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Onset of Depressed Heart Work is Correlated with the Increased Heart Rate and Shorten QT-Interval in High-Carbohydrate Fed Overweight Rats

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Abstract

Mechanical activity of the heart is adversely affected with metabolic syndrome (MetS) characterized with increased body-mass and marked insulin-resistance. Herein, we examined effects of high-carbohydrate intake on cardiac functional abnormalities via evaluating in situ heart-work, heart-rate and electrocardiograms (ECG) in rats. MetS is induced in Wistar male rats by adding 32% sucrose for 22-24 weeks and confirmed with insulin-resistance, increased body-weight, blood glucose and insulin, systolic and diastolic blood pressures besides significant left ventricular integrity-lost and increased connective-tissue around myofibrils. Analysis of in situ ECG-recordings showed markedly shorten QT-interval and depressed QRP with increased heart-rate. We also observed augmented oxidative stress and decreased antioxidant defense characterized with decreases in serum total thiol-level and attenuated paraoxonase and arylerase activities. Our data clearly indicate that increased heart-rate and shortened QT-interval concomitant with higher left ventricular developed pressure responses to β-adrenoreceptor stimulation as a result of less cAMP-release could be regarded as natural compensation mechanisms in overweight MetS rats. Since MetS leads further to persistent insulin-resistance and obesity, one should get into consideration these important facts associated with onset of the depressed heart-work, the increased heart-rate and shorten QT-interval in high-carbohydrate intake, which will possible lead to more deleterious effects on mammalian heart.

Key words: heart work, electrocardiogram, insulin resistance, metabolic syndrome, paraoxonase, arylerase, oxidative stress.
Introduction

Eating habit changes cause mainly overweightness and obesity in all over the world. High-carbohydrate intake with less physical exercise may increase the risk of cardiac problems while an imbalance in favour of high-carbohydrate intake causes weight gain and further obesity. Consequently, it is clear that this imbalance between energy intake/expenditure could be a key risk factor for cardiovascular disorders. Therefore, the World Health Organization (WHO) recommends to balance daily energy intake and expenditure. The WHO and the International Diabetes Federation (IDF) have defined MetS as a syndrome with multiple metabolic risk factors, including abdominal obesity, glucose intolerance, insulin resistance and heart function abnormalities (Alberti et al. 2006).

Excess carbohydrate consumption stimulates continuous insulin release, which then results in insulin resistance characterized with mild hyperglycemia (Volek et al. 2009). Metabolic syndrome (MetS) may occur during this process secondary to continuous insulin release and then it results to cardiac dysfunction. Many of these cardiac abnormalities could further lead to the development of atherosclerosis and increased risk of cardiovascular diseases (Hansen 1999; Matsuzawa et al. 2011; Reaven 1988). MetS has been found to be associated with many risk factors underlying several diseases including type-2 diabetes and obesity (Dinh et al. 2011). However, the exact relationship between MetS and cardiovascular heart disease has not been elucidated yet. Either MetS components of type-2 diabetes or MetS alone are characterized with abnormal cardiac structure and function, particularly left ventricular systolic and diastolic dysfunction (Aijaz et al. 2008).

It has been reported that MetS is also related to oxidative stress, which plays an essential role in multiple physiological systems basically contributing to cellular
dysfunction. Actually, it is clearly explained that loss of redox homeostasis contributes to a series of alterations in endogenous signaling pathways including promotion of impairments in metabolic signaling of insulin, which, in turn, leads to structural and functional abnormalities in cardiovascular system (Roberts and Sindhu 2009). We and others have shown that total oxidant status (TOS) and total antioxidant status (TAS) measured in serum in the presence of MetS were markedly changed in both animal and human studies (Galassetti 2012; Korkmaz-Icoz et al. 2016; Okatan et al. 2015). Increased oxidative stress together with reduction of antioxidant defense may impair insulin signaling leading to insulin resistance. Indeed, previous studies using high-sucrose diets confirmed the presence of glucose intolerance in experimental animals (Bremer et al. 2012; Pagliassotti et al. 1996). Since high-density lipoprotein (HDLs), one of the most important antioxidant defense systems in plasma, are typical biochemical markers for confirmation of MetS. The antioxidant properties of HDLs are, at least to some extent, attributable to serum paraoxonase and its arylesterase/paraoxonase activity (James 2006). They prevent oxidation of serum lipids in metabolic disorders. Studies have demonstrated that there is an association between low levels of paraoxonase (PON1) and organ dysfunction, particularly in terms of cardiovascular pathologies seen in MetS patients (Eren et al. 2014; Hashemi et al. 2011).

Although the significant heritability of the individual components of MetS has been well recognized, to our knowledge, there is no clear data how the heart work, the heart rate and the parameters of electrocardiogram (ECG) can correlate in high-carbohydrate fed MetS rats. Herein, we aimed to examine the effects of high-carbohydrate intake on functional abnormalities of the heart via evaluating in situ the heart work, heart rate and electrocardiogram (ECG) parameters in MetS rats with
peripheral insulin resistance. Therefore, we focused on to evaluate the effects of high-carbohydrate intake on *in situ* heart work and heart rate and also to demonstrate the alterations in the ECG parameters, which can be correlated with the altered heart function mentioned previously in MetS rats, which is induced by adding 32% sucrose into their drinking water. Additionally, in order to validate the relationship between increased oxidative stress and ultrastructural changes in the heart from these MetS rats, we also evaluated the oxidative stress status in MetS rats by measuring serum TOS and TAS levels together with serum PON1 activity (via measuring paraoxonase and arylesterase activities) and total thiol status besides light microscopy investigation of left ventricle stained with Masson’s trichrome.

**Materials and methods**

**Metabolic syndrome model in rats**

For metabolic syndrome (MetS) induction, we used 8-week old Wistar male rats. Induction is performed by adding 32% sucrose into their drinking water for 22-24 weeks, as described previously (Okatan et al. 2015; Ruiz-Ramirez et al. 2011). All animals are exposed to a 12-h light–dark cycle and the control group (CON group) had also free access to tap water. All animals are fed standard chow ad libitum and are housed in the standard rat cages.

All experimental procedures are performed in accordance with the standards of the European Community guidelines on the care and use of laboratory animals and had been approved by the local ethics committee of Ankara University (No:2015-12-137).

**Oral glucose tolerance test**

To perform oral glucose tolerance test, rats starved overnight with free access to tap water, then they are received 1 g/kg glucose in double-distilled water by orogastric
gavage, as described previously (Okatan et al. 2015). Blood samples are collected from tail at the beginning and then at 15th, 30th, 60th, and 120th minute following glucose administration. Blood glucose levels are assessed using standard glucose test strips (GlucoCheck Analyzer) and serum insulin levels are assessed using an enzyme immunoassay (EIA) kit (E-EL-R2466, Elabscience, China), according to the manufacturer’s instructions at 450 nm absorbance wavelength.

**Measurement of arterial pressure**

Systolic and diastolic blood pressures are measured by an indirect tail-cuff method via an NIBP200-A noninvasive blood pressure meter (BIOPAC Systems Inc, USA) as described previously (Krege et al. 1995).

**Biochemical analysis**

**A- Total antioxidant status (TAS) measurement in serum:** TAS levels are measured using commercially available kit (RL0024, Rel Assay Diagnostics, Turkey), as described previously (Erel 2004). Shortly, the novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The results are expressed as mmol Trolox equivalent/L.

**B- Total oxidant status (TOS) measurement in serum:** TOS levels are measured using commercially available kits (RL0024, Rel Assay Diagnostics, Turkey) as described previously (Erel 2004). Shortly, the oxidation reaction is enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, is related to the total
amount of oxidant molecules present in the sample. The assay is calibrated with \( \text{H}_2\text{O}_2 \) and the results are expressed in terms of \( \mu\text{M H}_2\text{O}_2 \) equivalent/L.

**C- Measurement of paraoxonase and arylesterase activities measurement in serum:** Paraoxonase and arylesterase activities are measured using commercially available kits (RL0031 and RL0055, *Rel Assay Diagnostics*, Turkey). The rate of paraoxon hydrolysis (diethylp-nitrophenylphosphate) is measured by monitoring the increase of absorption at 412 nm at 37°C. The amount of generated p-nitrophenol is calculated from the molar absorption coefficient at pH 8.5, which is 18.290 M\(^{-1}\) cm\(^{-1}\). Paraoxonase activity was expressed as U/L serum. Phenylacetate is used as a substrate to measure the arylesterase activity. Enzymatic activity is calculated from the molar absorption coefficient of the produced phenol, 1310 M\(^{-1}\) cm\(^{-1}\). One unit of arylesterase activity is defined as 1 \( \mu\text{mol} \) phenol generated per min under the above conditions and expressed as U/L.

**D- Total thiol measurement in serum:** The method depends on the process, in which thiols interact with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), forming a highly colored anion with maximum peak at 412 nm (Hu et al. 1993). The developed absorbance is proportional to total thiol content of the sample. We used 2 reagents as R1 (buffer solution 1X50 mL) and R2 (chromogen solution 1X 5 mL) and standard (liquid solution, stock calibrator, 1X 5 mL). We used a commercial kit (RL0178, *Rel Assay Diagnostics*, Turkey) and colorometric measurements are performed by using an autoanalyzer (Vital Selectra E). GSH (50-100 \( \mu\text{mol/L} \)) solution is used as calibrator.

**E- Serum cAMP measurement:** Serum cAMP level is measured using a cyclic AMP EIA Kit (#581001, *Cayman Chemical Company*, Ann Arbor, MI, USA). Briefly, serum samples are dried under a stream of nitrogen and then resuspended using 500
µL Elisa buffer. Then, cAMP level is detected according to the manufacturer’s instructions. The cAMP values are calculated using a multifunction microplate reader (Tecan, San Jose, CA, USA).

**In situ ECG measurement**

ECGs are recorded *in situ* through the animals’ paws using two home-made electrodes (MP150, BIOPAC Systems, Inc.) and ECGs are band-pass filtered (50-500 Hz). Briefly, two bipolar limb leads (20-gauge needles) are placed to paws with a third one on the tail as reference electrode under light ether anesthesia. All animals had their ECG’s recorded at least for 10 min. The peak-to-peak amplitude of the traces (QRS value) and durations of ECGs such as PR-, RR- and QT-intervals, and heart rate are measured.

**Heart Langendorff-perfusion measurement**

The rats are anesthetized with pentobarbital sodium (30 mg/kg by intraperitoneal injection) and hearts are prepared for Langendorff-perfusion apparatus, as described previously (Okatan et al. 2015). The hearts are electrically stimulated (DCS; Harward) at 300 beats/min with 1.5 ms square waves (at twice the threshold voltage). Changes in the left ventricular developed pressure (LVDP) are measured with a water-filled latex balloon inserted into the left ventricle and all data are recorded online then stored and processed (Model 1050BP; BIOPAC Systems, Goleta, California, USA). The LVDP response to β-adrenergic receptor (β-AR) agonist stimulation is obtained in the presence of a nonspecific β-AR agonist isoproterenol (ISO). The results are given as percentage changes in LVDP.

**Assessment of *in vivo* cardiac function through pressure-volume analysis**
Pressure volume analysis (PV loop analysis) is made under ketamine-xylasine (90mg/kg-10mg/kg) anesthesia. Body temperature of rats is stabilized at 37ºC. An incision is made on right carotid artery and the pressure-volume catheter (Transonic, NY, USA) is inserted through. First, arterial pressure is recorded, then the catheter is advanced to the left ventricle of the heart and cardiac parameters are recorded. The parameters calculated using Labscribe 2 acquisition software are as follows; heart rate (HR), end systolic pressure (ESP), end diastolic pressure (EDP), the rate of contraction and relaxation (dp/dtmax and dp/dtmin), end systolic volume (ESV), end diastolic volume (EDV), stroke volume (SV), cardiac output (CO), ejection fraction (EF), isovolumic relaxation constant (Tau), end systolic elastance (Ees), cardiac stiffness, preload recruitable stroke work (PRSW) and dp/dt-EDV relation. Preload recruitable cardiac parameters are calculated by inferior vena cava occlusion. Parameters such as ESV, EDV, SV, CO are normalized to body weight to eliminate body weight differences (Arioglu-Inan et al. 2013).

**Histological examination**

For structural investigation, we used light microscopic evaluation. The excised hearts are fixed in phosphate buffer 10% formaldehyde and the samples are dehydrated through series of alcohol solutions. Following clearing and paraffin infiltration processes, the embedded-samples are sectioned to 4-thickness by Leica RM 2125RT model microtome. The sections are stained with Masson’s trichrome. All samples are photographed by AxioCam MRc5 (Carl Zeiss) digital vision system.

**Chemicals and statistics**

Chemicals are obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. The results are expressed as means ± SEM. Statistical significance is evaluated by
one-way ANOVA followed by Tukey post-test. The probability level of p<0.05 is considered statistically significant.

**Results**

**General parameters of rats**

All rats are 8-month old at the end of the experimental protocol. The weight gain of MetS group is significantly higher (~20%) compared to control group (490±12 g vs. 414±13 g) whereas the ratio for the heart weight to body weight did not differ between these groups (Fig. 1A). This is a clear implication of absence of hypertropy in rats with MetS. The blood glucose and serum insulin levels in MetS group are significantly higher (115.80±3.57 mg/dL and 8.02±1.30 ng/mL, respectively) compared to those of controls (85.15±3.02 mg/dL and 3.69±0.67 ng/mL) (Fig. 1B, left and right, respectively).

To test whether there is insulin resistance in MetS rats, we applied oral glucose tolerance test. As seen in Fig. 1C, glucose tolerance is significantly impaired in rats with MetS, which also demonstrates the development of insulin resistance.

Arterial blood pressure (systolic and diastolic blood pressures) is measured by indirect tail-cuff method and the mean (±SEM) values are given in Fig. 1D. The systolic and diastolic blood pressures in MetS group are significantly higher (185±6 and 116±11 mmHg) compared to those of controls (126±2 and 85±12 mmHg), as shown previously (Okatan et al. 2015).

**Oxidative stress and antioxidant defense parameters in MetS rats**

To examine the systemic oxidative status in MetS rats, we monitored the serum levels of total oxidant status (TOS) and total antioxidant status (TAS). As seen in Fig. 2A
The TOS level is significantly higher in MetS group compared to CON group, while the TAS level is changed oppositely (Fig. 2A, right).

We also measured serum paraoxonase (PON1) activities by means of traditional (paraoxonase and arylesterase activity measurement) assay in MetS rats. As seen in Fig. 2B, both activities are markedly lower in MetS group compared to CON group (right and left, respectively). These data strongly confirmed the attenuated antioxidant defense in MetS rats. It is known that PON1 gene is located on chromosome 7q21.3-22.11 (Primo-Parmo S L et al. 1996), and exclusively associated with HDLs. It protects LDLs from oxidation by the hydrolysis of biologically active lipoperoxides (Mackness et al. 1991).

Since advanced oxidation protein products have been regarded as novel markers of oxidant-mediated protein damage (Witko et al. 1992), we aimed to evaluate the relationship of advanced oxidation protein products in serum of MetS rats and other oxidative stress markers. We measured serum total thiol level in MetS rats compared to control rats. As seen in Fig. 2C, the serum total thiol in MetS group is significantly lower compared to that of CON group.

**Changes in ECG parameters**

Fig. 3A (inset) shows the representative original electrocardiographs from MetS and CON groups. Comparison of the peak-to peak amplitude of the traces shows that the QRS value (59.6±2.6 µV) in MetS group is significantly small (~17%) compared to control (70.2±2.4 µV). The durations of the electrocardiographic parameters, PR-, RR- and QT-intervals are measured and compared between these groups. Interestingly, the QT-interval is markedly shorter (~23%) in MetS group.
(0.056±0.009 s) compared to CON group (0.069±0.008 s) while the others did not differ, significantly (Fig. 3B).

The heart rate is also significantly higher (~15%) in MetS group compared to the CON group (390±16 beat/min vs. 340±11 beat/min) (Fig. 3C). Previously, we have shown that as a basal mechanical activity of the heart prepared in Langendorff-perfusion system, left ventricular developed pressure (LVDP) in MetS rats is markedly depressed while the aortic pressure is increased significantly (Okatan et al. 2015). In the present study, we measured LVDP responses mediated by nonspecific β-adrenergic receptor stimulation (isoproterenol, ISO) in MetS group. As seen in Fig. 3D, ISO mediated contraction response at the doses between $10^{-6} - 10^{-5}$ M were significantly higher in MetS group comparison to the CON group. The maximal response obtained with $10^{-5}$ M ISO in MetS group is over 50% high with respect to the CON group while EC50 values are similar between these groups (6.97±0.47 vs. 7.28±0.49 for MetS vs. CON).

To test whether release of cAMP via sympathetic system is changed due to insulin resistance in overweight MetS rats, we measured serum cAMP levels. As seen in Fig. 3E, the cAMP level in MetS group (0.155±0.009 pmol/L) is significantly less than the CON group (0.532±0.051 pmol/L).

**In vivo basal hemodynamic parameters**

Cardiac function is evaluated by measuring basal hemodynamic parameters as *in situ* by using pressure-volume catheter. The original representative and superimposed heart-work diagrams are given in Fig. 4A. Both end systolic pressure (ESP) and end diastolic pressure (EDP) are increased in MetS group (106.5±2.8 mmHg and 6.9±0.7
mmHg vs. 92.1±3.7 mmHg and 4.8±0.5 mmHg) while the increase in EDP (~45%) is higher than that of ESP (~15%) (Fig. 4B).

Another marker of the heart function, the cardiac output (CO) is reduced significantly in MetS group (45.6±1.0 L/min) compared to CON group (60.4±5.6 L/min) while the isovolumetric relaxation constant of this group is slightly but significantly shorter (~15%) than CON group (Fig. 4C). When we calculated the total area from the pressure-volume graphs, the heart-work is found to be less about 30% in MetS group compared to CON group.

**Heart histopathology**

Light microscopy examination of left ventricular sections following Masson’s trichrome staining of MetS rats showed marked loss of integrity of muscle myofibrils, significant increases in connective tissue around myofibrils and marked fibrous increases in interstitial regions (Fig. 4D, upper part). Additionally, significant irregular, heterogeneous and differentiated appearances in cytoplasm are observed in heart sections of MetS rats (Fig. 4D, lower part).

**Discussion**

In the present study, we examined the effects of high-carbohydrate intake on functional abnormalities of heart via evaluating *in situ* preparations and have shown that onset of depressed heart work is correlated with the increased heart rate and shorten QT-interval in high-carbohydrate fed overweight rats. We also have demonstrated that overweightness is closely associated with development of metabolic syndrome (MetS) in mammalians, which is basically characterized with increased body-mass, increased blood glucose and serum insulin levels, marked insulin resistance and markedly increased systemic oxidative stress parallel to
decreased antioxidative defence system. MetS is increasingly common serious syndrome in all over the world, while its prevalence varies related to definition, ethnicity and gender. People with MetS have about 50-60% higher cardiovascular risk than the others (Hansen 1999; Matsuzawa et al. 2011; Qiao et al. 2007). Although several different factors have been involved, the signaling-pathways underlying the pathogenesis of cardiovascular risk factors remain to be not clear yet. Since MetS in mammalians is a multifactorial pathology, strategies for reducing cardiovascular risk should, therefore, involve the management of multiple risk factors.

MetS in mammalians is associated with a generalized metabolic disorder characterized with insulin resistance, increased body weight, significant high blood glucose and serum insulin levels, markedly increased systolic and diastolic blood pressures (Dutta et al. 2001; Isomaa et al. 2001; Okatan et al. 2015). Our previous studies and others emphasize that MetS is also characterized with increased oxidative stress, due to an imbalance between production and scavenging of oxidants (Bhatti et al. 2016; Bonomini et al. 2015; Gregorio et al. 2016; Okatan et al. 2015; Vendemiale et al. 1995), which also play important role in the pathogenesis of various diseases including MetS (Bonomini et al. 2015; Roberts and Sindhu 2009). Similar to other studies (Galassetti 2012; Korkmaz et al. 2013; Okatan et al. 2015), our findings, related with significantly increased total oxidative status (TOS) and decreased total antioxidant status (TAS) in serum of MetS rats, support these statements. These changes may later affect the impairment of insulin signaling, which further leads to insulin resistance. Indeed, previous studies using high-sucrose diet have shown a marked glucose intolerance in MetS modeled experimental animals (Brenner et al. 2003; Pagliassotti et al. 1996).
Additionally, we found significantly decreased serum paraoxonase and arylesterase activities in MetS group. Since the antioxidant properties of HDLs are attributable to serum paraoxonase and arylesterase activities (James 2006), these activities seem to be crucial in the relation of increased oxidative stress and organ dysfunction, particularly in terms of cardiovascular pathologies in MetS rats (Eren et al. 2014; Kagota et al. 2013). Indeed, we also observed marked loss of integrity of muscle myofibrils and significant increases in connective tissue around myofibrils, and marked fibrous increases in interstitial regions in left ventricular tissue sections of MetS rats by light microscopy investigation. These changes are closely associated with changes induced by increased oxidative stress, similar to previous findings (Ansley and Wang 2013; Butterfield et al. 1998; Kayama et al. 2015; Okatan et al. 2015).

Furthermore, we observed marked insulin resistance in MetS rats. This observation, in one hand, can be explained with an associated with increased oxidative status in MetS rats. Indeed, previous findings, which mentioned that an increased oxidative stress together with a reduction of antioxidant defense may impair insulin signaling leading to insulin resistance, support this idea. In this regard, previous studies using high-sucrose diets have shown marked glucose intolerance in experimental animals (Brenner et al. 2003; Sumiyoshi et al. 2006), which is supported due to previously shown data on an association with promotion of insulin signaling and high H$_2$O$_2$ giving a fact on typical metabolic actions of insulin linking to ROS and insulin relation (Czech et al. 1974). It seems that oxidation-induced disruption of cellular redistributed signaling molecules in response to insulin stimulation is associated with impaired insulin action in case of many pathological conditions such as obesity (Svegliati-Baroni et al. 2006). Indeed, any reduction in tissue levels of glutathione, a
cellular antioxidant, can induce increases of oxidative stress markers and impaired glucose homeostasis (Svegliati-Baroni et al. 2006). Some studies in humans have also confirmed a pivotal role of oxidative stress in insulin resistant states such as MetS, obesity, and type 2 diabetes (Keaney et al. 2004; Roberts and Sindhu 2009; Vincent et al. 2010; Kizhakekuttu and Widlansky 2010; Roberts and Sindhu 2009).

The clustering of metabolic abnormality is closely related to the progression of cardiovascular disorders (Balderas-Villalobos et al. 2013; Merabet et al. 2015; Okatan et al. 2016). Indeed, a correlation between obesity, insulin resistance and contractile dysfunction at most due to defect in intracellular Ca\textsuperscript{2+} handling in rat heart with MetS have been observed, previously (Balderas-Villalobos et al. 2013; Nevelsteent et al. 2013). ECG is one of the standard technologies used to monitor and assess cardiac function, and provide insight into the mechanisms driving myocardial pathology. In the present study, we evaluated \textit{in situ} ECG in rats with MetS and showed that there is significantly decreased QRS amplitude and shortened QT-interval together with markedly increased heart rate, similar to previously mentioned for both animals and humans (Korkmaz-Icoz et al. 2016; Merabet et al. 2015; Okatan et al. 2015). It is accepted that increased heart rate observed in MetS contributes to the deterioration of LV function via impaired LV filling and relaxation, reduced coronary perfusion and cardiac output. These findings are correlated with depressed heart work in individuals with MetS.

Interestingly, we observed a marked shortened QT-interval (23%), significantly decreased heart work (~30%) and cardiac output (32%) with significantly increased heart rate (~15%). The significant increases in both systolic and diastolic pressures together with increased end systolic and diastolic volumes suggest the induction of hypertension for compensation the decreased heart work and cardiac output in MetS.
rats. Another compensation mechanism may be induction of QT shortening during our experiments as initial response, instead of prolonged QT observed in obese patients (Pietrobelli et al. 1997). Indeed, this prolongation could be almost normalized when patients had even short-term weight loss (Bai et al. 2007; Pietrobelli et al. 1997). It is well accepted that MetS is a risk factor for prolonged QT, which may further increase cardiovascular morbidity and mortality in these subjects. A short QT-interval is defined as short QT syndrome, generally a genetic disease of the electrical system of the heart and mutations in the \textit{KCNH2}, \textit{KCNJ2}, and \textit{KCNQ1} genes. These genetic modifications seem to be closely associated with sudden death, most likely due to ventricular fibrillation (Chen et al. 2003; Gaita et al. 2003; Gussak et al. 2000; Laitinen et al. 2001). However, the cause of short QT syndrome is unclear and it has been hypothesized that the QT shortens when heart rate increases (Maltret et al. 2014). It has been also proposed that short QT-interval may be acquired (Gaita et al. 2004), because some drugs or conditions such as hyperkalemia, acidosis, and hyperthermia could cause induction of short QT-interval in even normal subjects (Patel and Pavri 2009). These hypothesis and data are in line with our present findings.

Another novelty of our data is to observe significantly increased cardiac response to a nonspecific β-adrenergic receptor (β-AR) stimulation with low serum cAMP level, despite of significantly decreased protein levels of β₁-AR- and β₂-AR in MetS group (Okatan et al. 2015). Most studies agree that the cAMP-pathway is stimulated through β-ARs and the β₁-subtype confers greater functional effects in cardiomyocytes (Brodde 1993; Steinberg 1999). As the major component of the interface between the sympathetic nervous system and the cardiovascular system, the β-AR signaling pathway emerges as a key actor during the progression of heart dysfunction.
Supporting these facts, in our MetS group, a decreased level of serum cAMP may be compensated with an increase cardiac response to the β-AR stimulation. Of note, regardless of the etiology, compensatory and adaptive changes occur in the heart to preserve cardiac output (Leimbach et al. 1986), which is decreased in our MetS group including changes in release of neurotransmitters via sympathetic system. Indeed, it seems less cAMP release can prevent desensitization of β-ARs in the heart, which are common characteristics in the development of cardiac dysfunction. Indeed, previous studies have shown regulation of heart rate by cAMP, mostly due to hyperinsulinemia driven sympathetic nervous system activity (Alig et al. 2009).

In conclusion, our present data demonstrated that either a short-term (relative to the life span of human) or mild overweight status can induce MetS, which includes significant changes in the electrical activity of the heart with markedly decreased cardiac output, most probably, due to a short QT-interval although existence of compensations with high heart rate and overstimulation in cardiac β-ARs. Since MetS leads to persistent insulin resistance and obesity as further-period responses, one should get into consideration these important facts associated with onset of the depressed heart work, the increased heart rate and shorten QT-interval in high-carbohydrate intake, which will possible lead to more deleterious effects on the heart in mammalians.

Acknowledgements

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Conflict of interest

The authors declare that there is no potential conflict of interest associated with this study.
References


Figure legends

Fig. 1. General parameters related to high sucrose intake of rats. (A) Body weight (left) and ratio of heart weight to body weight (right) of MetS rats compared to the control (CON group). The ratio is given as (g/g)×10^{-3}. (B) Blood glucose (left) and insulin (right) levels of the groups. (C) Demonstration of impaired glucose tolerance in MetS group measured with oral glucose tolerance test. (D) The systolic (left) and diastolic (right) pressure changes in MetS group compared to age-matched controls. Data presenting mean (±SEM) values. The total number of rats in each group; n_{CON}=30, n_{MetS}=34. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.

Fig. 2. Validation of systemic oxidative status in rats with MetS. Total oxidant status (TOS) and total antioxidant status (TAS) measured in serum of MetS rats (A; left and right, respectively). The paraoxonase and arylesterase activities measured in serum (B; left and right, respectively) and serum total thiol levels (C). Data presenting mean (±SEM) values. The total number of rats in each group; n_{CON}=10, n_{MetS}=10. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.

Fig. 3. Electrocardiogram (ECG) parameters measured in vivo in rats. (A) The amplitude of QRS complex with original ECG records (inset) and the PP, PR and QT intervals (B). (C) The heart rate values of MetS groups compared to those of controls (CON). (D) The responses of left ventricular developed pressure (LVDP) changes to nonspecific β-adrenergic receptor stimulation, isoprotoreanol (ISO) in a concentration-dependent manner. (E) Serum cAMP levels of the groups measured by using a conventional kit (Cayman, No.581001) spectrophotometrically measured at 412 nm. Data presenting mean (±SEM) values. The total number of rats in each group; n_{CON}=10, n_{MetS}=10. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.
Fig. 4. Mechanical activity and histology of the heart from MetS rats. (A) Representative pressure-volume (P-V) loops measured by vena cava occlusion. (B) Calculated parameters such as end systolic pressure (ESP) and end diastolic pressure (EDP) (B, left and right, respectively) as well as cardiac output and the isovolumetric relaxation constant (C; left and right, respectively). (D) Light microscopy examination of left ventricular sections following Masson’s trichrome staining. Bold arrows: irregular, heterogeneous and differentiated appearance in cytoplasm. The total number of rats in each group; n_{CON}=10, n_{MetS}=14. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.
Caption: Fig. 1 a-d. General parameters related to high sucrose intake of rats. (A) Body weight (left) and ratio of heart weight to body weight (right) of MetS rats compared to the control (CON group). The ratio is given as (g/g) \times 10^{-3}. (B) Blood glucose (left) and insulin (right) levels of the groups. (C) Demonstration of impaired glucose tolerance in MetS group measured with oral glucose tolerance test. (D) The systolic (left) and diastolic (right) pressure changes in MetS group compared to age-matched controls. Data presenting mean (±SEM) values. The total number of rats in each group; nCON= 30, nMetS= 34. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.
Caption: Fig. 2 a-c. Validation of systemic oxidative status in rats with MetS. Total oxidant status (TOS) and total antioxidant status (TAS) measured in serum of MetS rats (A; left and right, respectively). The paraoxonase and arylesterase activities measured in serum (B; left and right, respectively) and serum total thiol levels (C). Data presenting mean (±SEM) values. The total number of rats in each group; nCON=10, nMetS=10. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.
Caption: Fig. 3 a-e. Electrocardiogram (ECG) parameters measured in vivo in rats. (A) The amplitude of QRS complex with original ECG records (inset) and the PP, PR and QT intervals (B). (C) The heart rate values of MetS groups compared to those of controls (CON). (D) The responses of left ventricular developed pressure (LVDP) changes to nonspecific β-adrenergic receptor stimulation, isoproterenol (ISO) in a concentration-dependent manner. (E) Serum cAMP levels of the groups measured by using a conventional kit (Cayman, No. 581001) spectrophotometrically measured at 412 nm. Data presenting mean (±SEM) values. The total number of rats in each group; nCON=10, nMetS=10. Significant at *p<0.05 vs. CON group, with unpaired student's t-test.
Caption: Fig. 4 a-d. Mechanical activity and histology of the heart from MetS rats. (A) Representative pressure-volume (P-V) loops measured by vena cava occlusion. (B) Calculated parameters such as end systolic pressure (ESP) and end diastolic pressure (EDP) (B, left and right, respectively) as well as cardiac output and the isovolumetric relaxation constant (C; left and right, respectively). (D) Light microscopy examination of left ventricular sections following Masson’s trichrome staining. Bold arrows: irregular, heterogeneous and differentiated appearance in cytoplasm. The total number of rats in each group; nCON=10, nMetS=14. Significant at *p<0.05 vs. CON group, with unpaired student’s t-test.

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