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Effects of Metformin Treatment on Myocardial and Endothelial Function in Insulin Resistance Patients: A Metabolomic Study

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Abstract

Introduction: Insulin resistance (IR) adversely affects cardiac performance and peripheral vasodilation reserve. Metformin, prescribed to prevent the progression of IR resistance into diabetes, has been shown to improve myocardial performance and endothelial function in insulin resistant individuals. Metabolomics in the study of the metabolite profile of a biological organism proved its efficacy in detecting metabolites changes as a result of a therapeutic intervention and, so, predicting the response.

Aim: To evaluate myocardial and endothelium-dependent vasodilatory functions in an IR population subsequent to treatment with metformin and to determine the metabolic changes associated.

Methods: Twenty consecutive patients recently diagnosed with IR were studied. All subjects underwent echocardiography with Speckle Tracking technique, peripheral arterial tonometry to measure the endothelial flow reserve (EFR) and metabolomic analysis by ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy and multivariate analysis at baseline and after metformin treatment.

Results: Inter-test comparison performed at baseline and after 3 months of metformin showed a significant reduction in weight (79 ± 15.4 vs 80.9 ± 16.2, P<0.05) and BMI (29.7 ± 5.3 vs 30.8 ± 5.2, p<0.05). Moreover we evidenced a significant increase in EFR (2.1 ± 0.43 vs 1.88 ± 0.47, p<0.05), and of the Global Longitudinal Strain (20.2 ± 4.21% vs 15.4 ± 3.06%, p<0.001).

PLS-DA analysis of the metabolic profile detected by NMR identified two groups significantly different (cross validation p-value=0.005). More significant discriminating metabolites were: lactate, lipids, N.acetyl glycoproteins, valine, choline, betaine,creatine.

Conclusions: The data obtained show that in IR subjects metformin improves myocardial and endothelial function. This effect was associated to significant metabolic changes characterized by means of a metabolomic approach.

Keywords: Metformin; Insulin resistance; Myocardial function; Endothelial function; Metabolomics

Introduction

It is an established fact that insulin produces multiple physiological effects, not only on the glucose metabolism, but also on various structures and organs, such as vascular endothelium, heart, liver, adipose tissue and skeletal muscles [1].

Insulin resistance (IR), a condition in which elevated levels of this hormone produce a sub-optimal biological response, is considered a primary etiologic factor in the development of non-ischemic heart failure [1]. Accordingly, IR is associated with a form of cardiomyopathy in which the myocardium is incapable of responding to injuries by altering substrate metabolism to increase energy efficiency. Moreover, recent studies have shown that IR plays an important role in determining a reduction in cardiovascular performance [2,3]. A high prevalence of IR has been found in the non-ischemic heart failure population. It predates the development of the disease, and independently defines a worse prognosis [4]. In a large community-based sample of elderly men, IR predicted the incidence of congestive heart failure independently of the established risk factors, including diabetes [5]. Several studies have shown that a reduction in endothelial function [6] may represent the link between IR and decline in cardiovascular performance.

Treatment with metformin (MTF), an insulin-sensitizing agent, has been reported to prevent the above-mentioned cardiac abnormalities [7] and the evolution of IR diabetes [8]. Moreover we recently evidenced a beneficial effect induced by MTF on cardiopulmonary performance and on vascular endothelial function in IR patients with high HOMA-IR [9].

Metabolomics is the study of the small-molecule metabolite profile of a biological organism. Unlike previous experimental tools that have focused on a limited number of enzymatic reactions or a single path, metabolmics may provide a functional view of an organism as determined by the sum of its genes, RNA, proteins and environmental factors, including nutrition and drug therapies [10,11]. Metabolomics

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represents an innovative approach in terms of metabolic research and has been shown to produce a marked impact on the investigation of various cardiovascular diseases [10,11].

The present study investigated a population of individuals affected by IR without clinically evident cardiovascular diseases. The aim was to examine the possible effects of early MTF treatment on Left Ventricular (LV) and endothelial function and the underlying metabolic changes.

Materials and Methods

The study was conducted as an open, non-randomized trial; it was approved by the Institutional Ethics Committee (University Hospital, University of Cagliari), and written informed consent was obtained from all subjects. The study was performed in accordance with the Declaration of Helsinki.

Twenty consecutive untrained individuals of both sexes (9 women and 11 men; mean age 47 ± 13years) with impaired glucose tolerance (IGT), and impaired fasting glycaemia (IFG)) referring to the Diabetic Center of our University Hospital were enrolled in the trial. All subjects had a recent diagnosis of IR calculated according to the homeostatic model assessment (HOMA) index ([insulin (μU/mL) x glycaemia (mmol/L)] /22) and defined according to the values of Bonora et al. [12]

Inclusion criteria were the following: patients aged 20-75 years; an echocardiographic LVEF value ≥55% and absence of echocardiographic wall motion abnormalities at rest; normal hepatic and renal function (bilirubin ≤1.5 mg/dl, creatinine ≤2.0 mg/dl); availability of the patient to follow the study protocol. Patients were not eligible if they had a history of cardiac disease, hypertension with evidence of cardiac hypertrophy, diabetes mellitus and/or had been previously treated with insulin sensitizers.

A full baseline routine cardiovascular assessment, including physical examination and bi-dimensional Doppler echocardiogram with Speckle Tacking (ST) technique was performed. At enrolment, all patients underwent a complete series of blood chemistry tests, including oral glucose tolerance test (baseline glycaemia and 120' after a 75 g glucose load) and insulinaemia dosage. Moreover the collection of blood samples for metabolomics analysis was performed in all patients.

At baseline, all patients underwent a digital tonometry of upper limbs for endothelial flow reserve (EFR) measurement. After enrolment MTF treatment was commenced at a standard dose of 850 mg twice a day for the following 3 months. Out of the 20 patients originally enrolled, only 15 completed the study following 3-months MTF administration. Patients were excluded from the study as a result of severe diarrhea (n=2) and as a consequence of voluntary early drop out (n=3).

Over the last 3 days of MTF therapy patients once again underwent echocardiographic and EFR evaluation with the collection of blood samples for metabolomics analysis. Patients were familiarized with the medical environment and the day before the experimental session, all patients were asked to refrain from consuming alcohol, coffee, or aspirin, and to abstain from severe physical exercise.

On the morning of the experiments, patients reported to our laboratory at approximately 9:00 am after a light breakfast and were weighed and measured. They spent at least 20 minutes acclimatizing by lying in a quiet room at a controlled temperature of 22°C and relative humidity of 65%. On reaching a steady state of relaxation, the vascular reactivity test was performed. Subsequently, all patients underwent the Echocardiographic evaluation with acquisition of RAW data for ST analysis.

Endothelial function

Endothelial function was assessed by means of a peripheral arterial tone (PAT) device (Endo-PAT2000, Itamar Medical, Caesarea, Israel) [13]. A probe is placed on the tip of both index fingers to measure pulse volume changes by applying uniform pressure to the skin surface working as a pneumatic plethysmograph. Baseline pulse amplitude was measured from each fingertip for 5 min. By inflating a cuff placed around the proximal forearm with a pressure 60 mmHg above systolic pressure, arterial flow was interrupted for 5 min. Pulse amplitudes were recorded for another 5 min during hyperemia following deflation of the cuff. The natural logarithm of the ratio of post deflation to baseline pulse amplitude in the hyperemic finger divided by the same ratio in the control contralateral finger was used to estimate vascular function (lnPAT).

Conventional and ST echocardiography

Echocardiographic images were recorded using a system equipped with ST 2D imaging and raw data acquisition (Toshiba Artida; Toshiba Corp., Tochigi, Japan). Basal standard 2D measurements were carried out before and after MET treatment.

LV ejection fraction was obtained from the apical 4- and 2-chamber views according to Simpson's rule. Pulsed wave Doppler (PWD) was performed in apical 4-chamber view with the sample volume placed between the mitral leaflet tips and the early (E) and late (A) diastolic peak velocities determined; E deceleration time (DecT) was measured and then the E/A ratio was derived.

A four-chamber view clip was acquired at each evaluation at the apical level. LV longitudinal function was calculated offline from raw data. (Toshiba Corp., Tochigi, Japan). Global systolic strain (GLS) and Strain Rate (GLSR) were obtained by averaging the Strain and Strain Rate of all the segments at the 4-chamber view.

GLS and GLSR values were averaged over 3 cardiac cycles. A single investigator who was unaware of the status of the study patients performed all off-line measurements.

Metabolomics analysis

Chemicals: Deuterium oxide (D2O, 99.9%) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Sodium 3 Dimethyl-Silyl-Sulphonic acid (DSS, 98 atom % D) was acquired from Sigma-Aldrich (Milan, Italy).

Sample collection: Heparinized blood samples were immediately centrifuged at 4000 rpm for 15 minutes; the supernatant was then divided into 800 μL aliquots and stored at – 80°C. At the moment of analysis, specimens were thawed and centrifuged at 4500 rpm for 5 min at 4°C; 400 μL of supernatant was added to a standard solution [consisting of 100μL deuterated water (D_2O) and 150μL of 50 mM DSS] and then transferred in 5-mm O.D NMR tubes.

^{1}NMR spectroscopy: NMR experiments were carried out using a Varian UNITY INOVA 500 spectrometer operating at 500 MHz for proton and equipped with a 5 mm double resonance probe (Varian Inc., Palo Alto, CA).All samples were submitted to identical standard acquisition parameters and pulse sequences. Broad signals were removed and overlapping reduced by applying a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence [14] with a spectral width of 8 kHz, acquisition time 1.5 s, relaxation delay 3 s, and tau=500μs.

The residual water signal was suppressed by applying a pre-saturation technique with low power radiofrequency irradiation for 2 s during relaxation.

^{1}NMR data processing and multivariate statistical analysis: Spectra were imported in Mnova (Mnova Release 7, Mestrelab Research http://mestrelab.com/) and pre-processed with line broadening lb=0.5 Hz, zerofilling to 64k, and Fourier transformed. Each spectrum was phased, baseline corrected and the DSS signal aligned to 0.00 ppm. The ^{1}H-NMR spectra were reduced into consecutive integrated spectral regions (bins) of equal width (0.04 ppm) corresponding to the region 0.7 -8.7 ppm. The spectral regions 3.26 - 3.94 ppm and 4.38 - 5.30 ppm containing the resonances of residual water and glucose resonances were excluded from statistical analysis prior to integration. Normalization of the total area of the spectrum was applied to each sample data set in order to minimize the effects of variable concentration among different samples.

Pattern recognition. In order to recognize metabolic patterns data were analysed using: Principal Component Analysis (PCA), projection to latent structures by Partial Least Squares (PLS), and PLS-Discriminant Analysis (PLS-DA) within the SIMCA-P+ package (Version 13.0, Umetrics, Umeå, Sweden) [14]. PCA is a technique that transforms the variables in a data set into a smaller number of new latent variables known as principal components, which are uncorrelated with each other and account for decreasing proportions of the total variance of the original variables. Each new principal component is a linear combination of the original variables; in such a manner, a compact description of the variation within a data set is generated. When PCA proved inadequate to define a clustering, a supervised approach was used. PLS-DA is a supervised extension of PCA capable of distinguishing two or more classes by searching for variables (X matrix) correlated to class membership (Y matrix). By means of this approach, the axes are calculated to maximize separation between groups and can be used to examine separation that would otherwise be across three or more principal components. PLS, another supervised extension of PCA, is used to maximize the correlation between two data sets to allow for prediction of the response variable Y from X [15]. For each model built, the loading scores and the Variable Influence on Projection (VIP) parameters were examined in conjunction with the original spectra to identify metabolites more significantly contributing to data clustering. Loading scores describe the correlation between original variables and new component variables, whereas VIP parameters are essentially a measure of the degree to which a particular variable explains Y variance (class membership or linear trend). Model performance was evaluated using R^2 and Q^2 parameters, both of which vary between 0 and 1 [15]. R^2 provides an indication of the extent to which a variation within a data set can be explained by the various components of the model. The first three components of a PCA can typically represent 60–90% of the total variation found in an NMR-based metabolomics data set. Q^2 indicates how accurately the data, either classed or non-classed, can be predicted; Q^2 is more relevant for supervised than unsupervised pattern recognition processes. A Q^2 score >0.5 indicates a model that is better than chance, whereas a score between 0.7 and 1.0 demonstrates a highly robust trend.

Statistical analysis

Continuous variables between baseline and post-treatment were assessed using one way ANOVA for repeated measures. Categorical variables were compared by means of Chi square or Fischer exact test, as appropriate. A two-tailed value of $p<0.05$ was considered statistically significant.

Results

Biophysical, clinical, and metabolic characteristics of patients are reported in Table 1. HOMA index revealed the presence of IR in all patients, 6 of whom were overweight and 9 obese.

At baseline, conventional ST echocardiography attested a normal left ventricular (LV) systolic and diastolic function (Table 2).

Three months of MTF treatment produced a significant reduction in weight (79 ± 15.4 vs. 80.9 ± 16.2, P<0.05) and BMI (29.7 ± 5.3 vs. 30.8 ± 5.2, p<0.05). Conventional echo parameters remained unchanged (Table 2). Most interestingly we observed an improvement in longitudinal systolic function at TDI (Sm 7.58 ± 1 vs. 6.97 ± 1.19 cm/sec, p<0.01) and with the deformation indexes at ST (GLS 20.2 ± 4.21% vs. 15.4 ± 3.06%, p<0.001; GLSR 1.3 ± 0.33 s^{-1} vs. 1.06 ± 0.26

	Patients (N=20)
Age (years ± SD)	47 ± 13
Weight (Kg ± SD)	80.9 ± 16.2
BMI (Kg/m^2 ± SD)	30.8 ± 5.2
Hypertension	27%
Total Cholesterol (mg/dl ± SD)	219 ± 17
HDL (mg/dl ± SD)	52 ± 15
LDL (mg/dl ± SD)	129 ± 21
Triglycerides(mg/dl ± SD)	153 ± 57
Basalglycemia (mg/dl ± SD)	115 ± 14
OGTT 120 min (mg/dl ± SD)	142 ± 41
Carbohydratemetabolism	
IFG	72%
IGT	28%
Insulinaemia	20.2 ± 12.2
HOMA (<2.7)	6.2 ± 3.2

Abbreviations: BMI: Body Mass Index; NG: Normoglycaemic; IFG: impaired fasting glucose; IGT: Impaired Glucose Tollerance; HOMA: Homeostasis Model Assessment

Table 1: Basal characteristics.

	Basal	3 Months FU	P value
Weight(kg)	80.9 ± 16.2	79 ± 15.4	P<0.05
BMI	30.8 ± 5.2	29.7 ± 5.3	P<0.05
Conventionalechocardiography			
LVEF(%)	65.8 ±5.6	68.4 ± 7.4	ns
EDV(mL)	73.8 ± 15	67.5 ± 12.6	ns
ESV(mL)	25.2 ± 6.8	21.4 ± 6.6	ns
DecT (s)	0.22 ± 0.04	0.19 ± 0.02	ns
E/A	1.23 ± 0.38	1.11 ± 0.36	ns
TDI			
Em(cm/s)	10.6 ± 2.8	9.7 ± 1.9	ns
E/Em	8.87 ± 2.39	8.56 ± 2.49	ns
Sm(cm/s)	6.97 ± 1.19	7.58 ± 1	*P<0.01*
Speckle Tracking Echocardiography			
GLS(%)	15.4% ± 3.1%	20.2% ± 4.2%	*P<0.001*
GLSR(s^{-1})	1.06 ± 0.26	1.3 ± 0.33	*P<0.05*
Endothelial function			
EFR	1.88 ± 0.47	2.10 ± 0.43	*P<0.05*

Abbreviations: BMI: Body Mass Index; LVEF: Left Ventricular Ejection Fraction; EDV: End Diastolic Volume; ESV End Systolic Volume; DecT: Deceleration Time; E/A: Early and Late Diastolic Peak Velocity Ratio; E_m: TD Early Diastolic Peak Velocity; E_m/A_m: TD Early and Late Diastolic Peak Velocity Ratio; E/E_m: Early Diastolic Peak Flow Velocity and TD Velocity Ratio; GLS: Global Longitudinal Strain; GLSR: Global Longitudinal Strain Rate; EFR: Endothelial Flow Reserve

Table 2: Endothelial and myocardial echocardiographic parameters at baseline and after 3-months metformin treatment.

s^{-1}, $p<0.05$).Moreover mean EFR values were significantly increased by MTF therapy (2.1 ± 0.43 vs. 1.88 ± 0.47, $p<0.05$) .

Metabolomics analysis

Out of fifteen patients who performed all the 3 months of MTF therapy, two patients were excluded from metabolomics analysis for the impossibility to carry out the ^{1}HNMR analysis due to the blood samples haemolysis. A representative ^{1}H NMR spectrum of the blood is shown in Figure 1. Assignment of major resonances has been performed on the basis of data published in the literature [16]. The dominant metabolites identified in the majority of serum samples are reported in Table 3.

In order to determine the metabolic differences between the two groups pre- and post MTF therapy, the spectral data of the corresponding samples were subjected to multivariate analysis. An unsupervised PCA was initially applied to the NMR data matrix in order to detect any inherent separation between the groups and identify possible outliers; a significant separation was found (the two first PC's explain 67% of the total variance; $R^2x=0.898$; $Q^2=0.606$; data not shown). The application of PLS-DA classification model to ^{1}H-NMR data identified two metabolic clusters corresponding to pre and post-MTF treatment. To avoid over-fitting two latent variables were used in the PLS-DA model. The scores plot of the first and the second latent variable is reported in Figure 2. As shown, a good separation is observed between the two groups of samples. The first two latent variables explain 43.2 and 12.5 % of the total variation, respectively. Statistical values obtained with were: $R^2x = 0.557$; $R^2y = 0.802$ and $Q^2 = 0.592$. PLS-DA model validation was performed using y-table permutation testing (n = 200) and CV-ANOVA {Eriksson 2008}. Significance of the model was assessed trough 200 applications in which all Q^2 values of permuted Y vectors were lower than original one and the regression of Q^2 line had an intercept below zero (-0.25). Furthermore, CV-ANOVA gives a p-value of 0.004. The analysis of the loading plot of the latent variables indicates which regions of the NMR spectra are responsible for the separation between the two groups of samples. In particular, the basal time was characterized by higher levels oflactic acid, lipids, N-acetyl-glycoproteins compared to the post-MTF group, whereas the post-MTF fingerprint depended by an increase in choline, betaine, TMAO, creatine and valine (Table 4). These data are similar to those

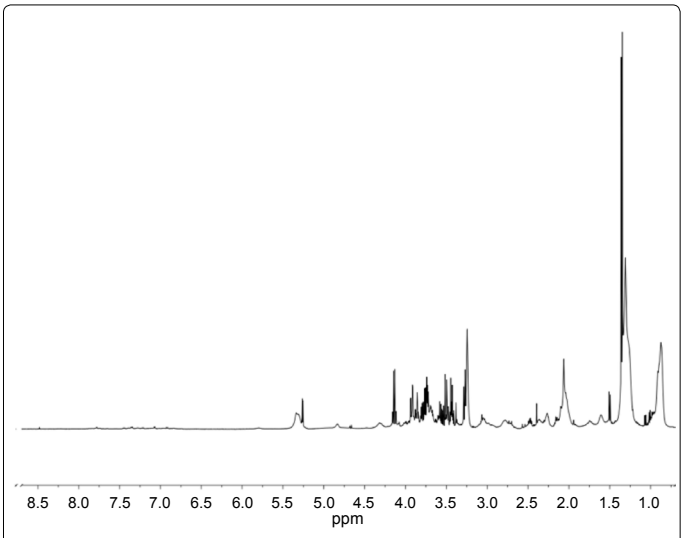

Figure 1: Representative ^{1}H NMR spectrum of a blood sample.

Compound	Group	^{1}H (ppm)[a]	^{1}H Multiplicity[b]
3-hydroxybutyrate	CH_3	1.22	d
	CH_2	2.48	dd
	CH	4.16	m
Acetate	CH_3	1.94	s
Alanine	CH_3	1.50	d
	CH	3.80	q
Betaine	N $(CH_3)_3$	3.25	s
	$-CH_2$	3.90	s
Choline	N-$(CH_3)_3$	3.23	s
	βCH_2	3.53	m
	αCH_2	4.06	m
Citrate	γCH_2	2.55	d
	$\gamma' CH_2$	2.72	d
Creatine	N-CH_3	3.06	s
	N-CH_2	3.95	s
Creatinine	N-CH_3	3.07	s
	N-CH_2	4.08	s
Cysteine	βCH_2	3.03	dd
	αCH	3.97	t
Formate	$HCOO^-$	8.48	s
α-Glucose	C4H	3.43	m
	C2H	3.56	dd
	C3H	3.78	m
	$C6H_2$	3.79	m
	C5H	3.86	m
	C1H	5.26	d
β-Glucose	C2H	3.27	dd
	C4H	3.43	m
	C5H	3.49	m
	C3H	3.50	m
	$C6H_2$	3.93	dd
	C1H	4.67	d
Glutamic acid	βCH_2	2.10	m
	γCH_2	2.38	m
	αCH	3.76	dd
Glutamine	βCH_2	2.15	m
	γCH_2	2.47	m
	αCH	3.77	t
Lactate	βCH_3	1.35	d
	αCH	4.14	q
Lipids in LDL	CH_3-$(CH_2)n$	0.87	m
	-$(CH_2)n$	1.26	m
Lipids in VLDL	CH_3-CH_2-CH_2-C=	0.90	m
	CH_2-CH_2-CH_2-CO	1.30	m
	CH_2-CH_2-CO	1.60	m
Lysine	γCH_2	1.43	m
	δCH_2	1.71	m
	βCH_2	1.90	m
	εCH_2	3.05	t
N-acetylglicoproteins	$NHCOCH_3$	2.05	s
Succinate	CH_2	2.41	s
Triglycerides	CH_3-$(CH_2)n$	0.91	m
	-$(CH_2)n$	1.30	m
	CH_2-CH_2-CO	1.60	m
	CH_2-C=C	2.04	m
	CH_2-C=O	2.25	m
	=CH CH_2 CH=	2.78	m
	CH=CH	5.33	m
Trimethylamine-N-oxide (TMAO)	N-$(CH_3)_3$	3.27	s
Valine	γCH_3	1.01	d
	γCH_3	1.06	d
	βCH	2.27	m
	αCH	3.62	m

[a]^{1}H chemical shift are reported with respect to DSS signal (0.00 ppm)
[b]Multiplicity definitions: s: singlet; d: doublet; t: triplet; q: quartet; dd: doublet of doublets; m: multiplet

Table 3: ^{1}H NMR chemical shift of the metabolites identified.

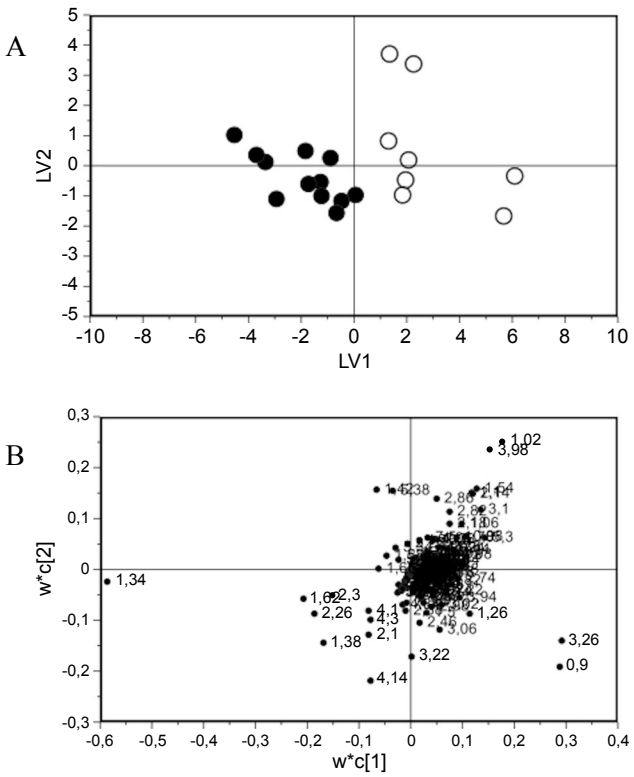

Figure 2: (A) PLS-DA scores plot of pre (T1, filled circles) vs post 3-months MTF therapy (T2, empty circles) groups. (B) Corresponding loadings plot.

Metabolite	Loading (bin)	Change trend
3-hydroxybutyrate	1.26, 2.46, 2.50, 4.22	↑
Alanine	1.50, 1.54	↑
Betaine/Choline/ Trimethylamine-N-oxide (TMAO)	3.26	↑
Citrate	2.74	↑
Creatine	3.1, 3.98	↑
Glutamine	2.14, 2.18	↑
Valine	1.02, 1.06, 1.10	↑
Lipids in LDL/VLDL (CH_3)	0.90	↑
Lactate	1.38, 4.14	↓
-(CH_2)*n* in LDL/VLDL/ Triglycerides	1.34	↓
Lipids in VLDL/ Triglycerides	1.58, 1.62, 1.66	↓
N-acetylglicoproteins	2.1	↓
Triglycerides	2.26, 2.30, 5.34, 5.38, 5.42	↓

Table 4: Change of metabolites identified by ^{1}H NMR in IR patients after MTF treatment.

found by Huo et al. in a study that analyzed the metabolic profiles of type 2 diabetes mellitus patients pre and post treatment with MTF [17].

Discussion

Much interest has been shown in the use of metabolomics to identify biomarkers of cardiovascular and metabolic disease. Diabetes increases the risk of mortality from cardiovascular disease [18,19] and, although atherosclerosis has a major role in the causes of death, a subpopulation of patients with type 2 diabetes develop myocardial dysfunction that is independent of vascular disease. How heart failure develops in this subgroup, especially in the absence of ischemic disease, is still unknown.

MTF is the most worldwide prescribed biguanide for the treatment of hyperglycemia and type 2 diabetes. In Diabetes Prevention Program, MTF displayed an ability to slow the progression from a glucose intolerant state towards diabetes [20,21]. *In vitro* studies have demonstrated that MTF exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain (electron transfer from the NADH to the Q 10 coenzyme) [22-26]. This inhibition may slow down the transfer of reducing equivalent during the Krebs cycle and limit the capacity of oxidative metabolism. In myocardial cells, the mitochondrial reserve may be used to some extent during stress; however, if an inhibition of the complex 1 reduces this reserve, a critical decrease in myocardial performance has been supposed. On the basis of the findings presented to date, it is of particular importance the influence of MTF treatment on longitudinal myocardial function in insulin-resistant subjects. Indeed, in view of their non-diabetic status, these subjects are generally deemed to be "healthy"; however, it may be beneficial to prescribe treatment with this drug to prevent the onset of diabetes.

This study investigated the metabolic effects of MTF in relation to its capacity to improve myocardial and endothelial function.

Our study examined the effects of three months MTF therapy on myocardial and endothelial function verifying the related metabolic changes by means of a metabolomics approach. The major findings were: i. MTF confirmed its high efficacy on reducing weight and BMI in IR subjects; ii. Longitudinal function after MTF significantly improves as shown by S wave at TDI and by the deformation indexes at ST echocardiography as GLS and GLSR; iii. Subjects with IR showed a significant improvement in peripheral endothelial function; iv. Metabolomic analysis showed a specific metabolic profile before treatment (higher lactic acid, lipids, n-acetylglicoproteins); v. After three months of MTF was observed a significant increase of valina, alanine, creatine, choline, 3-hydroxybutyrate, betaine and TMAO.

An inefficient energy metabolism in presence of IR has been demonstrated in animal models [4]. In sucrose-fed rats, serial echocardiographic assessments revealed that early abnormalities in diastolic function, followed by late systolic dysfunction, were associated with IR. A concurrent depressed sarcoplasmic reticulum function was demonstrated by a significant reduction in Ca^{2+} uptake [27]. In IR subjects abnormal glucose homeostasis was found correlated with impaired LV diastolic function [28], and this relationship has been shown to be independent of blood pressure, LV geometry, total plasma lipids and obesity [29].

In our study, we evidenced normal ventricular function at baseline but a positive effect of three months of MTF on longitudinal function has been found. Recent studies showed a latent longitudinal dysfunction in IR subjects, evidenced by a reduced longitudinal contractile reserve during exercise [30] or after a dobutamine stress test [3]. These evidences have not been confirmed in diabetic subjects with a good pharmacologic metabolic compensation [31]. More recently an insulin sensitizer as MTF showed to be associated with a reduced mortality with a lower hospitalization rate of heart failure patients with new-onset diabetes mellitus. Nevertheless, MTF was not associated with an improved prognosis of HF patients with a good metabolic compensation [32].

The early decline of longitudinal contractile reserve in IR patients has been related to several factors as insulin inhibition of nitric oxide production at the myocardial level [33], with potential endothelial dysfunction. More recently, IR that has been reported in association

with exercise intolerance in heart failure patients has been partly attributed to reduced coronary flow reserve [34]. Moreover, alteration of the myocardial energetic metabolism caused by IR can lead to ATP production impairment and have a major role in the early longitudinal dysfunction [35-38].

The presence of vascular endothelial dysfunction has been demonstrated in IR states, where it may represent an important early event in the development of atherosclerosis [39]. IR may be linked to endothelial dysfunction by a number of molecular and pathophysiological mechanisms, such as disturbances of subcellular signaling pathways and the PI-3-kinase/Akt pathway, common to both insulin action and nitric oxide production [40]. In subjects affected by metabolic syndrome a reduction of EFR [41] has been reported, thus confirming a compromised activity of the eNOS enzyme in case of IR. On the other hand, previous results obtained by our group in IR subjects underlined an improvement of EFR following 3 months therapy with MTF [7] and correlated with basal levels of HOMA-IR [9,42]. In the present study we amplified and defined this finding, showing that the MTF-induced improvement of EFR was closely linked with changes tomolecules such as betaine, choline [43,44] and creatine [45,46], that are implicated in NO-modulation, in radical-scavenger function and in oxidative stress reduction. Moreover, in myocardial hypertrophy and chronic HF there is a fall in total Creatine due to the mismatch between the need for ATP and its production. The loss of creatine is myocardial-specific, and is approximately an order of magnitude faster than that of ATP [47]. The resulting decrease in the PCr:ATP ratio [48] correlates with both NYHA classes [49] and the indices of systolic and diastolic function [50].

Higher levels of lipids-CH_2 groups (LDL/VLD, triglycerides) at baseline were observed compared to post MTF-treatment; in rats overweight MTF induced a reduction in triglycerides levels with a related raise in ketone bodies output by liver [51]. Our data, after MFT-treatment showed a decrease in lactate and an increase in citrate, alanine and ketone bodies levels, suggesting a shift towards a more balanced and healthy energetic metabolism, as demonstrated in diabetes patients.

In conclusion, the present study confirms how in a population with an impaired carbohydrate metabolism and IR, MTF therapy is capable of positively influencing myocardial and endothelial function. The improvement in the cardiac function was correlated to significant metabolic changes. We hypothesize that a better knowledge of the metabolic changes occurring during IR could be a positive target for a better therapy. Given the increasing importance attributed to early treatment of IR, further studies in larger population should be warranted.

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