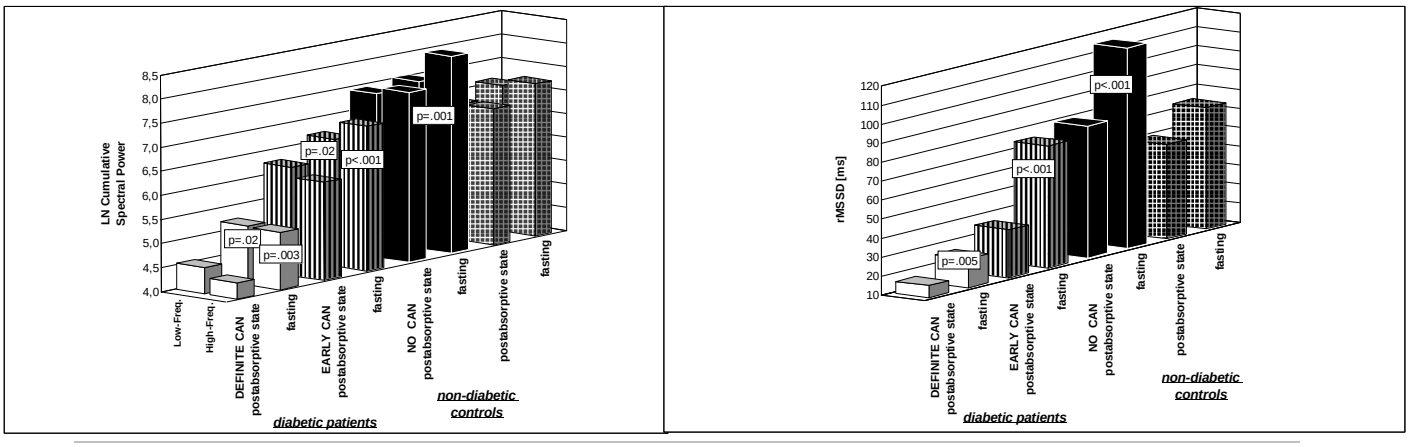
4.2. Praktická aplikace: výzkum reverzibility CAN a kvantifikace efektu nefarmakologických opatření - lačnění

Úvod: Z literatury je známo, že krátkodobé lačnění vyvolává snížení srdeční frekvence pravděpodobně zvýšením vlivu parasympatiku *(Clabough 1989)*, případně centrálně inhibičním efektem na sympatické regulace oběhového systému *(Young 1977, Einhorn 1982)*. Obdobně, racionalitu dlouhodobě kaloricky kontrolovaného/redukovaného příjmu stravy podporuje rovněž řada publikací (např. *Stein 2012)*.

C**ílem studie** bylo ověřit účinnost další nefarmakologické procedury – lačnění -- na autonomní regulace, sledované krátkodobou analýzou HRV. Sledovali jsme vliv v průměru 13-hodinového (1072±22 minut) lačnění na parametry časové a frekvenční analýzy HRV a srovnali jej s postprandiálními (146±11 minut po jídle) výsledky u 56 pacientů s diabetem a různými stupni autonomní dysfunkce, a 15 kontrol bez diabetu.

Výsledkem studie byl statisticky významný nárůst kumulativního spektrálního výkonu celého spektra, jakož i části nízko- a vysokofrekvenční ($p<0.001$), s odpovídajícím nárůstem parametru časové domény (MSSD), viz **Obr.19**. V kontrastu k této odpovědi, kontrolní probandi bez diabetu vykázali při tomto typu intervence pouze statisticky nevýznamnou změnu ($p=0.19$). Při celkovém posouzení výsledků studie **uzavíráme**, že lačnění zvyšilo variabilitu srdeční frekvence zvláště vlivem této vagální nefarmakologické stimulace. S ohledem na sledované změny i známé cirkadiánní variace v kardiovaskulárních autonomních regulacích je vhodné při měření HRV standardizovat odstup od posledního jídla.

Obr. 19: Analýza HRV ve frekvenční doméně (obrázek vlevo, LF a HF frekvenční spektrum) a časové doméně (obrázek vpravo, rMSSD) při lačnění *u pacientů s diabetem a odlišnými stupni autonomní dysfunkce (NO, EARLY a DEFINITE), jakož i u kontrolních probandů bez diabetu.*



4.2.1. Howorka K, Pumprla J, Schabmann A: Influence of fasting on heart rate variability in diabetic patients with different degrees of cardiovascular autonomic neuropathy. Diabetes, Nutrition & Metabolism 10(6), 1997, pp. 288-295

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Influence of fasting on heart rate variability in diabetic patients with different degrees of cardiovascular autonomic neuropathy

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Influence of fasting on heart rate variability in diabetic patients with different degrees of cardiovascular autonomic neuropathy

K. Howorka*,**, J. Pumprla** and A. Schabmann***

ABSTRACT. **Effects of fasting on the cardiovascular system in diabetes are not sufficiently described. We assessed possible influence of fasting periods exceeding 13 hrs on heart rate variability (HRV) as compared to a short postprandial period in diabetic patients and controls. Short-term spectral analysis of HRV was used either in a common postprandial/postabsorptive state (interval since the last meal 146±11 min) or during the fasting day (interval 1072±22 min) in 56 diabetic patients with different degrees of cardiovascular autonomic neuropathy and in 15 non-diabetic control persons comparable in age, body mass index and gender. Fasting resulted in diabetic patients with all degrees of cardiovascular autonomic neuropathy (including its definite form) in an increase of cumulative spectral power of total frequency band from 7.4±0.2 to 7.9±0.2 ln [ms^2], $p<0.001$, of low-frequency band from 6.6±0.2 to 6.9±0.2 ln [ms^2], $p=0.004$, and of high-frequency band from 6.5±0.2 to 7.3±0.2 ln [ms^2], $p<0.001$, and in an increase of parameters of time-domain analysis ($p<0.001$) as well. In contrast, non-diabetic control group displayed only a non-significant increase of HRV ($p=0.19$). We conclude that fasting increases HRV in diabetic patients, mainly due to an increase of vagal tone. In any interpretation of short-term spectral analysis of HRV the interval since the last meal should be considered. We propose a standardization of this interval.**

Diab. Nutr. Metab. 10: 288-295, 1997.

INTRODUCTION

Spectral analysis of heart rate variability (HRV) permits the evaluation of cardiovascular autonomic neuropathy (CAN) in diabetes (1) and risk stratification in congestive heart failure and/or coronary heart disease (2). Clinical evidence was found for the association between incidence of lethal arrhythmias and signs of an increased sympathetic and reduced vagal activity. A reduced HRV is an indicator of increased mortality (3-6). Therefore, since assessment of HRV became more popular, it is evident that it requires better standardization of measurement to allow appropriate clinical applications and interpretation of its pathophysiological correlates (7).

The immediate variability of short-term spectral measures of HRV was found to be low (8). Moreover, short, 2- to 15-minute samples were reported to be excellent predictors of mortality and were correlated with prognostically important data from sustained recording periods (9). In this way, short-term spectral analysis of consecutive, well-defined examination positions (supine-standing-supine) of a few minutes each (10) was assumed to deliver representative diagnostic information. However, neither the reproducibility of short-term recordings nor factors which could affect the results of measurements of HRV are sufficiently delineated. American Diabetes Association recommends in its Consensus Statement on Diabetic Neuropathy (11) that "...in an ideal situation, studies should be performed with the patient having [...] no [...] food for 8 hrs. Moreover, the studies should be performed in the morning...". It is evident that in

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Key words: Human diabetes mellitus, cardiovascular autonomic neuropathy, heart rate variability, fasting.

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Table 1 - *Clinical characteristics of diabetic patients with various degrees of CAN and of non-diabetic controls (means±SEM).*

	all	Diabetic patients			Non-diabetic controls
		no CAN	early CAN	definite CAN	
N (m/f)	56 (23/33)	28 (12/16)	18 (6/12)	10 (5/5)	15 (6/9)
Age (yr)	36±2	33±2	39±5	40±3	39±4
Diabetes duration (yr)	12±1.3	10±4	12±3	19±2	
BMI (kg/m²)	23.5±0.4	23.6±1.5	24.0±0.7	22.3±0.9	23.3±0.6
Blood glucose (mmol/l)					
fasting	6.6±0.3	6.2±0.3	6.4±0.5	7.9±1.3	
non-fasting	6.9±0.3	6.5±0.4	7.5±0.5	7.2±1.0	

insulin requiring patients even if the investigations were performed before noon or in the morning, food abstinence exceeding eight hours (*eg* no food intake till noon) will simply be not practicable in the majority of conventionally treated cases who are preprogrammed for food intake every several hours and who are trained to inject their insulin for breakfast immediately after awaking.

Fasting induces a decrease in resting heart rate apparently due to an increase in parasympathetic tone (12) and centrally mediated inhibition of sympathetic influences (13-15). Effects of fasting on HRV in humans have not been reported till now either in non-diabetic or in diabetic subjects.

Our aim was to evaluate the effects of fasting periods exceeding 13 hours as compared to a usual short postprandial/postabsorptive period (1-3 hours) on HRV assessed by its short-term spectral analysis in groups of diabetic patients and non-diabetic controls.

MATERIALS AND METHODS

The investigation conforms with the principles outlined in the Declaration of Helsinki (16). All participating subjects gave their informed consent. Patients were informed that they could interrupt fasting day by food intake at any time, but the experience of fasting together with other patients was generally well accepted. The team involved in patient care and rehabilitation, and in conducting the study was approved for conformity with European quality management and assurance standards ISO 9001 (17).

Study design

Intraindividual comparison (random order) of baseline values recorded in a common postprandial state (60-180 min) to values after a period of uninterrupted fasting of at least 800 min. Fasting was performed on occasion of outpatient group education programme for functional insulin treatment discriminating between prandial, basal and correctional use of insulin (18). It requires intensive blood glucose self-monitoring and multiple daily injections but allows full flexibility in food intake. One fasting day is a practical part of this educational programme; this exercise is a necessary teaching tool for learning blood glucose control without usual food intake and for estimating basal insulin need (18). Drinking water, tea and consumption of small amounts of oral glucose (12 to 18g) for hypoglycaemia prevention with blood glucose levels below 5.6 mmol/l were allowed. As the sympathetic/parasympathetic balance depends on a circadian rhythm, the intraindividual control measurements of HRV have been carried out on comparable time of the day (±60 min). Time interval between both investigations did not exceed 72 hours. The time point of the day and the amount of food for the last meal before the fasting day were kept as usual. Concomitant medication of associated and/or other chronic diseases as well as basal insulin replacement remained constant during both study days.

Patients and non-diabetic controls

Clinical data of 56 diabetic patients (nine Type II insulin requiring diabetic patients were equally distributed in all three neuropathy groups) and of 15 control subjects, comparable in age, body mass index (BMI) and gender are summarised in Table 1. Patients selected for the study had different degrees of CAN. Those displaying any electrocardiographic or clinical signs of coronary artery disease or taking β-blockers were excluded from the investiga-

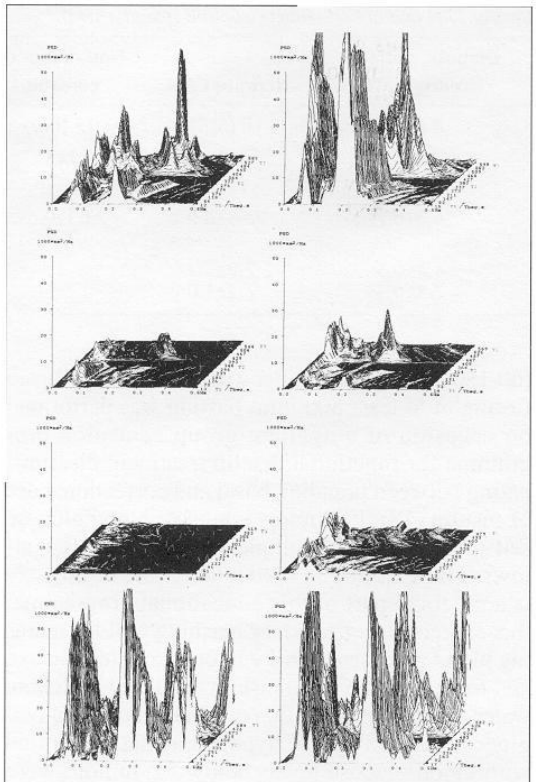

Fig. 1 - *Examples of typical impact of fasting (right panel) as compared to a short postprandial/postabsorptive period (left panel) on heart rate variability assessed by its short-term spectral analysis in patients with various degrees of CAN. Diabetic subject with no CAN (upper panel), with early involvement, one with definite involvement, and non-diabetic subject (lowest panel). Vertical axis: power spectral density (ms^2/Hz), horizontal axis: centre frequency (Hz), z-axis: time (s); positions: T1 supine, T2 standing, T3 supine.*

tion. The category of CAN was assessed initially by standard battery of cardiovascular reflex tests to standardised stimuli (19) including deep breathing, the Valsalva manoeuvre, orthostatic load and sustained handgrip and then by cumulative spectral power of total frequency band as well. Each single test of Ewing's battery was scored with 0 when normal, 0.5 when borderline and with 1 when out of age-related normal values range (1), summing up a total Ewing score. Diabetic patients with total score of 0-0.5 were considered as those without CAN, patients with score of 1-2.5 as with early CAN and those with score 3-5 as patients with definite/severe CAN (19). Patients with acute concomitant diseases and those with signs of hypoglycaemia and/or blood glucose levels below 3.3 mmol/l were excluded. In those cases the investigation of HRV was postponed.

Fasting day

To avoid hypoglycaemia, frequent blood glucose monitoring (12-18 measurements, at least every 2 hours, including at least three nocturnal measurements of blood glucose) was performed during the fasting time. All values below 5.6 mmol/l were corrected through immediate intake of oral glucose. Symptomatic or biochemical hypoglycaemia (blood glucose values below 3.3 mmol/l) was an exclusion criterion and in this case the study was postponed. Basal insulin substitution was kept unchanged and usually included twice daily long-acting insulin and a small amount of regular insulin (3±2 U) in the morning according to individual algorithms (18). Physical activity patterns were kept as usual and the educational programme was performed as on other days. The intake of at least 2.5 l of liquids (excluding caffeine-containing ones) was recommended and encouraged. The energy input on fasting day resulted almost exclusively from glucose intake for hypoglycaemia prevention and included 100-200 kcal from carbohydrates as a maximum.

Food intake on non-fasting day

Usual pattern of nutrition was kept on non-fasting days. Recommendations for meal composition were as previously reported (18): more than 50% of caloric intake from complex or fibre-rich carbohydrates, less than 20% from protein and less than 30% from fat. In lean patients, approximately 30 kcal/kg body weight/day were recommended, but individual adjustments according to the pattern of physical activity and to other factors were accepted as long as no tendency to weight gain has been recorded. On average, four to five meals a day were chosen by patients as a routine pattern for food intake. Thus, these recommendations were not different from those for the non-diabetic population.

Outcome variables and their assessment

Short-term spectral analysis of HRV included a recording consisting of three time segments (in positions supine-standing-supine), 256 artefact-free heart beats each, using VariaPulse TF3 system [Sima

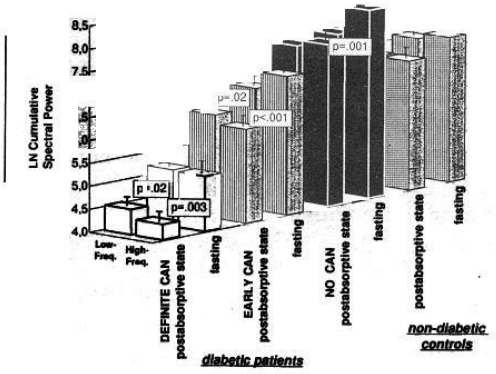

Fig. 2 - *Frequency-domain analysis of HRV: LN cumulative spectral power (x±SEM) in low-frequency (left bars) and high-frequency bands (right bars) in diabetic patients with definite, early and no CAN as well as in non-diabetic controls, in usual postprandial/postabsorptive state (front bars) and after prolonged fasting (rear bars). p describes significant differences vs postprandial/postabsorptive state in each subject category (intraindividual comparison).*

Media Olomouc, Ltd, Czech Republic, (20, 21)]. A surface ECG was continuously monitored with a time resolution of 1 ms. R-R intervals were telemetrically transferred into a receiver connected to a PC-compatible computer and displayed on-line together with an instantaneous spectral curve on a monitor. Computational method was based on fast Fourier transform modified by algorithm of Coarse-graining spectral analysis (22). That allowed to extract a broad-band non-harmonic "noise" contaminating particularly the lower frequencies (1/f component). Each dataset was filtered automatically by excluding recorded artefacts using a recognition algorithm, and manually, as well. The final results were immediately displayed on the monitor as three-dimensional running spectra (Fig.1). Parameters of *frequency-domain* were observed in every position within the low-frequency band (0.05-0.15 Hz) and high-frequency band (0.15-0.50 Hz). Main outcome variables were spectral power (units [ms^2]) in both frequency bands. To assess even small intraindividual changes in global autonomic tone during the trial, we used cumulative indices (spectral power of total frequency band with its low and high frequency components over all three positions, (21)]. Standard deviations were calculated for every parameter as this information was necessary for assessment of a stationarity of the examination. We excluded the findings bearing more than 30% relative deviation in any of the positions recorded. In those cases the examinations were repeated. *Time-domain* analysis also included the averaged R-R interval (ms) and rMSSD (root of mean square of difference of successive R-R intervals [ms^2]). Because of the skewness of the frequency-domain data distribution, log (LN) transformation was performed to produce a normal distribution before the final results were considered.

Statistical analysis

Statistical analysis was performed using standard statistical packages (SPSS, Statistical Package for the Social Sciences V7.0, SPSS Inc., Chicago, USA). Analysis of variance with repeated measures (General Lineal Model) was used to evaluate differences between groups (two-way interaction group X state) for the impact of fasting. Two-tailed paired Student's *t* test was applied to estimate intraindividual differences between investigations. Data are presented as mean±SEM, unless otherwise indicated.

RESULTS

The intraindividual control measurements in postprandial/postabsorptive state and on fasting day have been carried out under comparable blood glucose levels (Table 1, all $p>0.1$). HbA_{1c} level in diabetic patients (during the examination before the training in functional insulin treatment) was 158±2% of upper reference limit for glycosylated haemoglobin (two methods). In diabetic cohort the time interval since the last meal on baseline day (postprandial/postabsorptive conditions) was 146±11 min, on fasting days 1072±22 min, and in non-diabetic probands 142±25 min and 962±61 min, respectively.

Frequency-domain analysis revealed that fasting resulted in an increase of cumulative spectral power of total frequency band of HRV (Figs. 1 and 2), for all diabetic patients from 7.4±0.2 to 7.9±0.2 ln [ms^2], $p<0.001$, with no differences for impact of fasting between groups ($p=0.08$). Cumulative spectral power of total frequency band increased during fasting in patients with no CAN from 8.4±0.2 to 8.8±0.2 ln

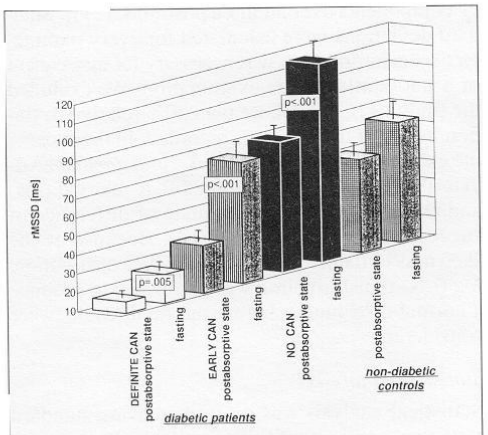

Fig. 3 - *Time-domain analysis of HRV: rMSSD (root of mean square of differences of successive R-R intervals) in diabetic patients with definite, early and no CAN as well as in non-diabetic controls, in usual postprandial/postabsorptive state (front bars) and after prolonged fasting (rear bars). p describes significant differences vs postprandial/postabsorptive state in each subject category (intraindividual comparison).*

increased to a somewhat lesser degree but significantly for the whole cohort of diabetic patients (from 6.6±0.2 to 6.9±0.2 ln [ms^2], p=0.004) and the differences in impact of fasting in particular neuropathy groups did not reach the level of significance (p=0.07), although the respective fasting-induced increases were more pronounced and significant in both groups with CAN. Increases in cumulative spectral power in low-frequency band were in patients with no CAN from 7.5±0.2 to 7.5±0.2 ln [ms^2], p=0.5, in those with *early* CAN from 6.3±0.2 to 6.7±0.2 ln [ms^2], p=0.02, and in those with *definite* CAN from 4.5±0.3 to 5.2±0.4 ln [ms^2], p=0.02. Non-diabetic group showed no significant increase of cumulative spectral power in low-frequency band from 6.9±0.3 to 7.1±0.2 ln [ms^2], p=0.32.

The increase of HRV in diabetic cohort was evidenced also using traditional parameters of analysis in time-domain. Significant changes were found for rMSSD (root of mean square of differences of successive R-R intervals; Fig.3) in diabetic patients (whole cohort increased from 54.1±5.3 to 86.2±8.2 ms; p<0.001) with no significant differences for impact of fasting in different neuropathy subgroups [ms^2], p=0.003, in those with *early* CAN from 7.0±0.2 to 7.7±0.2 ln [ms^2], p<0.001, and in those with *definite* CAN from 5.2±0.2 to 6.0±0.3 ln [ms^2], p=0.003. In contrast, non-diabetic controls demonstrated only a non-significant change of cumulative total spectral power during fasting: 7.7±0.2 *vs* 8.0±0.3 ln [ms^2]; p=0.19.

In general, an increase in cumulative power of high-frequency band was more pronounced (for all patients from 6.5±0.2 to 7.3±0.2 ln [ms^2], p<0.001) than that of low-frequency band and was significant in all neuropathy subgroups, including that with definite neuropathy (Fig. 2). No significant differences between groups were found for impact of fasting on changes in power in high frequency band (p=0.19): increases in those with no CAN from 7.6±0.1 to 8.2±0.2 ln [ms^2], p=0.004, with *early* CAN from 6.1±0.3 to 7.1±0.3 ln [ms^2], p<0.001, and in those with *definite* CAN from 4.3±0.2 to 5.2±0.3 ln [ms^2], p=0.003. Non-diabetic subjects showed only a non-significant increase of cumulative spectral power in high-frequency band from 6.9±0.3 to 7.2±0.3 ln [ms^2], p=0.15.

Cumulative spectral power in low-frequency band

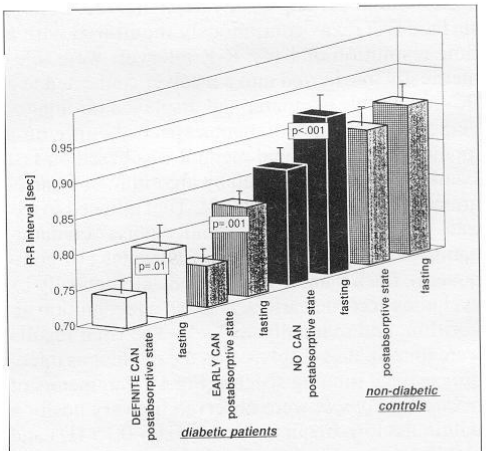

Fig. 4 - *Time-domain analysis of HRV: averaged R-R interval in diabetic patients with definite, early and no CAN as well as in non-diabetic controls, in usual postprandial/postabsorptive state (front bars) and after prolonged fasting (rear bars). p describes significant differences vs postprandial/postabsorptive state in each subject category (intraindividual comparison).*

(p=0.07): in patients with *definite* (increase from 16.3±1.6 to 25.3±3.0 ms; p=0.005), *early* (from 35.4±3.5 to 74.4±10.8 ms; p<0.001) and in those with no CAN (from 79.6±6.9 to 115.5±11.6 ms; p<0.001) but in non-diabetic controls only non-significant influences were registered (from 59.5±8.4 to 73.7±10.2 ms; p=0.1). Similar contrast was found for the averaged R-R interval (Fig. 4; no differences for the effect of fasting between groups, p=0.85) which increased during fasting for the whole diabetic cohort from 0.81±0.02 to 0.87±0.02 s; p<0.001, for patients with definite CAN from 0.74±0.02 to 0.79±0.03 s; p=0.01, for those with early CAN from 0.76±0.02 to 0.82±0.02 s; p=0.001, and for those with no CAN from 0.86±0.03 to 0.92±0.03 s; p<0.001. No significant differences for non-diabetic controls were assessed (0.89±0.03 *vs* 0.91±0.03 s; p=0.56).

Blood pressure values during fasting in a subcohort of patients equally representing all neuropathy groups have not been statistically different as compared to the values measured in a non-fasting state (113.7±4.1/73.2±3.0 *vs* 111.1±4.6/71.8±2.4 mmHg; p=0.10/0.44).

DISCUSSION

The present study documents the evidence that fasting increases heart rate variability in diabetic patients with all degrees of cardiovascular autonomic neuropathy, including its definite form. This phenomenon was caused by an increase of parasympathetic tone (23), although in patients with cardiovascular autonomic neuropathy significant increases of indices mainly attributed to sympathetic components (24) were also documented. The study was designed to evaluate effects of a fasting period exceeding 13 hours compared to a shorter, usual interval starting from the last meal in a group of diabetic patients and controls. The mentioned time interval was chosen as clinically relevant as it may occur in diabetic outpatients on flexible, functional treatment in cases of postponed meals. The practical exercise of one-day fasting was used to motivate patients for this somewhat prolonged fasting period necessary for the purposes of this study.

Observations published so far on prolonged fasting and its influence on autonomic innervation are scarce (25). To our knowledge, no relevant observations concerning the impact of prolonged fasting on autonomic function in humans have been reported till now.

Although the mechanisms of fasting-induced regulatory changes have still not been completely delineated, fasting is thought to increase vagal tone. Gastrointestinal reflexes may play a role but central control mechanisms are probably more important. In rats, bradycardia induced by fasting was shown to be due to suppression of norepinephrine turnover (13). It is known that increases in plasma β endorphins and endogenous opioids may be induced by exercise or by fasting (14). As they have a tonic inhibitory effect on sympathetic tone, they have been implicated in the pathophysiology of vaso-vagal syncope (15). Similarly, our study indicates that fasting provides a relevant reconditioning of autonomic balance of the heart supply with significant enhancement of vagal activity which was also illustrated by the decrease of heart rate. Changes of blood pressure during fasting, although clinically not relevant in our patients might also have some influences.

Our study showed a stronger influence of fasting in diabetes than in control non-diabetic persons. That might be a demonstration of an already known phenomenon that pathologic values (including blood lipids, blood pressure values, values associated with incipient CAN) are stronger influenced towards normal by physiological stimuli [among others by physical training (21, 26)] than normal values. Moreover, our diabetic subjects, conventionally treated till the time point of the investigation (*ie*, till their educational programme for functional insulin treatment), were not used to fast for a longer time period than about 8 hours during the night rest only, as under conventional insulin treatment a late evening snack is often used to prevent nocturnal hypoglycaemia. On the contrary, fasting for longer periods than 8 to 12 hours are nothing unusual in healthy subjects. The fasting period used in our study represents therefore a much stronger stimulus for diabetic subjects than for healthy persons. In diabetic patients a postponed gastric emptying and the persistence of food in the stomach during the control investigation on non-fasting day could also modify the cardiovascular autonomic control.

The changes in spectral indices of HRV observed in our study can be attributed neither to different levels of blood glucose, nor to different time points of the day during both investigations, as those were carefully matched for intraindividual comparison.

Although we did not directly control the respiration rate during the investigation - patients were asked to breathe in a fully relaxed manner - an indirect index of respiration rate (central frequency of high frequency band, $p=0.3$ for intraindividual comparison; data not shown) revealed comparable respiration frequency during both recordings. Insulin and other hormone levels were not assessed in our study and common relative hyperinsulinaemia during the postprandial investigation cannot thus be excluded. However, hyperinsulinaemia could contribute solely to an increase of sympathetic tone and to the increase of power in low frequency band (27). As in our study higher values of parasympathetic tone during fasting were shown and on those days patients used less than 50% of usual daily insulin consumption, the differences in insulin level thus cannot be responsible for the demonstrated increase of autonomic tone. The main consequence of our study should be *a standardization of the time intervals since last meal*, when short-term spectral analysis of HRV will be performed for diagnostic reasons in diabetes. The recommendation of ADA consensus statement on diabetic neuropathy (11) that patients should have no food for 8 hours is applicable only for patients under functional insulin treatment, where the food intake according to a scheduled plan is not necessary any more. In conviction that this group of patients unfortunately represents still a minority of the whole diabetic population, we propose the acceptance of an interval of 2 to 4 hours since last meal, which would allow the performance of the investigation in the morning after breakfast till before noon and then after lunch during the afternoon. However, as circadian fluctuations of HRV are known, we would prefer to recommend a morning or before-lunch diagnostic assessment of heart rate variability by short-term spectral analysis.

Further studies should be performed on therapeutic use of decreased food intake such as very-low-calorie-diet and prolonged fasting periods in diabetes. All factors which might increase heart rate variability should be welcomed, as it becomes apparent that reduced heart rate variability is associated with highly increased mortality rate. Diminished HRV was correlated with obesity (28) and other risk factors for increase of mortality (29); fasting can be assumed to positively influence all other risk factors associated with increased mortality such as high blood pressure, hyperglycaemia and hyperlipidaemia, and obesity. The question whether the increased heart rate variability and increased vagal tone induced by fasting in diabetes may decrease the risk of mortality, should be considered in future studies.

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4.3. Praktická aplikace: výzkum reverzibility CAN a kvantifikace efektu nefarmakologických opatření - efekt spinální stimulace

Úvod: Další relevantní aplikační oblastí pro vyšetřování HRV jsou kardiovaskulární onemocnění. V literatuře je popsána redukce kardiální ischemie *(Hautvast 1998)* a potenciálně i zvýšené uvolňování beta endorfinů při spinální neurostimulaci u pacientů s chronickým postižení koronárních arterií a chronickou refraktorní anginou pectoris *(Anderson 1998)* .

Cílem naší práce bylo pomocí testu HRV posoudit vliv této stimulace na autonomní regulace u 14 pacientů na této léčbě. Pro tyto účely byl testovací protokol modifikován, a pacienti byli vyšetřeni vsedě 3x po 5 minutách: (a) s vypnutou stimulací, (b) s maximální snesitelnou stimulací a (c) s poloviční intenzitou předchozí stimulace.

Výsledky: Ve srovnání s výsledkem 5-minutového záznamu bez stimulace (a) byla pozice (b) spojena s významným poklesem poměru LF/HF ($p=0.02$) jakož i celkovým poklesem LF části spektrálního výkonu ($p=0.001$). Výsledky této studie poprvé potvrzují, že během spinální stimulace dochází k poklesu LF spektrálního výkonu, který dominantně reflektuje vliv sympatikotonie.

4.3.1 Moore R, Groves D, Nolan J, Scutt D, Pumprla J, Chester MR: Altered short term heart rate variability with spinal cord stimulation in chronic refractory angina: Evidence for the presence of procedure related cardiac sympathetic blockade. Heart, 2004 ,90 (2): 211-212 IF 3.1

(Originální publikace této práce in extenso začíná na následující straně)

SCIENTIFIC LETTER

Altered short term heart rate variability with spinal cord stimulation in chronic refractory angina: evidence for the presence of procedure related cardiac sympathetic blockade

R Moore, D Groves, J Nolan, D Scutt, J Pumprla, M R Chester

Heart 2004;90:211–212. doi: 10.1136/hrt.2002.002998

The first spinal cord stimulator was implanted for intractable angina in 1987. Following this innovation there followed extensive scrutiny of neurostimulation in patients suffering from coronary artery disease, with several observational trials demonstrating the ability of spinal cord stimulation (SCS) to diminish angina, reduce the frequency of hospital admissions, and improve quality of life.

SCS moderates the symptoms of angina by inhibition of pain perception through the release of spinal inhibitory neurotransmitters at the level of the dorsal horn, and also potentially by liberation of cardiac β endorphins. In addition it has been established that neurostimulation also achieves its benefit in part by reduction in cardiac ischaemia.[1] The proposed mechanism for this observation is altered cardiac autonomic balance.

Heart rate variability (HRV) has been used to verify this proposed influence on cardiac autonomic tone. The two trials published to date, however, have failed to demonstrate any such effect.[2 3] Both studies used long term Holter (24 and 48 hour) ECG recording intervals in which baseline conditions were not controlled and spinal cord stimulators were activated only for a minority of the post-implant recording periods. The aim of this study was to use short term HRV recordings to assess directly the influence of SCS on HRV in a controlled experimental setting.

METHODS

Sixteen consecutive patients with chronic refractory angina that was successfully controlled with SCS were recruited from the neurostimulation clinic at the National Refractory Angina Centre, The Cardiothoracic Centre, Liverpool, from May to July 2001.

All patients had a Medtronic Pisces quad plus electrode and a Medtronic XTREL II pulse generator implanted. Two patients were ineligible for the study on the basis of multiple atrial and ventricular ectopic beats, which prevented assessment of short term HRV.

Each patient was isolated for a period of 30 minutes before HRV recording. A five minute high resolution 1000 Hz HRV recording was taken with the SCS set at zero output, maximum comfortable output and half maximum output with the pulse width, frequency, and electrode settings left unaltered from their usual treatment positions. To investigate whether the order of recording was capable of biasing results, seven patients underwent repeat recording without altering the SCS output from its original settings for the three sequential five minute measurements. All the recordings were made between 2–4 pm in a secluded room with an ambient temperature maintained at approximately 21°C and the patient in a seated position.

The data were acquired and processed using the VariaCardio TF4 HRV monitor, which complies with the recommendations of the joint American and European task force.[4] All datasets were checked for ectopic beats/noise and only datasets, which required three RR intervals or less to be deleted, were included in the analysis. The power spectrum density was calculated using an established coarse grain fast Fourier transformation method.

Differences between the HRV parameters at each SCS output setting were evaluated using the paired Student's *t* test. Variables with skewed distribution were normalised using logarithmic transformation ($\log_e$) before analysis. A probability value of $p < 0.05$ was considered significant. Values are expressed as mean (SD).

RESULTS

A total of 14 male patients, aged 40–77 years, were investigated. All patients had been previously diagnosed as suffering from refractory angina, and the majority of study participants (93%) had previously undergone either coronary artery bypass surgery or percutaneous coronary angioplasty.

The mean low frequency/high frequency (LF/HF) ratio with full power SCS was significantly reduced when compared to recordings taken during no stimulation ($p = 0.024$) (table 1). The mean LF/HF ratio was reduced, although not significantly, with stimulation at half usual output when compared to baseline ($p = 0.498$).

The mean natural logarithm of low frequency ($\log_e$ LF) and mean normalised LF power were both significantly lower with no stimulation than with full power stimulation ($p = 0.001$ and $p = 0.01$, respectively) and with half power stimulation ($p = 0.001$ and $p = 0.01$, respectively).

There was no significant difference comparing full power to half power SCS ($p = 0.493$, $p = 0.939$).

There were no significant differences in absolute HF power with SCS calibrated at the three different output settings. There was no significant alteration in heart rate, or arterial blood pressure at the three SCS settings. No patient reported angina during the study.

Seven patients underwent three, five minute recordings without altering the SCS settings (full output). In this group there was no significant difference between any of the HRV parameters.

DISCUSSION

SCS in this study significantly altered spectral power parameters in HRV. The LF/HF ratio decreased significantly

Abbreviations: HF, high frequency; HRV, heart rate variability; LF, low frequency; SCS, spinal cord stimulation

Table 1 Mean spectral power at different SCS settings

	LF/HF ratio			LF			HF			Normalised LF			Normalised HF		
Recording	Off	Half	Full	Off	Half	Full	Off	Half	Full	Off	Half	Full	Off	Half	Full
Mean	1.59	1.41	0.83	381	188	209	400	542	458	0.47	0.34	0.35	0.53	0.66	0.65
SD	2.00	2.46	1.08	688	298	369	677	1091	879	0.25	0.26	0.21	0.25	0.26	0.21

HF, high frequency; LF, low frequency.

with spinal stimulation, as did absolute LF and normalised LF spectral power density. These observations are consistent with reduced cardiac sympathetic activity during SCS.

Researchers have previously demonstrated indirect evidence of reduced cardiac sympathetic activity with SCS in man, and in a recent publication Foreman and colleagues[5] reported a suppressive effect of SCS on intrinsic cardiac sympathetic activity in dogs undergoing coronary artery ligature. SCS is maximised at the lower cervical and upper thoracic spinal segments, which correspond directly with the portion of the cord responsible for sympathetic outflow. Investigation with SCS has revealed a generalised local "field effect" with reduced neural activity mediated by alteration in the balance of inhibitory and excitatory neurotransmitters. Therefore, although SCS could alter parasympathetic activity both directly and through interspinal neurons, its dominant influence would be anticipated to be directly through diminution of spinal sympathetic activity.

This study also demonstrated that LF spectral components were significantly diminished in the absence of perceived paraesthesia with SCS. It may be postulated that prolonged stimulation at this lower level could offer lessening in the total ischaemic burden and possibly altered arrhythmia thresholds for this patient group.

The order of SCS settings was not randomised, which could provide a confounding bias. The study was only partially blinded, as patients were aware of neurostimulation at full output. In addition the changes in cardiac autonomic balance observed with SCS in this study were evident immediately after switching between output settings. Further work is required to see if these effects are more persistent with increasing periods of stimulation.

SCS is becoming more and more widely used to treat patients suffering with chronic refractory angina. This study shows for the first time that stimulation of the spinal cord interferes with cardiac autonomic nerve traffic and suggests a possible mechanism of action through reduced resting cardiac sympathetic tone. Further clinical studies into the effect of low output stimulation on angina and ischaemia are warranted.

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4.4. Praktická aplikace: výzkum reverzibility CAN a kvantifikace efektu nefarmakologických opatření - řízené zpomalení dechové frekvence pomocí bio-feedbacku

Úvod: Pozitivní účinek řízeného dýchání HRV ve smyslu zlepšení pocitu zdraví a well-being je známý např. z výzkumů pěvců v hudebních tělesech *(Vickhoff 2013)*.

Cílem naší studie bylo ověřit účinnost nefarmakologického ovlivnění výše krevního tlaku pomocí řízeného zpomalení respirační frekvence domácím systémem RespeRate u 32 pacientů s metabolickým syndromem, v cross-over designu ve skupinách bez a s 8 týdenní domácí léčbou. Principem léčby je řízené zpomalení respirační frekvence za pomoci bio-feedbacku na 6/min pomocí 4-6 krátkodobých v průměru 12-minutových sezení týdně, po dobu 1-2 měsíců. Systém tvoří hrudní pás registrující respirační exkurze, propojený s počítačovou jednotkou, udávající do sluchátek dechovou frekvenci v podobě hudebních akordů. Dechová frekvence se tak z původních 10-15 cyklů za minutu sníží až na cca 6/min.

Výsledky: Vzhledem k již vstupně dostatečně kompenzovaným hodnotám krevního tlaku byl efekt domácí aplikace RespeRate na hodnoty krevního tlaku u těchto pacientů menší než u těch, kteří vstupovali do studie s vyššími hodnotami TK. Po 8-týdenním používání systému řízeného dýchání RespeRate došlo u uživatelů k signifikantnímu poklesu systolického krevního tlaku a hodnoty pulse pressure v 24h ABPM (p=0.01), zatímco u kontrol nebyly změny statisticky významné. Rovněž došlo ke statisticky významnému poklesu centrální frekvence v HF spektru HRV (x=0.13±0.11 Hz vs 0.08±0.07 Hz, p=0.04), a posílení spektrální energie v LF (p=0.02), což odpovídá principu použití systému, řízenému zpomalení respirační frekvence.

Závěrem lze konstatovat, že i u optimálně kompenzovaných pacientů s metabolickým syndromem je 8-týdenní řízené zpomalení respirační frekvence účinné ve smyslu mírného poklesu krevního tlaku. V případě systematické aplikace lze metodu považovat za efektivní nefarmakologickou intervenci k podpože reverzibility kardiovaskulární autonomní dysfunkce.

4.4.1. Howorka K, Pumprla J, Tamm J et al. Effects of guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients. Auton Neurosci 2013, 179: 131-137. IF 1.4

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Effects of guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients☆,☆☆

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ABSTRACT

Objective: Our aim was to investigate medium-term effects of device-guided breathing on blood pressure (BP) and its capacity to improve the cardiovascular autonomic balance in hypertensive diabetic patients. This feasibility study was conceived as a proof-of-concept trial under real life conditions for justification of further investigations.

Methods: A randomized, controlled study (RCT) of the effects of device-guided slow breathing on top of usual care against usual care alone (including non-pharmacological and pharmacological treatment). The intervention included 12-min sessions of guided breathing performed daily for 8 weeks. Treatment effects were assessed with ambulatory blood pressure monitoring (24 h ABPM) and with spectral analysis of short-term heart rate variability (HRV) obtained during standardized modified orthostatic load. Thirty-two subjects with diabetes and antihypertensive therapy were randomly assigned to both study groups.

Results: After 8 weeks of guided breathing, significant reductions were demonstrated in 24 h systolic BP ($\bar{x} \pm$ SEM: 126.1 ± 3.0 vs 123.2 ± 2.7 mm Hg, $p = 0.01$), and in 24 h pulse pressure (PP, 53.6 ± 2.6 vs. 51.3 ± 2.5 mm Hg, $p = 0.01$), whereas no significant impact in the control group was shown. The differences in treatment effects (delta mm Hg, RESPeRATE® vs control) were significant only for PP (−2.3 ± 0.8 vs +0.2 ± 1.2 mm Hg, $p < 0.05$). Strong baseline dependence of treatment effects (delta systolic BP) was observed ($p < 0.01$). Guided breathing showed a stronger treatment effect in terms of an increase in HRV, predominantly in low frequency band ($p < 0.03$ vs. usual care).

Conclusion: Even in well controlled hypertensive diabetic patients, guided breathing induced relevant effects on BP and HRV, finding which should be investigated further.

1. Introduction

Hypertension is a serious cardiovascular risk factor, with a prevalence of up to 28% in North America and 44% in Europe. As only 28% of patients are adequately controlled (Wolf-Maier et al., 2004), additional non-pharmacological blood pressure lowering might save complications and postpone death.

Slow, device-guided breathing has been shown to effectively reduce blood pressure. In several studies (Rosenthal et al., 2001; Viskoper et al., 2003; Elliot et al., 2004; Meles et al., 2004; Logtenberg et al., 2007; Altena et al., 2009; Schein et al., 2009), a 12-minute daily use over 8 weeks resulted in consistent lowering of systolic and diastolic blood pressure. The pathophysiological mechanism of BP lowering with slow breathing is not fully elucidated yet. Inappropriately high sympathetic nervous outflow from the central nervous system is believed to be an important component in the development of hypertension, inducing an increase in cardiac output and peripheral resistance (Smith et al., 2004). A better understanding of the guided breathing mechanism of action would allow for an appropriate target group selection and hence for a more appropriate allocation of resources and reduction of clinical side effects when pharmacological intervention becomes necessary.

Three-dimensional spectral analysis of short-term heart rate variability using fast Fourier transform offers a unique instrument for an instantaneous quantification of sympathetic and parasympathetic cardiovascular autonomic control (Howorka et al., 1998; Pumprla et al.,

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2002; Howorka et al., 2010). Autonomic dysfunction is common in diabetes and hypertension (Gerritsen et al., 2001) and worsens the life prognosis (Ewing et al., 1985). Hypertension and diabetes mellitus are interrelated diseases, and dominate in metabolic syndrome. It is estimated that more than 60% of diabetic patients suffer from hypertension (Arauz-Pacheco et al., 2002), which increases morbidity and mortality (Morrish et al., 2001). In diabetes, a near-normalization of blood pressure towards values below 130/80 mm Hg is recommended (Mogensen, 2003; ADA, 2004), since it remains the main risk factor for micro- and macrovascular diabetes complications.

Diabetic autonomic neuropathy is related to an increased all-cause mortality risk in diabetic patients (Ewing et al., 1985; Ziegler et al., 1992; Vinik and Erbas, 2001; Gerritsen et al., 2001; Wheeler et al., 2002). The most life threatening condition is an advanced cardiovascular autonomic neuropathy (CAN). Heart rate variability (HRV) is a measure of CAN and is partly related to the breathing rate. A lower breathing rate is associated with an increase in HRV (Pitzalis et al., 1998). Furthermore, a prolonged exhalation also results in an increased HRV (Strauss-Blasche et al., 2000). Short-term spectral analysis of HRV recorded under standardized conditions over three time segments during a modified orthostatic test, provides a fast, objective, non-invasive and reproducible method to detect even early stages of CAN and their dynamics in diabetes (Howorka et al., 1998; Pumprla et al., 2002).

Several studies have shown that controlled slow breathing results in a decrease of systolic, diastolic and mean arterial blood pressure, and also in an increase of HRV (Patel et al., 1985; Joseph et al., 2005; Pinheiro et al., 2007). Our hypothesis was that guided breathing with RESPeRATE® improves autonomic function. More specifically, we hypothesized that it would reduce blood pressure values and increase heart rate variability in diabetic patients with hypertension. We wanted to investigate guided breathing-related effects within a controlled trial (intervention vs usual care) to filter out potential placebo- (study-) related effects. More specifically, our aim was to estimate its therapeutic potential as an additional non-pharmacological treatment option in ambulatory diabetic patients with hypertension already treated by pharmacological agents.

This feasibility study was conceived as a proof-of-concept trial under real life conditions as a justification for further and more profound (and costly) investigation.

2. Methods

The study was carried out in accordance with the EU-GCP guideline and in accordance with the guidelines of the Declaration of Helsinki (1964), including recent revisions (Declaration of Helsinki, amended, 2008). Study protocol was approved by the local Ethics Committee (EudraCT No 2011-003839-53). Each subject signed an informed consent form before inclusion in the study.

2.1. Study intervention: device guided breathing with RESPeRATE® in addition to "usual care"

Guided breathing using the RESPeRATE® device was used as study intervention procedure. The device consists of a breathing sensor positioned on the chest, headphones and a computerized unit (Fig. 1). The breathing sensor analyzes the individual breathing pattern and creates a personalized melody composed of two distinct inhale and exhale guiding tones. The exhalation tone is gradually prolonged, thereby slowing down the breathing, which finally leads to less than 10 breaths per minute. An internal memory is included in the device, which registers and stores date, time, duration and effective use in each treatment session. The time the user spends with a breathing rate of less than ten breaths/min is called "effective time" and is quantified. The target time period for the use of the device is one session (at least 12 min) per day according to the recommendation of the manufacturer (based on experience from previous clinical investigations).

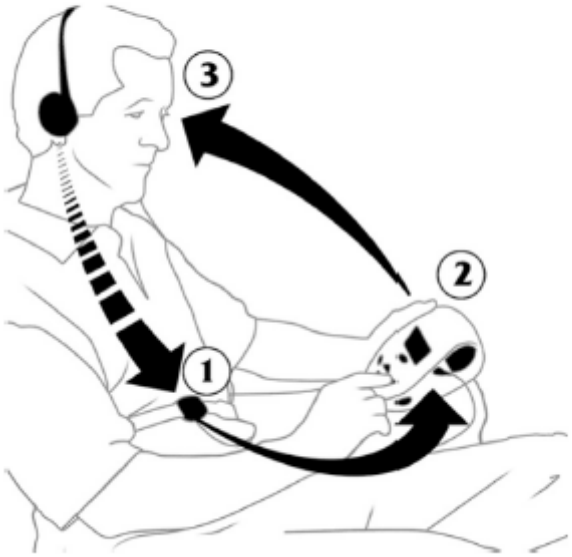

Fig. 1. Principles of device-guided paced breathing: (1) monitoring breathing movements, (2) composing breathing-guiding tones, and (3) synchronizing breathing movements with the guiding tones (by courtesy of Intercure Inc.).

In our study, RESPeRATE® was used as an add-on on top of the usual pharmacological and non-pharmacological treatment. All study subjects had a history of participation in a structured patient group education on diabetes and hypertension (Howorka et al., 2009; Pumprla, 2008).

2.2. Control intervention: "usual care"

The control intervention included the usual treatment as taught during structured hypertension education (Howorka et al., 2009), which includes the 'classical' recommended non-pharmacological measures (salt reduction,/DASH diet, weight reduction, endurance and or muscle hypertrophy training, Chobanian et al., 2003), and – if necessary – individual pharmacotherapy (usually 1–3 agents; mainly ACE inhibitors and/or sartans, if necessary other agents), and at least two self-BP-measurements per week.

2.3. Research design

Randomized, controlled trial (RCT) of effects of guided breathing with RESPeRATE® on top of usual care against usual care alone, while "standard" treatment and usual care remained unchanged (non-pharmacological and pharmacological treatment based on structured group education and individual counseling). Intraindividual and intergroup comparison of ambulatory blood pressure monitoring (24 h ABPM) as well as HRV target parameters between baseline and values obtained after 8 weeks of either intervention (systematic, daily use of guided breathing) or no intervention ("usual care"). Randomization was performed using www.randomization.com. Patients were randomly allocated to one of the two groups as described in study protocol submitted to the local Ethics Committee. The study was conducted in a way that patients were kept blinded regarding their group assignment.

2.4. Inclusion and exclusion criteria

2.4.1. Inclusion criteria

Clinical manifestation of diabetes mellitus (types 1 and 2) for at least three months, age 18–78 years, history of hypertension

(>130/80 mm Hg) and concomitant antihypertensive medication, acceptable level of hypertension control (at least 50% of BP self measurements within target limits, i.e. below 130/80 mm Hg in diabetes), structured education, at least sedentary compliance, capability of self-monitoring and recording the values, and understanding the informed consent.

2.4.2. Exclusion criteria

Uncontrolled hypertension with acute need for medication adjustment, insufficiently controlled diabetes with acute need for additional pharmacological intervention, inability for systematic use of guided breathing, severe autonomic neuropathy (total cumulative power below 100 ms^2/total Ewing Score > 3), pregnancy planning or current pregnancy, extraordinary circumstances and/or treatment which might severely influence blood pressure control and/or heart rate and/or heart rate variability during the study, circumstances which might interfere with applicability of study instruments, any history of disease which could interfere with the objectives of the study, e.g. severe psychological disorders including drug abuse and/or self-destructive behavior, deafness, severe symptomatic cardiac insufficiency, atrial fibrillation or clinically apparent coronary heart disease.

2.5. Concomitant medication and study related restrictions

Study participants could not receive any other treatment than that for diabetes, hypertension and hyperlipidemia or for another stable chronic condition (such as thyroid replacement for hypothyroidism) throughout the whole study period. Concomitant medication for all chronic conditions remained unchanged during the study.

In accordance with the study protocol, subjects had to abstain from atypical strenuous physical activity or atypical life circumstances for at least one day before the investigation days, as well as from other activities which might interfere with the study-related questions. Patients kept their usual dietary habits consistent throughout the study. They were educated in intensified treatment of diabetes (Functional Insulin Treatment, Howorka, 1996) and hypertension as described (DiabetesFIT® Curriculum, Howorka et al., 2009). A usual frequency of blood glucose self-monitoring (approx. five daily measurements in insulin-treated diabetes) and at least two blood pressure self-measurements per week were asked for during the study. Patients' records including documentation of self-monitoring and interventional sessions with RESPeRATE® were kept. The primary compliance endpoint quantifying "appropriate use" of the device, was the "effective time" accumulated over the 8-week treatment period, using the data-logging capability of the device.

2.6. Patients

Thirty two diabetic patients with hypertension and/or already using hypertensive treatment were recruited. The potential study participants who met inclusion criteria were selected by the principal investigator and co-investigators. The final study population included n = 32 diabetic patients with hypertension and antihypertensive treatment (male: 17, age: 49.3 ± 11.7/SD/, diabetes duration: 25.8 ± 12.6 year, HbA1c: 7.7 ± 0.9%, type 1 diabetes n = 25, insulin therapy and FIT-educated n = 31, hypertension-educated n = 32, number of antihypertensive agents/day 1.6 ± 1.1, normoalbuminuria: n = 30, history of previous hyperalbuminuria n = 4, currently increased albumin excretion rate n = 2).

2.7. Main methods of assessment

Ambulatory blood pressure monitoring (ABPM) over a 24 h period, and spectral analysis of short-term heart rate variability (HRV) in time- and frequency-domaINS as obtained with VariaCardio TF5 during standardized modified orthostatic load (supine1–standing–supine2).

2.8. Ambulatory blood pressure monitoring (ABPM)

ABPM was performed using a BoSo TM-2420 PC system according to the recommendations of the Austrian Hypertension Society (Slany et al., 2008). Additional outcome variables such as mean systolic and diastolic blood pressure, pulse pressure, mean heart rate, and night-time dipping were assessed as described.

2.9. Spectral analysis of HRV

Short-term spectral analysis of HRV was calculated from recordings obtained during the modified orthostatic load consisting of 15 min of artifact-free records, using a VariaCardio TF5® system (Advanced Medical Diagnostics Group, UK) (Howorka et al., 1998; Salinger et al., 1999; Pumprla et al., 2002; Howorka et al., 2010). R–R intervals were recorded with sampling rate of 1000 Hz. Computational method was based on modified fast Fourier transform (FFT). Recordings were obtained in three consecutive positions (supine–standing–supine), each lasting 300 s in recording time. Artifacts were labeled, and a specific algorithm imputed beat-to-beat intervals throughout an artifact period to preserve time relationships of the adjacent, uncorrupted heart rate data (Pumprla et al., 2002). The final results were immediately displayed on the monitor as three-dimensional running spectra (see Fig. 2).

Parameters of frequency-domain HRV were measured in every position within the very-low frequency (VLF, 0.01–0.05 Hz), correlating among others with activity of baro- and thermoreceptors, low frequency (LF, 0.05–0.15 Hz) mirroring the combined sympathetic and parasympathetic control, and high-frequency bands (HF, 0.15–0.40 Hz), corresponding exclusively to parasympathetic tone, as well as total spectral power (TF, 0.01–0.40 Hz) representing the degree of cardiac autonomic dysfunction in diabetes (Malik and Task Force, 1996; Howorka et al., 1998). Main outcome variables were total spectral power [unit ms^2] and spectral power in all three frequency bands individually. To assess even small intraindividual changes in global autonomic tone during the trial, we used cumulative indices (spectral power of frequency bands with its main frequency components obtained over all three positions during the orthostatic load, Howorka et al., 1998). Time-domain analysis included the averaged R–R intervals (ms), RMSSD, root of the mean square of difference of successive R–R intervals (ms) as well as SDNN, standard deviation of all normal intervals. Because of the skewness of the frequency-domain data distribution, a natural log (LN) transformation was performed to produce a normal distribution before the final results were analyzed.

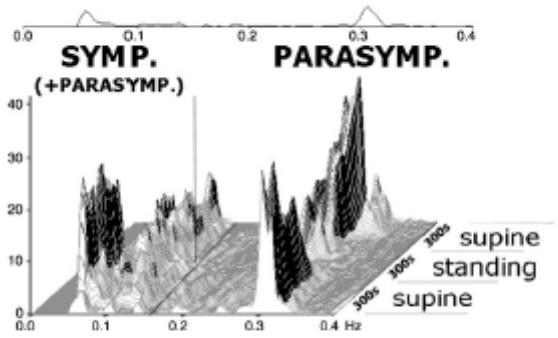

Fig. 2. Three-dimensional display of HRV pattern was obtained during a modified orthostatic load (standardized assessment by VariaCardio TF4® in positions supine–standing–supine, each position 300 s). Horizontal axis: frequency of fluctuations of R–R intervals (Hz); vertical axis: power spectral density (ms^2/Hz); z-axis: time relapsed (s), modified after Salinger et al., 1999; Pumprla et al., 2002.

2.10. Statistical methods

Treatment effects were quantified by generation of delta values, calculated intraindividually as the arithmetic difference between values after and before treatment. Intraindividual intervention-related differences to baseline values were determined using paired t-tests, differences between study groups were detected through unpaired t-tests or Wilcoxon tests where appropriate. One-tailed t-tests were applied to detect differences in treatment effects between groups (superiority comparison, Sackett, 2004; Schein et al., 2009). Normality of frequency distributions was assessed by Kolmogorov–Smirnov goodness-of-fit tests. Correlations between baseline values and intervention effects were described with a linear correlation model using Pearson coefficients. All statistical tests used an alpha level of $p < 0.05$. Initial data processing and calculations were run with Microsoft Excel 2007, statistical analyses were performed with IBM SPSS Statistics 18.0 and SYSTAT 12 (SYSTAT Software Inc. San Jose, CA, USA).

3. Results

3.1. Guided breathing performance and compliance analysis

Patients in the intervention group demonstrated sufficient compliance with the prescribed method for use of the guided breathing device. We found that for a stable, reproducible session performance, patients' experience with five and more guided breathing sessions was needed. Patients spent most of their time (86%) at slow breathing rate (in average around 6 breaths per minute, while values below 10 breaths per minute were indicated earlier as the therapeutic effect threshold, Elliott et al., 2004.). In a typical session, taking at least 12 min, a trend of slowing down the breathing rate from 9–10 to 5–6/min could be consistently observed, which finding corresponds with recorded prolonged exhalation time and excellent compliance. An average exhalation time was about 50% longer than inhalation time. The average degree of synchronization between breathing movements and the guiding tones, as provided by the device, was 72%. These results indicate good performance with the device guiding instructions.

3.2. ABPM

With exception for heart rate, no differences between treatment groups were found at baseline (see Table 1 and Fig. 3). Subjects already displayed a fair or good control of their blood pressure even before the study.

In the intervention group, the guided breathing induced a significant overall reduction of systolic 24 h BP ($x \pm$ SEM: 126.1 ± 3.0 vs 123.2 ± 2.7 mm Hg, $p = 0.01$), non-significant reduction of systolic day time blood pressure (129.3 ± 3.0 vs. 127.1 ± 2.7, $p = 0.06$), a significant reduction of pulse pressure (PP) in 24 h (53.6 ± 2.6 vs. 51.3 ± 2.5 mm Hg, $p = 0.01$), and daytime PP (54.8 ± 2.8 vs. 52.8 ± 2.6 mm Hg, $p = 0.04$), as well as non-significant reduction of 24 h mean arterial pressure (90.3 ± 1.7 vs. 89.0 ± 1.5 mm Hg, $p = 0.08$),

Table 1
Patient characteristics at baseline in both treatment groups.

	Resperate + usual care	Usual care	p=
N	16	16	
Age (years)	49.9 ± 11.3	48.8 ± 12.4	0.78
Sex	M = 8/F = 8	M = 9/F = 7	0.75
Type of diabetes	t1d = 11/t2d = 5	t1d = 14/t2d = 2	0.19
Weight (kg)	80.0 ± 16.9	76.3 ± 17.0	0.54
Waist circumference (cm)	92.1 ± 15.2	90.0 ± 11.5	0.67
Diabetes duration (years)	26.6 ± 11.5	25.1 ± 14.0	0.73
Proteinuria (median/Q1/Q3; µg)	5/5/8.25	5.5/5/7.5	0.57
Initial heart rate in 24 h ABPM (bpm)	71 ± 7	78 ± 9	0.02

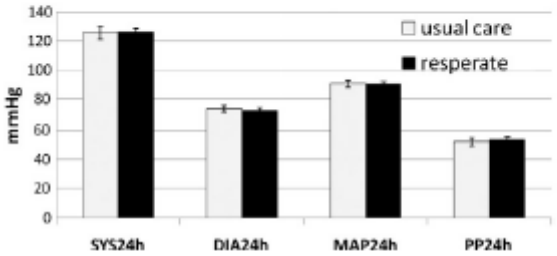

Fig. 3. Comparison of baseline values ($x \pm$ SEM) between treatment groups. No differences for systolic (SYS 24 h), diastolic (DIA 24 h) BP, mean arterial pressure (MAP 24 h) and pulse pressure (PP 24 h) were found before the study.

whereas in the control group the non-intervention ("usual care" alone) induced no significant changes. The effects of both intervention types – i.e. RESPeRATE® use vs. usual care alone – are summarized in Fig. 4.

Even though guided breathing induced more pronounced effects on systolic blood pressure, the differences between groups for the impact of treatment (delta mm Hg, Fig. 4) were not significant, the only exception being pulse pressure (-2.3 vs. $+0.2$ mm Hg, $p < 0.05$). Similarly, no differences between group-specific treatment effects were found neither for daytime nor for night-time blood pressure, as well as for night-time dipping, probably due to the high variability of these target variables. A strong dependence of treatment effect (delta systolic mm Hg) on baseline blood pressure values in both groups was found ($r = 0.59$, $n = 32$, $p = 0.01$), see Fig. 5. Significant baseline dependence was given for 24 h daytime systolic ($p < 0.001$), 24 h total diastolic blood pressure ($p = 0.013$), and 24 h total and daytime pulse pressure (both $p = 0.008$).

3.3. Heart rate variability

At baseline no significant differences between treatment groups were found for all target variables of heart rate variability (all $p > 0.13$).

3.3.1. Frequency domain analysis

In comparison with 'usual care' alone, guided breathing induced a significantly stronger treatment effect in low-frequency (LF, $p = 0.02$), in combined low and high frequency bands (LFHF, $p < 0.05$) as well as a non-significant effect in total spectral power over all three frequency bands ($p = 0.06$), always as cumulative value over three positions supine–standing–supine. Fig. 6 summarizes treatment effects (delta

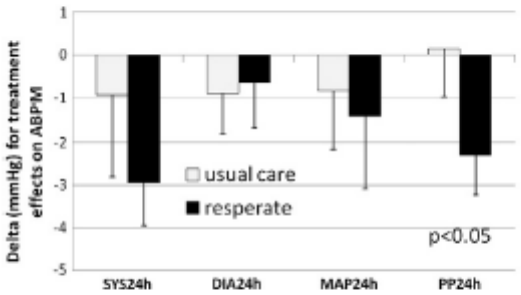

Fig. 4. Comparison of treatment effects ($x \pm$ SEM) after 8 weeks of guided breathing vs. usual care alone. The pulse pressure was significantly reduced (PP 24 h, $p < 0.05$), while the differences between the treatment impacts on systolic blood pressure (SYS 24 h, $p = 0.18$), diastolic BP (DIA 24 h, $p = 0.43$) and mean arterial pressure (MAP 24 h, $p = 0.36$), did not reach significance in ambulatory BP monitoring.

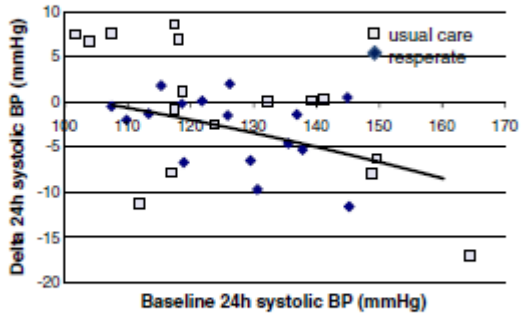

Fig. 5. Baseline dependence: Correlation between treatment effect as delta (mm Hg) and 24 h baseline systolic BP values, $p = 0.01$ for both groups, $y = -0.0022x^2 + 0.328x - 8.3058$, $r = 0.59$.

'after' – 'baseline' LN spectral power in individual frequency bands) in both study groups.

The intervention group showed a significant reduction of centroid frequency in the high frequency band (HF, $x = -13 \pm 11$ mHz vs. $+8 \pm 7$ mHz, $p = 0.04$) which correlates with a slower spontaneous respiration rate induced by the guided breathing intervention.

3.3.2. Time domain analysis

At baseline, no significant differences for time domain parameters (mean R–R interval, SDNN, RMSSD) were found, although the intervention group displayed non-significantly longer mean R–R intervals 906 ± 137 ms as compared to the non-intervention group 836 ± 97 ms, corresponding to slightly slower heart rate in the intervention group in 24 h ABPM. No significant differences were found for treatment effects between both study groups.

4. Discussion

Our study is the first randomized controlled trial that reports effects of device-guided breathing in diabetic patients, using 24 h ABPM. In previous trials, either only a casual/office BP was measured (Schein et al., 2009) or ABPM was used in non-diabetic individuals (Rosenthal et al., 2001). Our hypotheses, that guided breathing would positively influence autonomic control and further improve of blood pressure control, have been confirmed in our setting: in already well controlled hypertensive diabetic patients, a consistent use of the device for a

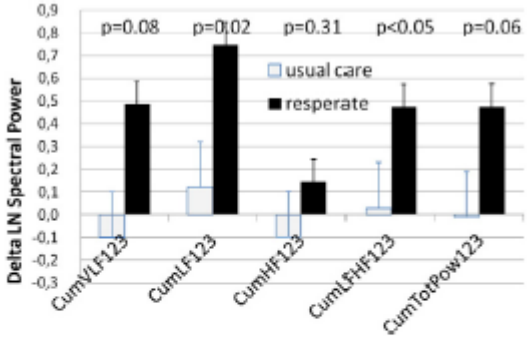

Fig. 6. Comparison of treatment effects (delta LN spectral power ± SEM) between groups. Target variables: cumulative spectral power over 3 positions – CumVLF123 (very low frequency band), CumLF123 (low frequency band), CumHF123 (high frequency band), CumLFHF123 (low and high frequency bands), and CumTotPow123 (total frequency band).

time period of eight weeks resulted in small but measurable effects. Systolic blood pressure was further lowered by −2.9 and the pulse pressure −2.3 mm Hg by guided breathing whereas no significant effect could be seen in the control group with usual care alone. Since 24 h ambulatory BP is not biased by the white coat effect, the observed reduction by 3 mm Hg is clinically important (Mancia et al., 1995). In contrast to office BP, mean 24 h ABPM values are generally accepted to be mostly free of placebo effect (Mancia et al., 1995) and of the statistical artifact called "regression to the mean" (O'Brien et al., 1991; Coats et al., 1992) due to the large number of measurements involved.

Our study was conceived as a RCT to filter out a possible placebo effect in "usual care" group. Study-related effects (placebo of the "usual care" group) did not reach statistical significance, whereas the systolic BP and PP reductions in the treatment group are significant and not related to placebo effect. According to JNC7 (JNC 7th report, 2003) "every mm Hg counts": a 3 mm Hg reduction in systolic BP is associated with 8% reduction in the mortality from stroke and with 5% reduction from coronary artery disease. In diabetes, the gains seem even more relevant. Therefore, the 3 mm Hg systolic BP reduction observed in the treatment group is both clinically and statistically significant, placebo-free benefit. As elevated pulse pressure is increasingly being recognized as a risk factor for cardiovascular – particularly coronary – disease (Dart and Kingwell, 2001), the beneficial effect of guided breathing in the intervention group is particularly relevant.

These results are supported by increase of cumulative power in HRV predominantly in low frequency corresponding to combined sympathetic and parasympathetic components. This finding correlates with the observed shift-to-left of centroid frequency component of the (parasympathetic) HF band which implies slowing down of respiration rate by guided breathing.

These significant effects have been achieved for both, blood pressure and heart rate variability despite the fact that diabetic patients recruited for this study already displayed almost optimal blood pressure control at baseline. Recent recommendations (Mogensen, 2003, JNC 7th report 2003) for diabetes target high normal or even (in younger patients) normal blood pressure values. In a similar study about guided-breathing (Elliott et al., 2004) where the group using the RESPeRATE® device achieved a blood pressure lowering of -15.0 ± 1.8 compared to the non-intervention group with a blood pressure lowering of "only" -9.2 ± 1.6 mm Hg, while the initial blood pressure values were around $150 \pm 9/86 \pm 9$ mm Hg, with only 77% of patients using pharmacological treatment. According to these studies, and taking into account the usual standard deviations of blood pressure values, sample size of 30 patients appeared sufficient (Lenth, 2006). However, the pre-study blood pressure control in our diabetes and hypertension educated patients was much better than in previous studies. Further blood pressure lowering effect was small but still achievable in our patients who were already using 1.6 pharmacological agents per day and were educated (Howorka et al., 2009) about another means for non-pharmacological blood pressure reduction like endurance and muscle hypertrophy training, DASH diet, weight control and sodium restriction (JNC 7th report 2003). This fact underlines the efficacy of the method and – considering the demonstrated baseline dependence of therapeutic effects – illustrates its potential for patients with insufficient blood pressure control. Adjuvant non-pharmacological treatment is simply a need. In many patients, simple life style modification provides a potential for sufficient initial treatment. Guided breathing seems to provide such an additional and complementary option for treatment.

As demonstrated previously, only two non-pharmacological measures are known to improve heart rate variability in various stages of autonomic neuropathy in diabetes so far: food restriction or fasting (Howorka et al., 1997b) and systematic endurance training (Howorka et al., 1997a). As shown in our current study, guided breathing provides one more option for influencing autonomic nervous control in diabetes in a positive way. As many patients require multiple combinations of

pharmacological agents, which per se could diminish heart rate variability, guided breathing seems to provide an important, complementary adjuvant treatment affecting both, blood pressure as well as autonomic function, in an advantageous way. In our study, the 8-week systematic use of guided breathing induced a slowing of spontaneous breathing rate in intervention group, which is declared to be the main treatment effect of the device (Schein et al., 2001, Viskoper et al., 2003; Elliot et al., 2004).

Beyond the specific necessity for optimal control for diabetes, international guidelines recommend applying lifestyle modification in hypertension therapy (JNC 7th report, 2003). Additional non-pharmacological treatment could enhance antihypertensive treatment strategy, as they could be used to reduce the amount of drugs needed to control hypertension and therefore reduce side effects. In diabetes, hypertension after decades of therapy duration requires usually 3–4 pharmacological agents. Treatment adherence remains very low (Poluzzi et al., 2007), so that further approaches to complement the complex pharmacological therapy are necessary to reach the therapeutic goal.

5. Conclusions

Even in already well controlled diabetic patients, guided breathing had beneficial influences on cardiovascular autonomic control and a measurable impact on BP control. It enriches available options for non-pharmacological blood pressure and pulse pressure reduction. If consistently used, guided breathing enhances restoration of physiological autonomic balance in patients with diabetes and hypertension. Our initial investigation reveals findings relevant enough to justify further investigation of the impact of guided breathing in hypertensive diabetic patients.

List of abbreviations

ABPM	Ambulatory blood pressure monitoring
ACEI	Angiotensin-converting enzyme inhibitor
ANOVA	Analysis of variance
BP	Blood pressure
CAN	Cardiovascular autonomic neuropathy
DASH	Dietary approaches to stop hypertension
DIA	Diastolic (blood pressure)
ECG	Electrocardiogram
FIT	Functional Insulin Treatment
HbA1c	Hemoglobin A1c
HF band	High frequency band
HRV	Heart rate variability
LF band	Low frequency band
LN	Natural logarithm
MAP	Mean arterial pressure
RMSSD	Root of the mean square of difference of normal successive R–R intervals
PP	Pulse pressure
R–R interval	Interval between two normal successive R waves
RCT	Randomized controlled trial
SDNN	Standard deviation of all normal R–R intervals
SYS	Systolic (blood pressure)
VLF band	Very low frequency band

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4.5. Praktická aplikace: výzkum reverzibility CAN a kvantifikace efektu nefarmakologických opatření - radiofrekvenční redukci podkožního tuku

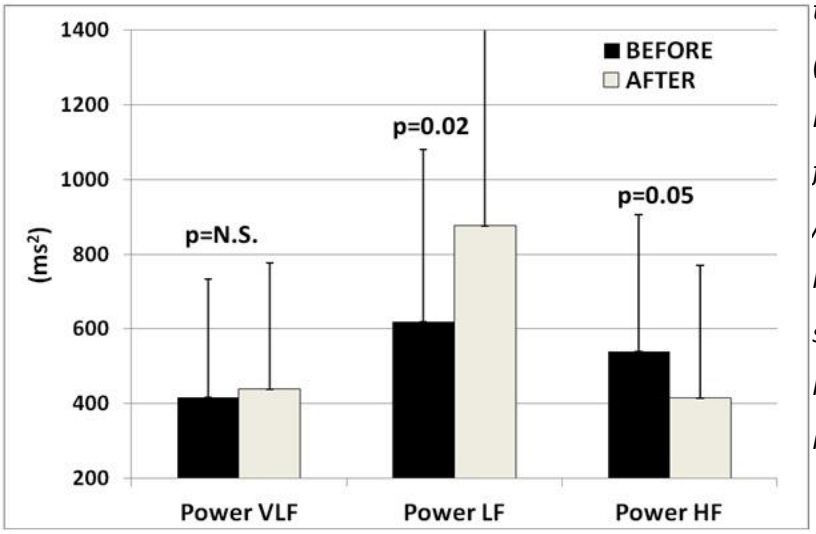
Úvod: Selektivní redukce subkutánního abdominálního tuku pomocí neinvazivních fyzikálních procedur typu radiofrekvenčního elektromagnetického záření nebo kryolipolýzy je jedna z nejžádanějších procedur v neinvazivní estetické medicíně. Nicméně, bezpečnost a účinnost nabízených metod dle akceptovaných vědeckých standardů byla ověřena a publikována pouze u minima technologií.

Cílem naší práce bylo ověřit bezpečnost a účinnost nové bezkontaktní radiofrekvenční technologie Vanquish® při redukci podkožního tuku, která se používá v neinvazivním tvarování těla u pacientů s elementy metabolického syndromu s BMI 25-30 kg/m^2. Provedli jsme retrospektivní analýzu 20 klientek našeho centra, které absolvovaly ošetření dle protokolu výrobce (4 sezení po 30 minutách) a byly ochotné dostavit se k vyšetření/follow-up vizitám 1 a 3 měsíce po ošetření. Kromě vyšetření kardiovaskulárních autonomních regulací jsme rovněž vyšetřili laboratorní parametry včetně adiponektinu a leptinu, C-peptidu a dalších ukazatelů inzulinoresistence.

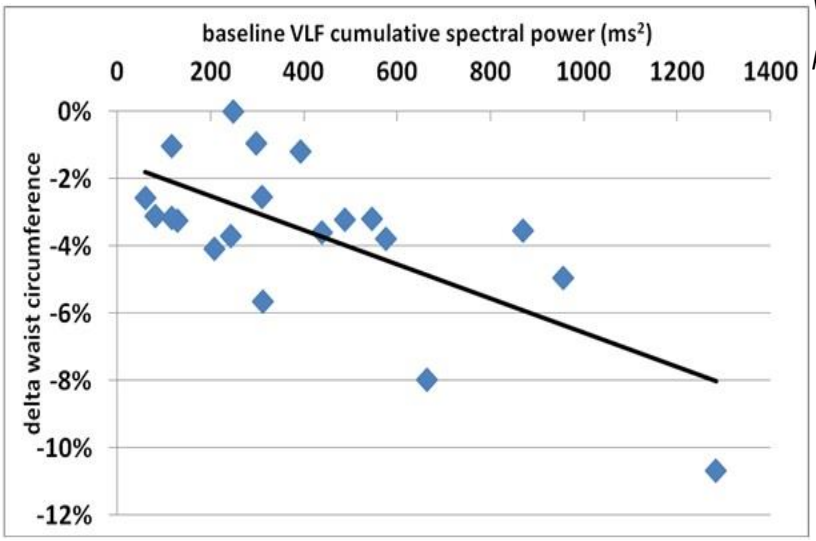
Výsledky: Série čtyř 30-minutových ošetření systémem Vanquish vedla k statisticky významné redukci obvodu pasu, měřeného na predefinovaných místech v období ihned po ukončení ošetřování, 1 a 3 měsíce poté ($p<0.02$-0.001). Signifikantní korelace ($r=-0.58$, $p=0.007$) byla nalezena mezi redukcí obvodu pasu a vstupní hodnotou VLF (very-low-frequency) spektrálního výkonu, který odpovídá sympatiko-termoregulačním aktivitám organismu *(Task Force 1996)*, viz **Obr.21.** Ošetření mělo okamžitou (20±14min po ukončení) přechodnou odezvu v elevaci sympatikotonu ($p=0.02$) a poklesu tonu parasympatiku ($p=0.05$). Nicméně dlouhodobě (39±18 dnů po ukončení) nevedla série ošetření k žádným změnám v kardiovaskulárních autonomních regulacích, viz **Obr.20**. Statisticky významná korelace byla vysledována mezi vstupní hodnotou adiponektinu a okamžitou autonomní odezvou po ošetření v VLF a celkovém spektrálním výkonu jakož i delta obvodu pasu po ukončení ošetřování ($r=0.59$, $p=0.006$ and $r=0.45$, $p=0.04$, resp.), viz **Obr.22**. Jako vysvětlení těchto asociací se zde nabízí např. antilipolytický vliv u hyperinzulinních/ inzulinorezistentnějších klientek. Tato retrospektivní analýza slouží jako proof-of-concept pro prospektivní studii na větší populaci.

Závěr: Tento nález je v souladu se studií Matsumota et al, *(Matsumoto 2001)* který si povšiml, že autonomní odpověď na termogenní impuls nebo kapsaicinem kořeněnou potravinu, sledovaná v HRV spektru o velmi nízké frekvenci, byla u obézních jedinců nižší než u neobézních. Hodnocení spektrálního výkonu v VLF pásmu by mohlo přinést bližší informace o podílu autonomního systému na regulaci energetické bilance organismu. Hypoteticky by tedy snížený vstupní výkon ve VLF pásmu mohl implikovat nižší sympatikotermogenní odpověď na terapeutická opatření zaměřená na redukci nadváhy a abdominální podkožní tukové vrstvy.

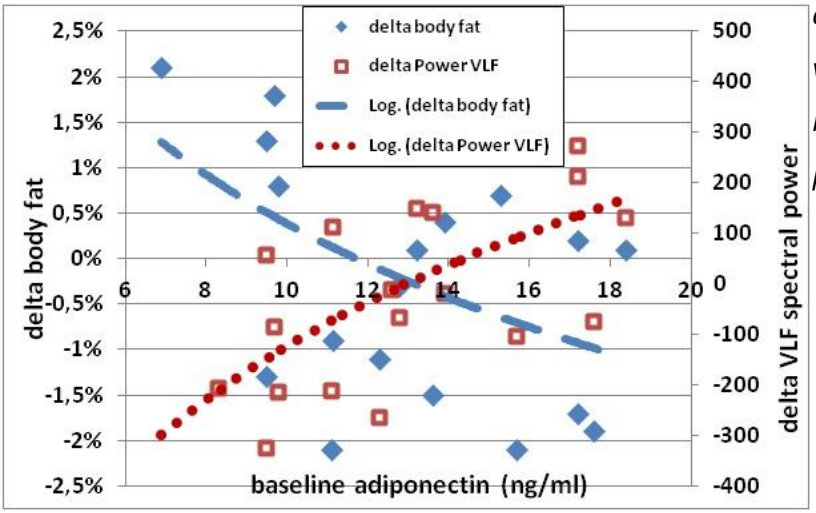
Obr. 20: Okamžitý efekt radiofrekvenční léčby *systémem Vanquish® za účelem redukce podkožního tuku v abdominální oblasti (20±14min po ukončení léčby). Kumulativní spektrální výkon ve frekvenčních pásmech very low /Power VLF/, low /Power LF/ a high /Power HF/: došlo k signifikantnímu nárůstu sympatikotonní odpovědi (LF pásmo) a k redukci parasympatické (HF).*



Obr. 21. Úspěšnost léčby (redukce obvodu pasu) *významně koreluje se vstupní hodnotou spektrálního výkonu v VLF pásmu (r=-0.69, p<0.001).*



Obr. 22: Obdobně, pokles procenta tělesného tuku *při bioimpedančním měření kompozice těla po čtyřech ošetřeních Vanquishem významně koreluje se vstupní hodnotou adiponektinu (r=0.45, p=0.045).*



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Non-contact radiofrequency-induced reduction of subcutaneous abdominal fat correlates with initial cardiovascular autonomic balance and fat tissue hormones: safety analysis

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Author's contributions:
Jiri Pumprla and Kinga Howorka contributed equally to the paper.

Running head: Radiofrequency fat reduction correlates with initial autonomic balance

Key words: Metabolic syndrome, insulin resistance, selective-field radiofrequency, body contouring, subcutaneous fat reduction, heart rate variability, autonomic control

Abstract

Background and objective:

The non-invasive reduction of subcutaneous abdominal fat became popular in the last decade. Radiofrequency (RF), non-non-contact, selective-field device Vanquish® has been developed to selectively induce deep fat tissue heating to reduce waist circumference. Our analysis was designed to evaluate immediate and sustained effects of this treatment on cardiovascular autonomic function and on selected metabolic parameters.

Study Design/patients and methods:

A retrospective, single site proof-of-concept analysis of selective-field RF treatment effects was conducted in 20 individuals with metabolic syndrome, wishing to reduce the subcutaneous abdominal fat. Four 30-minutes treatment sessions (manufacturer´s standard protocol) were performed in 1-week intervals. Vital signs, ECG, lab screening, body composition, subcutaneous fat thickness and spectral analysis of heart rate variability (HRV) have been examined before, after the 1st and 4th treatment, and at follow-up visits 1 month and 3 months after the final treatment.

Results:

The RF treatment lead to a significant reduction of abdominal circumference after the 4th session ($p<0.001$), and during follow-up after 1 month ($p<0.001$), as well as after 3 months ($p<0.02$). There was a significant correlation ($r=-0.58$, $p=0.007$) between reduction of abdominal circumference and initial very-low frequency (VLF) spectral power at follow-up after 1 month. In terms of immediate effects of the treatment, a significant increase of cumulative spectral power in low frequency ($p=0.02$, LF) and reduction in high frequency ($p=0.05$, HF) band have been observed immediately (20 ± 14 minutes) after the treatment. On the contrary, no sustained impact on autonomic balance has been recorded 39 ± 18 days after the treatment. A significant correlation was observed between the initial adiponectin values and immediate autonomic response to one treatment, as observed in VLF and total spectral band ($r=0.59$, $p=0.006$ and $r=0.45$, $p=0.04$, resp.).

Conclusions:

Our analysis shows that the selective-field RF treatment is safe and efficient for reduction of subcutaneous abdominal fat. While the treatment intervention increases the immediate sympathetic response of the body to deep tissue heating, no sustained change in autonomic function could be recorded 1 month after the treatment series. The observed correlation between initial VLF spectral power and waist circumference reduction after the treatment, as well as the association of initial adiponectin values and immediate autonomic response to the treatment might be instrumental for decisions on therapeutic strategies in body contouring.

Introduction

Obesity considerably impairs individual health and aesthetic appearance. In addition to its known health impacts -- reduction of life expectancy and quality of life[1] -- obesity leads to numerous problems including disadvantages in employment[2], in social interactions and decreased satisfaction with own body image[3]. These aspects lead to social pressure and subsequently to increased demand for effective procedures for weight reduction, body contouring and beauty enhancement.

Central obesity is associated with insulin resistance and related components of metabolic syndrome that can be typically treated by nutritional, behavioural and lifestyle changes[1]. Although the reduction of subcutaneous fat alone does not lead directly to reduction of cardiovascular risk in obese subjects[4], there is some evidence that the large-volume liposuction might positively influence the insulinemia[5] and thus insulin sensitivity. Furthermore, clinical experience shows that aesthetic procedures leading to improved patient´s self esteem[6] often significantly enhance the motivation to further lifestyle changes towards healthier goals.

Various invasive and particularly non-invasive body contouring procedures for reduction of subcutaneous fat layers have been introduced in the last decade. While the surgical liposuction still counts for the most effective gold-standard procedure in this respect[7], due to its invasiveness, downtime and side effects, a bunch of non- or semi-invasive procedures became available as its indirect alternative on the quickly growing (often called "lunch-time-procedure") market[8]. However, despite many individual – often only anecdotal – user reports, only a minority of methods is proven according to the evidence-based medicine standards. Such evidence is available for efficacy of chemical lipolysis, based on injection of phosphatidylcholine and deoxycholic acid[9], and for selected energy-based technologies using focused ultrasound[10], cryolipolysis[11] and/or thermal/radiofrequency for lipolysis[12]. Despite the broad use in the practice, clinical safety data of these aesthetic procedures are scarce, with only a few publications available[13]. Although these intensive procedures might have a significant impact on autonomic homeostasis and individual health, to our knowledge, no immediate or sustained effects of such treatments on autonomic function have been investigated.

Analysis of beat-to-beat fluctuations of heart rate (heart rate variability, HRV) is an established tool to non-invasively quantify cardiac autonomic function[14]. The frequency (spectral) decomposition and quantification of irregular course of heart rate into three main frequency bands allows a detailed view of different domains of the cardiovascular control. The short-term HRV spectral analysis is proven useful for assessment of impact of various physiological stimuli on the body such as food restriction[15], endurance physical training[16] or guided breathing[17]. In particular, the very low frequency (VLF) spectral band has been shown to reflect thermoregulatory vasomotor mechanisms, changes in peripheral chemoreceptor activity and fluctuations in renin-angiotensin systems[14,18]. In this respect, analysis of the VLF band enables the quantification of sympatho-thermogenic autonomic responses related to energy metabolic control, as it has been demonstrated e.g. by an acute cold exposure, spicy food containing capsaicin and green tea extract or low-calorie diet[19-22].

The selective-field radiofrequency device Vanquish®, using electromagnetically induced rapid oscillations of electrical dipoles to heat up the fatty tissue[23], is increasingly being used for reduction of subcutaneous abdominal fat. Its efficacy has already been demonstrated[24]. However, although the reported patient acceptance of these treatments was well to superlative[24], no metabolic and/or safety data have been published yet. Our aim was therefore to evaluate the safety and efficacy of this novel technology in a proof-of-concept retrospective data analysis of all clients who attended our clinic and were subjected to the treatment including follow-up within the 5-months time period. This paper focuses on the immediate and sustained effects of the treatment on the autonomic balance of the body and related metabolic values. The analysis is thought as a preparation for a further controlled prospective observation.

Patients and methods

Study design

A retrospective, uncontrolled, single site proof-of-concept analysis of the impact of selective-field radiofrequency treatment on cardiovascular autonomic control and on selected metabolic data (insulin resistance parameters and fat tissue hormones) was conducted in overweight individuals with components of metabolic syndrome and visually detectable excess of subcutaneous fat who wished to reduce the abdominal circumference. Data have been routinely acquired before, at visits immediately after the 1st and 4th treatments, and at follow-up visits in 1-month and 3-months after the last treatment. For assessment of metabolic data, blood sampling was performed before, on the next morning after the 1st and 4th treatments, and 1 and 3 months after the last treatment. Assessment of the intervention effect on the autonomic balance, using the standardized analysis of short-term heart rate variability as obtained during the modified orthostatic load[18,25], was performed before, immediately (acute effect) after the 1st treatment, and 1 month (sustained effect) after the last treatment. These data have been acquired during routine services of the clinic.

Inclusion and exclusion criteria

The selective-field RF treatment protocol has been offered to all individuals with visually excessive subcutaneous fat wishing to reduce their waist circumference. In the retrospective evaluation of efficacy and safety all patients have been included who accepted the necessity of follow-up investigations. Attendance of follow-up visits was a prerequisite for waiving their treatment fees. No reimbursement or coverage of travel expenses have been offered to these patients.

The following standard routine criteria of our clinic for exposition to RF treatment were applied: *Inclusion* criteria were age 20-70 years, both genders, BMI over 25kg/m^2, abdominal circumference over 80 and 94 cm in women and men, respectively, with at least 20 mm of abdominal subcutaneous adipose tissue (as measured by calliper at predefined locations), stable weight over the last 6 months and signed informed consent on treatment. *Exclusion* criteria were pregnancy or insufficient contraceptive methods, surgical liposuction within the last 12 months, insufficiently controlled metabolic disease including diabetes mellitus of both types, untreated hypo- or hyperthyroidism, uncontrolled liver, kidney or cardiovascular

disease, implanted pacemaker or metal implant, acute or feverish disease, history of thrombophlebitis, any haematological disease, chronic medication of corticosteroids, beta-blockers, anticoagulants, insufficient treatment adherence or any other clinical or biochemical condition bearing potential to interfere with the treatment targets. Females in child-bearing age were educated about necessary contraceptive methods, and those planning pregnancy in the following 12 months were not subjected to the RF treatment.

Patients

The study population consisted of n=20 (f=18/m=2) subjects with age 47.8±7.2yr, BMI 28.2±3.6 kg/m^2, abdominal circumference 96±9 cm, insulin resistance HOMA2 index 1.49±0.80 with insulin sensitivity of 79.8±28.9%, fat percentage in body composition 38±7%, blood pressure 138±12/79±7 mmHg, and with reported insufficient aerobic activity/median 30/Q1=0, Q3=60/min weekly. Chronic treatment of concomitant diseases remained unchanged during the whole treatment period. Six female patients received substitution of hypothyroidism resulting in euthyroid values of TSH (x=1.2±0.8 mU/l), four subjects used antihypertensive medication (ACE inhibitors or sartans) and four subjects had lipid lowering agents (statins). Eight female patients received oral contraceptives. Further details can be found in **Table 1**.

Intervention

The non-non-contact, selective-field radiofrequency system Vanquish® (BTL Industries) has been used for treatment of subcutaneous fat layers[23]. All subjects underwent four 30 minutes treatment sessions in the abdominal area, with one week break between the sessions, as recommended in the standard treatment protocol by the manufacturer. Flat multipolar applicator panel was used for emitting the radiofrequency (27.12 MHz) energy for selective generation of deep tissue thermal heating of adipose tissue layers. The unit adjusts the parameters of the emitted energy in real time and shows the instantaneous value on display. This electromagnetic radiation is heating up the adipose tissue much more effectively then surrounding tissues, while limiting potential side effects due to minimized exposition of skin, muscles, or internal organs to this energy[23]. The treatment procedure consists of placing the emitting panel over abdomen and flanks close to the skin using a

spacer which standardizes distance between the panel and the body surface. Once it is in a proper position, treatment can be started while the intensity of the emitted energy is set according to the protocol and to tuning efficiency of the system. The skin temperature is measured before, in 10, 20 and 30 minutes during the treatment, while the subject is frequently asked to give feedback on subjective thermal perception and to immediately report any pain or unpleasant sensations. Operator adjusts the emitted energy intensity close to the tolerable level according to client´s feedback during the treatment procedure and to the measured skin temperature while the safety threshold is set to 42°C.

Routine clinical assessments

Blood sampling, autonomic balance evaluated by heart rate variability analysis, clinical assessment including vital signs, casual blood pressure, electrocardiogram (ECG), body composition evaluated by bioelectrical impedance measurements, abdominal circumference in three predefined points and anthropometric assessment by calliper were evaluated at predefined time points as indicated elsewhere.

Measurement of vital signs

Casual blood pressure has been measured in accordance with standard recommendations[26] in sitting position, using validated oscillometric automated monitor Omron M6 (Omron, Japan). The average from three measurements has been used for data analysis.

A calibrated, computer-assisted system ECG Seiva (Seiva, Czech Republic) has been used for 12-lead surface ECG recordings. Data have been electronically stored and evaluated by a single specialist experienced in ECG readings.

Height has been measured by validated ultrasound height measuring unit ADE MZ10020 (ADE, Germany) within standardized conditions as set by manufacturer.

Temperature before and during the treatment sessions has been measured by a calibrated non-non-contact infrared skin thermometer BaseTech IRT-350 (BaseTech, Germany) at predefined locations (around umbilicus and at upper and lower abdominal wall on both sides)

Weight has been measured within the body composition assessment as described below.

Assessment of weight and body composition

Body composition has been assessed by a calibrated scale, Omron BF 511 (Omron, Japan), a 8-sensor, one-frequency (50 kHz, 500 uA) bioelectrical body impedance analysis device, under strictly standardized conditions as set by the manufacturer. The device delivers along with weight and BMI also gender-specific percentage of body fat and muscle mass, basal metabolic rate (in kcal) and amount of visceral fat (arbitrary units). The declared weight measurement accuracy is 1%[27].

Assessment of waist circumference

Waist circumference was measured using a measuring tape with a spring handle (www.netzwerk-lipolyse.de), in order to control for the pressure exerted on the patient´s abdomen. Three measurements in different locations have been performed at the end of gentle expiration, in the standing position: horizontally around the patient's abdomen at its narrowest part (under the rib cage), at the level of the umbilicus, and 5 cm below the umbilicus. Data were recorded to the nearest millimetre.

Assessment of subcutaneous fat layer using calliper (skinfold thickness measurements)

All measurements were performed with the subject in standing position. The measurement points were selected as follows: above the iliac crest in the mid-axillary line, right and left, paraumbilically at 1/3 distance between the iliac crest and umbilicus, right and left, and 5 cm below umbilicus. The skinfold was pinched up firmly between the thumb and forefinger and pulled away from the underlying tissues. The measurements were performed with calibrated calliper of Harpenden type, ie. with a constant measuring pressure 10p/mm^2, in accordance with established guidelines[28]. The results are presented in mm, as average of five subsequent measurements per one point.

A standard blood sampling has been performed in the morning by venipuncture after an overnight 10 hours fasting. After clotting, the serum was separated and immediately explored for most analyses. For fat hormones, the serum was stored at -20°C until analysed. Insulin resistance was evaluated using the HOMA2 calculations based on fasting glycemia and C-peptide values (Homeostatic model assessment as described by Levy et al[29]).

Assessment of autonomic balance

A standardized analysis protocol of short-term HRV in time and frequency domain as obtained during a modified orthostatic load (5 minutes supine and 5 minutes in standing position) has been used for quantification of treatment effects on the autonomic control of the body[14,18,25]. The HRV measurements have been performed using the VariaCardio TF5 system (Advanced Medical Diagnostics Group, UK). The main principle of spectral analysis of HRV is a decomposition (using fast Fourier transform algorithms) of irregular fluctuations of heart rate into regular cycles that represent influences of various domains on the autonomic balance. Such resulting spectral power is then quantified within three standard frequency bands: (1) very-low frequency component (VLF, 0.01-0.04 Hz), its cycles occur with typical frequency of 0.01 Hz, corresponding to wavelength of 100 seconds. The VLF power is related to control of energy metabolism and thermoregulation, changes in peripheral chemoreceptor activity and fluctuations in renin-angiotensin system, (2) low-frequency component (LF, 0.04-0.15 Hz), with typical variations occurring at frequency 0.1 Hz, i.e. 6-times per minute. It represents predominantly sympathetic control with certain amount of vagal influence, (3) high-frequency component (HF, 0.15-0.4 Hz), with cycles fluctuating at average frequency 0.25 Hz, ie 15-times per minute. This power is related to respiratory activity and parasympathetic control[14]. While the VLF band is mediated primarily by sympathetic control and the HF by the parasympathetic one, the middle one, LF band, includes both, with predominance of the sympathetic branch of the autonomic control[14,18,30]. The main parameters of the analysis are the spectral power (area under the curve) in each of the individual bands and in the total frequency band, the centroid frequencies and the relative proportion of individual frequency bands contents in the total spectral power. We have shown previously that the cumulative numbers generated by summing up the individual frequency band spectral powers over both test positions increase the discrimination power/capability of respective parameters[32].

Statistical analysis

Statistical analysis was performed using standard statistical packages (SPSS, Statistical Package for the Social Sciences V10.0, SPSS Inc., Chicago, USA). Normality of data

distribution was verified by Kolmogoroff-Smirnoff test. A two-tailed paired Student's t-test was applied to estimate differences between groups in case of normal data distribution. Relations among variables were assessed using Pearson´s correlation analysis. Data are presented as means $\pm$ SD, unless indicated otherwise. The significance level was set a priori at $p \leq 0.05$.

Results

Treatment intensity

During all four sessions, the average skin temperature values before, at 10, 20 and 30 minutes of treatment were 31.8±1.1, 39.8±0.7, 39.6±0.6 and 39.2±1.0°C respectively, while the delivered total average maximum energy was 158.5±13.0 W and the total average effective energy was 156.2±13.1 W. While starting the treatment session at 160 W energy level as suggested by manufacturer, in 25 out of 84 (29,8%) sessions the energy intensity could be increased -- in accordance with subject´s heat sensation -- to 170-200 W within the first 10 minutes of treatment, and in 13 out of 84 (15.5%) sessions the energy intensity had to be reduced to 100-150 W due to excess heat perception. The average effective emitted energy in each of four treatment session was therefore 156±14, 160±17, 160±19, and 153±16 W respectively. A significant correlation between the averaged skin temperature after 30 minutes of treatment and reduction in abdominal circumference was observed 1 month after the last treatment (r=-0.49, p=0.03).

Vital signs

When compared with initial values, the average casual blood pressure was significantly lower after the 4th treatment session (134±12 vs. 127±10 mmHg, p=0.003) and raised to 129±9 mmHg (p=0.04 vs. initial value) 1 month after the treatment. The average heart rate has changed from 69±12 to 67±9/min (p=0.04) after 4th treatment session, and raised to 69±11/min (p=0.33, both p vs. initial value) after 1 month. No other significant changes have been observed ECG.

General effects

The radiofrequency selective-field treatment lead to a significant reduction of abdominal circumference as measured at 3 different locations after the 4th session (umbilicus, 96.1±9.3 vs 93.7±9.0 cm, p<0.001 vs. initial value), and during follow-up after 1 month (92.6±9.6 cm, p<0.001) as well after 3 months (93.3±10.1 cm, p<0.02). Despite the significant drop in body weight at follow-up 1 month after the treatment (from 78.8±12.4 to 78.0±12.1 kg, p=0.001), no significant correlation has been found between the deltas in body weight and abdominal circumference values vs. their respective initial values at this time point (r<0.41, p>0.07). The

weight increased to 78.4±12.0 kg after 3 months follow-up. No statistically significant change in body composition (in percentage of body fat and muscle mass) has been recorded during all three measurements vs. initial values.

Autonomic balance

Regarding the immediate effects of the treatment on autonomic balance, a significant increase in low frequency (p=0.02) and reduction in high frequency (p=0.05) band cumulative spectral powers have been observed in HRV 20±14 minutes after the treatment. No sustained effects on autonomic balance, however, have been observed during the follow-up period after the treatment. Figures 1 and 2 summarize the impact of the treatment on autonomic balance immediately after one treatment and 39±18 days (sustained effect) after the last treatment, respectively.

At follow-up after 1 month, there was a significant correlation between the reduction of abdominal circumference and the initial very-low frequency band cumulative spectral power (r=-0.58, p=0.007, **Fig. 3**). Moreover, in a subgroup comparison, subjects with a higher initial cumulative VLF power (6.4±0.4 LN ms^2) demonstrated a significantly bigger drop in abdominal circumference after the 4th treatment (4.1±1.9 vs. 2.6±0.9 cm, p=0.045) than those with lower initial cumulative VLF spectral power (5,1±0.5 LN ms^2).

Interrelationships between treatment effects and metabolic parameters

As expected, a significant correlation between weight and insulin resistance index based on HOMA2 calculations has been observed before the treatment (p=-0.53, p=0.016). Change of body weight correlated significantly with the initial HOMA2 indices after the 4th treatment (r=-0.54, p=0.014 for HOMA2, and r=-0.47, p=0.036 for % beta function) and 1 month after the last treatment (p=-0.57, p=0.009 for % beta function). There was a significant correlation between the initial adiponectin values and deltas of total body fat percentage (r=-0.45, p=0.05) and body weight (r=-0.52, p=0.02) observed after the 4th treatment.

Metabolic and autonomic interrelationships

The immediate autonomic response to one treatment (Fig. 4), as observed in the VLF band (r=-0:59, p=0.006) and in the total spectral power (r=-0.45, p=0.04) correlated significantly

with the initial adiponectin values. Furthermore, a subgroup analysis based on initial adiponectin values (cut-off value 13.0 ng/ml) revealed a significantly stronger acute autonomic response to one treatment in those with higher initial adiponectin level (15.8±1.8 ng/ml) than with a lower one (10.3±1.8 ng/ml). Similarly, in respect to sustained effects, there was a significant correlation between delta of adiponectin values 1 month follow-up vs. initial values and delta of autonomic response in VLF band 1 month follow-up vs. initial values (r=0.48, p=0.03).

Side effects and drop-outs

Overall, two drop-outs have been recorded. In two cases the local skin irritation led to interruption of the protocol after the 2nd treatment. These subjects were not included in the analysis. One subject underwent elective surgery at 1 month of follow-up and did not attend the planned visit. Four subjects did not attend the last follow-up visit after 3 months.

After first two treatments, one subject reported abdominal discomfort, and another one a hyperesthesia around umbilicus. These symptoms resolved within 1 week after the treatment session. No more adverse reactions have been observed after exchange of spacer used for proper positioning of the energy emitting panel at the 3rd treatment session.

Dataset 1. Data of non-contact radiofrequency-induced reduction of abdominal fat HRV. The heart rate variability data are provided.

Click here to access the data.

http://dx.doi.org/10.5256/f1000research.5708.d38309

Discussion

The principal findings of our study are threefold: the efficacy of selective-field radiofrequency treatment in terms of reduction of waist circumference up to 3 months after the treatment series was confirmed. The treatment is safe, as no clinically relevant side-effects were observed. The impact on autonomic cardiovascular balance is significant but transient, while being limited to an increased sympathetic response immediately after this energy-based treatment in abdominal area. No sustained effect of the intervention on autonomic balance has been observed 1 month after the last treatment. The treatment efficacy is inversely associated with insulin resistance and other features of metabolic syndrome and may be explained by the inhibitory effect of higher insulin levels on the (treatment-induced) lipolysis[32]. Whether the treatment efficacy could be better predictable using assessment of VLF spectral power and insulin resistance profile, and/or it could be further supported e.g. by pharmacological agents (such as metformin or insulin sensitisers[33]) or other means, this should be investigated in further prospective trials.

There is a pathogenic link among autonomic imbalance, insulin resistance and obesity. In addition to genetic background and lifestyle factors, autonomic imbalance could be a common root of obesity, hypertension and/or type 2 diabetes mellitus. At early stages of obesity/metabolic syndrome development, parasympathetic control is decreased while sympathetic overactivity usually occurs[34]. This dysfunction increases cardiovascular workload, hemodynamic stress and induces potentially significant cardiac pathology leading to serious arrhythmias. It remains open, however, whether elevated sympathetic tone is a *primary* feature that contributes to the development of obesity and metabolic syndrome or if it develops and/or changes *secondary* to the obese state.

It has been shown that sympathetic overactivity precedes the development of insulin resistance and type 2 diabetes mellitus[35]. Studies with genetically predisposed humans with insulin resistance have shown that early insulin resistance is already associated with increased sympathetic control, and it has been suggested that hyperinsulinemia is the initiating factor leading to increase of sympathetic neural activity[36]. Subsequently, adrenoceptor down-regulation and/or reduced sensitivity are likely to develop which situation results in a secondary reduction of sympathetic responsiveness. As adrenoceptors are involved in control of energy expenditure, their down-regulation leads further to

impaired food-induced thermogenesis and post-prandial fat oxidation, promoting the accumulation of body fat. In this way, the development of obesity can be seen as a consequence of inappropriate/insufficient sympathetic control, energy dissipation, gaining weight and then insulin resistance[37]. This theory also confirms the earlier popular Bray´s MONALISA hypothesis, stating that *"Most Obesities kNown Are Low In Sympathetic Activity"*[38]. It is also consistent with findings from population studies, e.g. in observation of 7000 individuals without hypertension at baseline, low heart rate variability predicted greater risk of incident hypertension over 9 years of follow-up[39]. Similarly, in almost 2000 participants of Framingham Offspring Study, LF power and LF/HF ratio were lower in diabetic subjects than in those with normal fasting glucose. HRV was inversely associated with plasma glucose levels and was reduced in diabetic individuals as well as in subjects with impaired fasting glucose levels[40].

Heart rate variability measurement is an established tool for the assessment of impact of intervention on autonomic balance[15-17,41,42]. While the HRV LF and HF frequency bands have been sufficiently studied in short- and long-term ECG recordings, interpretation of the VLF band -- particularly in short-term recordings – is less explored. Along with influences coming from sympatho-thermoregulation, renin-angiotensin system and chemoreceptors, a clear VLF response to excessive temperature variations has been demonstrated[19]. Further on, significant impacts of a spicy food[20] or capsaicin[21] on VLF spectral power have been reported. These findings are consistent with our results where a significant correlation has been observed between the initial adiponectin level and the immediate VLF band autonomic response to a single treatment, as well as between the initial adiponectin and reduction of percentage of body fat after the treatment series (Fig.4). Additionally to fat percentage, the initial VLF spectral power significantly correlated with change in waist circumference seen after the treatment series (Fig.3). These observations raise a question whether individuals with higher VLF spectral power and higher adiponectinemia/lower insulin resistance might enjoy a better sympatho-thermogenic capability to "burn" the available energy while more readily inducing lipolysis processes, than those individuals with lower VLF tone. This hypothesis might have clinical implications in weight management programs and/or body contouring treatments for subcutaneous fat layers reduction.

At present, there are some new therapeutic targets and procedures taking into account autonomic imbalance in obesity as an independent and sensitive marker of health. Autonomic dysfunction is reversible with lifestyle changes such as hypocaloric nutrition or fasting[15] and physical endurance training[16]. Recently, the approach of cold-induced, facultative thermogenesis aiming for sympathoadrenergically-mediated weight reduction by stimulation of brown fat tissue has been introduced[43,44]. A sympathetic stimulation of brown fat tissue leading to increased daily energy expenditure by 200-400 kcal[45] has been suggested as the main mechanism in this successful model, and there is some hope for success of this approach on the development of obesity[46]. The conversion of white adipose tissue to the highly thermogenic beige adipose tissue has been shown to be influenced by acute sympathetic activation, as well[47]. Since the autonomic imbalance is a marker of adverse risk[14,18], its improvement resulting from weight loss should be beneficial for the health of obese/diabetic individuals.

Our relatively short and limited observation could not deliver sufficient evidence on whether subcutaneous abdominal fat reduction using selective-field RF treatment improves obesity-related cardiovascular risk. However, despite even only little changes in body weight, patients with significantly reduced waist circumference are reported to have an improved metabolic profile[48]. It has been shown that the waist circumference is a proven marker of higher total mortality risk[49] as well as of a cardiovascular risk[50]. Therefore, reducing waist circumference and percentage of fat in body composition may represent an useful and clinically relevant target[48]. Moreover, the initial successful waist reduction may play a significant role in further stimulating the motivation of patients with metabolic syndrome for long-term adaptation and adherence to "healthier" lifestyle habits. As this is a proof-of-concept uncontrolled retrospective analysis, we cannot fully exclude external confounding factors that might have contributed to our observations. As a logical step, a randomized, controlled trial verifying the results in an appropriate patient sample would contribute to a better understanding of our findings.

In conclusion, our analysis provided a proof of concept for safety and efficacy of selective field RF treatment using the standard 4 × 30 minutes protocol for moderate reduction of subcutaneous fat tissue. Only transient and non-sustained effects on autonomic balance have been found during the follow-up after the treatment series. The efficacy of Vanquish RF

treatment in terms of waist circumference reduction was shown and it was significantly related to initial VLF spectral power and adiponectin levels. This implicates that less insulin resistance may offer better conditions for lipolytic action of the treatment. This body contouring procedure was the most efficient in moderate abdominal overweight with lower insulin resistance, and as such can well complement other, systemic clinical measures for weight reduction based on lifestyle and nutritional changes. As the measurements of HRV and fat hormone status are easily performed, we suggest considering the inclusion of these parameters into the clinical prescreening armamentarium in order to enhance the outcomes of aesthetic body contouring methods.

Consent

Written informed consent for publication of their anonymised clinical details was obtainedfrom all patients.

Data availability

F1000Research: Dataset 1. Data of non-contact radiofrequency-induced reduction of abdominal fat HRV, 10.5256/f1000research.5708.d38309[51]

Competing interests

JP received speaking honorarium from BTL Industries. No competing interests were disclosed for KH, ZK and ES.

Author contributions

JP and KH conceived the analysis. JP and KH designed the treatment protocol following the standard recommendation of the manufacturer. JP, ZK and KH carried out the research.
ES contributed to the analysis and provided expertise in cardiology. JP and KH prepared the first draft of the manuscript. ES contributed to the preparation of the manuscript. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

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5. ZÁVĚR

Předložená disertační práce shrnuje důkazy o tom, že rutinní hodnocení krátkodobé HRV – zvláště ve frekvenční doméně při standardizovaném modifikovaném ortostatickém testu – poskytuje u pacientů s autonomní dysfunkcí srovnatelný nebo dokonce lepší diagnostický přínos ve srovnání s výsledky zlatého standardu, Ewingovou baterií reflexnícх testů, přičemž při kratší vyšetřovací době vyžaduje menší nároky na spolupráci pacienta. Tato metoda poskytuje užitečný základ pro sledování účinnosti nefarmakologických i farmakologických intervencí, poskytovaných s cílem dosáhnout reverzibility autonomní dysfunkce při metabolickém syndromu.

Po stránce **metodologické**

(1) Definovali jsme optimální parametry využitelné pro deskripci odlišných stupňů autonomní dysfunkce, sestavili jsme věkově vztažené normy pro posuzování HRV během modifikovaného ortostatického pokusu v časové a frekvenční doméně, a ověřili přinos využití kumulovaných parametrů spektrálního výkonu při posuzování stupně autonomního postižení.

(2) Ověřili jsme, že zahrnutí ortostatické manipulace zpřesňuje diagnostiku CAN a odhaluje i časné parasympatické postižení, jinak neodlišitelné dříve užívanou robustní baterií reflexních testů dle Ewinga.

(3) Prokázali jsme dostatečnou reprodukovatelnost/opakovatelnost obou typů analýz HRV u pacientl s metabolickám syndromem.

Po stránce **klinické/aplikační**

(4) Prokázali jsme, že vytrvalostní fyzický trénink u pacientů s diabetem s různým stupněm autonomní dysfunkce významně zlepšuje HRV, vyjma těch s pokročilým postižením.

(5) Zjistili jsme, že 13-hodinové lačnění je vysoce efektivní ve smyslu zvýšení HRV u pacientů se všemi třemi stupni CAN, včetně těch s pokročilou autonomní dysfunkcí

(6) Řízené zpomalení dechové frekvence pomocí biofeedback-systému vedlo k významnému poklesu již vstupně uspokojivě korigovaných parametrů krevního tlaku, jakož i významnému nárůstu spektrálního výkonu v LF pásmu HRV.

(7) Při aplikaci série čtyř radiofrekvenčních ošetření subkutánního tuku u pacientů

s metabolickým syndromem jsme prokázali, že ač 20±14 minut po ukončení ošetření lze detekovat významnou sympatoadrenergní reakci (vzestup LF a pokles HF pásma), 39±18 dnů po posledním oišetření nejsou detekovatelné změny v autonomních regulacích.

(8) Při využití analýzy HRV během spinální stimulace u pacientů s chronickou ICHS a refrakterní anginou pectoris jsme prokázali signifikantní vliv intervence na autonomní funkci—zvýšení spektrálního výkonu v oblasti parasympatiku. Tento nález podporuje hypotézu vysvětlující klinický přínos spinální stimulace pozitivním ovlivněním autonomní balance ve prospěch ochranného vlivu vagu.

Analýzu variací srdeční frekvence -- zvláště pokud je provedena ve spektrální doméně – lze považovat za spolehlivou a citlivou diagnostickou metodu, umožňující kvantifikovatelný vhled do stavu pacienta, jakož i za prostředek k efektivnímu dlouhodobému sledování progrese a terapeutických úspěchů při péči o pacienty s metabolickým syndromem a diabetem.

Náš výzkum poskytnul významná data k potenciální reverzibilitě autonomní dysfunkce využitím efektů vytrvalostního tréninku, periodického lačnění nebo řízeného zpomalení dechové frekvence pomocí domácího biofeedback-systému. Uvedené nefarmakologické klinické strategie mohou být kombinovány s farmakologickými opatřeními s cílem další ochrany či zlepšení autonomní funkce. Zda by kombinace obou přístupů zajistila další reverzibilitu nálezu autonomní dysfunkce a potažmo prognozy našich pacientl s metabolickým syndromem a diabetem, by mělo být ověřeno v budoucích pozorováních.

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Ziegler D, Zentai CP, Perz S et al for the KORA Study Group: Prediction of Mortality Using Measures of Cardiac Autonomic Dysfunction in the Diabetic and Nondiabetic Population. The MONICA/KORA Augsburg Cohort Study. *Diabetes Care*, 2008 , 31 , 3: 556-561.

7. ŽIVOTOPIS

Name: Jiri Pumprla **Titles/Degrees:** MD, MBA, MPH
Date of birth: April, 23rd , 1962, in Olomouc, Czech Republic
Address: Domovina 13, CZ 77900 Olomouc, Czech Republic

Present professional engagement

- *Medical centre/outpatient offices, pharmacy and pharmaceuticals distribution, registered clinical trials centre, health education*, *PreventaMed Ltd.,* Owner and CEO, www.vilazdravi.cz
- *Research Group Functional Rehabilitation and Group Education,* Deputy Head, c/o Professor Howorka, *Centre of Medical Physics and Biomedical Engineering,* Medical University of Vienna, AKH 4L, Währinger Gtl 18-20, A 1090 Vienna, Austria; Phone +43 1 40 400 3981, Fax +43 1 40 400 3988. www.diabetesfit.org

Education

1981 - 1987 MD, School of Medicine, Palacky University in Olomouc, Czech Republic
1990 specialisation in internal medicine/board examination
2002 - 2008 Professional MBA in Applied Biomedicine, Donau University Krems, Austria
2006 – 2007 MPH / Master of Public Health, Medical University Vienna, Austria
2006 – ongoing postgraduate PhD studies, Palacky University in Olomouc, Czech Republic

Professional history

1987 - 1989 1st Dept. of Internal Medicine, District hospital, Prerov, CZ
1989 - 1993 2nd Dept. of Internal Medicine, University Hospital, Olomouc, CZ
1991 – ongoing Research group Functional rehabilitation – medical research at Med Univ. Vienna, A
1995 – ongoing Private outpatient medical practice, pharma business, PreventaMed Ltd., Olomouc, CZ
1993 - 2000 Sima Media Ltd, Specialised Devices and Medical Engineering, Olomouc, CZ
2000 - 2005 Advanced Medical Diagnostics Group, Beaconsfield/London & Leeds, UK
2010 – ongoing Aesthetics and anti-aging clinic, Olomouc, CZ

Study stays and clinical experience

1992 – ongoing Research Group Functional Rehabilitation and Group Education, Center of Medical Physics and Biomedical Engineering, Medical University Vienna, Austria, and Private Medical Practice, Professor Howorka, Vienna, Austria.
1992 – ongoing 15 clinical trials as co-investigator at Research Group Functional Rehab., Med Univ. Vienna, 5 clinical trials as principal investigator at PreventaMed Ltd. Good clinical practice certificate.

Other short professional stays/visits

1985 School of medicine, University Volgograd, RU / **1987** School of medicine, University Katowice, PL / **1989** Dept. internal med., District hospital, Neu-Ulm, D / **1990** Institute of Diabetes Research and Treatment, Karlsburg, D / **1990** Dept. of internal med., Univ. hospital Michallon, Grenoble, F / **1991** Dept. of internal med., St.Thomas' Hospital, Dept. of internal med., Charing Cross Hospital, London, UK / **1991** Dept. of endocrinology, NiGuarda Hospital, Milano, I , District hospital, Bergamo, I / **1992** endocrinologist practice, Dr.A.de Ryff-Leche, Basel, CH / **1993** Clinic of diabetes and cardiovasc. diseases, Bad Nauheim, D / **1995** Dept. of endocrinology & nutrition, University of Düsseldorf, D / **1995** Dept. of endocrinology, University Hospital, Aarhus, DK / **1998** Dept. of internal medicine, University Krakow, PL / **1998** Institute of physiology, University Zurich, CH / **1998** Dept. of internal medicine, University Athens, GR / **1999** Dept. of endocrinology, University of Minnesota, USA / **1999** Dept. of anaesthesiology, Charite Hospital, Humboldt University Berlin, D / **2000** Dept of cardiovascular sciences, St George‘s Hospital, London University, UK / **2000** Dept of medicine, Cardiothoracic centre, North Staffordsh Hosp., Stoke on Trent, UK/ **2002** National refractory angina centre, Cardiothoracic centre, University Liverpool, UK / **2005** Minnesota Center for Obesity, Metabolism and Endocrinology, Minneapolis, Minnesota, USA / **2005** Diabetes Research Institute, School of Medicine, Miami, USA

Grants

1992 Study Grant Medical Helpline Foundation, Vienna, A / **1993, 1994** Hoechst DDG Reisesti-pendium, Ulm, Berlin, D / **1995** Travel Grant Diabetes Care and Research Europe, Athens, GR / **1994-1995** Postgraduate Study Grant, University Vienna, A / **1997** European Association for Study of Diabetes Young Scientists Training Course, Perugia, I

Special areas of professional interest

- Research in cardiovascular autonomic dysfunction (spectral- & time-analysis of biosignals) in chronic diseases; risk stratification in heart disease, hypertension & macroangiopathy
- Therapeutic Patient Education, influencing social determinants of health by group education of patients with diabetes and hypertension, co-development of educational media
- Management and optimization of processes in outpatient medical offices, conversion of health, beauty and anti-aging, running clinical trials at non-universitary outpatient setting
- Quality management system in a small private health care organization (SME)

Publications (co-authorship or first author)

- 1 book - translation of „Insulinabhängig?..." (Howorka, Kirchheim publishers) into Czech
- approx. 120 international/national congress presentations
- approx. 100 articles & abstracts in top ranking biomedical journals
- 30 papers in peer-reviewed impacted medical journals (listed e.g. in Medline)

Other professionally relevant activities

- Founder and president of „*Preventa Foundation*", Olomouc, Czech Republic, group education and rehabilitation of patients with cardiovascular disease and metabolic syndrome (since 1996)
- Clinical co-developer of a computer-aided system for risk stratification of patients with cardiovascular disease and/or evaluation of autonomic neuropathy in diabetes. Co-owner of manufacturing companies (1994-2007)
- Article reviewer for top medical journals *Cardiovascular Research*, *American Journal of Cardiology* and *International Journal of Cardiology* (since 1998)
- Expert/Projects reviewer for *European Commission, ICT for Health units* (since 2009, ongoing)

Patent applications

2004 Apparatus for Processing an ECG Signal / Ellis,Vychodil,Pumprla (**EP 1608265**) / **2004** Method and Apparatus for Identifying Features in an ECG Signal / Ellis,Vychodil,Pumprla (**WO 2004084722**)

Languages/Skills

English and German - fluent knowledge, Russian - communication level, Czech as mother tongue.
PC skills - MS Office, Internet/Search engines, Recherche/Information synthesis & analysis, Basic statistics (SPSS), Data protection, Web-based medical services, Telemedicine applications in internal medicine and medical clinical research. Experience with management and data communications for clinical trials. Practical experience with medical marketing, pharmaceutical business. Training in Good Clinical practice

Hobbies

Family, sports, active music, international travelling, learning, meeting & communicating with people

References

- *Malcolm Ellis,* BSc, PhD, CEng, CEO, MIE Medical Research Ltd, Leeds, UK, Phone +44 113 2793710, m.ellis@mie-uk.com
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- *Sheryl Hill*, Founder of FIT USA Foundation, a non-profit diabetes education organisation, Mound, Minnesota, USA, Phone +1 612 472 0202
- *Kinga Howorka*, Prof, MD, Centre of Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria, Phone +43 1 40 400 39810, kinga.howorka@meduniwien.ac.at
- *Robert Ireland,* CEO, Kowa Pharmaceuticals Europe, London, UK, Phone +44 118 944 3803, bireland@kowa.co.uk
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- *Ivo Krc*, Prof. MD, former head of 2nd Department of Medicine, University Hospital Olomouc, Czech Republic, Phone +420 585 85 1111
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8. PUBLIKAČNÍ A VĚDECKOVÝZKUMNÁ ČINNOST AUTORA K XI/2014

1. Publikace související s disertační prací

a) Původní vědecké publikace in extenso uveřejněné v recenzovaných vědeckých časopisech s IF:

1. Howorka K, Pumprla J, Haber P, Koller-Strametz J, Mondrzyk J, Schabmann A: Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy. *Cardiovasc Res*, 1997,34: 206-214.
   IF: 5.2

2. Howorka K, Pumprla J, Schabmann A: Influence of fasting on heart rate variability in diabetic patients with different degrees of cardiovascular autonomic neuropathy. *Diab Nutr Metab/Clin Exp*, 1997, 10: 288-295.
   IF: 0.6

3. Howorka K, Pumprla J, Schabmann A: Optimal parameters of short-term heart rate spectrogram for routine evaluation of diabetic cardiovascular autonomic neuropathy. *J Auton Nerv Syst*, 1998, 69: 164-172.
   IF: 1.4

4. Howorka K, Heger G, Schabmann A, Skrabal F, Pumprla J: Weak relationship between symptom perception and objective hypoglycaemia induced changes of autonomic function in hypoglycaemia unawareness in diabetes. *Acta Diabetol*, 1998, 35 (1): 1-8.
   IF: 3.7

5. Birner P, Heinzl H, Schindl M, Pumprla J, Schnider P: Cardiac autonomic function in patients suffering from primary focal hyperhidrosis. *Eur Neurol*, 2000, 44/2: 112-116.
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6. Moore R, Groves D, Nolan J, Scutt D, Pumprla J, Chester MR: Altered short term heart rate variability with spinal cord stimulation in chronic refractory angina: Evidence for the presence of procedure related cardiac sympathetic blockade. *Heart,* 2004, 90 (2): 211-214.
   IF: 3.1

7. Howorka K, Pumprla J, Jirkovska A, Lacigova S, Nolan J: Modified orthostatic load for spectral analysis of short-term heart rate variability improves the sensitivity of autonomic dysfunction assessment. *J Diab Compl*, 2010, 24: 48-54.
   IF: 1.9

8. Howorka K, Pumprla J, Tamm J, Schabmann A, Klomfar S, Kostineak E, Howorka N, Sovova E: Effects of guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients. *Auton Neurosci,* 2013; 179(1-2):131-7. Doi: 10.1016/j.autneu. 2013.08.065. Epub 2013 Aug 27.

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b) Původní vědecké publikace in extenso uveřejněné v ostatních recenzovaných vědeckých časopisech

9. Jirkovská A, Boucek P, Pumprla J, Hosová J, Skibová J, Wosková V: The Ewing test for autonomic neuropathy and spectral analysis of heartrate variability aid in the diagnosis of Charcot's osteoarthropathy | Vysetrení autonomní neuropatie Ewingovými testy i spektrální analýzou variability srdecní frekvence pomáhá diagnostikovat Charcotovu osteoartropatii. *Vnitr Lek*, 1999, 45 (7): 403-409.

10. Hosová J, Jirkovská A, Boucek P, Pumprla J, Skibová J: Parameters of power spectral analysis of heart rate variability for use in clinical evaluation of various stages of diabetic cardiovascular autonomic neuropathy | Návrh parametrů spektrální analýzy variability srdecní frekvence vhodných pro klinické hodnocení různých stadií diabetické kardiovaskulární autonomní neuropatie. *Vnitr Lek,* 2001, 47 (10): 682-688.

11. Hosová J, Jirkovská A, Bouček P, Pumprla J, Hejnová J, Lacigová S, Skibová J: Normal values of parameters of power spectral analysis of heart rate variability for clinical evaluation of autonomic neuropathy in patients with diabetes. *DMEV*, 2001, 4 (2): 103-108.

12. Pumprla J, Howorka K, Kolackova Z, Sovova E: Non-contact radiofrequency-Induced reduction of subcutaneous abdominal fat correlates with Initial cardiovascular autonomic balance and fat tissue hormones: safety analysis. *F1000Research*, 2014 (accepted for publication)

c) Přehledné/souborné vědecké publikace in extenso uveřejněné v recenzovaných vědeckých časopisech s IF

13. Pumprla J, Howorka K, Groves D, Chester M, Nolan J: Functional assessment of heart rate variability: Physiological basis and practical applications. *Intl J Cardiol*, 2002, 84 (1): 1-14.
    IF: 1.9

d) Přehledné/souborné vědecké práce uveřejněné v ostatních recenzovaných vědeckých časopisech

14. Opavský J, Pumprla J, Salinger J, Howorka K, Thoma H, Vychodil R: Spektrální analýza variability srdeční frekvence - přínos pro diagnostiku diabetické autonomní neuropatie. *Scr Med Fac Med Univ Bru Mas*. 1994, 67, Suppl. 1: 29-32. ISSN: 1211-3395.

15. Salinger J, Vychodil R, Novotny J, Pumprla J, Opavsky J, Stejskal P, Vaverka F, Bula J: Telemetric, computer aided system for noninvasive examination of heart rate variability, type VariaPulse TF 3. *Comput Cardiol*, 1995: 437-441.

16. Pumprla J: Variabilita srdeční frekvence: význam měření pro praxi. *Kapitoly z kardiol*, 2001, 3: 66-70.

17. Pumprla J, Sovová E, Howorka K: Variabilita srdeční frekvence: Využití v interní praxi se zaměřením na metabolický syndrom. *Interní med*, 2014, 16/5: 205-208.

e) Kapitoly v monografiích

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f) Publikovaná abstrakta

18. Pumprla J, Howorka K, Heger G, Schmid R, Thoma H: Erfassung der autonomen Neuropathie: Trennschärfe der Spektralanalyse der Herzfrequenz gegenüber Ewing Standardbatterie von Herzkreislauf-Reflextests. *Diabetes Stoffw* 1994, 3: 109.

19. Pumprla J, Howorka K, Heger G: Fasten erhöht die Herzratenvariabilität bei Diabetikern. 22. Jahrestagung der Österr. Diabetesgesellschaft, Graz, September 1994. *Diab Stoffw*, 1994, 3: 323.

20. Howorka K, Pumprla J, Heger G, Thoma H: Erfassung der autonomen Neuropathie: Trennschärfe der Spektralanalyse der Herzfrequenz gegenüber Ewing Standardbatterie von Herzkreislauf-Reflextests. Jahrestagung der Österr. Diabetesgesellschaft, Graz, September 1994.*Diab Stoffw*, 1994, 3: 317.

21. Pumprla J, Howorka K, Heger G, Thoma H: Selectivity of parameters of power spectral analysis of heart rate variability in autonomic neuropathy. 30th Annual Meeting EASD, Düsseldorf, September 1994. *Diabetologia* 1994: A186.

22. Pumprla J, Howorka K, Salinger J, Opavsky J, Thoma H: Spectral analysis of heart rate variability in autonomic neuropathy. "Cardiovascular Alterations in Diabetes Mellitus" - Post-Congress Workshop of the XV World Congress of the International Society for Heart Research, Budapest, July 1995. *J Mol Cel Card*, 1995, 27: A429.

23. Pumprla J, Howorka K, Heger G, Thoma H, Salinger J, Opavsky J: Evaluation of short-time heart rate variability in cardiovascular autonomic neuropathy in diabetes: which parameter have the highest diagnostic impact. *Comput Cardiol*, 1995 (Proceedings): 82.

24. Pumprla J, Howorka K, Schabmann A, Salinger J, Opavsky J, Thoma H: Untersuchung der kardiovaskulären autonomen Neuropathie mittels Spektralanalyse der Herzraten-variabilität: Welche Parameter haben den größten diagnostischen Einfluß? *Diab Stoffw* 1995, 4: 24.

25. Salinger J, Vychodil R, Pumprla J, Novotny J, Opavsky J, Stejskal P, Vaverka F, Bula J, Howorka K: Diagnostisches System zur nicht-invasiven Untersuchung der Herzfrequenzvariabilität mittels telemetrischer Datenübertragung, Typ Varia Pulse TF3. *Diab Stoffw,* 1995, 4: 18.

26. Pumprla J, Howorka K, Opavsky J, Salinger J, Vychodil R: Spektrální analyza variability srdecní frekvence v detekci pocínající a pokrocilé diabetické autonomní neuropatie. Sborník abstrakt,XXXI. Diabetologické Dny v Luhacovicích, 1995.

27. Pumprla J, Howorka K, Salinger J, Opavsky J, Vychodil J: Diabetická autonomní neuropatie:

srovnání rutinního hodnocení pomocí casové a spektrální analýzy variability srdecní frekvence systémem VariaPulse TF3. XVIII. Endokrinologické dny v Olomouci, Sborník abstrakt, 1995.

28. Pumprla J, Howorka K, Heger G, Opavsky J, Salinger J, Thoma H: Spectral analysis of heart rate variability in the routine assessment of cardiac autonomic neuropathy in diabetes. Abstracts/Proceedings. *Comp Signal Process Clin Med Biomed Engin*, Bern, June 9-10, 1995.

29. Howorka K, Pumprla J, Heger G, Thoma H, Opavsky J, Salinger J: Computerised assessment of autonomic influences of yoga using spectral analysis of heart rate variability. Engineering in Medicine and Biology Society, 1995 and 14th Conference of the Biomedical Engineering Society of India. An International Meeting, Proceedings of the First Regional Conference, New Delhi. *IEEE* P*roceed,* 1995.

30. Howorka K, Pumprla J, Heger G, Thoma H, Opavsky J, Salinger J: Computerised assessment of autonomic influences of yoga using spectral analysis of heart rate variability. *IEEE Engineering in Medicine and Biology Society Annual Conference,* 1995.

31. Pumprla J, Šimek I, Opavský J, Salinger J: Vyšetření autonomního nervového systému u některých onemocnění GIT metodou spektrální analýzy variability R - R intervalů: *Zobrazovací a výpočetní techniky v gastroenterologii*. Prac. den České gastroent. spol. a Sekce gastrointest. radiodiagnostiky Radiodiagnost. spol., Praha, 22.9.1994. *Čes Slov Gastr* 1995, 49,2: 76-77.

32. Pumprla J, Howorka K, Thoma H: Fasting increases heart rate variability in diabetes. Proceedings of the 6th meeting of the Diabetic Neuropathy Study Group of the EASD, August 30th – 03 September 1996, Baden(Vienna). *Diabetologia* 1996, *39* (Suppl. 1): A17.

33. Pumprla J, Howorka K, Schabmann A: Reproducibility of staging of cardiovascular autonomic dysfunction in diabetes using spectral analysis of heart rate variability and battery of reflex tests. *1st Virtual Congress of Cardiology on Internet*, 1996. Http:// pcvc. Sminter. com.ar/ cvirtual/ tlibres3/tnn2608i.htm.

34. Pumprla J, Howorka K, Haber P, Mondrzyk J, Podolsky A, Thoma H, Salinger J, Opavsky J: Vliv pravidelneho telesneho treninku na variabilitu srdecni frekvence u diabetiku s ruznym stupnem kardiovaskularni autonomni neuropatie. Abstrakta. XXXII. Diabetologicke dny, Luhacovice, 1996.

35. Pumprla J, Howorka K, Thoma H, Salinger J, Opavsky J: Lačnění zvyšuje variabilitu srdeční frekvence u diabetiků. Abstrakta. XXXII. Diabetologicke dny, Luhacovice, 1996.

36. Pumprla J, Howorka K, Schabmann A et al: Reprodukovatelnost vyšetření diabetické kardiovaskulární autonomní neuropatie pomocí spektrální analýzy variability srdeční frekvence a Ewingovy baterie funkčních testů. XXXIII. Diabetologické dny, Luhačovice, 1997.

37. Howorka K, Pumprla J, Schabmann A, Thoma H: Development of an analytic standard for evaluating heart rate variability in cardiovascular autonomic neuropathy in diabetes: comparison of short-term spectral analysis with Ewing's standard battery of reflex tests as reference method (Entwicklung der Analysestandards zur Beurteilung der Herzratenvariabilität in kardiovaskulärer autonomen Neuropathie bei Diabetes: Vergleich der Kurzzeit-Spektralanalyse mit Ewing's Standardbatterie der Reflextests als Referenzmethode). *Biomed Eng*, 1998, 43 Suppl : 568-569.

38. Pumprla J, Howorka K, Schabmann A: Routine-Erfassung der kardiovaskulären autonomen Neuropathie bei Diabetes: diagnostische Zuordnungsfähigkeit mittels Spektralanalyse der Herzratenvariabilität. *Diab Stoffw,* 1998, 7 (Suppl. 1): 91.

39. Pumprla J, Howorka K, Anderer P, Saletu B, Krieger M, Schabmann A: Reduction of low-frequency band spectral power and centroid frequency in analysis of heart rate variability in diabetic patients with hypoglycaemia unawareness. *Diabetologia* 1998, 41 (Suppl. 1): A306.

40. Salinger J, Pumprla J, Vychodil R, Stejskal P, Opavsky J, Novotny J, Bula J: Microcomputer system for telemetric assessment of short term heart rate variability in time and frequency domain, type VariaCardio TF4. *Comput Cardiol*, 1999: 599.

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g) Seznam přednášek/posterů přednesených uchazečem na veřejných odborných fórech (*viz seznam abstrakt)*

102. Co-chairman a speaker na Workshopu Functional insulin treatment and modular education. EASD congress, Vienna, 28-09-2009: „International performance benchmarking of diabetes outcomes: modular education counts! "Skeleton key" for self-competence: FIT-Update – annual educational weekend for re-motivation. Success stories."

h) nezařazeno

Postgraduální MBA Professional Biomedicine Degree (Postgraduální univerzita Krems) se zaměřením na farmacii. Diplomová práce na téma *„Calculating the economic impact of outpatient diabetes modular education programme".* 2002-2008

Postgraduální MPH Degree (Master of Public Health, Lékařská univerzita Vídeň) se zaměřením na sekundární prevenci při metabolickém syndromu. Diplomová práce na téma *„Secondary and tertiary prevention with therapeutic education in subjects with hypertension and hyperlipidemia in the context of increasing prevalence of metabolic syndrome"*. 2005-2007

Investigátor v: White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F; EXAMINE Investigators: Alogliptin after acute coronary syndrome in patients with type 2 diabetes. New England J Medicine 2013 Oct 3;369(14):1327-35. doi: 10.1056/NEJMoa1305889

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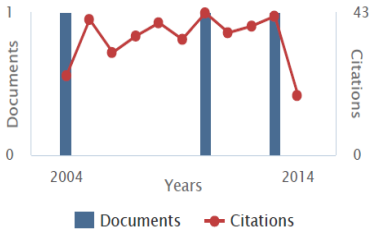

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International Journal of Cardiology
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Functional assessment of heart rate variability: Physiological basis and practical applications (Review)

Pumprla, J.[a], Howorka, K.[a], Groves, D.[b], Chester, M.[b], Nolan, J.[c]

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View references

Abstract

The autonomic nervous system dynamically controls the response of the body to a range of external and internal stimuli, providing physiological stability in the individual. With the progress of information technology, it is now possible to explore the functioning of this system reliably and non-invasively using comprehensive and functional analysis of heart rate variability. This method is already an established tool in cardiology research, and is increasingly being used for a range of clinical applications. This review describes the theoretical basis and practical applications for this emerging technique. © Elsevier Science Ireland Ltd. All rights reserved.

Author keywords

Autonomic system; Heart rate variability; Parasympathetic; R-R interval; Spectral analysis; Sympathetic

Indexed keywords

EMTREE medical terms: analytic method; autonomic nervous system function; cardiology; cardiovascular function; cardiovascular reflex; clinical research; electrocardiogram; functional assessment; heart electrophysiology; heart rate variability; human; medical informatics; non invasive measurement; nonhuman; physiology; priority journal; QT interval; reliability; review; RR interval; theoretical study; time

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1993, 1994 Hoechst DDG Reisesti-pendium, Ulm, Berlin, D
1995 Travel Grant Diabetes Care and Research Europe, Athens, GR
1994-1995 Postgraduate Study Grant, University Vienna, A
1997 European Association for Study of Diabetes Young Scientists Training Course, Perugia, I

- **Stáže a kurzy**

Dlouhodobá stáž na Institutu pro biomedicínskou techniku a fyziku (přednosta: Prof. Ing. Dr. Herwig Thoma), Výzkumná skupina Funkční rehabilitace a skupinová edukace (vedoucí: Prof. Dr. Med. Kinga Howorka, MBA, MPH, MSc)
Krátkodobé stáže viz CV

- **Patenty**

Evropský patent (aplikace): **2004** Apparatus for Processing an ECG Signal / Malcolm Ellis, Rostislav Vychodil, Jiri Pumprla (**EP 1608265**)
Světový patent (aplikace): **2004** Method and Apparatus for Identifying Features in an ECG Signal / Malcolm Ellis, Rostislav Vychodil, Jiri Pumprla (**WO 2004084722**)

- **Ostatní**

Expertní činnost/Review EU projektů v rámci Framework P7 a Horizon 2020 v oblasti Personal health systems a Telemedicínské aplikace, ICT for Health/ DG Connect, Components / REA (Research Executive Agency). Evropská Komise, Brusel. 2009 – dosud

Principal investigator s cca 20-letou zkušeností v provádění klinických hodnocení v interní medicíně (Sub- a Principal inv.), Lékařská univerzita Vídeň (Prof. Howorka, investigator-initiated trials) a následně PreventaMed s.r.o, certifikace dle Good Clinical Practice, úspěšně absolvovaný externí audit 2013 (Takeda), spolupráce s několika CRO. 1993 - dosud

Klinická činnost – soukromá praxe Ordinace vnitřního lékařství/PreventaMed s.r.o. (ordinace, lékárna, edukace, klinické studie, estetická medicína). Od 1995 – dosud

Vývoj a klinický výzkum HRV: Sima Media Olomouc s.r.o., CZ, společník a jednatel. Společnost měla zásadní podíl na klinickém vývoji a iniciálním rozšíření systémů pro měření variability srdeční frekvence VariaPulse TF3/VariaCardio TF4 do ambulantní rutinní praxe a do řady českých a zahraničních odborných pracovišť. 1993-2001

Vývoj a klinický výzkum HRV: Advanced Medical Diagnostics Group, Londýn/Leeds, UK, společník a Medical Director. Společnost měla zásadní podíl na vývoji nové generace předchozí technologie HRV a jejím šíření hlavně v anglicky a německy mluvících oblastech včetně USA a Jižní Afriky. Podány 2 patenty, podána aplikace 510k k FDA pro systém VariaCardio TF5 ke vstupu na trh v USA. Péče o aplikace v 12 zemích světa/4 kontinentech. 2002-2007