International Journal of Obesity
https://doi.org/10.1038/s41366-018-0137-7

ARTICLE

Biochemistry/Physiology

Altered tricarboxylic acid cycle flux in primary myotubes from severely obese humans

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Received: 10 January 2018 / Revised: 27 April 2018 / Accepted: 10 May 2018

Abstract

Background/objective The partitioning of glucose toward glycolytic end products rather than glucose oxidation and glycogen storage is evident in skeletal muscle with severe obesity and type 2 diabetes. The purpose of the present study was to determine the possible mechanism by which severe obesity alters insulin-mediated glucose partitioning in human skeletal muscle.

Subjects/methods Primary human skeletal muscle cells (HSkMC) were isolated from lean (BMI = 23.6 ± 2.6 kg/m^2, $n = 9$) and severely obese (BMI = 48.8 ± 1.9 kg/m^2, $n = 8$) female subjects. Glucose oxidation, glycogen synthesis, non-oxidized glycolysis, pyruvate oxidation, and targeted TCA cycle metabolomics were examined in differentiated myotubes under basal and insulin-stimulated conditions.

Results Myotubes derived from severely obese subjects exhibited attenuated response of glycogen synthesis (20.3%; 95% CI [4.7, 28.8]; $P = 0.017$) and glucose oxidation (5.6%; 95% CI [0.3, 8.6]; $P = 0.046$) with a concomitant greater increase (23.8%; 95% CI [5.7, 47.8]; $P = 0.004$) in non-oxidized glycolytic end products with insulin stimulation in comparison to the lean group (34.2% [24.9, 45.1]; 13.1% [8.6, 16.4], and 2.9% [−4.1, 12.2], respectively). These obesity-related alterations in glucose partitioning appeared to be linked with reduced TCA cycle flux, as 2-[^{14}C]-pyruvate oxidation (358.4 pmol/mg protein/min [303.7, 432.9] vs. lean 439.2 pmol/mg protein/min [393.6, 463.1]; $P = 0.013$) along with several TCA cycle intermediates, were suppressed in the skeletal muscle of severely obese individuals.

Conclusions These data suggest that with severe obesity the partitioning of glucose toward anaerobic glycolysis in response to insulin is a resilient characteristic of human skeletal muscle. This altered glucose partitioning appeared to be due, at least in part, to a reduction in TCA cycle flux.

Introduction

Severe obesity (BMI ≥ 40 kg/m^2) is associated with an elevated incidence of metabolic disorders such as insulin resistance and type 2 diabetes (T2D) [1–3]. A primary culprit linked with impaired glucose homeostasis is insulin resistance in peripheral tissues, with skeletal muscle accounting for the majority of glucose disposal under

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Published online: 11 June 2018

insulin-stimulated conditions (~70–90%) [4]. The intracellular fates of glucose also appear to be altered with obesity and/or T2D in a manner which preferentially partitions sugar entering the muscle cell toward glycolytic end products rather than oxidation and/or glycogen storage [5–9]. For example, Friedman et al. [8] reported decreases in insulin-stimulated glycogen synthesis and glucose oxidation rates in conjunction with an increase in non-oxidized glycolytic products (increased lactate production) in intact skeletal muscle strips from severely obese humans. An increase in circulating lactate, a gluconeogenic precursor, could hypothetically increase blood glucose production by the liver and contribute to hyperglycemia [10]. To date, the origin of impaired glucose partitioning in skeletal muscle with severe obesity is still unclear, but may be linked with defective oxidative characteristics of substrate metabolism.

There is emerging evidence supporting a link between impaired mitochondrial respiratory capacity and dysregulated glucose metabolism [11, 12]. However, we reported that mitochondrial respiratory capacity is normal in primary myotubes derived from severely obese subjects thus suggesting an upstream lesion [13, 14]. The tricarboxylic acid (TCA) cycle is a key metabolic pathway of glucose oxidation that generates NADH and $FADH_2$ for ATP production via respiration. However, it is uncertain whether severely obese individuals have a reduction in TCA cycle flux in skeletal muscle that could subsequently partition glucose toward the formation of glycolytic end products. Therefore, the purpose of this study was to determine if the TCA cycle flux is altered in skeletal muscle with severe obesity with a resultant increase in glycolytic end products. The fates of glucose (i.e., glucose oxidation, glycogen synthesis, and non-oxidized glycolysis products) were determined in cultured primary human myotubes under basal and insulin-stimulated conditions with radioisotope-labeled glucose followed by targeted TCA cycle metabolomic analysis. Our findings suggest that the TCA cycle is a potential site responsible for the altered glucose partitioning evident with severe obesity.

Materials/subjects and methods

Human subjects

Skeletal muscle was obtained from the vastus lateralis of lean (body mass index $\leq 25\,kg/m^2$, $n = 9$) and severely obese (body mass index $\geq 40\,kg/m^2$, $n = 8$) Caucasian females with the percutaneous needle biopsy technique. Participants were excluded if they had a diagnosis of diabetes, heart disease, or history of cancer within the last 5 years. Participants were nonsmokers and not taking medications known to alter metabolism. The protocol was approved by the East Carolina University Policy and Review Committee on Human Research, and informed consent was obtained. Subject characteristics are presented in Table 1.

Primary human skeletal muscle cells

Satellite cells were isolated from 50 to 100 mg of fresh muscle tissue and cultured into myoblasts as described previously [15]. In brief, muscle tissue was immediately transferred to ice-cold DMEM, minced and cleaned free of adipose and connective tissues. Satellite cells were isolated by trypsin digestion for 30 min, pre-plated 2 h in 3.0 ml growth media on an uncoated T25 tissue culture flask to remove fibroblasts, and then transferred to a type I collagen-coated T25 flask for attachment. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in growth media supplemented with 10% FBS, 0.5 mg/ml BSA, 0.5 mg/ml fetuin, 20 ng/ml human epidermal growth factor, 0.39 µg/ml dexamethasone, 100 U/ml penicillin, and 100 µg/ml streptomycin. For experiments, myoblasts were sub-cultured onto 12-well type I collagen-coated plates at densities of 60×10^3 cells per well. At 80–90% confluence, differentiation was induced by changing to low-serum differentiation media containing 2% heat-inactivated horse serum. Experiments were performed on day 7 of differentiation. For all experiments, the *N* represents the number of subjects examined, each with triplicate observations.

Glucose oxidation and non-oxidized glycolysis

Following 3-h serum starvation, cells were incubated in a sealed 12-well plate containing 600 µl serum-free DMEM, including 12.5 mmol/l HEPES, 0.5% BSA, 5.0 mM glucose, 1.5 µCi/ml D-[1-^{14}C] glucose (American Radiolabeled Chemicals, MO), 100 U/ml penicillin, and 100 µg/ml streptomycin in the presence or absence of 100 nM insulin for 2-h at 37 °C. Following incubation, 500 µl reaction media was transferred to a modified 48-well microtiter plate with fabricated grooves between two adjoining wells to allow for acid-driven $^{14}CO_2$ from media to be trapped by

Table 1 Subject characteristics

	Lean ($n = 9$)	Severely obese ($n = 8$)
Age (yrs)	29.6 ± 2.5	35.0 ± 2.3
Weight (kg)	57.0 ± 2.3	122.4 ± 7.6*
BMI (kg/m^2)	23.6 ± 2.6	48.8 ± 1.9*
Fasting glucose (mg/dl)	88.4 ± 8.8	88.7 ± 3.2
Insulin (µIU/ml)	7.8 ± 0.6	17.6 ± 1.9*
HOMA-IR	1.7 ± 0.1	3.8 ± 0.3*

*<0.05 vs. lean

200 µl of 1 M NaOH [16]. Incorporation of ^{14}C-labeled glucose into CO_2 was determined with liquid scintillation. Data were normalized to total protein content. The relative change of glucose oxidation in response to insulin stimulation was calculated as (insulin-stimulated condition − basal condition)/basal condition × 100. The same equation was used to calculate relative change of non-oxidized glycolysis and glycogen synthesis rates in response to insulin stimulation.

Non-oxidized glycolytic metabolites were measured by using Whatman, Grade DE81 ion-exchange cellulose paper (GE Healthcare Life Sciences, PA). Briefly, the remaining 100 µl of incubation media was collected and centrifuged for 5 min at 10,000 rpm. An aliquot of the supernatant was added to the ion-exchange cellulose paper, followed by 30 min of drying and washed four times (10 min each) with dH_2O. After washing, the paper was collected in 4 ml liquid scintillation fluid. ^{14}C-Labeled glucose into non-oxidized glycolytic metabolites was quantified by liquid scintillation counting. The non-oxidized glycolytic metabolites are the ionized intermediates from glucose metabolism (lactate, pyruvate, and alanine) remaining on the ion-exchange paper. The unionized glucose was not retained, which was validated by applying ^{14}C-labeled glucose directly to the filter and scintillation counting. Water after each wash was collected and tested to verify that there was no unionized product left on papers after four washes. Data were normalized to total protein content.

Glycogen synthesis

The rate of glycogen synthesis was determined using previously described methods [17]. Briefly, after 2 h of incubation with reaction media in the presence or absence of insulin (100 nM), the cells were washed with ice-cold PBS twice and solubilized in 250 µl of 0.05% SDS. An aliquot was transferred to a 2 ml tube containing carrier glycogen (2 mg) and heated for 1-h at 100 °C. The remaining lysate was used to assess protein concentration (bicinchoninic acid assay, Pierce Biotechnology, Rockford, IL). Glycogen was precipitated by the addition of 100% ethanol and overnight incubation at 4 °C. Glycogen pellets were centrifuged (11,100 × *g* for 15 min at 4 °C), washed once with 70% ethanol, and resuspended in dH_2O. Incorporation of ^{14}C-labeled glucose into glycogen was determined with liquid scintillation. Data were normalized to total protein content.

Pyruvate oxidation

To determine the site of glucose partitioning that may be altered with severe obesity, we examined the oxidation of both 1-^{14}C-pyruvate and 2-^{14}C-pyruvate in primary myotubes. Following 3-h serum starvation, HSkMC were treated with reaction media containing either 1-^{14}C-pyruvate or 2-^{14}C-pyruvate (0.5 µCi/ml, 1 mM sodium pyruvate) for 2 h. Two different carbon position radiolabeled pyruvates (1-^{14}C-pyruvate and 2-^{14}C-pyruvate) were used to determine the site responsible for the altered glucose oxidation with severe obesity. Briefly, 1-^{14}C-pyruvate oxidation measures $^{14}CO_2$ produced from the conversion of pyruvate to acetyl-CoA via pyruvate dehydrogenase complex (PDH), which indicates PDH activity; 2-^{14}C-pyruvate oxidation measures $^{14}CO_2$ exclusively produced from TCA cycle, which indicates TCA cycle activity. Following the 2-h incubation, acid-driven CO_2 production was determined as described above.

Targeted TCA cycle metabolomics

Myotubes were treated in the same manner as the glucose oxidation experiments, except radiolabeled glucose was not added. Following the 2-h incubation, cell pellets were washed with 1× PBS twice, collected in 1 ml 1× PBS, spun down at 2000 × *g* speed to discard the supernatant and stored at −80 °C freezer for metabolomics analyses.

TCA cycle-targeted metabolomics was performed at Mayo Clinic Metabolomics Core Facility. Briefly, before analysis, the cell pellets were lysed in 50 µl of 1×PBS after spiking in 20 µl of internal solution containing U-^{13}C-labeled analytes. The proteins were removed by adding 250 µl of chilled methanol and acetonitrile solution to the sample mixture. After drying the supernatant, the sample was derivatized as described previously [18] before it was analyzed on an Agilent 5975C GC/MS (gas chromatography/mass spectrometry). Concentrations of lactic acid (*m/z* 261.2), fumaric acid (*m/z* 287.1), succinic acid (*m/z* 289.1), oxaloacetic acid (*m/z* 346.2), ketoglutaric acid (*m/z* 360.2), malic acid (*m/z* 419.3), *cis*-aconitic acid (*m/z* 459.3), citric acid (*m/z* 591.4), isocitric acid (*m/z* 591.4), and glutamic acid (*m/z* 432.4) were measured against a seven-point calibration curves that underwent the same derivatization. Data were normalized to total protein content.

Statistical analysis

We have calculated the minimum sample size necessary for our design based on a previous study [19]. We achieved a power of greater than 80% for detecting the effects that we anticipate at a significance level of $P < 0.05$ with eight subjects. Data are expressed as means ± SEM. To determine the significance, Student's *t* test and two-way ANOVA followed by appropriate *t*-test were used. For correlation studies, Pearson correlation analysis was performed. All calculations were performed with SPSS statistical software (23.0; SPSS, Inc., Chicago, IL). Significance was set as $P \leq 0.05$.

Results

Myotubes raised in culture retain the metabolic phenotypes of the donor

The severely obese subjects had a higher body mass and increased plasma insulin concentrations and HOMA-IR compared to their lean counterparts (Table 1). In the myotubes, insulin stimulation increased glycogen synthesis in both lean and severely obese myotubes (Fig. 1a, $P < 0.05$), but such response was significantly attenuated (~41%) in myotubes from severely obese individuals when compared to lean individuals (Fig. 1b, $P < 0.05$), indicating insulin resistance.

Glucose is partitioned toward non-oxidized glycolytic end products in myotubes from severely obese humans

Basal glucose oxidation rates were similar in the myotubes from severely obese and lean subjects; however, while insulin increased the glucose oxidation rate in myotubes from lean individuals (~13%), there was no significant change in myotubes from the severely obese (Fig. 2a). The relative change in the glucose oxidation rate with insulin stimulation was significantly higher (~57%) in myotubes from the lean compared to the severely obese subjects (Fig. 2b, $P < 0.05$). Furthermore, there were no differences in basal non-oxidized glycolysis rates between the groups (Fig. 2c). However, the rate of non-oxidized glycolysis increased significantly with insulin stimulation in myotubes from severely obese (Fig. 2c, $P < 0.05$), but this response was significantly dampened lean subjects (Fig. 2d, $P < 0.05$). The ratio of complete glucose oxidation to non-oxidized glycolysis was calculated to further assess the partitioning of glucose. The basal rates were similar between groups but insulin significantly increased the ratio in the lean (10%), and decreased the ratio in the severely obese (−13%) (Fig. 2e, $P < 0.05$). This finding suggests that with insulin stimulation, myotubes from lean subjects partitioned a greater proportion of glucose toward oxidation while the myotubes from severely obese subjects partitioned toward non-oxidized glycolytic products.

Indices of glucose partitioning are related to whole-body insulin sensitivity

The relative changes in insulin-stimulated glycogen synthesis (Fig. 3a) and glucose oxidation (Fig. 3b) rates in myotubes were negatively correlated with HOMA-IR ($P < 0.05$). The relative changes in non-oxidized glycolysis with insulin correlated positively with HOMA-IR (Fig. 3c, $P < 0.05$).

Myotubes from severely obese humans exhibit impaired TCA cycle flux

To further dissect out the mechanism underlying the dysregulated glucose partitioning in response to insulin stimulation in the myotubes from severely obese individuals, we performed pyruvate oxidation assay in the cultured human myotubes by using pairs of radiolabeled pyruvate ([1-^{14}C] pyruvate and [2-^{14}C] pyruvate) [20]. By utilizing different radiolabeled pyruvates, it allowed us to determine whether defects in glucose oxidation were due to impairments in: (1) pyruvate dehydrogenase complex activity (e.g., 1-^{14}C-pyruvate oxidation) or (2) TCA cycle flux (e.g., 2-^{14}C-pyruvate oxidation). There were no differences in 1-[^{14}C]-pyruvate oxidation rates under any condition (Fig. 4a), while 2-[^{14}C]-pyruvate oxidation rates were significantly lower (~21%) in myotubes from severely obese when compared to lean subjects regardless of conditions (Fig. 4b, $P < 0.05$). Further, the ratio of 2-[^{14}C]- to 1-[^{14}C]-pyruvate oxidation was lower in myotubes from severely

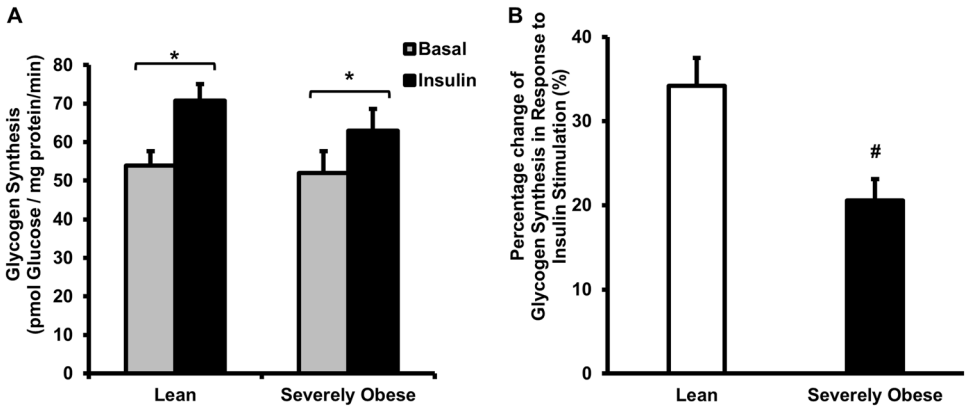

Fig. 1 Basal and insulin-stimulated glycogen synthesis rates in myotubes derived from lean ($n = 9$) and severely obese subjects ($n = 8$). **a** Absolute glycogen synthesis rates; **b** Percent change of glycogen synthesis in response to insulin. *$P < 0.05$ vs. basal; $^{\#}P < 0.05$ vs. lean

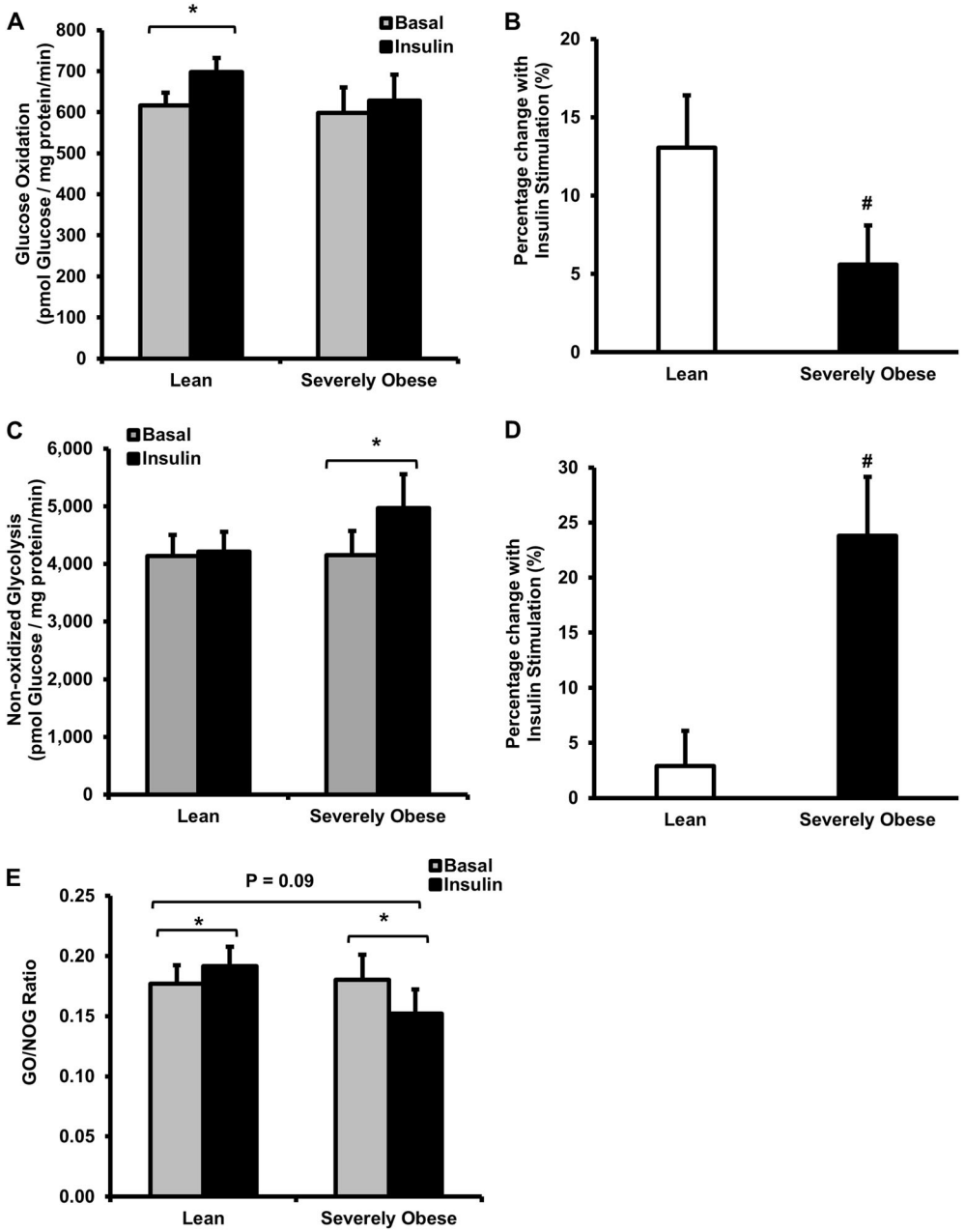

Fig. 2 Basal and insulin-stimulated glucose oxidation rate, non-oxidized glycolysis rate, and glucose oxidation to non-oxidized glycolysis (GO/NOG) ratio in myotubes derived from lean ($n = 9$) and severely obese subjects ($n = 8$). **a** Absolute values of glucose oxidation. **b** Percent change of glucose oxidation rate in response to insulin. **c** Absolute values of non-oxidized glycolysis production. **d** Percent change of non-oxidized glycolysis production in response to insulin. **e** Glucose oxidation to non-oxidized glycolysis (GO/NOG) ratio. $^{*}P < 0.05$ vs. basal; $^{\#}P < 0.05$ vs. lean

obese (~30%) compared to lean subjects (Fig. 4c, $P < 0.05$), suggesting that TCA cycle flux is impaired with severe obesity. In addition, there was a relationship between 2-[^{14}C]-pyruvate oxidation rates and insulin-stimulated glucose oxidation rates (Fig. 4e, $r = 0.675$, $P = 0.01$). There was no relationship between 1-[^{14}C]-pyruvate oxidation and glucose oxidation (Fig. 4d).

Myotubes derived from lean and severely obese humans have differing TCA cycle intermediates profiles

In the myotubes, insulin significantly increased citrate and isocitrate production ($P < 0.05$). Primary myotubes derived from severely obese humans exhibited reductions or trends

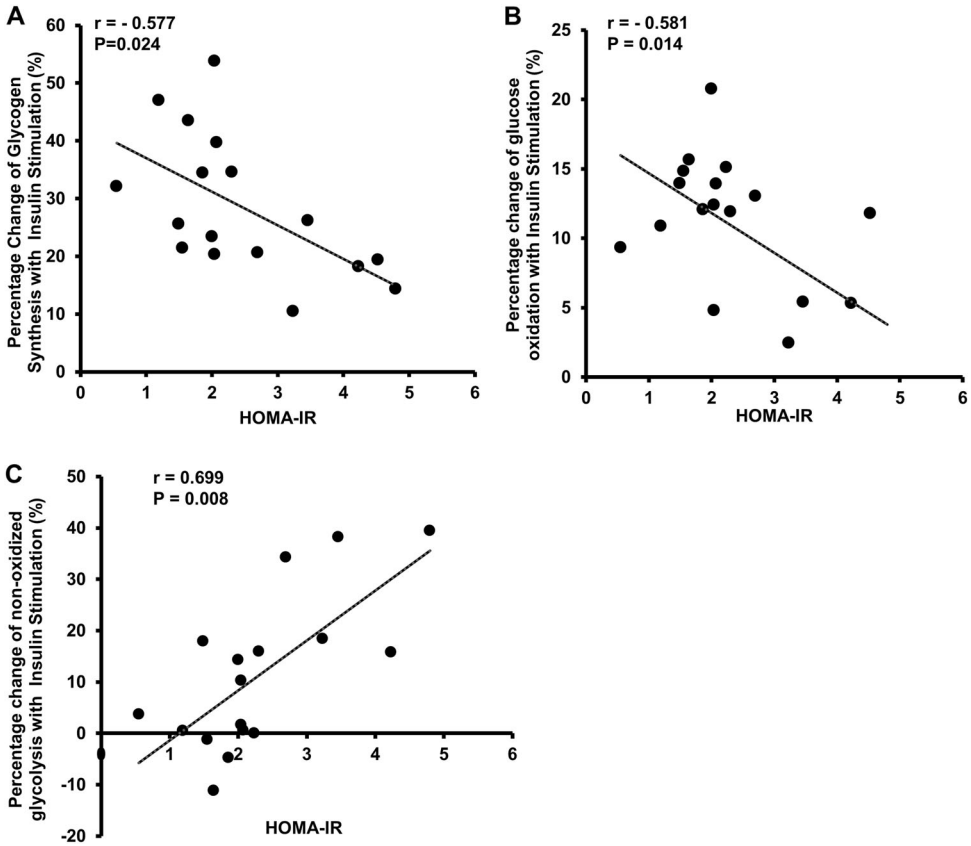

Fig. 3 Relationships of HOMA-IR with **a** relative increases in glycogen synthesis rates with insulin stimulation, **b** relative increases in glucose oxidation rates with insulin stimulation and **c** relative increases in non-oxidized glycolysis rates with insulin stimulation

toward reductions in TCA cycle intermediates when compared to the lean group (main effect of severe obesity): citrate ($P = 0.042$), *cis*-aconitic acid ($P = 0.066$), isocitrate ($P = 0.017$), α-ketoglutarate ($P = 0.05$), and succinate ($P = 0.088$) (Fig. 5).

Discussion

The ability of insulin to stimulate glucose oxidation and glycogen storage in skeletal muscle is depressed with obesity and insulin resistance, resulting in an increased production of non-oxidized glycolytic end products (e.g., lactate), which can serve as gluconeogenic substrates [8, 21, 22]. Together, these detriments in glucose partitioning can have severe consequences on whole-body metabolism, resulting in the progression of various metabolic disorders, including type 2 diabetes. In the current study, we observed that skeletal muscle from severely obese individuals retained the altered glucose partitioning at the muscle cell level, with a depressed ability to stimulate glucose oxidation and glycogen storage with insulin. This altered glucose partitioning appeared to be due, at least in part, to a reduction in TCA cycle flux. Our data suggest the TCA cycle is altered in human skeletal muscle with severe obesity in a manner which shunts glucose toward the production of non-oxidized glycolytic end products. In myotubes from individuals with T2D, Gaster and colleagues [23] reported depressed TCA cycle flux; the current data provide the additional information that depressed TCA cycle flux in skeletal muscle is not confined to solely T2D but also present with obesity.

The pyruvate dehydrogenase complex (PDC) controls the entry of glucose-derived pyruvate into the mitochondria [24] and plays a critical role in metabolic dysfunction and insulin resistance in skeletal muscle [25, 26]. However, in the present study, 1-(^{14}C)-pyruvate oxidation, a surrogate for PDC activity, did not differ between the lean and severely obese subjects (Fig. 4a). Conversely, 2-(^{14}C)-pyruvate oxidation and its ratio to 1-(^{14}C)-pyruvate oxidation, surrogate markers of TCA cycle flux, were lower in myotubes derived from severely obese subjects (Fig. 4b, c). These data suggest that TCA cycle dysfunction is a potential lesion responsible for the altered glucose partitioning in skeletal muscle with severe obesity.

To better understand the specific sites within the TCA cycle impaired with severe obesity, we utilized targeted metabolomics analysis of TCA cycle intermediates. In agreement with a depressed capacity for 2-[^{14}C] pyruvate oxidation, TCA cycle intermediates (isocitrate and α-ketoglutarate) generated from steps (isocitrate to α-ketoglutarate and α-ketoglutarate to succinyl-CoA) that are responsible

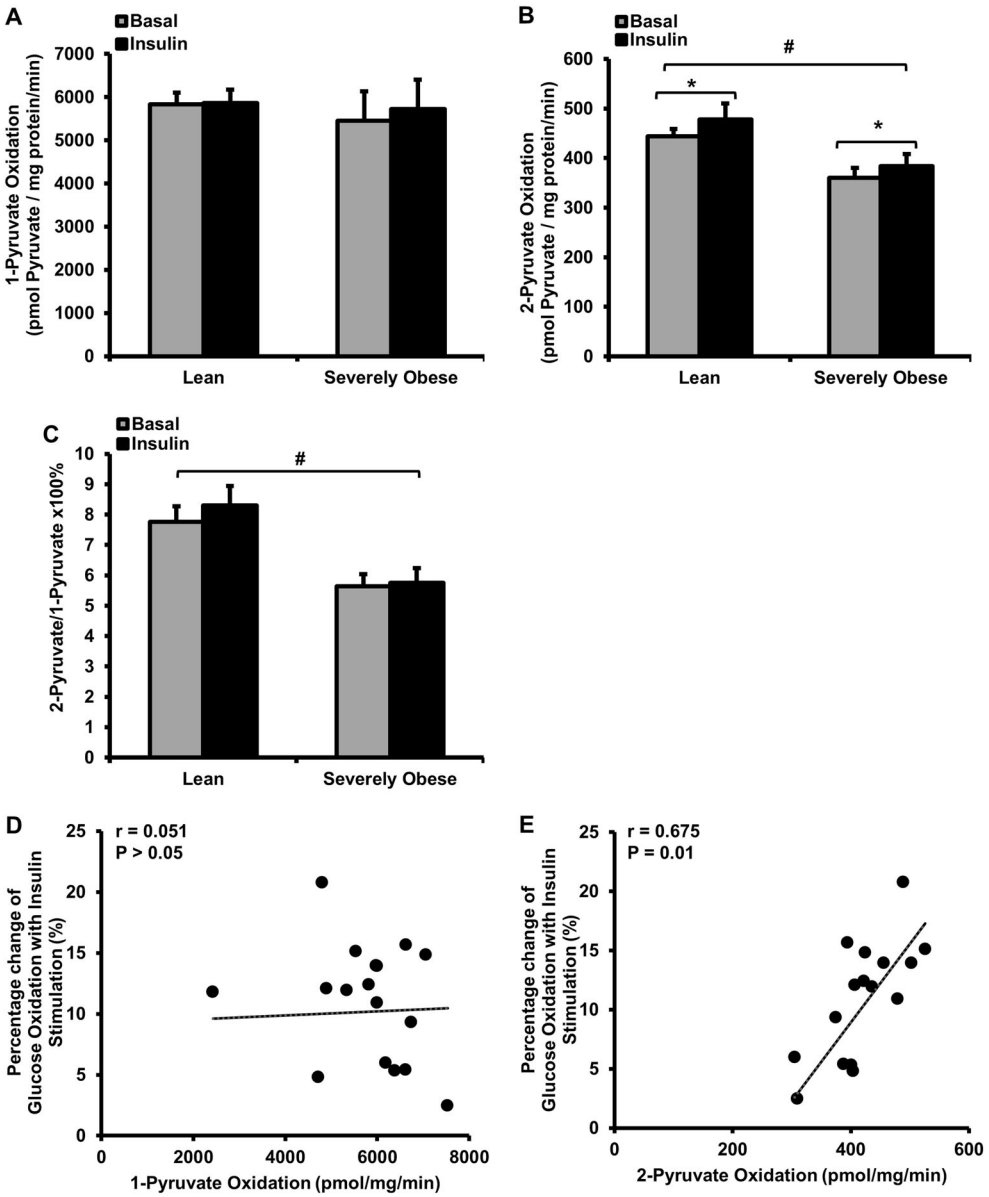

Fig. 4 Basal and insulin-stimulated pyruvate oxidation rates in myotubes derived from lean ($n = 8$) and severely obese ($n = 8$) subjects. **a** 1-[^{14}C] pyruvate oxidation rates. **b** 2-[^{14}C] pyruvate oxidation rates. **c** Ratio of 2-[^{14}C] pyruvate oxidation to 1-[^{14}C] pyruvate oxidation. **d** Relationship of 1-[^{14}C] pyruvate oxidation rates with relative changes in glucose oxidation rate in response to insulin stimulation. **e** Relationship of 2-[^{14}C] pyruvate oxidation rates with relative changes in glucose oxidation rate in response to insulin stimulation.*$P < 0.05$ vs. basal; $^{\#}P < 0.05$ vs. lean

for CO_2 production were lower in myotubes from severely obese humans (Fig. 5). Further, there was also a reduced concentration of citrate with severe obesity, which can be interpreted as indicating that either: (1) the step of conversion from acetyl-CoA to citrate (i.e., citrate synthase) is reduced and/or (2) the amount of acetyl-CoA available for entry into the TCA cycle is lowered with obesity. While we did not measure acetyl-CoA concentration, 1-[^{14}C] pyruvate oxidation rates were not different between lean and severely obese groups (Fig. 4a), suggesting no defect at the level of acetyl-CoA production. This is consistent with data from our previous study where citrate synthase activity was significantly reduced in skeletal muscle of severely obese humans [27]. However, another study reported that citrate synthase activity was not different in myotubes derived from lean and obese subjects [28]. This is not surprising given that the severity of obesity was different between that study (moderately obese; BMI = 33.7 ± 1.4) and ours (severely obese; BMI = 48.8 ± 1.9). We have shown that defective skeletal muscle oxidative capacity is evident in skeletal muscle with severe obesity, but not with moderate obesity [29]. Interestingly, a reduction in intermediates with

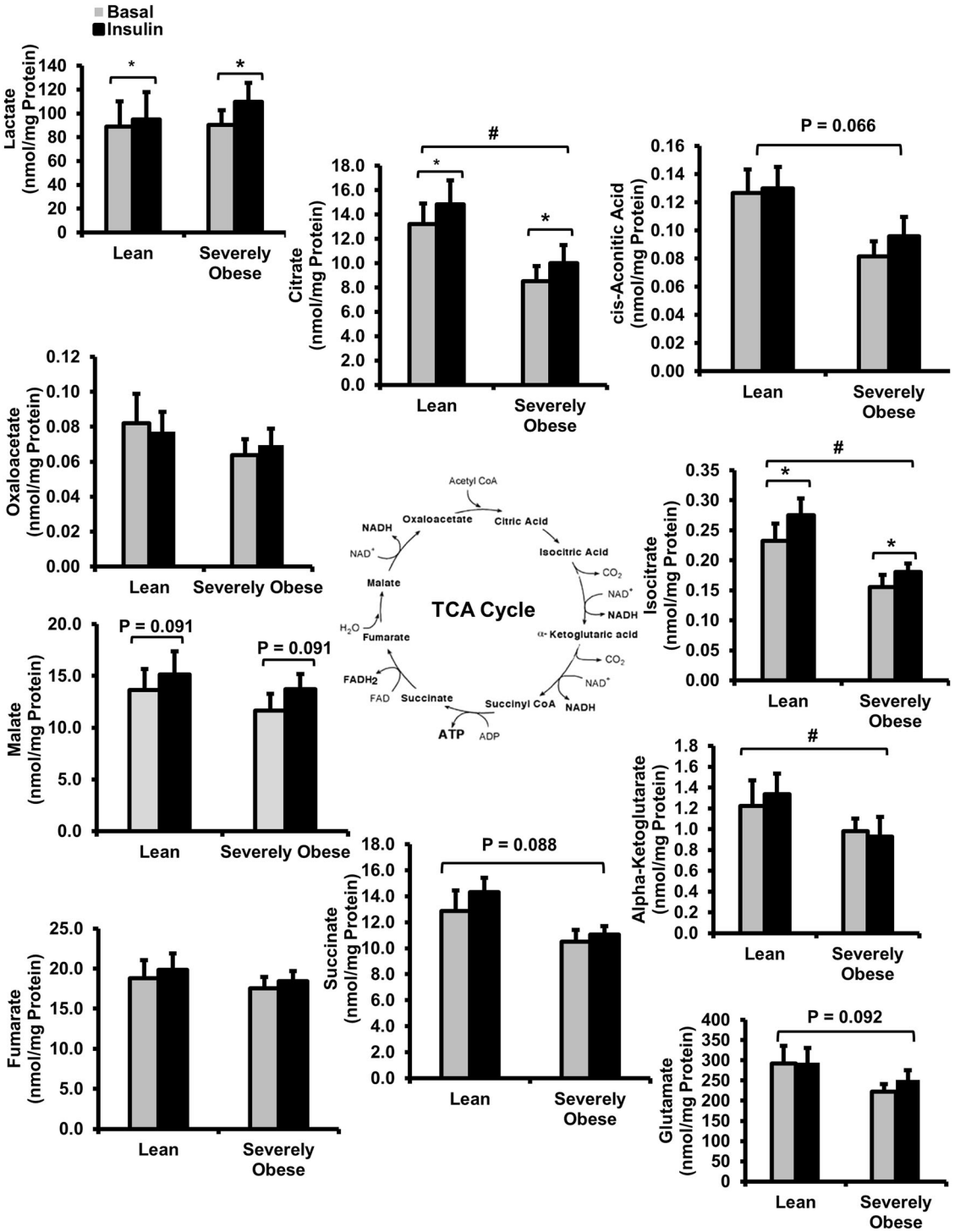

Fig. 5 Targeted TCA cycle metabolomics under basal (gray) and insulin-stimulated conditions in myotubes derived from lean ($n = 8$) and severely obese subjects ($n = 8$). $^{*}P < 0.05$ vs. basal; $^{\#}P < 0.05$ vs. lean

obesity was not evident in the more distal steps of the TCA cycle (i.e., fumarate, malate, and oxaloacetate). This is consistent with data indicating greater anaplerosis from amino acids in skeletal muscle from obese humans [30]. We thus hypothesize that amino acids may have been used to replenish those latter steps of the TCA cycle in myotubes from severely obese humans. Taken together, our metabolomics and oxidation data suggest that dysregulated steps early in the TCA cycle, such as citric acid production, may be responsible, at least in part, for the impaired TCA cycle

flux in myotubes with severe obesity. Future studies using stable isotope tracers in human skeletal muscle cell culture to further analyze TCA cycle flux are needed in order to identify the specific reaction(s)/enzymes responsible for the altered TCA cycle flux in skeletal muscle from severely obese individuals.

The mechanism(s) underlying impaired TCA cycle function at muscle cell level is unclear, but may be due to several factors. As the alterations were observed in muscle cells raised under uniform culture conditions, derangements in TCA cycle function with severe obesity may be linked with a genetic/epigenetic origin. For example, our group has reported differing methylation signatures near the transcription start site of several metabolic enzymes in myotubes from severely obese humans [31, 32] that could subsequently alter gene expression. Gaster and colleagues also hypothesized that posttranslational modifications (i.e., phosphorylation and acetylation) of TCA cycle enzymes differ with metabolic disorders in a manner which could impair flux [23, 33]. A limitation of the current study was not directly examining either of these factors. It could also be argued that TCA cycle flux was lower with severe obesity due to insulin resistance (Figs. 1, 2, and 3) and less potential substrate entering the cell. However, if this was the case then pyruvate oxidation would have been consistently depressed, rather than only being lower when using 2-[^{14}C] pyruvate as the substrate (Fig. 5). In addition, TCA cycle occurs in the mitochondria of skeletal muscle cells. Therefore, it has been hypothesized that decreased mitochondrial content may contribute to the dysregulated TCA cycle flux evident in skeletal muscle from obese and type 2 diabetic humans [34, 35]. While we did not measure mitochondrial content in this study, a prior study from our group has demonstrated that mitochondrial content was lower in myotubes derived from severely obese humans in comparison to the leans [36], suggesting the reduced mitochondrial content may, at least in part, contribute to the deranged TCA cycle flux in the myotubes from the severely obese humans.

A cellular origin of skeletal muscle metabolic dysfunction appears to be a hallmark characteristic with severe obesity. Previous reports have shown that this cell culture model reflects what is observed in vivo, as similar impairments in lipid oxidation and insulin signaling in severe obesity were evident at the whole body [37], intact muscle tissue [27, 38, 39], and cell culture levels [40–42]. The results of the current study extend these findings to glucose metabolism and insulin action and suggest impaired glucose metabolism in response to insulin, another characteristic of severe obesity, also originates at skeletal muscle cell level. The magnitude difference between lean and severely obese in insulin-stimulated glycogen synthesis (~50% decrease in severely obese group) (Fig. 1b) is similar to the previous report examining glucose metabolism in intact muscle strips [8]. The indices of insulin action in the primary myotubes (changes of glycogen synthesis and glucose oxidation in response to insulin stimulation) were correlated to a whole-body index of insulin sensitivity (HOMA-IR) (Fig. 3a–c). Collectively, these data suggest metabolic disorders, such as insulin resistance and reduced complete glucose oxidation rate, can be observed from whole body, and down to the level of the cell in severely obese humans.

In conclusion, the results of the current study reveal myotubes derived from severely obese non-diabetic subjects present impaired glucose partitioning in response to insulin stimulation that originates from the defective TCA cycle flux. Further, the impairments observed at the level of muscle cell may be a key determinant of whole-body measures of glucose metabolism. In sum, these findings are in agreement with the growing notion that skeletal muscle contains an inherent metabolic program that is influenced by severe obesity. Future studies should focus on targeting TCA cycle flux using the human skeletal muscle cell culture model to prove if this approach can effectively improve glucose oxidation and insulin sensitivity in skeletal muscle from severely obese humans.

Acknowledgements We thank the research volunteers for their dedication and effort to this study. We also thank Gabe Dubis and Angela Clark for assisting in specimen collection. This study was supported by grants from Janssen Research & Development, LLC, Golden LEAF Foundation, National Institutes of Health (DK56112, JAH) and American Heart Association Postdoctoral Fellowship (15POST25080003, KZ). This publication was made possible by Mayo Clinic Metabolomics Resource Core through grant number U24DK100469 from the National Institute of Diabetes and Digestive and Kidney Diseases and originates from the National Institutes of Health Director's Common Fund.

Compliance with ethical standards

Conflict of interest P.J.H. and J.L. are employed by Johnson & Johnson. G.L.D. and W.J.P. received research grants from Janssen Research & Development, LLC.

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