European Journal of Heart Failure (2012) **14**, 754–763
doi:10.1093/eurjhf/hfs080

Availability of energetic substrates and exercise performance in heart failure with or without diabetes

Vojtech Melenovsky[1]*, Martin Kotrc[1], Jan Polak[1,2], Terezie Pelikanova[3], Bela Bendlova[4], Monika Cahova[5], Ivan Malek[1], Petr Jarolim[5], Ludmila Kazdova[6], and Josef Kautzner[1]

[1]Department of Cardiology, Institute of Clinical and Experimental Medicine–IKEM, Prague 4, 140 28, Czech Republic; [2]Division of Pulmonary and Critical Care Medicine, The Johns Hopkins University, Baltimore, MD, USA; [3]Diabetes Center, Institute of Clinical and Experimental Medicine–IKEM, Prague, Czech Republic; [4]Institute of Endocrinology, Prague, Czech Republic; [5]Center for Experimental Medicine, Institute of Clinical and Experimental Medicine–IKEM, Prague, Czech Republic; and [6]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Received 22 December 2011; revised 15 April 2012; accepted 8 April 2012; online publish-ahead-of-print 12 June 2012

Aims The goal of the study was to examine whether resting or post-exercise metabolic substrate levels are associated with differential exercise performance and long-term outcome in control subjects or heart failure (HF) patients with or without type 2 diabetes mellitus (DM).

Methods and results Twenty five healthy controls matched with 97 patients with stable advanced HF were prospectively enrolled. Exercise capacity, age, gender, and HF aetiology were balanced between HFDM− and HFDM+ groups. Subjects underwent maximal bicycle spiroergometry with blood sampling to measure metabolites and neurohormones before and immediately after the exercise. HFDM+ patients had increased free fatty acids, glucose, and β-hydroxybutyrate compared with controls. HFDM+ patients had higher baseline copeptin (24 ± 16 vs. 17 ± 13 pmol/L, $P < 0.05$) but otherwise showed similar neurohumoral activation and exercise response to HFDM− patients. Peak oxygen consumption (VO_2) was unrelated to post-exercise free fatty acids, glucose, lactate, or glycerol, but strongly correlated with post-exercise pyruvate (in all: $r = 0.62$, $P < 0.001$). During the next 17 ± 10 months, 36% of HF patients experienced an adverse event (death, urgent transplantation, or assist device insertion). From metabolic factors, only post-exercise glucose [hazard ratio (HR) 1.28, $P = 0.04$), total body fat (HR 0.58, $P < 0.001$), and the presence of DM (HR 1.98, $P = 0.04$) were predictive of the outcome.

Conclusions With the exception of pyruvate, acute changes of metabolic substrates are not related to cardiac performance in HF, regardless of diabetic status. Inhibition of body fat depletion, attenuation of stress-related hyperglycaemia, or increasing dynamics of plasma pyruvate may represent therapeutic targets in advanced HF.

Keywords Heart failure • Diabetes mellitus • Insulin resistance • Free fatty acids • Glucose • Substrate metabolism

Introduction

The heart has only a minimal capacity to store energy, so it is dependent on sufficient and timely delivery of metabolic substrates from the blood. Alterations of systemic substrate metabolism might thus contribute to the pathophysiology of cardiac dysfunction, especially during stress.[1,2] Fluctuations in blood levels of metabolic substrates do not affect cardiac function or exercise capacity in healthy subjects,[2,3] but it is not known whether the same applies in patients with advanced heart failure (HF), particularly if they simultaneously have type 2 diabetes mellitus (DM).

The chief metabolic substrate for the heart are free fatty acids (FFAs), but cardiac muscle can also utilize glucose, lactate, ketone bodies, glycerol, and amino acids, depending on substrate

* Corresponding author. Tel: +420 739 938 477, Fax: +420 261 362 486, E-mail: vojtech.melenovsky@ikem.cz

availability, haemodynamic loading, metabolic state, or presence of HF.[2] Myocardial metabolism of advanced HF is characterized by down-regulation of FFA oxidation,[4,5] by a shift towards the use of glucose,[7] and by less efficient coupling between substrate utilization and cardiac mechanical work.[6] At the same time, neurohumoral activation accompanying HF[7] enhances FFA release from adipose tissue into the blood,[8] creating a mismatch between myocardial substrate preference and availability.[2] Adverse effects of deregulated metabolite levels on cardiac function could be further potentiated in diabetic HF patients, where flexibility of substrate utilization is further restrained by insulin resistance.[9]

Cardiopulmonary exercise testing with respiratory gas analysis serves as an objective measure of exercise intolerance and cardiopulmonary reserve.[10,11] At peak exercise, oxygen consumption (peak VO_2) is determined by maximal cardiac output and maximal oxygen extraction. Because the latter is relatively constant among subjects, peak VO_2 reflects maximum cardiac output and the upper limit of heart pump function.[12] Theoretically, if availability of a metabolic substrate influences maximal cardiac pumping capacity, then a significant correlation between peak VO_2 and metabolite level immediately after the exercise should be found. Similarly, chronic effects of an adverse metabolic profile could be discerned by examining its link to long-term outcome in HF.

The aim of the study was to examine whether resting or post-exercise neurohumoral and metabolic substrate profiles are associated with differential exercise performance and long-term outcome in control subjects or HF patients with or without DM. Understanding the regulation of substrate utilization in the heart is important for further development of novel therapies targeting energetic efficiency in HF.[13]

Methods

We prospectively enrolled patients with chronic (>6 months), stable advanced HF, electively hospitalized in our institution for pre-transplant assessment or implantable cardioverter defibrillator (ICD)/cardiac resynchronization therapy (CRT) device insertion. Patients with recent decompensation or with reversible cardiac dysfunction (planned valve surgery, revascularization, or tachycardia-induced cardiomyopathy) were excluded. From 108 consecutively screened patients, 12 were excluded in order to balance age, sex, body composition, HF aetiology, and exercise capacity between HF subjects with or without DM (HFDM+; HFDM−). Healthy controls were recruited by advertisement to match the age, gender, and body composition of the HF cohort. DM was defined as pre-existing treated disease (antidiabetic drugs or insulin) or by significant elevation of haemoglobin A_{1C} (>6.5%, Diabetes Control and Complications Trial reporting).[14] Subjects with type 1 DM were excluded. All subjects were studied in the fasted (>12 h) state (before the morning insulin dose in insulin-treated HFDM+). Biochemistry, questionnaires, echocardiographic (Vivid-7, GE Healthcare, Wauwatosa, WI, USA), and anthropometric evaluations were performed. Skinfold thickness was measured in triplicate using Best's caliper in biceps, triceps, subscapular, and suprailiacal areas, and the sum was converted into total body fat (% of body weight) using the gender-specific Womersley's formula.[15]

All subjects underwent symptom-limited upright bicycle spiroergometry using calibrated equipment (V_{max} Encore 29S, Sensormedics-CareFusion, San Diego, CA, USA). Manual flow calibration was performed with a syringe; gas calibrations were performed with a manufacturer-recommended gas mixture. Baseline load was set to 25 W and was followed by 25 W stepwise increments every 3 min till exhaustion. Respiratory gas was sampled continuously from the mouthpiece, and minute ventilation (VE), VO_2, carbon dioxide output (VCO_2), and respiratory quotient (RQ) were calculated. Peak VO_2 was determined by the highest VO_2 achieved during exercise. Ventilatory efficiency was assessed by calculating the slope of the VE/VCO_2 relationship. Peak cardiac mechanical function was also quantified by calculating a non-invasive surrogate of cardiac power (circulatory power = peak VO_2 × peak systolic blood pressure).[11,16]

For sampling metabolites and hormones, an i.v. cannula was inserted into the forearm vein >1 h before the study and the line was then flushed with saline without heparin. The pre-exercise samples were taken after supine rest (>15 min); post-exercise samples were taken immediately (1–3 min) after the termination of exercise. Samples were placed on ice, immediately spun, and the aliquots were stored at −80°C until analysis. Serum pyruvate and lactate were measured by colorimetric assays from Biovision, San Francisco, USA [pyruvate, sensitivity 1–200 μM/L, coefficient of variation (CV) 3.8%; lactate, sensitivity 0.001–10 mM/L, CV 2.8%]. Serum glycerol and β- hydroxybutyrate were measured by colorimetric assays from Cayman Chemical Company, Ann Arbor, USA (glycerol, sensitivity 0–20 mg/L, CV 7.5%; β-OH butyrate, sensitivity 0–0.5 mM/L, CV 4.2%). Free fatty acids were measured by NEFA colorimetric assay (WAKO Diagnostics, Richmond, USA). Plasma catecholamines were determined using a Cat-Combi kit (RIA-1303, DRG Instruments, Germany) with an interassay CV of 12.8–13.6% for epinephrine and 14.9–18.5% for norepinephrine. Serum insulin was measured using IRMA (Immunotech, Prague, Czech Republic), glucose was measured using a glucose oxidase colorimetric assay (ERBA-Lachema, Brno, Czech Republic), and the HOMA (homeostatic model assessment) index of insulin resistance was calculated as described before.[17] N-terminal pro brain natriuretic peptide (NT-proBNP) was measured using the proBNP II assay (Roche Diagnostics, Indianapolis, USA; sensitivity 5–35 000 ng/L, CV 2.7–3.1%). Copeptin (C-terminal provasopressin, CT-proAVP) was measured using the copeptin assay and the Kryptor Compact analyzer (both BRAHMS AG, Hennigsdorf, Germany); assay range 4.8–500 pmol/L, CV 9.0% at 24 pmol/L and 3.7% at 97 pmol/L.

Data were analysed using SPSS 19 software (IBM, Inc.). Differences among groups were tested with χ^2 test (proportions) or analysis of variance (ANOVA) and Sheffe's post-hoc test (continuous variables). Changes within groups were tested with paired *t*-test. Correlations were tested using Pearson's *r*. An adverse outcome was defined as combined endpoint of death without transplantation, urgent heart transplantation, or ventricular assist device insertion. Because time to non-urgent transplant reflects donor availability rather than the recipient's condition, those patients were censored as having no outcome on the day of transplantation, as done previously.[18] Time to event was analysed using univariate Cox's proportional hazard model. Variables were z-standardized and presented as hazard ratios (HRs) ± 95% confidence intervals (CIs). The protocol was approved by the institutional ethics committee and subjects gave signed informed consent.

Results

Baseline characteristics

The study included 97 predominantly middle-aged male patients with stable, advanced HF [70% New York Heart Association

Table 1 Characteristics of study subjects

	Controls ($n = 25$)	HFDM– ($n = 61$)	HFDM+ ($n = 36$)	*P*-value
Anthropometry				
Age, years	50 ± 8	52 ± 9	55 ± 6	0.07
Male gender, %	88	84	89	0.73
BMI, kg/m^2	28 ± 3	27 ± 4	29 ± 4	0.07
Body weight, kg	90 ± 9	84 ± 14	90 ± 17	0.06
Total body fat, %	20 ± 8	22 ± 9	22 ± 7	0.60
Heart failure and co-morbidities				
Ischaemic aetiology, %	–	45	50	0.58
HF duration, years	–	8 ± 9	7 ± 8	0.74
NYHA functional class, (I–IV)	1 ± 0	2.7 ± 0.6*	2.9 ± 0.5*	<0.0001
MLHFQ score sum	1 ± 2	45 ± 24*	47 ± 23*	<0.0001
Leg oedema present, %	0%	32%	45%*	<0.0001
LV ejection fraction, %	60 ± 0	24 ± 6*	23 ± 6*	<0.0001
LV end-diastolic diameter, mm	50 ± 5	71 ± 9*	70 ± 8*	<0.0001
RV end-diastolic diameter, mm	27 ± 3	29 ± 7	35 ± 10*†	<0.0001
Inferior vena cava diameter, mm	15 ± 4	19 ± 5*	22 ± 7*†	<0.0001
Tricuspid regurgitation gradient, mmHg	14 ± 7	34 ± 10*	45 ± 13*†	<0.0001
RV dysfunction grade, (0–3)	0 ± 0	1.4 ± 1.0*	1.9 ± 0.9*†	<0.0001
Serum creatinine, μmol L^{-1}	83 ± 11	104 ± 38*	113 ± 39*	0.003
Serum triglycerides, mmol/L	1.6 ± 0.8	1.6 ± 0.9	1.8 ± 1.2	0.72
Haemoglobin A_{1C} (DCCT), %	5.6 ± 2.4	5.9 ± 2.5	8.3 ± 4.5*†	<0.0001
Medication				
Furosemide use, %	–	95	100	0.18
Daily dose, mg	–	82 ± 56	148 ± 102	<0.0001
Beta-blocker use, %	–	98	97	0.70
Daily dose (0–3)	–	1.5 ± 0.7	1.5 ± 0.7	0.93
ACEI/ARB use, %	–	91	97	0.20
Aldosterone antagonist use, %	–	82	92	0.20
DM diet/OAD/insulin, %	–	–	39/39/22	–

Analysis of variance or χ^2 test used for comparison.
Values are means ± SD. Tricuspid regurgitation gradient was measurable in 8/46/23 subjects.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CO_2, body carbon dioxide production; DCCT, Diabetes Control and Complications Trial; DM, diabetes mellitus; HF, heart failure; LV, left ventricular; MLHFQ, Minnesota Living with Heart Failure Questionnaire; NYHA, New York Heart Association; OAD, oral antidiabetic drug; RV, right ventricular; VE, minute ventilation. VO_2, body oxygen consumption..
* $P < 0.05$ vs. controls, † $P < 0.05$ vs. HFDM–, Sheffe's post-hoc test.

(NYHA) class ≥ III] and 25 matched controls. Baseline characteristics are given in *Table 1*. In HF patients, 37% had type 2 DM. Controls and HF patients had similar sex, age, and body composition. HFDM+ and HFDM− subgroups were additionally balanced in disease stage, HF aetiology, and exercise tolerance. Both HF groups demonstrated a similar degree of left ventricular (LV) dysfunction and remodelling, but patients in the HFDM+ group had a more dilated and dysfunctional right ventricle, a higher tricuspid regurgitant gradient, and a larger diameter of the inferior vena cava, indicating more systemic congestion and pulmonary hypertension. Both HF groups had similar HF therapy, with the exception of a higher furosemide dose in the HFDM+ patients. In HFDM+ subjects, diabetes was treated with diet in 14, with insulin in 9, and with oral antidiabetic drugs (OADs) in 14 subjects (metformin in 5, sulfonylureas in 9 subjects). Characteristics of HFDM+ subjects according to DM therapy are given in the Supplementary material online, *Table S1*. Patients on OADs were more obese and had a higher tricuspid regurgitant gradient than those on a DM diet. Insulin-treated patients were more symptomatic (higher Minnesota Living With Heart Failure Questionnaire score), had the worst long-term glycaemic control, and more often had leg oedema.

Cardiopulmonary exercise results

The results of cardiopulmonary exercise tests are summarized in *Table 2*. Both HF groups have a similar response in heart rate, blood pressure, and ventilation to exercise. Both HF groups have similarly reduced peak VO_2 and circulatory power, confirming severe exercise limitation. Diabetes therapy had no impact on peak VO_2 (Supplementary material online, *Table S1*), but patients

Table 2 Cardiopulmonary exercise variables

	Controls ($n = 25$)	HFDM– ($n = 61$)	HFDM+ ($n = 36$)	*P*-value
Heart rate, s^{-1}				
Rest	74 ± 9	79 ± 14	81 ± 11	0.13
Peak	161 ± 16	124 ± 25*	118 ± 20*	<0.001
Systolic BP, mmHg				
Rest	118 ± 15	95 ± 17*	97 ± 14*	<0.001
Peak	194 ± 27	121 ± 22*	121 ± 21*	<0.001
Diastolic BP, mmHg				
Rest	85 ± 12	66 ± 11*	68 ± 12*	<0.001
Peak	92 ± 14	72 ± 13*	75 ± 14*	< 0.001
Exercise time, min	21 ± 6	8.6 ± 2.8*	7.5 ± 2.4*	<0.001
Peak work rate, W	172 ± 50	72 ± 24*	63 ± 20*	<0.001
Peak respiratory rate, per min	30 ± 10	28 ± 8	30 ± 10	0.43
Peak VE, L/min	94 ± 33	55 ± 15*	56 ± 17*	<0.001
Peak VO_2, mL/kg/min	29.2 ± 7	14.6 ± 3.3*	13.3 ± 2.6*	<0.001
Peak respiratory quotient	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.36
VE/VCO_2 slope	24.2 ± 3	35 ± 8.9*	39.3 ± 12*	<0.001
Circulatory power, mL/kg/min/mmHg	5664 ± 1458	1803 ± 613*	1635 ± 441*	<0.001

Analysis of variance or χ^2 test used for comparison. Values are means ± SD.
BP, blood pressure; CO_2, body carbon dioxide production; DM, diabetes mellitus; HF, heart failure; VE, minute ventilation; VO_2, body oxygen consumption.
* $P < 0.05$ vs. controls, † $P < 0.05$ vs. HFDM–, Sheffe's post-hoc test.

on OADs had a lower VE/VCO_2 slope than the other HFDM+ subgroups.

Metabolites

The levels of metabolites sampled before and after exercise are summarized in *Figure 1* (graphs and statistics) and in Supplementary material online, *Table S2* (numerical data). Controls and HFDM– patients had similar pre-exercise levels of metabolites. In contrast, HFDM+ patients had significantly elevated FFAs, glucose, and β-OH butyrate compared with controls; the former two metabolites were also significantly higher than in the HFDM– group. Baseline lactate and pyruvate were similar in all groups. Plasma glycerol, which reflects the net balance between lipolysis and FFA re-esterification in adipose tissue, was similar in all three groups. Exercise led to no change in FFAs in controls, but to a significant decrease in both HF groups. The exercise-induced drop in FFAs was relatively larger in the HFDM+ (−39%) than in the HFDM– (−28%) group. Glycaemia mildly increased in controls (+15%), but it did not change in either HF group. Insulin-treated HFDM+ patients had significantly higher post-exercise glycaemia than HFDM+ patients on a DM diet (Supplementary material online, *Table S1*). Serum β-OH butyrate marginally decreased in those with HFDM–. Lactate rose in parallel in all groups by 9–14%. The most pronounced difference between HF groups and controls was in the exercise dynamics of pyruvate—it increased by 255% in controls, but only by 59% in HFDM– and by 102% HFDM+ groups. The post-exercise pyruvate level was significantly higher in controls than in both HF groups. In the pooled HF cohort, β-OH butyrate correlated with baseline glycerol ($r = 0.63$, $P < 0.001$), haemoglobin A_{1C} ($r = 0.49$, $P < 0.001$), pyruvate, glucose, and FFAs ($r = 0.43$, 0.38, and 0.35; all $P < 0.001$).

Hormones

Hormonal levels are summarized in *Figure 2*, and in Supplementary material online, *Table S2*. At baseline, HF patients had similar norepinephrine and epinephrine levels, but increased copeptin and NT-proBNP compared with controls. Copeptin was higher in HFDM+ than in HFDM– patients. In all HF patients, baseline copeptin correlated with norepinephrine ($r = 0.47$, $P < 0.001$) and creatinine ($r = 0.57$, $P < 0.001$), but not with insulin ($P = 0.9$), haemoglobin A_{1C} ($P = 0.2$), or metabolite concentrations. After exercise, catecholamines and copeptin reached similar levels in all three groups, despite different exercise duration. NT-proBNP increased similarly in both HF groups by 10–14%. Both HF groups had elevated baseline insulin, but the HOMA index of insulin resistance was increased only in the HFDM+ group. With exercise, insulin did not change in controls and it decreased in the HF groups; the HOMA index remained elevated in the HFDM+ group. In the pooled HF cohort at baseline, FFAs did not correlate with hormones (insulin, HOMA index of insulin resistance, NT-proBNP, or norepinephrine: $r = -0.1$ to 0.04, all $P > 0.5$), but FFAs were predicted by measures of body fatness [body mass index, $r = 0.35$, $P = 0.001$; waist circumference, $r = 0.32$, $P = 0.008$; percentage total body fat, $r = 0.24$, $P = 0.04$), by furosemide daily dose ($r = 0.24$, $P = 0.03$), and by baseline glycerol ($r = 0.44$, $P < 0.0001$) and β-OH butyrate ($r = 0.35$, $P = 0.0001$) levels.

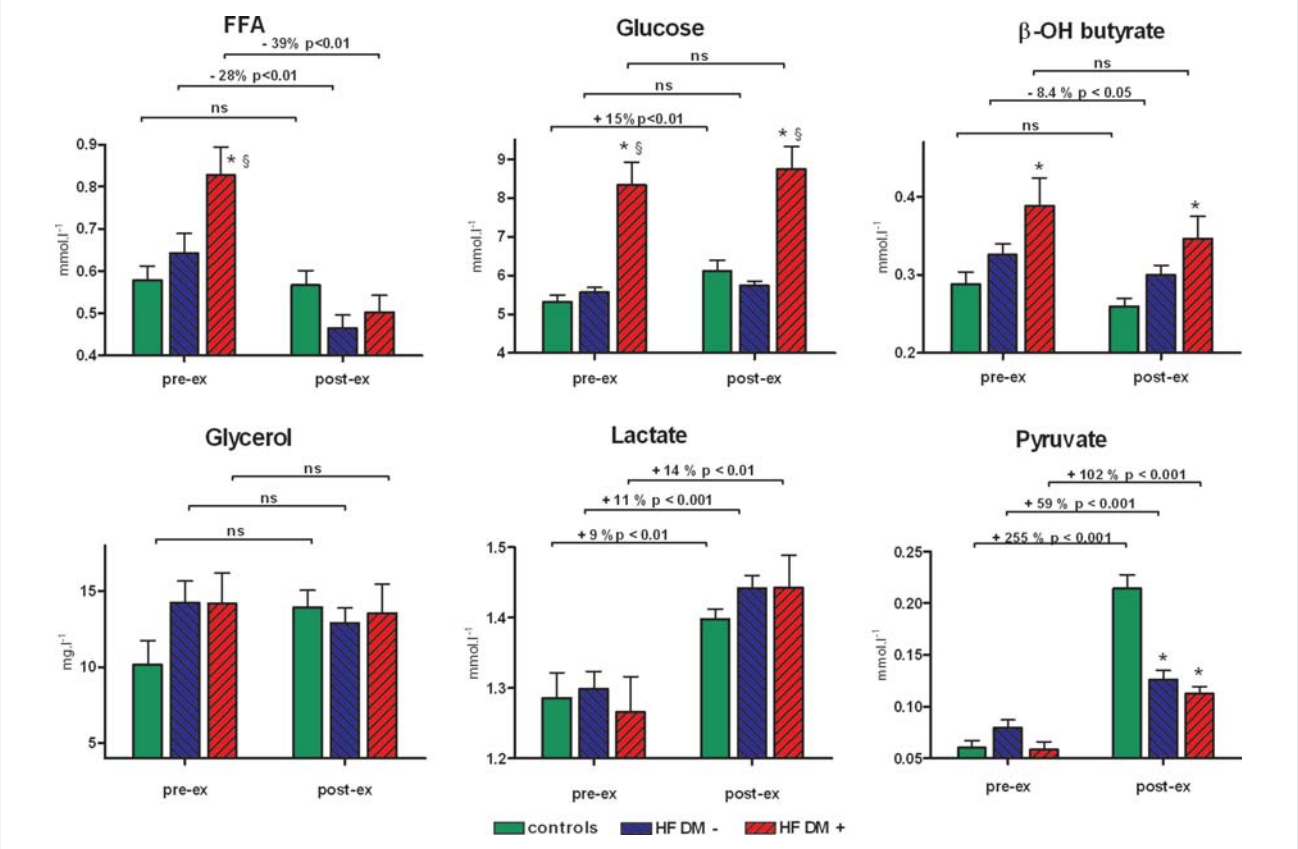

Figure 1 Concentration of substrates of cardiac energetic metabolism in peripheral blood sampled before and immediately after maximal symptom-limited bike exercise in controls and in patients with heart failure with (HFDM+) or without (HFDM−) diabetes mellitus. Bars represent the mean ± SE. Differences among groups (before and after exercise) were tested with analysis of variance (ANOVA) and Sheffe's post-hoc test (*$P < 0.05$ vs. controls, §$P < 0.05$ vs. HFDM−). Changes within groups were tested with paired *t*-test (brackets). FFA, free fatty acids.

Metabolic response and exercise capacity

The relationships between post-exercise levels of metabolites and peak performance parameters are given in *Table 3*. In controls but not in HF subjects, post-exercise glucose correlated with cardiac power ($r = 0.57$, $P < 0.01$) and marginally with peak VO_2. In HF patients and in all pooled subjects, peak VO_2 and circulatory power were unrelated to peak FFAs, lactate, β-OH butyrate, glucose, or glycerol, but were strongly related to post-exercise pyruvate ($P < 0.001$; *Figure 3*). The VE/VCO_2 slope was weakly related to post-exercise pyruvate. The relationship of pre-exercise metabolite levels to peak exercise capacity is summarized in the Supplementary material online, *Table S3*, and correlations of LV ejection fraction and resting NT-proBNP with metabolites are presented in the Supplementary material online, *Table S4*.

Metabolic response and outcomes

During 16.5 ± 10 months of follow-up, 35 out of a total of 97 HF subjects (36%) experienced an adverse event. The z-standardized HRs of hormones and metabolites for the adverse outcome are summarized in *Figure 4*. From metabolites, only post-exercise glucose was associated with significantly increased risk of adverse outcome (HR 1.28, 95% CI 1.01–1.60, $P = 0.04$). From humoral factors, only NT-proBNP and copeptin levels were predictive. The presence of DM (HR 1.98, 95% CI 1.01–3.85, $P = 0.04$) and reduced body fat (percentage of body weight) (HR 0.58, 95% CI 0.40–0.86, $P < 0.001$) were associated with significantly higher risk of an adverse outcome.

Discussion

The results of this study can be summarized as follows: HFDM+ patients had a more irregular metabolic profile but showed similar neurohumoral activation and exercise responses to those with HFDM−; only copeptin was more elevated in the HFDM+ group. Secondly, post-exercise FFA, glucose, lactate, glycerol, and β-OH butyrate were not related to the indexes of peak cardiopulmonary performance in HF patients, regardless of their diabetic status, implicating that either decreased or excessive availability of these substrates is not imposing a limitation on cardiac function. However, post-exercise serum pyruvate was substantially lower in both HF groups than in controls and it was strongly positively correlated with peak VO_2. Thirdly, post-exercise glucose level was the

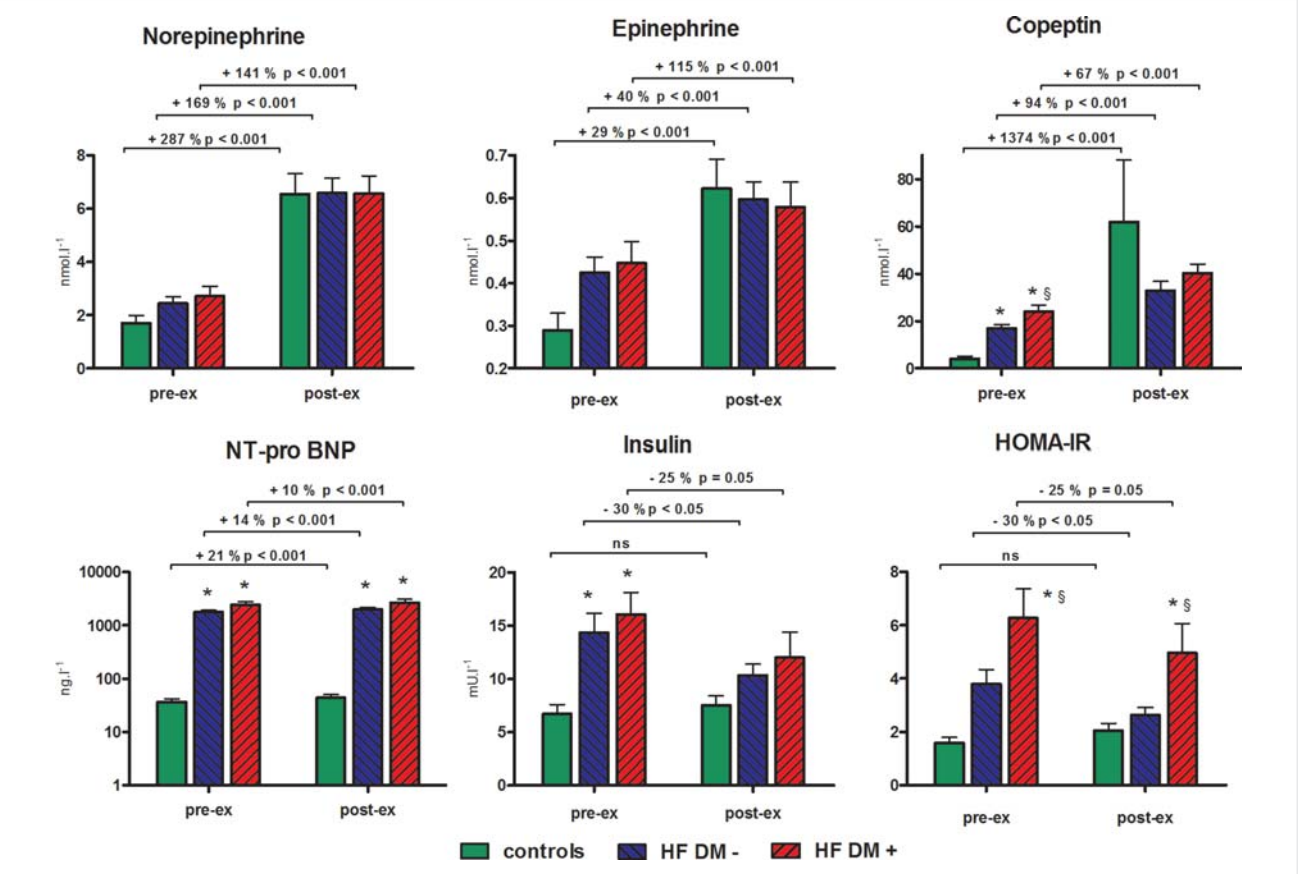

Figure 2 Concentration of hormones and homeostatic model assessment index of insulin resistance (HOMA-IR) before and immediately after maximal symptom-limited bike exercise in controls and in patients with heart failure with (HFDM+) or without (HFDM−) diabetes mellitus. Bars represent the mean ± SE. Differences among groups (before and after exercise) were tested with analysis of variance (ANOVA) and Sheffe's post-hoc test (*$P < 0.05$ vs. controls, §$P < 0.05$ vs. HFDM−). Changes within groups were tested with paired *t*-test (brackets). NT-proBNP, N-terminal pro brain natriuretic peptide.

only blood metabolite to be associated with an increased risk of adverse outcome. A higher risk of adverse events was associated with reduced total body fat, but not with increased FFA levels.

Nerohumoral and metabolic profile in heart failure patients with and without diabetes mellitus

In both HF groups, levels of circulating catecholamines were not different from those of controls. This is in contrast to older studies (from the pre-beta-blocker era) of similarly symptomatic HF subjects.[19–22] The suppression of catecholamine levels in our HF patients probably reflects more intensive pharmacotherapy with beta-blockers and angiotensin-converting enzyme (ACE) inhibitors. Patients with HFDM+ and HFDM− have a generally similar profile of neurohormones, which is in agreement with the study of van der Horst *et al.*[22] who addressed this issue in a large unselected HF cohort. The only hormone that separated HFDM− and HFDM+ patients in our study was copeptin, a stable C-terminal fragment of arginine−vasopressin prohormone that was higher in the HFDM+ group. This novel observation is in agreement with other recent data, indicating that the arginine−vasopressin system plays a role in the pathophysiology of DM, by affecting glycogenolysis, and glucagon, insulin, and corticotropin release.[23] The HOMA index of insulin resistance was also more increased in HFDM +, but it did not correlate with copeptin.

The levels of FFAs in HF patients, particularly in the non-diabetic subgroup, were less abnormal than reported earlier[20,24] and correlated with body adiposity rather than with norepinephrine concentrations. HFDM+ patients had a more irregular metabolic profile than controls, with elevated FFAs, glucose, and β-OH butyrate. Increased β-OH butyrate in HF subjects reflects an increased FFA availability for ketone body production in the liver.[20] Increased fasting glucose in HF is a consequence of impaired glucose disposal[25] and increased glucose hepatic output;[9,26] both these abnormalities are more pronounced in diabetic HF.[27]

Metabolites and exercise capacity

Both HF groups have severe exercise limitation, decreased peak VO_2, diminished ventilatory efficiency, and diminished peak circulatory power, an index of cardiac mechanical function that integrates both pressure and flow generated by the left ventricle.[16] With the

Table 3 Correlation of post-exercise metabolites and peak exercise performance parameters

	All (n = 122)	All HF (n = 97)	Controls (n = 25)	HF DM– (n = 61)	HF DM + (n = 36)
Peak VO_2					
FFAs	0.09	−0.18	0.15	−0.16	−0.19
Glucose	−0.10	−0.13	0.40§	−0.10	0.03
β-OH butyrate	−0.21§	−0.15	0.05	−0.14	−0.10
Lactate	0.03	0.20	−0.36	0.15	0.32
Pyruvate	0.62**	0.40**	0.38	0.39*	0.41§
Glycerol	0.07	0.02	0.23	0.02	0.04
Circulatory power (peak VO_2 × peak SBP)					
FFAs	0.14	−0.07	0.09	−0.02	−0.16
Glucose	−0.09	−0.11	0.57*	−0.25	0.10
β-OH butyrate	−0.16	0.00	0.19	−0.03	0.11
Lactate	−0.09	0.09	−0.35	0.09	0.13
Pyruvate	0.60**	0.37**	0.34	0.38*	0.28
Glycerol	0.09	0.07	0.31	0.15	−0.04
VE/VCO_2 slope					
FFAs	−0.15	−0.08	0.04	0.08	−0.36§
Glucose	0.10	0.05	−0.01	−0.06	−0.10
β-OH butyrate	0.22§	0.13	0.41§	0.02	0.16
Lactate	0.04	−0.03	0.37	−0.14	0.05
Pyruvate	−0.36**	−0.16	0.01	−0.28§	0.18
Glycerol	0.01	0.01	0.39	−0.05	0.03

Pearson's correlation coefficient (r), *$P < 0.01$; **$P < 0.001$; §$P = 0.01–0.05$.
CO_2, body carbon dioxide production; DM, diabetes mellitus; HF, heart failure; SBP, systolic blood pressure; VE, minute ventilation; VO_2, body oxygen consumption.

exception of pyruvate, no relationship of metabolites to peak exercise capacity was observed in patients with HF, indicating that peak performance of the failing heart is not limited by low or high substrate levels. It has been suggested that catecholamine and FFA surges occurring during stress or exercise might lead to less effective substrate utilization, because high FFAs may acutely inhibit glucose oxidation in the cardiac and skeletal muscle by reciprocal regulation of substrate use (Randle's glucose–fatty acid cycle).[13,28] Our data speak rather against this mechanism, because the FFA level decreased post-exercise in all HF subjects and was unrelated to cardiac performance, even in HFDM+ patients. Acute effects of metabolic substrate fluctuation on the failing heart are apparently negligible and are likely to be overridden by stronger intracellular homeostatic mechanisms, such as myocardial AMP kinase activation.[28]

Our findings are in line with those of Halbirk *et al.* who showed that a 27% long-term reduction of circulating FFAs with acipimox had no consequences on systolic and diastolic function at rest or cardiac output during exercise.[17] Similarly, Wiggers *et al.* demonstrated that either artificial elevation (>1.4 mmol/L) or almost total suppression (<0.1 mmol/L) of circulating FFAs had no effect on regional ventricular function or exercise capacity in non-diabetic patients with HF due to coronary artery disease,[29] indicating preserved ability of the heart to adapt to extreme, short-term changes in myocardial substrate supply. Riley *et al.* also found no evidence of depletion of circulating metabolic substrates during steady-state submaximal[30] or graded maximal[31] exercise in non-diabetic HF patients. In contrast, Tunnanen *et al.* used positron emission tomography (PET) imaging and echocardiography to evaluate the acute effects of FFA reduction after acipimox in non-diabetic non-ischaemic HF patients. Acute FFA reduction (−84%) led to mild reduction of stroke volume (−8%) and cardiac work (−10%) that was not paralleled, in contrast to the controls, by reduction of oxygen demand, indicating deterioration of cardiac efficiency.[6] The observed exercise-related FFA decrease could be explained by relative hypoperfusion of adipose tissue during exercise[32] and/or by increased FFA utilization in working skeletal muscles[19,33] of HF subjects compared with controls. This conclusion is supported by our findings of preserved plasma norepinephrine response to exercise and preserved norepinephrine responsiveness of adipose tissue in patients with HF.[8]

The differences in the dynamics of plasma pyruvate between HF and control groups were more pronounced than in the dynamics of lactate. Post-exercise pyruvate significantly correlated with peak VO_2 both in the pooled cohort ($r = 0.62$) and in the individual subgroups ($r = 0.39–0.41$). The relationship between exercise dynamics of pyruvate and peak VO_2 has not been previously reported in HF. In healthy subjects, lactate increases more steeply during exercise; however, it drops immediately with the onset of recovery. This is in contrast to pyruvate that continues to rise in recovery, reflecting its intramuscular concentration and intracellular NADH/NAD^+ ratio.[34] Blood pyruvate is determined by the balance between pyruvate production (by glycolysis) and clearance. Pyruvate is either metabolized into acetyl-CoA, or is

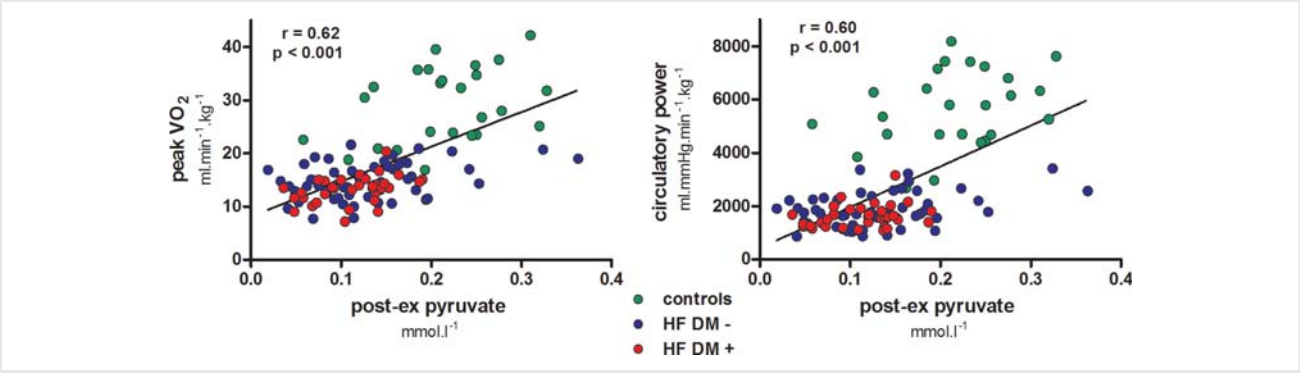

Figure 3 Correlations (Pearson's *r*) between maximal exercise capacity (peak VO_2), circulatory power (peak VO_2 × peak systolic blood pressure), and post-exercise pyruvate levels in controls and in patients with heart failure with (HFDM+) or without (HFDM−) diabetes mellitus.

converted into lactate, depending on the cytosolic redox state. Low post-exercise pyruvate in HF subjects can be a consequence of skeletal muscle myopathy accompanying HF, although histochemistry and magnetic resonance spectroscopy of skeletal muscle from HF patients showed rather enhanced glycolytic capacity, and more prevalent glycolytic (type II) fibres. Low post-exercise pyruvate in HF could also be due to increased pyruvate consumption by the mitochondrial pyruvate dehydrogenase complex, uninhibited by products of fatty acid oxidation due to falling FFA levels at peak exercise. Whether insufficient pyruvate dynamics in HF is a mere marker of exercise intolerance, or it has direct effects on the heart and muscles cannot be resolved from these data. Interestingly, intravascular administration of pyruvate has strong positive inotropic effects in the failing human heart.[35]

Metabolites, neurohormones, and long-term outcome

Of the metabolites, only post-exercise glycaemia was associated with an increased risk of the adverse outcome, which is a novel observation in HF patients. Stress-induced hyperglycaemia reflects higher glucose production than utilization, probably due to norepinephrine-, glucagon-, and cortisol-mediated stimulation of glucose hepatic output, increased in HF.[26] Hyperglycaemia induced by an acute illness (infection or myocardial infarction) is a known independent predictor of mortality, even in non-diabetic subjects.[36] Of the hormones, only NT-proBNP and copeptin levels were predictive, more than plasma norepinephrine. Of the clinical variables, adverse outcome was strongly predicted by decreased total body fat content, confirming previous reports.[37] Therefore, excessive fat depletion due to cardiac cachexia, rather than high FFA levels per *se*, confers adverse prognosis in advanced HF.

Study limitations

The presented study has several limitations that do not violate its main results. First, circulating triglycerides were not measured, but their contribution to myocardial energy provision in human HF is minor.[38] Secondly, females were underrepresented due to gender imbalance in case referral. Thirdly, we did not measure metabolites throughout the entire exercise study but only at two time points, which limits the temporal resolution of changes in

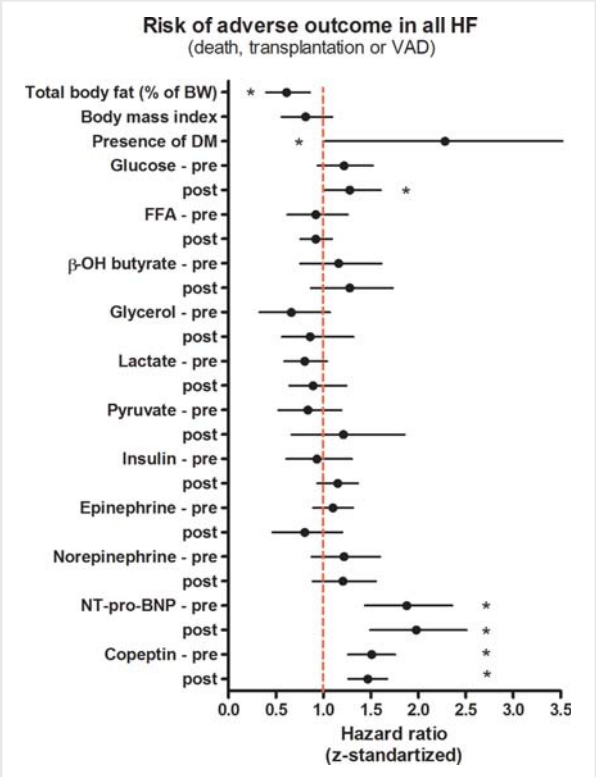

Figure 4 The impact of metabolic, humoral and clinical factors on the risk of adverse outcome (combined endpoint of death without transplant, urgent transplantation, or ventricular assist device insertion) in an univariate Cox's model. Hazard ratios are expressed per 1 − SD change (z-standardized). *$P < 0.05$. BW, body weight; DM, diabetes mellitus; FFA, free fatty acids; HF, heart failure; NT-proBNP, N-terminal pro brain natriuretic peptide; VAD, ventricular assist device.

metabolites. Fourthly, the peak exercise blood sample was not taken during, but immediately after, the exercise, which might have lessened the observed dynamics of lactate. Fifthly, because of the study design that aimed to compare metabolic response in HFDM+ and HFDM− groups at a similar level of achieved exercise, we cannot address the question of how DM itself affects exercise performance in HF. Instead, we focused on the relationship between metabolites and exercise cardiac indexes within groups.

Conclusions

In conclusion, this study shows that energetic substrate levels in the blood do not play a major role in exercise limitation of HF patients, regardless of their diabetic status. HFDM+ patients had higher copeptin, but otherwise similar neurohumoral profile to HFDM− patients. Acute changes of metabolites are not related to cardiac performance, with the exception of post-exercise plasma pyruvate that was positively correlated with exercise capacity. Importantly, diabetic status, post-exercise glycaemia, and body fat depletion were related to adverse long-term outcome in HF. Acute manipulations in substrate availability are thus unlikely to change the cardiac functional status or prognosis of HF patients, either with or without DM. An inhibition of body fat depletion, attenuation of stress-induced hyperglycaemia, or increasing the dynamics of plasma pyruvate may represent a therapeutic target in advanced HF.

Supplementary material

Supplementary material is available at *European Journal of Heart Failure* online.

Acknowledgements

We would like to thank Josef Halaxa, Hana Podlesakova, Ivana Kamenarova, and Jaroslava Kulhava from the Department of Cardiology, Institute for Clinical and Experimental Medicine in Prague for their excellent work.

Funding

The Ministry of Health (MZO-00023001 to V.M., NS10497-3/2009 to V.M. and J.P.); the Ministry of Education (MSMT-1MO510 and KONTAKT LH12052 to V.M.); the Grant Agency of the Academy of Sciences of the Czech Republic (305/09/1390 to V.M.).

Conflict of interest: J.K. is a member of Advisory Board for GE Healthcare. P.J. has received a research grant from Roche Diagnostics. Otherwise, there are no relationships with industry posing a conflict of interest in this paper.

References

1. Korvald C, Elvenes OP, Myrmel T. Myocardial substrate metabolism influences left ventricular energetics *in vivo*. *Am J Physiol Heart Circ Physiol* 2000; **278**:H1345–H1351.
2. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 2005;**85**:1093–1129.
3. Hawley JA, Burke LM, Angus DJ, Fallon KE, Martin DT, Febbraio MA. Effect of altering substrate availability on metabolism and performance during intense exercise. *Br J Nutr* 2000;**84**:829–838.
4. Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. *Circulation* 1996; **94**:2837–2842.
5. Melenovsky V, Benes J, Skaroupkova P, Sedmera D, Strnad H, Kolar M, Vlcek C, Petrak J, Benes J Jr., Papousek F, Oliyarnyk O, Kazdova L, Cervenka L. Metabolic characterization of volume overload heart failure due to aorto-caval fistula in rats. *Mol Cell Biochem* 2011;**354**:83–96.
6. Tuunanen H, Engblom E, Naum A, Nagren K, Hesse B, Airaksinen KE, Nuutila P, Iozzo P, Ukkonen H, Opie LH, Knuuti J. Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. *Circulation* 2006;**114**:2130–2137.
7. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. *N Engl J Med* 1999;**341**:577–585.
8. Polak J, Kotrc M, Wedellova Z, Jabor A, Malek I, Kautzner J, Kazdova L, Melenovsky V. Lipolytic effects of B-type natriuretic peptide 1–32 in adipose tissue of heart failure patients compared with healthy controls. *J Am Coll Cardiol* 2011;**58**:1119–1125.
9. Paolisso G, De Riu S, Marrazzo G, Verza M, Varricchio M, D'Onofrio F. Insulin resistance and hyperinsulinemia in patients with chronic congestive heart failure. *Metabolism* 1991;**40**:972–977.
10. Shelton RJ, Ingle L, Rigby AS, Witte KK, Cleland JG, Clark AL. Cardiac output does not limit submaximal exercise capacity in patients with chronic heart failure. *Eur J Heart Fail* 2010;**12**:983–989.
11. Woods PR, Bailey KR, Wood CM, Johnson BD. Submaximal exercise gas exchange is an important prognostic tool to predict adverse outcomes in heart failure. *Eur J Heart Fail* 2011;**13**:303–310.
12. Chaitman BP. Exercise stress testing. In: Zipes DP, Braunwald E, eds. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. 7th ed. Philadelphia, PA: W.B. Saunders; 2005. p.153–177.
13. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. *Circulation* 2007;**116**:434–448.
14. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2011;**34**:S62–S69.
15. Durnin JV, Womersley J. Total body fat, calculated from body density, and its relationship to skinfold thickness in 571 people aged 12–72 years. *Proc Nutr Soc* 1973;**32**:45A.
16. Cohen-Solal A, Tabet JY, Logeart D, Bourgoin P, Tokmakova M, Dahan M. A non-invasively determined surrogate of cardiac power ('circulatory power') at peak exercise is a powerful prognostic factor in chronic heart failure. *Eur Heart J* 2002;**23**:806–814.
17. Halbirk M, Norrelund H, Moller N, Schmitz O, Gotzsche L, Nielsen R, Nielsen-Kudsk JE, Nielsen SS, Nielsen TT, Eiskjaer H, Botker HE, Wiggers H. Suppression of circulating free fatty acids with acipimox in chronic heart failure patients changes whole body metabolism but does not affect cardiac function. *Am J Physiol Heart Circ Physiol* 2010;**299**:H1220–H1225.
18. Aaronson KD, Schwartz JS, Chen TM, Wong KL, Goin JE, Mancini DM. Development and prospective validation of a clinical index to predict survival in ambulatory patients referred for cardiac transplant evaluation. *Circulation* 1997;**95**: 2660–2667.
19. Paolisso G, Gambardella A, Galzerano D, D'Amore A, Rubino P, Verza M, Teasuro P, Varricchio M, D'Onofrio F. Total-body and myocardial substrate oxidation in congestive heart failure. *Metabolism* 1994;**43**:174–179.
20. Lommi J, Kupari M, Koskinen P, Naveri H, Leinonen H, Pulkki K, Harkonen M. Blood ketone bodies in congestive heart failure. *J Am Coll Cardiol* 1996; **28**:665–672.
21. Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med* 1984;**311**:819–823.
22. van der Horst IC, de Boer RA, Hillege HL, Boomsma F, Voors AA, van Veldhuisen DJ. Neurohormonal profile of patients with heart failure and diabetes. *Neth Heart J* 2010;**18**:190–196.
23. Enhorning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G, Struck J, Morgenthaler NG, Bergmann A, Lindholm E, Groop L, Lyssenko V, Orho-Melander M, Newton-Cheh C, Melander O. Plasma copeptin and the risk of diabetes mellitus. *Circulation* 2010;**121**:2102–2108.
24. Lommi J, Kupari M, Yki-Jarvinen H. Free fatty acid kinetics and oxidation in congestive heart failure. *Am J Cardiol* 1998;**81**:45–50.
25. Doehner W, Rauchhaus M, Ponikowski P, Godsland IF, von Haehling S, Okonko DO, Leyva F, Proudler AJ, Coats AJ, Anker SD. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. *J Am Coll Cardiol* 2005;**46**:1019–1026.
26. Paolisso G, Gambardella A, Marrazzo G, Verza M, Teasuro P, Varricchio M, D'Onofrio F. Metabolic and cardiovascular benefits deriving from beta-adrenergic blockade in chronic congestive heart failure. *Am Heart J* 1992;**123**:103–110.
27. Masoudi FA, Inzucchi SE. Diabetes mellitus and heart failure: epidemiology, mechanisms, and pharmacotherapy. *Am J Cardiol* 2007;**99**:113B–132B.

28. Hue L, Taegtmeyer H. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab* 2009;**297**:E578–E591.
29. Wiggers H, Norrelund H, Nielsen SS, Andersen NH, Nielsen-Kudsk JE, Christiansen JS, Nielsen TT, Moller N, Botker HE. Influence of insulin and free fatty acids on contractile function in patients with chronically stunned and hibernating myocardium. *Am J Physiol Heart Circ Physiol* 2005;**289**:H938–H946.
30. Riley M, Elborn JS, Bell N, Stanford CF, Nicholls DP. Substrate utilization during exercise in chronic cardiac failure. *Clin Sci (Lond)* 1990;**79**:89–95.
31. Riley M, Bell N, Elborn JS, Stanford CF, Buchanan KD, Nicholls DP. Metabolic responses to graded exercise in chronic heart failure. *Eur Heart J* 1993; **14**:1484–1488.
32. Smiseth OA, Riemersma RA, Steinnes K, Mjos OD. Regional blood flow during acute heart failure in dogs. Role of adipose tissue perfusion in regulating plasma-free fatty acids. *Scand J Clin Lab Invest* 1983;**43**:285–292.
33. Norrelund H, Wiggers H, Halbirk M, Frystyk J, Flyvbjerg A, Botker HE, Schmitz O, Jorgensen JO, Christiansen JS, Moller N. Abnormalities of whole body protein turnover, muscle metabolism and levels of metabolic hormones in patients with chronic heart failure. *J Intern Med* 2006;**260**:11–21.
34. Wasserman K, Beaver WL, Davis JA, Pu JZ, Heber D, Whipp BJ. Lactate, pyruvate, and lactate-to-pyruvate ratio during exercise and recovery. *J Appl Physiol* 1985; **59**:935–940.
35. Hermann HP, Arp J, Pieske B, Kogler H, Baron S, Janssen PM, Hasenfuss G. Improved systolic and diastolic myocardial function with intracoronary pyruvate in patients with congestive heart failure. *Eur J Heart Fail* 2004;**6**: 213–218.
36. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. *Lancet* 2000;**355**:773–778.
37. Futter JE, Cleland JG, Clark AL. Body mass indices and outcome in patients with chronic heart failure. *Eur J Heart Fail* 2011;**13**:207–213.
38. Funada J, Betts TR, Hodson L, Humphreys SM, Timperley J, Frayn KN, Karpe F. Substrate utilization by the failing human heart by direct quantification using arterio-venous blood sampling. *PloS ONE* 2009;**4**:e7533.