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Free fatty acid availability is closely related to myocardial lipid storage and cardiac function in hypoglycemia counterregulation

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Winhofer Y, Krššák M, Wolf P, Anderwald C, Geroldinger A, Heinze G, Baumgartner-Parzer S, Marculescu R, Stulnig T, Wolzt M, Trattnig S, Luger A, Krebs M. Free fatty acid availability is closely related to myocardial lipid storage and cardiac function in hypoglycemia counterregulation. *Am J Physiol Endocrinol Metab* 308: E631–E640, 2015. First published February 10, 2015; doi:10.1152/ajpendo.00371.2014.—Hypoglycemia, a major side effect of intensive glucose-lowering therapy, was recently linked to increased cardiovascular risk in patients with diabetes. Whether increased circulating free fatty acids (FFA) owing to catecholamine-induced lipolysis affect myocardial energy metabolism and thus link hypoglycemia to cardiac vulnerability is unclear. Therefore, this study investigated the impact of hypoglycemia counterregulation (± inhibition of lipolysis) on myocardial lipid content (MYCL) and left ventricular function in healthy subjects. Nine healthy men were studied in randomized order: *1*) insulin/hypoglycemia test (IHT; ins+/aci−), *2*) IHT during inhibition of adipose tissue lipolysis by acipimox (ins+/aci+), *3*) normoglycemia with acipimox (ins−/aci+), and *4*) normoglycemia with placebo (ins−/aci−). MYCL and cardiac function were assessed by employing magnetic resonance spectroscopy/imaging at baseline and at 2 and 6 h. In response to acute hypoglycemia, plasma FFA ($P < 0.0001$) and ejection fraction (EF; from 63.2 ± 5.5 to 69.6 ± 6.3%, $P = 0.0001$) increased significantly and were tightly correlated with each other ($r = 0.68$, $P = 0.0002$); this response was completely blunted by inhibition of adipose tissue lipolysis. In the presence of normoglycemia, inhibition of lipolysis was associated with a drop in EF (from 59.2 ± 5.5 to 53.9 ± 6.9%,$P = 0.005$) and a significant decrease in plasma FFA, triglycerides, and MYCL (by 48.5%, $P = 0.0001$). The present data indicate that an intact interorgan cross-talk between adipose tissue and the heart is a prerequisite for catecholamine-mediated myocardial contractility and preservation of myocardial lipid stores in response to acute hypoglycemia.

hypoglycemia; insulin/hypoglycemia test; catecholamine-mediated lipolysis; free fatty acids; myocardial lipid content; cardiac function; acipimox; cardiac magnetic resonance imaging and spectroscopy

HYPOGLYCEMIA REMAINS A FREQUENT AND SEVERE COMPLICATION of glucose-lowering therapies in patients with diabetes (38). Sympathoadrenal activation during hypoglycemia counterregulation leads to a chain of reactions that impact the cardiovascular system in multiple ways. Besides its proarrhythmogenic and proinflammatory potential (25, 37), the effects of catecholamine-mediated lipolysis and the consequent increase in circulating free fatty acids (FFA) on cardiac substrate metabolism are unclear; elevated plasma FFA concentrations have been linked to an increased risk of heart failure and cardiovascular mortality (4, 23). Although FFA serve as one of the main substrates for cardiac work, their oversupply may result in increased myocardial lipid content (cardiac steatosis), lipotoxicity, and consequent alterations in left ventricular function (27, 29).

Myocardial lipid content (MYCL) can be noninvasively assessed by magnetic resonance (MR) spectroscopy simultaneously with cardiac function (33). We recently observed acute changes in MYCL following combined hyperglycemia-hyperinsulinemia, proving that rapid changes in myocardial substrate metabolism can be monitored employing this technique (36).

Prior studies have shown that lipolysis secondary to prolonged fasting and the consequent increase in FFA result in increased MYCL associated with impaired left ventricular diastolic function in healthy subjects and patients with type 2 diabetes (12, 34). These effects can be ascribed to direct effects of FFA, since the inhibition of adipose tissue lipolysis by acipimox abolishes myocardial lipid accumulation (11). On the other hand, catecholamine-mediated lipolysis during hypoglycemic counterregulation was linked to posthypoglycemic insulin resistance (19). Previous data also suggest that circulating triglycerides might contribute to myocardial lipid accumulation (during fasting) in experimental animals (31).

Whether an acute increase in FFA due to catecholamine-induced lipolysis in the course of hypoglycemia counterregulation impacts myocardial substrate metabolism and function is unclear. Therefore, the aim of the study was to investigate associations between FFA, MYCL, and cardiac function under dynamic conditions in the course of hypoglycemia counterregulation.

RESEARCH DESIGN AND METHODS

Study Population

Nine healthy young men (age 24.7 ± 2.2 yr, body mass index 24.2 ± 3.6 kg/m^2) were included in this study after the following criteria had been excluded: acute or chronic inflammatory, heart, liver, or kidney disease, impaired glucose tolerance or diabetes, history of seizures,

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and general MR contraindications (i.e., claustrophobia, metal agents, in/on the body).

All subjects underwent a screening examination, including blood chemistry, an electrocardiogram (ECG), and assessment of blood pressure and medical history. Study participants were asked to refrain from intensive physical training and stop regular moderate exercising, and they were instructed to ingest an isocaloric diet (30 $kcal \cdot kg^{-1} \cdot day^{-1}$, carbohydrate/protein/fat: 55/15/30%) for 3 days prior to the study as well as in the time between 480 min of the study protocol and the next morning.

The study conformed to the Declaration of Helsinki, and the Human Ethics Committee of the Medical University of Vienna approved the study protocol (EK no. 125/2011); all subjects gave written informed consent. One subject underwent only 2 study days and was thereafter withdrawn from the study because of concurrent participation in another trial (noninterventional, but additional blood were samples taken); these data were used for analyses.

Study Protocol

Subjects underwent 4 study days in randomized order, with a minimum interval of 14 days between the study days: *1*) standard insulin/hypoglycemia test (IHT; ins+/aci−), *2*) IHT during inhibition of lipolysis by acipimox (ins+/aci+; 250 mg of acipimox at 0 and 180 min), *3*) normoglycemia with acipimox (ins−/aci+), and *4*) normoglycemia with administration of placebo (ins−/aci−).

Each subject was studied after an overnight fast for ≥8 h. After placing a venous catheter into an antecubital vein, initial blood samples were taken at baseline (−15 to 0 min). Thereafter, the baseline MR examination (0–60 min of study protocol) took place, followed by the second (180 to 240 min) and third MR examination (420–480 min). Blood samples for the measurement of counterregulatory hormones (see below) were drawn at 0, 75, 90, 105, 120, 135, 165, 270, 330, and 405 min, and in between, additional blood samples for assessment of plasma glucose were taken every 5 min between 60 and 105 min and then every 15 min until 480 min. At 480 min, after the third MR examination, subjects left the study laboratory and returned for a control visit the next morning for a fourth MR examination. In the time between the end of the study protocol (480 min) and the MR examination on the following morning, diet and physical activity were not controlled; MR data of this measurement are reported in the text but not shown in the figures or tables.

Insulin/Hypoglycemia Test

To assess the impact of an acute hypoglycemic event, standardized hypoglycemia was induced following the protocol of the IHT, which is clinically used to evaluate hypothalamic-pituitary function and was proven to be safe in a broad spectrum of patients (5, 6). Hypoglycemia was induced by bolus injection of 0.1 IU insulin aspart/kg body wt (Novo Nordisk, Bagsvaerd, Denmark) at 60 min of the study protocol. Subjects were under constant supervision by a physician and had ECG monitoring.

Administration of Acipimox

To suppress adipose tissue lipolysis during hypoglycemic counterregulation, acipimox (250 mg at 0 and 180 min; Pfizer, Ascoli Piceno, Italy) was administered orally in ins+/aci+ and ins−/aci+ and a placebo in ins−/aci− and ins+/aci−. Acipimox inhibits adenylyl cyclase, resulting in suppression of hormone-sensitive lipase activity (28).

ECG Monitoring

Continuous ECG monitoring was applied to all subjects for the duration of the whole protocol, lasting for 24 h. ECG monitoring was discontinued only during the MR sessions, during which a MR-compatible monitor monitored the heart rate. Two blinded physicians analyzed the ECGs for pathological arrhythmias, which were not detected in any subject (data not shown). The mean heart rate per hour as well as heart rate change compared with the baseline value on that study day (ΔHR) was used for analyses.

Plasma Metabolites

Glucose concentrations were analyzed immediately (Biosen C_line; EKF Diagnostic, Barleben/Magdeburg, Germany). Plasma FFA (microfluorimetric assay; Wako, Neuss, Germany), cortisol (35), glucagon (RIA Kit; Millipore), insulin (RIA Kit), and somatotropine (Liaison HGH Kit; CLIA, DiaSorin, Italy) were analyzed in the Laboratory of Endocrinology and Metabolism and triglycerides (by enzymatic-colorimetric GPO/PAP method) and catecholamines (noradrenalin and adrenalin by high-performance liquid chromatography) analyzed at the Department for Laboratory Medicine of the Medical University of Vienna. A blood gas analysis, including measurement of pH and electrolytes, was performed at baseline and 135 min of the study protocol.

Calculations of Metabolic Parameters

Although an insulin hypoglycemia test was originally designed and is used in clinical routine to evaluate hypothalamic-pituitary function (growth hormone and cortisol release), we also decided to extrapolate metabolic insulin action by dividing the plasma glucose fall by the circulating insulin concentrations assessed in ins+/aci−. This result is termed insulin-mediated hypoglycemic glucose disappearance (IHGD). The gold standard to measure whole body insulin sensitivity is the euglycemic hyperinsulinemic clamp test. In this test, hepatic glucose production is reduced in the presence of defined hyperinsulinemia by ~90%, and the major amount of glucose infused is taken up by skeletal muscle. In our approach (IHGD), insulin action was estimated by considering circulating insulin's ability to decrease plasma glucose concentrations. However, we are aware that hypoglycemic conditions were present, which results in elevated hepatic glucose output due to counterregulation. Nevertheless, we believe that our calculations on the action of insulin to induce hypoglycemia reflect at least the amount of insulin's effect in glucose-utilizing tissues, predominantly and particularly the skeletal muscle.

Area under the curves was calculated by using the trapezoidal rule and given for different time intervals.

MRI and Spectroscopy

All MR measurements were performed on the 3.0-T Tim Trio System (Siemens, Erlangen, Germany).

^{1}H-MRS for the quantification of myocardial and hepatic lipid content. MYCL was measured by employing recently published protocols (17, 36). Anatomic imaging was used to guide two acquisitions of water-suppressed Point RESolved Spectroscopy sequence [echo time = 30 ms; repetition time was according to individual heartbeat; no. of scans (NS) = 8, no. of dummy scans (ND) = 2] during separate breath holds. The volume of interest (VOI; ~6–8 cm^3) was placed over the interventricular septum. The spectral signal was acquired following ECG, and the start of the signal acquisition was paced into the mid-diastole. Two additional spectra without water suppression (NS = 4, ND = 2) were used as internal reference. The spectra were processed offline using AMARES time domain line fitting, as implemented with the jMRUI software package. MYCL was determined from processed spectra as a ratio of the intensities of CH_2 (1.25 ppm) and CH_3 (0.8–0.9 ppm) group resonances to the intensity of the water resonance from non-water-suppressed spectra of the same VOI. The coefficient of variation (test-retest) of this method was 20%.

Hepatic lipid content was measured in analogy to previously published protocols (16).

^{1}H-MRI for myocardial function. Visualization of cardiac function was performed using prospective ECG-gated cine true fast imaging

with steady-state precession (TrueFISP) sequences in two-chamber, four-chamber, and short-axis orientation. Short-axis images were used to quantify left ventricular global (end-diastolic and end-systolic volume, stroke volume, ejection fraction, and myocardial mass) via ARGUS software (Siemens). These volumetric parameters were normalized to body surface area using the Dubois-formula. Ejection fraction was used as primary index of left ventricular systolic function. Additionally, we determined left ventricular diastolic function using Flash-based (fast low-angle shot) prospective ECG-gated cine phase contrast sequence and postprocessing via ARGUS software to determine the early (E) and atrial (A) diastolic filling phase (E/A) ratio of mitral inflow (1, 2, 20, 33).

Statistical Analysis

Sample size calculation. For the sample size calculation, we concentrated on the assumed changes in myocardial lipid content during hypoglycemia counterregulation. We found that a sample size of eight subjects will have 80% power to detect a relevant difference in the mean myocardial lipid content of 0.07% of water signal (WS), assuming a standard deviation of differences of 0,06% of WS [as observed in our previous study (36)], using a paired *t*-test with a 0.05 two-sided significance level.

Data analyses. Comparisons between study days at single time points and changes in the course of time (within and between the study days) were analyzed using linear mixed models. For the parameters EF, MYCL, hepatic lipid content (HCL), and end-diastolic volume being measured only three times on each study day (and on the next morning), time was treated as class variable, and the linear mixed model with the smallest AIC value was chosen among the following candidates. In addition to the fixed of effects time, study day, and their interaction, we considered either subjects or subjects and time as random factors or a repeated effect with the block structure defined by subjects. We also considered these models, with the covariance parameters allowed to vary by study day. The covariance structure was chosen among the following: unstructured, autoregressive, compound symmetry, Toeplitz, and diagonal. For the variables glucose, insulin, cortisol, glucagon, human growth hormone (HGH), noradrenalin, adrenalin, FFA and triglycerides, and furthermore, heart rate, which were measured 10 or even 31 times (glucose) at each study day, the time effects were modeled using a natural cubic or B-spline basis. Again, we chose the mixed model with the smallest AIC value, where we considered the models with subjects or subjects and time as random factors, as described above. In order to obtain approximately normal distributions of residuals in the mixed models, we have replaced (in the mixed-model analysis) EF by its square and the variables MYCL, HCL, insulin, glucagon, HGH, FFA, noradrenalin, and adrenalin by their logarithms.

Values of the parameters, which have been logarithmized in the mixed models, are described by geometric mean (range), whereas values of all the other variables are described by means ± SD. Similarly, in Figs. 1–3, the behavior over time of parameters that have been logarithmized in the mixed model is illustrated using geometric mean with geometric SD on a logarithmic scale. For other parameters, the behavior over time is summarized by means ± SD. Associations between different variables are described by Pearson's correlation coefficient, where we have either restricted ourselves to a certain study day or computed partial correlation coefficients adjusted for the subjects (3).

All statistical analysis were performed using SAS 9.3 software (SAS Institute, Cary, NC). Graphics were prepared with R software (http://www.r-project.org). Two-sided P values ≤ 0.05 were interpreted as indicating statistical significance.

RESULTS

Baseline characteristics of study subjects are given in Table 1.

Table 1. *Baseline characteristics of study subjects*

Subject Characteristics	Value
Age, yr	24.7 ± 2.2
Body mass index, kg/m^2	24.2 ± 3.6
Body surface area, m^2	2.0 ± 0.2
Triglycerides, mg/dl	99.1 ± 35.9
Total cholesterol, mg/dl	155.1 ± 24.3
HDL cholesterol, mg/dl	52.0 ± 10.1
Hb A_{1c}, %	5.1 ± 0.2

Values are means ± SD; $n = 9$ subjects (all male).

Metabolic and Endocrine Changes in the Course of Hypoglycemic Counterregulation

The insulin dose was sufficient to induce acute hypoglycemia in all subjects with comparable plasma glucose nadirs of 2.0 ± 0.4 mmol/l in ins+/aci− and 1.9 ± 0.3 mmol/l in ins+/aci+ at 20 and 25 min, respectively, after insulin administration (not sigificant; Fig. 1*A*). No subject had neuroglycopenic symptoms necessitating glucose administration. Glucose and insulin concentrations (except insulin at 270, 330, and 405 min; Fig. 1*B*) were independent of acipimox administration. Following hypoglycemia, no significant changes in plasma sodium or potassium concentrations or pH were observed (data not shown).

The expected changes in plasma concentrations of counterregulatory hormones were observed. Comparable increases (compared to baseline: $P < 0.0001$) in glucagon concentrations (Fig. 1*C*) were observed in ins+/aci− and ins+/aci+. In addition, cortisol (Fig. 1*D*) increased significantly following hypoglycemia ($P < 0.0001$). Only at 405 min were cortisol concentrations significantly higher in ins+/aci+ compared with ins+/aci− ($P = 0.035$). Serum concentrations of HGH (Fig. 1*E*) were comparably increased in ins+/aci− and ins+/aci+ from 0 to 135 min ($P = 0.0003$ and $P < 0.0001$, respectively). Only between 165 and 405 min was HGH higher in ins+/aci+ compared with ins+/aci− ($P < 0.006$).

Catecholamine and Lipid Response to Hypoglycemia and Inhibition of Lipolysis

As expected, plasma concentrations of noradrenalin (Fig. 2*A*) and adrenalin (Fig. 2*B*) increased in ins+/aci− and ins+/aci+ (from baseline to 90 min, $P < 0.0001$) without significant differences between these 2 study days (i.e., acipimox administration).

In ins+/aci−, plasma FFA (Fig. 2*C*) increased significantly, by 2.5-fold from baseline to 270 min ($P < 0.0001$), whereas triglycerides decreased after 270 min compared with the earlier time points. However, during acipimox administration, FFA concentrations were comparably suppressed in ins+/aci+ by 82.9% [95% confidence interval (CI), 74.4–88.6%, $P < 0.0001$] and in ins−/aci+ by 89.7% (95% CI, 85.1–92.9%, $P < 0.0001$) compared with baseline. Triglycerides were decreased significantly in ins−/aci+ compared with baseline; in ins+/aci+, their decline started at 135 min of the study protocol. In ins−/aci−, FFA and triglyceride concentrations remained unchanged during the study day (Fig. 2*D*).

The decline in plasma FFA is accompanied by a decrease in myocardial lipid content and left ventricular systolic function.

In parallel with the FFA suppression, MYCL (Fig. 3, *left*) declined during acipimox administration, in ins+/aci+ by 40.5%

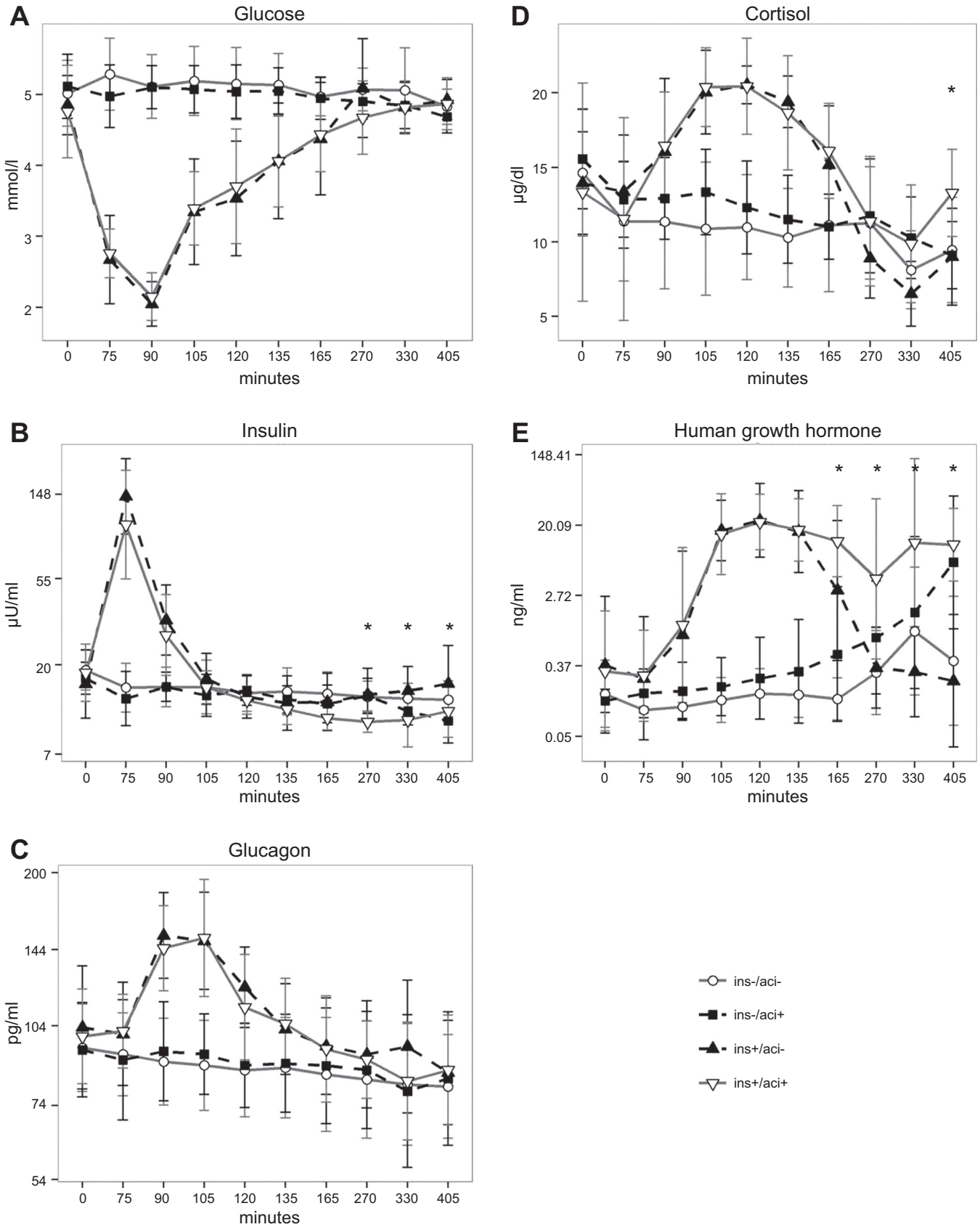

Fig. 1. Plasma concentrations of glucose (*A*), insulin (*B*), glucagon (*C*), cortisol (*D*), and human growth hormone (HGH; *E*) on each study day. Glucose and cortisol are described by means ± SD and insulin, glucagon, and HGH by geometric means ± geometric SD on logarithmic scale. *Differences between aci+ and aci−.

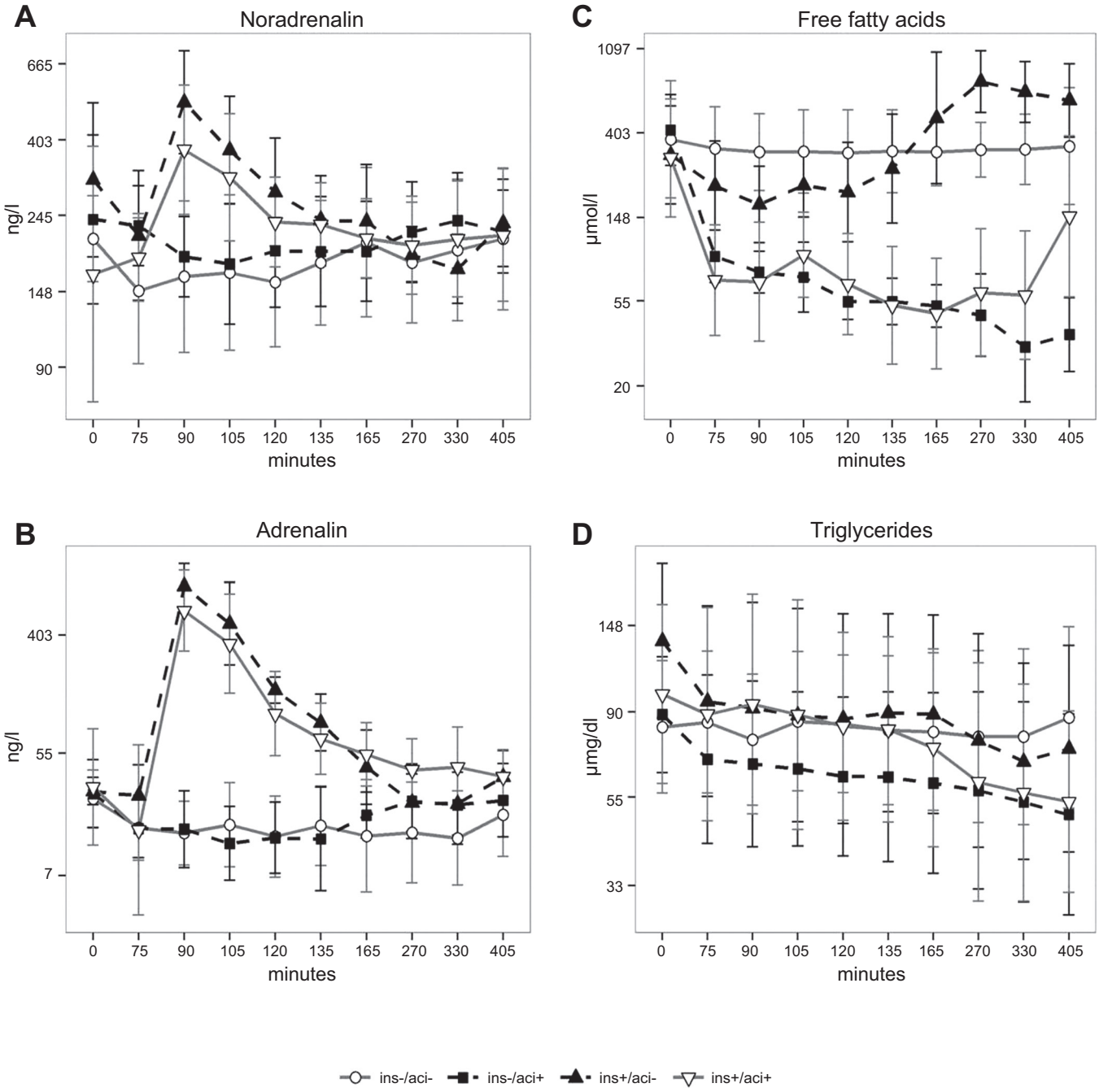

Fig. 2. Plasma concentrations of noradrenaline (*A*), adrenaline (*B*), free fatty acids (FFA; *C*), and triglycerides (*D*) on each study day described by geometric means ± geometric SD on logarithmic scale.

of the baseline value (95% CI, 13.7–58.9%, $P = 0.007$), and in ins−/aci+ by 48.5% of the baseline value (95% CI, 28.5.2–62.9%, $P = 0.0001$). MYCL remained unchanged in ins−/aci− as well as in ins+/aci− (i.e., normal hypoglycemic counterregulation).

The EF (Fig. 3, *right*) increased significantly in response to hypoglycemia in ins+/aci− (i.e., normal hypoglycemic counterregulation) from 63.2 ± 5.5% at baseline to 69.6 ± 6.3% at 180–240 min (2nd MR), $P = 0.0001$ and was still higher compared with baseline at the third MR examination ($EF_{420–480\ min}$: 66.8 ± 6.7%, $P = 0.025$). In ins+/aci+, EF did not increase and remained unchanged compared with baseline. In ins−/aci+, EF decreased from 59.2 ± 5.5% at baseline to 53.9 ± 6.9% at the third MR examination ($P = 0.005$) in parallel with the depletion of MYCL.

Heart rate, as assessed by continuous ECG monitoring, did not differ between the study days, and in particular no difference was observed whether acipimox or placebo was given (Table 2).

Correlation Analyses

Whereas baseline values of the logarithm of MYCL and EF and the logarithm of FFA concentrations showed no significant partial

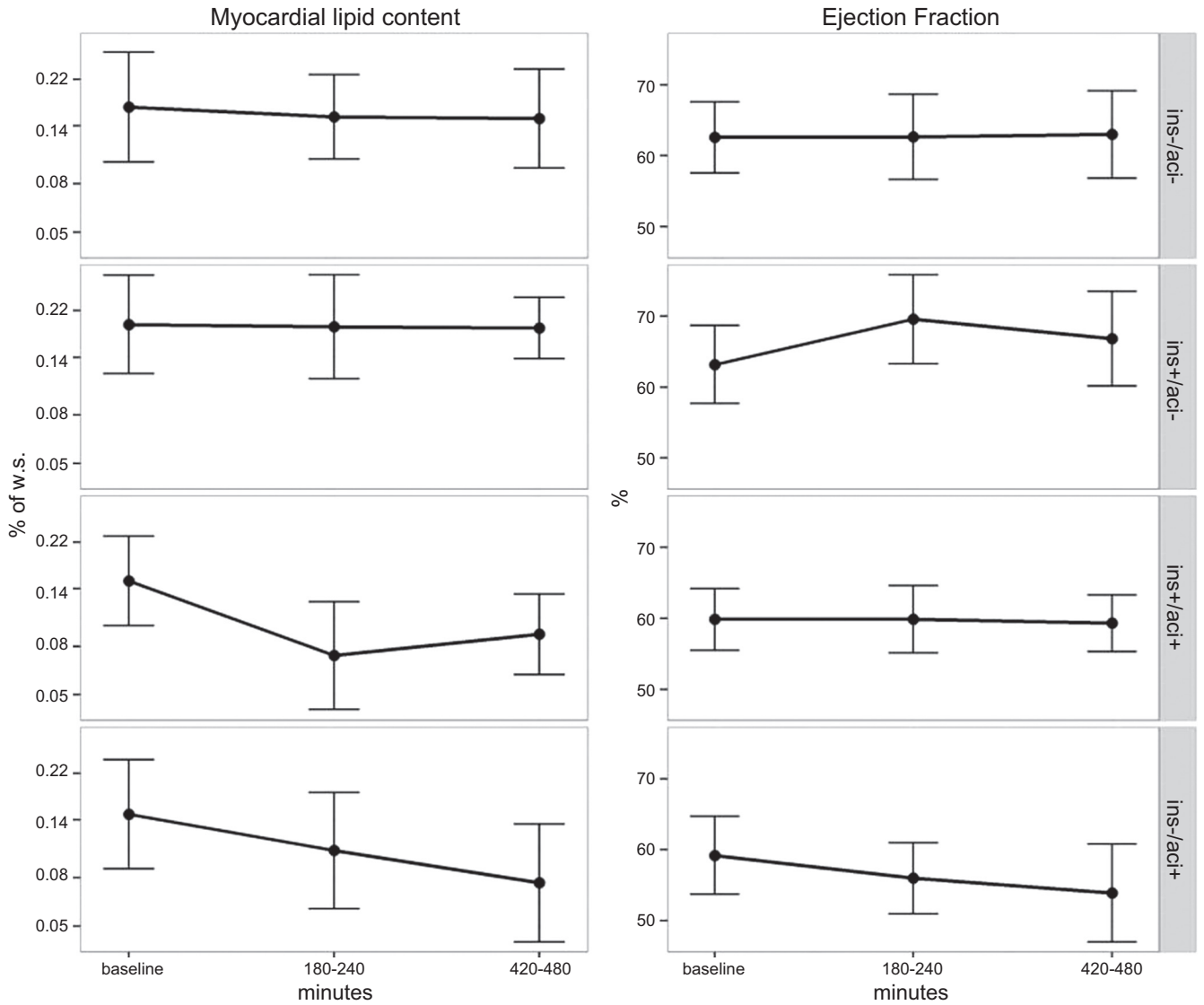

Fig. 3. Myocardial lipid content (MYCL; *left*) decreased significantly in ins+/aci+ and ins−/aci+. The ejection fraction (EF; *right*) increased significantly under normal hypoglycemic counterregulation in ins+/aci− and decreased in ins−/aci+. EF is described by means ± SD and MYCL by geometric means ± geometric SD on logarithmic scale. WS, water signal.

correlation adjusted for the subjects (adj. sub.) with each other, strong correlations were observed under dynamic conditions; e.g., the logarithm of area under the curve $(AUC)_{FFA\ 0-405\ min}$ was significantly correlated (adj. sub.) with the logarithm of $MYCL_{180-240\ min}$ (r_{part} = 0.60, P = 0.001) and the logarithm of $MYCL_{420-480\ min}$ (Fig. 4*A*). Moreover, the logarithm of $AUC_{FFA\ 0-405\ min}$ was strongly correlated (adj. sub.) with EF at the same time points: $EF_{180-240\ min}$ (r_{part}=0.69, P = 0.0001) and $EF_{420-480\ min}$ (see Fig. 4*B*).

Table 2. *Heart rate according to continuous ECG monitoring*

	Heart Rate, beats/min			
Study Protocol, min	ins−/aci−	ins+/aci−	ins+/aci+	ins−/aci+
60–120	66.3 ± 7.9	73.0 ± 9.3	71.7 ± 9.0	66.6 ± 9.7
120–180	66.0 ± 8.3	65.0 ± 8.7	65.5 ± 4.6	65.7 ± 9.1
240–300	62.0 ± 7.4	63.3 ± 5.8	64.0 ± 5.7	62.1 ± 6.0
300–360	62.4 ± 7.9	64.8 ± 6.4	61.0 ± 6.0	65.3 ± 8.2
360–420	67.6 ± 6.4	70.8 ± 5.7	64.0 ± 5.8	67.1 ± 7.8

Values are means ± SD. ECG, electrocardiogram. There was no difference in heart rate between the study days and especially no difference between acipimox or placebo administration independent of glycemia (ins+/ins−). From 180 to 240 min, continuous ECG monitoring was interrupted due to the magnetic resonance (MR) examination.

In ins+/aci−, insulin-mediated hypoglycemic glucose disappearance (IHGD) was correlated with the stroke volumes (SV) at all three MR examinations: baseline (see Fig. 4*C*), $SV_{180-240\ min}$ (r = 0.84, P = 0.01), and $SV_{420-480\ min}$ (r = 0.87, P = 0.005). In addition, IHGD was inversely correlated with the logarithm of MYCL at baseline (Fig. 4*D*) and positively correlated with the logarithm of noradrenalin at 90 min (Fig. 4*E*) and the logarithm of the AUC of noradrenalin from 0 to 165 min (r = 0.77, P = 0.03).

Furthermore, we observed a moderate inverse correlation between MYCL and parameters of left ventricular systolic function ($MYCL_{baseline}$ with $SV_{baseline}$: r = −0.45, P = 0.009; $MYCL_{420-480\ min}$ with $SV_{420-480\ min}$: r = −0.41, P < 0.02; $MYCL_{baseline}$ with end-diastolic $volume_{baseline}$: r =

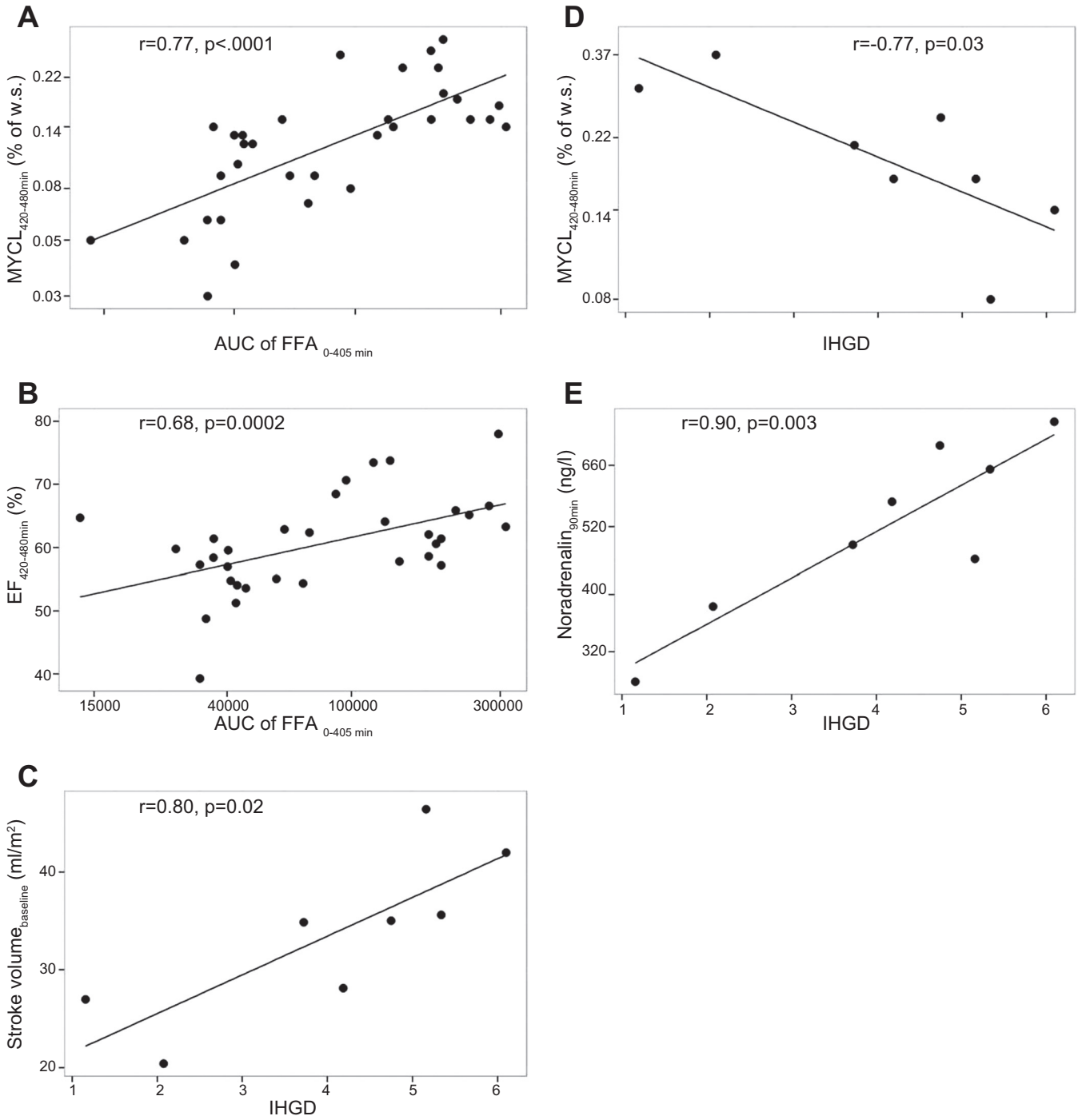

Fig. 4. The logarithm of the area under the curve (AUC) of FFA was partially correlated, adjusted for the subjects with the logarithm of MYCL (*A*) as well as the EF (*B*). In ins+/aci− (i.e., normal hypoglycemic counterregulation), insulin-mediated hypoglycemic glucose disappearance (IHGD) was strongly associated with stroke volume (*C*), inversely with the logarithm of myocardial lipid content (both shown at baseline; *D*) and positively with the logarithm of noradrenaline-plasma concentrations at 90 min (i.e., peak concentration of noradrenalin following hypoglycemia; *E*).

-0.45, $P < 0.009$), which lost significance after adjustment for subjects.

Impact of Hypoglycemia on HCL and Diastolic Function (E/A Ratio)

HCL and the E/A ratio, a marker of diastolic function, remained unchanged during all experimental conditions (data not shown).

Baseline values of MYCL, EF, HCL, and E/A ratio were comparable between the 4 study days and are given in Table 3.

Followup Next Morning

On the next morning after each study day (1,440–1,500 min), subjects returned for a physical control as well as a MR examination. $MYCL_{1,440–1,500\ min}$, $HCL_{1,440–1,500\ min}$, and $EF_{1,440–1,500\ min}$ were comparable between the 4 study conditions.

Table 3. *Baseline MR data*

	ins−/aci−	ins+/aci−	ins+/aci+	ins−/aci+	*P* Value
MYCL (%WS)	0.17 (0.08, 0.31)	0.19 (0.08, 0.37)	0.15 (0.08, 0.23)	0.15 (0.08, 0.29)	NS
EF, %	62.59 ± 5.02	63.18 ± 5.50	59.86 ± 4.36	59.2 ± 5.52	NS
HCL (%WS)	1.35 (0.27,5.60)	2.23 (0.95, 7.56)	1.64 (0.44, 9.76)	1.39 (0.31, 5.59)	NS
E/A ratio	1.69 ± 0.34	1.60 ± 0.38	1.63 ± 0.39	1.69 ± 0.45	NS

Values are means ± SD. MYCL, myocardial lipid content; WS, water signal; EF, ejection faction; HCL, hepatic lipid content; E/A, early (E) and atrial (A) diastolic filling phase; NS, not significant. Baseline values of MYCL, EF, HCL, and the E/A ratio did not differ between the study days.

DISCUSSION

To summarize, the main findings of the current study were that *1*) the rise in circulating FFA during hypoglycemia counterregulation is closely related to the increase in EF; *2*) conversely, acute suppression of plasma FFA concentrations during pharmacological inhibition of lipolysis is accompanied by decreases in EF as well as MYCL; and *3*) although baseline values of FFA, EF, and MYCL were not correlated with each other, we observed tight correlations between these variables under stimulated conditions.

The increase in EF following hypoglycemia has been described previously, and several mechanisms have been proposed to contribute to this physiological response. Initial studies on the effect of insulin-induced hypoglycemia on cardiac performance have demonstrated that the rise the EF is mediated by β-adrenergic receptors (8). Furthermore, it has been shown that an increase in EF is observed immediately after insulin administration before glucose concentrations are affected (7), indicating that insulin might impact myocardial function directly. In line with this hypothesis, not only hypoglycemic hyperinsulinemia but also euglycemic hyperinsulinemia is associated with an increase in EF in healthy subjects as well as in patients with type 1 diabetes (26). Hence, sympathetic stimulation as well as insulin are potential stimulators of cardiac action. However, in the present study insulin did not affect EF in the presence of low circulating FFA (ins+/aci+). Furthermore, despite the well-documented effect of FFA on the sympathetic tone (9, 22), heart rate following hypoglycemia was independent of prevailing FFA concentrations (there was no difference whether acipimox or placebo was given). In addition, the AUC of FFA concentrations was tightly correlated to MYCL and EF. Hence, our observation that adipose tissue lipolysis and the subsequent rise in FFA are closely related to the increase in the EF stresses the importance of a functioning interplay between adipose tissue lipolysis and the heart during hypoglycemia counterregulation.

Previous studies have indicated that the heart seems to be able to adapt to prolonged alterations in FFA availability. Acute effects of acipimox on EF have already been observed in patients with dilated cardiomyopathy and in healthy subjects (32), whereas no changes in cardiac function were observed during long-term treatment (28 days) in patients with chronic heart failure (10). Furthermore, pharmacological inhibition of lipolysis with acipimox for 1 wk was associated with a decrease in EF, but MYCL was unchanged in another study (18).

So far, to our best knowledge, no data indicating potential effects of acipimox on intramyocardial lipolysis are available. Furthermore, the observed acute decrease in MYCL during acipimox administration is not compatible with an acute inhibition of intramyocardial lipolysis by this drug.

The observed rapid decline in MYCL and EF during inhibition of lipolysis could indicate a failure of the myocardium of healthy men to adapt to rapid changes in substrate availability. Myocardial glucose and FFA metabolism are tightly coupled, with increased FFA metabolism inhibiting myocardial glucose metabolism and vice versa (24). It has been shown that decreased availability of FFA in response to acipimox increases myocardial glucose uptake (14, 21). Since acipimox administration decreases myocardial FFA uptake by 80%, it has been estimated that the rise in myocardial glucose uptake is not sufficient to compensate for the depletion in FFA supply in healthy humans (18, 32). In contrast to our initial hypothesis that the consequent rise in FFA during hypoglycemia counterregulation is associated with increased myocardial lipid accumulation, we did not observe changes in MYCL following hypoglycemia (ins+/aci−). Thus, the observations in the present study are in line with the assumption that the heart utilizes available lipid stores for energy production during acutely increased demands and/or decreased availability of circulating FFA.

The assumption that the observed deterioration of EF during inhibition of lipolysis is caused by impaired energy production contradicts previous in vitro studies of myocardial substrate metabolism (18, 30). Therefore, the heart is considered a metabolic omnivore that can rapidly switch between lipid and glucose oxidation, which has been shown to be associated with a marked energetic advantage (15).

One further important observation of the present study was that FFA, MYCL, and EF were tightly associated under dynamic rather than baseline conditions. Of note is that the calculation of areas under the curve used to describe the extent of changes in plasma concentrations of hormones and substrates limits the possibility to fully account for the temporal sequence of changes in these parameters.

So far, we can only speculate on potential consequences for patients with diabetes mellitus who are at risk for recurrent hypoglycemia. Altered hypoglycemia counterregulation in patients with long-standing type 2 diabetes might be accompanied by alterations within the adipose tissue-myocardium crosstalk. Morbidity and mortality associated with hypoglycemia in various clinical trials has been attributed to an increase in cardiovascular events, an effect that could relate to sympathetic activation. Our results indicate that drugs that modulate adipose tissue lipolysis and thus limit the supply of FFA to the myocardium, including nicotinic acid analogs and insulin, might impair the ability of the heart to adequately cope with situations of increased energy demand.

Besides MYCL, HCL was also measured. HCL was unaffected by the current protocols, confirming that the myocardial triglyceride pool is much more flexible compared with hepatic

lipids in response to short-term metabolic alterations, including insulin treatment (13, 36). In general, acipimox administration did not affect circulating concentrations of insulin or cortisol following hypoglycemia; also, glucose concentrations were independent of FFA inhibition. Only HGH pertained longer elevated in ins+/aci+ compared with ins+/aci− (from 165 to 405 min). This is in line with prior observations indicating that low FFA concentrations induce HGH release (19).

To roughly estimate metabolic insulin action, we calculated IHGD under ins+/aci− and found that IHGD was correlated with stroke volume as well as the peak in noradrenalin concentrations after acute hypoglycemia, whereas IHGD was inversely correlated with MYCL. The association between IHGD and the noradrenalin peak could indicate that insulin action is a prerequisite for adequate stress response following hypoglycemia.

There are some limitations to address. We are not able to deliver data on myocardial energy metabolism since we did not perform phosphorus MR spectroscopy. Furthermore, no female subjects were included due to logistical reasons (difficult scheduling due to limited availability of the NMR scanner and the necessity to coordinate examinations with the individual menstrual cycle). Our recent observation that myocardial lipid accumulation did not differ between male and female subjects following combined hyperglycemia-hyperinsulinemia does not indicate important sex differences in myocardial lipid metabolism (36). In addition, we could not include patients with type 2 diabetes in the present protocol because it is impossible to induce standardized hypoglycemia by an insulin/hypoglycemia test (bolus injection) in patients with varying hyperglycemia and severe insulin resistance.

In summary, the current study clearly demonstrates the importance of a tightly regulated metabolic interplay between adipose tissue and the myocardium during stress conditions with catecholamine release by demonstrating that inhibition of adipose tissue lipolysis results in acute depletion of myocardial lipid stores and impairment of left ventricular systolic function.

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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

Y.W. and M. Krebs conception and design of research; Y.W., M. Krssak, P.W., R.M., and M. Krebs performed experiments; Y.W., M. Krssak, P.W., C.-H.A., A.G., G.H., T.S., and M.W. analyzed data; Y.W., M. Krssak, P.W., C.-H.A., A.G., G.H., S.B.-P., S.T., A.L., and M. Krebs interpreted results of experiments; Y.W. and A.G. prepared figures; Y.W. and M. Krebs drafted manuscript; Y.W., M. Krssak, P.W., C.-H.A., A.G., G.H., S.B.-P., R.M., T.S., M.W., S.T., A.L., and M. Krebs edited and revised manuscript; Y.W., M. Krssak, P.W., C.-H.A., A.G., G.H., S.B.-P., R.M., T.S., M.W., A.L., and M. Krebs approved final version of manuscript.

REFERENCES

1. **Baer FM, Theissen P, Schneider CA, Voth E, Schicha H, Sechtem U.** [Magnetic resonance tomography imaging techniques for diagnosing myocardial vitality]. *Herz* 19: 51–64, 1994.
2. **Barkhausen J, Ruehm SG, Goyen M, Buck T, Laub G, Debatin JF.** MR evaluation of ventricular function: true fast imaging with steady-state precession versus fast low-angle shot cine MR imaging: feasibility study. *Radiology* 219: 264–269, 2001.
3. **Bland JM, Altman DG.** Calculating correlation coefficients with repeated observations: Part 1—Correlation within subjects. *BMJ* 310: 446, 1995.
4. **Djousse L, Benkeser D, Arnold A, Kizer JR, Zieman SJ, Lemaitre RN, Tracy RP, Gottdiener JS, Mozaffarian D, Siscovick DS, Mukamal KJ, Ix JH.** Plasma free fatty acids and risk of heart failure: the Cardiovascular Health Study. *Circ Heart Fail* 6: 964–969, 2013.
5. **Erturk E, Jaffe CA, Barkan AL.** Evaluation of the integrity of the hypothalamic-pituitary-adrenal axis by insulin hypoglycemia test. *J Clin Endocrinol Metab* 83: 2350–2354, 1998.
6. **Finucane FM, Liew A, Thornton E, Rogers B, Tormey W, Agha A.** Clinical insights into the safety and utility of the insulin tolerance test (ITT) in the assessment of the hypothalamo-pituitary-adrenal axis. *Clin Endocrinol* 69: 603–607, 2008.
7. **Fisher BM, Gillen G, Dargie HJ, Inglis GC, Frier BM.** The effects of insulin-induced hypoglycaemia on cardiovascular function in normal man: studies using radionuclide ventriculography. *Diabetologia* 30: 841–845, 1987.
8. **Fisher BM, Gillen G, Hepburn DA, Dargie HJ, Frier BM.** Cardiac responses to acute insulin-induced hypoglycemia in humans. *Am J Physiol Heart Circ Physiol* 258: H1775–H1779, 1990.
9. **Florian JP, Pawelczyk JA.** Non-esterified fatty acids increase arterial pressure via central sympathetic activation in humans. *Clin Sci* 118: 61–69, 2010.
10. **Halbirk M, Nørrelund H, Møller N, Schmitz O, Gøtzsche L, Nielsen R, Nielsen-Kudsk JE, Nielsen SS, Nielsen TT, Eiskjær H, Bøtker 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* 299: H1220–H1225, 2010.
11. **Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A, Romijn JA, Smit JW.** Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 295: E714–E718, 2008.
12. **Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA.** Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 93: 497–503, 2008.
13. **Jankovic D, Winhofer Y, Promintzer-Schifferl M, Wohlschlager-Krenn E, Anderwald CH, Wolf P, Scherer T, Reiter G, Trattnig S, Luger A, Krebs M, Krssak M.** Effects of insulin therapy on myocardial lipid content and cardiac geometry in patients with type-2 diabetes mellitus. *PLoS One* 7: e50077, 2012.
14. **Knuuti MJ, Maki M, Yki-Jarvinen H, Voipio-Pulkki LM, Harkonen R, Haaparanta M, Nuutila P.** The effect of insulin and FFA on myocardial glucose uptake. *J Mol Cell Cardiol* 27: 1359–1367, 1995.
15. **Korvald C, Elvenes OP, Myrmel T.** Myocardial substrate metabolism influences left ventricular energetics in vivo. *Am J Physiol Heart Circ Physiol* 278: H1345–H1351, 2000.
16. **Krssak M, Hofer H, Wrba F, Meyerspeer M, Brehm A, Lohninger A, Steindl-Munda P, Moser E, Ferenci P, Roden M.** Non-invasive assessment of hepatic fat accumulation in chronic hepatitis C by 1H magnetic resonance spectroscopy. *Eur J Radiol* 74: e60–e66, 2010.
17. **Krssak M, Winhofer Y, Gobl C, Bischof M, Reiter G, Kautzky-Willer A, Luger A, Krebs M, Anderwald C.** Insulin resistance is not associated with myocardial steatosis in women. *Diabetologia* 54: 1871–1878, 2011.
18. **Lehto HR, Parkka J, Borra R, Tuunanen H, Lepomaki V, Parkkola R, Knuuti J, Nuutila P, Iozzo P.** Effects of acute and one-week fatty acid lowering on cardiac function and insulin sensitivity in relation with myocardial and muscle fat and adiponectin levels. *J Clin Endocrinol Metab* 97: 3277–3284, 2012.
19. **Lucidi P, Rossetti P, Porcellati F, Pampanelli S, Candeloro P, Andreoli AM, Perriello G, Bolli GB, Fanelli CG.** Mechanisms of insulin resistance after insulin-induced hypoglycemia in humans: the role of lipolysis. *Diabetes* 59: 1349–1357, 2010.

20. **Mohiaddin RH, Amanuma M, Kilner PJ, Pennell DJ, Manzara C, Longmore DB.** MR phase-shift velocity mapping of mitral and pulmonary venous flow. *J Comput Assist Tomogr* 15: 237–243, 1991.
21. **Nuutila P, Knuuti MJ, Raitakari M, Ruotsalainen U, Teräs M, Voipio-Pulkki LM, Haaparanta M, Solin O, Wegelius U, Yki-Järvinen H.** Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans. *Am J Physiol Endocrinol Metab* 267: E941–E946, 1994.
22. **Paolisso G, Manzella D, Rizzo MR, Ragno E, Barbieri M, Varricchio G, Varricchio M.** Elevated plasma fatty acid concentrations stimulate the cardiac autonomic nervous system in healthy subjects. *Am J Clin Nutr* 72: 723–730, 2000.
23. **Pilz S, Scharnagl H, Tiran B, Seelhorst U, Wellnitz B, Boehm BO, Schaefer JR, Marz W.** Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease. *J Clin Endocrinol Metab* 91: 2542–2547, 2006.
24. **Randle PJ, Garland PB, Hales CN, Newsholme EA.** The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1: 785–789, 1963.
25. **Reno CM, Daphna-Iken D, Chen YS, VanderWeele J, Jethi K, Fisher SJ.** Severe hypoglycemia-induced lethal cardiac arrhythmias are mediated by sympathoadrenal activation. *Diabetes* 62: 3570–3581, 2013.
26. **Russell RR 3rd, Chyun D, Song S, Sherwin RS, Tamborlane WV, Lee FA, Pfeifer MA, Rife F, Wackers FJ, Young LH.** Cardiac responses to insulin-induced hypoglycemia in nondiabetic and intensively treated type 1 diabetic patients. *Am J Physiol Endocrinol Metab* 281: E1029–E1036, 2001.
27. **Schaffer JE.** Lipotoxicity: when tissues overeat. *Curr Opin Lipidol* 14: 281–287, 2003.
28. **Stirling C, McAleer M, Reckless JP, Campbell RR, Mundy D, Betteridge DJ, Foster K.** Effects of acipimox, a nicotinic acid derivative, on lipolysis in human adipose tissue and on cholesterol synthesis in human jejunal mucosa. *Clin Sci* 68: 83–88, 1985.
29. **Szczepaniak LS, Victor RG, Orci L, Unger RH.** Forgotten but not gone: the rediscovery of fatty heart, the most common unrecognized disease in America. *Circ Res* 101: 759–767, 2007.
30. **Taegtmeyer H, Golfman L, Sharma S, Razeghi P, van Arsdall M.** Linking gene expression to function: metabolic flexibility in the normal and diseased heart. *Ann NY Acad Sci* 1015: 202–213, 2004.
31. **Trent CM, Yu S, Hu Y, Skoller N, Huggins LA, Homma S, Goldberg IJ.** Lipoprotein lipase activity is required for cardiac lipid droplet production. *J Lipid Res* 55: 645–658, 2014.
32. **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* 114: 2130–2137, 2006.
33. **van der Meer RW, Diamant M, Westenberg JJ, Doornbos J, Bax JJ, de Roos A, Lamb HJ.** Magnetic resonance assessment of aortic pulse wave velocity, aortic distensibility, and cardiac function in uncomplicated type 2 diabetes mellitus. *J Cardiovasc Magn Reson* 9: 645–651, 2007.
34. **van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ.** Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 56: 2849–2853, 2007.
35. **Vila G, Krebs M, Riedl M, Baumgartner-Parzer SM, Clodi M, Maier C, Pacini G, Luger A.** Acute effects of hydrocortisone on the metabolic response to a glucose load: increase in the first-phase insulin secretion. *Eur J Endocrinol* 163: 225–231, 2010.
36. **Winhofer Y, Krssak M, Jankovic D, Anderwald CH, Reiter G, Hofer A, Trattnig S, Luger A, Krebs M.** Short-term hyperinsulinemia and hyperglycemia increase myocardial lipid content in normal subjects. *Diabetes* 61: 1210–1216, 2012.
37. **Wright RJ, Newby DE, Stirling D, Ludlam CA, Macdonald IA, Frier BM.** Effects of acute insulin-induced hypoglycemia on indices of inflammation: putative mechanism for aggravating vascular disease in diabetes. *Diabetes Care* 33: 1591–1597, 2010.
38. **Zoungas S, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP, Marre M, Heller S, Group AC.** Severe hypoglycemia and risks of vascular events and death. *N Engl J Med* 363: 1410–1418, 2010.