Assessment of myocardial metabolic flexibility and work efficiency in human Type 2 diabetes using 16-18F-fluoro-4-thiapalmitate, a novel PET fatty acid tracer

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Running Head: Myocardial metabolic flexibility in Type 2 Diabetes

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Abstract

Altered myocardial fuel selection likely underlies cardiac disease risk in diabetes, affecting oxygen demand and myocardial metabolic flexibility. We investigated myocardial fuel selection and metabolic flexibility in human Type 2 Diabetes (T2DM), using positron emission tomography to measure rates of myocardial fatty acid oxidation ($^{16-18}$F-fluoro-4-thia-palmitate, FTP), and myocardial perfusion and total oxidation ($^{11}$C-acetate). Participants underwent paired studies under fasting conditions, comparing 3-hour insulin+glucose euglycemic clamp conditions (120 mU/m$^2$/min) to 3-hour saline infusion. Lean controls (n=10) were compared with glycemically controlled volunteers with T2DM (n=8). Insulin augmented heart rate, blood pressure and stroke index in both groups (all p<0.01), and significantly increased myocardial oxygen consumption (p=0.04) and perfusion (p=0.01) in both groups. Insulin suppressed available non-esterified fatty acids (p<0.0001), but fatty acid concentrations were higher in T2DM under both conditions (p<0.001). Insulin-induced suppression of fatty acid oxidation was seen in both groups (p<0.0001). However, fatty acid oxidation rates were higher under both conditions in T2DM (p=0.003). Myocardial work efficiency was lower in T2DM (p=0.006), and decreased in both groups with the insulin-induced increase in work and shift in fuel utilization (p=0.01).

Augmented fatty acid oxidation is present under baseline and insulin-treated conditions in T2DM, with impaired insulin-induced shifts away from fatty acid oxidation. This is accompanied by reduced work efficiency, possibly due to greater oxygen consumption with fatty acid metabolism. These observations suggest that improved fatty acid suppression, or reductions in myocardial fatty acid uptake and retention, could be therapeutic targets to improve myocardial ischemia tolerance in type 2 diabetes.

Keywords: Myocardial, heart, diabetes, metabolism, metabolic flexibility
Introduction

Alterations in metabolic substrate uptake and metabolism are part of the phenotype that defines Type 2 diabetes mellitus (T2DM)(3, 4, 33, 39, 74). The phenomenon of ‘metabolic flexibility’ is the capacity of an organism, a tissue bed, or a cell system to switch readily among fuel types. Impaired metabolic flexibility is another phenotypic feature of T2DM (33, 66). Impaired metabolic flexibility has been demonstrated in skeletal muscle in human diabetes (32, 66). This whole-body effect likely arises due to effects of impaired insulin-stimulated glucose uptake, together with abnormalities in availability, uptake and metabolism of fatty acid fuels (33, 66, 75).

The myocardium is also affected by T2DM. The fuel needs of the heart are dramatically different than those of skeletal muscle, supporting continuous work even under resting conditions. Abnormalities in myocardial fuel selection in animal models of diabetes have been described (42, 54, 75), including impairments in insulin-stimulated glucose uptake and impaired suppression of myocardial fatty acid uptake by insulin. The capacity for flexible selection among fuel sources has implications for ischemia tolerance, as the oxygen requirements for metabolizing fatty acids are higher than those for carbohydrates (43), and may contribute to efficiency of supplying fuel for contractile function more generally. Most studies to date that evaluated metabolic flexibility of the diabetic heart have studied the insulin deficient state, i.e. models of Type 1 diabetes (5, 42, 54). Very little data exist exploring effects of Type 2 diabetes on myocardial fuel selection, and data is particularly lacking in human T2DM (75). Here we describe studies in non-obese non-diabetic control and obese Type 2 diabetic humans, using positron emission tomography (PET) to quantify the effect of insulin to drive myocardial fuel selection away from fatty acids. We present evidence for metabolic inflexibility in the diabetic heart in Type 2 diabetes mellitus, owing to diabetes-related abnormalities in fatty acid uptake and in fatty acid availability.
Methods

Participant selection: Participants were recruited from the general public, by advertising in local newspapers and by word of mouth. Participants were recruited into two groups, lean and Type 2 diabetes mellitus (T2DM). Lean status was defined as BMI <25 kg/m² and percent fat by dual energy x-ray absorptiometry (Lunar DXA, GE, Madison WI) <28% in men and <30% in women. Lean volunteers were required to have normal glucose control by ADA 2002 criteria on screening 75g oral glucose tolerance test. T2DM participants could be treated with any combination of oral agents and insulin except agents of the PPARgamma agonist class; diabetes management was standardized for a 1-week period in advance of measurements to insulin only as detailed below. Inclusion criteria included age 18-45, in good general health (other than T2DM), without recognized diabetic complications. Lean subjects were required to be taking no regular medications. For T2DM volunteers the use of blood pressure and cholesterol medications was allowed, but these medications were discontinued for 3-5 days in advance of the PET studies. Exclusion criteria were chronic illnesses other than T2DM, extreme hypertension or hyperlipidemia that would preclude brief discontinuation of medications for these conditions, musculoskeletal conditions which would not allow comfortable performance of the 3-hour supine glucose clamp and PET imaging procedure, claustrophobia, history of radiation exposure that would preclude the additional dosing with the PET imaging, and current pregnancy. This study was overseen by the Indiana University Institutional Review Board, and all participants gave written informed consent for their participation. This study was registered on clinicaltrials.gov as NCT02563834

Study Design: Participants were studied on two consecutive days, under the protocol outlined in Figure 1. Participants were admitted to the Indiana University Clinical Research Center (CRC) between 8 and 10 PM the evening prior to the first planned study and fasted overnight. No cigarette smoking was permitted starting 24 hours prior to the planned study. A urine pregnancy test was performed on admission in female participants to ensure a negative result prior to proceeding with the study.
PET studies were performed in the fasting state on two consecutive days. On both days a 2-tracer protocol was used to measure cardiac perfusion and oxidative metabolism ($^{11}$C-acetate) (52, 63, 68) and fatty acid uptake ($^{16}$-$^{18}$F-fluoro-4-thiapalmitate, FTP, a synthetic 16 carbon fatty acid) (20, 21). FTP was selected because the presence of the thiol group at position 4 effectively traps the tracer in the mitochondrion after uptake and initial handling by the β-oxidation pathway without opportunity to redistribute into other intracellular fatty acid pools, therefore providing a direct measure of fatty acid oxidation rate (19-21). Blood samples for later measurement of circulating levels of relevant metabolites (glucose, insulin, non-esterified fatty acid) were taken at planned intervals across the study (Figure 1). Noninvasive measurements of systemic hemodynamics were taken simultaneously during the PET studies, at similar intervals (impedance cardiography, BioZ.com, CardioDynamics, San Diego) using surface electrodes placed on the thorax and neck, and an automated blood pressure cuff.

On one study day, a control infusion of normal saline was applied in order to match the timing of PET imaging and the volume loading associated with the insulin/glucose clamp procedure. On the other study day, generally the second day, a hyperinsulinemic (120 mU/m²/min) euglycemic clamp procedure was initiated by 0800h as we have previously done, according to the method of DeFronzo and Andres (18). The PET imaging procedure began after 120 min of the saline or insulin infusion. The insulin/glucose infusion continued throughout the PET imaging sequence, maintaining euglycemia by continuous adjustment to the glucose infusion rate according to the arterialized blood samples every 5 minutes. At the end of the first study day, participants were returned to the CRC, fed a 2000 kcal/d lunch and supper, then fasted again from 8PM until the end of the PET imaging procedure on the second day.

**Glucose Management:** Participants with T2DM underwent a 7-day standardization of glycemic control, using only long- and short-acting insulin and discontinuing all other diabetes medicines from 7 days prior to PET measurements. These changes were individualized by the team physician (KM), and adjusted every 1-2 days based
on self-monitored blood glucose values, targeting AM glucose levels 90-130 mg/dL.
(5.0- 7.2 mmol/L). The goal of this intervention was to achieve near-normal levels of
glycemia at the time of the studies with only long-acting insulin carrying into the
interval of the glucose clamp and PET procedure. To the degree that improvement in
glycemia and exposure to insulin also mitigated any adaptations or chronic effects of
hyperglycemia on myocardial fuel selection, uptake, or metabolism, such secondary
effects were anticipated to bias toward measuring the underlying state of the heart
independent of chronic hyperglycemia. Similarly, such effects could improve insulin
responses in other target tissues and thereby help isolate effects of diabetes per se
independent of hyperglycemia and under-insulinization.

PET Methodology: Analytical grade source chemicals were used for the synthesis of
labeled tracers. Carbon-11 (\(^{11}\)C) and Fluorine-18 (\(^{18}\)F) were generated using a
Siemens CTI RDS 112 medical cyclotron system. 16-[\(^{18}\)F]fluoro-4-thia-palmitate
(FTP) was synthesized as described previously (21) with radiochemical purity
>98%. FTP was formulated for intravenous administration in 1% human serum
albumin and sterile filtered using a 0.2 \(\mu\) filter. Acetate was labeled as previously
described (31).

Imaging was performed using a Siemens ECAT EXACT HR+ whole body PET scanner.
This system has an intrinsic in-plane image resolution of approximately 4.2 mm
FWHM (Full Width at Half-Maximal). The system simultaneously measures 63 image
planes with an axial coverage of 15.5 cm.

Conventional data acquisition procedures were used to acquire and reconstruct the
PET images. This included a transmission measurement to enable correction for
attenuation by body mass. The image data was reconstructed using conventional
filtered back-projection algorithms and a Hanning smoothing filter, which produces
an image resolution of approximately 1.0 cm FWHM. This filter function was
selected because it is consistent with the observed resolution degradation observed
by the motion of the heart as determined with cardiac gated imaging studies.
Custom software was used to identify the entire left ventricle as a single volume of
interest, and then apply mathematical modeling to the resulting VOI. The left
ventricle cavity, concurrently identified, served as an arterial blood pool from which the input function was derived. Following acetate infusion, serial timed peripheral blood samples were taken (at minutes 0, 3, 6, 10, 15, 20, 25 and 30) and immediately analyzed for circulating concentrations of labeled CO$_2$ and later used as a correction for the image-derived input function (11, 53). Similarly, timed samples were taken at these same intervals following FTP infusion and assayed for total and FTP-specific radioactivity in order to determine metabolite correction curves for the input function.

Compartmental modeling using a 2-compartment model was applied to the metabolite-corrected acetate data to derive separate terms for influx and efflux kinetics. Myocardial blood flow was measured directly from the influx kinetic parameter $k_1$ (31, 47). Myocardial oxygen consumption was derived from the efflux parameter $k_2$ using $k_2 = 0.0071 + 0.0074(MVO_2)$ (67), as we have done previously (47). For FTP kinetics we applied Patlak analysis to derive uptake kinetics using metabolite-corrected input functions (55). In exploratory analyses we applied a variety of 2- and 3-compartment models, but owing to the monotonic uptake of FTP in many individual datasets were unable to derive kinetic constants beyond simple uptake kinetics; therefore data are only presented for Patlak-derived results.

The slope of the Patlak plot ($K_i$) for FTP represents the net rate at which the radiotracer is trapped, incorporating rates of fatty acid uptake and efflux prior to metabolic trapping. Multiplying $K_i$ values by ambient non-esterified fatty acid concentrations at the time of fatty acid imaging provided net rates of fatty acid oxidation ($FAO = K_i \times NEFA/LC$), where a lumped constant (LC) value of 2.10 was applied to account for the difference of FTP kinetics to natural fatty acid kinetics as based on studies in isolated rat hearts under normoxic conditions (19).

**Impedance Cardiography:** Systemic hemodynamic parameters were measured in order to assess cardiac work during the measurements of metabolic rates. Conventional automated cuff-derived blood pressures were measured concurrently with impedance cardiography (BioZ.com, CardioDynamics, San Diego CA). Impedance cardiography provides a non-invasive measure of cardiac output and
other systemic hemodynamic parameters, deriving parameters from cyclical fluctuations in the electrical resistance (impedance) of the with the cardiac cycle. The waveform directly reflects stroke volume, allowing derivation of cardiac output and (together with blood pressure measures) cardiac work (17, 27). The use of this system allowed repeated measurements to be made noninvasively during the course of the PET imaging with the subject positioned in the scanner. Steady state measurements were taken twice, at 120 minutes (steady state, start of PET scanning) and at the end of the scan sequence, and the mean of these readings was taken to reflect cardiac status at the time of PET imaging.

**Analyte Measurement** The concentrations of glucose in arterialized blood samples was measured by a glucose oxidase method (Model 2300 Yellow Springs Instruments, Yellow Springs, OH). Blood for determination of other analytes was frozen at −80°C. Insulin determinations were made using a dual-site radioimmunoassay (Millipore, Billerica, MA). Non-esterified fatty acid measurements were performed using a colorimetric assay (Wako Diagnostics, Richmond VA). Cholesterol and triglyceride measures were made using standard methodologies through our local hospital’s clinical laboratory.

**Statistical Approach**

Descriptive statistics were presented as mean ± standard deviation; other results were presented as mean±SEM. Normality of distribution of variables was assessed graphically, and by statistical testing using a Kolmogrov Smirnov test; no variables required transformation in order to apply parametric testing. Baseline characteristics were compared between groups using unpaired t-tests.

The overall comparison of interest is in the set of measurements made at the time of PET imaging, comparing the two study groups. We used a mixed model approach, comparing effects of the insulin/glucose clamp versus saline on parameters measured at the time of PET imaging across groups. For parameters where pre-PET values were measured (e.g. baseline and steady state values on each study day), these were also incorporated into the analyses. Analyses were also performed comparing the two treatment groups under steady state insulin conditions, in order
to highlight differences in the achieved metabolic or hemodynamic effects. Regression analyses were used to evaluate the determinants of myocardial fuel consumption, efficiency, and blood flow under these treatment conditions. Statistical analyses were performed using SPSS version 23 (IBM, Chicago IL). Statistical power for this study was based on the PET endpoints of blood flow, oxidation rate, and fatty acid utilization. For the acetate-derived measures the population variability observed by us and by others is on the order of 15% (coefficient of variation), i.e. 1SD ~0.15 of mean values (23, 36). At the time of this study no estimate of variability for the FTP imaging in humans was available; we assumed it was similar to that of the acetate parameters. Based on this estimate, we calculated that 9 participants per group would be sufficient to demonstrate between-group differences on the order of 1SD at 83% power. To allow for dropouts and incomplete data, we planned to recruit 10 subjects per group.
Results

Characteristics of the participants are described in Table 1. At the time of enrollment the diabetic participants had an average HbA1c of 8.3±3.5%, and their fasting glucose was 8.3±2.6 mmol/L. Following the pre-study period of standardization of therapy using basal/bolus insulin without oral agents, the 7 day average fasting glucose was 5.8±1.0 mmol/L, and 7 day average pre-supper glucose was 6.7±1.1 mmol/L.

Metabolic measures: The approach to matching glucose concentrations for fasting study conditions was acceptably achieved. Lean control subjects had normal range glucose values the morning of both study days (Table 2; ~5 mmol/L). Following short-term improvement of their glucose control in preparation for the study procedures, diabetic subjects had glucose values within the target range (Table 2; 6.3±0.3 control day, 7.0±0.5 clamp day). These values were significantly higher than those of the lean controls (p<0.0001) but not different within diabetic subjects across the two study days (p=0.08). On the control day, no study-imposed change in these values was undertaken, but on the clamp day we targeted a steady-state glucose value of 5 mmol/L.

The non-esterified fatty acid (NEFA) concentrations were higher in the diabetic participants (fasting saline control day p=0.46, insulin-glucose clamp steady state p=0.001) and were less completely suppressed by the high-dose insulin infusion than was achieved in the lean control subjects (Table 1). This reflects insulin resistance in adipose tissue for suppression of lipolysis. One lean participant had unexpectedly high fatty acid concentrations, greater than 3 SD above the mean for the lean group (BMI 21.0, fasting NEFA 1310 µmol/L, fasting glucose 5.1 mmol/L). In fact this NEFA value was higher than all the values from diabetic participants. Excluding that individual, the NEFA concentrations in the lean group (N=9) were 314±72 µmol/L, and suppressed to 30±10 with insulin (p=0.002 at steady state). Analyses of metabolic and PET measurements were not overall affected by excluding this individual otherwise (not shown), and the results presented in the Tables and Figures include this individual.
Under control and insulin treated conditions, lean subjects had numerically but not statistically lower insulin concentrations than the diabetic subjects (Table 1). The clamp-derived glucose disposal rate differed as expected between groups, with steady state values in lean subjects 10.2±0.7 and in diabetic subjects 5.5±0.7 mg/kg/min (p<0.0001). Expressed per kg fat-free mass these values were 0.18±0.02 and 0.10±0.02 respectively (p=0.004).

Hemodynamics and Cardiac Work: We observed expected effects of the insulin-glucose clamp on systemic hemodynamics and cardiac work (Table 2). Heart rate (p=0.04) and blood pressures (p=0.01 – 0.007) were modestly increased at steady state of the insulin infusion compared to the equivalent time point of the control study day. The hemodynamic measures derived from the transthoracic impedance measures were also affected in expected ways by the insulin clamp. The stroke index was significantly increased (p=0.03); cardiac index and systemic vascular resistance were unchanged; and the increase in left cardiac work index approached the threshold of significance (p=0.07). Only the blood pressure effects of the insulin clamp differed between control and T2DM participants.

PET results: The main parameters of interest for this study were myocardial blood flow and oxygen consumption (both derived from the acetate tracer) and myocardial fatty acid utilization (Patlak rate constant (K) and fatty acid oxidation rates). The results of these analyses are presented in Figures 2 and 3. The effects of insulin on systemic hemodynamics and cardiac work (Table 2) were also evident in the PET measures as an increase in perfusion and an increase in oxygen consumption (Figure 2). These effects were observed in both groups of participants, and did not differ between groups.

Values for K and fatty acid oxidation rate were different between groups under control conditions (higher among diabetic participants; Figure 3). Insulin significantly suppressed fatty acids in both groups, although the achieved fatty acid concentrations remained elevated among diabetic participants under steady state insulin exposure (NEFA 28.4±9.1 µmol/L lean, 85.0±10.2 T2DM, p=0.001). Under these insulin-stimulated, low NEFA conditions, net fatty acid uptake (Patlak K) was
significantly increased in both groups (**Figure 3**), numerically but not significantly higher in T2DM. Together these factors produced a persisting significantly elevated fatty acid oxidation rate in T2DM even under high-dose insulin exposure. Although the between-group differences in fatty acid oxidation rates did not achieve significance when comparing all values ($p=0.07$), the rates were significantly different under steady state insulin clamp conditions (FAO at steady state $0.13\pm0.14 \mu\text{mol}/100\text{g/min lean}$ vs $0.49\pm0.15$ T2DM, $p=0.005$).

There were modest between-group differences in the measures of cardiac work, not achieving statistical significance (**Table 2, Figure 2**); there were no group differences in the effects of insulin on cardiac work. Adjusting FAO for cardiac work did not materially change the overall result regardless of whether the selected measure of work was $\text{MVO}_2$ (**Figure 3**), CI or LCWI (not shown), with higher work-adjusted FAO values in T2DM than controls.

We explored a number of potential contributors to the baseline and insulin-induced changes in fatty acid oxidation, including age, sex, race/ethnicity, baseline fatty acid and glucose concentrations, insulin-stimulated glucose disposal rate (i.e. insulin resistance), myocardial oxygen consumption and myocardial blood flow. Including these parameters in a sequence of multivariable regression analyses (not shown), the only significant independent determinants of fatty acid oxidation were NEFA concentrations and glucose disposal rate (both $p<0.001$). These parameters exerted effects in addition to the effect of insulin in the analyses of insulin-induced change in FAO (not shown).

One expected effect of changes in fuel substrate is a change in myocardial efficiency, i.e. work done per unit oxygen consumed. We therefore analyzed measures of cardiac work relative to the insulin-induced shift in fuel utilization. **Figure 4** presents these analyses using cardiac index and left ventricular work index derived from the impedance cardiography measures, which were correlated with $\text{MVO}_2$. Under saline and insulin-treated conditions there were differences in these measures of work between control and T2DM participants (**Table 2, Figure 2**), in the setting of nonsignificantly higher myocardial oxygen consumption in T2DM.
Reduced myocardial efficiency in T2DM compared to controls was observed in both saline and insulin conditions (Figure 4). Fatty acid oxidation rates normalized by MVO2 were elevated in T2DM compared to controls in both saline and insulin conditions (Figure 3), suggesting enhancement of fatty acid oxidation rates in T2DM in disproportion to the metabolic requirement. However, acute fuel shifts away from fatty acid utilization and changes in cardiac function and work induced by the insulin/glucose clamp reduced myocardial efficiency to a greater extent in T2DM (Figure 4).
Discussion

Using positron emission tomography and a metabolically trapped probe of mitochondrial \( \beta \)-oxidation (FTP), we have demonstrated elevated rates of fatty acid oxidation in type 2 diabetes under saline-treated and insulin/glucose clamp conditions. Insulin treatment resulted in reductions in fatty oxidation in both control and T2DM participants, but the achieved rates of myocardial fatty oxidation remained elevated in T2DM compared to controls. This arose due to combined effects of higher rates of FTP trapping (\( K_i \)) and less effective insulin-induced suppressions of fatty acid availability in T2DM. We also observed impaired myocardial work efficiency in type 2 diabetes.

Metabolic flexibility in the heart. The concept of metabolic inflexibility was originally proposed as a phenomenon of impaired fuel switching in skeletal muscle in obesity and diabetes, but applies equally to fuel selection in the heart. Given the energy needs of the heart and an existing preference for fatty acid fuel sources, it is not obvious \textit{a priori} that obesity-related differences in fatty acid availability and uptake would be detrimental to the myocardium. Nevertheless, many prior reports have linked obesity and insulin resistance with increased myocardial fatty acid uptake and utilization (6, 8, 10, 13, 26, 37, 38, 48, 49, 56-58, 62). Further, it is apparent from a number of experimental studies limiting or augmenting fatty acid uptake that the increased fatty acid uptake can contribute to impairments in myocardial function (2, 22, 25, 28, 34, 44, 48, 50, 51, 59, 73).

Our data implicate abnormalities in myocardial fatty acid transport in addition to failure to suppress adipose fatty acid release in the excess fatty acid uptake and oxidation in type 2 diabetes. Diabetes-related differences were observed in the fatty acid uptake kinetic as well as in the availability of fatty acid substrate, under control and insulin-stimulated conditions, and the net difference between groups results from combined effects of both of these phenomena. These data are consistent with observations of increased myocardial fatty acid uptake in animal models of insulin resistance (12-14, 16, 37, 50, 54), and in obesity/insulin resistance in humans (40, 49, 56, 57), here extended into observations in human Type 2 diabetes. In humans,
PPARgamma agonists and weight loss can improve the abnormally increased fatty acid uptake (29, 41, 72), suggesting this phenomenon is subject to remodeling and not a fixed feature of the diabetic state. Experimental data supports diabetes-related abnormalities in myocardial content and function of CD36 and FATP, the major sarcolemmal fatty acid transport proteins (1, 9, 15, 24, 37, 46, 64, 73). Consistent with experimental evidence in animal models (1, 9, 22, 45, 73), the importance of CD36 for human myocardial fatty acid uptake was recently demonstrated in a family recognized to have CD36 deficiency (30). Lipoprotein lipase, functioning in adipocytes or in vasculature more proximal to the myocardium, has also been implicated as a contributor to the detrimental phenotype of elevated fatty acid uptake (35). Overall the presence of combined defects in fatty acid uptake and availability presents an opportunity and a need to address both factors concurrently in order to rescue the obese/diabetic heart from the effects of increased fatty acid uptake.

**Myocardial efficiency:** We observed reduced myocardial work efficiency in type 2 diabetes compared to lean controls. This observation is in line with data from animal models (50, 61) and from obese humans (49, 56, 58), and augments the sparse human data evaluating this feature of myocardial function in human diabetes.

We observed a further reduction in work efficiency (i.e. more oxygen consumed per unit work) with the euglycemic hyperinsulinemic clamp. This reduction was seen in both study groups, while maintaining the between-group difference noted under baseline conditions. This was concurrent with an observed reduction in fatty acid oxidation, and with an insulin-induced increase in cardiac work. This result is paradoxical since the shift from fat oxidation (requiring 0.24 mole O₂ per mole ATP generated) to glucose (0.16 mole O₂ per mole ATP generated) (43) was expected to produce an improvement in metabolic efficiency. Direct changes in cardiac work have been shown to improve efficiency (dilated cardiomyopathy treated with vasodilator) (7), reduce efficiency (healthy humans subjected to right ventricular pacing tachycardia) (71), and leave efficiency unchanged (healthy humans subjected...
Experimentally imposed fuel shifts have similarly produced contradictory results: In an ovine model of type 1 diabetes, insulin-induced fuel shifts were associated with a reduction in work and in oxygen consumption, producing no change in the underlying relationship of these factors (60). In humans, acute pharmacologic reduction of fatty acid availability produced a parallel reduction in work in control but not in patients with dilated cardiomyopathy (DCM), resulting in impaired work efficiency in DCM (69). This was interpreted as evidence that the fatty acid-dependent metabolism of the DCM state was intolerant of the depletion of fatty acid fuel. Also in DCM, trimetazidine induced a shift away from fatty acid dependence and a concurrent reduction in work, with no net change in the work efficiency (70). From this we conclude that the results of this sort of experiment on integrated work and metabolism at the organ level cannot be predicted \textit{a priori} based on knowledge of cellular metabolism. Further studies are needed to better define the relationships of work, fuel selection, and myocardial efficiency in obesity and diabetes.

Limitations

Our study has a number of limitations. In our assessment of metabolic flexibility we used 2 tracers to evaluate total oxidative metabolism ($^{11}$C-acetate) and rates of fatty acid oxidation ($^{18}$F-FTP), but did not include a 3rd tracer to allow measurement of carbohydrate metabolism. We therefore do not have concurrent direct evidence of fuel shifts toward carbohydrate use, but have inferred this from the large measured reductions in fatty acid uptake and utilization and from the known effects of insulin to drive glucose uptake and utilization in the heart. We did not include a group of obese non-diabetic individuals, to evaluate whether the observed effects were specific to the diabetic state. We studied a comparatively small number of participants, and therefore were not powered to demonstrate potentially important but small differences between groups. Nevertheless we observed important and highly statistically significant effects of parameters related to our main study design questions. We had an unbalanced sex distribution between groups, which was
potentially important given that others have demonstrated sex-specific differences in myocardial fuel selection (57). Our population had too few female participants to allow meaningful post-hoc analyses by sex, and in particular female participants were uniquely present in the T2D group which could have contributed to the observed between-group differences; this will require further study with sufficient sample sizes in both sexes. The specialized fatty acid tracer FTP produced a more direct measure of fatty acid oxidation rate than is provided by traditional $^{11}$C-palmitate tracer methodology, but conversely did not allow us to derive rates of transport, esterification, storage or reverse transport out of the mitochondrion from compartmental modeling such as can be done with unmodified palmitate tracers. The FTP-derived rate of fatty acid oxidation is, however, more directly and simply measured and provides a complementary set of observations that are concordant with measures that have been previously published using palmitate modeling approaches.

Conclusion

Elevated rates of myocardial fatty acid oxidation in type 2 diabetes arise due to combined effects of elevated rates of transport and greater substrate availability. Myocardial metabolic inflexibility was observed as a failure to fully suppress myocardial fatty acid oxidation during a hyperinsulinemic euglycemic clamp, and reflected persistently elevated rates of myocardial fatty acid transport as well as impairment of insulin-induced suppression of fatty acid availability. This was associated with reduced work efficiency in diabetes, which persisted with the shift away from fatty acid metabolism. These phenomena likely contribute to the abnormalities in myocardial function and ischemia tolerance that are seen in type 2 diabetes.

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Disclosures:

None
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**Figure Headings**

Figure 1. Study protocol diagram. Arrows indicate time points where measurements and blood sampling took place across the study protocol. FTP, 16-[\(^{18}\)F]fluoro-4-thiopalmitate; ICG, impedance cardiography.

Figure 2. Effects of Insulin on Cardiac Work and Perfusion. Statistical comparisons indicate main effects (Horizontal line: Insulin versus saline, combining study groups; Vertical line: Lean versus diabetes, combining treatments) or the interaction term (evaluating whether the insulin effect differed by study group). MVO2, myocardial oxygen consumption; T2DM, Type 2 diabetes.

Figure 3. Effects of Insulin on Myocardial Fatty Acid Dynamics. Statistical comparisons indicate main effects (Horizontal line: Insulin versus saline, combining study groups; Vertical line: Lean versus diabetes, combining treatments) or the interaction term (evaluating whether the insulin effect differed by study group). FAO, fatty acid oxidation; FTP, 16-[\(^{18}\)F]fluoro-4-thiopalmitate; MVO2, myocardial oxygen consumption; NEFA, non-esterified fatty acids; T2DM, Type 2 diabetes.

Figure 4. Cardiac Work Efficiency. Statistical comparisons indicate main effects ('Insulin' denotes insulin versus saline, combining study groups; 'Groups' indicates lean versus T2DM, combining treatments). MVO2, myocardial oxygen consumption; T2DM, Type 2 diabetes.
**Table 1. Population Description**

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<tr>
<td><strong>LDL Cholesterol (mmol/L)</strong></td>
<td>2.71 ± 0.71</td>
<td>3.20 ± 0.84</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mmol/L)</strong></td>
<td>1.06 ± 0.27</td>
<td>1.03 ± 0.27</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>TG (mmol/L)</strong></td>
<td>0.81 ± 0.37</td>
<td>1.25 ± 0.42</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>HbA1c Enrollment (%)</strong></td>
<td>8.3 ±3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glucose Enrollment (mmol/L)</strong></td>
<td>4.9 ± 0.2</td>
<td>8.3 ± 2.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Glucose AM Control (mmol/L)</strong></td>
<td>4.8 ± 0.2</td>
<td>6.3 ± 1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Glucose AM Insulin (mmol/L)</strong></td>
<td>5.1 ± 0.5</td>
<td>7.0 ± 1.3</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Glucose SS Clamp (mmol/L)</strong></td>
<td>5.1 ± 0.5</td>
<td>5.0 ± 0.6</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>NEFA Control (umol/L)</strong></td>
<td>414 ± 356</td>
<td>528 ± 252</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>NEFA Clamp (umol/L)</strong></td>
<td>28 ± 23</td>
<td>85 ± 35</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Insulin Control (uU/mL)</strong></td>
<td>8 ± 3</td>
<td>27 ± 29</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Insulin Clamp (uU/mL)</strong></td>
<td>204 ± 76</td>
<td>240 ± 67</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Glucose Disposal Rate (mg/kg fat free mass/min)</strong></td>
<td>8.33 ± 0.55</td>
<td>3.49 ± 0.61</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 2. Hemodynamic Responses to Insulin-Glucose Clamp.

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Type 2 DM</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Day Baseline</td>
<td>Control Day 3hr Saline</td>
<td>Clamp Day Baseline</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>119±4</td>
<td>116±5</td>
<td>124±5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70±4</td>
<td>69±3</td>
<td>71±4</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>84±4</td>
<td>83±4</td>
<td>87±4</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>55±3</td>
<td>56±3</td>
<td>60±4</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>97±6</td>
<td>97±7</td>
<td>93±8</td>
</tr>
<tr>
<td>SI (mL*m⁻²)</td>
<td>51±3</td>
<td>51±3</td>
<td>48±3</td>
</tr>
<tr>
<td>CO (L*min⁻¹)</td>
<td>5.3±0.4</td>
<td>5.3±0.4</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td>CI (L<em>min⁻¹</em>m⁻²)</td>
<td>2.8±0.1</td>
<td>2.8±0.1</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>SVR (dyne<em>s</em>cm⁻²)</td>
<td>1188±109</td>
<td>1190±120</td>
<td>1183±137</td>
</tr>
<tr>
<td>SVRI (dyne<em>s</em>cm⁻³*m⁻²)</td>
<td>2042±266</td>
<td>2049±289</td>
<td>2025±294</td>
</tr>
<tr>
<td>LCW (kg*m)</td>
<td>5.7±0.4</td>
<td>5.6±0.5</td>
<td>6.1±0.5</td>
</tr>
<tr>
<td>LCWI (kg*m⁻²)</td>
<td>3.0±0.2</td>
<td>2.9±0.2</td>
<td>3.1±0.2</td>
</tr>
</tbody>
</table>
Table 2 Note: Results are presented from analyses using mixed modeling, evaluating overall differences by participant group, whether the overall results differ by study day, and the effect of insulin versus saline adjusted for study day differences. Statistically significant comparisons are bolded for convenience; * indicates variables where the insulin effect was statistically significant (p<0.05) within a specific group. CI, cardiac index; CO, cardiac output; DBP, diastolic blood pressure; HR, heart rate; LCW, left cardiac work; LCWI, left cardiac work index; MAP, mean arterial pressure; SBP, systolic blood pressure; SI, stroke index; SS, Steady State, the stage at approximately 3 hours where the insulin effect has stabilized; SV, stroke volume; SVR, systemic vascular resistance, SVRI, systemic vascular resistance index.
Figure 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICG</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Labs</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

11C-Acetate, 11C decay, 18FP 7.5mCi

Hyperinsulinemic Clamp (120 mU/m²/min)

Days 1 and 2

Day 2 only
Figure 2

Cardiac Index (L/min m$^{-2}$)

- Lean
- T2DM

Saline: p=0.011
Insulin: p=0.10 group

p=0.75 insulin*group

MVO$_2$ (mL*100g$^{-1}$*min$^{-1}$)

- Lean
- T2DM

Saline: p=0.004
Insulin: p=0.11 group

p=0.83 insulin*group

Myocardial Blood Flow (mL*100g$^{-1}$*min$^{-1}$)

- Lean
- T2DM

Saline: p=0.04
Insulin: p=0.27 group

p=0.74 insulin*group
Figure 3

- **FTP Paille-Ki (ml plasma g^-1 min^-1)**
  - Saline vs Insulin: Lean vs T2DM
  - 
P=0.001

- **NEFA (µmol/L)**
  - Saline vs Insulin: Lean vs T2DM
  - 
P<0.0001
  - Group: p=0.02 insulin group

- **FA Oxidation Rate (µmol/100g^-1 min^-1)**
  - Saline vs Insulin: Lean vs T2DM
  - 
P<0.0001
  - Group: p=0.03 insulin group

- **FAO/MVO2 (µmol/ml)**
  - Saline vs Insulin: Lean vs T2DM
  - 
P<0.0001
  - Group: p=0.06 insulin group

* p-values indicated with asterisks (*)
Figure 4

- Cardiac Index/MVO₂
  - p=0.01 Insulin
  - p=0.006 Groups
  - Lean
  - T2DM

- Left Cardiac Work Index/MVO₂
  - p=0.01 Insulin
  - p=0.06 Groups
  - Lean
  - T2DM
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-120</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICG</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>↑</td>
<td></td>
</tr>
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<td>Labs</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>¹¹C-Acetate</th>
<th>¹¹C decay</th>
<th>²²F-TP 7.5mCi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1 and 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2 only</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:**

- Hyperinsulinemic Clamp (120 mU/m²/min)
Saline Insulin

Cardiac Index (L/min m$^{-2}$)

- Saline: 2.0
- Insulin: 3.5

- p=0.011
- p=0.10 group
- p=0.75 insulin*group

MVO$_2$ (mL*$100g^{-1}$*min$^{-1}$)

- Saline: 15
- Insulin: 30

- p=0.004
- p=0.11 group
- p=0.83 insulin*group

Myocardial Blood Flow (ml*$100g^{-1}$*min$^{-1}$)

- Saline: 35
- Insulin: 55

- p=0.04
- p=0.27 group
- p=0.74 insulin*group
**Saline Insulin**

- FTP Patlak Ki (ml plasma*g^-1*min^-1)
  - Lean: 0.00, 0.05, 0.10, 0.15
  - T2DM: 0.00, 0.05, 0.10, 0.15
  - p=0.10 group
  - p=0.79 insulin*group

- NEFA (µmol*L^-1)
  - Lean: 0.00, 0.5, 1.0, 1.5, 2.0, 2.5
  - T2DM: 0.00, 0.5, 1.0, 1.5, 2.0, 2.5
  - p<0.0001
  - p=0.003 group
  - p=0.07 insulin*group

- FA Oxidation Rate (µmol*100g^-1*min^-1)
  - Lean: 0.00, 0.5, 1.0, 1.5, 2.0, 2.5
  - T2DM: 0.00, 0.5, 1.0, 1.5, 2.0, 2.5
  - p<0.0001
  - p=0.003 group
  - p=0.07 insulin*group

- FAO / MVO2 (µmol/ml)
  - Lean: 0.00, 0.5, 1.0, 1.5, 2.0, 2.5
  - T2DM: 0.00, 0.5, 1.0, 1.5, 2.0, 2.5
  - p<0.0001
  - p=0.01 group
  - p=0.06 insulin*group
Saline Insulin

Cardiac Index/MVO$_2$

Lean

T2DM

$p=0.01$ Insulin

$p=0.006$ Groups

Saline

Insulin

Left Cardiac Work Index/MVO$_2$

$p=0.01$ Insulin

$p=0.06$ Groups

Saline

Insulin