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Metabolic Flexibility in the Postprandial Period is Attenuated in Healthy Aging

by

Adam D. Osmond

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor George A. Brooks, Chair
Professor José Pablo Vázquez-Medina
Professor Gregory W. Aponte

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Abstract

Metabolic Flexibility in the Postprandial Period is Attenuated in Healthy Aging

by

Adam D. Osmond

Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor George A. Brooks, Chair

Aging-related decrements in metabolic flexibility have been reported during supraphysiological conditions, but responses to physiological perturbations are less studied. The purpose of this research was to assess metabolic flexibility by determining changes in the rates of whole-body lipolysis, fatty acid (FA) oxidation, carbohydrate (CHO) oxidation, and FA reesterification in the postprandial period. Further, we sought to examine how lactate controls energy substrate partitioning in the postprandial period. After a 12-hr overnight fast, 15 young (21-35 yr; 7 men and 8 women) and 14 older (60-80 yr; 7 men and 7 women) participants had the forearm vein catheterized for primed, continuous infusion of [1,1,2,3,3-^{2}H]glycerol. A contralateral warmed hand vein was catheterized for arterialized blood sampling. Indirect calorimetry was performed simultaneously to determine total FA and CHO oxidation rates (Rox). Total FA reesterification rates (Rs) were estimated from tracer-measured lipolytic and FA oxidation rates. After a 90-min equilibration period, participants underwent a 120-min, 75-g oral glucose tolerance test (OGTT).

In young participants, glycerol rate of appearance (Ra) (an index of lipolysis) decreased from baseline 5 min post-challenge ($p = 0.03$), remained steady until 15 min, decreased until 30 min ($p < 0.001$), and then continuously declined until the end of observation at 120 min ($P < 0.001$). In older participants, Ra decreased 30 min post-challenge ($p = 0.002$) and then remained low until 120 min ($p \leq 0.003$). There were no differences in glycerol Ra between groups at any time ($p \geq 0.14$). Blood lactate concentrations were inversely correlated with glycerol Ra in both groups, but the correlation was higher in older participants ($r = -0.93$ vs. $r = -0.71$).

At 60 min post-challenge, FA Rox decreased from baseline in both groups ($p < 0.001$), but FA Rox was higher in older participants ($p = 0.04$). In young participants, CHO Rox increased 5 min post-challenge ($p = 0.02$) and then remained elevated until 120 min ($p \leq 0.01$). In older participants, CHO Rox increased 30 min post-challenge ($p = 0.02$) and then continuously rose until 120 min ($p < 0.001$). In both groups, FA Rs decreased between 5–30 min post-challenge ($p \leq 0.002$) and then increased, but FA Rs was lower in older participants at 60 min ($p = 0.02$) and 90 min ($p = 0.03$). The AUC for FA Rox was greater than that for FA Rs in older ($p = 0.008$), but not young participants ($p = 0.34$). Blood lactate concentrations and lactate oxidation rates were inversely correlated with FA Rox in both groups, but the correlations were higher in older participants ($r \geq -0.83$ vs. $r \leq 0.73$).

In young participants, plasma insulin concentrations increased from baseline 5 min post-challenge ($p = 0.01$), continuously rose until 30 min ($p = 0.001$), and then remained elevated until 120 min ($p < 0.001$). In older participants, [Insulin] increased 15 min post-challenge ($p < 0.001$) and then remained elevated until 120 min ($p < 0.001$). Further, [Insulin] was higher in young participants at 5 min ($p = 0.05$) and 30 min ($p = 0.01$) post-challenge. Plasma [Insulin] was inversely correlated with glycerol Ra in both groups, but the correlation was higher in older participants ($r = -0.98$ vs. $r = -0.78$). Further, the correlations between [Lactate], [Insulin], glycerol Ra, and FA Rox were more significant in older participants ($p \leq 0.02$ vs. $p \geq 0.04$).

Our results indicate that metabolic flexibility to oral glucose consumption is delayed in "healthy" aging. Specifically, we report the following: (1) the suppression of lipolysis was delayed, (2) the fall in FA oxidation and rise in CHO oxidation rates were delayed; (3) the suppression of FA oxidation was attenuated such that FA oxidation was favored over CHO oxidation and FA reesterification, and (4) lactate is involved in the suppression of lipid metabolism in aging.

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CHAPTER 1

Introduction

Metabolic flexibility describes the ability to switch back and forth between carbohydrate (CHO) and fatty acid (FA) utilization in response to changing physiological conditions. Such conditions include the transition from rest to exercise (1), or from postabsorptive to postprandial states (2). Metabolic flexibility has previously been demonstrated in the ability to alter substrate utilization in response to insulin stimulation (3-5) and exogenous fuel administration in resting (6) and exercising (7-9) humans. The loss of metabolic flexibility, or metabolic inflexibility, is observable in metabolic disease states as well as in "healthy" aging (10-13). Central to the dogma of metabolic inflexibility is substrate competition induced by the excess availability of free fatty acids (FFA) in plasma during hyperinsulinemia and the subsequent elevation of FA oxidation at the expense of CHO oxidation (6, 14, 15). Physiologically, the persistence of FFA availability during hyperinsulinemia has been interpreted to indicate the presence of a defect in the insulin-mediated suppression of lipolysis (16).

The oral glucose tolerance test (OGTT) is a standard physiological method for assessing the influence of a glucose load on parameters of metabolic flexibility. Typically, an OGTT lasts 2 hr (17) or 3 hr (18). After oral glucose consumption, the rise in blood glucose concentrations is followed by an increase in insulin release, with subsequent effects of insulin stimulation on substrate partitioning. Reductions in the plasma concentrations of glycerol alone (19, 20) or concurrently with FFA (21, 22) during an OGTT have been interpreted as a reduction in lipolysis. Although changes in the concentrations of glycerol and FFA typically follow the lipolytic rate (23), they do not provide information on the turnover of those metabolites. Reductions in lipid/FA oxidation (24-26) with concurrent increases in CHO oxidation (27-29) have also been documented during an OGTT in young, but not older participants. Consequently, at present, it is unknown if there are aging-related modifications to substrate partitioning in the postprandial period, and if such modifications promote metabolic inflexibility in aging through the induction of substrate competition.

We have recently reviewed the role of lactate in signaling and its effects on energy substrate partitioning (30, 31). Briefly, intracellular lactate accumulation from increased glycolytic flux increases the production of malonyl-CoA and reactive oxygen species (ROS), which collectively inhibit FA transporters CPT I (32, 33) and CPT II (34) to limit mitochondrial FA transport and oxidation. Additionally, lactate binding to G-protein coupled receptor GPR-81, later renamed hydroxycarboxylic acid receptor 1 (HCAR-1) on adipose cells inhibits lipolysis similarly to insulin (35-37) to limit FA availability for oxidation. Although we have provided evidence of this lactate signaling during exercise (38-40), we aim to demonstrate lactate control of metabolic flexibility during the postprandial period.

To assess how parameters of metabolic flexibility are affected in aging, we recruited 15 young (21-35 yr; 7 men and 8 women) and 14 older (60-80 yr; 7 men and 7 women) individuals for studies using stable isotope tracer infusion and indirect calorimetry during an OGTT. Following oral glucose consumption, we hypothesized the following:

1. Rates of lipolysis, total FA oxidation, and total FA reesterification will decrease
2. Rates of total CHO oxidation will increase
3. Changes in FA and CHO rates will be delayed in aging

4. Rates of lipolysis and FA oxidation will be inversely correlated to blood lactate concentrations
5. The effects of increased blood lactate concentrations will be exaggerated in aging

CHAPTER 2

Aging delays the suppression of lipolysis in the postprandial period

INTRODUCTION

Metabolic flexibility describes the ability to switch back and forth between carbohydrate (CHO) and fatty acid (FA) utilization in response to changing physiological conditions. Such conditions include the transition from rest to exercise (1), or from postabsorptive to postprandial states (2). Metabolic flexibility has previously been demonstrated in the ability to alter substrate utilization in response to insulin stimulation (3-5) and exogenous fuel administration in resting (6) and exercising (7-9) humans. Intrinsically, metabolic flexibility is ultimately regulated by several mechanisms that similarly control substrate partitioning, including controls of adipose tissue lipolysis.

The loss of metabolic flexibility, or metabolic inflexibility, is observable in metabolic disease states as well as in "healthy" aging (10-13). Central to the "Randle Cycle" theory is that an abundance of FA determines energy substrate partitioning (14). From there, it follows that metabolic inflexibility is induced by an excess availability of free fatty acids (FFA) in plasma during hyperinsulinemia and the subsequent elevation of FA oxidation that occurs at the expense of CHO oxidation (6, 15). Bonadonna and colleagues (41) reported that FFA availability during insulin infusion was higher in older compared to younger individuals and resulted in greater total lipid oxidation. Although higher rates of lipolysis were suspected to have increased FFA availability, it was unclear if the lipolytic rate was different or altered by their experimental conditions. Greater plasma FFA availability in aging has been hypothesized to be a consequence of aging-related increases in adiposity, which would provide a greater volume and source of FA to be released into circulation (41). However, previous investigations have reported no differences in the lipolytic rate between older and younger participants during prolonged fasting (42) or during exercise performed at a given power output before (43) and after (44) endurance training. To our knowledge, the lipolytic response during physiological hyperinsulinemia has not yet been investigated in aging. Consequently, at present, it is unclear if there are aging-related modifications to the lipolytic rate in the postprandial period, and if such modifications promote metabolic inflexibility in aging through the induction of substrate competition by increasing FFA availability.

The oral glucose tolerance test (OGTT) is a standard physiological method for assessing the influence of a glucose load on parameters of metabolic flexibility. Historically, the OGTT has been used to determine the effects of a single bout of exercise on glucose tolerance (45), differences in glucose tolerance in young and elderly individuals (46), the effects of exercise training on glucose tolerance in aging (47), and in other settings. After oral glucose consumption, the rise in blood glucose concentrations is followed by an increase in insulin release to restore euglycemia. Reductions in the plasma concentrations of glycerol alone (19, 20) or concurrently with FFA (21, 22) have also been documented during an OGTT and have been interpreted as a reduction in lipolysis due to the rise in insulin. Although changes in the concentrations of glycerol and FFA typically follow the lipolytic rate (23), they do not provide information on the turnover of those metabolites. To our knowledge, only one investigation has utilized a glycerol isotope tracer to determine changes in the lipolytic rate during an OGTT. Behn and colleagues (48) reported a reduction in the glycerol rate of appearance (Ra), a marker of lipolysis, during an OGTT in very young (12-21 years old) overweight or obese women. Unfortunately, the metabolic health of their participants limits the application of their results for the general population and as such, our understanding of the changes in the lipolytic rate during an OGTT is incomplete.

A rise in blood lactate concentrations above baseline (i.e., lactatemia) during an OGTT is also documented (20, 49, 50). We have recently reviewed the role of lactate in signaling and its effects on energy substrate partitioning (31). Briefly, lactate binding to hydroxycarboxylic acid receptor 1 (HCAR-1) on adipose cells inhibits lipolysis similarly to insulin by activating the phosphodiesterase enzyme and reducing levels of cAMP (35-37). Evidence of the anti-lipolytic effect of lactate in humans was first provided by Boyd and colleagues (51), who demonstrated that lactate infusion prevented an increase in plasma glycerol and FFA concentrations during mild exercise. Similar results were obtained earlier in resting (52) and exercising (53) dogs receiving lactate infusions. In humans, lactate production by adipose cells following oral glucose consumption (54-57) provides an autocrine mechanism for the anti-lipolytic effects of lactate. Just as our understanding of the changes in the lipolytic rate during an OGTT are incomplete, it is not known if physiological lactatemia induced by oral glucose consumption influences lipolysis and further, if lactatemia influences aging-related modifications to the postprandial lipolytic rate.

The purpose of this investigation was to assess metabolic flexibility by determining changes in the rate of lipolysis as estimated from the glycerol Ra during an OGTT in healthy young and older individuals. Additionally, we sought to determine if physiological lactatemia influences the rate of lipolysis in the postprandial period. We hypothesized that lipolysis would decrease in response to oral glucose consumption, and that the rate of lipolysis would be inversely related to blood lactate concentrations. Further, we hypothesized that the lipolytic rate would be comparable between young and older individuals, but that the inhibitory effects of lactatemia would be exasperated in aging.

METHODS

Study participants. This study was approved by the University of California, Berkeley Committee for the Protection of Human Subjects (CPHS 2018-08-11312) and conformed to the standards set by the Declaration of Helsinki. Fifteen younger (21-35 years) and 14 older (60-80 years) men and women were recruited. Potential participants were interviewed and received verbal and written information on study purposes and procedures. After giving verbal and written consent, participants were screened for metabolic and cardiovascular diseases. Screening tests included a health history questionnaire, a blood draw for a basic metabolic panel, an electrocardiogram (ECG) and pulmonary function assessment via spirometry, three-site skin fold measurements (men: chest, abdomen, and thigh; women: triceps, suprailiac, thigh) to assess body density, and a physical examination. Measurements of body density were converted to body composition and expressed as percentage body fat according to the guidelines provided by the American College of Sports Medicine (58). To assess physical fitness, screening also included a continuous, progressive cycle ergometer test to determine peak oxygen consumption ($\dot{V}O_2peak$) and ventilatory threshold (VT). For dietary controls, participants provided 3-day dietary records that were analyzed for caloric intake and macronutrient consumption (Diet Analysis Plus Version 6.1, ESHA Research, Salem, OR). To qualify for participation, participants were required to be diet and weight stable; to have a body mass index (BMI) in the range of $\geq$18.5 and <30.0 kg/m^2; to be non-smokers; to have normal pulmonary function (FEV_1/FVC of >70%); to have a fasting blood glucose concentration of <100 $mg \cdot dL^{-1}$; to have a hemoglobin A1c percentage of <5.7 % of total hemoglobin; to have a fasting total cholesterol concentration of <200 $mg \cdot dL^{-1}$, LDL cholesterol concentration of <100

$mg \cdot dL^{-1}$, HDL cholesterol concentration of ≥40 $mg \cdot dL^{-1}$, and total triglyceride concentration of <150 $mg \cdot dL^{-1}$; to pass a physical examination; and to be cleared for participation by a licensed physician. Moreover, women were required to have a regular (28- to 35-day) menstrual cycle, to not be pregnant, and to not be taking oral contraceptives.

Screening procedures preceded experimental procedures by at least one week. Participants who were entered into the study were provided with verbal and written information on freedom to withdraw from the study as well as the contact information of the laboratory manager, principal investigator, and physician if adverse effects of the study procedures had occurred.

Exercise testing protocol. All participants underwent a graded exercise test (GXT) conducted on an electronically braked leg cycle ergometer (Lode Corival CPET, Gronigen, Netherlands). Open-circuit indirect calorimetry was performed simultaneously to continuously monitor expired gases throughout the GXT with a "metabolic cart" (Parvo Medics TrueOne 2400 System, Salt Lake City, UT). The "metabolic cart" was calibrated according to the manufacturer's instructions prior to each GXT. Each GXT began with 5 min of seated rest followed by a 2 min warm-up period at 25 W at a set cadence of 60 RPM. Participants then completed three, 4-min stages of 35 W, 60 W, and 85 W at a set cadence of 60 RPM. The cadence was dictated by a metronome and reinforced visually using a tachometer displayed on the ergometer screen. Participants subsequently pedaled at a self-selected cadence for the remainder of the test as the power output increased by 30 W per min. Young participants continued until volitional exhaustion. Older participants continued until achieving VT, which was identified by a respiratory exchange ratio (RER, or $R = \dot{V}CO_2/\dot{V}O_2$) of 0.97–1.00. For older participants, $\dot{V}O_2peak$ was estimated from VT using guidelines provided by the American College of Sports Medicine (58).

Experimental procedures. Participants underwent a 120-min OGTT with primed, continuous infusion of [1,1,2,3,3-^{2}H]glycerol (or D_5-glycerol) and [3-^{13}C]lactate (Cambridge Isotope Laboratories, Inc., Andover, MA). Data on lactate kinetics in young participants have been reported separately (59), and data on lactate kinetics in older participants will be reported separately (60), but the use of this stable isotope tracer is mentioned in this report for its relevancy to the present data.

Participants reported to the laboratory in the morning following a 12-hr overnight fast. Women who participated did so during the mid-follicular phase of their menstrual cycle. For the 24 hr preceding the experimental trial, participants were asked to maintain their standard dietary pattern and refrain from strenuous physical exercise. On the morning of the trial, a catheter was placed in a warmed hand vein for "arterialized" blood sampling and a contralateral arm vein catheter was placed for tracer infusion. Background blood samples were then taken for the determination of endogenous isotopic enrichment (IE) of glycerol. Subsequently, a priming bolus of 40 $mg \cdot 15\ mL^{-1}$ D_5-glycerol was given, and then the participants rested for 90 min while the tracer glycerol was infused continuously at a rate of 0.32 $mg \cdot min^{-1}$. Arterialized blood sampling was repeated at 75 and 90 min after continuous infusion.

After 90 min of continuous infusion, participants drank a solution containing 75 g of D-glucose in 296 mL (#10-O-075, Azer Scientific Inc., Morgantown, PA). Participants consumed the

solution in ≤2 min. Arterialized blood sampling was subsequently completed at 5, 15, 30, 60, 90, and 120 min after the consumption of glucose.

Blood sample collection. Arterialized blood samples for the determination of lactate and glucose concentrations and glycerol IE were immediately deproteinized in 2 volumes of cold 7% perchloric acid (PCA) after collection. Blood samples for the determination of plasma glycerol, FFA, and triglyceride (TG) concentrations were collected in tubes containing K_3EDTA (Vacuette, Greiner Bio-One, Monroe, NC). All samples were immediately placed on ice after collection and then centrifuged at 3,000 g for 10 min at 4°C. The supernatants were then separated and stored at -80°C until analysis.

Metabolite analyses. Plasma glycerol concentrations were determined enzymatically (61) and also concurrently with TG concentrations using a prepared set of reagents (Wako L-Type Triglyceride M, Fujifilm Healthcare Solutions, Lexington, MA). The average glycerol concentrations determined from the assays were not different for any data point ($p \geq 0.33$); the average values are reported. Plasma FFA concentrations were determined using a prepared set of reagents and standards (Wako HR Series NEFA-HR(2), Fujifilm Healthcare Solutions). Blood lactate concentrations were determined enzymatically (62) from PCA extracts of whole blood. Blood glucose concentrations were determined from PCA extracts using a prepared reagent (Pointe Scientific Glucose (Hexokinase), Fisher Scientific, Hampton, NH). Standards were prepared from stock preparations of glycerol (#13,487-2, Aldrich, Milwaukee, WI), sodium L-lactate (#71718, Sigma-Aldrich, St. Louis, MO), and D-glucose (#G7528, Sigma-Aldrich) diluted in ultrapure water.

D_5-glycerol analysis. Glycerol IE were determined by gas chromatography/mass spectrometry (GC/MS; GC Model 6890 Series and MS Model 5973N, Agilent Technologies) of the triacetate derivative, as previously described (23). PCA extracts of whole blood were neutralized with 2 N KOH, transferred to ion exchange columns that were previously washed with double deionized water (ddH_2O) through a cation resin (Analytical Grade 50W-X8, 50-100 Mesh H^+ Resin, Bio-Rad Laboratories, Hercules, CA) and with ddH_2O followed by 2 N formic acid through an anion resin (Analytical Grade 1-X8, 100-200 Mesh Formate Resin). Glycerol was eluted through the cation column with ddH_2O. The eluent was lyophilized, reconstituted in methanol, and then centrifuged at 3,000 g for 10 min at 4°C. The clear supernatant was collected and then dried under nitrogen (N_2) gas. Samples were then derivatized with a 2/1 mixture of acetic anhydride/pyridine and heated at 65°C for 10 minutes. The mixture was dried under N_2 gas and the samples were reconstituted in ethyl acetate for GC/MS analysis. Methane was used for chemical ionization. Selective ion monitoring was performed for mass-to-charge (M/Z) ratios of 159 for unlabeled glycerol and 164 for labeled [1,1,2,3,3-^{2}H]glycerol tracer.

Calculations. Glycerol Ra, rate of disappearance (Rd), and metabolic clearance rate (MCR) were calculated using the equations of Steele as modified for use with stable isotopes (63):

$$IE = \text{abundance of } D_5\text{-glycerol} / (\text{abundance of endogenous glycerol} + D_5\text{-glycerol})$$

$$Ra\ (\mu mol \cdot min^{-1}) = [F - V[(C_1 + C_2) / 2] \times [(IE_2 - IE_1) / (t_2 - t_1)]] / [(IE_2 + IE_1) / 2]$$

$$Rd\ (\mu mol \cdot min^{-1}) = Ra - V[(C_2 - C_1) / (t_2 - t_1)]$$

$$MCR\ (L \cdot min^{-1}) = Rd / [(C_1 + C_2) / 2]$$

where: F represents the tracer infusion rate (0.32 $mg \cdot min^{-1}$), V is the estimated volume distribution for glycerol (270 $mL \cdot kg^{-1}$), C_1 and C_2 are concentrations at sampling times t_1 and t_2 respectively, and IE_1 and IE_2 are isotopic enrichments at sampling times t_1 and t_2 respectively. For calculations of Ra, Rd, and MCR, plasma glycerol concentrations were first converted to units of $mg \cdot mL^{-1}$ using the molecular weight of glycerol (92.09 $g \cdot mol^{-1}$). Calculated Ra and Rd values were then converted back to units of μmol.

Statistical analyses. Data were analyzed using GraphPad Prism 10 (Version 10.1.2 for Windows, GraphPad Software, Boston, MA). Statistical significance was set at $\alpha \leq 0.05$. Repeated-measures two-way ANOVA with the Geisser-Greenhouse correction and Tukey's multiple comparisons test was used to assess the significance of mean differences between groups and across time points. Data are presented as mean ± standard error of the mean (SE). Differences across time points are expressed relative to baseline (i.e., before the consumption of glucose at 0 min). Area under the curve (AUC) was determined using GraphPad Prism 10. To assess the significance of differences in AUC, an unpaired t-test with Welch's correction was used. Data are presented as mean ± SE.

Pearson correlation coefficients were used to assess the significance of relationships among select variables studied. The magnitude of correlations are interpreted according to the guidelines provided by Mukaka (64). The Lactate Shuttle concept originally described in 1984 (65) has recently been updated to describe a postprandial mechanism (Postprandial Lactate Shuttle, or PLS) by which lactate regulates energy substrate partitioning following CHO consumption. The PLS is comprised of two phases: (1) a fast enteric phase of lactate production from glycolysis in the gut that is followed by (2) a prolonged systemic phase of lactate production as systemic glucose is disposed of via glycolysis in peripheral tissues (59). Consequently, correlations of blood lactate concentrations with selected variables were assessed throughout the observation period (0-120 min), during the enteric phase of the PLS only (0-30 min), and during the systemic phase of the PLS only (60-120 min). Correlations of variables with one another (i.e., not with blood lactate concentrations) were assessed for the entirety of the observation period only.

RESULTS

Participant characteristics. Comparisons between young and older participants are presented in Table 1. Older participants' mean age ($p < 0.001$), BMI ($p = 0.03$), body fat percentage ($p = 0.02$), and body fat mass (FM) ($p = 0.005$) were significantly higher than young subjects. In contrast, absolute ($p = 0.007$) and relative ($p < 0.001$) $\dot{V}O_2$peak, peak power output ($p = 0.002$), and VT ($p < 0.001$) were significantly higher in young participants. There were no significant differences in total body mass (TBM) ($p = 0.22$), fat free mass (FFM) ($p = 0.79$), pulmonary function (FEV_1/FVC) ($p = 0.09$), daily caloric intake ($p = 0.10$), or percentage of caloric intake from carbohydrates ($p = 0.12$), fat ($p = 0.68$), or protein ($p = 0.06$). There were no significant

differences in the size of the glucose load when expressed relative to TBM ($p = 0.36$) or per unit of FFM ($p = 0.95$), but when expressed per unit of FM, the relative glucose load was greater for young participants ($p = 0.007$).

Metabolite concentrations. Plasma glycerol concentrations ([Glycerol]) initially increased from baseline between 5-15 min in both groups, but the changes were not significant for either young ($p \geq 0.58$) or older ($p = 0.61$) participants (Fig. 1). In young participants, [Glycerol] decreased 30 min after glucose consumption (50.58 ± 3.01 vs. 69.19 ± 2.50 μM, $p < 0.001$) and then continuously declined thereafter ($p < 0.001$). Similarly, in older participants, [Glycerol] decreased at 30 min (48.84 ± 4.19 vs. 72.76 ± 5.12 μM, $p < 0.001$) and then continuously declined thereafter ($p < 0.001$). There were no significant differences between groups at any time point ($p \geq 0.24$).

The changes in plasma FFA concentrations ([FFA]) were similar to the changes in [Glycerol] (Fig. 2). Plasma [FFA] initially increased from baseline between 5-15 in both groups, but the changes were not significant for either young ($p \geq 0.60$) or older ($p \geq 0.67$) participants. In young participants, [FFA] decreased 30 min after glucose consumption (247.14 ± 32.78 vs. 464.71 ± 27.01 μM, $p < 0.001$) and then continuously declined thereafter ($p < 0.001$). Similarly, in older participants, [FFA] decreased at 30 min (326.87 ± 37.75 vs. 457.93 ± 35.52 μM, $p < 0.001$) and then continuously declined thereafter ($p < 0.001$). Additionally, [FFA] was higher in older participants between 30-120 min, but the difference was significant between 60-120 min ($p \leq 0.05$). Changes and differences in [FFA] were similar when expressed per unit of FFM (Fig. 3A). Additionally, changes in [FFA] were similar when expressed relative to FM for both groups, but [FFA] per unit of FM was higher at baseline ($p = 0.01$) and at 5 and 15 min ($p \leq 0.03$) in young participants (Fig. 3B).

Blood lactate concentrations ([Lactate]) changed in a biphasic pattern in both groups (Fig. 4). In young participants, [Lactate] increased 5 min after glucose consumption (0.71 ± 0.05 vs. 0.60 ± 0.05 mM, $p = 0.04$), continued to rise until 15 min (1.09 ± 0.09 mM, $p < 0.001$), decreased at 30 min (0.87 ± 0.09 mM, $p = 0.009$), increased at 60 min (1.19 ± 0.09 mM, $p < 0.001$), and then steadily declined thereafter ($p \leq 0.002$). In older participants, [Lactate] increased at 15 min (0.90 ± 0.06 vs. 0.65 ± 0.02 mM, $p = 0.02$), decreased at 30 min (0.84 ± 0.05 mM, $p = 0.04$), and then continuously rose thereafter ($p < 0.001$). There were no significant differences between groups at any time point ($p \geq 0.08$).

Blood glucose concentrations ([Glucose]) remained unchanged at 5 min in both groups ($p \geq 0.97$) (Fig. 5). Blood [Glucose] increased 15 min after glucose consumption in both young (7.02 ± 0.23 vs. 4.72 ± 0.10 mM, $p < 0.001$) and older (7.31 ± 0.34 vs. 5.04 ± 0.19 mM, $p < 0.001$) participants, continued to rise until 60 min ($p < 0.001$), and then steadily declined thereafter ($p < 0.001$). Additionally, [Glucose] was overall higher in older participants, but the difference was significant at 90 min (8.94 ± 0.46 vs. 7.29 ± 0.46 mM, $p = 0.02$).

Plasma TG concentrations ([TG]) remained unchanged between 5-15 min in both young ($p \geq 0.98$) and older ($p \geq 0.81$) participants (Fig. 6). In young participants, [TG] increased 30 min after glucose consumption (0.74 ± 0.10 vs. 0.66 ± 0.10 mM, $p < 0.001$), decreased at 60 min (0.61 ± 0.10 mM, $p = 0.03$) and then continuously declined thereafter ($p \leq 0.01$). In contrast, in older

participants, [TG] increased at 30 min (0.87 ± 0.08 vs. 0.75 ± 0.07 mM, $p = 0.01$) and then steadily declined until 120 min (0.65 ± 0.08 mM, $p = 0.03$). Although [TG] was generally higher in older participants, there were no significant differences between groups at any time point ($p \geq 0.11$).

Glycerol isotopic enrichment. Glycerol IE is presented as mole percent excess (MPE) (Fig. 7). Glycerol MPE increased 30 min after glucose consumption in both young (3.85 ± 0.18 vs. 2.66 ± 0.12 %, $p < 0.001$) and older (3.86 ± 0.20 vs. 2.73 ± 0.20 %, $p = 0.002$) participants, and then continuously rose thereafter ($p \leq 0.002$). There were no significant differences between groups at any time point ($p \geq 0.18$).

Glycerol rate of appearance. In young participants, glycerol Ra per unit of body mass decreased 5 min after glucose consumption (1.83 ± 0.10 vs. 1.98 ± 0.11 μmol·kg^{-1}·min^{-1}, $p = 0.03$), remained steady at 15 min ($p = 0.22$), decreased at 30 min (1.62 ± 0.10 μmol·kg^{-1}·min^{-1}, $p < 0.001$), and then continuously declined thereafter ($p < 0.001$) (Fig. 8). In contrast, in older participants, Ra decreased at 5 min ($p = 0.25$) and 15 min ($p = 0.08$), but the changes were not significant. Glycerol Ra then decreased at 30 min (1.39 ± 0.10 vs. 1.78 ± 0.12 μmol·kg^{-1}·min^{-1}, $p = 0.002$) and then continuously declined thereafter ($p \leq 0.003$). The changes in Ra were similar for both groups when expressed relative to FFM (Fig. 9) and FM (Fig. 10) and as an absolute rate (Fig. 11). Additionally, the changes in Ra per unit of body mass were similar when expressed as a percentage decrease from baseline (Fig. 12). There were no significant differences between groups when Ra was expressed relative to TBM ($p \geq 0.14$) or FFM ($p \geq 0.43$), as an absolute rate ($p \geq 0.15$), or as a percentage decrease from baseline ($p \geq 0.14$). However, Ra per unit of FM was higher in young participants at all time points ($p \leq 0.01$). There was no significant difference ($p = 0.67$) in the AUC for the glycerol Ra between groups (Fig. 13).

Glycerol rate of disappearance. The changes in glycerol Rd were similar to the changes in glycerol Ra. In young participants, glycerol Rd decreased 5 min after glucose consumption (1.83 ± 0.10 vs. 1.98 ± 0.11 μmol·kg^{-1}·min^{-1}, $p = 0.03$), remained steady at 15 min ($p = 0.19$), decreased at 30 min (1.62 ± 0.10 μmol·kg^{-1}·min^{-1}, $p < 0.001$), and then continuously declined thereafter ($p < 0.001$) (Fig. 14). In contrast, in older participants, Rd decreased at 5 min ($p = 0.27$) and 15 min ($p = 0.07$), but the changes were not significant. Glycerol Rd then decreased at 30 min (1.10 ± 0.10 vs. 1.78 ± 0.12 μmol·kg^{-1}·min^{-1}, $p = 0.002$) and then continuously declined thereafter ($p \leq 0.003$). The changes in Rd were similar for both groups when expressed relative to FFM (Fig. 15) and FM (Fig. 16) and as an absolute rate (Fig. 17). There were no significant differences between groups when Rd was expressed relative to TBM ($p \geq 0.13$) or FFM ($p \geq 0.44$) or when expressed as an absolute rate ($p \geq 0.17$). However, Rd per unit of FM was higher in young participants at all time points ($p \leq 0.01$).

Glycerol metabolic clearance rate. Glycerol MCR initially decreased from baseline between 5-30 min in both groups, but the change was not significant for either young ($p \geq 0.54$) or older ($p \geq 0.54$) participants (Fig. 18). In young participants, MCR steadily increased at 60 min until 120 min (35.98 ± 2.37 vs. 27.96 ± 1.91 mL·kg^{-1}·min^{-1}, $p < 0.001$). In contrast, in older participants, MCR increased at 60 min (32.74 ± 1.94 vs. 26.36 ± 1.57 mL·kg^{-1}·min^{-1}, $p = 0.01$) and then continuously rose thereafter ($p \leq 0.004$). The changes in MCR were similar for both groups when expressed relative to FFM (Fig. 19) and FM (Fig. 20) and as an absolute rate (Fig. 21). However, when expressed as a percentage increase from baseline, MCR was increased from

baseline at 60 min (11.42 ± 6.18 %, $p = 0.007$) and 90 min (23.84 ± 6.61 %, $p = 0.006$) in young participants and at 90 min (26.28 ± 6.50 %, $p = 0.04$) and 120 min (38.83 ± 6.87 %, $p = 0.01$) in older participants (Fig. 22). Additionally, there were no significant differences between groups when MCR was expressed relative to TBM ($p \geq 0.46$) or FFM ($p \geq 0.64$), as an absolute rate ($p \geq 0.22$), or as a percentage increase from baseline ($p \geq 0.25$). However, when expressed relative to FM, MCR was higher in young participants at baseline (183.84 ± 24.39 vs. 120.12 ± 10.61 $mL \cdot kg^{-1} \cdot min^{-1}$, $p = 0.03$) and between 5-30 min ($p \leq 0.04$).

Correlation of blood lactate concentrations with rates and markers of lipolysis. Results of correlations of [Lactate] with glycerol Ra, [Glycerol], and [FFA] are summarized in Table 2. There was an overall high inverse relationship between [Lactate] and glycerol Ra per unit of body mass in both groups, but the correlation was higher ($r = -0.93$ vs. $r = -0.71$) in older participants (Fig. 23). Additionally, the correlation was low ($r = -0.50$) in young participants but high ($r = -0.71$) in older participants during the enteric phase. The correlation was very high in both groups during the systemic phase, but it was positive ($r = 0.99$) in young participants and negative ($r = -0.94$) in older participants. The relationships were similar when glycerol Ra was expressed relative to FFM and FM and as an absolute rate. However, in young participants, the correlation was more significant during the systemic phase when Ra was expressed relative to FM and as an absolute rate.

There was an overall inverse relationship between [Lactate] and [Glycerol] in both groups, but the correlation was higher ($r = -0.78$ vs. $r = -0.54$) in older participants (Fig. 24). Similarly, the overall relationship between [Lactate] and [FFA] was higher ($r = -0.90$ vs. $r = -0.65$) in older participants (Fig. 25). The correlations were low or negligible during the enteric phase. However, the correlations were very high in both groups during the systemic phase, but it was positive ($r = 0.99$) in young participants and negative ($r \geq -0.95$) in older participants.

Correlation of variables related to substrate availability and utilization. There was a very high positive correlation between [FFA] and [Glycerol] in both groups ($r \geq 0.95$) (Fig. 26). In contrast, the relationship between [FFA] and [TG] was only moderate in young participants and low in older participants (Fig. 27). Glycerol Ra was highly and positively correlated with both [Glycerol] (Fig. 28) and [FFA] (Fig. 29) in young ($r \geq 0.96$) and older ($r \geq 0.94$) participants. In contrast, glycerol MCR was inversely correlated with plasma metabolite concentrations. In young participants, glycerol MCR was highly ($r \geq -0.83$, $p \leq 0.02$) correlated with [Glycerol] (Fig. 30), [FFA] (Fig. 31), and [TG] (Fig. 32). Similarly, in older participants, glycerol MCR was highly ($r \geq -0.83$) correlated with both [Glycerol] and [FFA], but only moderately ($r = -0.63$) correlated with [TG]. Results are summarized in Table 3.

Correlations of glycerol rate of appearance to parameters of physical fitness. Relationships between baseline glycerol Ra, the minimal glycerol Ra, the change in Ra from baseline to the minimal Ra (Δ Ra), the Δ Ra expressed as a percentage decrease, and the slope of the Δ Ra to $\dot{V}O_2peak$ and VT were assessed. In young participants, the minimal glycerol Ra was moderately and inversely correlated with VO_2peak when either variable was expressed per unit of body mass (Fig. 33) and as an absolute rate (Fig. 35). Similarly, in older participants, the minimal Ra was moderately and inversely correlated with VT when Ra was expressed per unit of body mass (Fig. 34) and as an absolute rate (Fig. 36). Baseline Ra, Δ Ra, Δ Ra expressed as a percentage decrease,

and the slope of the Δ Ra were not correlated with $\dot{V}O_2$peak or VT in either group. Results are summarized in Tables 4-7.

DISCUSSION

The purpose of this investigation was to determine changes in the rate of lipolysis following oral glucose consumption in healthy young and older individuals. Additionally, we sought to determine if physiological lactatemia influences the rate of lipolysis in the postprandial period. Although the lipolytic rates were comparable between groups, we observed notable differences in the immediate response to oral glucose consumption. In brief, following oral glucose consumption, we report the following: (1) the postprandial suppression of lipolysis is delayed in aging, (2) lactatemia has a greater influence on lipolysis in aging, and (3) postprandial glycerol clearance is accelerated in aging. Our results are discussed sequentially.

Glycerol rate of appearance and glycerol and FFA concentrations. In postabsorptive or exercising humans, glycerol is produced from lipolysis, but not recycled in adipose cells. Consequently, the glycerol Ra is representative of the rate of lipolysis (66). To our knowledge, only one other investigation has utilized a glycerol isotope tracer to determine changes in the rate of lipolysis during an OGTT. Behn and colleagues (48) reported a reduction in glycerol Ra during an OGTT in very young (12-21 years old) overweight or obese women. Similarly using a glycerol isotope tracer and paired with arterialized blood sampling, we expand on their data by demonstrating that the rate of lipolysis also decreases in healthy young and older individuals during an OGTT (Fig. 8).

Changes in [Glycerol] (Fig. 1) and [FFA] (Fig. 2) were closely related in both groups (Fig. 24). Both metabolites remained relatively unchanged within the first 15 min, abruptly decreased at 30 min, and then steadily declined until the end of observation. Similarly, glycerol Ra was reduced from baseline at 30 min in both groups and steadily declined between 30-120 min ($p \leq 0.05$ between time points). Results in our present study agree with previous investigations that have interpreted the reduction of plasma concentrations of glycerol alone (19, 20) or concurrently with FFA (21, 22) at 30 min and beyond as the suppression of lipolysis. Moreover, the very high positive correlations between Ra and [Glycerol] (Fig. 26) and [FFA] (Fig. 27) suggests that the reduced availability of these metabolites in plasma during an OGTT result largely from the suppression of lipolysis.

The blood sampling protocol employed in the present study differs from a traditional OGTT due to sampling at 5 and 15 min after glucose consumption. Importantly, we sampled arterialized blood that had passed through the lung parenchyma and blood compartments, but not muscle, adipose, or the integument as results from arm vein blood sampling. Our data reveals that the rate of lipolysis decreased immediately (i.e., 5 min) after glucose consumption in young participants. Thus, because they remained relatively unchanged at 5 min ($p \geq 0.60$) and 15 min ($p \geq 0.58$), changes in [Glycerol] and [FFA] were incomplete surrogates of the lipolytic rate during an OGTT. Changes in the concentrations of these metabolites may not be adequate to determine, for example, the effects of a single bout of exercise on postprandial lipid substrate partitioning. Importantly, they are unable to identify aging-related differences. Both [Glycerol] and [FFA]

remained unchanged from baseline at 5 min in both young ($p \geq 0.60$) and older ($p \geq 0.67$) participants. However, although glycerol Ra decreased immediately after glucose consumption, the reduction was only significant in young participants ($p = 0.03$ vs. $p = 0.25$). This reduction was a 7.53 ± 1.84 % decrease from baseline ($p = 0.02$) compared to a 4.37 ± 1.78 % decrease ($p = 0.29$) in older participants (Fig. 12). Moreover, because the absolute Ra (Fig. 11) at baseline ($p = 0.98$) became less similar between groups at 5 min ($p = 0.68$), our results suggest that the immediate control of lipolysis in the postprandial period is negatively affected in aging.

Notably, in young participants, glycerol Ra at 15 min was not different from baseline (1.82 ± 0.11 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $p = 0.22$) despite being similar to Ra at 5 min (1.83 ± 0.10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $p > 0.99$), presumably due to a higher variation in Ra at 15 min. A paired t-test comparing the mean difference in Ra from 0 min to 15 min yielded a p-value of 0.02. The present data is therefore interpreted to indicate a rapid and sustained reduction in the rate of lipolysis in young but not older participants.

Although glycerol Ra per unit of body mass was similar between groups at all time points, there are notable differences that are most apparent during the transition from the enteric (0-30 min) to systemic (60-120 min) phases of the PLS. Glycerol Ra was marginally higher in young participants at baseline ($p = 0.25$) and for the duration for the enteric phase ($p \geq 0.14$) and during the systemic phase, Ra was more similar between groups ($p \geq 0.73$). Accordingly, the reduction in Ra from 30 min to 60 min was significant in young ($p < 0.001$) but not older ($p = 0.24$) participants. Moreover, the continual reduction in Ra between 60-120 min was significant in young ($p \leq 0.05$) but not older ($p \geq 0.13$) participants, suggesting that a minimal rate was approaching or had been reached by 60 min in older participants. When expressed relative to FFM (Fig. 9) or as an absolute rate (Fig. 11), a crossover occurred such that the Ra of young participants fell slightly below that of older participants ($p \geq 0.17$) during the systemic phase.

Previous investigations of arterial-venous glycerol concentration differences alone (19, 67) or with a glycerol tracer (68) indicate that skeletal muscle does not release glycerol as does adipose cells during lipolysis. Consequently, we expressed changes in glycerol Ra per unit of FM (Fig. 10). Because body fat % and FM were lower in young participants, Ra was predictably and consistently higher in young participants throughout observation. We interpret the higher Ra to indicate greater rates of lipolysis in young participants strictly because of lower body FM, not lesser suppression of lipolysis (42). Contrarily, differences in the Ra became more apparent when expressed per unit of FM. The reduction in Ra from 30 min to 60 min was significant in young ($p = 0.001$) but not older ($p = 0.81$) participants, and the continual reduction between 60-120 min was significant in young ($p \leq 0.05$) but not older ($p \geq 0.13$) participants. When [FFA] was expressed per unit of FM (Fig. 3B), [FFA] in young participants was distinctly higher at baseline and between 5-15 min, but then sharply declined to values similar to that of older participants ($p \geq 0.59$). These results and the higher absolute [FFA] in older participants between 60-120 min collectively led to the speculation that the extent to which lipolysis was reduced (i.e., % decrease) was greater in young participants. However, we observed no differences in the % decrease in Ra between groups at any time point. Our findings are consistent with previous reports of comparable lipolytic rates between young and older individuals during prolonged fasting (42) and during exercise at a given power output before (43) and after endurance training (44).

Still, our results suggest that the postprandial control of lipolysis is impacted in aging and further, maybe be negatively affected by aging-related conditions. Metabolic flexibility was exemplified in our young participants in their ability to rapidly suppress lipolysis in response to oral glucose consumption. Presently, it is unclear why [FFA] was higher in our older participants between 60-120 min if not the result of lesser suppression of lipolysis. Because the AUC for 3 times the glycerol Ra ($p = 0.67$) (Fig. 13) and absolute glycerol Ra ($p \geq 0.15$) were not different between groups, we cannot conclude that FFA mobilization was greater in older participants. Potentially, older participants exhibited quantitatively less or slower peripheral uptake of plasma FFA (discussed below) to maintain higher [FFA]. Alternatively, and more likely, young participants exhibited greater rates of local, intracellular FA reesterification (i.e., within adipose tissue) (69). Future similar investigations might utilize D_5-glycerol in conjunction with a FA isotope tracer (e.g., [1-^{13}C]palmitate) to explore this hypothesis.

Influence of physical fitness on lipolytic rates. Basal, resting lipolytic rates were not related to physical fitness as defined by $\dot{V}O_2$peak or VT in either group. Although our results disagree with the results of Romijn and colleagues (70), who reported that basal lipolytic rates were higher in endurance-trained individuals, they can be explained by the differences in training status. Our participants were physically active, but not athletes in training; therefore, our population was most likely comparable to the control volunteers in Romijn et al (70). Similarly, the ability to suppress lipolysis as estimated from the Δ Ra from baseline to the minimal Ra, the Δ Ra as a percentage decrease, and the slope of the Δ Ra was not related to physical fitness. However, the minimal Ra (i.e., lowest Ra achieved during the OGTT) was inversely correlated with $\dot{V}O_2$peak in young participants (Fig. 30 and 32) and with VT in older participants (Fig. 31 and 33). Thus, higher levels of physical fitness may reduce the need for elevated rates of lipolysis and consequently, FA availability in the postprandial period to support energy expenditure. In this sense, physical fitness may promote metabolic flexibility by altering the lipolytic rate to favor CHO utilization. Future investigations might utilize endurance-trained individuals to confirm our speculation and determine if higher levels of physical fitness affect the ability to decrease lipolysis in response to oral glucose consumption.

Influence of lactatemia on lipolytic rates. We have recently reviewed the role of lactate in signaling and its sequential effects on energy substrate partitioning (31). Briefly, lactate binding to its receptor HCAR-1 on adipose cells inhibits lipolysis (35, 36). The anti-lipolytic effects of lactate have been documented in exercising humans (51) and resting (52) and exercising (53) dogs. Presently, the inverse correlations between [Lactate] and glycerol Ra (Fig. 21), [Glycerol] (Fig. 22), and [FFA] (Fig. 23) support the role of lactate signaling in the inhibition of lipolysis. Notably, the correlations were higher and more significant in older participants, suggesting that the inhibitory effects of lactatemia are exaggerated in aging.

Presently, lactatemia was induced by oral glucose consumption and subsequent glycolysis (Fig. 4). Interestingly, [Lactate] increased in a biphasic pattern in both groups, but the pattern of change differed. In young participants, [Lactate] increased immediately (i.e., 5 min) after glucose consumption and before an increase in [Glucose] (Fig. 5), thereby implicating the existence of the PLS. In both groups, [Lactate] was increased from baseline at 15 min and then suddenly decreased from 15 to 30 min ($p \geq 0.13$) while remaining elevated above baseline ($p \leq 0.04$). Subsequently,

[Lactate] rapidly increased from 30 to 60 min ($p \leq 0.04$), thereby dividing the PLS into enteric (0-30 min) and systemic (60-120 min) phases (59).

The rapid and dynamic changes in [Lactate] during the enteric phase apparently limited the influence of lactatemia on lipolysis. Although varying in size and direction, the correlations of [Lactate] with markers of lipolysis were not significant in either group (Table 4), thereby reducing the size of the overall correlations and suggesting that [Lactate] minimally influenced lipolysis during the enteric phase. However, the concurrent increase in [Lactate] and decrease in Ra at 5 min in young participants is notable, especially since in older participants, there was no change in [Lactate] at 5 min ($p = 0.96$) and the corresponding decrease in Ra was not significant. Thus, despite the non-significant correlations during the enteric phase, we contend that lactatemia might have a role in the immediate control of lipolysis in the postprandial period.

An important difference in [Lactate] between groups is apparent during the systemic phase. In young participants, [Lactate] steadily declined from 60-120 min, but in older participants, [Lactate] steadily increased. Our laboratory previously demonstrated that lactatemia represents a limitation in lactate clearance by oxidation and gluconeogenesis (71). Presently, substrate oxidation rates were determined simultaneously with arterialized blood sampling, but the data will be reported in Chapter 2 of this dissertation. Briefly, in young participants, CHO oxidation rates ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) remained steady between 60-120 min ($p \geq 0.87$ between time points) and above baseline values ($p < 0.001$) while [Lactate] steadily decreased. In contrast, in older participants, CHO oxidation rates increased in tandem with the increase in [Lactate] between 60-120 min. Our data of lactate kinetics in this investigation indicate overall greater rates of lactate oxidation in young participants (60). Thus, the divergent responses of CHO oxidation were driven by differences in lactate oxidation, which are reflected in the divergent responses of [Lactate] during the systemic phase.

In young participants, the correlations of [Lactate] with markers of lipolysis were nearly linear but positive ($r = 0.99$) during the systemic phase, suggesting that lactatemia did not have an inhibitory effect. Because correlations do not provide evidence of causation, we contend that this positive relationship reflects the greater rates of lactate oxidation freeing the restraints of lactatemia on lipolysis. Alternatively, the relationship suggests that other signaling mechanisms (e.g., insulin) might be more responsible for the suppression of lipolysis. In contrast, in older participants, [Lactate] was very highly and inversely ($r \geq -0.93$) correlated with markers of lipolysis. Although the correlations were not significant ($p \geq 0.16$), they suggest that lactatemia contributed to but was not solely responsible for the suppression of lipolysis. Thus, the divergent changes in [Lactate] between groups during the systemic phase further demonstrates that the inhibitory effect of lactatemia is exaggerated in aging.

Glycerol metabolic clearance rates and potential fates of glycerol and free fatty acids in plasma. Circulating glycerol can be taken up by the liver to be converted to glycerol-3-phosphate by glycerol kinase, which is subsequently used for esterification to FA as TG (72, 73). The newly synthesized TG can be stored as lipid droplets or released into circulation (74). Alternatively, glycerol can be taken up by the liver for gluconeogenesis, by other tissues expressing glycerol kinase (e.g., skeletal muscle) for conversion to lactate (75), or by skeletal muscle for the synthesis of intramuscular triglycerides (IMTG). Although we were not able to precisely identify the fate(s)

of circulating glycerol and FFA in the present study, changes in the glycerol MCR and in the concentrations of these metabolites allow for reasonable discussion.

As with the glycerol Ra, glycerol MCR was similar between groups at all time points when expressed per unit of body mass (Fig. 18) or FFM (Fig. 19) and as an absolute rate (Fig. 21). Although glycerol is not consumed by adipose, changes in MCR were expressed relative to FM since adipose was the source of circulating glycerol (19, 67, 68). Unlike the glycerol Ra, MCR was higher from baseline in young participants at 120 min only, while MCR was elevated above baseline between 60-120 min in older participants, suggesting that the extent of glycerol clearance was greater in older participants. However, we observed no differences in the % increase in MCR between groups ($p \geq 0.25$) during this period (Fig. 22). Still, our data indicates that glycerol clearance in the postprandial period is accelerated in aging. However, it is unclear why the increase in MCR occurred sooner in our older participants.

Total FA reesterification rates (Rs) were estimated as the difference between the rate of lipolysis (i.e., 3 times the glycerol Ra) and the rate of FA oxidation; the data will be reported in Chapter 2 of this dissertation. Briefly, total FA Rs ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) decreased below baseline ($p \leq 0.002$) between 5-30 min in both groups. In young participants, FA Rs increased to baseline values at 60 min, increased above baseline at 90 min ($p = 0.003$), and then decreased to baseline values at 120 min. In older participants, FA Rs steadily increased from 30 min to 90 min and then increased above baseline at 120 min ($p < 0.001$). These results in conjunction with changes in [Glycerol], [FFA], and [TG] suggest that reesterification in the liver was not a major fate of circulating glycerol or FFA during our observation period in either group. Accordingly, the relationships between [FFA] and [TG] were not high (Fig. 27), and glycerol MCR and [TG] were inversely related (Fig. 32), suggesting that circulating glycerol and FFA were not incorporated into newly synthesized TG. The increase in [TG] from 15 min to 30 min observed in both groups ($p \leq 0.01$) instead suggests that TG stored in the liver were released into circulation (Fig. 6).

Skeletal muscle uptake of circulating glycerol during hyperglycemia has been documented previously (76). Although the reduction in [Glycerol] and [FFA] between 30-120 min reflects the suppression of lipolysis, it also reflects muscle uptake of both glycerol and FFA. Increased rates of muscle glycerol uptake and subsequent conversion to glycerol-3-phosphate coupled with the uptake of FFA would promote intramuscular triglyceride (IMTG) synthesis. We observed high inverse relationships between glycerol MCR and [FFA] (Fig. 31) in both groups. Additionally, total FA Rs as described reached baseline or above baseline values by 120 min as [FFA] reached their lowest values. Consequently, we cannot eliminate the possibility of FA reesterification as IMTG synthesis during our observation period. Contemporary perspectives suggest that storage of circulating FFA as IMTG is protective against insulin resistance (77). Thus, accelerated glycerol clearance as observed in our older participants might be an adaptive response in aging. Because IMTG synthesis presumably preceded by glycerol uptake is lower in insulin-resistant individuals (78), future investigations might determine if glycerol clearance is hampered during the postprandial period in insulin-resistant individuals.

Alternatively, glycerol could have been taken up by the liver for conversion to glucose via gluconeogenesis, or by skeletal muscle for conversion to lactate via glycolysis (75) to serve as a

substrate for oxidative phosphorylation or glycogen synthesis (i.e., glyconeogenesis). The former is unlikely given the observed elevation of [Glucose] throughout our observation period.

CONCLUSION

Our results demonstrate that the rate of lipolysis, as indicated from the glycerol Ra, decreased in response to oral glucose consumption in healthy, young and older individuals. Moreover, because glycerol Ra, [Glycerol], and [FFA] were inversely correlated with [Lactate], we provide support for lactate control of metabolic flexibility. Although further investigation is required to confirm the exact mechanism by which lactate influences metabolic flexibility, we interpret these correlations to reflect lactate inhibition of lipolysis through HCAR-1 signaling. In our older participants, the suppression of lipolysis was delayed, and the correlations of [Lactate] with markers of lipolysis were higher, suggesting that metabolic flexibility of lipid substrate partitioning in the postprandial period is negatively affected in aging. Contrarily, our older participants quickly increased glycerol clearance, but the reason for this is not clear. Future, similar investigations should utilize glycerol and FFA isotope tracers in tandem to clarify the fates of circulating FFA during an OGTT. Additionally, future investigations might observe the beneficial effects of endurance exercise training on postprandial lipolytic rates in metabolic disease states.

FIGURE LEGENDS

Figure 1. Plasma glycerol concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 2. Plasma free fatty acid (FFA) concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 3A. Plasma free fatty acid (FFA) concentrations per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 3B. Plasma free fatty acid (FFA) concentrations per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 4. Blood lactate concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 5. Blood glucose concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 6. Plasma triglyceride (TG) concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 7. Isotopic enrichment (IE) of [1,1,2,3,3-^{2}H]glycerol, expressed as mole percent excess (MPE), in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 8. Glycerol rate of appearance (Ra) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 9. Glycerol rate of appearance (Ra) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 10. Glycerol rate of appearance (Ra) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 11. Glycerol rate of appearance (Ra) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 12. Glycerol rate of appearance (Ra) per unit of body mass, expressed as a percentage decrease from 0 min, in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 13. Area under the curve (AUC) for three times the glycerol rate of appearance (Ra) in young and older participants. Values are means ± SE.

Figure 14. Glycerol rate of disappearance (Rd) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 15. Glycerol rate of disappearance (Rd) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 16. Glycerol rate of disappearance (Rd) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 17. Glycerol rate of disappearance (Rd) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 18. Glycerol metabolic clearance rate (MCR) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 19. Glycerol metabolic clearance rate (MCR) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 20. Glycerol metabolic clearance rate (MCR) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 21. Glycerol metabolic clearance rate (MCR) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 22. Glycerol metabolic clearance rate (MCR) per unit of body mass, expressed as a percentage increase from 0 min, in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 23. Relationship between blood lactate concentrations and glycerol rate of appearance (Ra) per unit of body mass in young and older participants.

Figure 24. Relationship between blood lactate concentrations and glycerol concentrations in young and older participants.

Figure 25. Relationship between blood lactate concentrations and plasma free fatty acid (FFA) concentrations in young and older participants.

Figure 26. Relationship between plasma free fatty acid (FFA) and plasma glycerol concentrations in young and older participants.

Figure 27. Relationship between plasma free fatty acid (FFA) and plasma triglyceride (TG) concentrations in young and older participants.

Figure 28. Relationship between glycerol rate of appearance (Ra) and plasma glycerol concentrations in young and older participants.

Figure 29. Relationship between glycerol rate of appearance (Ra) and plasma free fatty acid (FFA) concentrations in young and older participants.

Figure 30. Relationship between glycerol metabolic clearance rate (MCR) and plasma glycerol concentrations in young and older participants.

Figure 31. Relationship between glycerol metabolic clearance rate (MCR) and plasma free fatty acid (FFA) concentrations in young and older participants.

Figure 32. Relationship between glycerol metabolic clearance rate (MCR) and plasma triglyceride (TG) concentrations in young and older participants.

Figure 33. Relationship between maximal rate of oxygen consumption ($\dot{V}O_2$peak, $mL \cdot kg^{-1} \cdot min^{-1}$) and the minimal glycerol rate of appearance (Ra) per unit of body mass attained in young and older participants.

Figure 34. Relationship between ventilatory threshold (VT, % $\dot{V}O_2$peak, $mL \cdot kg^{-1} \cdot min^{-1}$) and the minimal glycerol rate of appearance (Ra) per unit of body mass attained in young and older participants.

Figure 35. Relationship between maximal rate of oxygen consumption ($\dot{V}O_2$peak, $L \cdot min^{-1}$) and the lowest glycerol rate of appearance (Ra) attained in young and older participants.

Figure 36. Relationship between ventilatory threshold (VT, % $\dot{V}O_2$peak, $L \cdot min^{-1}$) and the lowest glycerol rate of appearance (Ra) attained in young and older participants.

Table 1. Characteristics of young and older participants.

Variable	Young	Older
Age, yr	26.40 ± 1.08	71.14 ± 1.48 *
Body mass, kg	66.63 ± 2.70	73.21 ± 4.18
Body mass index, kg/m^2	23.47 ± 0.77	26.32 ± 0.92 *
Body fat, %	17.27 ± 1.83	23.13 ± 1.29 *
Fat mass, kg	11.24 ± 1.16	16.83 ± 1.27 *
Fat free mass, kg	55.39 ± 2.93	56.39 ± 3.48
Relative glucose load,		
$g \cdot kg^{-1}$ body mass	1.15 ± 0.04	1.07 ± 0.07
$g \cdot kg^{-1}$ fat mass	7.67 ± 0.83	4.81 ± 0.35 *
$g \cdot kg^{-1}$ fat free mass	1.41 ± 0.07	1.41 ± 0.09
FEV_1/FVC, %	84.52 ± 1.98	79.84 ± 1.64
$\dot{V}O_2peak$,		
$L \cdot min^{-1}$	2.84 ± 0.25	1.91 ± 0.17 *
$mL \cdot kg^{-1} \cdot min^{-1}$	41.80 ± 2.93	26.18 ± 1.59 *
Peak Power Output, W	267.00 ± 21.06	170.42 ± 17.45 *
VT, % $\dot{V}O_2peak$	73.35 ± 0.64	60.99 ± 0.94 *
3-day Diet Records		
Energy, $kcal \cdot day^{-1}$	2573.32 ± 105.74	2305.52 ± 105.27
Carbohydrate, %	61.25 ± 0.34	60.08 ± 0.61
Fat, %	29.69 ± 0.52	29.99 ± 0.44
Protein, %	9.06 ± 0.26	9.93 ± 0.34

Values are means ± SE. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $\dot{V}O_2peak$, peak O_2 consumption; VT, ventilatory threshold. *Significantly different between groups, $p < 0.05$.

Table 2. Summary of correlations of blood lactate concentrations with markers of lipolysis.

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Ra, μmol·kg^{-1}·min^{-1}				
Overall	-0.71	0.07	-0.93	0.003
Enteric	-0.50	0.50	-0.71	0.29
Systemic	0.99	0.07	-0.93	0.24
Ra, μmol·kg FFM^{-1}·min^{-1}				
Overall	-0.71	0.07	-0.92	0.003
Enteric	-0.49	0.51	-0.69	0.30
Systemic	0.99	0.09	-0.94	0.23
Ra, μmol·kg FM^{-1}·min^{-1}				
Overall	-0.72	0.07	-0.92	0.003
Enteric	-0.52	0.48	-0.72	0.28
Systemic	0.99	0.009	-0.93	0.24
Ra, μmol·min^{-1}				
Overall	-0.73	0.07	-0.93	0.003
Enteric	-0.57	0.43	-0.72	0.28
Systemic	0.99	0.03	-0.95	0.20
[Glycerol]				
Overall	-0.54	0.21	-0.78	0.04
Enteric	0.12	0.88	-0.13	0.87
Systemic	0.99	0.09	-0.95	0.21
[FFA]				
Overall	-0.65	0.12	-0.90	0.005
Enteric	-0.15	0.85	-0.44	0.56
Systemic	0.99	0.05	-0.97	0.16

Overall, 0-120 min; Enteric, 0-30 min; Systemic, 60-120 min; Ra, glycerol rate of appearance; FFM, fat free mass; FM, fat mass; [Glycerol], plasma glycerol concentrations; [FFA], plasma free fatty acid concentrations.

Table 3. Summary of correlations of variables related to substrate availability and utilization.

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
[FFA] and [Glycerol]	0.99	<0.001	0.95	0.001
[FFA] and [TG]	0.52	0.24	0.34	0.43
Glycerol Ra and [Glycerol]	0.96	0.001	0.94	0.002
Glycerol Ra and [FFA]	0.97	<0.001	0.95	0.001
Glycerol MCR and [Glycerol]	-0.87	0.01	-0.83	0.02
Glycerol MCR and [FFA]	-0.85	0.02	-0.91	0.004
Glycerol MCR and [TG]	-0.83	0.02	-0.63	0.13

[FFA], plasma free fatty acid concentrations; [Glycerol], plasma glycerol concentrations; [TG], plasma triglyceride concentrations; Ra, rate of appearance per unit of body mass; MCR, metabolic clearance rate per unit of body mass.

Table 4. Summary of correlations of glycerol rate of appearance to relative peak oxygen consumption rates ($\dot{V}O_2$peak, mL $O_2 \cdot kg^{-1} \cdot min^{-1}$).

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Ra at 0 min	-0.22	0.44	-0.27	0.37
Minimal Ra	-0.52	0.04	-0.44	0.13
Δ Ra (0 min – Minimal Ra)	0.17	0.55	0.03	0.91
Δ Ra as % Decrease	0.37	0.17	0.19	0.54
Slope of Δ Ra	-0.07	0.81	0.13	0.68

Ra, glycerol rate of appearance per unit of body mass.

Table 5. Summary of correlations of glycerol rate of appearance to ventilatory threshold (VT, % $\dot{V}O_2$peak, mL $O_2 \cdot kg^{-1} \cdot min^{-1}$).

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Ra at 0 min	0.12	0.67	-0.22	0.46
Minimal Ra	-0.12	0.68	-0.48	0.09
Δ Ra (0 min – Minimal Ra)	0.27	0.33	0.14	0.66
Δ Ra as % Decrease	0.35	0.20	0.36	0.23
Slope of Δ Ra	-0.25	0.38	-0.33	0.27

Ra, glycerol rate of appearance per unit of body mass.

Table 6. Summary of correlations of glycerol rate of appearance to maximal oxygen consumption ($\dot{V}O_2$peak, L $O_2 \cdot min^{-1}$).

	Young		Older	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Ra at 0 min	0.19	0.49	0.07	0.83
Minimal Ra	-0.50	0.06	-0.36	0.22
Δ Ra (0 min – Minimal Ra)	0.42	0.12	0.26	0.39
Δ Ra as % Decrease	0.44	0.10	0.34	0.26
Slope of Δ Ra	-0.32	0.24	0.12	0.69

Ra, glycerol rate of appearance per unit of body mass.

Table 7. Summary of correlations of glycerol rate of appearance to ventilatory threshold (VT, % $\dot{V}O_2$peak, L $O_2 \cdot min^{-1}$).

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Ra at 0 min	0.12	0.66	-0.14	0.65
Minimal Ra	-0.29	0.29	-0.65	0.02
Δ Ra (0 min – Minimal Ra)	0.26	0.35	0.18	0.56
Δ Ra as % Decrease	0.34	0.21	0.36	0.23
Slope of Δ Ra	-0.24	0.39	-0.35	0.24

Ra, glycerol rate of appearance per unit of body mass.

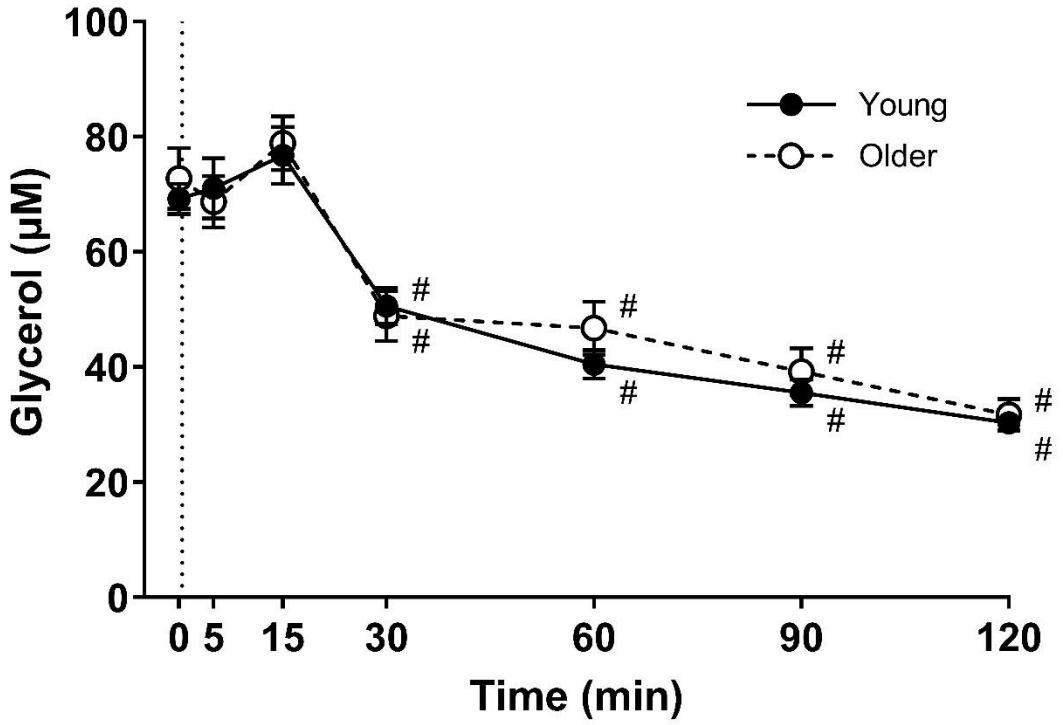

Figure 1. Plasma glycerol concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

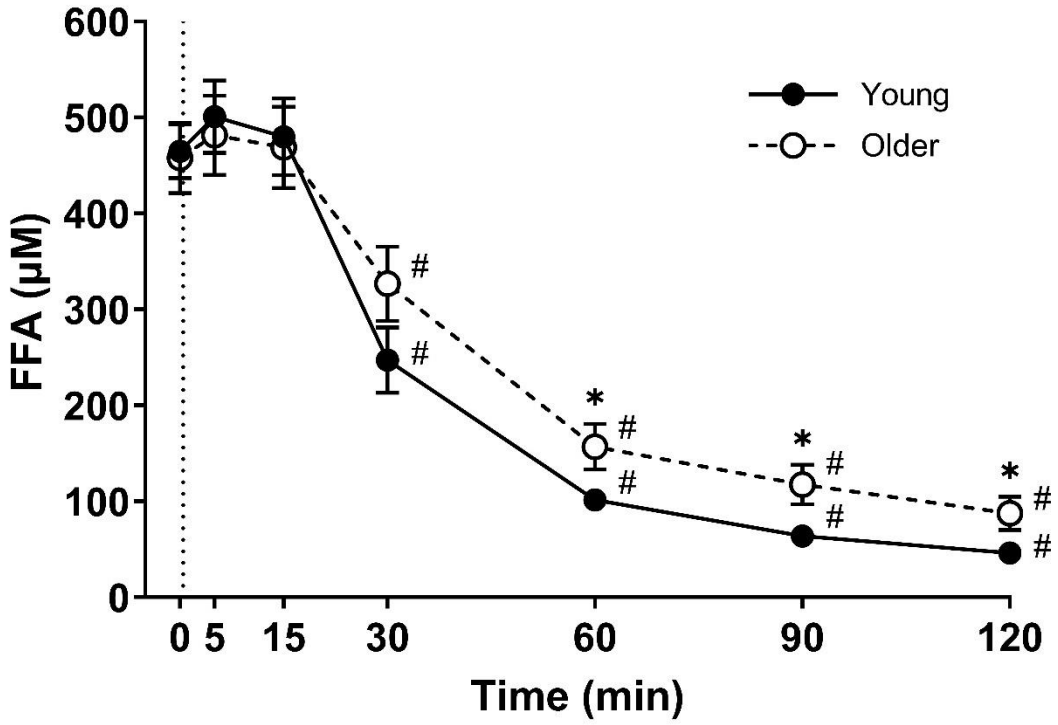

Figure 2. Plasma free fatty acid (FFA) concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

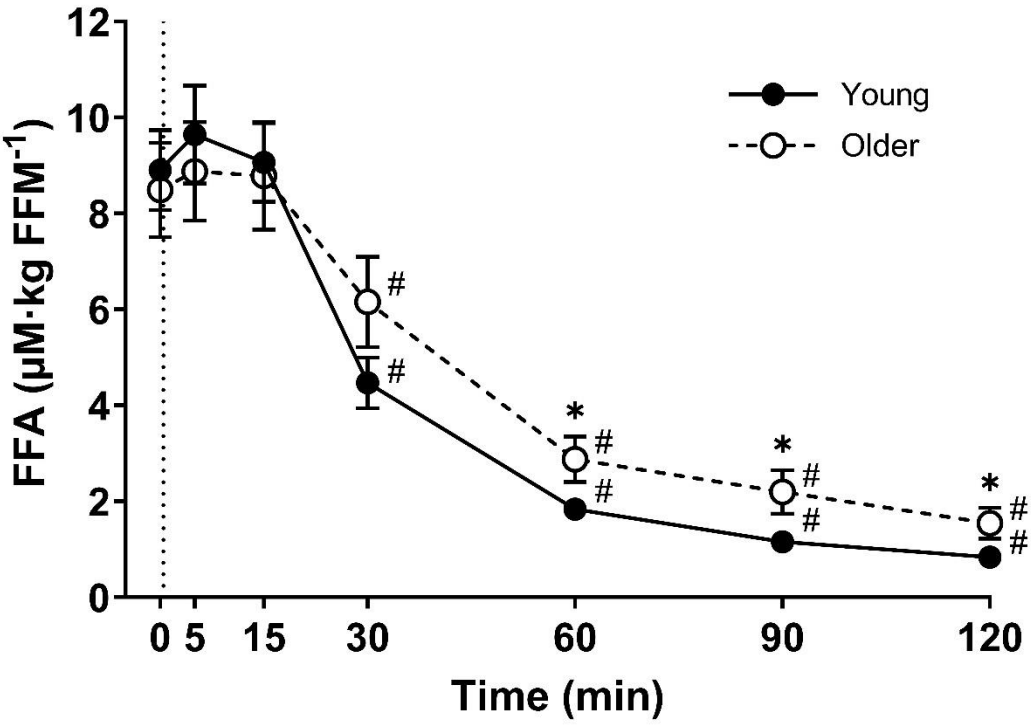

Figure 3A. Plasma free fatty acid (FFA) concentrations per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

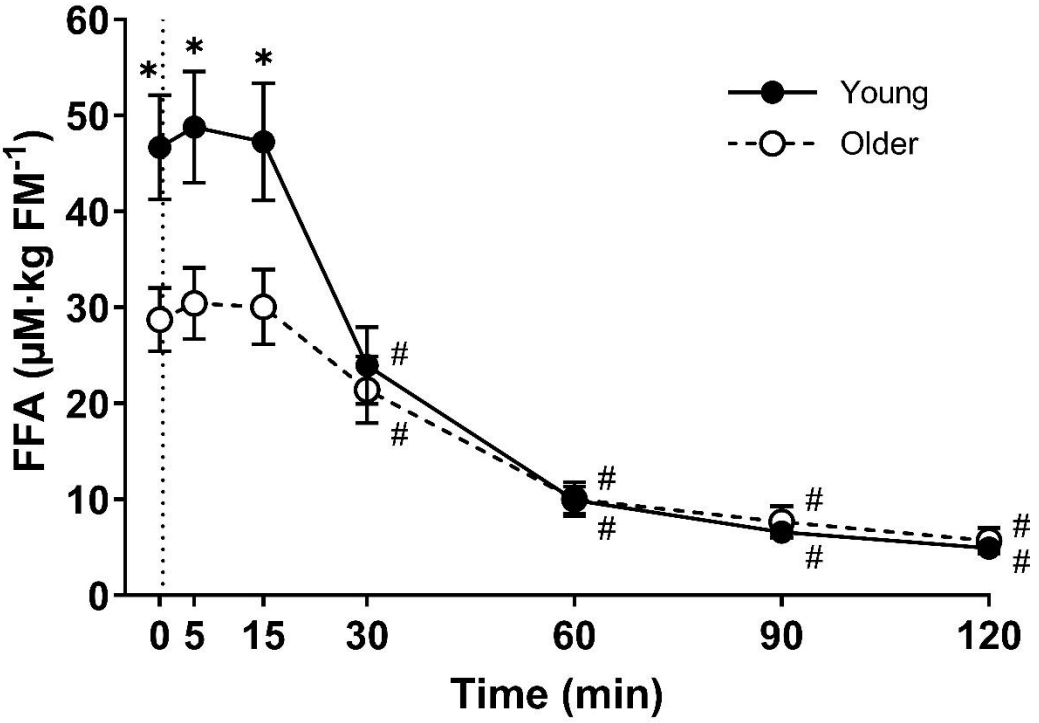

Figure 3B. Plasma free fatty acid (FFA) concentrations per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

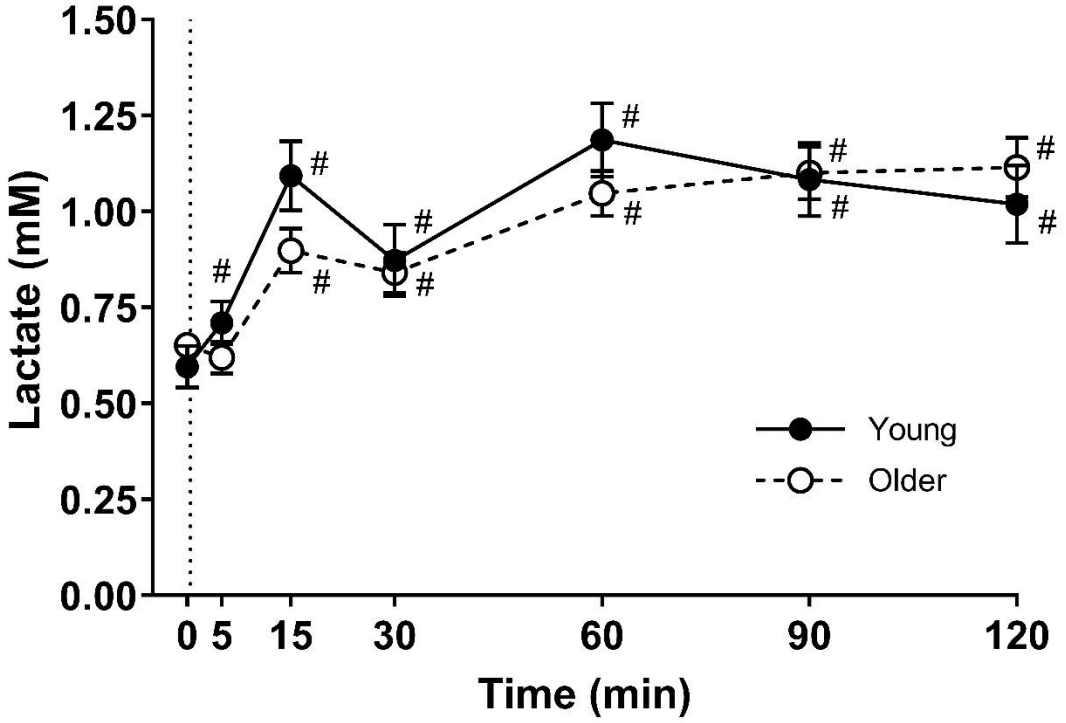

Figure 4. Blood lactate concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

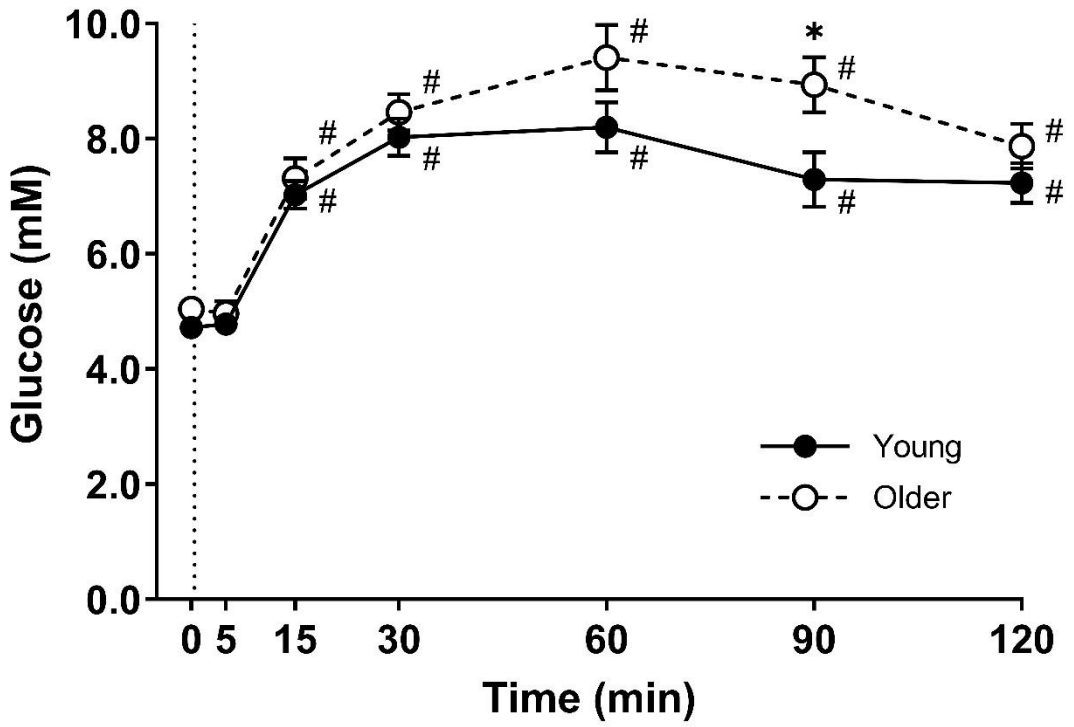

Figure 5. Blood glucose concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

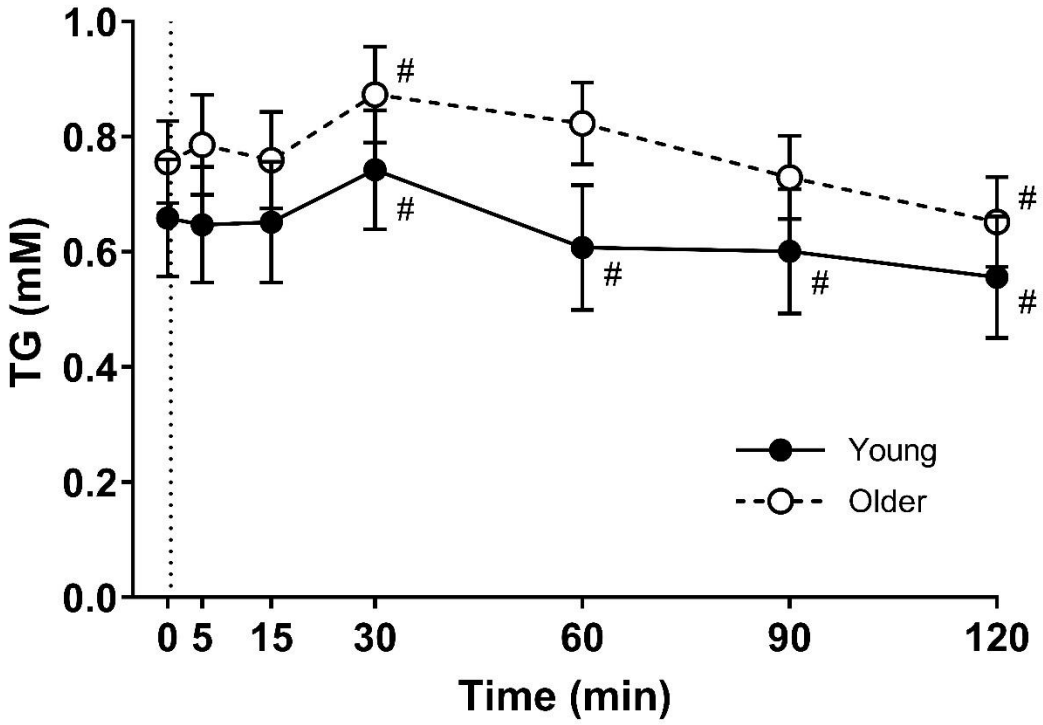

Figure 6. Plasma triglyceride (TG) concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

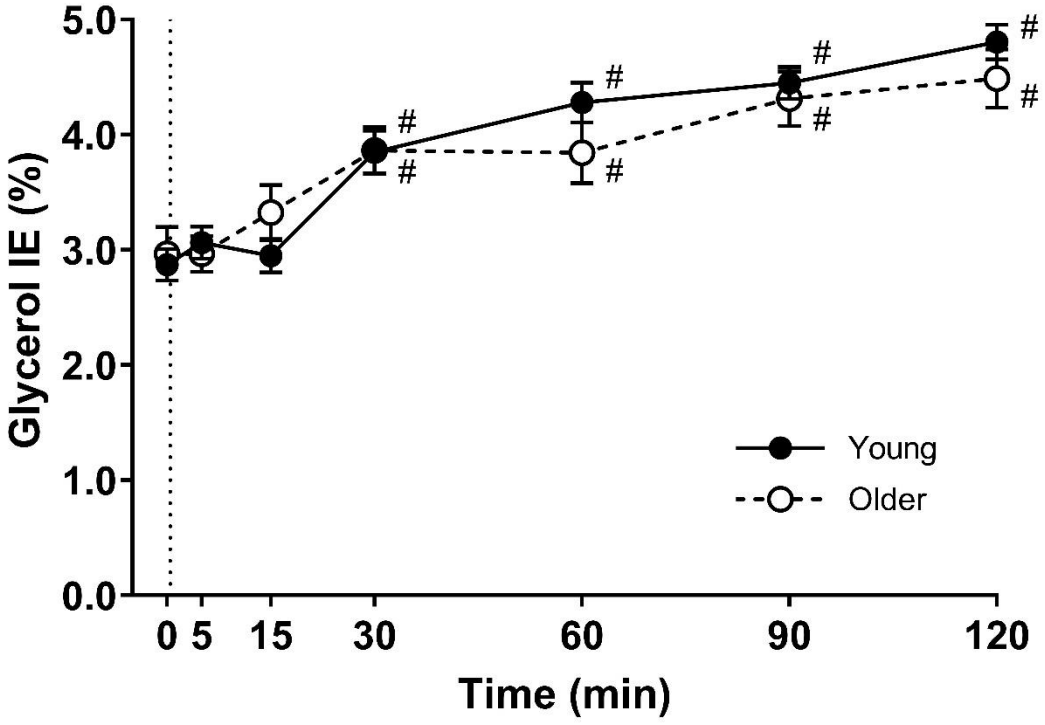

Figure 7. Isotopic enrichment (IE) of [1,1,2,3,3-^{2}H]glycerol, expressed as mole percent excess (MPE), in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

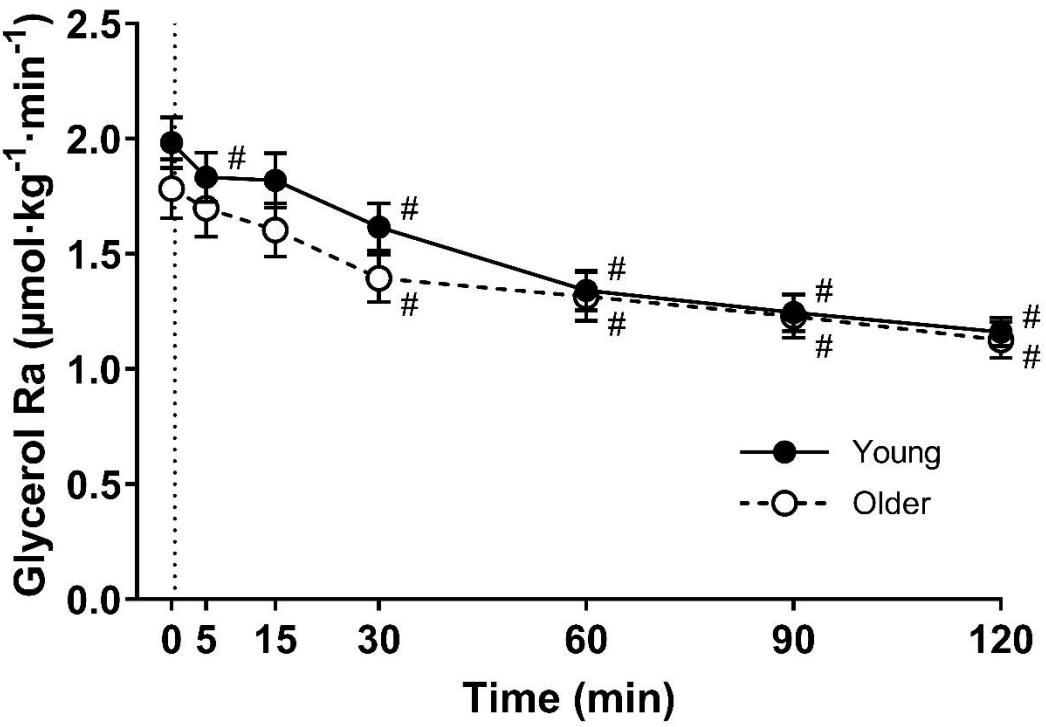

Figure 8. Glycerol rate of appearance (Ra) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

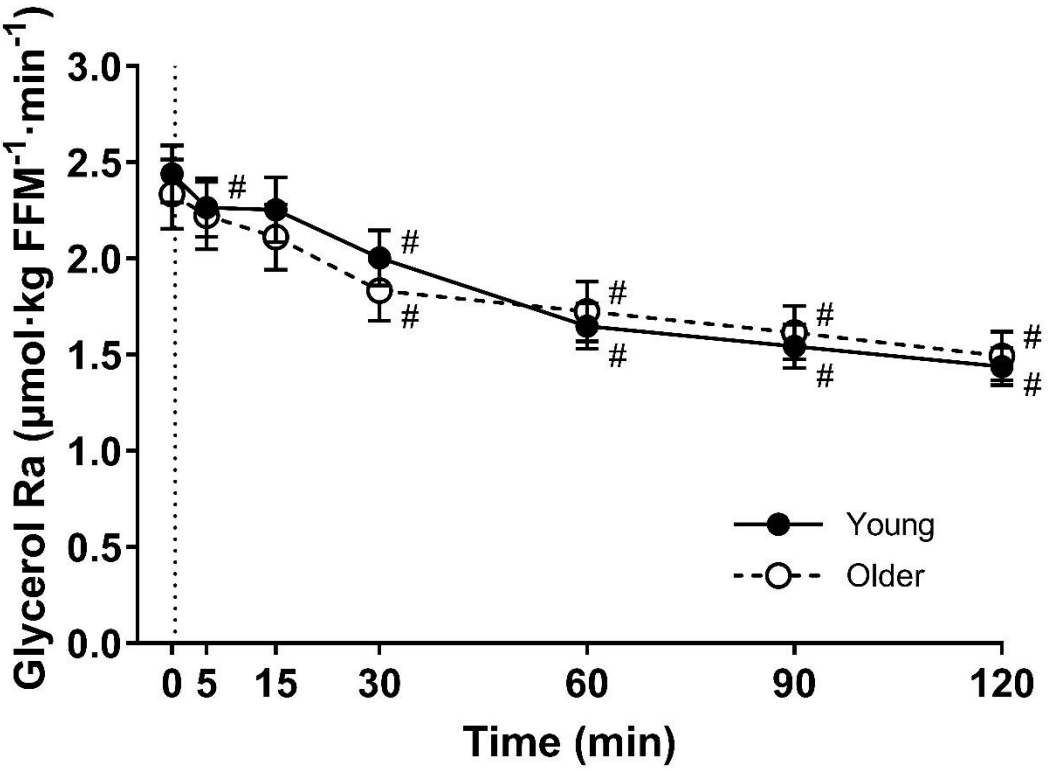

Figure 9. Glycerol rate of appearance (Ra) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

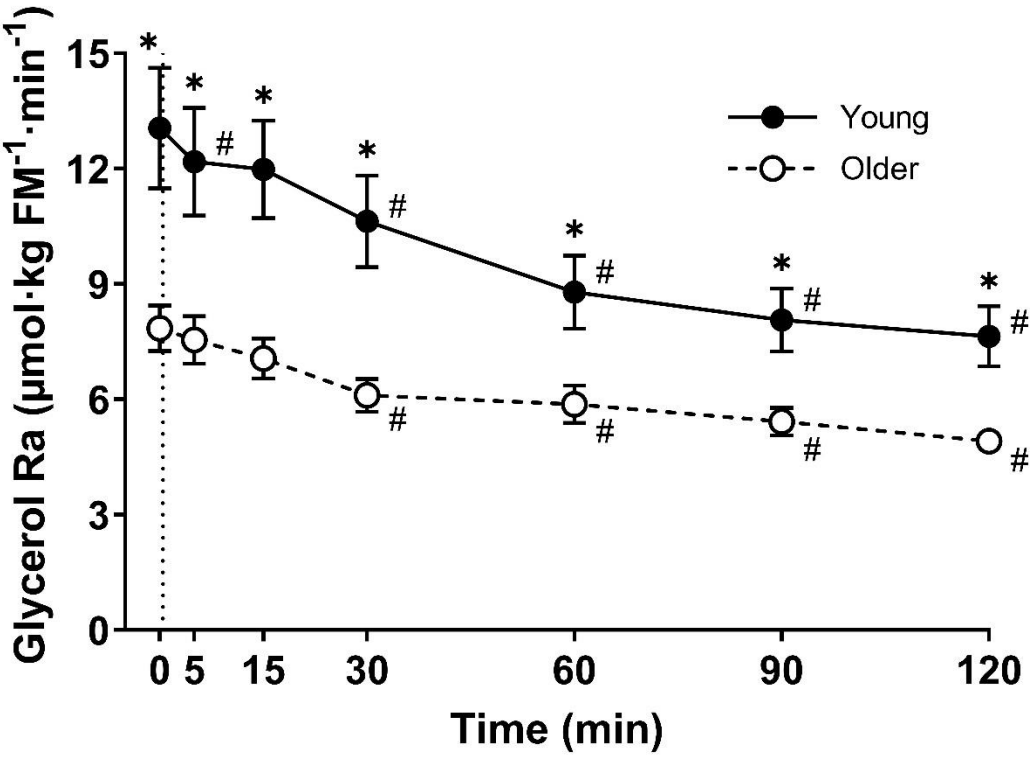

Figure 10. Glycerol rate of appearance (Ra) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

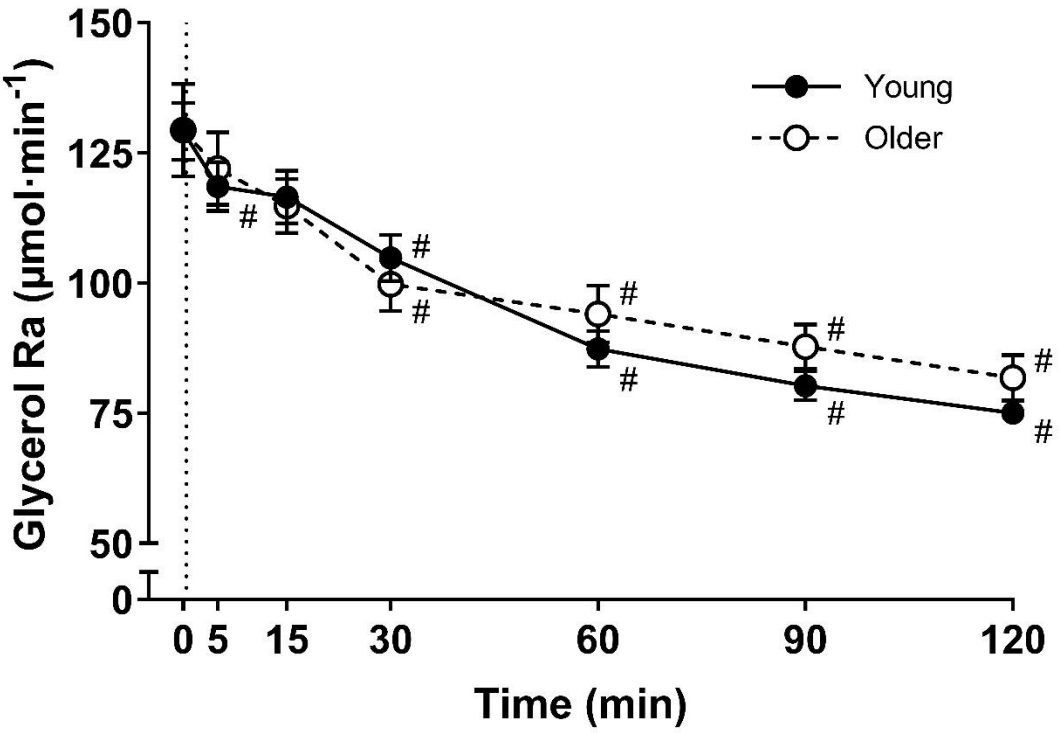

Figure 11. Glycerol rate of appearance (Ra) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

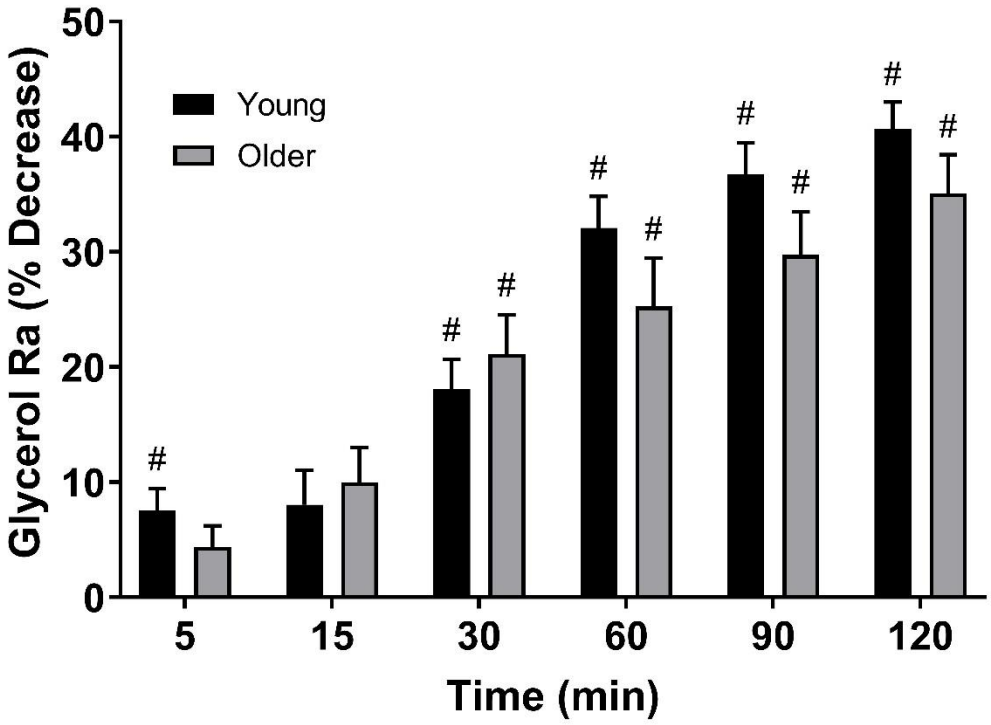

Figure 12. Glycerol rate of appearance (Ra) per unit of body mass, expressed as a percentage decrease from 0 min, in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

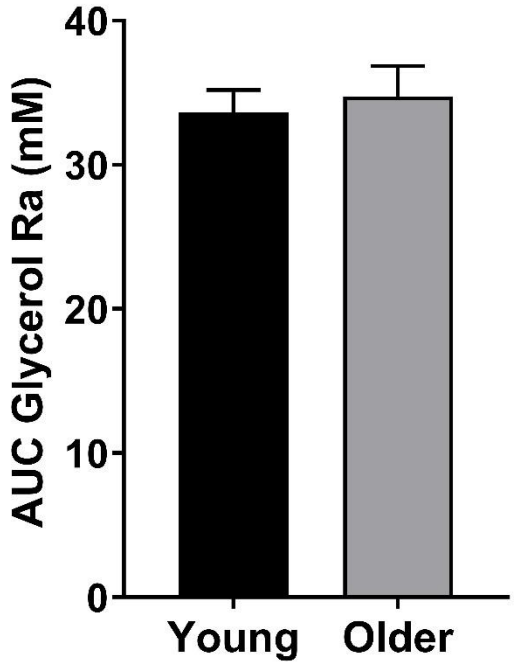

Figure 13. Area under the curve (AUC) for three times the glycerol rate of appearance (Ra) in young and older participants. Values are means ± SE.

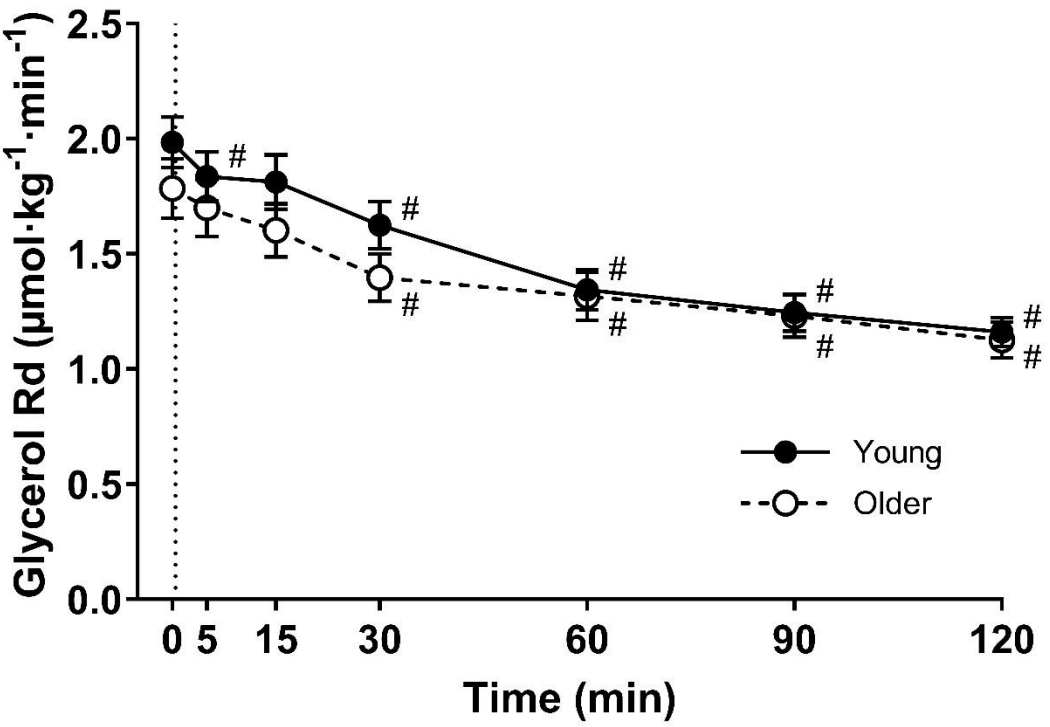

Figure 14. Glycerol rate of disappearance (Rd) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

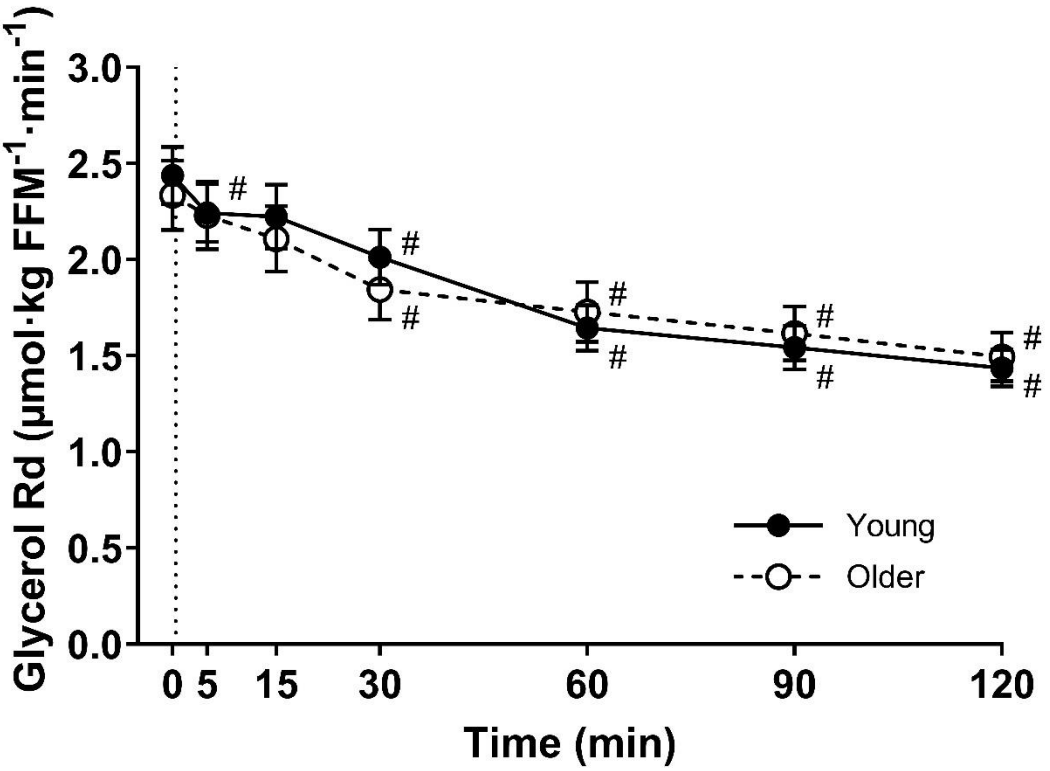

Figure 15. Glycerol rate of disappearance (Rd) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

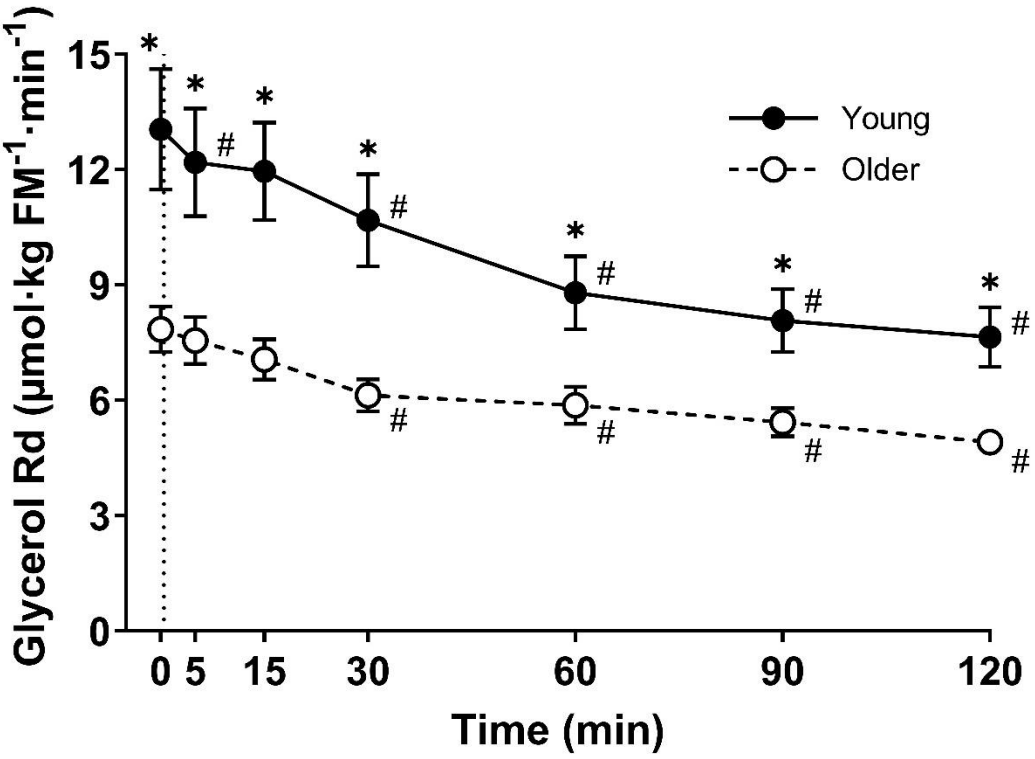

Figure 16. Glycerol rate of disappearance (Rd) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

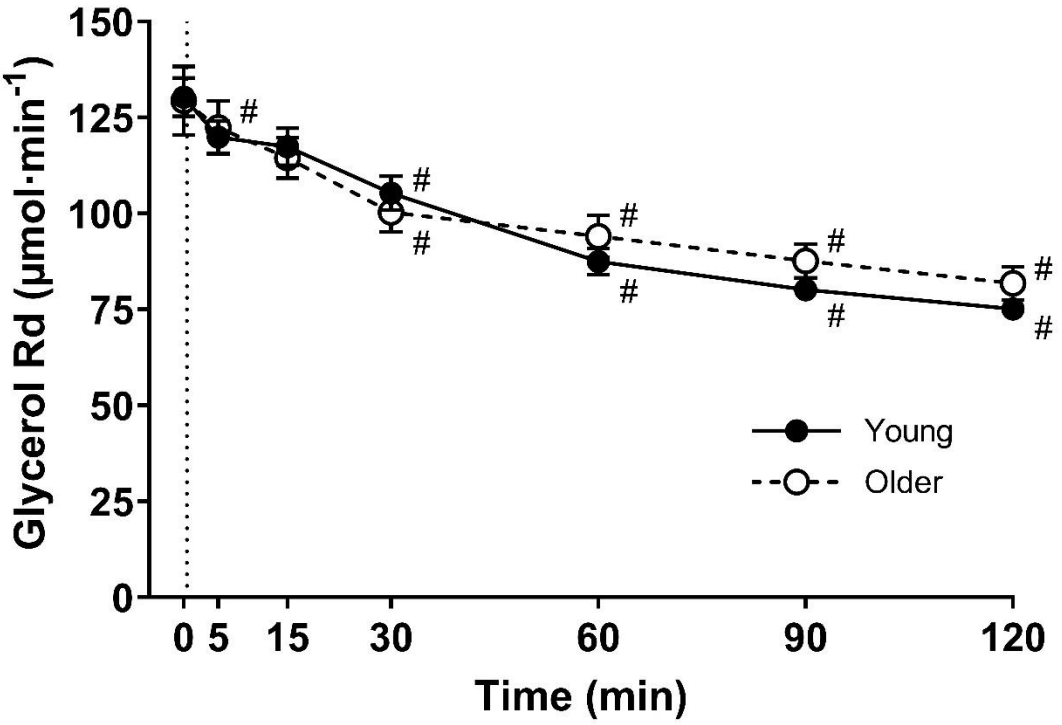

Figure 17. Glycerol rate of disappearance (Rd) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

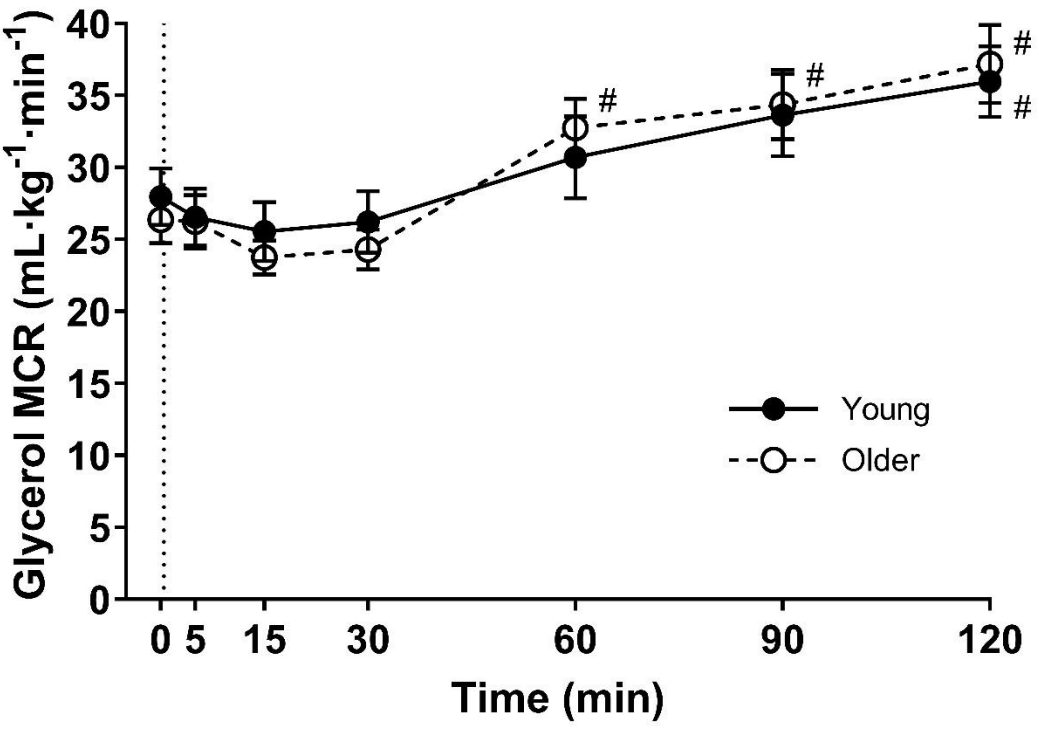

Figure 18. Glycerol metabolic clearance rate (MCR) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

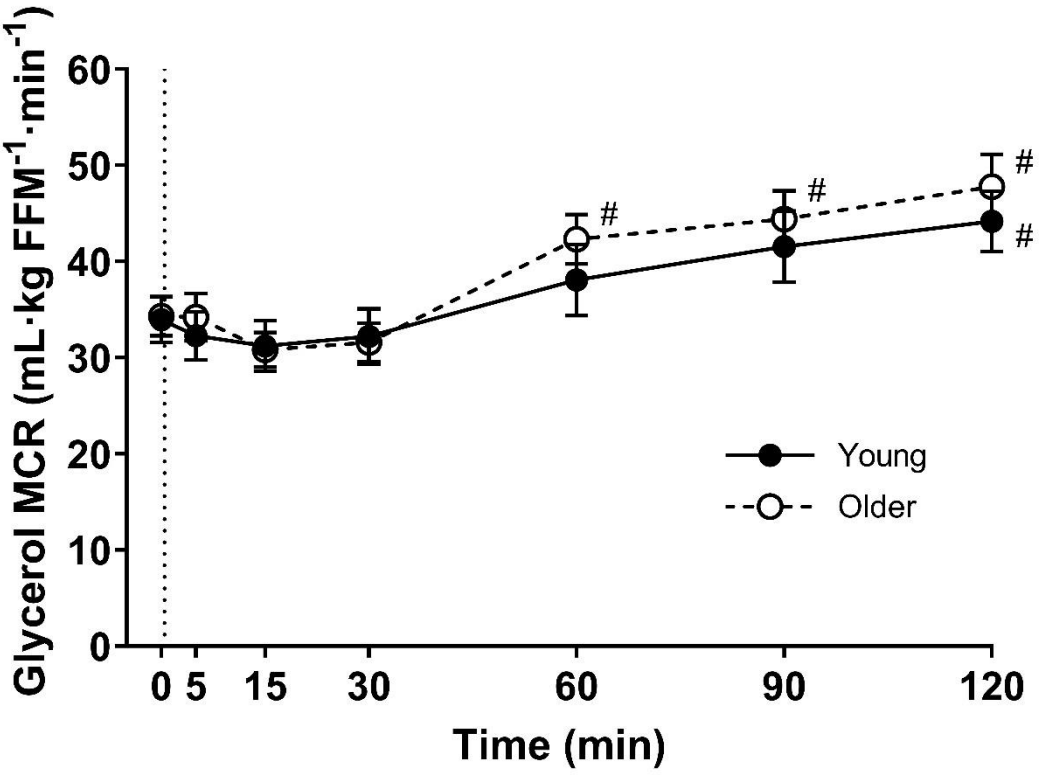

Figure 19. Glycerol metabolic clearance rate (MCR) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

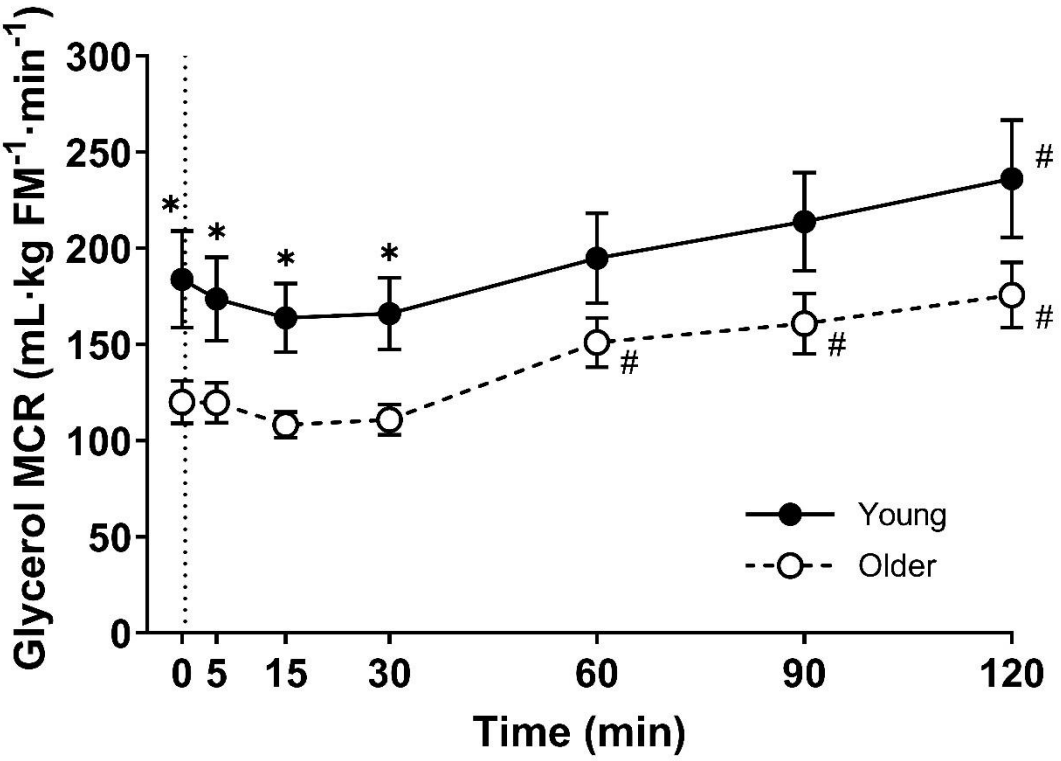

Figure 20. Glycerol metabolic clearance rate (MCR) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

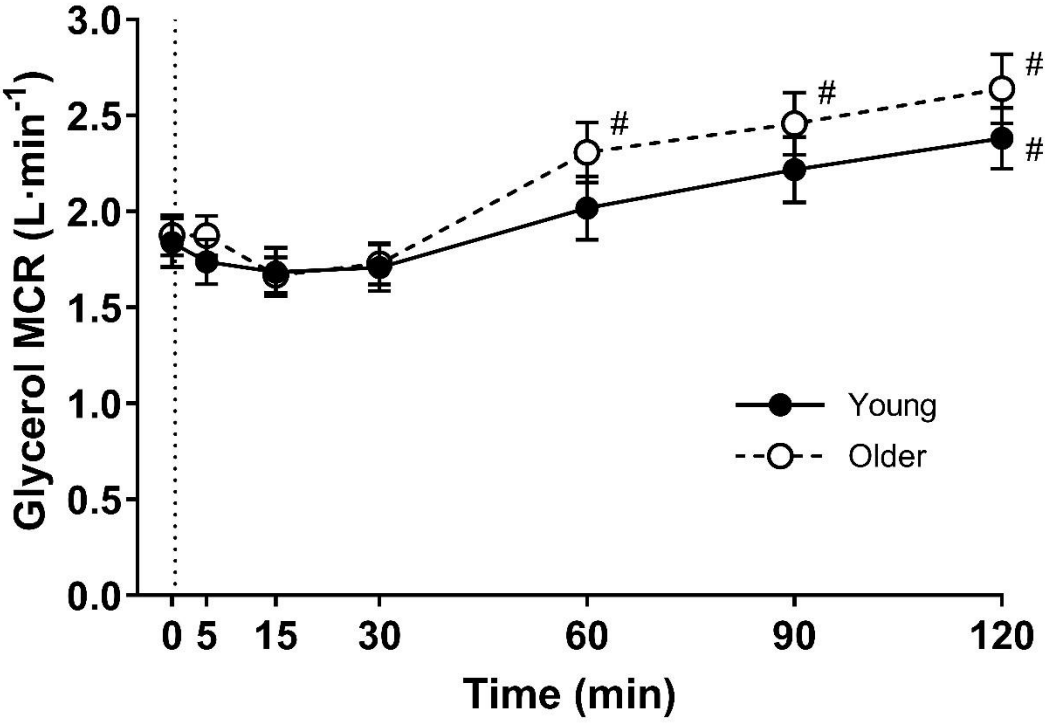

Figure 21. Glycerol metabolic clearance rate (MCR) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

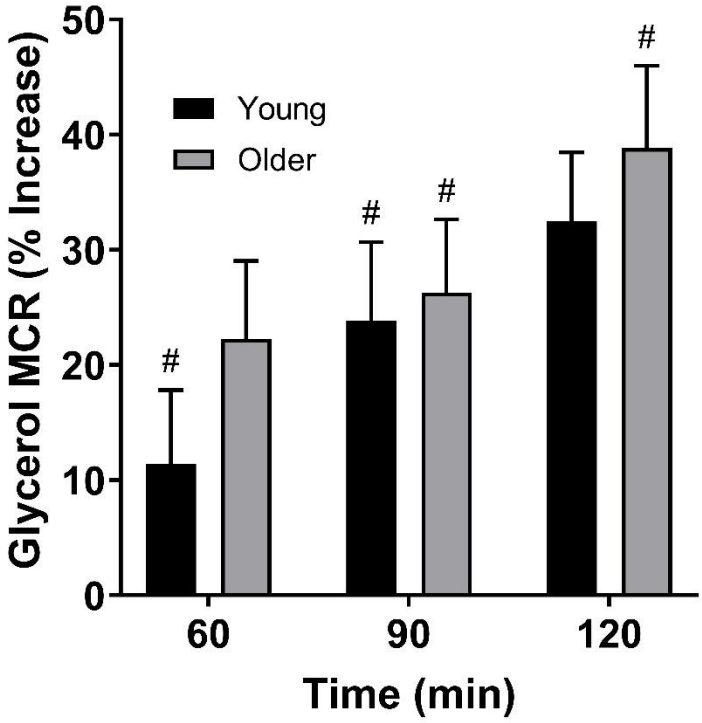

Figure 22. Glycerol metabolic clearance rate (MCR) per unit of body mass, expressed as a percentage increase from 0 min, in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

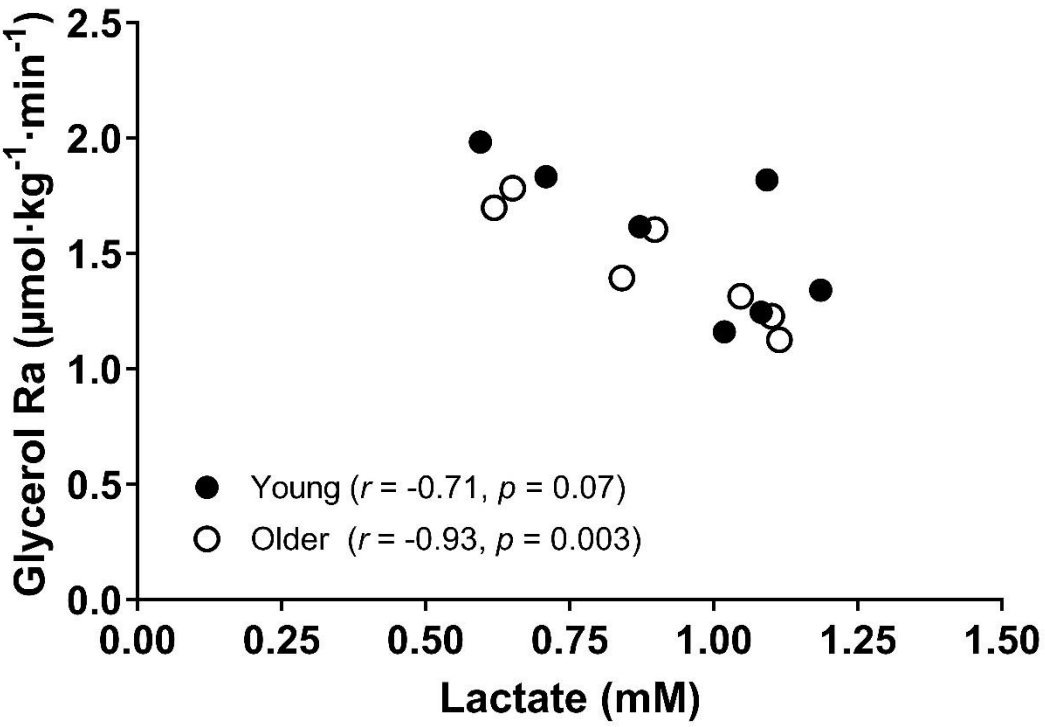

Figure 23. Relationship between blood lactate concentrations and glycerol rate of appearance (Ra) per unit of body mass in young and older participants.

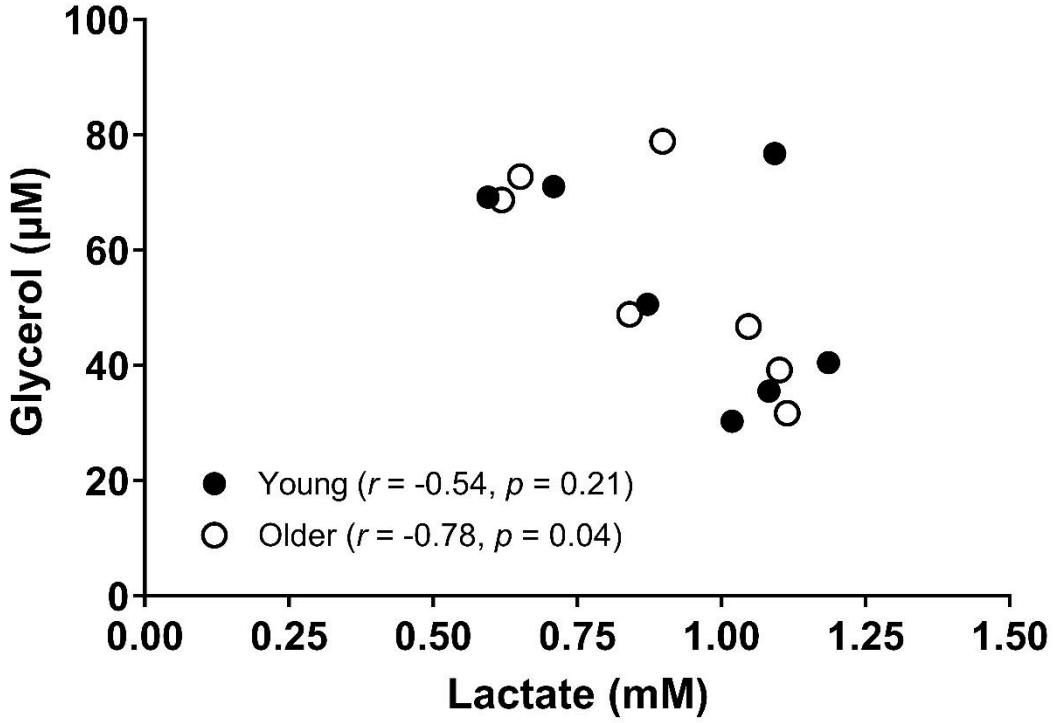

Figure 24. Relationship between blood lactate concentrations and glycerol concentrations in young and older participants.

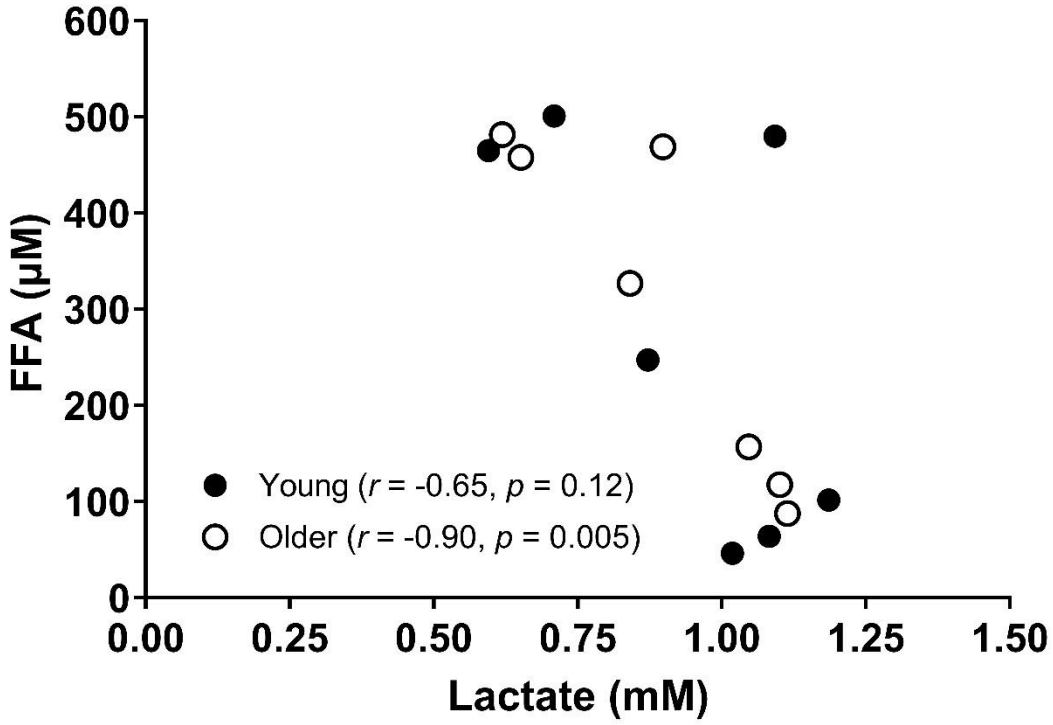

Figure 25. Relationship between blood lactate concentrations and plasma free fatty acid (FFA) concentrations in young and older participants.

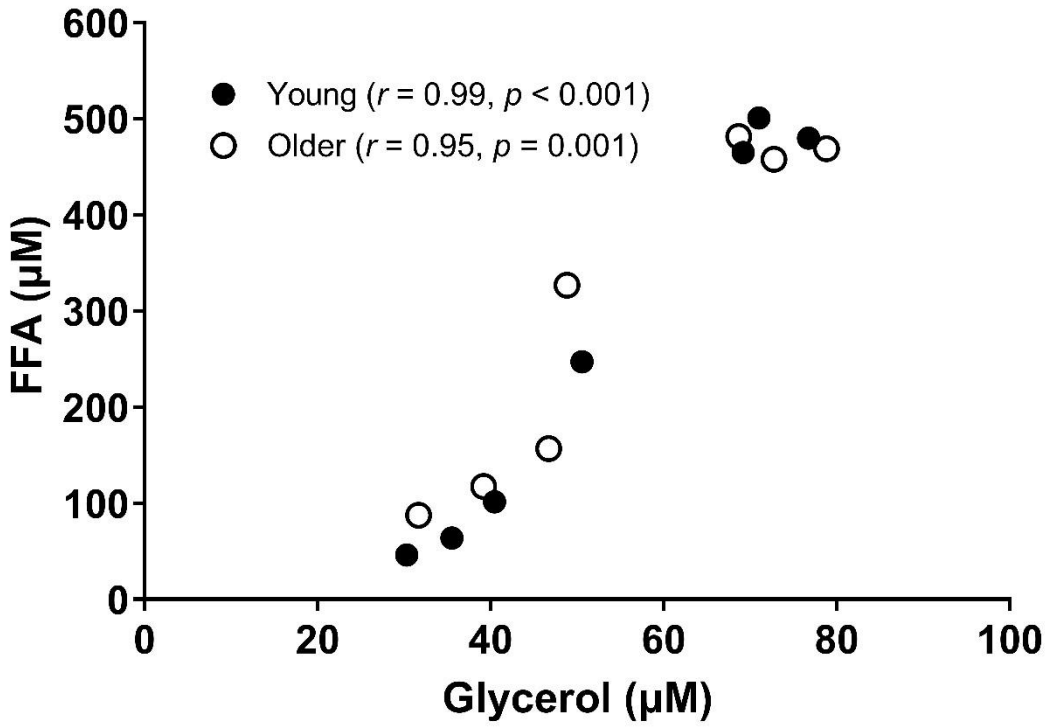

Figure 26. Relationship between plasma free fatty acid (FFA) and plasma glycerol concentrations in young and older participants.

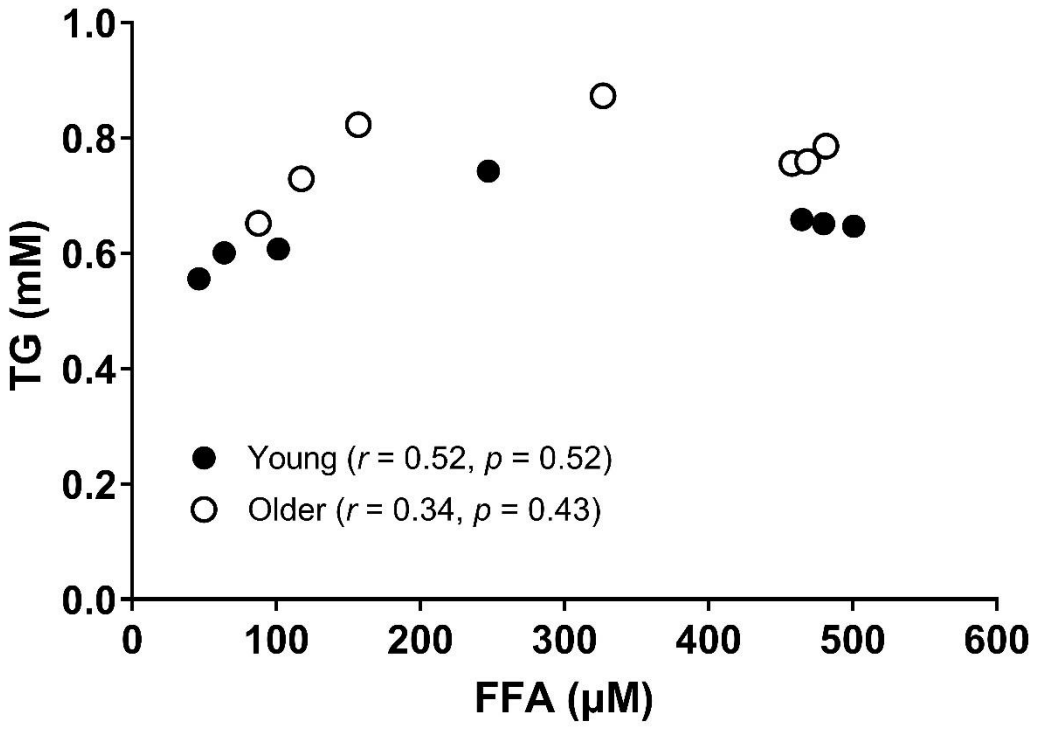

Figure 27. Relationship between plasma free fatty acid (FFA) and plasma triglyceride (TG) concentrations in young and older participants.

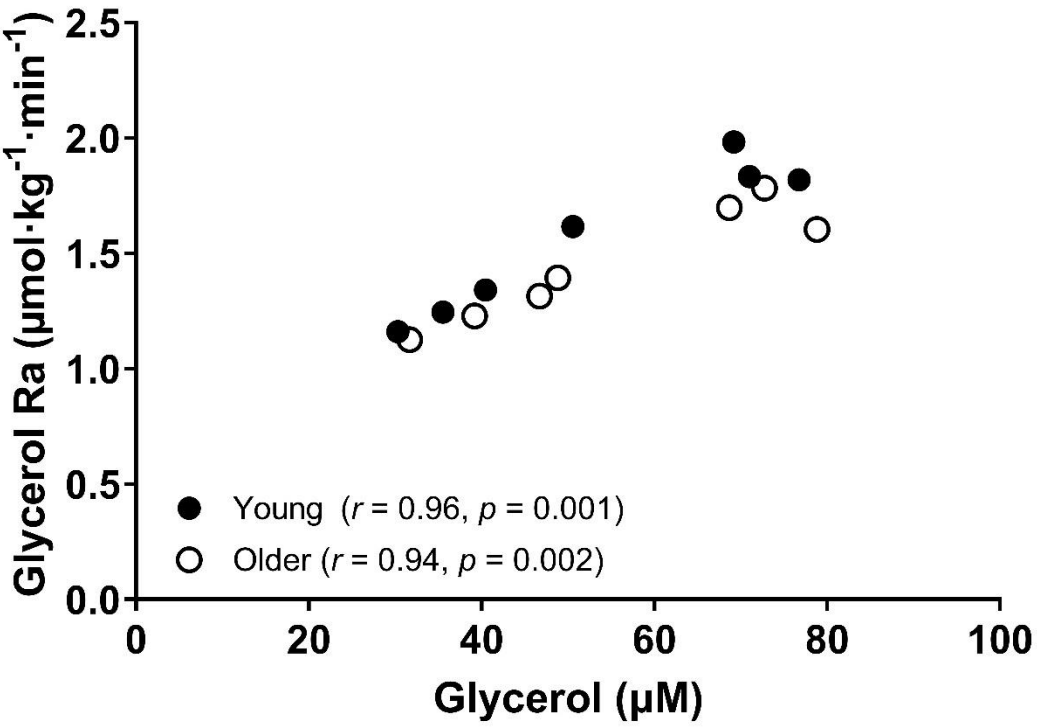

Figure 28. Relationship between glycerol rate of appearance (Ra) and plasma glycerol concentrations in young and older participants.

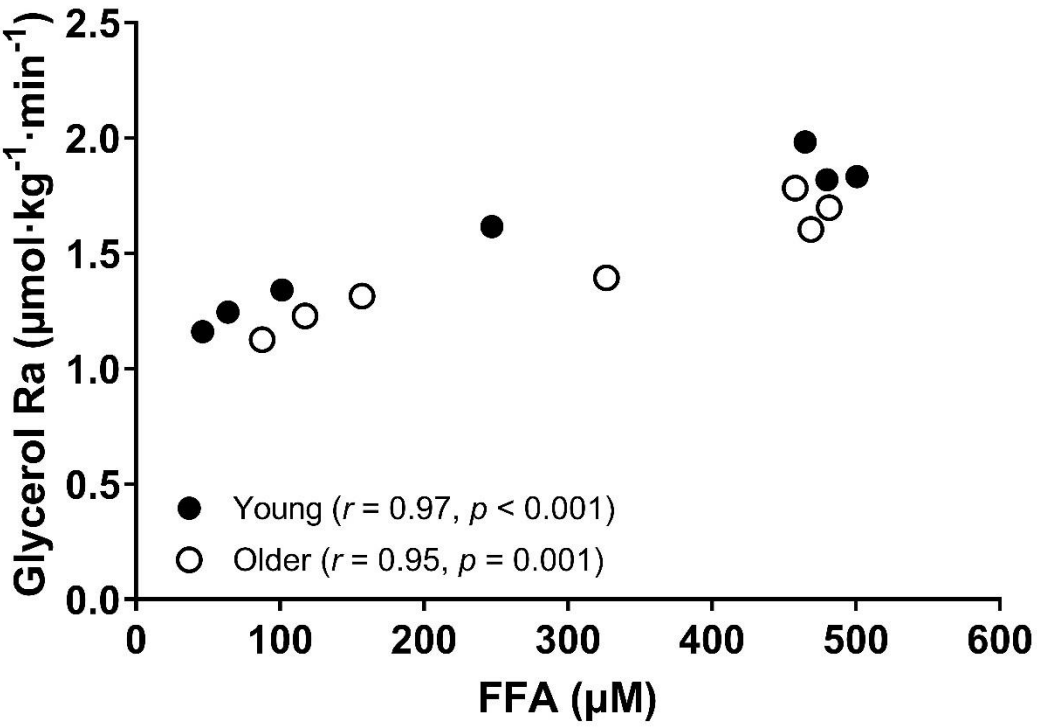

Figure 29. Relationship between glycerol rate of appearance (Ra) and plasma free fatty acid (FFA) concentrations in young and older participants.

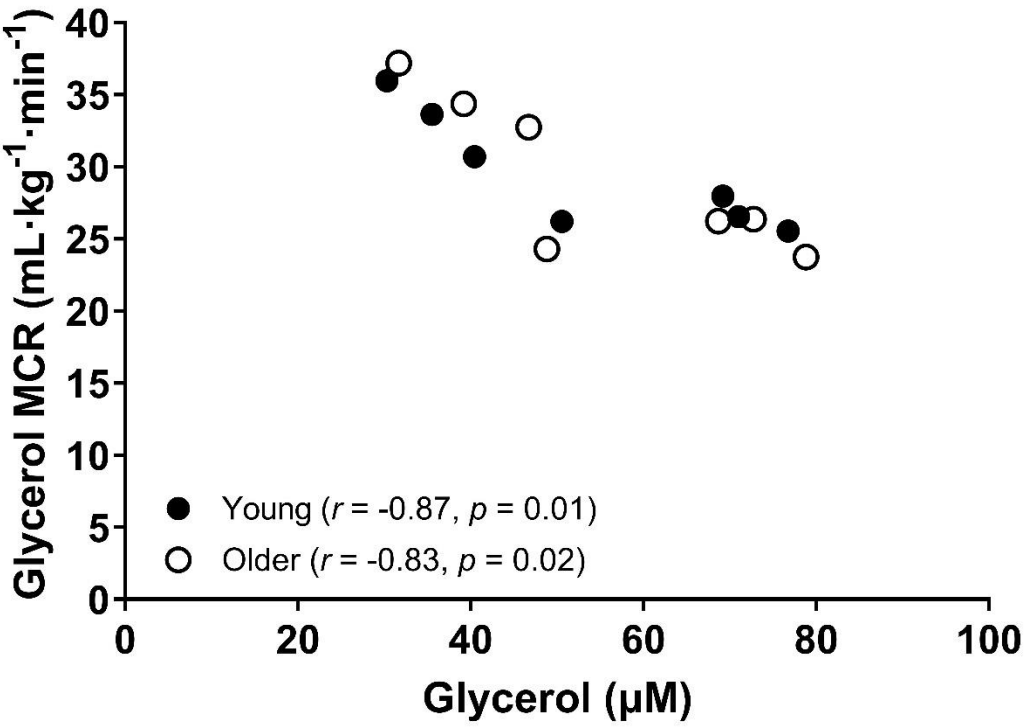

Figure 30. Relationship between glycerol metabolic clearance rate (MCR) and plasma glycerol concentrations in young and older participants.

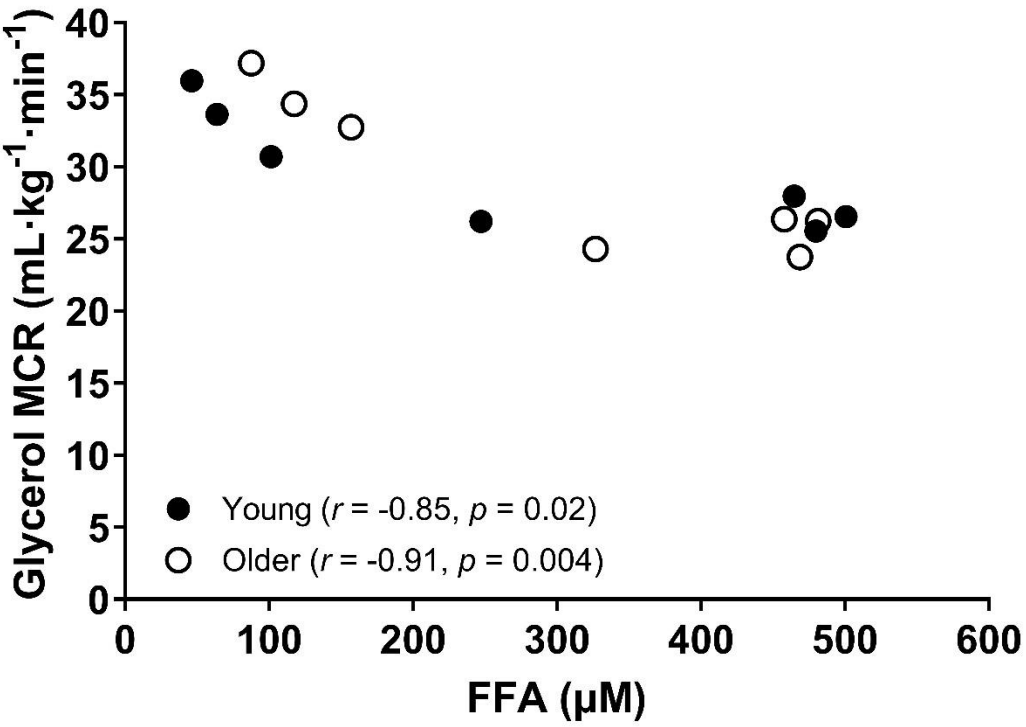

Figure 31. Relationship between glycerol metabolic clearance rate (MCR) and plasma free fatty acid (FFA) concentrations in young and older participants.

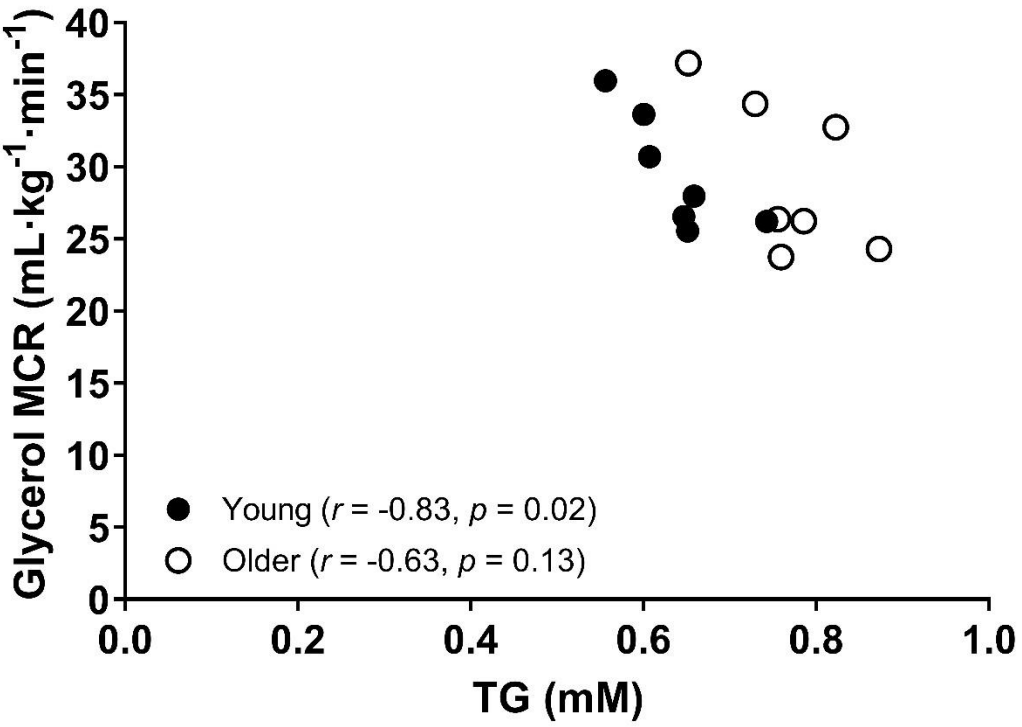

Figure 32. Relationship between glycerol metabolic clearance rate (MCR) and plasma triglyceride (TG) concentrations in young and older participants.

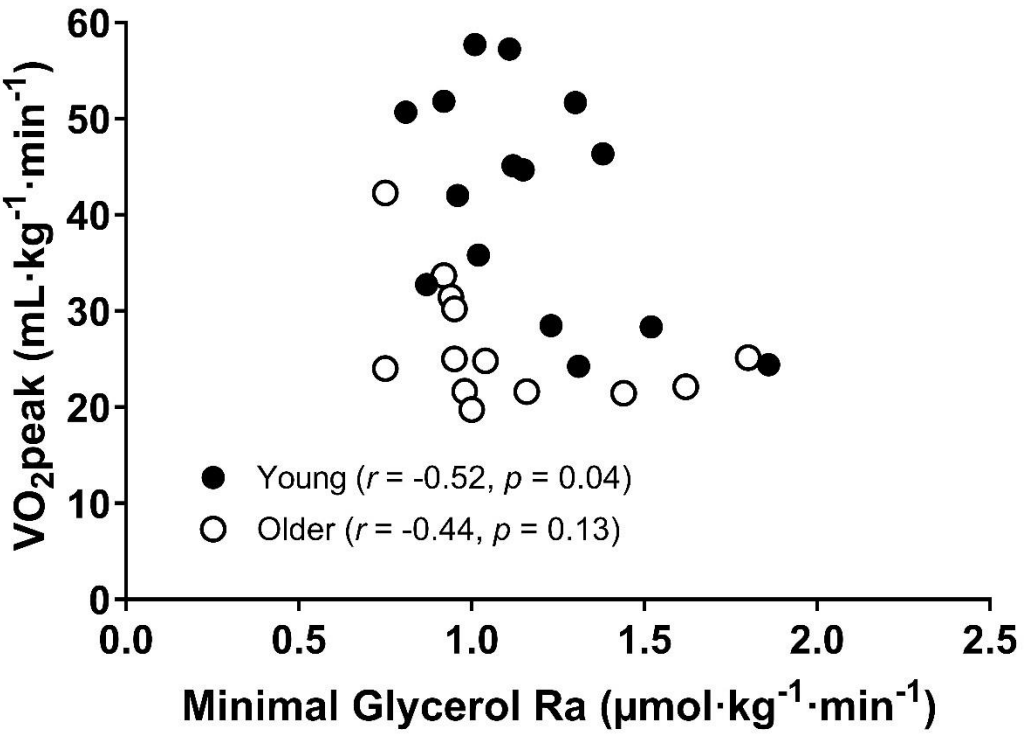

Figure 33. Relationship between maximal rate of oxygen consumption ($\dot{V}O_2$peak, $mL \cdot kg^{-1} \cdot min^{-1}$) and the minimal glycerol rate of appearance (Ra) per unit of body mass attained in young and older participants.

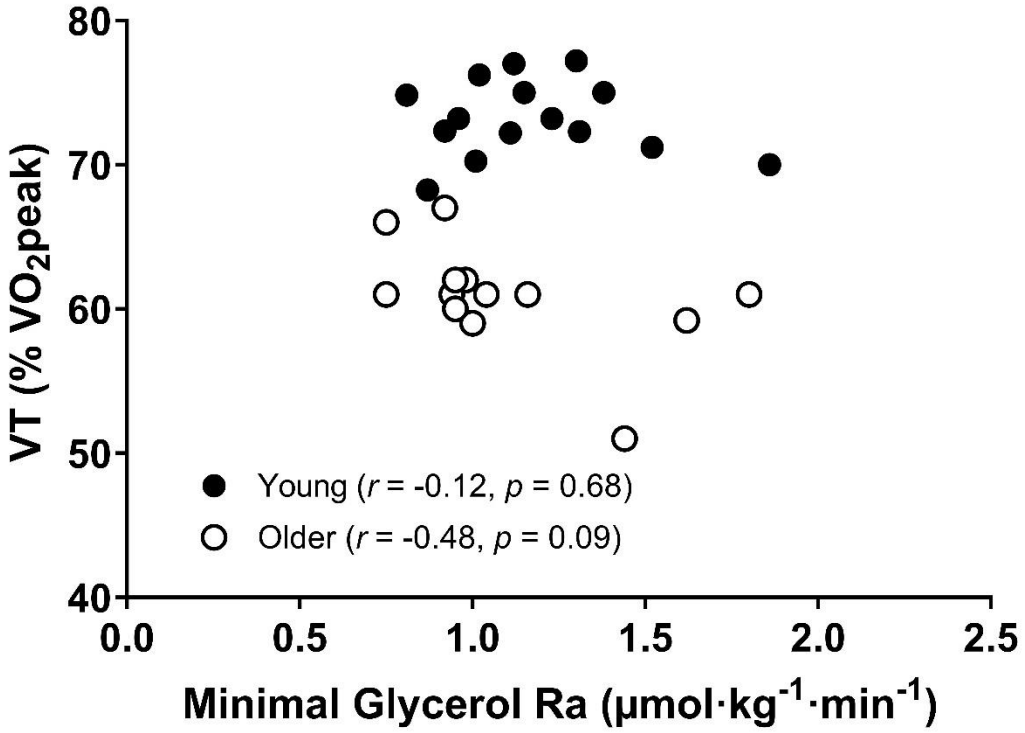

Figure 34. Relationship between ventilatory threshold (VT, % $\dot{V}O_2$peak, $mL \cdot kg^{-1} \cdot min^{-1}$) and the minimal glycerol rate of appearance (Ra) per unit of body mass attained in young and older participants.

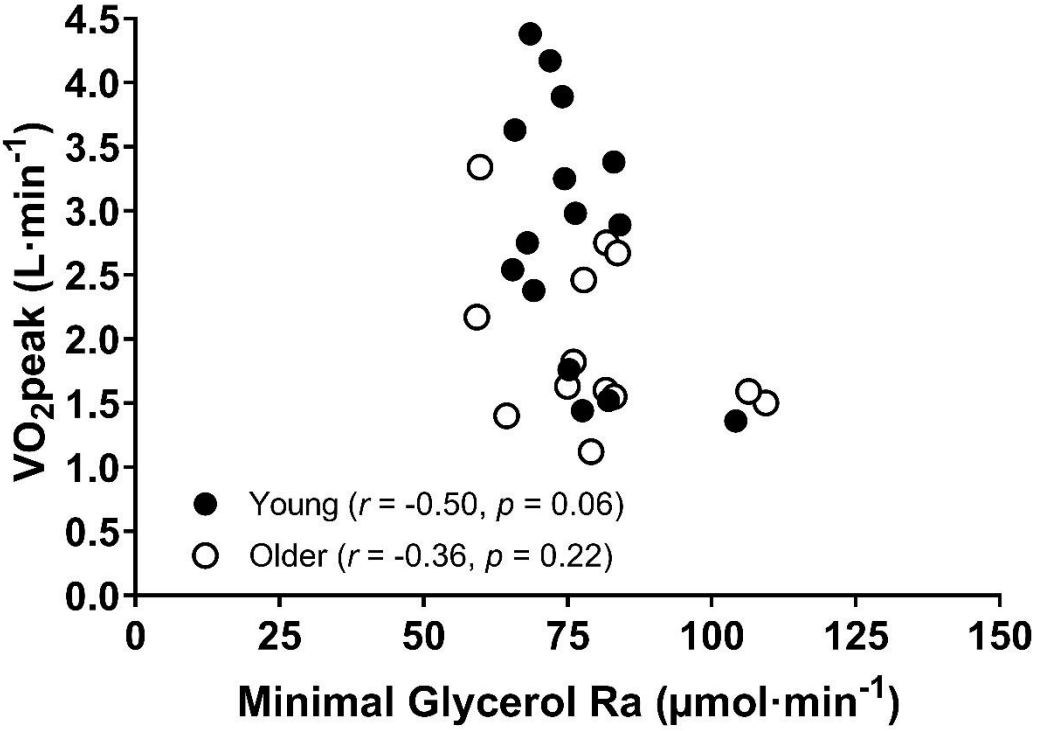

Figure 35. Relationship between maximal rate of oxygen consumption ($\dot{V}O_2$peak, L·min^{-1}) and the lowest glycerol rate of appearance (Ra) attained in young and older participants.

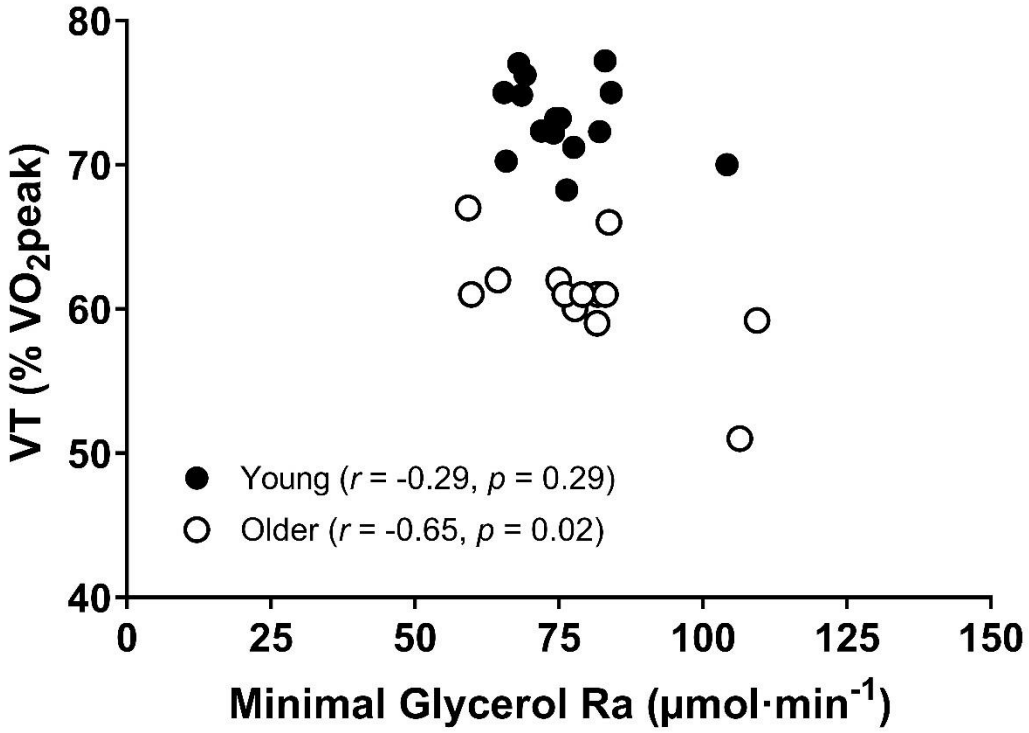

Figure 36. Relationship between ventilatory threshold (VT, % $\dot{V}O_2$peak, L·min^{-1}) and the lowest glycerol rate of appearance (Ra) attained in young and older participants.

CHAPTER 3

Postprandial fatty acid oxidation, but not reesterification is increased in aging

INTRODUCTION

Metabolic flexibility describes the ability to alter the pattern of substrate utilization in response to changes in energy substrate availability, as occurs in the transition from the postabsorptive to postprandial state (2). Following oral carbohydrate (CHO) consumption in resting (28, 29) and exercising (8, 9) humans, metabolic flexibility is illustrated by an increase in whole-body CHO oxidation. During postprandial rest, the systemic rise in CHO oxidation is mediated by an increase in insulin secretion to facilitate peripheral glucose uptake. Several previous investigations have utilized an OGTT to model physiological changes in CHO oxidation rates during hyperglycemia and hyperinsulinemia and demonstrated a rise in CHO oxidation within the first hour after glucose consumption (27-29). Other previous investigations also documented simultaneous reductions in lipid or fatty acid (FA) oxidation rates and plasma free fatty acid (FFA) concentrations (24-26). Because FA serve as the primary energy substrate during postabsorptive rest (79, 80), metabolic flexibility to oral CHO is also illustrated by the concurrent suppression of whole-body FA oxidation.

The loss of metabolic flexibility, or metabolic inflexibility, is rooted in the inability to suppress FA oxidation during hyperinsulinemia due to an excess availability of plasma FFA (6). Physiologically, the persistence of FFA availability during hyperinsulinemia would suggest a defect in the insulin-mediated suppression of lipolysis (16). Several previous investigations have utilized combined infusions of insulin and lipid and heparin to simulate intravascular lipolysis and observe the effects of elevated FFA on substrate oxidation rates. As predicted by the "Randle Cycle" (14), FA oxidation increased and CHO oxidation decreased when FFA availability was elevated (81-84). Plasma FA-induced alterations to insulin signaling are implicated to promote metabolic inflexibility by this mechanism (85, 86). Accordingly, the loss of metabolic flexibility is observable in metabolic disease states and aging (10-13). Bonadonna and colleagues (41) previously reported that FFA availability during hyperinsulinemia was higher in elderly compared to younger individuals and resulted in greater total lipid oxidation. Although higher rates of lipolysis were suspected to have increased FFA availability, it was unclear if the lipolytic rate was altered by their experimental conditions.

In Chapter 2 of this dissertation, we demonstrate that the rate of lipolysis (glycerol rate of appearance, Ra) decreases during an OGTT, and that the reduction is delayed in aging (30 min vs. 5 min in young). To our knowledge, simultaneous changes in lipolysis and FA oxidation rates have not been investigated during an OGTT, and changes in substrate oxidation rates during an OGTT have not been documented for older individuals. Fatty acids released from lipolysis are either oxidized in different tissues or reesterified into triglycerides (TG) in the liver, adipose tissue (87), or skeletal muscle (77, 88). Because FAs taken up by skeletal muscle have the potential to alter insulin signaling if not oxidized (85), growing our understanding of FA metabolism during the postprandial period is imperative. At present, it is unclear if there are aging-related modifications to FA oxidation or reesterification rates in the postprandial period, and if such modifications promote metabolic inflexibility in aging.

In opposition to the purported role of FA in affecting parameters of metabolic flexibility, Sidossis et al. (89, 90) reported that the intracellular availability of glucose, not FA, determined substrate oxidation rates. They proposed that hyperglycemia and hyperinsulinemia mutually

promoted the formation of malonyl-CoA, which inhibits carnitine palmitoyl transferase (CPT) I to limit mitochondrial FA transport (32, 33, 90). The results of Sidossis et al. (89, 90) importantly support the effects of lactate on energy substrate partitioning. We recently reviewed how lactate accumulation from increased glycolytic flux influences energy substrate partitioning (31). Intracellular lactate accumulation increases the production of malonyl-CoA and reactive oxygen species (ROS), which collectively inhibit FA transporters CPT I (32, 33) and CPT II (34) to limit mitochondrial FA transport and oxidation. Additionally, lactate binding to hydroxycarboxylic acid receptor 1 (HCAR-1) on adipose cells inhibits lipolysis (35-37) to limit FA availability for oxidation. In Chapter 2 of this dissertation, we also demonstrate that blood lactate concentrations are inversely related to lipolytic rates during an OGTT, and that the inhibitory effect of lactatemia is stronger in aging. Presently, we seek to determine if physiological lactatemia induced by oral glucose consumption influences aging-related modifications to postprandial FA oxidation rates.

The purpose of this investigation was to assess metabolic flexibility by determining changes in total CHO and FA oxidation rates and FA reesterification rates during an OGTT in healthy young and older individuals. Additionally, we sought to determine how lipolysis, lactatemia, and lactate oxidation influence substrate utilization in the postprandial period. We hypothesized that CHO oxidation would increase, and FA oxidation and reesterification would decrease in response to oral glucose consumption. We hypothesized that FA oxidation would be inversely related to both blood lactate concentrations and lactate oxidation. Further, we hypothesized that the rise in CHO oxidation would be delayed in aging, and that the inhibitory effects of lactatemia and lactate oxidation on FA oxidation would be exaggerated in aging.

METHODS

Study participants. This study was approved by the University of California, Berkeley Committee for the Protection of Human Subjects (CPHS 2018-08-11312) and conformed to the standards set by the Declaration of Helsinki. Fifteen younger (21-35 years) and 14 older (60-80 years) men and women were recruited. Potential participants were interviewed and received verbal and written information on study purposes and procedures. After giving verbal and written consent, participants were screened for metabolic and cardiovascular diseases. Screening tests included a health history questionnaire, a blood draw for a basic metabolic panel, an electrocardiogram (ECG) and pulmonary function assessment via spirometry, three-site skin fold measurements (men: chest, abdomen, and thigh; women: triceps, suprailiac, thigh) to assess body density, and a physical examination. Measurements of body density were converted to body composition and expressed as percentage body fat according to the guidelines provided by the American College of Sports Medicine (58). To assess physical fitness, screening also included a continuous, progressive cycle ergometer test to determine peak oxygen consumption ($\dot{V}O_2peak$) and ventilatory threshold (VT). For dietary controls, participants provided 3-day dietary records that were analyzed for caloric intake and macronutrient consumption (Diet Analysis Plus Version 6.1, ESHA Research, Salem, OR). To qualify for participation, participants were required to be diet and weight stable; to have a body mass index (BMI) in the range of ≥18.5 and <30.0 kg/m^2; to be non-smokers; to have normal pulmonary function (FEV_1/FVC of >70%); to have a fasting blood glucose concentration of <100 $mg \cdot dL^{-1}$; to have a hemoglobin A1c percentage of <5.7 % of total hemoglobin; to have a fasting total cholesterol concentration of <200 $mg \cdot dL^{-1}$, LDL cholesterol concentration of <100

$mg \cdot dL^{-1}$, HDL cholesterol concentration of ≥40 $mg \cdot dL^{-1}$, and total triglyceride concentration of <150 $mg \cdot dL^{-1}$; to pass a physical examination; and to be cleared for participation by a licensed physician. Moreover, women were required to have a regular (28- to 35-day) menstrual cycle, to not be pregnant, and to not be taking oral contraceptives.

Screening procedures preceded experimental procedures by at least one week. Participants who were entered into the study were provided with verbal and written information on freedom to withdraw from the study as well as the contact information of the laboratory manager, principal investigator, and physician if adverse effects of the study procedures had occurred.

Exercise testing protocol. All participants underwent a graded exercise test (GXT) conducted on an electronically braked leg cycle ergometer (Lode Corival CPET, Gronigen, Netherlands). Open-circuit indirect calorimetry was performed simultaneously to continuously monitor expired gases throughout the GXT with a "metabolic cart" (Parvo Medics TrueOne 2400 System, Salt Lake City, UT). The "metabolic cart" was calibrated according to the manufacturer's instructions prior to each GXT. Each GXT began with 5 min of seated rest followed by a 2 min warm-up period at 25 W at a set cadence of 60 RPM. Participants then completed three, 4-min stages of 35 W, 60 W, and 85 W at a set cadence of 60 RPM. The cadence was dictated by a metronome and reinforced visually using a tachometer displayed on the ergometer screen. Participants subsequently pedaled at a self-selected cadence for the remainder of the test as the power output increased by 30 W per min. Young participants continued until volitional exhaustion. Older participants continued until achieving VT, which was identified by a respiratory exchange ratio (RER, or $R = \dot{V}CO_2/\dot{V}O_2$) of 0.97–1.00. For older participants, $\dot{V}O_2peak$ was estimated from VT using guidelines provided by the American College of Sports Medicine (58).

Experimental procedures. Participants underwent a 120-min OGTT with primed, continuous infusion of [1,1,2,3,3-^{2}H]glycerol (or D_5-glycerol) and [3-^{13}C]lactate (Cambridge Isotope Laboratories, Inc., Andover, MA). Data on lactate kinetics in young participants have been reported separately (59), and data on lactate kinetics in older participants will be reported separately (60), but the use of these stable isotope tracers is mentioned in this report for its relevancy to the present data.

Participants reported to the laboratory in the morning following a 12-hr overnight fast. Women who participated did so during the mid-follicular phase of their menstrual cycle. For the 24 hr preceding the experimental trial, participants were asked to maintain their standard dietary pattern and refrain from strenuous physical exercise. On the morning of the trial, a catheter was placed in a warmed hand vein for "arterialized" blood sampling and a contralateral arm vein catheter was placed for tracer infusion. Background blood samples were then taken for the determination of endogenous isotopic enrichment (IE) of glycerol. Subsequently, a priming bolus of 40 $mg \cdot 15\ mL^{-1}$ D_5-glycerol was given, and then the participants rested for 90 min while the tracer glycerol was infused continuously at a rate of 0.32 $mg \cdot min^{-1}$. Arterialized blood sampling was repeated at 75 and 90 min after continuous infusion.

After 90 min of continuous infusion, participants drank a solution containing 75 g of D-glucose in 296 mL (#10-O-075, Azer Scientific Inc., Morgantown, PA). Participants consumed the

solution in ≤2 min. Arterialized blood sampling was subsequently completed at 5, 15, 30, 60, 90, and 120 min after the consumption of glucose.

Indirect calorimetry. At each of the blood sampling time points, respiratory gas exchange was determined by open-circuit indirect calorimetry. The "metabolic cart" was calibrated according to the manufacturer's instructions prior to each experiment. Respiratory gases were collected with a mouthpiece, nose clip, two-way non-rebreathing valve, and headgear-type valve support (Hans Rudolph Inc., Shawnee, KS). Hereafter, the breathing apparatus will be referred to as the "mouthpiece". Expired gases were collected for at least 5 min before and after (i.e., for at least 10 min) and simultaneously with blood sample collection. The first 5 min of each collection period was used for acclimation, and the data was not used for analysis. After collection at baseline, participants briefly removed the mouthpiece only to consume the glucose solution and then immediately replaced it. Participants were asked not to remove the mouthpiece at the 5 min and 15 min collection points to eliminate the acclimation period before the 15-min collection point. Otherwise, participants were allowed to remove the mouthpiece between collection points.

Calculations. Respiratory data was exported from the Parvo Medics TrueOne program using 5-sec averaging. Subsequently, respiratory data was averaged over the last 5 min of each 10-min collection period; the average values were used in the following calculations. The rate of energy expenditure (EE), percentage of EE derived from CHO and lipid, rate of total CHO oxidation (CHO Rox), and rate of total lipid oxidation (Lipid Rox) were calculated using the following equations (91, 92):

$$\text{EE from CHO} = (\text{RER} - 0.71) / 0.29$$

$$\text{EE from Lipid} = 1 - \text{EE from CHO}$$

$$\text{Total CHO Rox (kcal·min}^{-1}\text{)} = (\text{EE from CHO} \times \dot{V}O_2) \times 5.05 \text{ kcal·L}^{-1}\ O_2$$

$$\text{Total Lipid Rox (kcal·min}^{-1}\text{)} = (\text{EE from Lipid} \times \dot{V}O_2) \times 4.70 \text{ kcal·L}^{-1}\ O_2$$

$$\text{Total EE (kcal·min}^{-1}\text{)} = [(\text{EE from CHO} \times \dot{V}O_2) \times 5.05 \text{ kcal·L}^{-1}\ O_2] + [(\text{EE from Lipid} \times \dot{V}O_2) \times 4.70 \text{ kcal·L}^{-1}\ O_2]$$

where: RER is the respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) and $\dot{V}O_2$ is the rate of oxygen consumption in $L \cdot min^{-1}$. Total CHO Rox was converted to units of $mg \cdot min^{-1}$ using the caloric equivalent of CHO (4.2 $kcal \cdot g^{-1}$) (93), and then to units of $\mu mol \cdot min^{-1}$ using the molecular weight of glucose (180.16 $g \cdot mol^{-1}$). Total Lipid Rox was converted to units of $mg \cdot min^{-1}$ using the caloric equivalent of lipid (9.5 $kcal \cdot g^{-1}$) (93), and then to units of $\mu mol \cdot min^{-1}$ using the molecular weight of a representative TG (860 $g \cdot mol^{-1}$). Further, Lipid Rox was converted to FA Rox by multiplying by 3 (3 moles of FA per mole of TG).

The rate of total FA reesterification (Rs) was calculated as the difference between the rate of lipolysis and the rate of total FA oxidation (92, 94):

$$\text{Total FA Rs } (\mu mol \cdot min^{-1}) = (3 \times \text{Glycerol Ra}) - \text{Total FA Rox}$$

where: glycerol Ra is the glycerol rate of appearance in μmol·min^{-1} and FA Rox is the rate of total FA oxidation in μmol·min^{-1}.

Statistical analyses. Data were analyzed using GraphPad Prism 10 (Version 10.1.2 for Windows, GraphPad Software, Boston, MA). Statistical significance was set at $\alpha \leq 0.05$. Repeated-measures two-way ANOVA with the Geisser-Greenhouse correction and Tukey's multiple comparisons test was used to assess the significance of mean differences between groups and across time points. Data are presented as mean ± standard error of the mean (SE). Differences across time points are expressed relative to baseline (i.e., before the consumption of glucose at 0 min). Area under the curve (AUC) was determined using GraphPad Prism 10. To assess the significance of differences in AUC, an unpaired t-test with Welch's correction was used. Data are presented as mean ± SE.

Pearson correlation coefficients were used to assess the significance of relationships among select variables studied. The magnitude of correlations are interpreted according to the guidelines provided by Mukaka (64). Correlations of blood lactate concentrations and lactate oxidation rates with selected variables were assessed throughout the observation period (0-120 min), during the enteric phase of the postprandial lactate shuttle (PLS) only (0-30 min), and during the systemic phase of the PLS only (60-120 min). Correlations of variables with one another (i.e., not with blood lactate concentrations or lactate oxidation rates) were assessed for the entirety of the observation period only.

RESULTS

Participant characteristics. Comparisons between young and older participants are presented in Table 1. Older participants' mean age ($p < 0.001$), BMI ($p = 0.03$), body fat percentage ($p = 0.02$), and body fat mass (FM) ($p = 0.005$) were significantly higher than young subjects. In contrast, absolute ($p = 0.007$) and relative ($p < 0.001$) $\dot{V}O_2$peak, peak power output ($p = 0.002$), and VT ($p < 0.001$) were significantly higher in young participants. There were no significant differences in total body mass (TBM) ($p = 0.22$), fat free mass (FFM) ($p = 0.79$), pulmonary function (FEV_1/FVC) ($p = 0.09$), daily caloric intake ($p = 0.10$), or percentage of caloric intake from carbohydrates ($p = 0.12$), fat ($p = 0.68$), or protein ($p = 0.06$). There were no significant differences in the size of the glucose load when expressed relative to TBM ($p = 0.36$) or per unit of FFM ($p = 0.95$), but when expressed per unit of FM, the relative glucose load was greater for young participants ($p = 0.007$).

Postabsorptive measurements. There were no differences in total EE, the percentage of EE derived from lipid (% Lipid) and CHO (% CHO) energy sources, RER, $\dot{V}O_2$, total Lipid Rox, total CHO Rox, or total FA Rs between young and older participants at baseline ($p \geq 0.12$) (Table 2). There were no differences in EE, FA Rox, CHO Rox, or FA Rs when expressed per unit of body mass ($p \geq 0.14$) or FFM ($p \geq 0.51$) (Table 3). However, when expressed per unit of FM, all variables were higher in young participants ($p \leq 0.01$).

Rates of total energy expenditure. Total EE increased 5 min after glucose consumption in both young (1.24 ± 0.04 vs. 1.13 ± 0.04 kcal·min^{-1}, $p < 0.001$) and older (1.24 ± 0.06 vs. 1.16 ±

0.06 kcal·min^{-1}, $p = 0.01$) participants (Fig. 1). In young participants, EE continuously rose until 30 min to a peak rate (1.39 ± 0.04 kcal·min^{-1}, $p < 0.001$) and then steadily declined until 120 min ($p < 0.001$). In older participants, EE continuously rose until 15 min, slowly increased until 60 min to a peak rate (1.35 ± 0.07 kcal·min^{-1}, $p < 0.001$), and then steadily declined until 120 min ($p < 0.001$). There were no significant differences between groups at any time point ($p \geq 0.45$).

The changes in EE were dissimilar when expressed relative to total body mass (Fig. 2). In young participants, EE per unit of body mass increased 5 min after glucose consumption (12.19 ± 0.39 vs. 10.15 ± 0.28 μmol·kg^{-1}·min^{-1}, $p = 0.01$), continuously rose until 60 min (21.74 ± 0.68 μmol·kg^{-1}·min^{-1}, $p < 0.001$), and then faintly decreased until 120 min ($p < 0.001$). In older participants, EE increased at 15 min, but the change was not significant ($p = 0.12$). Relative EE increased above baseline at 30 min (12.53 ± 0.58 vs. 9.57 ± 0.47 μmol·kg^{-1}·min^{-1}, $p = 0.01$), continuously rose until 60 min ($p < 0.001$), and then steadily rose thereafter ($p < 0.001$). Additionally, EE per unit of body mass was higher in young participants between 5-90 min ($p \leq 0.02$), and AUC for EE per unit of body mass was greater in young participants ($p = 0.02$) (Fig. 3). Changes in EE were similar for both groups when expressed relative to FFM (Fig. 4) and FM (Fig. 6). However, EE was higher in young participants at 30 min and 60 min ($p \leq 0.03$) when expressed per unit of FFM and at all time points ($p \leq 0.01$) when expressed per unit of FM. There was no difference in AUC for EE per unit of FFM (Fig. 5).

Relative energy contributions from lipids and carbohydrates. Changes in the percentage of EE derived from lipid (% Lipid) and CHO (% CHO) energy sources over time are summarized in Tables 4 and 5, respectively. In young participants, at 30 min after glucose consumption, % Lipid decreased below baseline while % CHO increased from baseline ($p = 0.05$). In contrast, in older participants, % Lipid decreased below baseline while % CHO increased from baseline ($p < 0.001$). After achieving these changes, % Lipid remained low and % CHO remained elevated until the end of observation in both groups. Additionally, % Lipid was higher in older participants at 5 min ($p = 0.05$) and 60 min ($p = 0.03$), while % CHO was higher in young participants at the same time points ($p = 0.05$ and $p = 0.006$, respectively).

Respiratory exchange ratio and rates of oxygen consumption. Changes in the RER over time are summarized in Table 6. RER increased from baseline 60 min after glucose consumption in both groups ($p < 0.001$), but RER was higher in young participants at this time point ($p = 0.02$). In young participants, RER then increased at 90 min to a peak ($p < 0.001$) and then remained elevated at 120 min ($p < 0.001$). In older participants, RER steadily increased until 120 min ($p < 0.001$).

Changes in the $\dot{V}O_2$ over time are summarized in Table 7. $\dot{V}O_2$ per unit of body mass increased 5 min after glucose consumption in both young ($p < 0.001$) and older ($p = 0.03$) participants and then rose until 30 min to a peak ($p < 0.001$). $\dot{V}O_2$ then declined until 120 min but remained elevated above baseline rates throughout ($p \leq 0.007$). In addition, $\dot{V}O_2$ was higher in young participants at baseline ($p < 0.001$), at 5 min ($p = 0.009$), and between 30-120 min ($p \leq 0.02$) when expressed relative to total body mass, but similar between groups at all time points ($p \geq 0.56$) when expressed as an absolute rate.

Total fatty acid oxidation rates. FA Rox per unit of body mass initially increased from baseline between 5-15 min in both groups, but the changes were not significant for either young ($p \geq 0.72$) or older ($p \geq 0.16$) participants (Fig. 7). However, when expressed as a percentage increase, the change at 5 min was significant in older participants (11.95 ± 3.90 %, $p = 0.05$) (Fig. 9). FA Rox returned to baseline values ($p > 0.99$) at 30 min in both groups. In young participants, FA Rox decreased below baseline 60 min after glucose consumption (1.64 ± 0.24 vs. 3.75 ± 0.10 μmol·kg^{-1}·min^{-1}, $p < 0.001$), steadily declined until 90 min (1.30 ± 0.16 μmol·kg^{-1}·min^{-1}, $p < 0.001$), and then remained low until 120 min ($p < 0.001$). In older participants, FA Rox decreased at 60 min (2.36 ± 0.22 vs. 3.51 ± 0.12 μmol·kg^{-1}·min^{-1}, $p < 0.001$) and then continuously declined thereafter ($p < 0.001$). Additionally, FA Rox was higher in older participants at 60 min ($p = 0.04$). There was no difference in AUC for FA Rox per unit of body mass (Fig. 8).

The changes in FA Rox were similar for both groups when expressed relative to FFM (Fig. 11) and FM (Fig. 13), as a percentage decrease from baseline (Fig. 10), and as an absolute rate (Fig. 14). As well, FA Rox was lower in young participants at 60 min when expressed relative to FFM ($p = 0.03$) and as an absolute rate ($p = 0.01$). However, FA Rox per unit of FM was higher in young participants at baseline ($p = 0.007$), between 5-30 min ($p \leq 0.03$), and at 120 min ($p = 0.03$). Additionally, the percentage decrease in FA Rox was higher in young participants at 60 min (58.71 ± 5.42 vs. 34.26 ± 5.01 %, $p = 0.004$) and 90 min (67.98 ± 3.45 vs. 49.98 ± 5.80 %, $p = 0.02$). There was no difference in AUC for FA Rox per unit of FFM (Fig. 12).

Total carbohydrate oxidation rates. In young participants, CHO Rox per unit of body mass increased 5 min after glucose consumption (10.89 ± 0.40 vs. 8.86 ± 0.30 μmol·kg^{-1}·min^{-1}, $p = 0.02$), continuously rose until 60 min (20.88 ± 0.94 μmol·kg^{-1}·min^{-1}, $p < 0.001$), remained elevated until 90 min ($p < 0.001$), and then faintly decreased until 120 min ($p < 0.001$) (Fig. 15). In older participants, CHO Rox remained unchanged at 5 min ($p = 0.99$) and then increased at 15 min, but the change was not significant ($p = 0.26$). CHO Rox increased above baseline at 30 min (11.34 ± 0.60 vs. 8.40 ± 0.51 μmol·kg^{-1}·min^{-1}, $p = 0.02$), continuously rose until 60 min (16.18 ± 0.83 μmol·kg^{-1}·min^{-1}, $p < 0.001$), and then steadily rose thereafter ($p < 0.001$). Additionally, CHO Rox was higher in young participants between 5-90 min ($p \leq 0.03$), and AUC for CHO Rox per unit of body mass was greater in young participants ($p = 0.04$) (Fig. 16).

The changes in CHO Rox were similar for both groups when expressed relative to FFM (Fig. 18) and FM (Fig. 20), as a percentage increase from baseline (Fig. 17), and as an absolute rate (Fig. 21). However, CHO Rox was higher in young participants at 30 min and 60 min ($p \leq 0.03$) when expressed per unit of FFM, at all time points ($p \leq 0.01$) when expressed per unit of FM, and at 60 min ($p = 0.01$) when expressed as an absolute rate. Additionally, the percentage increase in CHO Rox was higher in young participants at 5 min (20.78 ± 4.66 vs. 1.44 ± 6.24 %, $p = 0.02$) and 60 min (139.87 ± 14.37 vs. 93.43 ± 10.50 %, $p = 0.02$). There was no difference in AUC for CHO Rox per unit of FFM (Fig. 19).

Total fatty acid reesterification rates. In young participants, FA Rs per unit of body mass decreased 5 min after glucose consumption (1.74 ± 0.23 vs. 2.28 ± 0.14 μmol·kg^{-1}·min^{-1}, $p = 0.002$) and then continuously declined until 30 min (1.15 ± 0.05 μmol·kg^{-1}·min^{-1}, $p < 0.001$) (Fig. 22). FA Rs then increased to baseline rates at 60 min ($p = 0.98$), increased above baseline rates at 90 min (2.49 ± 0.11 μmol·kg^{-1}·min^{-1}, $p = 0.003$), and then decreased to baseline rates at 120 min ($p > 0.99$).

Similarly, in older participants, FA Rs decreased at 5 min (1.28 ± 0.16 vs. 1.93 ± 0.17 μmol·kg^{-1}·min^{-1}, $p < 0.001$) and then continuously declined until 30 min (0.83 ± 0.18 μmol·kg^{-1}·min^{-1}, $p < 0.001$). FA Rs then increased continuously between 60-120 min and reached above baseline rates at 120 min (2.21 ± 0.17 μmol·kg^{-1}·min^{-1}, $p < 0.001$). Additionally, FA Rs was higher in young participants at 60 min ($p = 0.02$) and 90 min ($p = 0.03$). There was no difference in AUC for FA Rs per unit of body mass (Fig. 23).

The changes in FA Rs were similar for both groups when expressed relative to FFM (Fig. 24) and FM (Fig. 26), and as an absolute rate (Fig. 27). Additionally, FA Rs was similarly higher in young participants at 60 min ($p = 0.003$) and 90 min ($p < 0.001$) when expressed as an absolute rate. However, FA Rs was similar between groups at all time points ($p \geq 0.07$) when expressed per unit of FFM and higher in young participants at all time points ($p \leq 0.004$) when expressed per unit of FM. There was no difference in AUC for FA Rs per unit of FFM (Fig. 25).

Total fatty acid oxidation versus reesterification rates. There was no difference between the AUC for FA Rox and FA Rs in young participants when expressed per unit of body mass (Fig. 28) or per unit of FFM (Fig. 29). However, the AUC for FA Rox was greater than the AUC for FA Rs in older participants when expressed relative to total body mass ($p = 0.008$) and FFM ($p = 0.01$).

Correlation of total fatty acid and carbohydrate oxidation rates. FA Rox and CHO Rox were highly but inversely correlated in both young ($r \geq -0.93$) and older ($r \geq -0.96$) participants. The relationships were comparable when rates were expressed in absolute terms (Fig. 30) or relative to total body mass (Fig. 31), FFM, or FM. Results are summarized in Tables 8 and 9.

Correlation of glycerol rate of appearance with total and fatty acid and carbohydrate oxidation rates. Glycerol Ra was highly and positively correlated with FA Rox in both groups, but the correlation was higher ($r = 0.94$ vs. $r = 0.87$) in young participants (Fig. 32). In contrast, glycerol Ra was very highly and inversely correlated with CHO Rox in both young ($r = -0.98$) and older ($r = -0.96$) participants (Fig. 33). The relationships were comparable when rates were expressed in absolute terms or relative to total body mass, FFM, or FM. Results are summarized in Tables 10-13.

Correlation of other variables related to substrate availability and utilization. FA Rox and [FFA] were very highly and positively correlated in both young ($r = 0.93$) and older ($r = 0.95$) participants (Fig. 34). In contrast, CHO Rox and [FFA] were very highly and inversely correlated in both young ($r = -0.97$) and older ($r = -0.99$) participants (Fig. 35). Additionally, FA Rs was inversely and moderately ($r \geq -0.50$) correlated with [FFA] (Fig. 36) and highly ($r \geq -0.73$) correlated with [TG] (Fig. 37) in both groups. The relationships were comparable when rates were expressed in absolute terms or relative to total body mass, FFM, or FM. Results are summarized in Table 14.

Correlation of blood lactate concentrations with total fatty acid oxidation rates. Results of correlations of [Lactate] with FA Rox are summarized in Table 15. There was an overall inverse relationship between [Lactate] and FA Rox per unit of body mass in both groups, but the correlation was higher ($r = -0.84$ vs. $r = -0.62$) in older participants (Fig. 38). Additionally, the correlation was positive and moderate ($r = 0.68$) in young participants and negligible ($r = 0.22$) in older participants

during the enteric phase. The correlation was very high in both groups during the systemic phase, but it was positive (r = 0.94) in young participants and negative (r = -0.95) in older participants. The relationships were similar for both groups when FA Rox was expressed relative to FFM and FM and as an absolute rate (Fig. 39). However, in young participants, the correlation was high during the enteric phase when FA Rox was expressed relative to FFM and as an absolute rate, but low when expressed relative to FM.

Correlation of total lactate oxidation rates with total fatty acid oxidation rates. Results of correlations of the rate of total lactate oxidation (Rox) with FA Rox are summarized in Tables 16-18. There was an overall inverse relationship between Lactate Rox and FA Rox per unit of body mass in both groups, but the correlation was higher (r = -0.83 vs. r = -0.73) in older participants (Fig. 40). The correlation was positive and moderate (r = 0.65) in young participants and very high (r = 0.91) in older participants during the enteric phase. However, the correlation was very high and negative ($r \geq$ -0.96) in both groups during the systemic phase. The relationships were similar for both groups when Lactate Rox and FA Rox were expressed relative to FFM and FM and as an absolute rate (Fig. 41). However, in young participants, the correlation was negligible (r = 0.25) during the enteric phase when Lactate Rox and FA Rox were expressed relative to FM.

DISCUSSION

We determined changes in FA and CHO oxidation rates and FA reesterification rates following oral glucose consumption in healthy young and older individuals. Additionally, we determined how lipolysis, lactatemia, and lactate oxidation rates influence substrate utilization in the postprandial period. In brief, following oral glucose consumption, we report the following: (1) the suppression of FA oxidation is delayed in aging, (2) the rise in CHO oxidation is delayed in aging, (3) FA reesterification is not the primary fate of FA released from lipolysis, (4) the lipolytic rate informs aging-related differences in postprandial energy substrate partitioning, and (5) lactatemia and lactate oxidation have a greater influence on FA oxidation in aging. These results are discussed sequentially.

Total fatty acid oxidation rates. Whole-body total FA oxidation rates decrease in response to oral glucose consumption due to the ensuing rise in blood glucose and plasma insulin concentrations that collectively reduce lipolysis (16), FA availability, and FA oxidation (89). In young participants, total FA Rox increased non-significantly from baseline within the first 30 min after glucose consumption (Fig. 7). These early changes in FA Rox mirrored the changes reported by Kruszynska et al. (26) and Gomez et al. (25) who similarly observed healthy young men and women. Our results also agree with other previous investigations that reported that the reduction in FA/lipid oxidation occurs beyond the first 30 min (24-27). However, we observed a stable and minimal FA Rox between 60-120 min ($p \geq$ 0.65 between time points), which disagrees with their results. These investigations observed a decline in FA/lipid oxidation rates between 60-120 min. It is unclear why this discrepancy exists. Leclerc et al. (27) similarly utilized a 75-g OGTT and a population with comparable body mass. Felber et al. (24) and Gomez et al. (25) used a larger 100-g glucose load which, theoretically, should have resulted in greater suppression of FA oxidation due to a greater insulin response (95, 96). At minimum, we confirm extant data on the changes in FA oxidation immediately following oral glucose consumption in healthy young individuals. We

additionally provide missing data on FA oxidation rates during an OGTT in healthy older individuals. And finally, with the simultaneous use of D_5-glycerol, we provide missing data on total FA reesterification rates during an OGTT.

Total FA Rox fell below baseline rates at 60 min in both groups. However, at 60 min, FA Rox was higher for older participants. FA Rox also trended to be higher in older participants at 90 min ($p = 0.07$) when expressed relative to FFM (Fig. 11) and as an absolute rate (Fig. 14). In contrast, between 60-120 min, FA Rox was relatively constant in young participants ($p \geq 0.77$ between time points), suggesting that a minimal rate had been reached by 60 min. This is supported by the small changes ($p \geq 0.85$) in % Lipid during this period. Our results therefore demonstrate that the postprandial suppression of FA oxidation was delayed in our older participants. Because FAs serve as the primary energy substrate during postabsorptive rest (79, 80), we demonstrate that metabolic flexibility to oral glucose consumption is delayed in "healthy" aging. Multiple factors can be implicated as contributors to the delayed response of FA Rox (discussed below). However, worth noting first is the reciprocal changes in CHO oxidation rates.

Total carbohydrate oxidation rates. Whole-body total CHO oxidation rates predictably increase in response to oral glucose consumption due to the ensuing rise in plasma insulin concentrations that facilitates glucose uptake and oxidation (89). In young participants, total CHO Rox rapidly increased from baseline until 60 min (Fig. 15). Those early changes in CHO Rox mirrored the changes reported in previous investigations that similarly observed healthy young men and women (24, 25, 28). Although we observed quantitative differences between 0-60 min, they can be explained by the dissimilar sizes of the glucose load used. Moreover, our results agree with other previous investigations that reported stable and maximal CHO oxidation rates between 60-120 min after glucose consumption in young individuals (27, 29). However, we observed a significant increase in CHO Rox 15 min and 30 min after glucose consumption, which disagrees with the results of Alcantara et al. (29) and Felber et al. (24), respectively. These investigations did not observe a change in CHO at those time points. Methodological considerations (discussed below) are not suspected to be responsible for these discrepancies. More likely, physiological differences in the populations studied might have contributed to this difference. Alcantara et al. (29) studied young men and women with greater body fat content (12.73 ± 2.13 vs. 20 ± 6 % and 21.92 ± 1.47 vs. 38 ± 4 %, respectively), which might have contributed to the delayed rise in CHO oxidation (97, 98). Unfortunately, Felber et al. (24) did not thoroughly describe the characteristics of their control participants, so we are unable to posit a rationale for the disagreements in our data. Still, we expand on extant data by demonstrating that CHO oxidation rates increase above baseline as early as 5 min after glucose consumption in healthy young individuals. Additionally, we provide missing data on CHO oxidation rates during an OGTT in healthy older individuals.

Our results demonstrate that the postprandial rise in CHO oxidation was delayed in our older participants. As described, CHO Rox per unit of body mass (Fig. 51) increased above baseline 5 min after glucose consumption in young but not older ($p = 0.99$) participants ($p = 0.008$ between groups). Remarkably, this change was a 20.78 ± 4.66 % increase (Fig. 17) from baseline ($p = 0.009$). In contrast, in older participants, an increase in CHO Rox above baseline was not observed until 30 min. Moreover, between 60-120 min, CHO Rox remained relatively steady in young participants ($p \geq 0.87$ between time points) but steadily increased in older participants ($p \geq 0.03$ between time points). Because these changes were also observed when CHO Rox was

expressed as an absolute rate (Fig. 21), the divergent responses of CHO Rox between groups cannot be completely explained by the small difference in TBM ($p = 0.22$) or in the relative size of the glucose load ($p = 0.36$).

The ability of skeletal muscle to suppress lipid oxidation during hyperglycemia is related to insulin sensitivity, percentage body fat, and aerobic fitness (97, 98). Because the total EE represents a balance of CHO and FA utilization, the ability to increase CHO oxidation during physiological hyperglycemia would be influenced by the same variables. Insulin has a primary role in the disposal and subsequent oxidation of an oral glucose load in skeletal muscle (3, 28). Changes in plasma insulin concentrations were determined simultaneously with respiratory gas collection, but the data will be reported in Chapter 4 of this dissertation. Because peripheral insulin sensitivity (99) and skeletal muscle mass (100) reportedly decline in aging, the delayed rise in CHO oxidation can be a hypothesized consequence of the combined effects of insulin insensitivity and reduced muscle mass. However, our data suggests that our older participants did not exhibit these aging-related decrements. Insulin sensitivity during the OGTT (101) was not significantly lower in older participants ($p = 0.15$). Fat-free mass was not different between groups ($p = 0.79$). Consequently, the size of the glucose load was more comparable when expressed relative to FFM ($p = 0.95$), and when CHO Rox was expressed relative to FFM (Fig. 18), CHO Rox was more similar between groups compared to when CHO Rox was expressed relative to TBM. Basu et al. (102) reported that insulin action was lower in their elderly than young participants due to greater body fat content. Presently, we similarly report greater body fat mass in older participants. Coincident with a higher plasma insulin concentration ($p = 0.02$), CHO Rox was higher in young participants 30 min after glucose consumption (Fig. 18). These results allow us to speculate greater glucose uptake and subsequent oxidation in young participants (95). The delayed rise in CHO Rox observed in older participants is therefore attributed, in part, to lower circulating insulin and lower insulin action associated with greater body fat mass, but not insulin "insensitivity" or less FFM for glucose uptake. The impact of this distinction is extended to partially explain the delayed suppression of FA oxidation.

In addition to the delayed increase from baseline, we report that CHO Rox per unit of body mass was significantly lower in older participants between 5-90 min. Resting metabolic rate (103) and the metabolic scope reportedly decrease with advancing age. Thus, our finding that post-challenge CHO Rox was lower in older participants is potentially unsurprising. However, we did not observe any significant differences in resting, postabsorptive EE whether expressed as an absolute rate or relative to TBM or FFM. Consequently, the ability to increase EE and alter the balance of CHO and lipid utilization in older participants is attributed to other factors. As previously described, greater body fat mass (104) and its associated effects on insulin action (102) are likely responsible for lower post-challenge CHO Rox. Consistently, when expressed as absolute instead of relative rates, there were no differences in EE (Fig. 1) and less differences in CHO Rox between young and older participants post-challenge, indicating that differences in body composition also contributed to lower post-challenge CHO Rox in older participants. Further, aging-related changes that cannot be accounted for within scope of our methodology, such as the slowing of gastric emptying (105, 106) or reduced skeletal muscle glucose transporter protein content (107) might further explain the delayed rise in CHO Rox in our older participants.

Total fatty acid reesterification rates. We have previously estimated total FA reesterification rates in young (92) and older (13) individuals at rest and during exercise (108, 109). Presently, total FA Rs was determined as the difference in the lipolytic rate (3 × glycerol Ra) and total FA oxidation rate. Our estimation therefore more specifically determines the rate of reesterification of FA released from lipolysis (69). Because the only fates of FA released from lipolysis are oxidation and reesterification, results of FA Rox and FA Rs will be discussed concurrently. Total FA Rox per unit of body mass increased from baseline between 5-30 min after glucose consumption in both groups. Although the changes were not significant, the increases in FA Rox were substantial enough to cause a simultaneous reduction in total FA Rs below baseline ($p \leq 0.002$) (Fig. 22). Therefore, our data indicates that FA released from lipolysis between 5-30 min were utilized to support total EE rather than being recycled. Consistently, comparisons of % Lipid and % CHO (Tables 4 and 5, respectively) indicate that total EE still comprised of mostly lipids at 5 min and 15 min (≥57.34 ± 1.96 %), and approximately 50% lipids at 30 min. Notably, the progressive decrease in % Lipid from 5 min to 30 min was not matched by an increase in FA Rs due to the simultaneous reduction in lipolysis and therefore, FA availability.

In contrast to FA Rox, total FA Rs was lower at 60 min and 90 min for older participants. Because the only fates of FA released from lipolysis are oxidation and reesterification (69), we estimated the division of FAs for each pathway by comparing the AUC for FA Rox to the AUC for FA Rs. There were no differences observed in young participants ($p \geq 0.34$), suggesting that a similar amount of FAs were allotted to oxidation and reesterification. In contrast, the AUC for FA Rox was greater than the AUC for FA Rs in older participants (Fig. 28 and 29). Our results therefore also suggest that postprandial lipid substrate partitioning was altered in our older participants such that FA reesterification was demoted in favor of FA oxidation.

Fatty acids derived from adipose tissue lipolysis serve as the primary energy substrate during postabsorptive rest (42, 79, 80, 110, 111) and at lower exercise intensities eliciting an increase in the resting metabolic rate (112). Consequently, we expressed changes in FA Rox (Fig. 13) and FA Rs (Fig. 26) per unit of FM. Because body fat mass was lower in young participants, FA Rs was predictably and consistently higher in young participants throughout observation. We do not suspect that a greater amount of FA were recycled in young participants as this difference suggests. However, this approach notably revealed that FA Rox per unit of FM was not higher in older participants at 60 min and 90 min. The similar FA Rox at 60 min ($p = 0.99$) is particularly notable because FA Rox was consistently higher in older participants at 60 min when expressed in other units. These results collectively led to the speculation that the extent to which FA Rox was reduced (i.e., % decrease) was greater in young participants. Accordingly, the % decrease in FA Rox (Fig. 10) was higher in young participants at 60 min and 90 min. These results highlight the delayed suppression of FA oxidation in our older participants.

The role of insulin in the suppression of FA oxidation is well-documented (3, 113). Notably, Thiebaud et al. (95) and Yki-Jarvinen et al. (96) demonstrated greater suppression of lipid oxidation with increasing plasma insulin concentrations. Because we observed no differences in FA Rox per unit of FFM and % Lipid at baseline ($p \geq 0.90$) and 120 min ($p \geq 0.83$), the suppression of FA Rox was truly delayed and not restricted in our older participants. The delay is regarded to be a consequence of lower circulating insulin as previously described and, by extension, a consequence of the delayed rise in CHO Rox. To reiterate, the differences in

FA Rox are not thought to be a result of differences in insulin sensitivity among our study population. Bonadonna et al. (41) similarly observed alterations in substrate oxidation in aging that were not attributed to changes in insulin sensitivity, but instead, to greater body fat mass allowing for greater FFA mobilization and subsequent oxidation. Presently, we cannot conclude that FFA mobilization was greater in older participants because the glycerol Ra was not different between groups. Still, the available evidence suggests that increased body fat mass in aging influences aging-related changes in substrate partitioning (102).

Skeletal muscle and adipose take up circulating glycerol and FFA, respectively, during hyperglycemia to serve as substrates for TG synthesis via reesterification (76). In adipocytes, TG synthesis occurs during insulin stimulation with glucose serving as the glycerol backbone following conversion to glycerol-3-phosphate (87). Importantly, in skeletal muscle FA taken up but not oxidized have the potential to alter insulin signaling (85). Reesterification in skeletal muscle therefore serves as a mechanism to protect against insulin resistance (77). Because FA Rox was elevated, the physiological impact of greater FA oxidation over reesterification in the present study is likely negligible in our healthy older participants. However, this result might have important implications for aging-related metabolic disease states in which elevated plasma FFA concentrations give rise to greater rates of FA uptake (114) into skeletal muscle with lower capacities for FA oxidation (115, 116). Because there were no differences in the AUC for FA Rs between groups, we do not suspect that the mechanism for FA reesterification is negatively affected in aging. Still, the unknown postprandial fate of FA released from lipolysis in "unhealthy" aging characterized by metabolic inflexibility warrants further investigation.

Insulin also has an essential role in the regulation of FA reesterification (94, 96, 113). As described, FA Rs decreased below baseline rates between 5-30 min in both groups due to the concurrent reduction in lipolysis and increase in FA Rox leaving little FAs available for reesterification. Later, FA Rs increased transiently above baseline rates at 90 min and 120 min in young and older participants, respectively. FA Rs also remained at baseline rates at 60 min and 120 min ($p \geq 0.98$) in young participants. Notably, plasma insulin concentrations were elevated above baseline ($p < 0.001$) at these time points. Because Yki-Jarvinen et al. (96) also demonstrated greater suppression of FA reesterification with increasing plasma insulin concentrations, this was an unexpected result. We initially hypothesized that FA Rs would be suppressed throughout the OGTT primarily due to the suppression of lipolysis (94), and we demonstrated that the rate of lipolysis decreased in both groups. Alongside the lower plasma glycerol and FFA concentrations at those time points, it is therefore unclear if these brief increases resulted in substantial TG synthesis via FA reesterification. FA Rs and [TG] were inversely correlated (Fig. 37), suggesting that the reesterification of FA in the liver into newly synthesized TG was not a major fate during our observation period in either group. In contrast, FA Rs and [FFA] were moderately but not significantly correlated (Fig. 36). This relationship raises the possibility of FA reesterification occurring in other tissues. Unfortunately, we are not able to determine rates of oxidation and disposal of plasma FFA, but our data allows us to speculate that reesterification was not the primary fate of FA released from lipolysis. Future similar investigations might utilize D_5-glycerol in conjunction with a FA isotope tracer (e.g., [1-^{13}C]palmitate) to explore this hypothesis.

Evidence of "healthy" aging. Impaired insulin sensitivity is regarded as the primary cause of glucose intolerance in aging (99). As described, insulin sensitivity during the OGTT (101) was not significantly different between groups ($p = 0.15$). Estimation of insulin sensitivity by the homeostatic model assessment method (HOMA-IR) (117) showed that our older participants had comparatively lower insulin sensitivity than young participants ($p = 0.02$), but not low enough to be associated with metabolic syndrome (118). Presently, we provide evidence of "healthy" aging in our older participants through parameters of substrate oxidation. Our 12-hr postabsorptive measurements revealed no differences in RER, % Lipid, or % CHO (Table 2), indicating that FA metabolism was not different in aging during fasting conditions. When expressed relative to FFM, FA Rox and CHO Rox were similar between groups at the end of observation ($p \geq 0.83$). Additionally, the % decrease in FA Rox was nearly equal at the end of observation ($p = 0.99$). Our results collectively indicate that our older participants did not exhibit metabolic inflexibility derived from insulin insensitivity or greater body fat mass as originally described by Kelley et al. (5). Unlike their obese volunteers, our older participants responded to the physiological rise in insulin by increasing CHO oxidation and suppressing FA oxidation. However, evidence of impaired metabolic flexibility in aging is provided by the delayed but not restricted changes in energy substrate partitioning.

Influence of lipolysis and plasma free fatty acid availability on substrate oxidation rates. Bonadonna et al. (41) reported that FFA availability and FA oxidation during hyperinsulinemia was higher in older compared to younger individuals. Although higher rates of lipolysis were suspected to have increased FFA availability, it was unclear if the lipolytic rate was altered by their experimental conditions. Presently, we observed no differences in the lipolytic rate between groups. However, the suppression of lipolysis did not occur until 30 min after glucose consumption in older participants, and [FFA] was higher in older participants between 60-120 min. The higher [FFA] at 60 min importantly coincided with higher FA Rox and lower CHO Rox.

The correlations of glycerol Ra and [FFA] to FA Rox (Fig. 32 and 34) and CHO Rox (Fig. 33 and 35) were comparable between groups in size and significance, indicating that age did not affect these relationships. Results of the correlations can be interpreted in at least two ways. Lipolysis and [FFA] are predictably inversely related to CHO Rox but positively related to FA Rox due to the respective effects of insulin. The rise in plasma insulin concentrations following oral glucose consumption facilitates peripheral glucose uptake and oxidation. Simultaneously, insulin suppresses lipolysis to reduce plasma FFA availability for oxidation. Because CHO Rox rose and FA Rox, glycerol Ra, and [FFA] declined in both groups over time, the correlations were consequently high in both groups.

Alternatively, results of the correlations can highlight the differences observed in our older participants. The correlations might suggest that the delayed suppression of lipolysis and resulting higher plasma FFA availability could have enabled higher FA Rox, thereby delaying the rise in CHO Rox. Consistently, in older participants, correlations of [FFA] to substrate oxidation rates were higher, suggesting that [FFA] had a larger influence than glycerol Ra on substrate oxidation rates. Previous investigations have also demonstrated greater rates of FA/lipid oxidation and lower rates of CHO oxidation when plasma FFA availability was elevated during hyperinsulinemia by lipid and heparin infusion (6, 81, 82). Kruszynska et al. (26) notably documented lower CHO oxidation rates following oral glucose consumption when plasma FFA availability was artificially

elevated. We observed similar results as these investigations and as Bonadonna et al. (41), but importantly, we observed similar results during physiological hyperinsulinemia in which lipolysis and [FFA] were allowed to fall below baseline levels. Other previous investigations determined that elevated plasma FFA availability induces transient changes in insulin action (119) to affect substrate oxidation rates (6, 81, 82). Although we are unable to determine if plasma FFA were elevated at a concentration high enough and for long enough (120) to have transiently affected insulin action in our older participants, interpretation of these correlations from this perspective are potentially meaningful in "unhealthy" aging or metabolic disease states in which postprandial lipolytic rates may be insensitive to insulin signaling such that plasma FFA concentrations remain elevated. Overall, our data are interpreted to suggest that the delayed suppression of lipolysis contributed to greater FFA availability and FA Rox at the expense of CHO Rox and FA Rs.

Influence of lactatemia and lactate oxidation rates on carbohydrate and fatty acid oxidation rates. We have recently reviewed the role of lactate in signaling and its sequential effects on energy substrate partitioning (31). Briefly, intracellular lactate accumulation limits mitochondrial FA uptake by increasing the production of malonyl-CoA and ROS, which collectively inhibit FA transporters CPT I (32) and CPT II (34). Additionally, lactate binding to its receptor HCAR-1 on adipose cells inhibits lipolysis (35-37). Lactate therefore controls lipid substrate partitioning at the beginning and end of the pathway, from FA mobilization to oxidation. The effects of lactatemia on FA oxidation are however indirect through its effects on lipolysis and FA availability. We have previously shown, in aggregate, reduced contribution of lipids to total energy expenditure when lactate oxidation rates were elevated (39, 40) and reduced FA oxidation rates when blood lactate concentrations were elevated (38) during exercise. Presently, the inverse correlations between FA Rox and [Lactate] (Fig. 38 and 39) and rates of lactate oxidation (Lactate Rox) (Fig. 40 and 41) support the role of lactate in substrate partitioning. The correlations were notably higher and more significant in older participants, suggesting that the inhibitory effects of lactate are exaggerated in aging.

Lactatemia is preceded by glycolysis and lactate production in different tissues, and represents a limitation in systemic lactate clearance by oxidation and gluconeogenesis (71, 121). Presently, lactatemia was induced by oral glucose consumption and subsequent glycolysis. The Lactate Shuttle concept originally described in 1984 (65) has recently been updated to describe a postprandial mechanism, or PLS, by which lactate regulates energy substrate partitioning following CHO consumption (59). The PLS is comprised of two phases: (1) a fast enteric phase of lactate production from glycolysis in the gut that is followed by (2) a prolonged systemic phase of lactate production as glucose is disposed of via glycolysis in peripheral tissues. Because lactate production occurred in the gut during the enteric phase, there was minimal substrate competition between lactate and FA in peripheral tissue. We consequently did not observe an inhibitory effect of Lactate Rox on FA Rox during the enteric phase in either group (Table 16). Contrarily, lipids still contributed approximately 50% to total EE in both groups (Table 4), indicating that FA derived from adipose tissue lipolysis were still being utilized. We therefore also did not observe an inhibitory effect of [Lactate] on FA Rox in either group during this period (Table 15).

As described in Chapter 2 of this dissertation, an important difference in [Lactate] between groups was apparent during the systemic phase. In young participants, [Lactate] steadily declined from 60-120 min, but in older participants, [Lactate] steadily increased. Our data of lactate kinetics

in this investigation indicate overall greater rates of lactate oxidation in young participants (60). The divergent responses of CHO oxidation during the systemic phase were therefore driven by differences in lactate oxidation, which are reflected in the divergent responses of [Lactate] during the systemic phase. These responses would ultimately determine the influence of lactate on lipid substrate partitioning.

In young participants, the correlation of [Lactate] with FA Rox was highly positive ($r \geq 0.89$) during the systemic phase, suggesting that lactatemia did not have an inhibitory effect. Because Lactate Rox and FA Rox were very highly and inversely correlated ($r \geq -0.92$), we contend that this positive relationship reflects greater lactate oxidation freeing the restraints of lactatemia on lipolysis and FFA availability, as opposed to a permissive effect of lactatemia on FA Rox. In contrast, in older participants, both [Lactate] and Lactate Rox were very highly and inversely ($r \geq -0.95$) correlated with FA Rox during the systemic phase. Although the correlations were not significant ($p \geq 0.13$), they suggest that lactate contributed to but was not solely responsible for the suppression of FA oxidation.

Results of the systemic phase correlations are particularly meaningful because they ultimately determined the size, direction, and significance of the overall correlations. Implications from the overall correlations are therefore similar to those of the systemic phase: the suppression of FA Rox was influenced by the rise in Lactate Rox in both groups, but in older participants, it was also indirectly affected by lactate inhibition of lipolysis. We therefore provide evidence of postprandial substrate competition that is determined, in part, by lactate metabolism. Further, because the overall correlations were only significant in older participants (Tables 15 and 16), we show that the inhibitory effects of lactate are exaggerated in aging.

Collectively, our results suggest that the changes in substrate oxidation rates in older participants were more dependent on changes in the lipolytic rate and [FFA]. As previously described, we hypothesize that lower circulating insulin is most likely responsible for these differences. However, there is some evidence that insulin action could have been transiently altered in skeletal muscle. Rates of plasma FFA uptake are reportedly higher when plasma FFA concentrations are elevated (114). Because [FFA] was higher between 60-120 min and FA Rox was higher at 60 min, FFA uptake by skeletal muscle was most likely higher in older participants. We do not suspect that a greater amount of FAs were oxidized in older participants because the AUCs for FA Rox (Fig. 8 and 12) were not different between groups ($p \geq 0.32$). Consequently, intracellular FA concentrations could have been transiently elevated and caused alterations in insulin signaling to reduce rates of glucose uptake in older participants (85, 86, 122-124). While this model would explain the delayed rise in CHO Rox, it does not completely explain the delayed suppression of FA Rox since FA Rox was influenced by lactate suppression of lipolysis. Still, the rise in CHO Rox and thus lactate production occurred simultaneously with the suppression of lipolysis but before the suppression of FA Rox in older participants. Therefore, lactate can be viewed as permissive to insulin in correcting postprandial lipid substrate partitioning in our older participants. From this perspective, lactate is not a hinderance to postprandial metabolic flexibility in aging. This proposed role of lactate is notably in contrast to its restrictive effects on metabolic flexibility during exercise in individuals with poor lactate clearance capacities (38).

Methodological considerations. In the present study, respiratory gases were collected with a mouthpiece. A face mask that covers the nose and mouth and a ventilated canopy hood (hereafter, "canopy") that is placed over the head of an individual laying supine have also been used historically to collect respiratory gases for the determination of EE and substrate oxidation rates during non-exercise conditions. Segal (125) and Isbell et al. (126) reported that respiratory gas values obtained from these three devices yielded comparable results. Our own pilot testing also yielded comparable results. Because we sought to determine immediate (i.e., 5 min after glucose consumption) changes in substrate oxidation rates, we employed the mouthpiece to minimize the amount of time needed for participants to acclimate to the experimental conditions. However, other recent investigations (29, 127) exclusively use the canopy, presumably because it is regarded as the "gold standard" method for the determination of EE since other devices purportedly overestimate EE (128). Despite its reputation as the "gold standard" method, the same recent investigations have employed correction procedures for values obtained using the canopy due to the variability in data obtained from different indirect calorimetry systems (29, 129). We opted not to use such correction procedures. Compared to extant resting data obtained from a canopy, we observed higher EE but comparable CHO Rox as Alcantara et al. (29) and comparable CHO Rox but higher FA Rox as Ritz et al. (130). We also observed lower relative CHO Rox and higher relative FA Rox as reported by Kruszynska et al. (26), who utilized heavier participants. Importantly, the observed changes in CHO Rox and FA Rox during the OGTT most closely resemble those reported by Leclerc et al. (27). Because much of our data generally agrees with extant data and given the amount of variability in extant data obtained from the canopy, we conclude that our use of a mouthpiece is not a limitation or major factor that explains any discrepancies in the data we report. Rather, variability in the populations studied (e.g., age, total body weight, body fat mass), in experimental conditions (e.g., amount of time fasted), or inherently present in the different measurement systems better explain the differences between our present data and extant data.

CONCLUSION

Our results demonstrate that the fall in total FA oxidation and rise in total CHO oxidation following oral glucose consumption are delayed in healthy older individuals compared to young individuals. We also demonstrate that reesterification is not the primary fate of FA released from lipolysis during an OGTT. In aging, FA reesterification is demoted in favor of FA oxidation. Moreover, because FA Rox was inversely correlated with Lactate Rox and [Lactate], we provide support for lactate control of metabolic flexibility through the direct (via intracellular inhibition of mitochondrial FA uptake) and indirect (via inhibition of lipolysis) suppression of FA oxidation, respectively. In our older but not young participants, the suppression of FA Rox was dependent on lactate suppression of lipolysis, suggesting that metabolic flexibility of energy substrate partitioning in the postprandial period is negatively affected in aging. Rephrased, our young participants suppressed FA Rox in response to oral glucose consumption, but our older participants suppressed FA Rox due to reduced FA availability. From this perspective, lactate rescues postprandial metabolic flexibility in aging by supporting the suppression of lipolysis and FA oxidation. Future similar investigations should utilize glycerol and FA isotope tracers in tandem to clarify the fates of circulating FFA during an OGTT. Notably, the changes in substrate oxidation rates in our healthy older participants were delayed, but not restricted. Therefore,

future investigations might observe the beneficial effects of endurance exercise training on postprandial substrate oxidation and reesterification rates in metabolic disease states.

FIGURE LEGENDS

Figure 1. Rate of energy expenditure (EE) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 2. Rate of energy expenditure (EE) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 3. Area under the curve (AUC) for rate of energy expenditure (EE) per unit of body mass in young and older participants. Values are means ± SE. *Significantly different between groups, $p < 0.05$.

Figure 4. Rate of energy expenditure (EE) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 5. Area under the curve (AUC) for rate of energy expenditure (EE) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

Figure 6. Rate of energy expenditure (EE) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 7. Rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 8. Area under the curve (AUC) for rate of total fatty acid (FA) oxidation (Rox) per unit of total body mass in young and older participants. Values are means ± SE.

Figure 9. Rate of total fatty acid (FA) oxidation (Rox) per unit of body mass, expressed as a percentage increase from 0 min, in young and older participants at 5 min and 15 min. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 10. Rate of total fatty acid (FA) oxidation (Rox) per unit of body mass, expressed as a percentage decrease from 0 min, in young and older participants between 60-120 min. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 11. Rate of total fatty acid (FA) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 12. Area under the curve (AUC) for rate of total fatty acid (FA) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

Figure 13. Rate of total fatty acid (FA) oxidation (Rox) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 14. Rate of total lipid oxidation (Rox) expressed in units of $kcal \cdot min^{-1}$ (A) and $mg \cdot min^{-1}$ (B) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 15. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 16. Area under the curve (AUC) for rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants. Values are means ± SE. *Significantly different between groups, $p < 0.05$.

Figure 17. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass, expressed as a percentage increase from 0 min, in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 18. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 19. Area under the curve (AUC) for rate of total carbohydrate (CHO) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

Figure 20. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 21. Rate of total carbohydrate (CHO) oxidation (Rox) expressed in units of $kcal \cdot min^{-1}$ (A) and $mg \cdot min^{-1}$ (B) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 22. Rate of total fatty acid (FA) reesterification (Rs) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 23. Area under the curve (AUC) for rate of total fatty acid (FA) reesterification (Rs) per unit of total body mass in young and older participants. Values are means ± SE.

Figure 24. Rate of total fatty acid (FA) reesterification (Rs) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 25. Area under the curve (AUC) for rate of total fatty acid (FA) reesterification (Rs) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

Figure 26. Rate of total fatty acid (FA) reesterification (Rs) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 27. Rate of total fatty acid (FA) reesterification (Rs) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 28. Area under the curve (AUC) for rates of total fatty acid (FA) oxidation (Rox) and reesterification (Rs) per unit of total body mass in young and older participants. Values are means ± SE. ^Significantly different between FA Rox and FA Rs, $p < 0.05$.

Figure 29. Area under the curve (AUC) for rates of total fatty acid (FA) oxidation (Rox) and reesterification (Rs) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE. ^Significantly different between FA Rox and FA Rs, $p < 0.05$.

Figure 30. Relationship between rates of total carbohydrate (CHO) and lipid oxidation in young and older participants.

Figure 31. Relationship between rates of total carbohydrate (CHO) and total fatty acid (FA) oxidation per unit of body mass in young and older participants.

Figure 32. Relationship between glycerol rate of appearance (Ra) and rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants.

Figure 33. Relationship between glycerol rate of appearance (Ra) and rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants.

Figure 34. Relationship between plasma free fatty acid (FFA) concentrations and rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants.

Figure 35. Relationship between plasma free fatty acid (FFA) concentrations and rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants.

Figure 36. Relationship between plasma free fatty acid (FFA) concentrations and rate of total fatty acid (FA) reesterification (Rs) per unit of body mass in young and older participants.

Figure 37. Relationship between triglyceride (TG) concentrations and rate of total fatty acid (FA) reesterification (Rs) per unit of body mass in young and older participants.

Figure 38. Relationship between blood lactate concentrations and rate of total fatty acid (FA) oxidation per unit of body mass in young and older participants.

Table 1. Characteristics of young and older participants.

Variable	Young	Older
Age, yr	26.40 ± 1.08	71.14 ± 1.48 *
Body mass, kg	66.63 ± 2.70	73.21 ± 4.18
Body mass index, kg/m^2	23.47 ± 0.77	26.32 ± 0.92 *
Body fat, %	17.27 ± 1.83	23.13 ± 1.29 *
Fat mass, kg	11.24 ± 1.16	16.83 ± 1.27 *
Fat free mass, kg	55.39 ± 2.93	56.39 ± 3.48
Relative glucose load,		
$g \cdot kg^{-1}$ body mass	1.15 ± 0.04	1.07 ± 0.07
$g \cdot kg^{-1}$ fat mass	7.67 ± 0.83	4.81 ± 0.35 *
$g \cdot kg^{-1}$ fat free mass	1.41 ± 0.07	1.41 ± 0.09
FEV_1/FVC, %	84.52 ± 1.98	79.84 ± 1.64
$\dot{V}O_2peak$,		
$L \cdot min^{-1}$	2.84 ± 0.25	1.91 ± 0.17 *
$mL \cdot kg^{-1} \cdot min^{-1}$	41.80 ± 2.93	26.18 ± 1.59 *
Peak Power Output, W	267.00 ± 21.06	170.42 ± 17.45 *
VT, % $\dot{V}O_2peak$	73.35 ± 0.64	60.99 ± 0.94 *
3-day Diet Records		
Energy, $kcal \cdot day^{-1}$	2573.32 ± 105.74	2305.52 ± 105.27
Carbohydrate, %	61.25 ± 0.34	60.08 ± 0.61
Fat, %	29.69 ± 0.52	29.99 ± 0.44
Protein, %	9.06 ± 0.26	9.93 ± 0.34

Values are means ± SE. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $\dot{V}O_2peak$, peak O_2 consumption; VT, ventilatory threshold. *Significantly different between groups, $p < 0.05$.

Table 2. Absolute postabsorptive measurements in young and older participants.

Variable	Young	Older	*p* value
EE, kcal·min^{-1}	1.13 ± 0.04	1.16 ± 0.06	0.74
% Lipid	61.54 ± 1.26	61.87 ± 2.15	0.90
% CHO	38.02 ± 1.37	38.13 ± 2.15	0.97
RER	0.82 ± 0.004	0.82 ± 0.006	0.93
$\dot{V}O_2$, L·min^{-1}	0.23 ± 0.01	0.24 ± 0.01	0.76
Lipid Rox,			
kcal·min^{-1}	0.66 ± 0.02	0.70 ± 0.05	0.47
mg·min^{-1}	69.59 ± 2.14	73.54 ± 4.86	0.48
CHO Rox,			
kcal·min^{-1}	0.44 ± 0.02	0.46 ± 0.03	0.68
mg·min^{-1}	105.09 ± 4.57	109.34 ± 7.76	0.65
FA Rs, μmol·min^{-1}	146.44 ± 4.52	133.92 ± 5.96	0.12

Values are means ± SE. EE, total energy expenditure; % Lipid, percentage of EE derived from lipid; % CHO, percentage of EE derived from carbohydrates; RER, respiratory exchange ratio; $\dot{V}O_2$, rate of oxygen consumption; Lipid Rox; rate of total lipid oxidation; CHO Rox, rate of total carbohydrate oxidation; FA Rs, rate of total fatty acid reesterification.

Table 3. Relative postabsorptive measurements in young and older participants.

Variable	Young	Older	*p* value
EE			
μmol·kg^{-1}·min^{-1}	10.15 ± 0.28	9.57 ± 0.47	0.32
μmol·kg FFM^{-1}·min^{-1}	12.50 ± 0.49	12.39 ± 0.59	0.90
μmol·kg FM^{-1}·min^{-1}	70.27 ± 9.00	43.14 ± 3.33 *	0.01
FA Rox			
μmol·kg^{-1}·min^{-1}	3.75 ± 0.10	3.51 ± 0.12	0.14
μmol·kg FFM^{-1}·min^{-1}	4.57 ± 0.17	4.58 ± 0.17	0.94
μmol·kg FM^{-1}·min^{-1}	25.41 ± 2.89	15.73 ± 0.96 *	0.007
CHO Rox			
μmol·kg^{-1}·min^{-1}	8.86 ± 0.30	8.40 ± 0.51	0.45
μmol·kg FFM^{-1}·min^{-1}	10.83 ± 0.43	10.87 ± 0.63	0.96
μmol·kg FM^{-1}·min^{-1}	61.62 ± 8.06	37.69 ± 3.08 *	0.02
FA Rs			
μmol·kg^{-1}·min^{-1}	2.28 ± 0.14	1.93 ± 0.17	0.15
μmol·kg FFM^{-1}·min^{-1}	2.82 ± 0.21	2.58 ± 0.26	0.51
μmol·kg FM^{-1}·min^{-1}	14.60 ± 1.34	8.23 ± 0.53 *	<0.001

Values are means ± SE. EE, total energy expenditure; FA Rox; rate of total fatty acid oxidation; CHO Rox, rate of total carbohydrate oxidation; FA Rs, rate of total fatty acid reesterification.
*Significantly different between groups, $p < 0.05$.

Table 4. Percentage of energy expenditure derived from lipids in young and older participants over time.

Time (min)	**Young** (%)	**Older** (%)
0	61.54 ± 1.26	61.87 ± 2.15
5	57.78 ± 1.54 *	63.98 ± 2.44
15	57.34 ± 1.96	61.02 ± 1.95
30	49.21 ± 2.94 #	55.43 ± 2.14
60	22.49 ± 3.01 # *	35.72 ± 3.06 #
90	18.98 ± 2.58 #	26.85 ± 3.76 #
120	18.81 ± 1.91 #	18.64 ± 3.18 #

Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Table 5. Percentage of energy expenditure derived from carbohydrates in young and older participants over time.

Time (min)	**Young** (%)	**Older** (%)
0	38.02 ± 1.37	38.13 ± 2.15
5	42.04 ± 1.47 *	36.02 ± 2.44
15	42.66 ± 1.96	38.77 ± 2.04
30	50.76 ± 2.93 #	44.36 ± 2.25
60	76.19 ± 3.84 # *	63.42 ± 3.58 #
90	80.83 ± 2.68 #	73.15 ± 3.76 #
120	80.42 ± 1.52 #	80.78 ± 3.47 #

Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Table 6. Changes in the respiratory exchange ratio (RER) in young and older participants over time.

Time (min)	Young	Older
0	0.82 ± 0.004	0.82 ± 0.006
5	0.83 ± 0.005	0.81 ± 0.007
15	0.83 ± 0.006	0.82 ± 0.006
30	0.86 ± 0.010	0.84 ± 0.007
60	0.93 ± 0.012 # *	0.89 ± 0.010 #
90	0.94 ± 0.008 #	0.92 ± 0.011 #
120	0.94 ± 0.005 #	0.94 ± 0.011 #

Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Table 7. Changes in the rate of oxygen consumption ($\dot{V}O_2$) in young and older participants over time.

Time (min)	Young		Older	
	$L \cdot min^{-1}$	$mL \cdot kg^{-1} \cdot min^{-1}$	$L \cdot min^{-1}$	$mL \cdot kg^{-1} \cdot min^{-1}$
0	0.23 ± 0.01	3.53 ± 0.05 *	0.24 ± 0.01	3.29 ± 0.03
5	0.26 ± 0.01 #	3.88 ± 0.08 # *	0.26 ± 0.01 #	3.55 ± 0.08 #
15	0.27 ± 0.01 #	4.08 ± 0.08 #	0.27 ± 0.01 #	3.79 ± 0.12 #
30	0.29 ± 0.01 #	4.34 ± 0.10 # *	0.28 ± 0.01 #	3.82 ± 0.08 #
60	0.27 ± 0.01 #	4.14 ± 0.09 # *	0.27 ± 0.01 #	3.76 ± 0.06 #
90	0.26 ± 0.01 #	3.98 ± 0.08 # *	0.27 ± 0.01 #	3.70 ± 0.07 #
120	0.25 ± 0.01 #	3.83 ± 0.07 # *	0.26 ± 0.01 #	3.57 ± 0.07 #

Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Table 8. Correlation matrix of total carbohydrate and total lipid/fatty acid oxidation rates in young participants.

Lipid/FA Rox	**CHO Rox**			
	kcal	μmol·kg TBM^{-1}	μmol·kg FFM^{-1}	μmol·kg FM^{-1}
kcal	r = -0.94 p = 0.002	r = -0.94 p = 0.002	r = -0.94 p = 0.002	r = -0.95 p = 0.001
μmol·kg TBM^{-1}	r = -0.94 p = 0.001	r = -0.94 p = 0.001	r = -0.94 p = 0.002	r = -0.96 p = 0.001
μmol·kg FFM^{-1}	r = -0.95 p = 0.001	r = -0.95 p = 0.001	r = -0.95 p = 0.002	r = -0.96 p = 0.001
μmol·kg FM^{-1}	r = -0.93 p = 0.002	r = -0.93 p = 0.002	r = -0.93 p = 0.003	r = -0.95 p = 0.001

All units are per minute, e.g., kcal·min^{-1}. CHO, carbohydrate; FA, fatty acid; Rox, rate of oxidation; TBM, total body mass; FFM, fat free mass; FM, fat mass.

Table 9. Correlation matrix of total carbohydrate and total lipid/fatty acid oxidation rates in older participants.

Lipid/FA Rox	**CHO Rox**			
	kcal	μmol·kg TBM^{-1}	μmol·kg FFM^{-1}	μmol·kg FM^{-1}
kcal	r = -0.98 p < 0.001	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.96 p < 0.001
μmol·kg TBM^{-1}	r = -0.97 p < 0.001	r = -0.96 p < 0.001	r = -0.97 p < 0.001	r = -0.96 p = 0.001
μmol·kg FFM^{-1}	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.96 p = 0.001
μmol·kg FM^{-1}	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.96 p = 0.001

All units are per minute, e.g., kcal·min^{-1}. CHO, carbohydrate; FA, fatty acid; Rox, rate of oxidation; TBM, total body mass; FFM, fat free mass; FM, fat mass.

Table 10. Correlation matrix of glycerol rate of appearance with total lipid and fatty acid oxidation rate in young participants.

Lipid/FA Rox	**Glycerol Ra**			
	µmol	µmol·kg TBM^{-1}	µmol·kg FFM^{-1}	µmol·kg FM^{-1}
mg	$r = 0.93$ $p = 0.002$	$r = 0.94$ $p = 0.002$	$r = 0.94$ $p = 0.001$	$r = 0.94$ $p = 0.002$
µmol·kg TBM^{-1}	$r = 0.94$ $p = 0.002$	$r = 0.94$ $p = 0.002$	$r = 0.95$ $p = 0.001$	$r = 0.94$ $p = 0.001$
µmol·kg FFM^{-1}	$r = 0.93$ $p = 0.002$	$r = 0.94$ $p = 0.002$	$r = 0.94$ $p = 0.002$	$r = 0.94$ $p = 0.002$
µmol·kg FM^{-1}	$r = 0.94$ $p = 0.001$	$r = 0.95$ $p = 0.001$	$r = 0.95$ $p = 0.001$	$r = 0.95$ $p = 0.001$

All units are per minute, e.g., µmol·min^{-1}. Ra, rate of appearance; FA, fatty acid; Rox, rate of oxidation; TBM, total body mass; FFM, fat free mass; FM, fat mass.

Table 11. Correlation matrix of glycerol rate of appearance with total lipid and fatty acid oxidation rate in older participants.

Lipid/FA Rox	**Glycerol Ra**			
	μmol	μmol·kg TBM^{-1}	μmol·kg FFM^{-1}	μmol·kg FM^{-1}
mg	$r = 0.88$ $p = 0.01$	$r = 0.89$ $p = 0.01$	$r = 0.89$ $p = 0.01$	$r = 0.89$ $p = 0.01$
μmol·kg TBM^{-1}	$r = 0.86$ $p = 0.01$	$r = 0.87$ $p = 0.01$	$r = 0.87$ $p = 0.01$	$r = 0.87$ $p = 0.01$
μmol·kg FFM^{-1}	$r = 0.86$ $p = 0.01$	$r = 0.87$ $p = 0.01$	$r = 0.87$ $p = 0.01$	$r = 0.87$ $p = 0.01$
μmol·kg FM^{-1}	$r = 0.85$ $p = 0.02$	$r = 0.87$ $p = 0.01$	$r = 0.87$ $p = 0.01$	$r = 0.86$ $p = 0.01$

All units are per minute, e.g., μmol·min^{-1}. Ra, rate of appearance; FA, fatty acid; Rox, rate of oxidation; TBM, total body mass; FFM, fat free mass; FM, fat mass.

Table 12. Correlation matrix of glycerol rate of appearance with total carbohydrate oxidation rate in young participants.

CHO Rox	**Glycerol Ra**			
	μmol	μmol·kg TBM^{-1}	μmol·kg FFM^{-1}	μmol·kg FM^{-1}
mg	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.98 p < 0.001	r = -0.98 p < 0.001
μmol·kg TBM^{-1}	r = -0.98 p < 0.001	r = -0.98 p < 0.001	r = -0.98 p < 0.001	r = -0.98 p < 0.001
μmol·kg FFM^{-1}	r = -0.98 p < 0.001	r = -0.98 p < 0.001	r = -0.98 p < 0.001	r = -0.98 p < 0.001
μmol·kg FM^{-1}	r = -0.97 p < 0.001	r = -0.97 p < 0.001	r = -0.98 p < 0.001	r = -0.98 p < 0.001

All units are per minute, e.g., μmol·min^{-1}. Ra, rate of appearance; CHO, carbohydrate; Rox, rate of oxidation; TBM, total body mass; FFM, fat free mass; FM, fat mass.

Table 13. Correlation matrix of glycerol rate of appearance with total carbohydrate oxidation rate in older participants.

CHO Rox	**Glycerol Ra**			
	μmol	μmol·kg TBM^{-1}	μmol·kg FFM^{-1}	μmol·kg FM^{-1}
mg	r = -0.95 p < 0.001	r = -0.96 p < 0.001	r = -0.96 p < 0.001	r = -0.95 p < 0.001
μmol·kg TBM^{-1}	r = -0.95 p < 0.001	r = -0.96 p < 0.001	r = -0.96 p < 0.001	r = -0.95 p < 0.001
μmol·kg FFM^{-1}	r = -0.95 p < 0.001	r = -0.96 p < 0.001	r = -0.96 p < 0.001	r = -0.95 p < 0.001
μmol·kg FM^{-1}	r = -0.95 p < 0.001	r = -0.96 p < 0.001	r = -0.96 p < 0.001	r = -0.96 p < 0.001

All units are per minute, e.g., μmol·min^{-1}. Ra, rate of appearance; CHO, carbohydrate; Rox, rate of oxidation; TBM, total body mass; FFM, fat free mass; FM, fat mass.

Table 14. Summary of correlations of variables related to substrate availability and utilization.

	Young		Older	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Lipid/FA Rox and [FFA]				
$mg \cdot min^{-1}$	0.93	0.002	0.96	0.001
$\mu mol\ FA \cdot kg^{-1} \cdot min^{-1}$	0.93	0.002	0.95	0.001
$\mu mol\ FA \cdot kg\ FFM^{-1} \cdot min^{-1}$	0.92	0.003	0.95	0.001
$\mu mol\ FA \cdot kg\ FM^{-1} \cdot min^{-1}$	0.95	0.001	0.95	0.001
CHO Rox and [FFA]				
$mg \cdot min^{-1}$	-0.96	<0.001	-0.98	<0.001
$\mu mol \cdot kg^{-1} \cdot min^{-1}$	-0.97	<0.001	-0.99	<0.001
$\mu mol \cdot kg\ FFM^{-1} \cdot min^{-1}$	-0.97	<0.001	-0.99	<0.001
$\mu mol \cdot kg\ FM^{-1} \cdot min^{-1}$	-0.97	<0.001	-0.99	<0.001
FA Rs and [FFA]				
$\mu mol \cdot min^{-1}$	-0.55	0.20	-0.60	0.16
$\mu mol \cdot kg^{-1} \cdot min^{-1}$	-0.50	0.25	-0.61	0.14
$\mu mol \cdot kg\ FFM^{-1} \cdot min^{-1}$	-0.48	0.27	-0.61	0.15
$\mu mol \cdot kg\ FM^{-1} \cdot min^{-1}$	-0.61	0.14	-0.60	0.15
FA Rs and [TG]				
$\mu mol \cdot min^{-1}$	-0.78	0.04	-0.75	0.05
$\mu mol \cdot kg^{-1} \cdot min^{-1}$	-0.80	0.03	-0.73	0.06
$\mu mol \cdot kg\ FFM^{-1} \cdot min^{-1}$	-0.82	0.03	-0.73	0.06
$\mu mol \cdot kg\ FM^{-1} \cdot min^{-1}$	-0.72	0.07	-0.75	0.05

FA, fatty acid; CHO, carbohydrate; Rox, rate of oxidation; [FFA], plasma free fatty acid concentrations; [TG], plasma triglyceride concentrations; Rs, rate of reesterification.

Table 15. Summary of correlations of blood lactate concentrations with total lipid and fatty acid oxidation rates.

	Young		Older	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
FA Rox, μmol·kg^{-1}·min^{-1}				
Overall	-0.62	0.14	-0.84	0.02
Enteric	0.68	0.32	0.22	0.78
Systemic	0.94	0.23	-0.95	0.19
FA Rox, μmol·kg FFM^{-1}·min^{-1}				
Overall	-0.61	0.14	-0.84	0.02
Enteric	0.85	0.15	0.22	0.78
Systemic	0.94	0.22	-0.95	0.21
FA Rox, μmol·kg FM^{-1}·min^{-1}				
Overall	-0.63	0.13	-0.83	0.02
Enteric	0.40	0.60	0.32	0.68
Systemic	0.89	0.30	-0.96	0.18
Lipid Rox, kcal·min^{-1}				
Overall	-0.61	0.15	-0.85	0.01
Enteric	0.74	0.26	0.03	0.97
Systemic	0.94	0.88	-0.96	0.19

Overall, 0-120 min; Enteric, 0-30 min; Systemic, 60-120 min; FA, fatty acid; Rox, rate of oxidation; FFM, fat free mass; FM, fat mass.

Table 16. Summary of correlations of total lactate oxidation rates with total lipid and fatty acid oxidation rates.

	Young		Older	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Lactate Rox and FA Rox, TBM				
Overall	-0.73	0.06	-0.83	0.02
Enteric	0.65	0.35	0.91	0.10
Systemic	-0.97	0.17	-0.96	0.18
Lactate Rox and FA Rox, FFM				
Overall	-0.71	0.07	-0.84	0.02
Enteric	0.76	0.24	0.92	0.08
Systemic	-0.97	0.16	-0.95	0.21
Lactate Rox and FA Rox, FM				
Overall	-0.79	0.03	-0.79	0.04
Enteric	0.25	0.75	0.87	0.13
Systemic	-0.92	0.26	-0.98	0.13
Lactate Rox and Lipid Rox, Abs				
Overall	-0.70	0.08	-0.84	0.02
Enteric	0.67	0.33	0.83	0.17
Systemic	-0.97	0.16	-0.97	0.16

Overall, 0-120 min; Enteric, 0-30 min; Systemic, 60-120 min; FA, fatty acid; Rox, rate of oxidation; TMB, relative to total body mass; FFM, relative to fat free mass; FM, relative to fat mass; Abs, absolute rate.

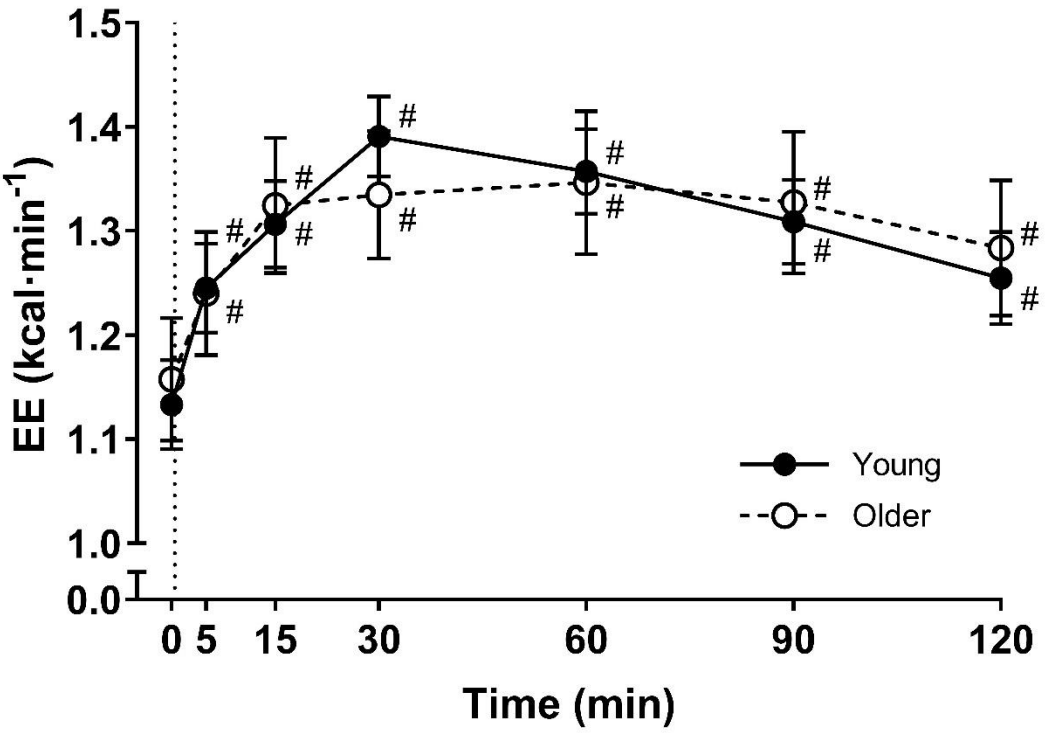

Figure 1. Rate of energy expenditure (EE) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

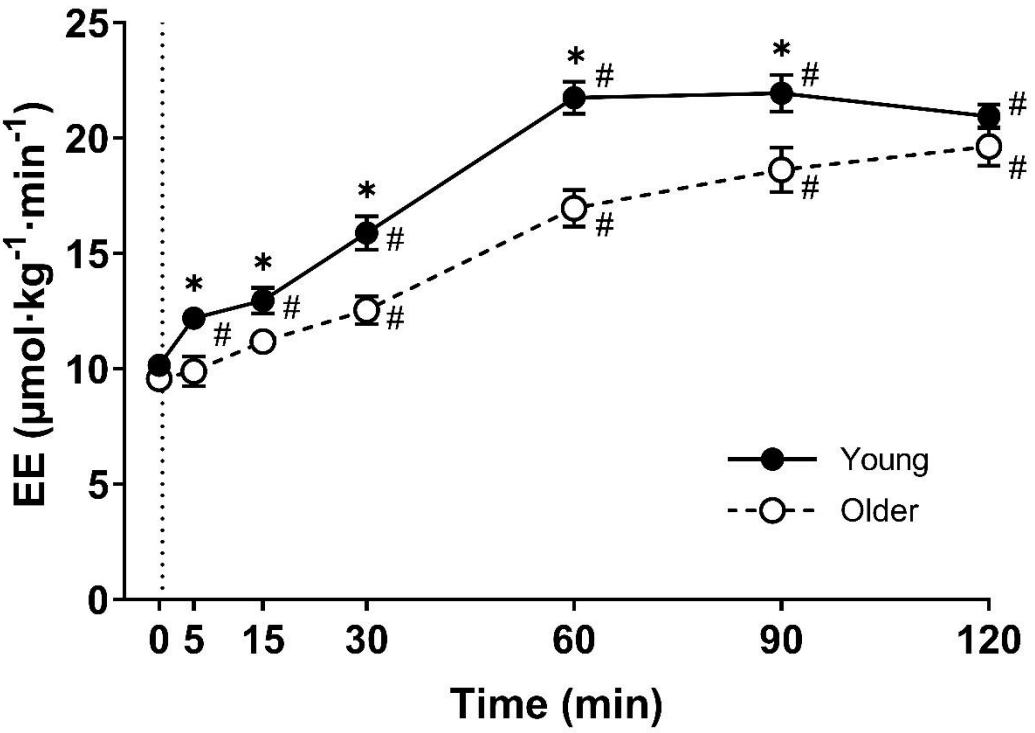

Figure 2. Rate of energy expenditure (EE) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

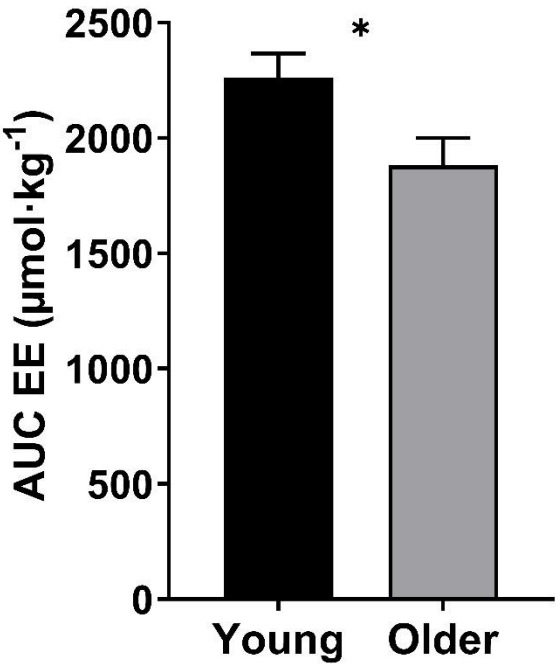

Figure 3. Area under the curve (AUC) for rate of energy expenditure (EE) per unit of body mass in young and older participants. Values are means ± SE. *Significantly different between groups, $p < 0.05$.

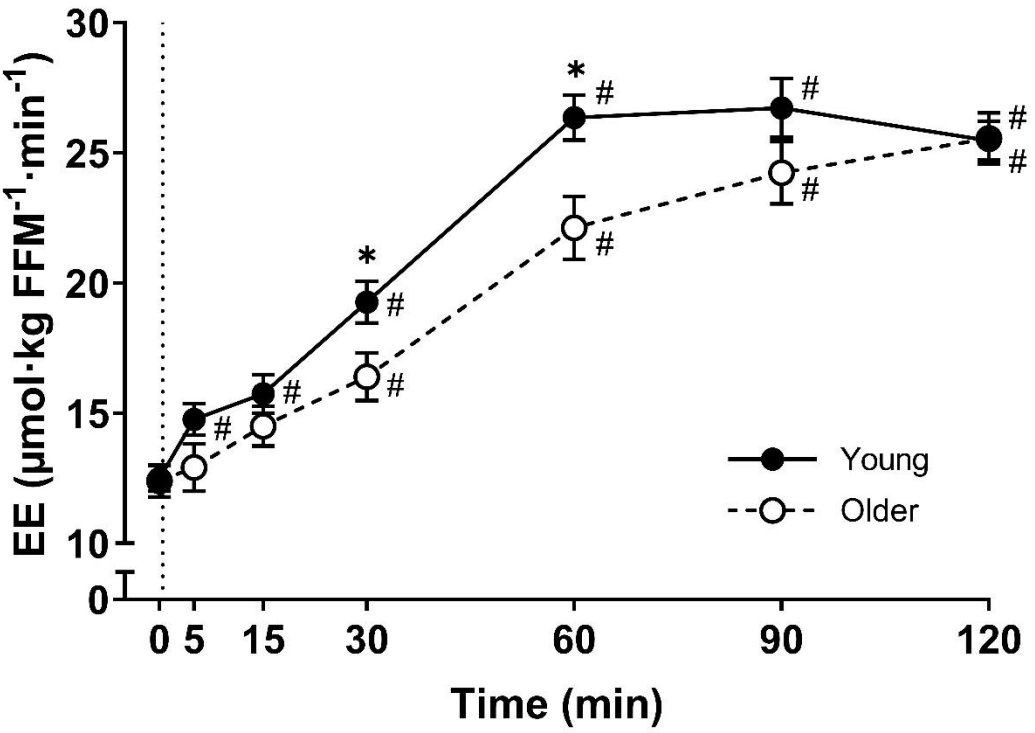

Figure 4. Rate of energy expenditure (EE) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

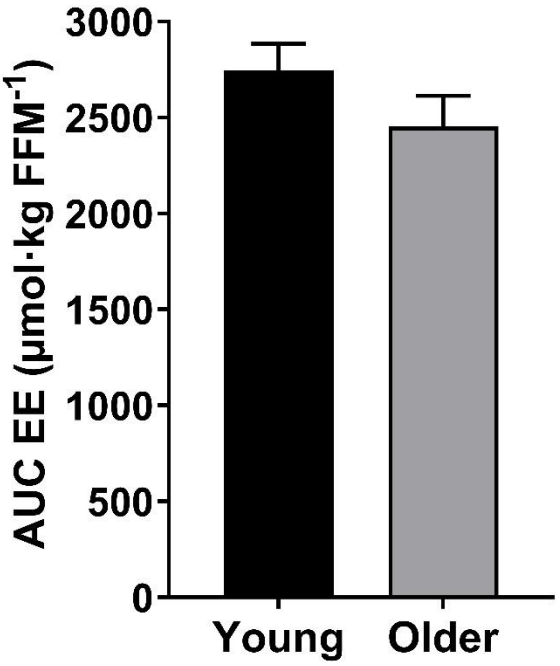

Figure 5. Area under the curve (AUC) for rate of energy expenditure (EE) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

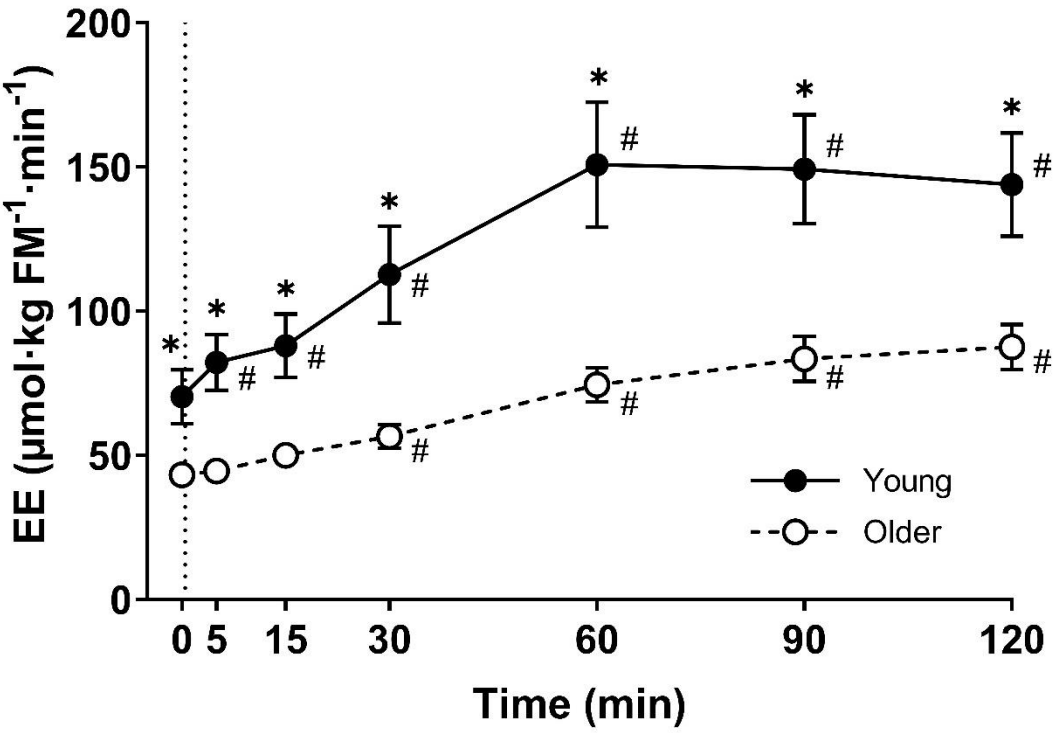

Figure 6. Rate of energy expenditure (EE) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

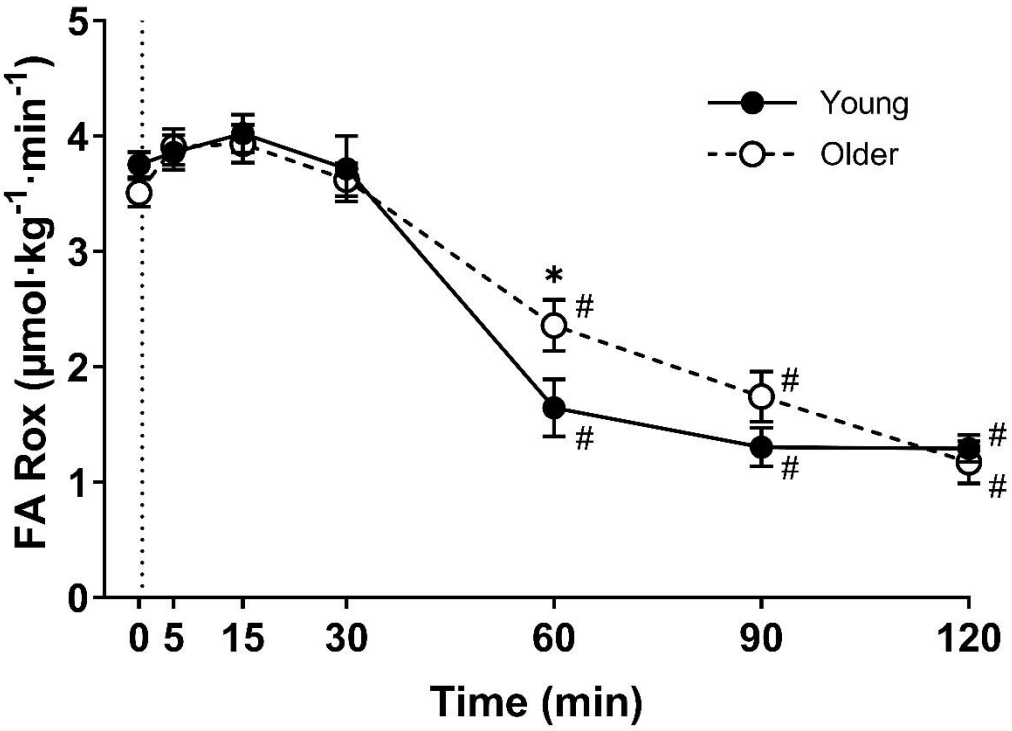

Figure 7. Rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

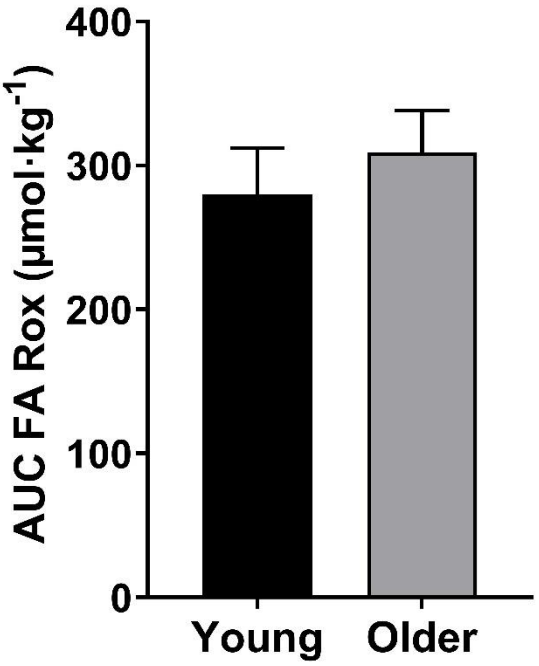

Figure 8. Area under the curve (AUC) for rate of total fatty acid (FA) oxidation (Rox) per unit of total body mass in young and older participants. Values are means ± SE.

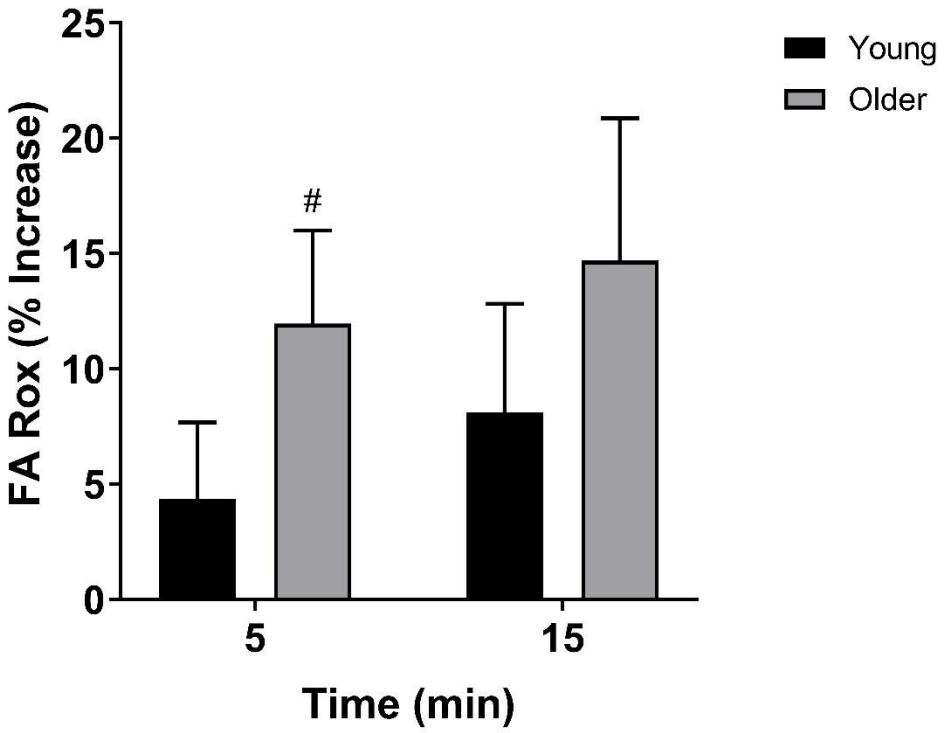

Figure 9. Rate of total fatty acid (FA) oxidation (Rox) per unit of body mass, expressed as a percentage increase from 0 min, in young and older participants at 5 min and 15 min. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

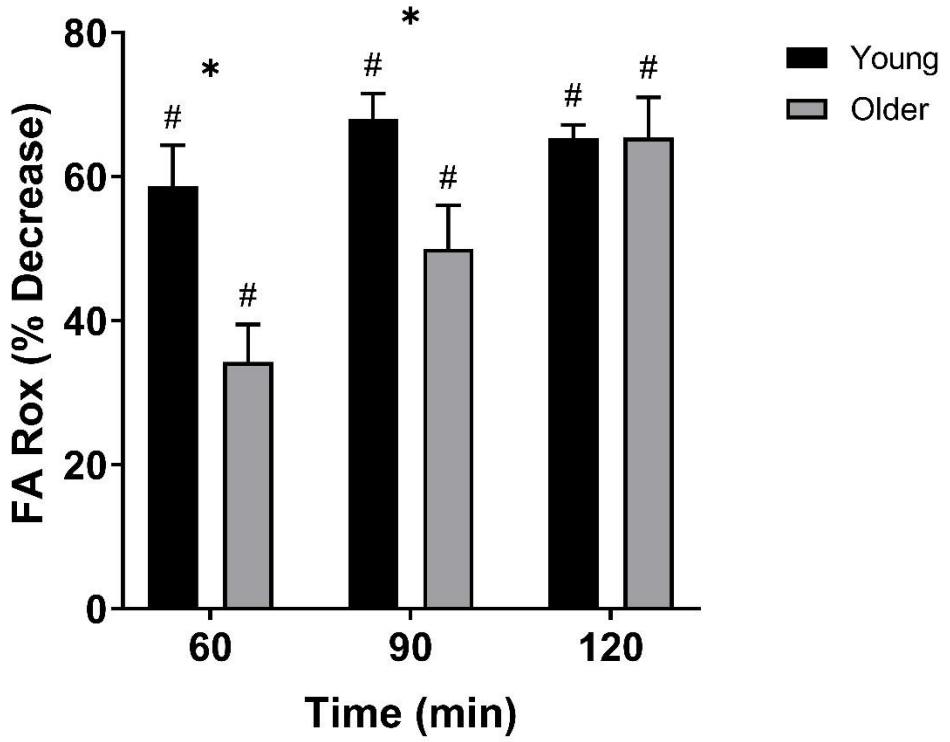

Figure 10. Rate of total fatty acid (FA) oxidation (Rox) per unit of body mass, expressed as a percentage decrease from 0 min, in young and older participants between 60-120 min. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

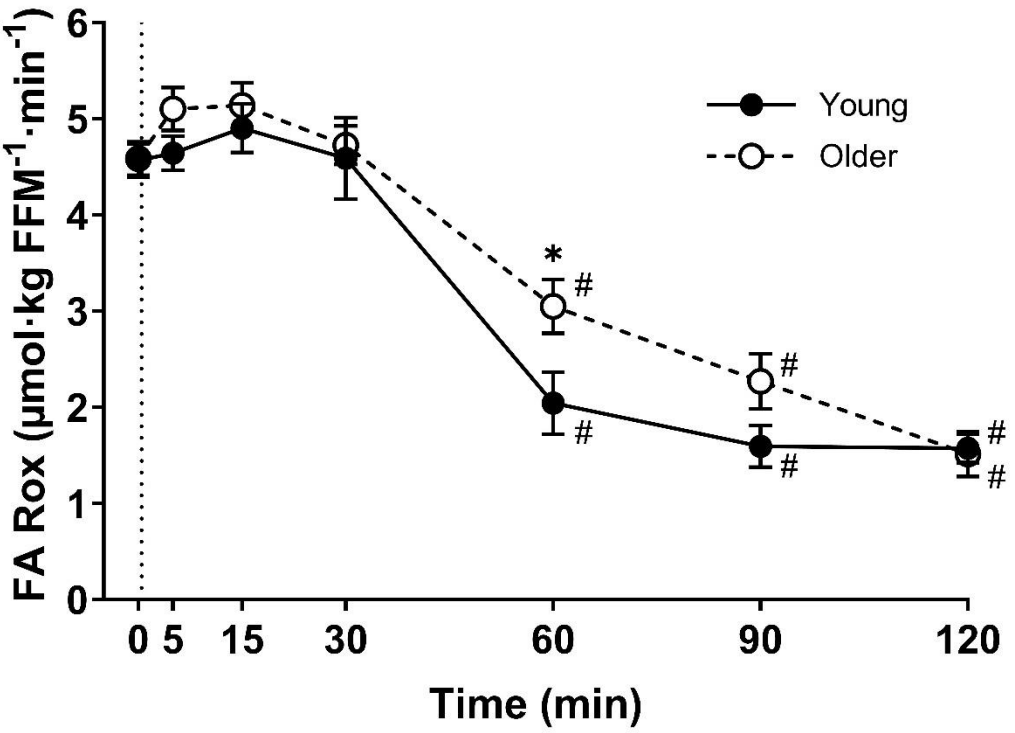

Figure 11. Rate of total fatty acid (FA) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

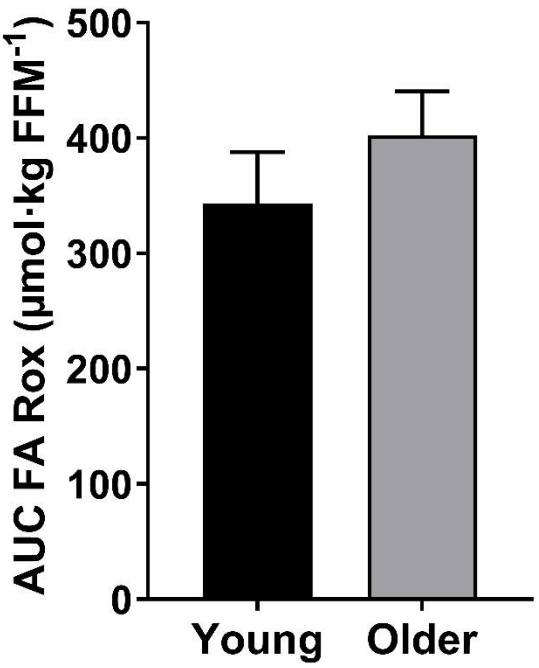

Figure 12. Area under the curve (AUC) for rate of total fatty acid (FA) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

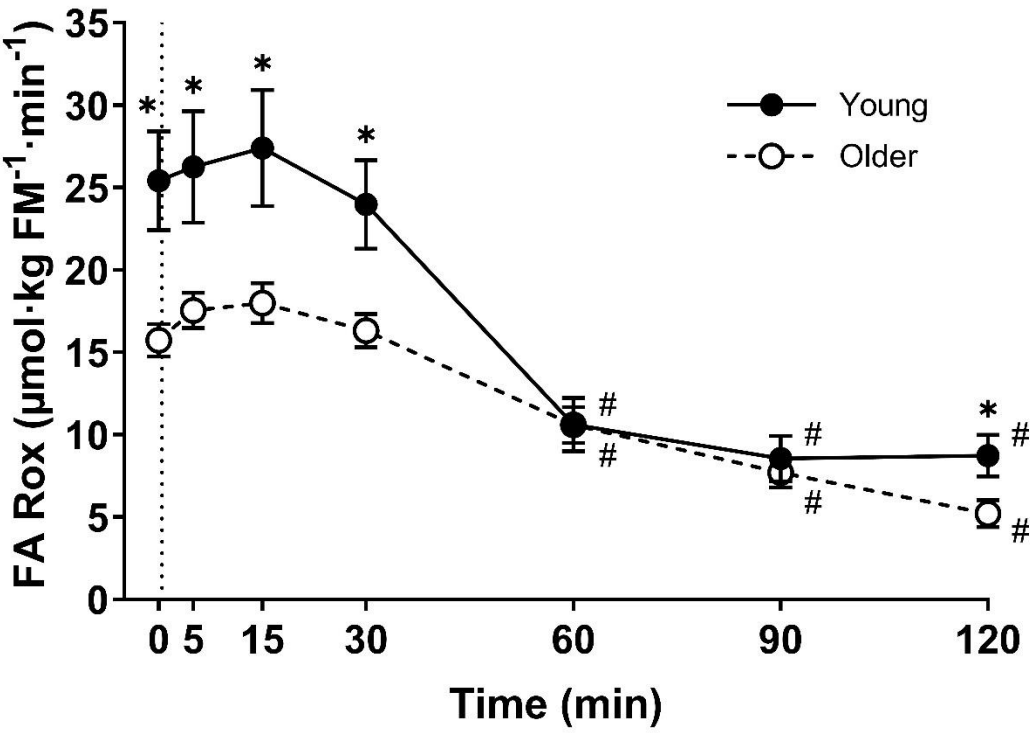

Figure 13. Rate of total fatty acid (FA) oxidation (Rox) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

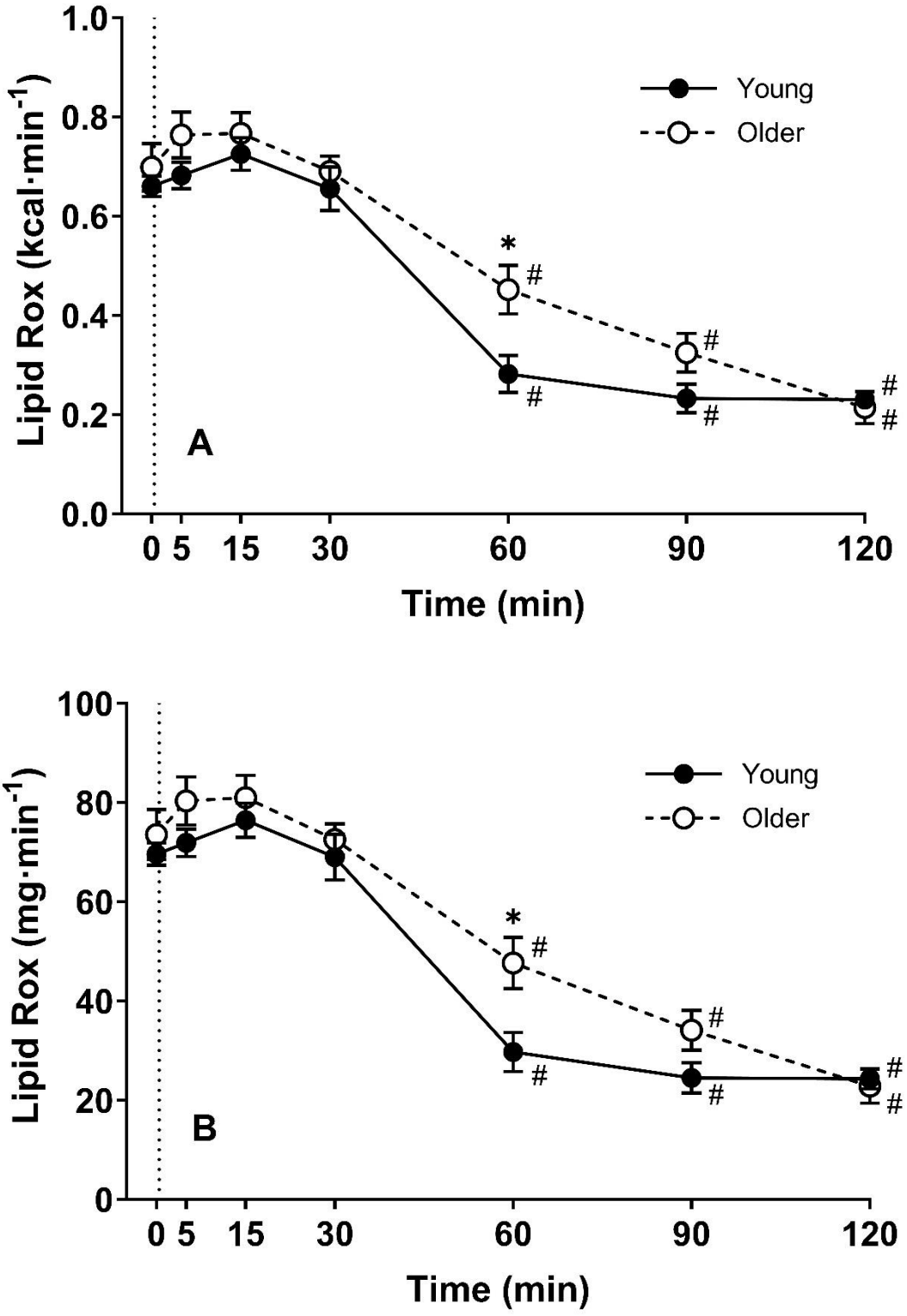

Figure 14. Rate of total lipid oxidation (Rox) expressed in units of kcal·min^{-1} (A) and mg·min^{-1} (B) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

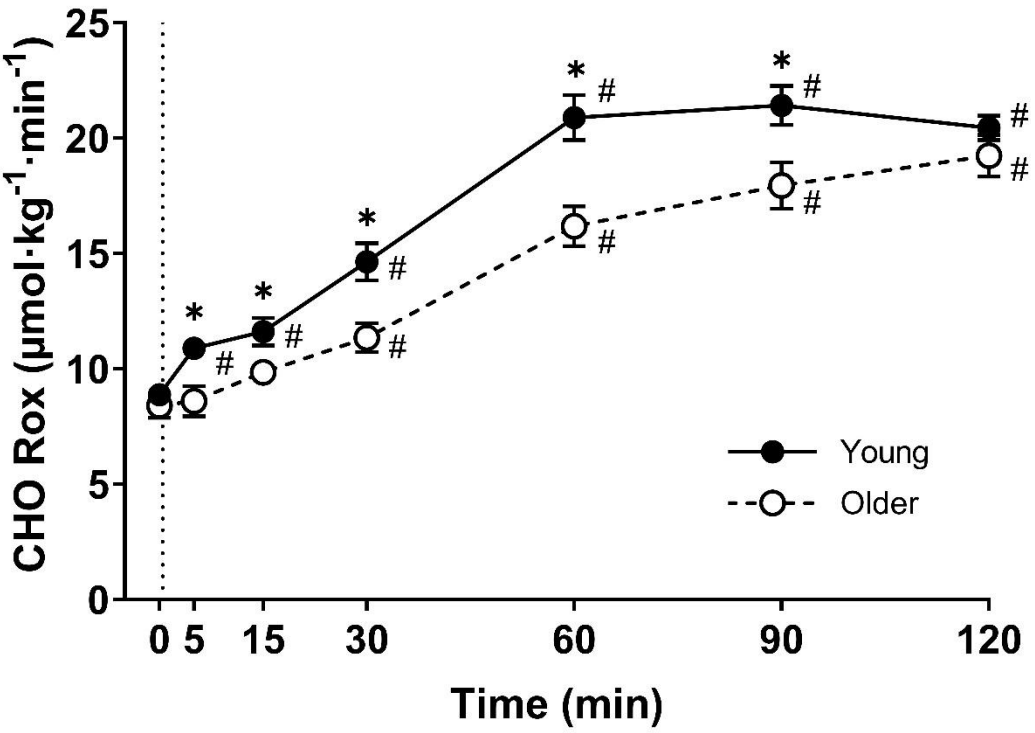

Figure 15. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

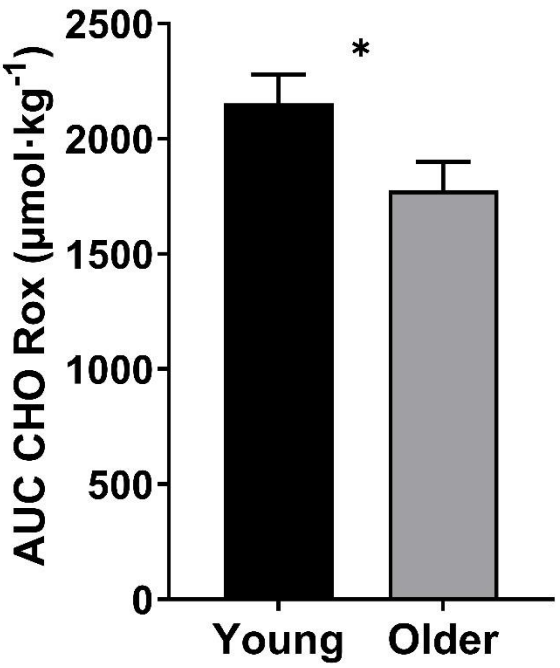

Figure 16. Area under the curve (AUC) for rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants. Values are means ± SE. *Significantly different between groups, $p < 0.05$.

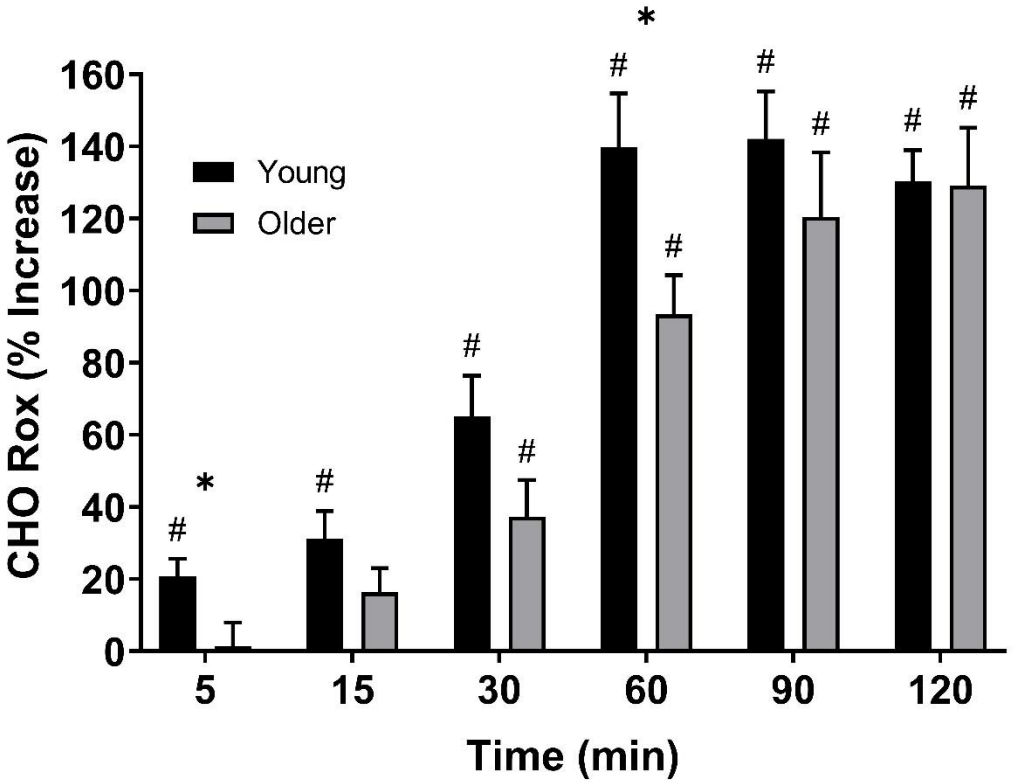

Figure 17. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass, expressed as a percentage increase from 0 min, in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

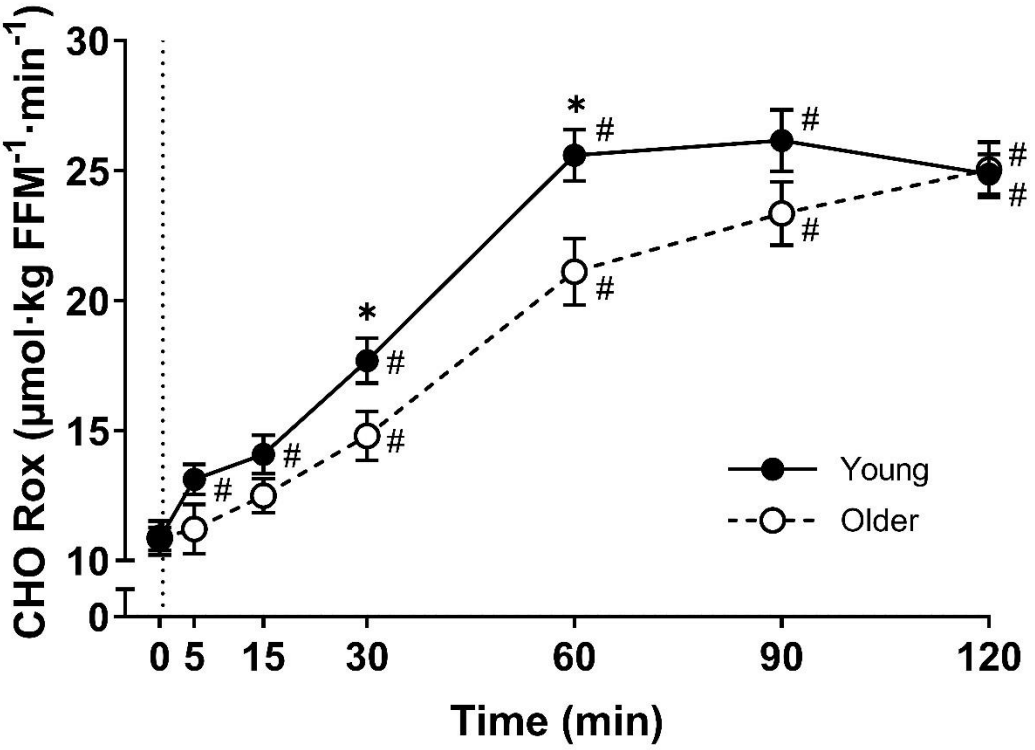

Figure 18. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

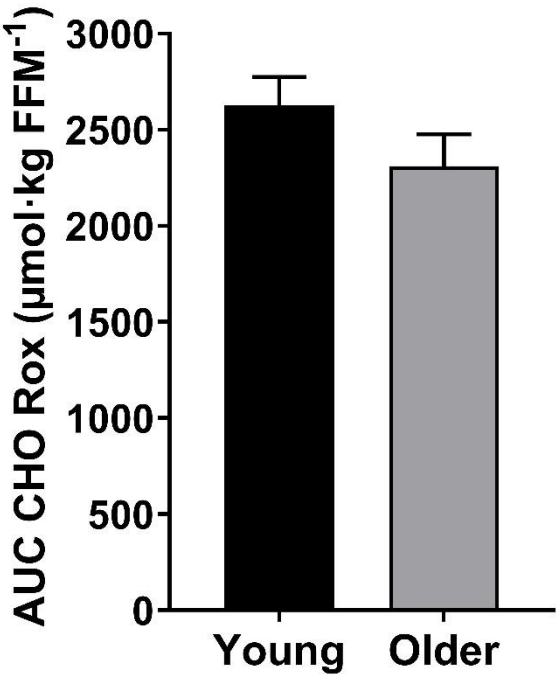

Figure 19. Area under the curve (AUC) for rate of total carbohydrate (CHO) oxidation (Rox) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

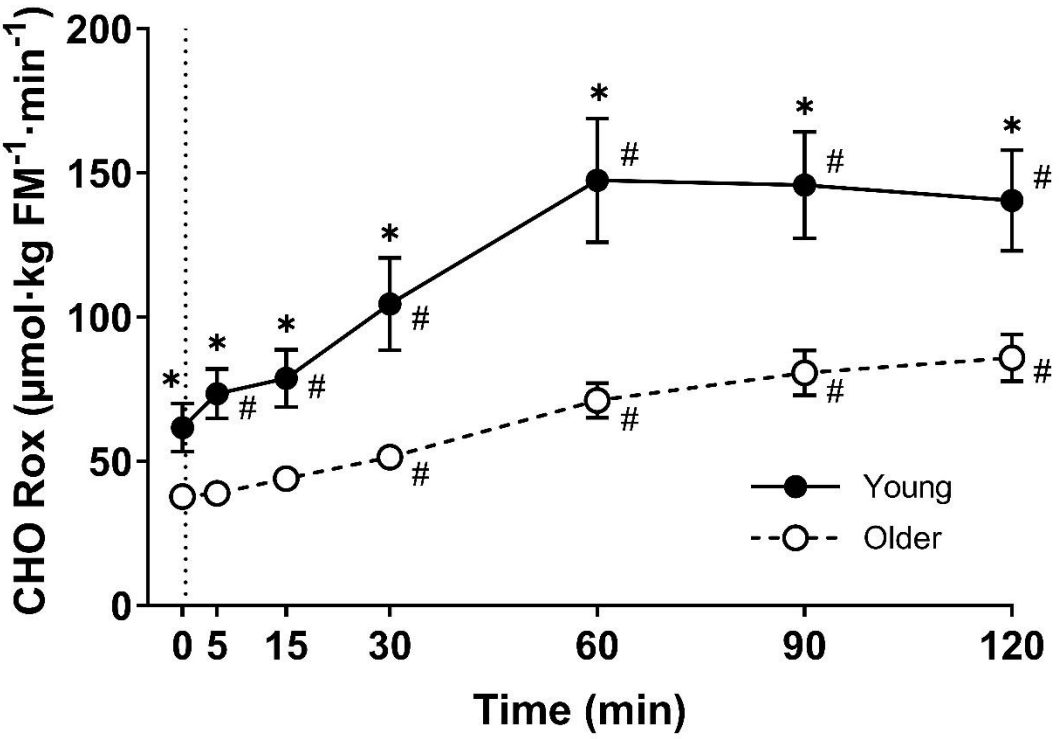

Figure 20. Rate of total carbohydrate (CHO) oxidation (Rox) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

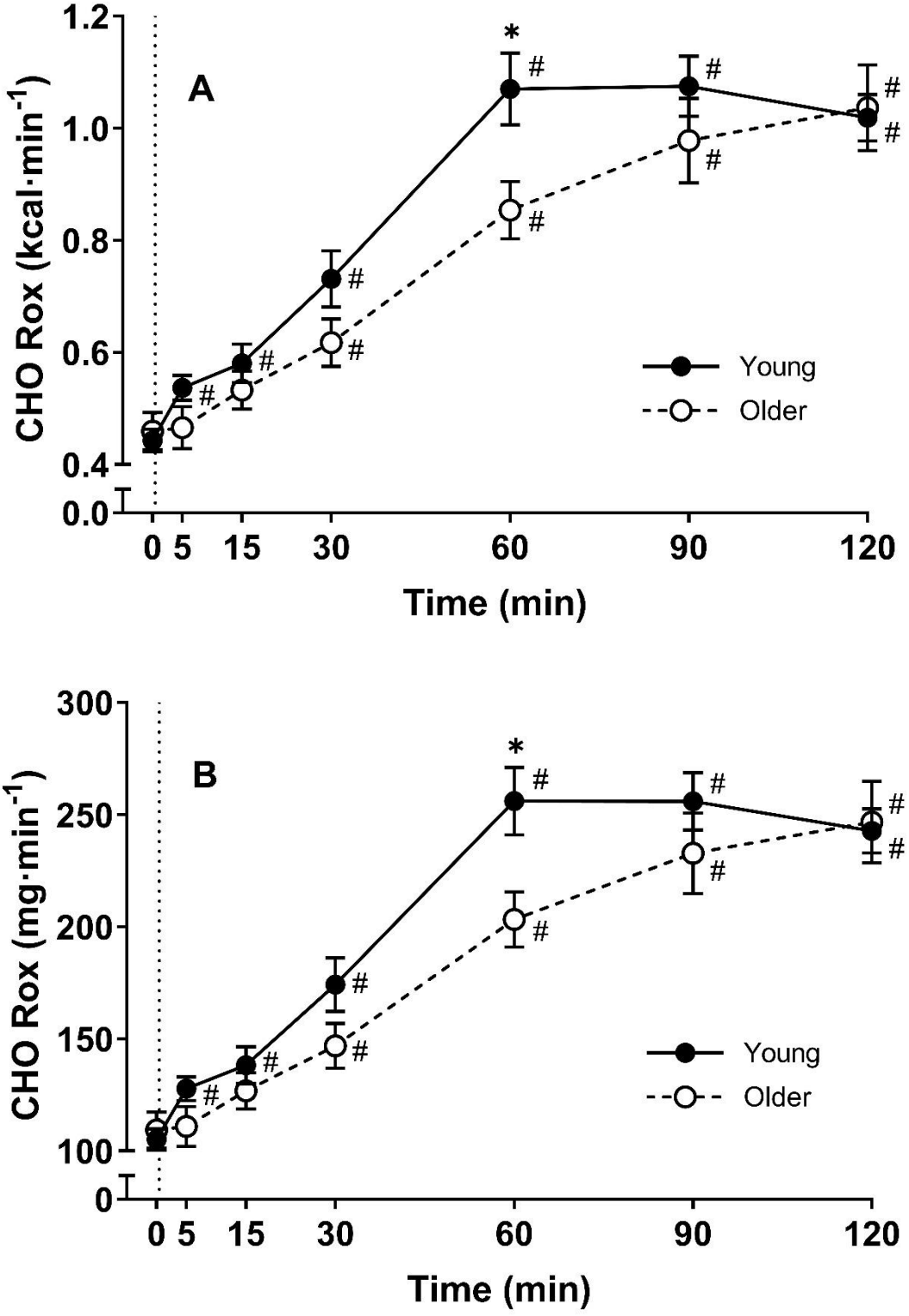

Figure 21. Rate of total carbohydrate (CHO) oxidation (Rox) expressed in units of kcal·min^{-1} (A) and mg·min^{-1} (B) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

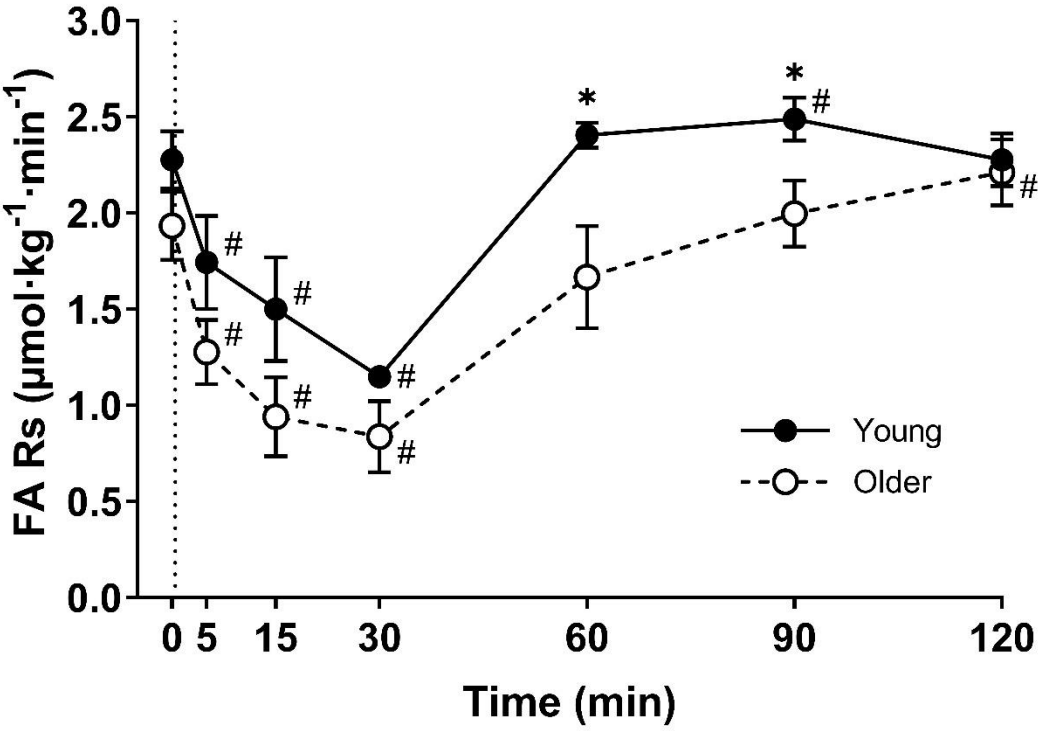

Figure 22. Rate of total fatty acid (FA) reesterification (Rs) per unit of body mass in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

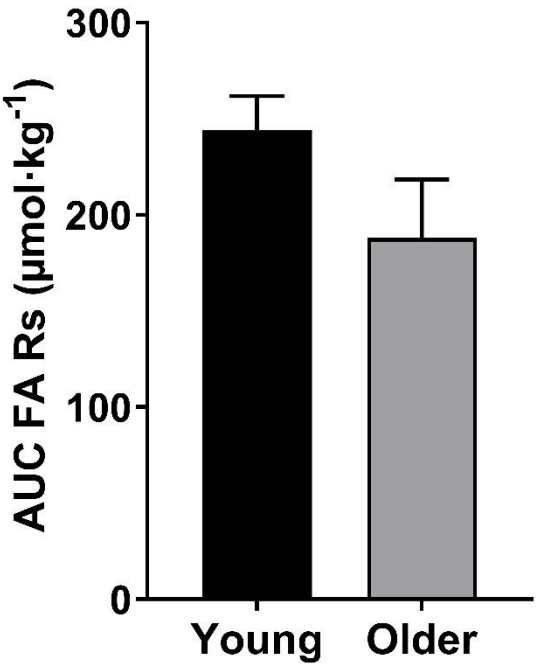

Figure 23. Area under the curve (AUC) for rate of total fatty acid (FA) reesterification (Rs) per unit of total body mass in young and older participants. Values are means ± SE.

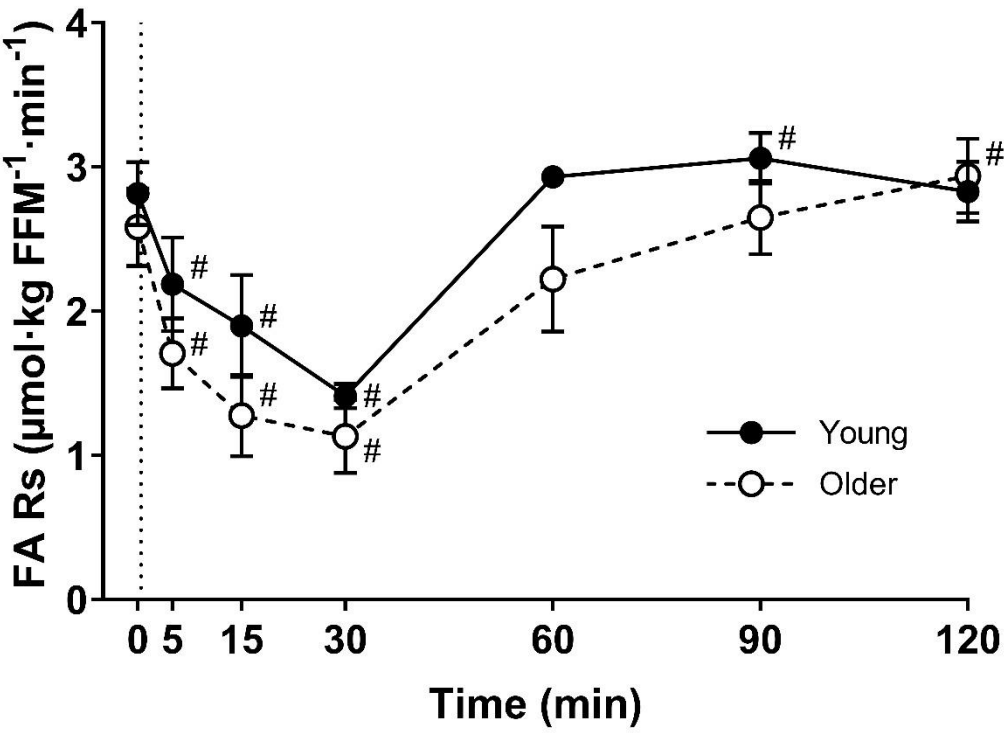

Figure 24. Rate of total fatty acid (FA) reesterification (Rs) per unit of fat free mass (FFM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

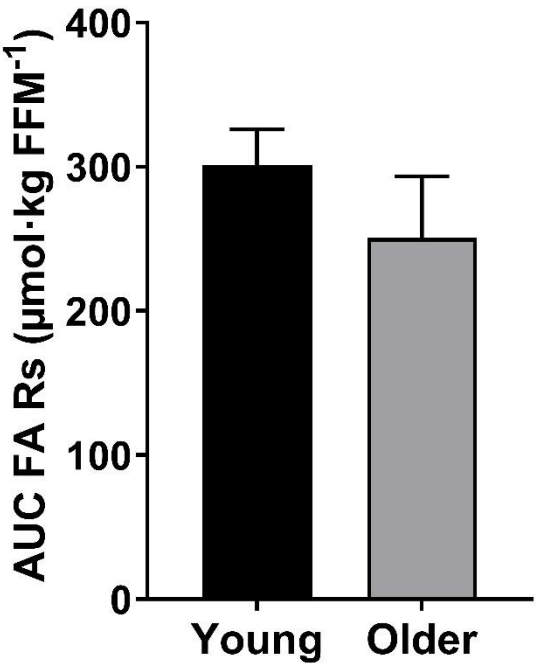

Figure 25. Area under the curve (AUC) for rate of total fatty acid (FA) reesterification (Rs) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE.

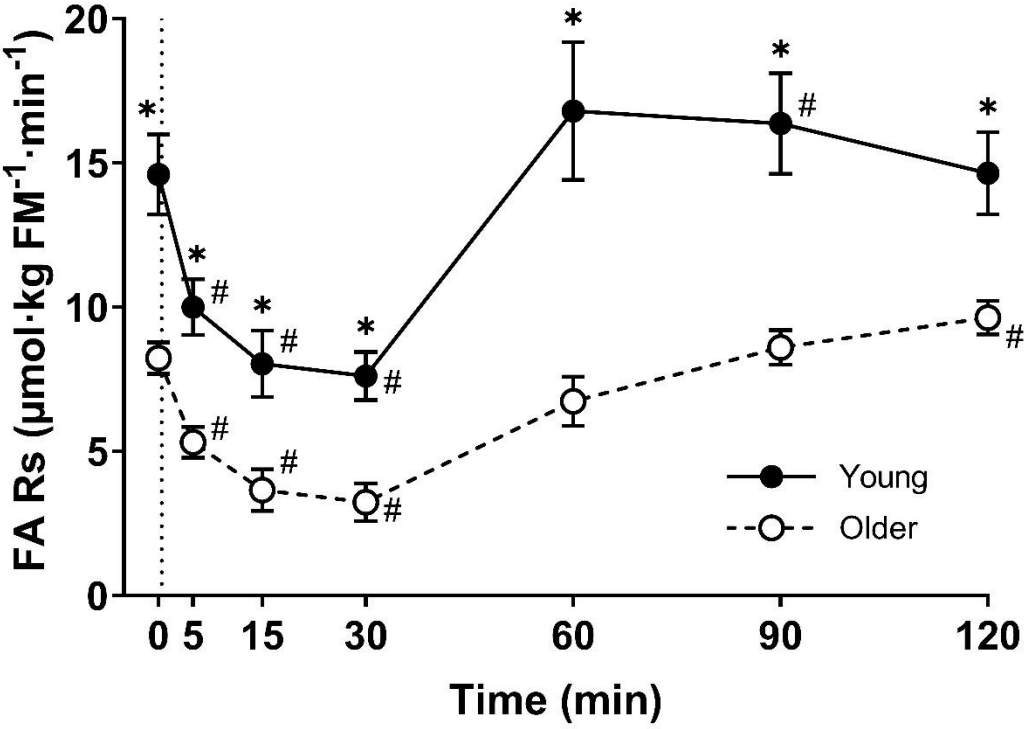

Figure 26. Rate of total fatty acid (FA) reesterification (Rs) per unit of fat mass (FM) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

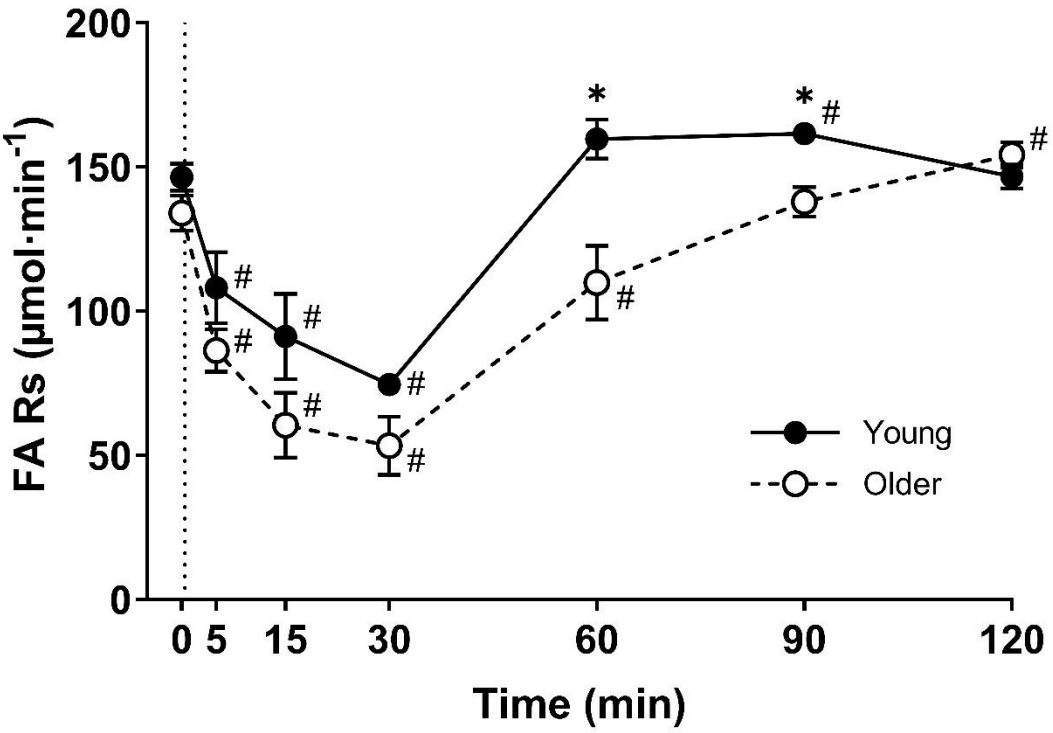

Figure 27. Rate of total fatty acid (FA) reesterification (Rs) in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

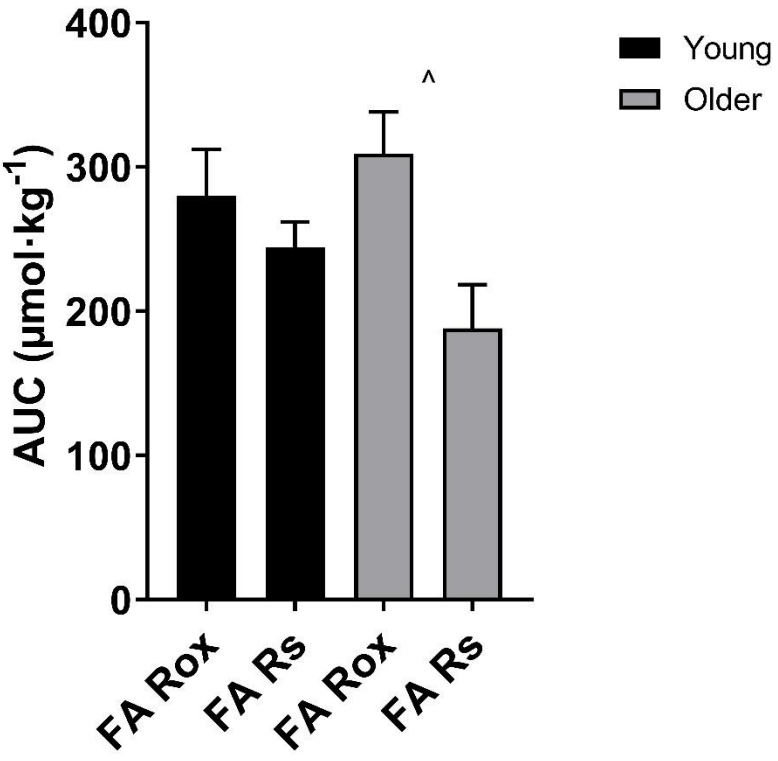

Figure 28. Area under the curve (AUC) for rates of total fatty acid (FA) oxidation (Rox) and reesterification (Rs) per unit of total body mass in young and older participants. Values are means ± SE. ^Significantly different between FA Rox and FA Rs, $p < 0.05$.

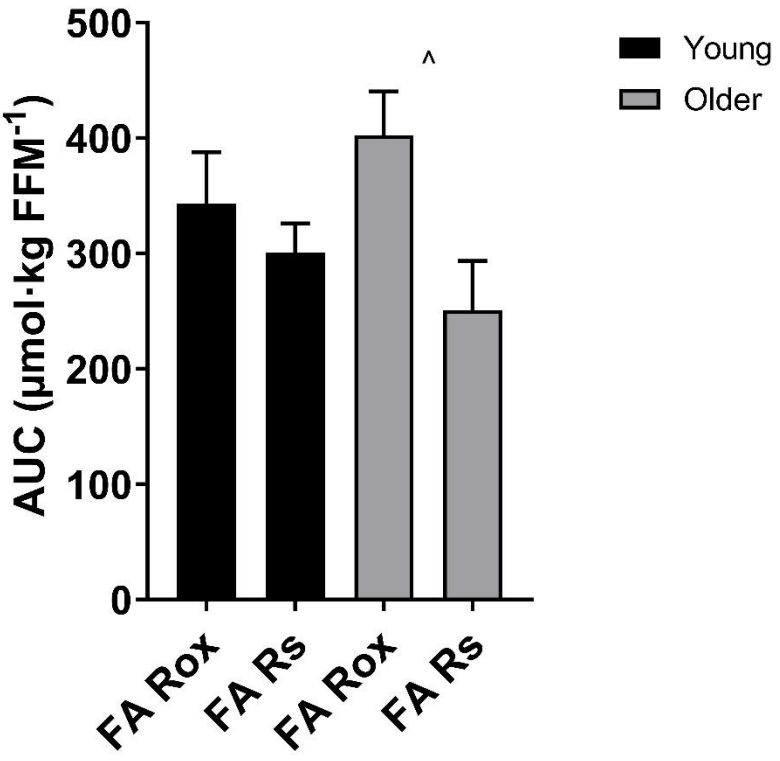

Figure 29. Area under the curve (AUC) for rates of total fatty acid (FA) oxidation (Rox) and reesterification (Rs) per unit of fat free mass (FFM) in young and older participants. Values are means ± SE. ^Significantly different between FA Rox and FA Rs, $p < 0.05$.

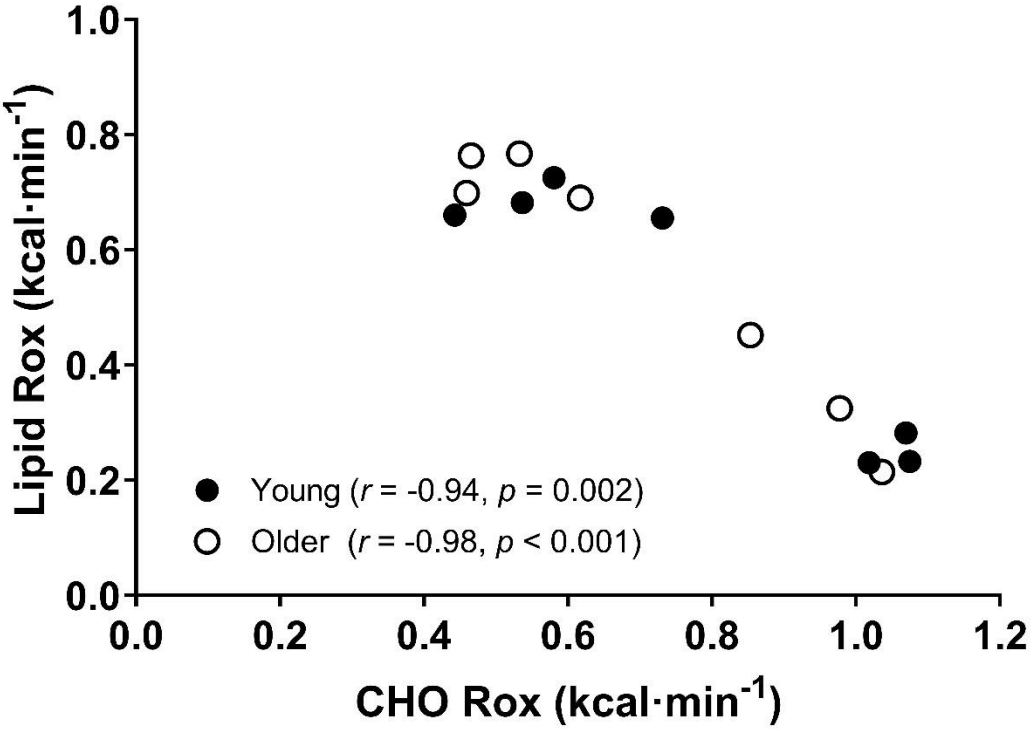

Figure 30. Relationship between rates of total carbohydrate (CHO) and lipid oxidation in young and older participants.

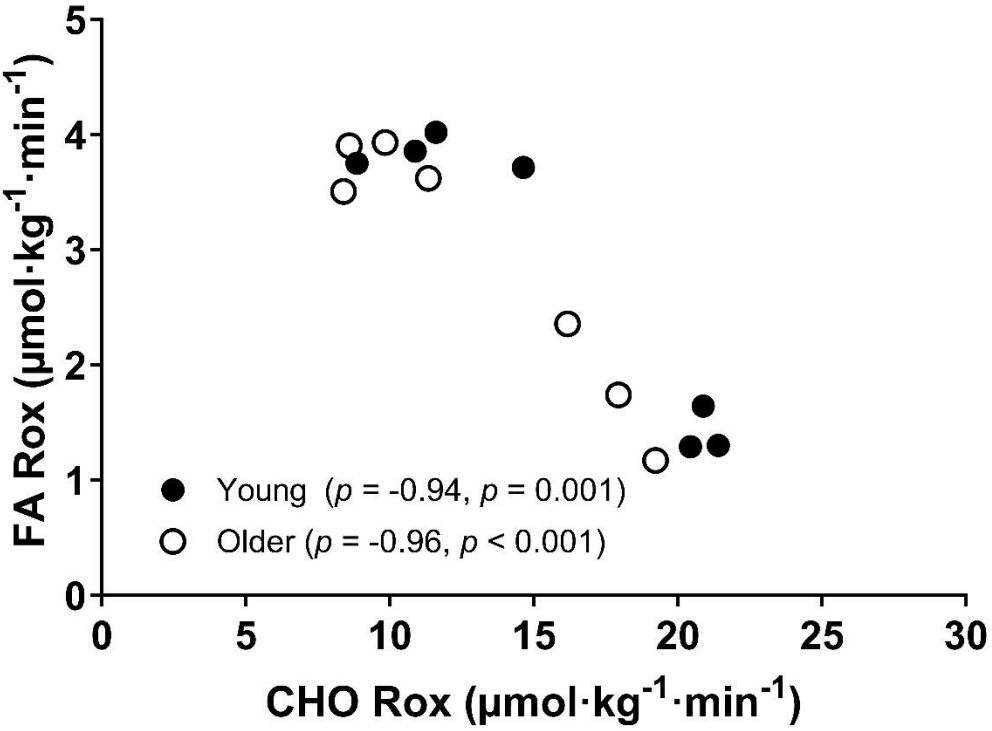

Figure 31. Relationship between rates of total carbohydrate (CHO) and total fatty acid (FA) oxidation per unit of body mass in young and older participants.

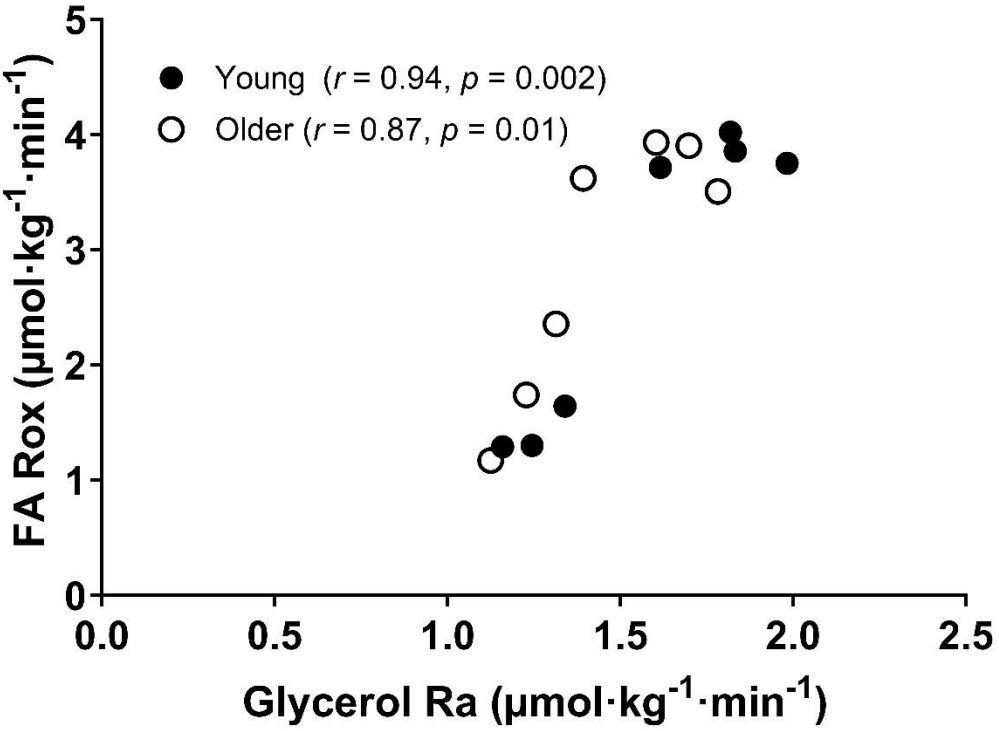

Figure 32. Relationship between glycerol rate of appearance (Ra) and rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants.

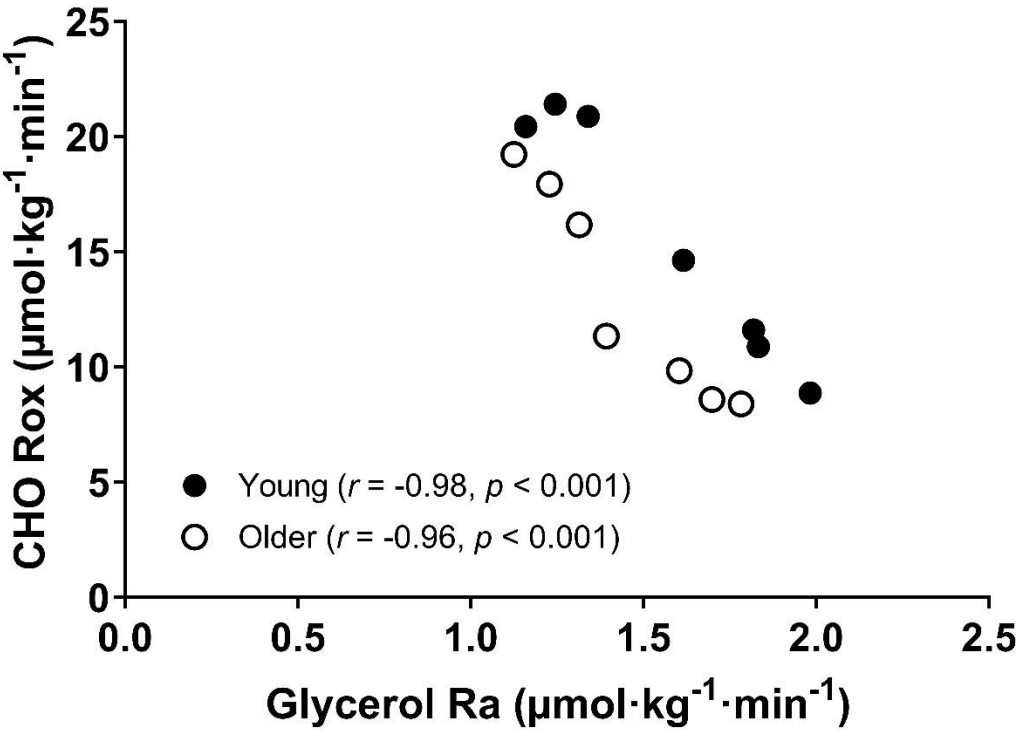

Figure 33. Relationship between glycerol rate of appearance (Ra) and rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants.

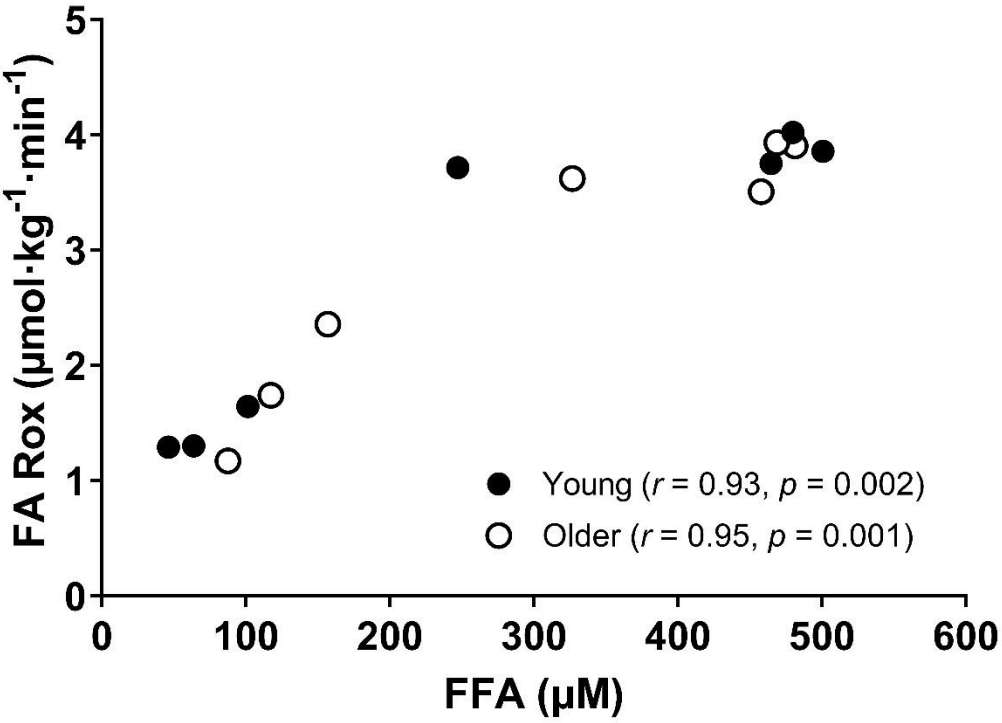

Figure 34. Relationship between plasma free fatty acid (FFA) concentrations and rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants.

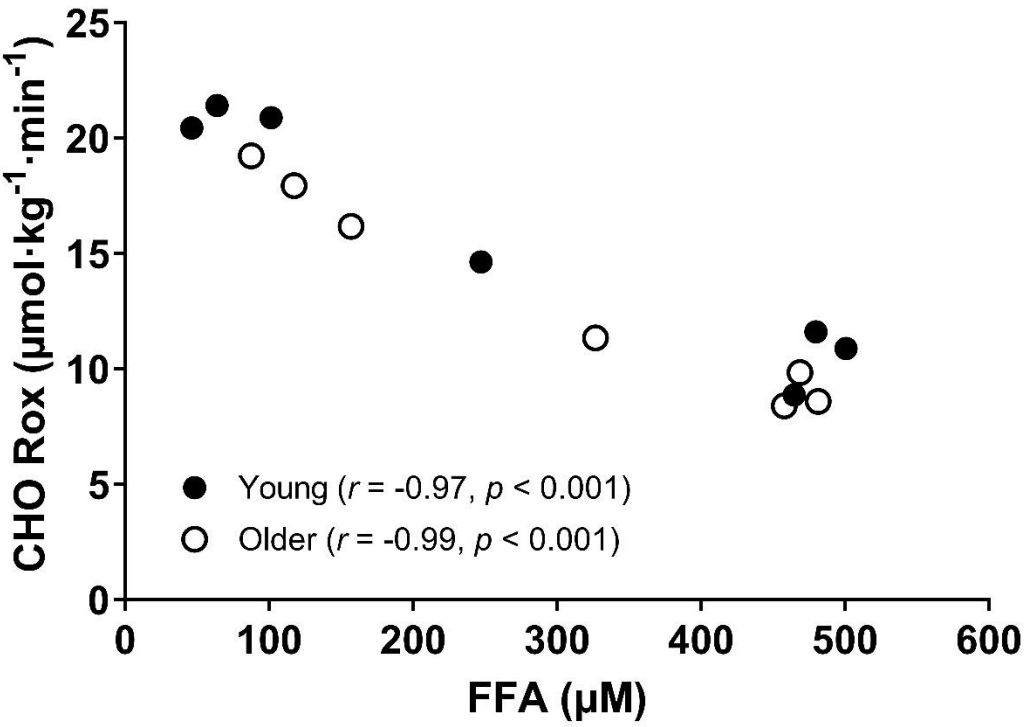

Figure 35. Relationship between plasma free fatty acid (FFA) concentrations and rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants.

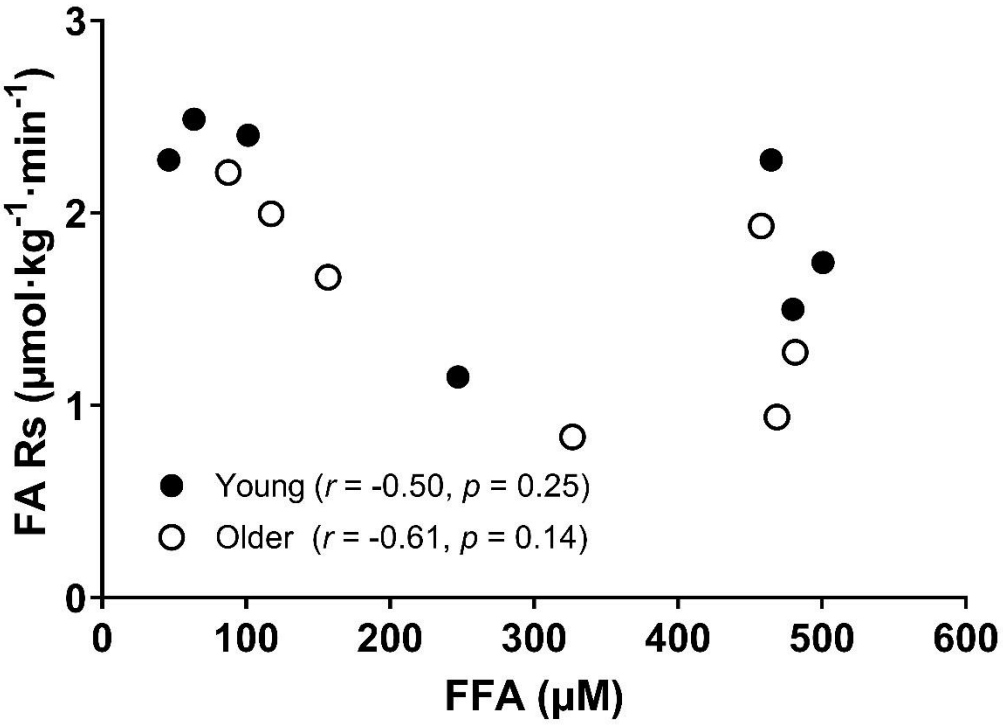

Figure 36. Relationship between plasma free fatty acid (FFA) concentrations and rate of total fatty acid (FA) reesterification (Rs) per unit of body mass in young and older participants.

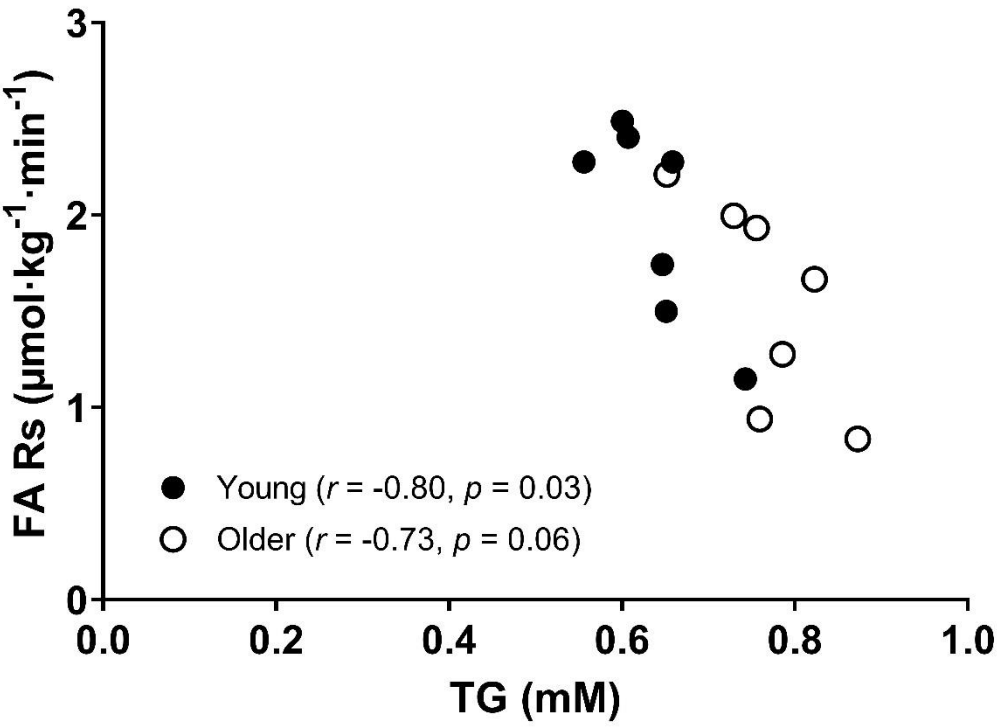

Figure 37. Relationship between triglyceride (TG) concentrations and rate of total fatty acid (FA) reesterification (Rs) per unit of body mass in young and older participants.

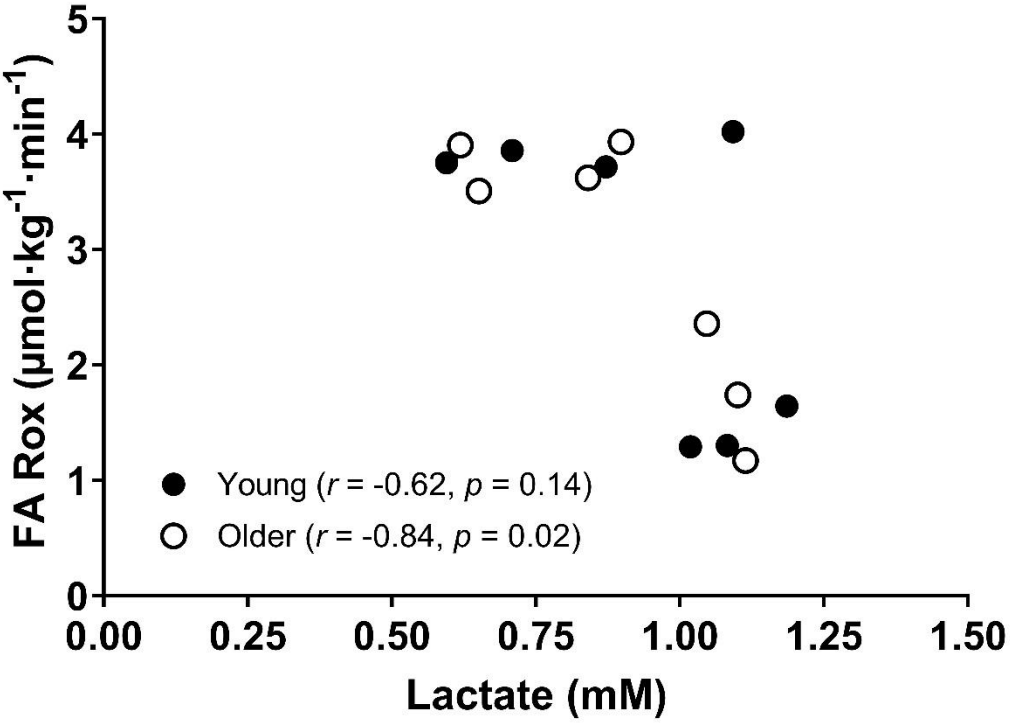

Figure 38. Relationship between blood lactate concentrations and rate of total fatty acid (FA) oxidation per unit of body mass in young and older participants.

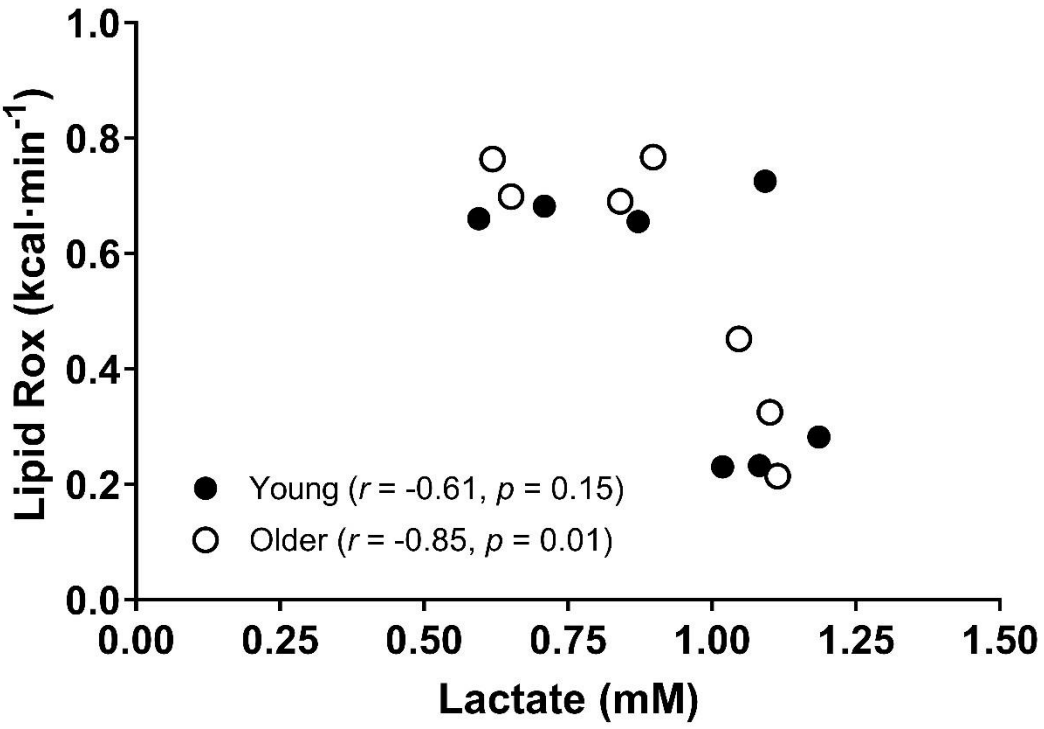

Figure 39. Relationship between blood lactate concentrations and rate of total lipid oxidation in young and older participants.

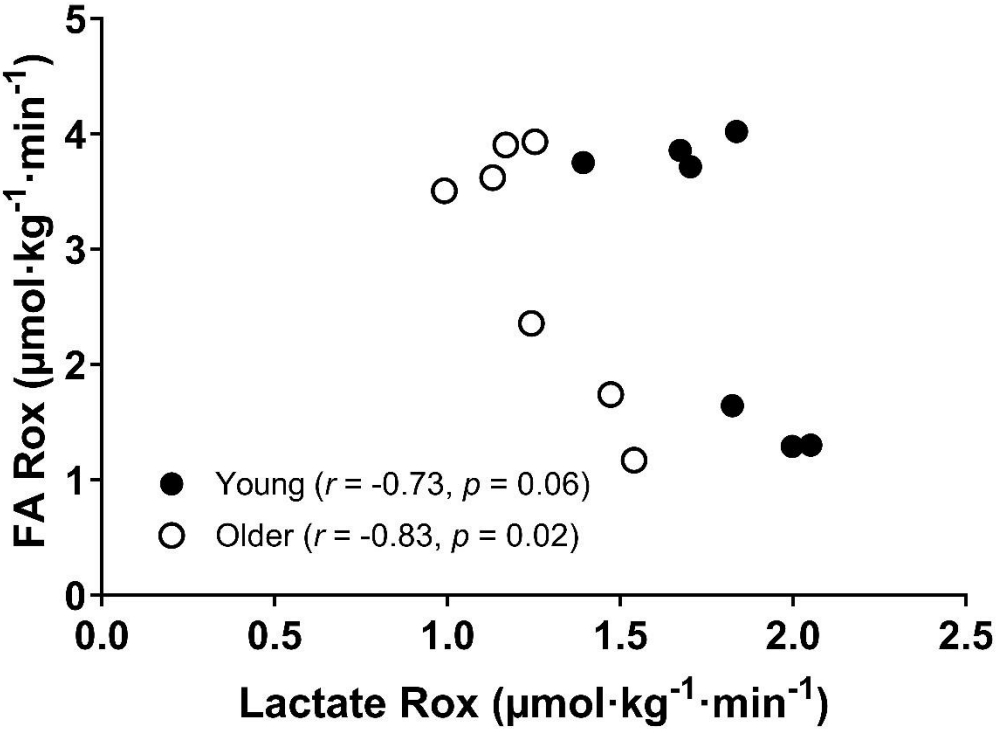

Figure 40. Relationship between rate of total lactate and total fatty acid (FA) oxidation per unit of body mass in young and older participants.

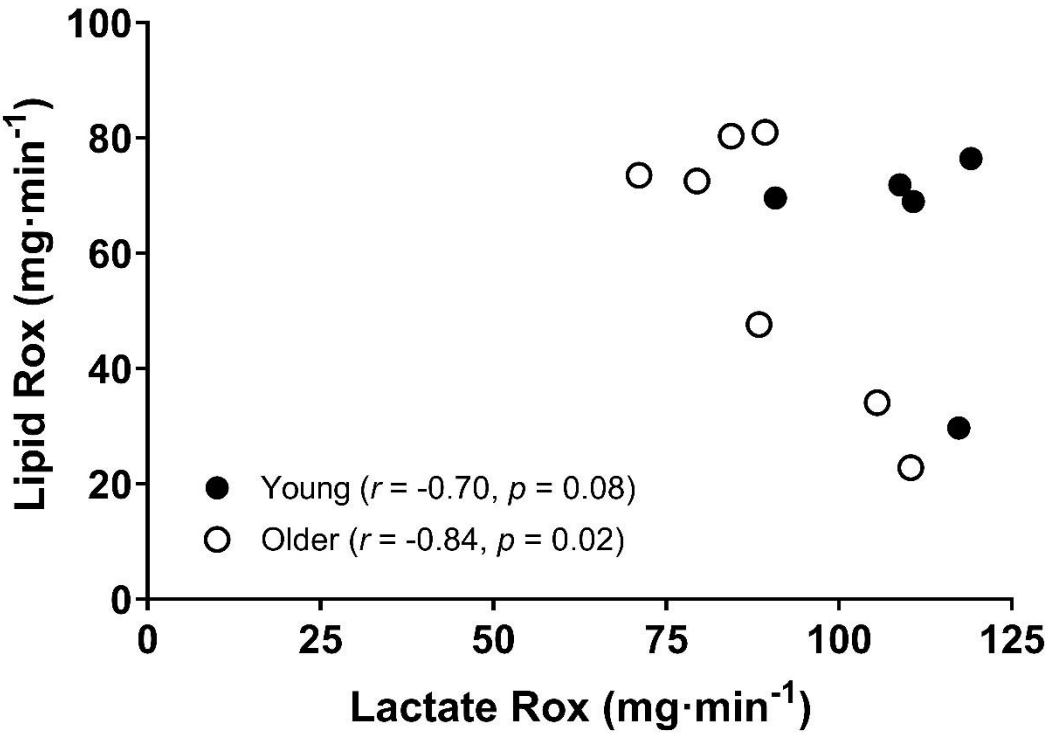

Figure 41. Relationship between rate of total lactate and total lipid oxidation in young and older participants.

CHAPTER 4

Insulin and lactate control postprandial energy substrate partitioning in aging

INTRODUCTION

Metabolic flexibility describes the ability to switch back and forth between carbohydrate (CHO) and fatty acid (FA) utilization in response to changes in energy substrate availability. Kelley and colleagues (3-6) initially described metabolic flexibility as the ability to alter the pattern of substrate utilization in response to insulin stimulation. Several previous investigations have utilized an oral glucose tolerance test (OGTT) to model physiological changes in substrate utilization during hyperinsulinemia. Following oral CHO consumption, metabolic flexibility is illustrated by an increase in whole-body CHO oxidation (27-29) that is mediated by an increase in insulin action to facilitate peripheral glucose uptake and utilization. Other investigations also documented simultaneous reductions in lipid or FA oxidation rates (24-26). Because FAs serve as the primary energy substrate during postabsorptive rest (42, 79, 80, 111), metabolic flexibility to oral CHO is also illustrated by the concurrent suppression of whole-body FA oxidation. Thus, metabolic flexibility is regulated by several mechanisms that control substrate partitioning.

Insulin has an essential role in regulating energy substrate availability. As an example, insulin is responsible for the suppression of adipose tissue lipolysis and subsequent reduction of plasma free fatty acid (FFA) availability (131-133). Consequently, insulin has an indirect role in suppressing FA oxidation rates (3, 90, 113, 134). The loss of metabolic flexibility, or metabolic inflexibility, is characterized by the inability to alter the pattern of substrate utilization during hyperinsulinemia. Consistent with the "Randle Cycle" theory that an abundance of FA determines energy substrate partitioning (14), several previous investigations have demonstrated that FA oxidation rates remain elevated during hyperinsulinemia if plasma FFA availability also remains elevated (6, 81-84). Plasma FA-induced alterations to insulin-stimulated glucose uptake are implicated to promote metabolic inflexibility by this mechanism (85, 86, 135). The loss of metabolic flexibility has therefore been implicated in the progression of insulin resistance (136) and is observable in aging (10-13), as advancing age is associated with reductions in insulin sensitivity (99, 137). As well, elevated plasma FFA availability is characteristic of metabolic disease states (138, 139), aging, and aging-related disease states (140).

Physiologically, the persistence of FFA availability during hyperinsulinemia has been interpreted to indicate a defect in the insulin-mediated suppression of lipolysis (16). Bonadonna and colleagues (41) previously reported that FFA availability during hyperinsulinemia was higher in elderly compared to younger individuals and resulted in greater total lipid oxidation. Although higher rates of lipolysis were suspected to have increased FFA availability, it was unclear if the lipolytic rate was altered by their experimental conditions. Similarly, several previous investigations have documented increased plasma insulin concentrations with concurrent reductions in plasma glycerol and FFA concentrations (21, 22) and FA oxidation rates (24-27, 130) during an OGTT and speculated that their results resulted from the suppression of lipolysis. However, these investigations were unable to demonstrate altered rates of lipolysis from their data.

In Chapters 2 and 3 of this dissertation, we reported aging-related differences in the rates of whole-body lipolysis, total FA oxidation, and total CHO oxidation. Notably, many of the reported responses were speculated to result from differences in lactate metabolism and signaling (59, 60). We have also reviewed the role of lactate in signaling and its effects on energy substrate partitioning (31). Overall, lactate controls lipid substrate partitioning at the beginning and end of

the pathway, from FA mobilization to oxidation. Lactate binding to hydroxycarboxylic acid receptor 1 (HCAR-1) on adipose cells inhibits lipolysis. This mechanism was first described by Ahmed et al. (36), who reported that lactate autocrine signaling via GPR81 (HCAR-1) potentiates the inhibitory effects of insulin on lipolysis. In humans, lactate production by adipocytes following oral glucose consumption (54-57) provides an autocrine mechanism for the anti-lipolytic effects of lactate. Additionally, intracellular lactate accumulation following increased glycolytic flux limits mitochondrial FA transport and oxidation (31, 32, 34). Lactate therefore shares inhibitory effects on FA metabolism with insulin (33, 90, 131-133).

We have also recently updated the Lactate Shuttle concept originally described in 1984 (65) to describe a postprandial mechanism (Postprandial Lactate Shuttle, or PLS) by which lactate regulates energy substrate partitioning following CHO consumption. The PLS is comprised of two phases: (1) a fast enteric phase of lactate production from glycolysis in the gut that is followed by (2) a prolonged systemic phase of lactate production as systemic glucose is disposed of via glycolysis in peripheral tissues (59). Changes in plasma insulin concentrations with concurrent changes in blood lactate concentrations, rates of lipolysis, and FA oxidation rates were omitted from Chapters 2 and 3 of this dissertation. Consequently, it is not known if insulin and lactate differentially affect FA substrate partitioning in aging through mechanisms of the PLS.

The purpose of this investigation was to assess metabolic flexibility by determining concurrent changes in plasma insulin and glucagon concentrations and rates of lipolysis, FA oxidation, and CHO oxidation during an OGTT in healthy young and older individuals. We sought to determine immediate (i.e., 5 min and 15 min after glucose consumption) changes in plasma hormone concentrations that corresponded to aging-related changes in the rate of lipolysis. Additionally, we sought to determine if physiological hyperinsulinemia and lactatemia (i.e., increased blood lactate concentrations) differentially affect FA substrate partitioning in the postprandial period. We hypothesized that the insulin and glucagon responses in the immediate period following glucose consumption would be attenuated in older individuals, but comparable during the later period. Further, we hypothesized that insulin and lactate would be inversely related to parameters of FA substrate partitioning, but that the inhibitory effects would be exaggerated in aging.

METHODS

Study participants. This study was approved by the University of California, Berkeley Committee for the Protection of Human Subjects (CPHS 2018-08-11312) and conformed to the standards set by the Declaration of Helsinki. Fifteen younger (21-35 years) and 14 older (60-80 years) men and women were recruited. Potential participants were interviewed and received verbal and written information on study purposes and procedures. After giving verbal and written consent, participants were screened for metabolic and cardiovascular diseases. Screening tests included a health history questionnaire, a blood draw for a basic metabolic panel, an electrocardiogram (ECG) and pulmonary function assessment via spirometry, three-site skin fold measurements (men: chest, abdomen, and thigh; women: triceps, suprailiac, thigh) to assess body density, and a physical examination. Measurements of body density were converted to body composition and expressed as percentage body fat according to the guidelines provided by the American College of Sports

Medicine (58). To assess physical fitness, screening also included a continuous, progressive cycle ergometer test to determine peak oxygen consumption ($\dot{V}O_2peak$) and ventilatory threshold (VT). For dietary controls, participants provided 3-day dietary records that were analyzed for caloric intake and macronutrient consumption (Diet Analysis Plus Version 6.1, ESHA Research, Salem, OR). To qualify for participation, participants were required to be diet and weight stable; to have a body mass index (BMI) in the range of ≥18.5 and <30.0 kg/m^2; to be non-smokers; to have normal pulmonary function (FEV_1/FVC of >70%); to have a fasting blood glucose concentration of <100 $mg \cdot dL^{-1}$; to have a hemoglobin A1c percentage of <5.7 % of total hemoglobin; to have a fasting total cholesterol concentration of <200 $mg \cdot dL^{-1}$, LDL cholesterol concentration of <100 $mg \cdot dL^{-1}$, HDL cholesterol concentration of ≥40 $mg \cdot dL^{-1}$, and total triglyceride concentration of <150 $mg \cdot dL^{-1}$; to pass a physical examination; and to be cleared for participation by a licensed physician. Moreover, women were required to have a regular (28- to 35-day) menstrual cycle, to not be pregnant, and to not be taking oral contraceptives.

Screening procedures preceded experimental procedures by at least one week. Participants who were entered into the study were provided with verbal and written information on freedom to withdraw from the study as well as the contact information of the laboratory manager, principal investigator, and physician if adverse effects of the study procedures had occurred.

Exercise testing protocol. All participants underwent a graded exercise test (GXT) conducted on an electronically braked leg cycle ergometer (Lode Corival CPET, Gronigen, Netherlands). Open-circuit indirect calorimetry was performed simultaneously to continuously monitor expired gases throughout the GXT with a "metabolic cart" (Parvo Medics TrueOne 2400 System, Salt Lake City, UT). The "metabolic cart" was calibrated according to the manufacturer's instructions prior to each GXT. Each GXT began with 5 min of seated rest followed by a 2 min warm-up period at 25 W at a set cadence of 60 RPM. Participants then completed three, 4-min stages of 35 W, 60 W, and 85 W at a set cadence of 60 RPM. The cadence was dictated by a metronome and reinforced visually using a tachometer displayed on the ergometer screen. Participants subsequently pedaled at a self-selected cadence for the remainder of the test as the power output increased by 30 W per min. Young participants continued until volitional exhaustion. Older participants continued until achieving VT, which was identified by a respiratory exchange ratio (RER, or $R = \dot{V}CO_2/\dot{V}O_2$) of 0.97–1.00. For older participants, $\dot{V}O_2peak$ was estimated from VT using guidelines provided by the American College of Sports Medicine (58).

Experimental procedures. Participants underwent a 120-min OGTT with primed, continuous infusion of [1,1,2,3,3-^{2}H]glycerol (or D_5-glycerol) (Cambridge Isotope Laboratories, Inc., Andover, MA). Data on glycerol kinetics was reported in Chapter 2 of this dissertation, but the use of this stable isotope tracer is mentioned in this report for its relevancy to the present data.

Participants reported to the laboratory in the morning following a 12-hr overnight fast. Women who participated did so during the mid-follicular phase of their menstrual cycle. For the 24 hr preceding the experimental trial, participants were asked to maintain their standard dietary pattern and refrain from strenuous physical exercise. On the morning of the trial, a catheter was placed in a warmed hand vein for "arterialized" blood sampling and a contralateral arm vein catheter was placed for tracer infusion. Background blood samples were then taken for the determination of endogenous isotopic enrichment (IE) of glycerol. Subsequently, a priming bolus

of 40 mg·15 mL^{-1} D_5-glycerol was given, and then the participants rested for 90 min while the tracer glycerol was infused continuously at a rate of 0.32 $mg \cdot min^{-1}$. Arterialized blood sampling was repeated at 75 and 90 min after continuous infusion.

After 90 min of continuous infusion, participants drank a solution containing 75 g of D-glucose in 296 mL (#10-O-075, Azer Scientific Inc., Morgantown, PA). Participants consumed the solution in ≤2 min. Arterialized blood sampling was subsequently completed at 5, 15, 30, 60, 90, and 120 min after the consumption of glucose.

Blood sample collection. Arterialized blood samples for the determination of plasma insulin and C-peptide concentrations were collected in tubes containing K_3EDTA (Vacuette, Greiner Bio-One, Monroe, NC). Samples for the determination of plasma glucagon concentrations were collected in tubes containing K_3EDTA and 10 µL of DPP IV inhibitor (#DPP4-M, Sigma-Aldrich, St. Louis, MO) per mL of whole blood. Additionally, 8 TIU of aprotinin per mL of whole blood was added to each tube prior to collection to prevent the degradation of hormones. All samples were immediately placed on ice and then centrifuged at 3,000 g for 10 min at 4°C. The plasma layers were separated and stored at -80°C until analysis.

Hormone analyses. Concentrations of insulin (#80-INSHU-E01.1), glucagon (#48-GLUHUU-E01), and C-peptide (#80-CPTHU-E01.1) were determined using enzyme-linked immunosorbent assay (ELISA) kits (ALPCO Diagnostics, Salem, NH). Standard curves were constructed using a software program (SoftMax Pro 4.3.1 LS, Molecular Devices, Sunnyvale, CA) and concentrations were calculated from a 4-parameter logistic fit.

Estimations of insulin sensitivity. The homeostatic model assessment (HOMA) method was used to estimate insulin resistance (IR) from baseline, fasting blood glucose and plasma insulin concentrations (117). The method is highly correlated with measurements of insulin resistance attained with the hyperinsulinemic-euglycemic (141) and hyperglycemic clamp techniques (142). Scores were calculated using the following equation:

$$\text{HOMA-IR} = (\text{BPI} \times \text{BBG}) / 22.5$$

where: BPI is the baseline plasma insulin concentration in $\mu IU \cdot mL^{-1}$ and BPG is the baseline blood glucose concentration in mM. A HOMA-IR score of 1 (unit less) indicates "normal" insulin sensitivity (142), and higher scores indicate greater degrees of insulin resistance in non-diabetic individuals (118).

The composite insulin sensitivity index (CISI) was used to estimate whole-body insulin sensitivity during the OGTT (101). The index is highly correlated with the direct measurement of insulin sensitivity (i.e., the rate of whole-body glucose disposal) attained with the euglycemic insulin clamp technique (143). Scores were calculated using the following equation:

$$\text{CISI} = 10{,}000 / \sqrt{[(\text{BPI} \times \text{BBG}) \times (\text{MPI} \times \text{MBG})]}$$

where: BPI is the baseline plasma insulin concentration in $\mu IU \cdot mL^{-1}$, BPG is the baseline blood glucose concentration in $mg \cdot dL^{-1}$, MPI is the mean plasma insulin concentration from 30-120 min

in $\mu IU \cdot mL^{-1}$ and MBG is the mean blood glucose concentration from 30-120 min in $mg \cdot dL^{-1}$. Prior to calculations, plasma insulin concentrations were converted from units of pM to $\mu IU \cdot mL^{-1}$ by dividing by 6 (144) and blood glucose concentrations were converted from units of mM to $mg \cdot dL^{-1}$ using the molecular weight of glucose (180.16 $g \cdot mol^{-1}$). A CISI score ≤2.5 (unit less) is associated with insulin resistance (145), while higher scores suggest greater degrees of insulin sensitivity.

Estimations of insulin secretion. The HOMA method was used to estimate pancreatic β-cell function (%β) from baseline, fasting blood glucose and plasma insulin concentrations (117). Wallace et al. (142) describes HOMA-%β as an index of β-cell activity, but not of overall β-cell health. Thus, the index reflects insulin secretory capacity. The method is positively correlated with estimates of β-cell activity attained with continuous infusion glucose model assessment, the hyperglycemic clamp technique, and the intravenous glucose tolerance test (142). Scores were calculated using the following equation:

$$\text{HOMA-\%}\beta = (20 \times \text{BPI}) / (\text{BPG} - 3.5)$$

A HOMA-%β score of 100% indicates "normal" β-cell function (142), and lower scores indicate lesser β-cell function.

The insulinogenic index (IGI) was also used to estimate β-cell function. The index utilizes the changes in plasma insulin and blood glucose concentrations to characterize "early-phase" (i.e., 0-30 min) (146, 147) and "late-phase" (i.e., 30-120 min) (148-150) insulin responses during an OGTT. Notably, the classically defined "late-phase" insulin response is analogous to the "enteric phase" of the PLS (59). The IGI has been described as an index of insulin secretion in response to the change in blood glucose concentrations (151, 152). Because factors other than glucose affect insulin secretion following oral glucose consumption (153), the index does not reflect glucose-stimulated insulin secretion. Alternatively, the index reflects insulin secretory capacity in response to glucose and other factors that promote insulin secretion. Scores were calculated using the following equations:

$$\text{IGI1} = \Delta I_{30\text{-}0} / \Delta G_{30\text{-}0}$$

$$\text{IGI2} = \text{AUC}(I)_{0\text{-}30} / \text{AUC}(G)_{0\text{-}30}$$

$$\text{IGI3} = \text{AUC}(I)_{30\text{-}120} / \text{AUC}(G)_{30\text{-}120}$$

where: $\Delta I_{30\text{-}0}$ is the change in plasma insulin concentration from baseline at 30 min in pM, $\Delta G_{30\text{-}0}$ is the change in blood glucose concentration from baseline at 30 min in mM (152), $\text{AUC}(I)_{0\text{-}30}$ is the area under the curve (AUC) for plasma insulin concentrations over the first 30 min of observation in pM·min, $\text{AUC}(G)_{0\text{-}30}$ is the AUC for blood glucose concentrations over the first 30 min of observation in mM·min, $\text{AUC}(I)_{30\text{-}120}$ is the AUC for plasma insulin concentrations over the last 90 min of observation in pM·min, and $\text{AUC}(G)_{30\text{-}120}$ is the AUC for blood glucose concentrations over the last 90 min of observation in mM·min (150). Although a "normal" IGI score has not been established, lower IGI scores have been reported in individuals with non-insulin dependent diabetes mellitus (154) and impaired glucose tolerance (155). Accordingly, a higher IGI score is interpreted to reflect greater insulin secretory capacity.

The oral disposition index (DI) was also used to estimate β-cell function (156). Scores were calculated using the following equations:

$$DI1 = IGI1 \times CISI$$

$$DI2 = (\Delta I_{120\text{-}0} / \Delta G_{120\text{-}0}) \times CISI$$

where: $\Delta I_{120\text{-}0}$ is the change in plasma insulin concentration from baseline at 120 min in pM and $\Delta G_{120\text{-}0}$ is the change in blood glucose concentration from baseline at 120 min in mM (150). Utzschneider et al. (157) describes the DI as a marker of the β-cell's ability to compensate for insulin resistance. Thus, the DI is alternatively termed the "insulin secretion/insulin resistance index" (158) as it describes the relationship between insulin secretion and insulin sensitivity. Although a "normal" DI score has not been established, lower DI scores have been reported in individuals at risk for developing diabetes (159). Accordingly, a higher DI score is interpreted to reflect greater insulin secretory capacity.

Statistical analyses. Data were analyzed using GraphPad Prism 10 (Version 10.1.2 for Windows, GraphPad Software, Boston, MA). Statistical significance was set at $\alpha \leq 0.05$. An unpaired t-test with Welch's correction was used to assess the significance of mean differences in indexes of insulin sensitivity and insulin secretion between groups. Repeated-measures two-way ANOVA with the Geisser-Greenhouse correction and Tukey's multiple comparisons test was used to assess the significance of mean differences between groups and across time points. Data are presented as mean ± standard error of the mean (SE). Differences across time points are expressed relative to baseline (i.e., before the consumption of glucose at 0 min). Area under the curve (AUC) was determined using GraphPad Prism 10. To assess the significance of differences in AUC, an unpaired t-test with Welch's correction was used. Data are presented as mean ± SE.

Pearson correlation coefficients were used to assess the significance of relationships among select variables studied. The magnitude of correlations are interpreted according to the guidelines provided by Mukaka (64). Correlations of plasma insulin concentrations with the glycerol rate of appearance (Ra), plasma metabolite concentrations, and total FA oxidation rates were assessed throughout the observation period (0-120 min), during the enteric phase of the PLS only (0-30 min), and during the systemic phase of the PLS only (60-120 min) (59). Correlations of plasma insulin concentrations with total CHO oxidation rates and of the plasma insulin-to-glucagon ratio with markers of lipolysis were assessed for the entirety of the observation period only.

RESULTS

Participant characteristics. Comparisons between young and older participants are presented in Table 1. Older participants' mean age ($p < 0.001$), BMI ($p = 0.03$), body fat percentage ($p = 0.02$), and body fat mass (FM) ($p = 0.005$) were significantly higher than young subjects. In contrast, absolute ($p = 0.007$) and relative ($p < 0.001$) $\dot{V}O_2$peak, peak power output ($p = 0.002$), and VT ($p < 0.001$) were significantly higher in young participants. There were no significant differences in total body mass (TBM) ($p = 0.22$), fat free mass (FFM) ($p = 0.79$), pulmonary function (FEV_1/FVC) ($p = 0.09$), daily caloric intake ($p = 0.10$), or percentage of caloric intake

from carbohydrates ($p = 0.12$), fat ($p = 0.68$), or protein ($p = 0.06$). There were no significant differences in the size of the glucose load when expressed relative to TBM ($p = 0.36$) or per unit of FFM ($p = 0.95$), but when expressed per unit of FM, the relative glucose load was greater for young participants ($p = 0.007$).

Indexes of insulin sensitivity and insulin secretion. The HOMA-IR score was significantly higher in older participants ($p = 0.02$) (Table 2). In contrast, the IGI1 ($p = 0.006$), IGI2 ($p = 0.01$), DI1 ($p < 0.001$), and DI2 ($p = 0.01$) scores were significantly higher in young participants. There were no differences in the CISI ($p = 0.18$), HOMA-%β ($p = 0.13$), or IGI3 ($p = 0.07$) scores between groups.

Plasma insulin concentrations. In young participants, plasma insulin concentrations ([Insulin]) increased 5 min after glucose consumption (77.82 ± 12.62 vs. 22.71 ± 2.50 pM, $p = 0.01$), continuously rose until 30 min (411.86 ± 65.40 pM, $p < 0.001$), remained steady at 60 min ($p < 0.001$), decreased at 90 min (318.36 ± 43.97 pM, $p < 0.001$), and then increased at 120 min (347.87 ± 44.63 pM, $p < 0.001$) (Fig. 1). In contrast, in older participants, [Insulin] increased at 15 min (151.40 ± 17.49 vs. 33.85 ± 3.60 pM, $p < 0.001$), continuously rose until 30 min (313.41 ± 35.48 pM, $p < 0.001$), and then remained elevated thereafter ($p < 0.001$). Additionally, [Insulin] was higher in older participants at baseline ($p = 0.02$) but higher in young participants at 5 min ($p = 0.05$) and 30 min ($p = 0.01$). There were no differences in the AUC for [Insulin] ($p = 0.47$) (Fig. 2). Similarly, there were no differences in the AUC for [Insulin] between 0–30 min ($p = 0.32$) or 30–120 min ($p = 0.64$) (Fig. 3).

Plasma C-peptide concentrations. In young participants, plasma C-peptide concentrations ([CP]) were increased from baseline at 5 min (666.40 ± 65.43 vs. 206.25 ± 14.93 pM, $p < 0.001$) and 60 min (1957.12 ± 171.70 pM, $p < 0.001$) after glucose consumption (Fig. 4). In contrast, in older participants, [CP] was increased from baseline at 60 min only (1501.41 ± 100.49 vs. 307.69 ± 19.52 pM, $p < 0.001$). Additionally, [CP] was higher in older participants at baseline ($p < 0.001$) but higher in young participants at 5 min ($p = 0.004$) and 60 min ($p = 0.04$).

Blood glucose concentrations. Blood glucose concentrations ([Glucose]) remained unchanged at 5 min in both groups ($p \geq 0.97$) (Fig. 5). [Glucose] increased 15 min after glucose consumption in both young (126.53 ± 0.23 vs. 84.98 ± 0.10 $mg \cdot dL^{-1}$, $p < 0.001$) and older (131.70 ± 0.34 vs. 90.78 ± 0.19 $mg \cdot dL^{-1}$, $p < 0.001$) participants, continued to rise until 60 min ($p < 0.001$), and then steadily declined thereafter ($p < 0.001$). Additionally, [Glucose] was overall higher in older participants, but the difference was significant at 90 min (161.04 ± 0.46 vs. 131.37 ± 0.46 $mg \cdot dL^{-1}$, $p = 0.02$). There were no differences in the AUC for [Glucose] ($p = 0.24$) (Fig. 6).

Plasma glucagon concentrations. In young participants, plasma glucagon concentrations ([Glucagon]) decreased from baseline between 5-15 min, but the changes were not significant ($p \geq 0.18$) (Fig. 7). [Glucagon] decreased 30 min after glucose consumption (5.87 ± 0.75 vs. 9.78 ± 0.80 pM, $p = 0.001$), continuously declined until 60 min (3.97 ± 0.47 pM, $p < 0.001$), and then remained low thereafter ($p < 0.001$). In older participants, [Glucagon] increased at 5 min ($p = 0.32$) and then decreased at 15 min ($p = 0.39$), but the changes were not significant. [Glucagon] similarly decreased at 30 min (5.24 ± 0.65 vs. 7.49 ± 0.79 pM, $p = 0.002$), continuously declined until 60 min (3.92 ± 0.37 pM, $p < 0.001$), and then remained low thereafter ($p < 0.001$). There were no

significant differences between groups at any time point ($p \geq 0.06$). There were no differences in the AUC for [Glucagon] ($p = 0.51$) (Fig. 8).

Plasma insulin-to-glucagon ratio. Changes in the plasma insulin-to-glucagon ratio (IGR) were similar to the changes in [Insulin]. In young participants, the IGR increased 5 min after glucose consumption (8.85 ± 1.29 vs. 2.34 ± 0.29, $p = 0.002$), continuously rose until 15 min (28.79 ± 4.64, $p = 0.004$), remained steady at 30 min ($p = 0.08$), increased at 60 min (96.48 ± 14.27, $p = 0.03$), decreased at 90 min (67.20 ± 7.03, $p = 0.007$), and then increased at 120 min (124.79 ± 28.88, $p = 0.03$) (Fig. 9). In contrast, in older participants, the IGR increased at 15 min (24.95 ± 2.81 vs. 4.60 ± 0.52, $p < 0.001$) and then continuously rose thereafter ($p \leq 0.002$). Additionally, IGR was higher in older participants at baseline ($p = 0.009$).

Correlation between plasma insulin concentrations and plasma glucagon concentrations. There was an inverse relationship between [Insulin] and [Glucagon] (Fig. 10) in both groups. However, the correlation was higher ($r = -0.98$ vs. $r = -0.90$) and more significant ($p < 0.001$ vs. $p = 0.006$) in older participants.

Correlation between plasma insulin concentrations with rates and markers of lipolysis. Results of correlations of [Insulin] with variables related to substrate availability and utilization are summarized in Table 4. There was an overall inverse relationship between [Insulin] and glycerol Ra per unit of body mass (Fig. 11), [Glycerol] (Fig. 12), and [FFA] (Fig. 13) in both groups. However, the correlations were higher ($r \geq -0.89$ vs. $r \geq -0.75$) and more significant ($p \leq 0.007$ vs. $p \leq 0.05$) in older participants. The relationships were similar when glycerol Ra was expressed relative to FFM and FM and as an absolute rate.

Correlation between plasma insulin concentrations with total carbohydrate and fatty acid oxidation rates. There was an overall inverse relationship between [Insulin] and FA Rox in both groups, but the correlation was higher ($r = -0.87$ vs. $r = -0.59$) in older participants (Fig. 14). Additionally, the relationship was only significant in older participants ($p \leq 0.01$ vs. $p \geq 0.14$). In contrast, there was an overall positive relationship between [Insulin] and CHO Rox in both groups, but the correlation was higher ($r = 0.96$ vs. $r = 0.80$) in older participants (Fig. 15). The relationships were similar when FA Rox and CHO Rox were expressed relative to FFM and FM and as an absolute rate.

Correlation between the plasma insulin to glucagon ratio with rates and markers of lipolysis. Results of correlations of the IGR with glycerol Ra, [Glycerol], and [FFA] are summarized in Table 5. There was a very high inverse relationship between the IGR and glycerol Ra (Fig. 16), [Glycerol] (Fig. 17), and [FFA] (Fig. 18) in both groups. The correlations were similar in size and significance. The relationships were similar when glycerol Ra was expressed relative to FFM and FM and as an absolute rate.

DISCUSSION

The purpose of this investigation was to assess metabolic flexibility by determining concurrent changes in plasma insulin and glucagon concentrations and rates of lipolysis, CHO

oxidation, and FA oxidation during an OGTT in healthy young and older individuals. Additionally, we sought to determine if physiological hyperinsulinemia and lactatemia differentially affect FA substrate partitioning in the postprandial period. Although we observed predictable hormone responses in both groups, we also observed notable aging-related differences in the immediate response to oral glucose consumption. In brief, following oral glucose consumption, we report the following: (1) the insulin response is attenuated in aging, (2) hyperinsulinemia contributes to the suppression of lipolysis, (3) the glucagon response is unaffected in aging, and (4) hyperinsulinemia and lactatemia are required for the suppression of lipolysis in aging. These results are discussed sequentially.

Indexes of insulin sensitivity. We utilized two methods to estimate insulin sensitivity. The HOMA-IR method estimates insulin sensitivity from baseline, fasting [Insulin] and [Glucose] (117, 142). The CISI method also estimates insulin sensitivity, but from changes in [Insulin] and [Glucose] obtained during the last 90 min of an OGTT (101). Both methods are highly correlated with the hyperinsulinemic-euglycemic (101, 141) and hyperglycemic clamp techniques (142). Accordingly, both methods have been cited by different investigators who studied populations with normal glucose tolerance (146), diabetic populations, and others (143). Presently, the mean HOMA-IR score was higher in our young participants ($p = 0.02$), which implies superior insulin sensitivity. Although this is consistent with the observation that glucose tolerance generally declines in aging (99, 137), we did not observe any indication of insulin "resistance" as observed in metabolic disease states in our older participants. The mean HOMA-IR score of our older participants was lower than the HOMA-IR scores previously reported in metabolic disease states (141, 160, 161), indicating greater insulin sensitivity among our older participants. Moreover, the CISI scores of young and older participants were similar ($p = 0.18$) and were greater than the scores previously reported in insulin resistant individuals (162-164). Notably, the baseline [Insulin] in our older participants (33.85 ± 3.60 pM, or 5.77 ± 0.63 $\mu IU \cdot mL^{-1}$) was lower than what has been previously reported in individuals with normal glucose tolerance (148, 158). Thus, despite higher [Insulin] ($p = 0.02$) at baseline (Fig. 3), we contend that there is no indication of insulin "resistance" in our older compared to young participants (46). At minimum, our older participants exhibited comparatively lower insulin sensitivity than young participants, but not insulin "resistance" as observed in metabolic disease states.

Glucose tolerance. Normal glucose tolerance is defined as having a venous blood glucose concentration of <140 $mg \cdot dL^{-1}$ at the end of an OGTT (165). Our older participants had a mean [Glucose] of 141.82 ± 6.82 $mg \cdot dL^{-1}$ at the end of observation (Fig. 5), which implies impaired glucose tolerance. However, because blood glucose concentrations are higher in arterial than venous blood (166), and because there was no difference ($p = 0.23$) in [Glucose] between groups at 120 min and no difference ($p = 0.24$) in the AUC for [Glucose] (Fig. 6), we conclude that our older participants exhibited normal glucose tolerance. Consequently, the possibilities of insulin resistance and impaired glucose tolerance will not be considered in the interpretation of the data.

Plasma insulin and C-peptide concentrations and indexes of insulin secretion during the "early-phase", or Enteric Postprandial Lactate Shuttle Phase. A key finding of the present investigation is the immediate increase in [Insulin] 5 min after glucose consumption in young ($p = 0.01$) but not older ($p = 0.62$) participants. Additionally, [CP] was increased in young ($p < 0.001$) but not older ($p = 0.32$) participants at 5 min (Fig. 4). C-peptide is secreted with insulin from

pancreatic β-cells following cleavage of their prohormone precursor, proinsulin (167), and is used as a measure of insulin secretion (142, 168). Thus, there was a rapid increase in insulin secretion in young participants only. To our knowledge, our investigation is the first to demonstrate an increase in [Insulin] and [CP] at 5 min during an OGTT (59). Notably, Knop et al. (169) similarly did not observe an increase in plasma insulin or C-peptide concentrations at 5 min after consumption of a 50-g glucose load in their healthy older (44-69 years) participants with normal glucose tolerance. We expand on their data by demonstrating that the size of the glucose load was likely not a major factor that influenced the secretion of insulin within the first 5 min of an OGTT.

Although we observed no differences in the AUC for [Insulin] between groups ($p = 0.32$) during the first 30 min of observation (Fig. 3), our data suggests that "early-phase" insulin secretion was greater in young participants. In young compared to older participants, [Insulin] was higher at 5 min and 30 min ($p \leq 0.05$), [Insulin] trended to be higher at 15 min ($p = 0.10$), and [CP] was higher at 5 min and 60 min ($p \leq 0.04$). Although insulin and C-peptide are secreted in equimolar amounts, the longer half-life of C-peptide allows it to remain in circulation longer than insulin (168). Consequently, the higher [CP] at 60 min reflects greater insulin secretion in the period preceding it (i.e., 0-30 min). Consistently, the IGI1, IGI2, and DI1 scores were higher in young participants ($p \leq 0.01$). Both the IGI and DI provide an indirect assessment of β-cell function because they describe the ability of the β-cell to secrete insulin in response to ambient changes in the blood glucose concentration (151, 152, 156). Collectively, these results suggest that the insulin response in the immediate period following oral glucose consumption was attenuated in our older participants due to lower β-cell function.

The hyperglycemic clamp technique originally described by DeFronzo et al. (170) in 1979 is regarded as the "gold standard" method for quantifying insulin secretion, or β-cell function. Since its introduction, the IGI and DI methods have been employed by DeFronzo and colleagues (101, 148-150, 156, 158, 159) and others (146, 152, 154, 155, 157) for estimating β-cell function during an OGTT. Thus, although we recognize that our calculations of IGI and DI are not perfect indicators of β-cell function, we regard them as appropriate surrogate estimations.

The secretion of insulin by β-cells during hyperglycemia occurs following cellular uptake of glucose and increased glycolytic flux, ultimately resulting in insulin release (171). Reports on the influence of age on β-cell function are not completely in agreement. DeFronzo (99, 137) reported that the insulin response to ambient changes in the blood glucose concentration is not affected in aging. Consistently, DeFronzo, Garduno-Garcia, et al. (150) later reported that the rate of insulin secretion during an OGTT was not different between young and older individuals with normal glucose tolerance. In contrast, Muller et al. (172) reported a delayed insulin response during an OGTT between young and older individuals. Basu et al. (102) reported impaired insulin secretion in elderly individuals following intravenous injection of glucose and the ingestion of a mixed meal. Pacini et al. also reported lower insulin secretion during an intravenous glucose tolerance test in elderly individuals with both lower (173) and normal (174, 175) insulin sensitivity. Hence, if β-cell function is impaired in aging, the β-cell response to factors associated with oral glucose consumption as well as increased blood glucose concentrations only are affected.

The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are rapidly secreted from intestinal cells following nutrient

consumption and act directly on β-cells to promote insulin secretion (176). They are responsible for approximately 50-70% of the total insulin secreted following nutrient consumption (177, 178). Because the increase in [Insulin] at 5 min was not accompanied by an increase in [Glucose] (p = 0.99), an incretin effect likely contributed to the immediate increase in [Insulin] we observed in young participants (179). Previous investigations have reported that the secretion of GLP-1 and GIP are greater in older compared to young individuals, potentially to compensate for reduced β-cell sensitivity to incretin stimulation (150, 180, 181). Presently, the non-significant increase in [Insulin] (p = 0.65) at 5 min and lower [Insulin] at 30 min in older participants supports the hypothesis that β-cell sensitivity to incretin stimulation is reduced in aging.

Insulin secretion during nutrient consumption is also regulated by neural mechanisms. Cephalic phase insulin release (CPIR) refers to preabsorptive insulin secretion induced by sensory stimulation during nutrient consumption (182). The response is mediated by the autonomic mechanisms affecting β-cells. Briefly, stimulation of pancreatic islets by acetylcholine and non-cholinergic signals, such as pituitary adenylate cyclase-activating polypeptide (PACAP) contained in parasympathetic nerves (183), induces insulin secretion (182, 184). The response occurs independently from GLP-1 and GIP (182) and is initiated within the first 5-10 min following nutrient consumption (185). The immediate increase in [Insulin] at 5 min in our young participants provides further evidence for CPIR in healthy humans (186). To our knowledge, aging-related changes in CPIR have not yet been identified. However, the non-significant increase in [Insulin] at 5 min in older participants suggests that β-cell sensitivity to CPIR-activating signals is reduced in aging. Although extant data on younger individuals indicates that CPIR is only responsible for a small percentage of the total insulin secreted in the postprandial period (186, 187), considering the present data, future investigations might choose to reevaluate the contribution of CPIR in aging.

Overall, the lower [Insulin] in our older participants during the "early phase" (i.e., 0-30 min) of the OGTT is attributed to lower β-cell function associated with lower sensitivity to incretin stimulation, stimulation by CPIR-activating signals, or both. Some (172, 188) but not all (150) previous investigations have reported a diminished insulin response following oral glucose consumption in older individuals (153). Notably, Jackson et al. (188) similarly reported lower plasma insulin concentrations in their healthy elderly (76.8 ± 3.4 years) participants within the first 30 min after consumption of a 100-g glucose load, indicating that the size of the glucose load was likely not a major factor that influenced the secretion of insulin in the "early phase" of the present investigation.

Plasma insulin concentrations and indexes of insulin secretion during the "late phase". In young participants, [Insulin] peaked at 30 min, but remained relatively unchanged from 30 min at all succeeding time points ($p \geq 0.77$). In contrast, in older participants, [Insulin] trended to increase from 30 min at all succeeding time points ($0.08 \leq p \leq 0.10$) and peaked at 120 min. Because [Insulin] was not different between groups at 120 min (p = 0.85), our results suggest that the attenuated insulin response in older participants persisted into the "late phase" (i.e., 30-120 min) of the OGTT. Consistently, in young participants, the IGI3 score trended to be higher (p = 0.06) while the DI2 score was significantly higher (p = 0.01). From these estimations, and as previously described, the overall slower rise in [Insulin] in older participants can be attributed to lower β-cell function associated with lower β-cell sensitivity to incretin stimulation (150, 180, 181). However,

as the meaning of the IGI and DI scores indicate, mechanisms related to β-cell glucose metabolism might also be responsible for the slower rise in [Insulin].

Following cellular uptake of glucose via glucose transporter GLUT2, β-cells increase ATP production through glycolysis and subsequent mitochondrial respiration. The resulting increase in the ATP/ADP ratio depolarizes β-cell membranes, allowing an influx of calcium ions (Ca^{2+}) that are required for insulin exocytosis (171, 189). Because multiple factors influence insulin secretion, the mechanism of glucose-stimulated insulin release (GSIR) has been studied in isolated islets to interrogate the effects of glucose stimulation alone. Previous investigations utilizing human islets reported lower ATP content (190) and impaired Ca^{2+} signaling (191) in islets from older donors, which contributed to lower GSIR (192, 193). Additionally, in islets of older animals, lower GLUT2 expression (194) and lower Ca^{2+} uptake (195) are theorized to contribute to reduced GSIR. Multiple components of the signal transduction pathway can therefore be implicated in the aging-related decline in GSIR. Presently, the IGI and DI scores suggest that GSIR was lower in older participants, but the exact mechanism cannot be determined from our data.

Influence of plasma insulin concentrations on lipolysis. Insulin has an essential role in the suppression of adipose tissue lipolysis. Briefly, insulin binding to its receptors on adipose cells inhibits lipolysis by activating the phosphodiesterase enzyme and reducing levels of cAMP, deactivating the regulatory hormone-sensitive lipase enzyme responsible for triglyceride hydrolysis (131-133). Previous investigations utilizing stable isotope tracers reported a reduction in the plasma glycerol (196) and free fatty acid (94) rates of appearance (Ra) during variable insulin infusion rates or during hyperinsulinemic-euglycemic clamp experiments (16, 197) to demonstrate a reduction in the rate of lipolysis. Presently, the inverse correlations between [Insulin] and glycerol Ra (Fig. 11), [Glycerol] (Fig. 12), and [FFA] (Fig. 13) and support the role of insulin in the inhibition of lipolysis. Although the correlations were higher and more significant in older participants (Table 3), we do not interpret these results to suggest that insulin had a less important role in the suppression of lipolysis in young participants. Alternatively, the size and significance of the correlations partially explain the aging-related differences in the suppression of lipolysis.

The results of Campbell et al. (94) suggest that the glycerol Ra is initially suppressed at a plasma insulin concentration of approximately 86 pM and maximally suppressed at approximately 386 pM. Other investigators similarly reported a serum insulin concentration (16) or insulin infusion rate (196) associated with the maximal suppression of the glycerol Ra. In Chapter 2 of this dissertation we demonstrated that in young participants, glycerol Ra decreased from baseline 5 min after glucose consumption. Presently, we show this occurred simultaneously with an increase in [Insulin] from baseline to 77.82 ± 12.62 pM ($p = 0.01$). In older participants, glycerol Ra and [Insulin] (47.52 ± 6.67 pM, $p = 0.62$) were not different from baseline at 5 min. However, glycerol Ra trended to decrease from baseline at 15 min ($p = 0.08$) while [Insulin] increased from baseline to 151.40 ± 17.49 pM ($p < 0.001$). Thus, the delayed suppression of lipolysis in older participants can be attributed, in part, to the slower rise in [Insulin] to a "threshold" concentration required to suppress lipolysis (94).

As described in Chapter 2 of this dissertation, glycerol Ra continuously declined in young ($p \leq 0.05$) but not older ($0.18 \geq p \geq 0.13$) participants between 60-120 min. Because [Insulin] did not change between 60-120 min in both groups ($p \geq 0.49$), the correlations were higher and more

significant in older participants. Rephrased, the correlations were lower and less significant in young participants because glycerol Ra was not maximally suppressed. The inability of insulin to maximally suppress lipolysis is not likely meaningful because [FFA] and FA Rox were simultaneously low. However, the results alternatively suggest that other factors responsible for the suppression of lipolysis (i.e., lactate) exhibited a greater contribution in older participants.

Influence of plasma insulin concentrations on substrate oxidation rates. The role of insulin in the suppression of FA oxidation is well-documented (3, 90, 113). Groop et al. (134) reported that the role of insulin is indirect through its inhibitory effects on lipolysis, i.e., insulin suppression of lipolysis reduces plasma FFA availability. Sidossis et al. (90) later reported that hyperglycemia and hyperinsulinemia mutually promoted the formation of malonyl-CoA, which limits mitochondrial FA transport to directly limit FA oxidation. Still, the role of insulin is most likely indirect through its promotion of glucose uptake to malonyl-CoA formation (33). Accordingly, insulin also has an essential role in promoting CHO oxidation by increasing glucose availability (3, 95, 96). Presently, the inverse correlations between [Insulin] and FA Rox (Fig. 14) supports the role of insulin in the suppression of FA oxidation. As well, the positive correlations between [Insulin] and CHO Rox (Fig. 15) supports an earlier contention that our older participants did not exhibit insulin "resistance" as observed in metabolic disease states (198).

Previous investigations have demonstrated greater rates of FA/lipid oxidation and lower rates of CHO oxidation when plasma FFA availability was elevated during hyperinsulinemia by lipid and heparin infusion (6, 81, 82). Elevated plasma FFA availability purportedly induces transient changes in insulin action (119) to affect substrate oxidation rates. Presently, [FFA] was significantly higher in older participants between 60-120 min. However, the higher [FFA] apparently did not affect the ability of insulin action to signal changes in substrate oxidation rates, as the relationships between [Insulin] and FA Rox and CHO Rox were higher than in young participants. With the correlation between [Insulin] and glycerol Ra, our data collectively suggests that the ability of insulin action to affect energy substrate partitioning in the postprandial period is preserved in "healthy" aging. Interpretation of these correlations from this perspective are potentially meaningful in "unhealthy" aging or metabolic disease states in which postprandial lipolytic rates may be insensitive to insulin signaling such that plasma FFA concentrations remain elevated at a concentration high enough and for long enough (120) to transiently affect insulin action.

Interestingly, the correlation between [Insulin] and FA Rox was not significant ($p = 0.17$) in young participants, indicating that insulin did not have a significant inhibitory effect. Because correlations do not provide evidence of causation, we contend that this relationship instead reflects overall greater control of metabolic flexibility in young participants. As described in Chapter 3 of this dissertation, the suppression of FA Rox was dependent on lactate suppression of lipolysis in our older but not young participants. The higher and more significant correlation between [Insulin] and FA Rox in older participants further supports this inference, collectively suggesting that older participants suppressed FA Rox due to reduced FA availability. In contrast, the significant ($p = 0.03$) correlation between [Insulin] and CHO Rox in young participants supports the inference that FA Rox was suppressed in response to the increased availability of glucose and consequent increase in CHO Rox.

Plasma glucagon concentrations. The primary role of glucagon is to increase hepatic glucose output by increasing glycogenolysis and gluconeogenesis to increase blood glucose concentrations (199). Because blood glucose concentrations increase during an OGTT, plasma glucagon concentrations are expected to decline. Previous investigations have demonstrated that plasma glucagon concentrations decreased 30 min after consumption of a glucose load in individuals with normal glucose tolerance (200, 201). However, Mitrakou et al. (201) reported that the decrease in glucagon was delayed to 60 min in individuals with impaired glucose tolerance. Presently, [Glucagon] decreased from baseline 30 min after glucose consumption in both groups ($p \leq 0.002$) (Fig. 7). Additionally, there were no differences in [Glucagon] between groups at any time point or in the AUC for [Glucagon] (Fig. 8). Our results agree with those of Simonson and DeFronzo (202), who reported that the progressive reduction in plasma glucagon concentrations was not different between their healthy young (24 ± 1 years) and older (62 ± 2 years) individuals during hyperglycemic clamp experiments. We expand on their data by demonstrating that the reduction in [Glucagon] following oral glucose consumption is similar in healthy young and older participants.

Although there were no significant differences in [Glucagon] between groups, there were apparent differences. First, at baseline, [Glucagon] trended to be higher in young participants ($p = 0.06$). There were no obvious effects of the marginally higher [Glucagon] on [Glucose], which was similar between groups at baseline ($p = 0.17$). The marginally higher [Glucagon] is also notable because postabsorptive plasma glucagon concentrations are occasionally reported to be higher in older individuals (202, 203) and in individuals with impaired glucose tolerance (200). Hence, the result provides more evidence of "healthy" aging. Second, in older participants, there was a non-significant ($p = 0.32$) increase in [Glucagon] from baseline at 5 min. Yabe et al. (204) reported that an increase in plasma glucagon concentrations during an OGTT contributes to hyperglycemia. Because the increase in [Glucagon] was not significant, and because the increase in [Glucagon] was not paralleled by an increase in [Glucose], the result is likely not meaningful. Similarly, the higher [Glucose] in older participants at 90 min ($p = 0.02$) is not likely to be a result of aging-related differences in glucagon signaling (202) since [Glucagon] was decreased from baseline ($p < 0.001$) at that time point.

Glucagon secretion by pancreatic α-cells is regulated by several mechanisms, including GLP-1 signaling (176, 205), regulation by other hormones, and regulation by autonomic mechanisms (206, 207). For brevity, only the mechanisms most relevant to the present data will be discussed. Previous investigations by Cryer et al. (208, 209) demonstrated that glucagon secretion is regulated by insulin signaling. Notably, Cooperberg and Cryer (210) demonstrated that plasma glucagon concentrations were reduced during insulin infusion despite prevailing hypoglycemia. Presently, we observed an inverse correlation between [Insulin] and [Glucagon] in both groups (Fig. 10). The relationship was higher in older participants ($r = -0.98$ vs. $r = -0.90$), most likely due to the gradual rise in [Insulin] that paralleled the gradual reduction in [Glucagon]. Interestingly, in young participants, we did not observe an immediate decrease in [Glucagon] from baseline at 5 min ($p = 0.95$) to match the increase in [Insulin], or lower [Glucagon] compared to older participants at 30 min ($p = 0.54$) to match the higher [Insulin]. We interpret our results to indicate that α-cell sensitivity to insulin stimulation is unaffected in aging.

Similar to insulin, glucagon secretion is also regulated by the ambient blood glucose concentration. Following cellular uptake of glucose via glucose transporter GLUT1, α-cells increase ATP production through glycolysis and subsequent mitochondrial respiration. The resulting increase in the ATP/ADP ratio repolarizes α-cell membranes, inhibiting an influx of Ca^{2+} that are required for glucagon exocytosis (206, 211, 212). Accordingly, the reduction in [Glucagon] at 30 min followed the rise in [Glucose] at 15 min in both groups, suggesting that hyperglycemia contributed to the suppression of [Glucagon].

To our knowledge, aging-related changes in α-cell function have not been studied extensively as β-cell function has. However, abnormalities in α-cell function and glucagon secretion have been identified in aging-related metabolic disease states, including impaired glucose tolerance (201) and type 2 diabetes (207, 213, 214). Our data on [Glucagon] collectively suggests that the α-cell response in the postprandial period is preserved in "healthy" aging. Further, our data suggests that malfunctions of the mechanisms that regulate glucagon secretion are not necessarily a feature of aging (202) but are likely exclusive to metabolic disease states.

Plasma insulin-to-glucagon ratio and its influence on lipolysis. Previous investigations utilizing glucagon infusions and stable isotope tracers (215) or microdialysis assessments in humans (216, 217) and investigations using isolated human adipocytes (218) indicate that the rate of lipolysis is not affected by glucagon signaling. However, the plasma IGR is regarded as a key regulator of anabolic and catabolic processes. Specifically, an increase in the plasma IGR following nutrient consumption is an important anabolic response in which the mobilization of stored nutrients would be inhibited (213, 219, 220). Hence, the suppression of glucagon secretion but not glucagon action might be an important response that contributes to the regulation of lipolysis in the postprandial period.

We observed a predictable increase in the plasma IGR in both groups during the observation period (Fig. 9) due to the increase in [Insulin] and decrease in [Glucagon]. Because of the changes in [Insulin], the IGR increased 5 min after glucose consumption in young ($p = 0.002$) but not older ($p = 0.45$) participants. Thus, similar to the changes in [Insulin], the rise in the IGR was attenuated in older participants. Interestingly, in young participants, the IGR at 30 min only trended to increase from baseline ($p = 0.08$), presumably due to a higher variation in [Insulin] at 30 min affecting the calculation of the IGR. The present data is nonetheless interpreted to indicate an immediate and sustained increase in the IGR in young but not older participants.

We also observed high and significant inverse correlations between the plasma IGR and glycerol Ra (Fig. 16), [Glycerol] (Fig. 17), and [FFA] (Fig. 18) in both groups. While the relationships of these variables to the IGR and [Insulin] were comparable for older participants, the relationships were notably higher and more significant in young participants. This was most likely due to the more gradual rise in the IGR compared to the rapid rise in [Insulin]. Still, the result underscores the importance of the IGR as a regulator of anabolic and catabolic processes (213, 219, 220).

While a number of previous investigations have demonstrated that the rate of lipolysis is not affected by glucagon signaling in humans (215-217), Gerich et al. (221) demonstrated that glucagon exerts a lipolytic effect when plasma insulin concentrations are low. Gerich et al. (221)

reported an increase in plasma glycerol and FFA concentrations (as markers to indicate an increased lipolytic rate) during glucagon infusion, but not during infusion of glucagon and insulin. Hence, lipolysis occurs when the IGR is low. The suppression of glucagon secretion might therefore be an important response that contributes to the suppression of lipolysis in the postprandial period. Although our data does not suggest that the delayed suppression of lipolysis in our older participants resulted from an attenuated IGR response or failure to suppress [Glucagon], future investigations might choose to investigate the postprandial lipolytic response when plasma glucagon concentrations are maintained at postabsorptive levels or elevated as in metabolic disease states (200). Such results might help identify the role of α-cell abnormalities in the control of lipid substrate partitioning and metabolic flexibility.

Influence of lactatemia versus hyperinsulinemia on lipolytic and fatty acid oxidation rates. We have recently reviewed the role of lactate in signaling and its sequential effects on energy substrate partitioning (31). Briefly, intracellular lactate accumulation limits mitochondrial FA uptake by increasing the production of malonyl-CoA and ROS, which collectively inhibit FA transporters CPT I (32) and CPT II (34). Additionally, lactate binding to its receptor HCAR-1 on adipose cells inhibits lipolysis by activating the phosphodiesterase enzyme and reducing levels of cAMP (35-37). Lactate therefore controls lipid substrate partitioning at the beginning and end of the pathway, from FA mobilization to oxidation. The effects of lactatemia on FA oxidation are however indirect through its effects on lipolysis and FA availability. Thus, lactate shares inhibitory effects on FA metabolism with insulin (33, 90, 131-133). However, our data suggests that hyperinsulinemia and lactatemia differentially affect FA partitioning in aging.

A comparison of correlations of [Insulin] and [Lactate] to variables related to FA availability and utilization in young participants is presented in Table 6. Although the overall relationship between glycerol Ra and [Lactate] only trended to be significant ($p = 0.07$), the size of the correlation between [Insulin] and [Lactate] and Ra were similar. Thus, both increased [Insulin] and [Lactate] contributed to the suppression of lipolysis, but [Insulin] might be more important. This is further supported by the overall relationships between [Insulin] and [Glycerol] and [FFA], which were higher and more significant than those with [Lactate]. Notably, during the enteric phase, the relationship between [Insulin] and Ra trended to be significant ($r = -0.94$, $p = 0.06$) while the relationship between [Lactate] and Ra was not high or significant ($r = -0.50$, $p = 0.50$). These results are explained, in part, by the biphasic response of [Lactate]. Still, because there was an increase in both [Insulin] and [Lactate] at 5 min that was matched by a decrease in Ra, both hyperinsulinemia and lactatemia have a role in the immediate control of lipolysis in the enteric phase.

In contrast, the correlations during the systemic phase suggest that neither [Lactate] nor [Insulin] exhibited an inhibitory effect on glycerol Ra. While the positive relationship between [Lactate] and Ra can be explained by greater rates of lactate oxidation freeing the restraints of lactatemia on lipolysis (60), we are unable to postulate a reason for the positive relationship between [Insulin] and Ra.

Ahmed et al. (36) described an autocrine mechanism in mouse adipocytes in which lactate signaling via GPR81 (HCAR-1) potentiates the inhibitory effects of insulin on lipolysis. In humans, lactate production by adipocytes following oral glucose consumption (54-57) provides an

autocrine mechanism for the anti-lipolytic effects of lactate. Presently, in older participants, the influence of [Insulin] and [Lactate] on glycerol Ra, [Glycerol], and [FFA] were similar (Table 7). Notably, the systemic phase correlations were nearly identical and linear ($r \geq -0.93$) but not significant ($p \geq 0.14$). Results of the overall and systemic phase correlations allow us to speculate that both insulin and lactate are required for the complete suppression of lipolysis in aging.

A minor discrepancy is apparent during the enteric phase. Similar to young participants, the relationship between [Insulin] and glycerol Ra during the enteric phase was significant ($r = -0.96$, $p = 0.04$) while the relationship between [Lactate] and Ra was not ($p = 0.29$). Thus, insulin exerted greater control over lipolysis during the enteric phase in both young and older participants. Physiologically, mediation of the anti-lipolytic effects of insulin by autocrine lactate signaling is preceded by insulin-mediated glucose uptake leading to glycolysis and lactate formation. Hence, according to the canonical mechanism described by Ahmed et al. (36), the absence of a high or significant enteric phase correlation in both groups is understood.

As previously described, the correlations between [Insulin] and [Lactate] and FA Rox in young participants reflect overall greater control of metabolic flexibility. To reiterate, we interpret the results of these correlations to indicate that the suppression of FA Rox was a response to the suppression of lipolysis and reduced FA availability in our older but not young participants. This was most likely a result of the attenuated insulin response contributing to the delayed suppression of lipolysis. Thus, lactate rescues postprandial metabolic flexibility in aging by supporting the suppression of lipolysis and FA oxidation. Accordingly, the correlations between [Insulin] and [Lactate] and FA Rox in older participants were identical in size and significance.

Overall, our comparison of correlations of [Insulin] and [Lactate] to glycerol Ra provides support for the autocrine mechanism described by Ahmed et al. (36) in aging, particularly during the systemic phase of the PLS. The apparent absence of this mechanism in our young participants most likely results from the greater rates of systemic lactate oxidation freeing the restraints of lactatemia on lipolysis (60).

CONCLUSION

Our results demonstrate that the insulin response following oral glucose consumption is attenuated in healthy older individuals compared to young individuals. This was most apparent in the immediate period after glucose consumption. Because the correlations between [Insulin] and glycerol Ra and FA Rox were higher and more significant in older participants, we demonstrate that the delayed suppression of FA metabolism can be explained by the differences in the insulin response. Moreover, a comparison of the correlations of [Insulin] and [Lactate] to glycerol Ra suggests that both insulin and lactate are required for the complete suppression of lipolysis in aging. From this perspective, lactate rescues postprandial metabolic flexibility in aging by supporting the suppression of lipolysis and FA oxidation. In contrast, the glucagon response and the insulin-to-glucagon ratio were not different. Future similar investigations should determine changes in the other counterregulatory hormones to determine if aging-related differences in how the plasma concentrations of these hormones change help explain the reported differences in FA substrate partitioning during an OGTT.

FIGURE LEGENDS

Figure 1. Plasma insulin concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 2. Area under the curve (AUC) for plasma insulin concentrations in young and older participants. Values are means ± SE.

Figure 3. Area under the curve (AUC) for plasma insulin concentrations between 0–30 min (A) and 30–120 min (B) in young and older participants. Values are means ± SE.

Figure 4. Plasma C-peptide concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 5. Blood glucose concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 6. Area under the curve (AUC) for blood glucose concentrations in young and older participants. Values are means ± SE.

Figure 7. Plasma glucagon concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

Figure 8. Area under the curve (AUC) for plasma glucagon concentrations in young and older participants. Values are means ± SE.

Figure 9. Plasma insulin-to-glucagon ratio in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Figure 10. Relationship between plasma insulin concentrations and plasma glucagon concentrations in young and older participants.

Figure 11. Relationship between plasma insulin concentrations and glycerol rate of appearance (Ra) per unit of body mass in young and older participants.

Figure 12. Relationship between plasma insulin concentrations and plasma glycerol concentrations in young and older participants.

Figure 13. Relationship between plasma insulin concentrations and plasma free fatty acid (FFA) concentrations in young and older participants.

Table 1. Characteristics of young and older participants.

Variable	Young	Older
Age, yr	28.1 ± 1.4	70.6 ± 2.4 *
Body mass, kg	66.64 ± 2.70	73.21 ± 4.18
Body mass index, kg/m^2	23.5 ± 1.8	26.2 ± 1.3 *
Body fat, %	17.63 ± 1.73	23.14 ± 1.29 *
Fat mass, kg	11.47 ± 1.10	16.83 ± 1.27 *
Fat free mass, kg	55.16 ± 2.92	56.39 ± 3.48
Relative glucose load,		
$g \cdot kg^{-1}$ body mass	1.15 ± 0.04	1.07 ± 0.07
$g \cdot kg^{-1}$ fat mass	7.67 ± 0.83	4.81 ± 0.35 *
$g \cdot kg^{-1}$ fat free mass	1.41 ± 0.07	1.41 ± 0.09
FEV_1/FVC, %	84.3 ± 2.2	79.8 ± 1.7
$\dot{V}O_2peak$,		
$L \cdot min^{-1}$	2.8 ± 0.3	1.9 ± 0.2 *
$mL \cdot kg^{-1} \cdot min^{-1}$	41.1 ± 3.2	26.1 ± 1.9 *
Peak Power Output, W	270 ± 20	170 ± 20 *
VT, % $\dot{V}O_2peak$	73 ± 2	61 ± 2 *
3-day Diet Records		
Energy, $kcal \cdot day^{-1}$	2570 ± 122	2277 ± 144
Carbohydrate, %	61 ± 2	60 ± 1
Fat, %	30 ± 1	30 ± 1
Protein, %	9 ± 1	10 ± 2

Values are means ± SE. FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; $\dot{V}O_2peak$, peak O_2 consumption; VT, ventilatory threshold. *Significantly different between groups, $p < 0.05$.

Table 2. Indexes of insulin sensitivity and insulin secretion.

	Young	Older
Insulin sensitivity		
HOMA-IR	0.80 ± 0.09	1.27 ± 0.15 *
CISI	7.45 ± 0.85	6.00 ± 0.58
Insulin secretion and β-cell function		
HOMA-%β, %	66.71 ± 7.29	100.99 ± 19.59
IGI1, $pM \cdot mM^{-1}$	134.31 ± 23.46	55.66 ± 6.75 *
IGI2, $pM \cdot mM^{-1}$	32.36 ± 4.18	19.26 ± 1.52 *
IGI3, $pM \cdot mM^{-1}$	47.41 ± 5.23	35.02 ± 3.41
DI1	803.86 ± 111.07	305.67 ± 35.38 *
DI2	1000.25 ± 137.32	581.56 ± 55.98 *

Values are means ± SE. HOMA, homeostatic model assessment method; IR, insulin resistance; CISI, composite insulin sensitivity index; %β, β-cell function; IGI, insulinogenic index; DI, disposition index. *Significantly different between groups, $p < 0.05$.

Table 3. Plasma insulin concentrations, expressed in units of $\mu IU \cdot mL^{-1}$, in young and older participants over time.

Time (min)	Young	Older
0	3.78 ± 0.42	5.64 ± 0.60 *
5	12.97 ± 2.10 #	7.92 ± 1.11 *
15	36.78 ± 5.83 #	25.23 ± 2.91 #
30	68.64 ± 10.90 #	35.83 ± 2.70 # *
60	67.75 ± 9.71 #	52.23 ± 5.91 #
90	53.06 ± 7.33 #	58.26 ± 7.91 #
120	57.98 ± 7.44 #	60.06 ± 7.87 #

Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

Table 4. Summary of correlations of plasma insulin concentrations with variables related to substrate availability and utilization.

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Glycerol Ra				
$\mu mol \cdot min^{-1}$	-0.78	0.04	-0.98	<0.001
$\mu mol \cdot kg^{-1} \cdot min^{-1}$	-0.78	0.04	-0.98	<0.001
$\mu mol \cdot kg\ FFM^{-1} \cdot min^{-1}$	-0.78	0.04	-0.98	<0.001
$\mu mol \cdot kg\ FM^{-1} \cdot min^{-1}$	-0.78	0.04	-0.98	<0.001
[Glycerol]	-0.75	0.05	-0.89	0.007
[FFA]	-0.81	0.03	-0.96	<0.001
Lipid/FA Rox				
$mg \cdot min^{-1}$	-0.58	0.17	-0.88	0.008
$\mu mol\ FA \cdot kg^{-1} \cdot min^{-1}$	-0.59	0.17	-0.87	0.01
$\mu mol\ FA \cdot kg\ FFM^{-1} \cdot min^{-1}$	-0.57	0.18	-0.87	0.01
$\mu mol\ FA \cdot kg\ FM^{-1} \cdot min^{-1}$	-0.62	0.14	-0.86	0.01
CHO Rox				
$mg \cdot min^{-1}$	0.81	0.03	0.96	<0.001
$\mu mol \cdot kg^{-1} \cdot min^{-1}$	0.80	0.03	0.96	<0.001
$\mu mol \cdot kg\ FFM^{-1} \cdot min^{-1}$	0.80	0.03	0.96	<0.001
$\mu mol \cdot kg\ FM^{-1} \cdot min^{-1}$	0.83	0.02	0.96	<0.001

Ra, rate of appearance; [Glycerol], plasma glycerol concentrations; [FFA], plasma free fatty acid concentrations; FA, fatty acid; CHO, carbohydrate; Rox, rate of oxidation.

Table 5. Summary of correlations of plasma insulin to glucagon ratio with markers of lipolysis.

	Young		**Older**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
Glycerol Ra				
μmol·min^{-1}	-0.90	0.005	-0.99	<0.001
μmol·kg^{-1}·min^{-1}	-0.91	0.005	-0.99	<0.001
μmol·kg FFM^{-1}·min^{-1}	-0.91	0.005	-0.99	<0.001
μmol·kg FM^{-1}·min^{-1}	-0.90	0.005	-0.99	<0.001
[Glycerol]	-0.90	0.005	-0.94	0.002
[FFA]	-0.92	0.004	-0.98	<0.001

Ra, rate of appearance; [Glycerol], plasma glycerol concentrations; [FFA], plasma free fatty acid concentrations.

Table 6. Comparison of correlations of plasma insulin concentrations and blood lactate concentrations with variables related to fatty acid availability and utilization in young participants.

	[Insulin]		**[Lactate]**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
[Glycerol], μM				
Overall	-0.75	0.05	-0.54	0.21
Enteric	-0.70	0.29	0.12	0.88
Systemic	0.64	0.56	0.99	0.09
[FFA], μM				
Overall	-0.81	0.03	-0.65	0.12
Enteric	-0.86	0.14	-0.15	0.85
Systemic	0.79	0.41	0.99	0.05
Glycerol Ra, $\mu mol \cdot kg^{-1} \cdot min^{-1}$				
Overall	-0.78	0.04	-0.71	0.07
Enteric	-0.94	0.06	-0.50	0.50
Systemic	0.68	0.53	0.99	0.07
FA Rox, $\mu mol \cdot kg^{-1} \cdot min^{-1}$				
Overall	-0.58	0.17	-0.62	0.14
Enteric	-0.13	0.87	0.68	0.32
Systemic	0.93	0.23	0.94	0.23

Overall, 0-120 min; Enteric, 0-30 min; Systemic, 60-120 min; [Insulin], plasma insulin concentrations; [Lactate], blood lactate concentrations; [Glycerol], plasma glycerol concentrations; [FFA], plasma free fatty acid concentrations; Ra, glycerol rate of appearance; FA, fatty acid; Rox, rate of oxidation.

Table 7. Comparison of correlations of plasma insulin concentrations and blood lactate concentrations with variables related to fatty acid availability and utilization in older participants.

	[Insulin]		**[Lactate]**	
Variables	Pearson *r*	*p* value	Pearson *r*	*p* value
[Glycerol], μM				
Overall	-0.89	0.007	-0.78	0.04
Enteric	-0.58	0.42	-0.13	0.87
Systemic	-0.96	0.19	-0.95	0.21
[FFA], μM				
Overall	-0.96	<0.001	-0.90	0.005
Enteric	-0.78	0.22	-0.44	0.56
Systemic	-0.97	0.14	-0.97	0.16
Glycerol Ra, $\mu mol \cdot kg^{-1} \cdot min^{-1}$				
Overall	-0.98	<0.001	-0.93	0.003
Enteric	-0.96	0.04	-0.71	0.29
Systemic	-0.94	0.23	-0.93	0.24
FA Rox, $\mu mol \cdot kg^{-1} \cdot min^{-1}$				
Overall	-0.88	0.01	-0.84	0.02
Enteric	0.05	0.95	0.22	0.78
Systemic	-0.96	0.18	-0.95	0.19

Overall, 0-120 min; Enteric, 0-30 min; Systemic, 60-120 min; [Insulin], plasma insulin concentrations; [Lactate], blood lactate concentrations; [Glycerol], plasma glycerol concentrations; [FFA], plasma free fatty acid concentrations; Ra, glycerol rate of appearance; FA, fatty acid; Rox, rate of oxidation.

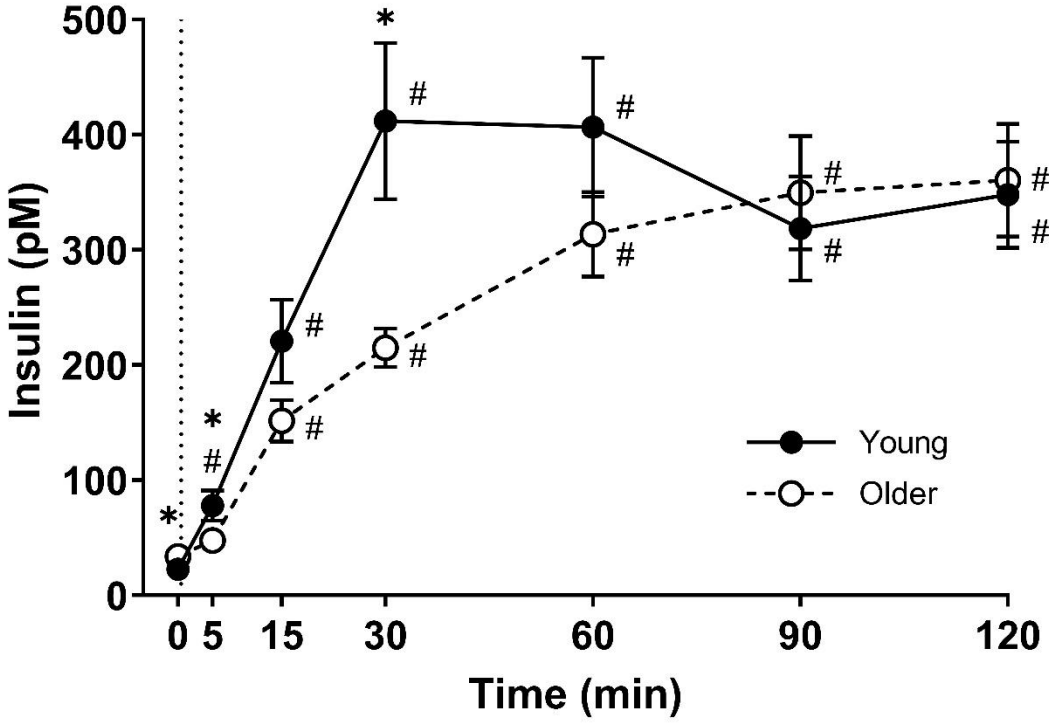

Figure 1. Plasma insulin concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

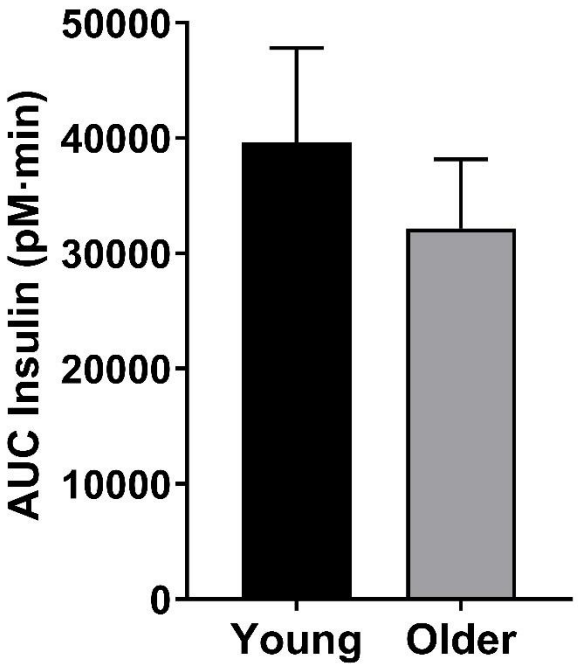

Figure 2. Area under the curve (AUC) for plasma insulin concentrations in young and older participants. Values are means ± SE.

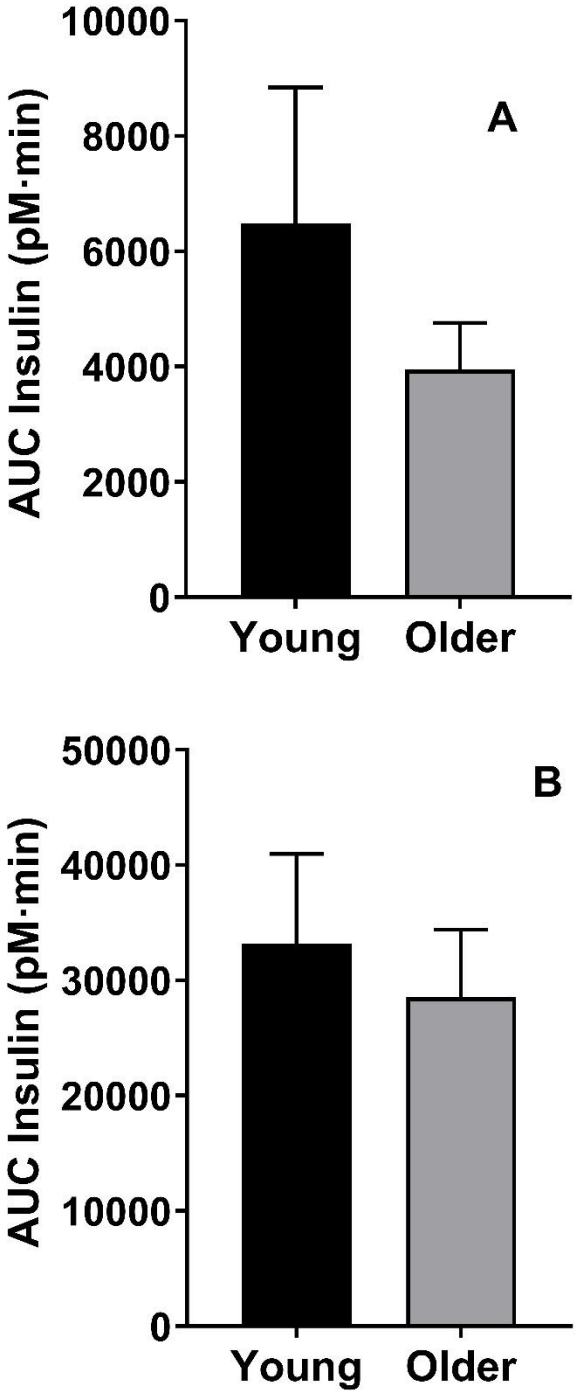

Figure 3. Area under the curve (AUC) for plasma insulin concentrations between 0–30 min (A) and 30–120 min (B) in young and older participants. Values are means ± SE.

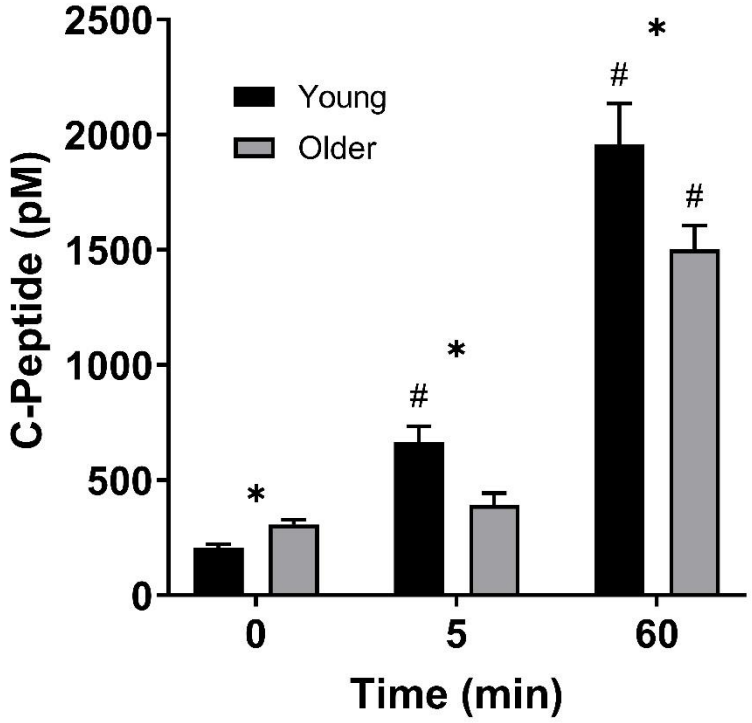

Figure 4. Plasma C-peptide concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

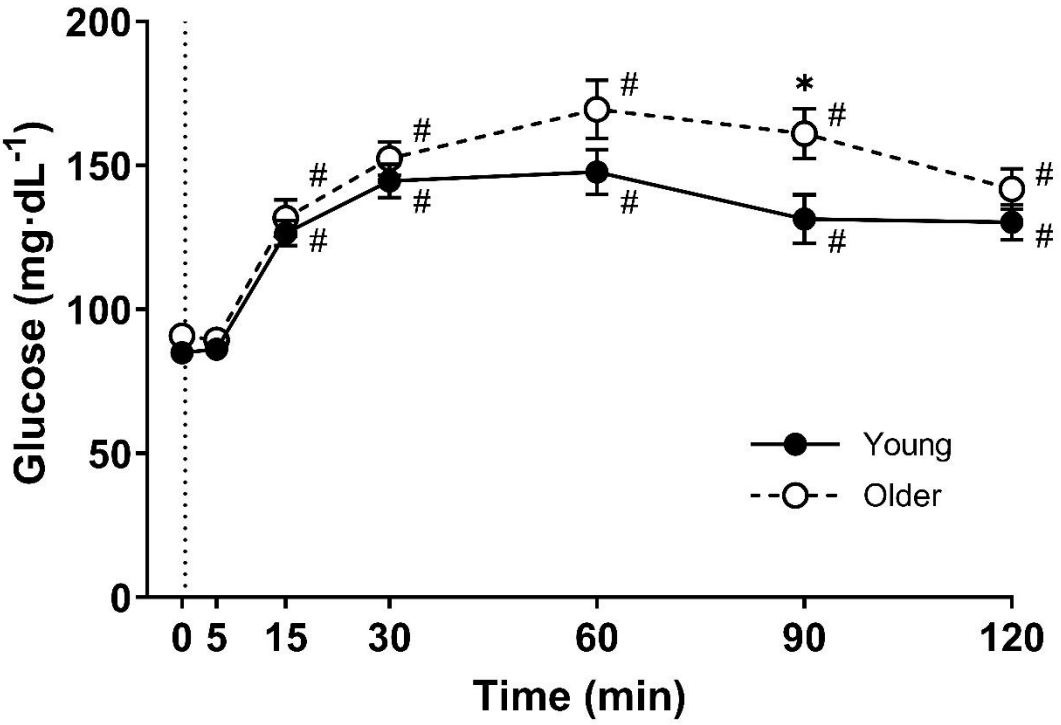

Figure 5. Blood glucose concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

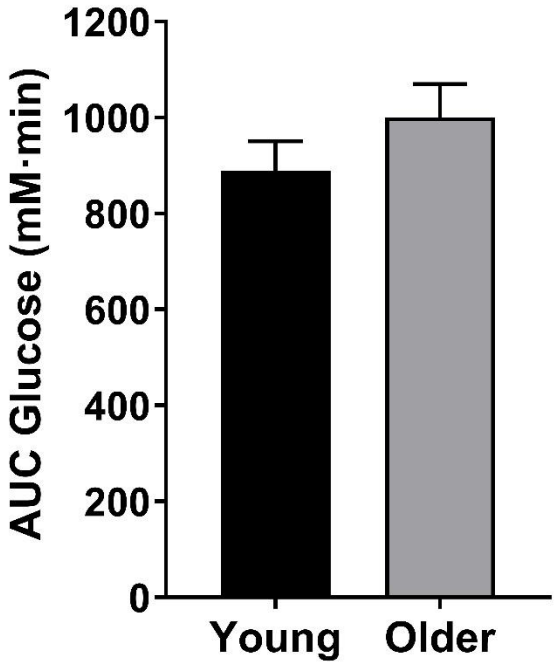

Figure 6. Area under the curve (AUC) for blood glucose concentrations in young and older participants. Values are means ± SE.

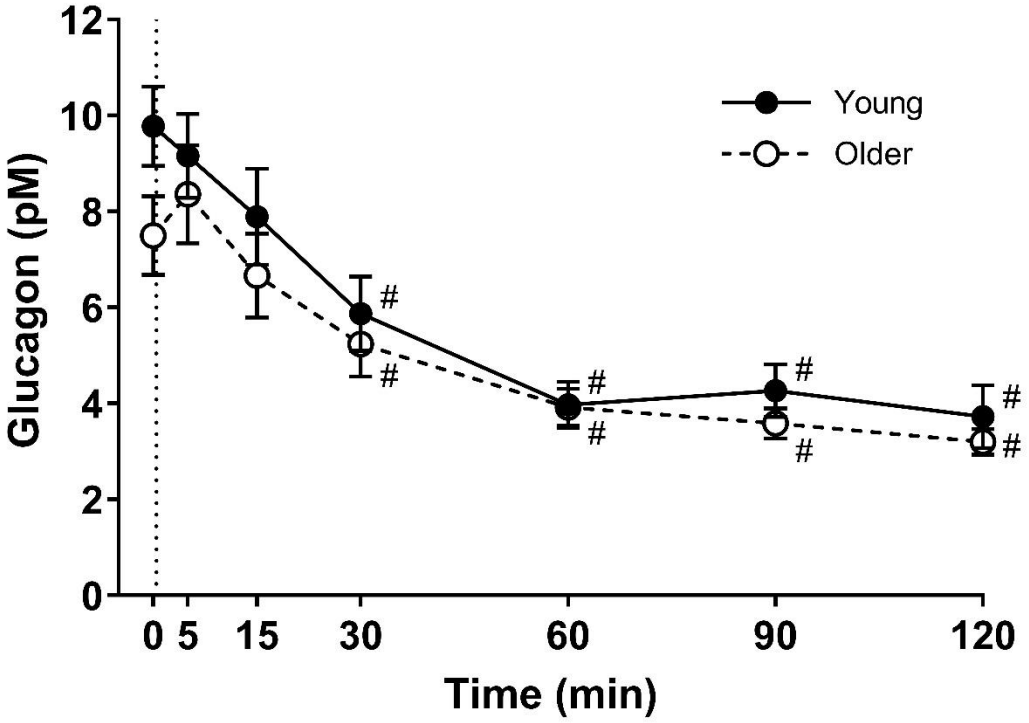

Figure 7. Plasma glucagon concentrations in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$.

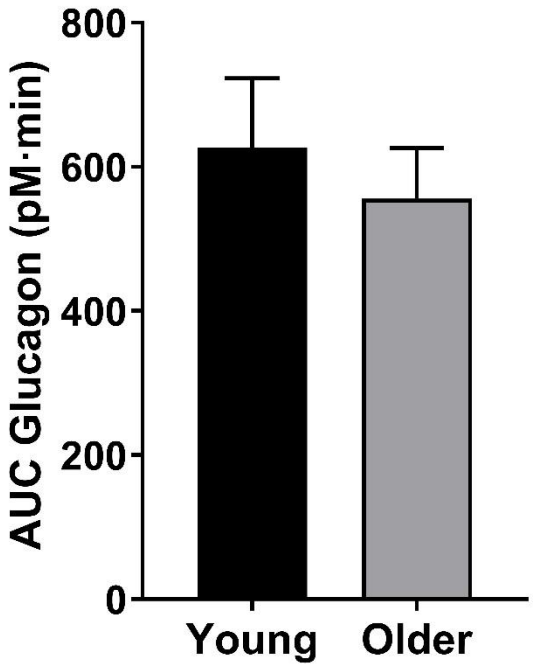

Figure 8. Area under the curve (AUC) for plasma glucagon concentrations in young and older participants. Values are means ± SE.

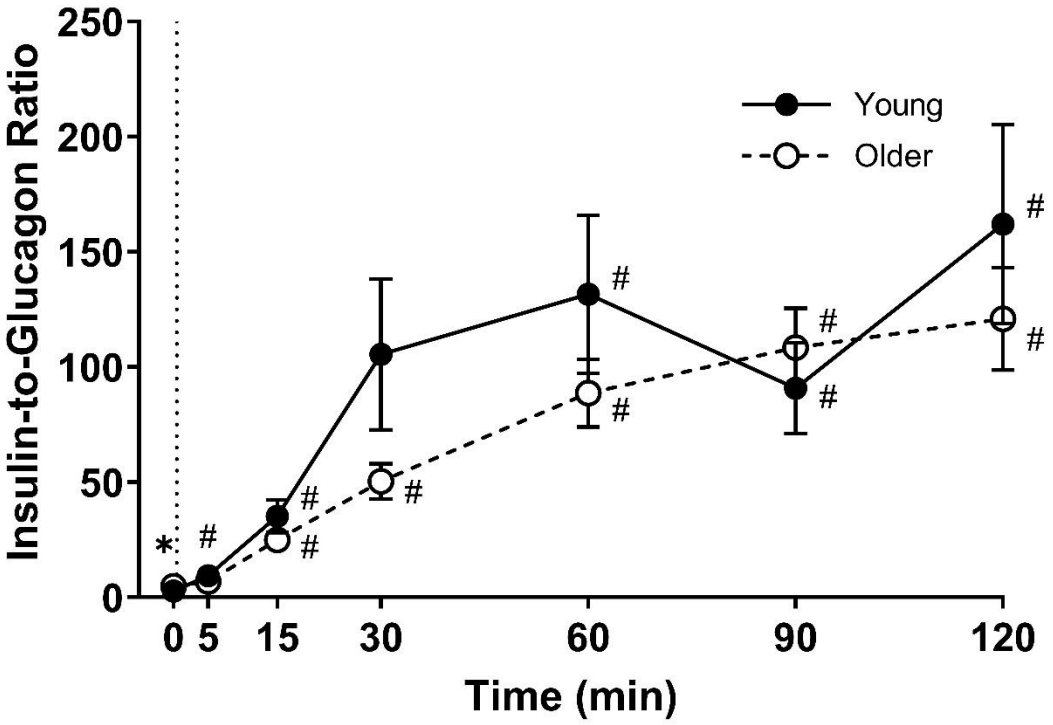

Figure 9. Plasma insulin-to-glucagon ratio in young and older participants over time. Values are means ± SE. #Significantly different from 0 min, $p < 0.05$. *Significantly different between groups, $p < 0.05$.

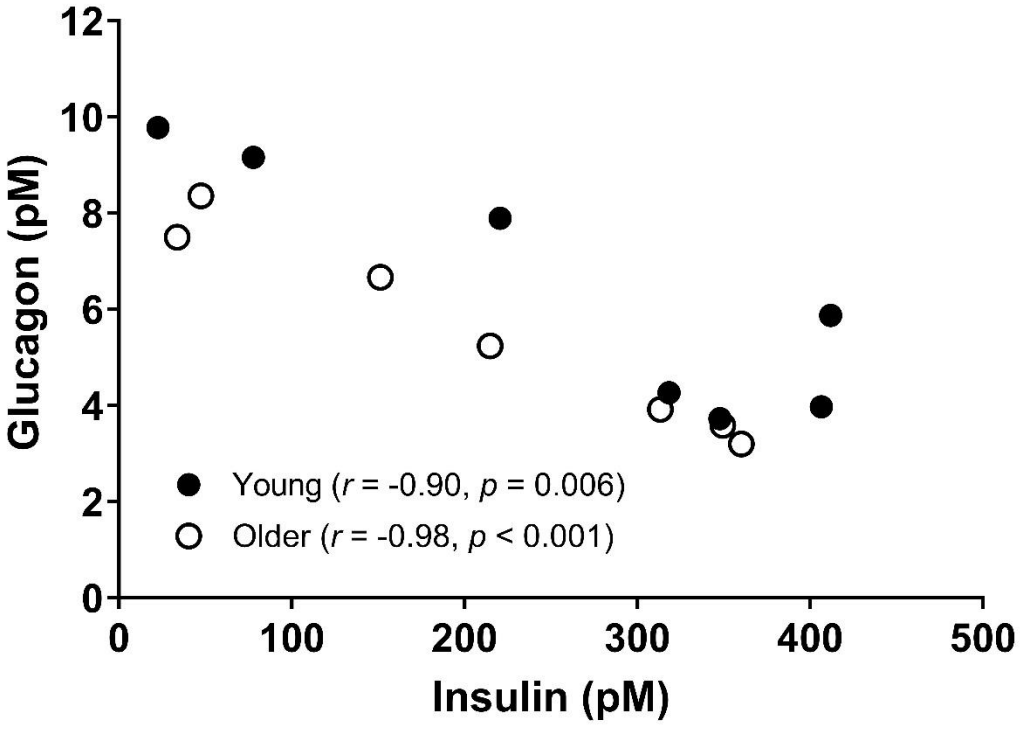

Figure 10. Relationship between plasma insulin concentrations and plasma glucagon concentrations in young and older participants.

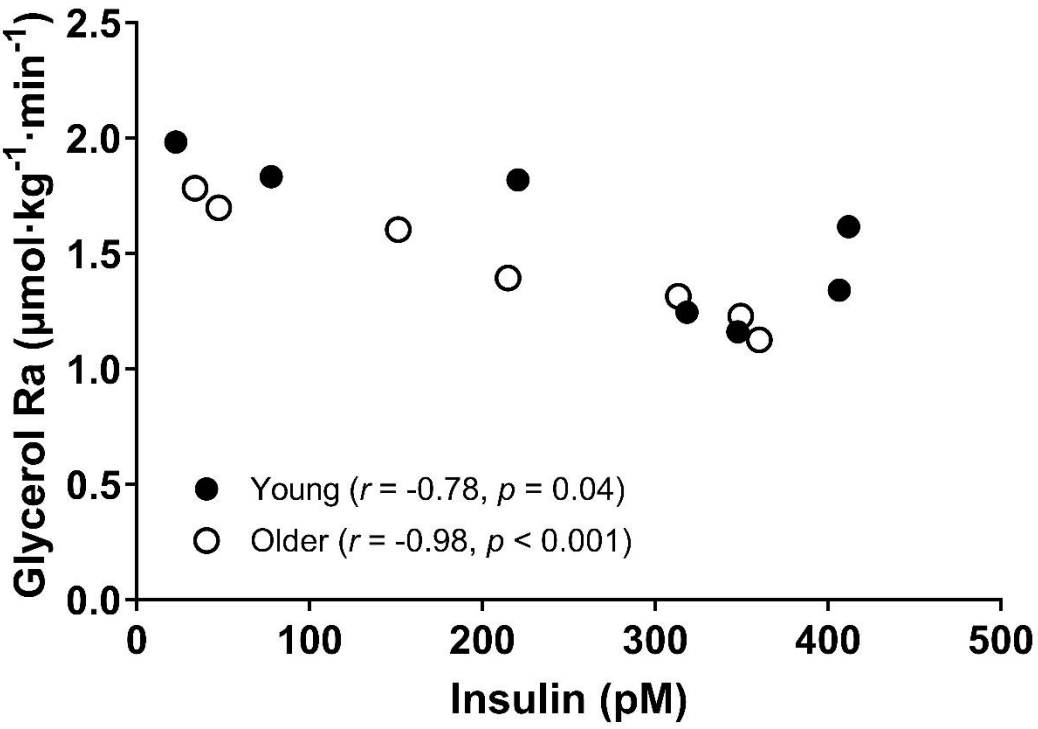

Figure 11. Relationship between plasma insulin concentrations and glycerol rate of appearance (Ra) per unit of body mass in young and older participants.

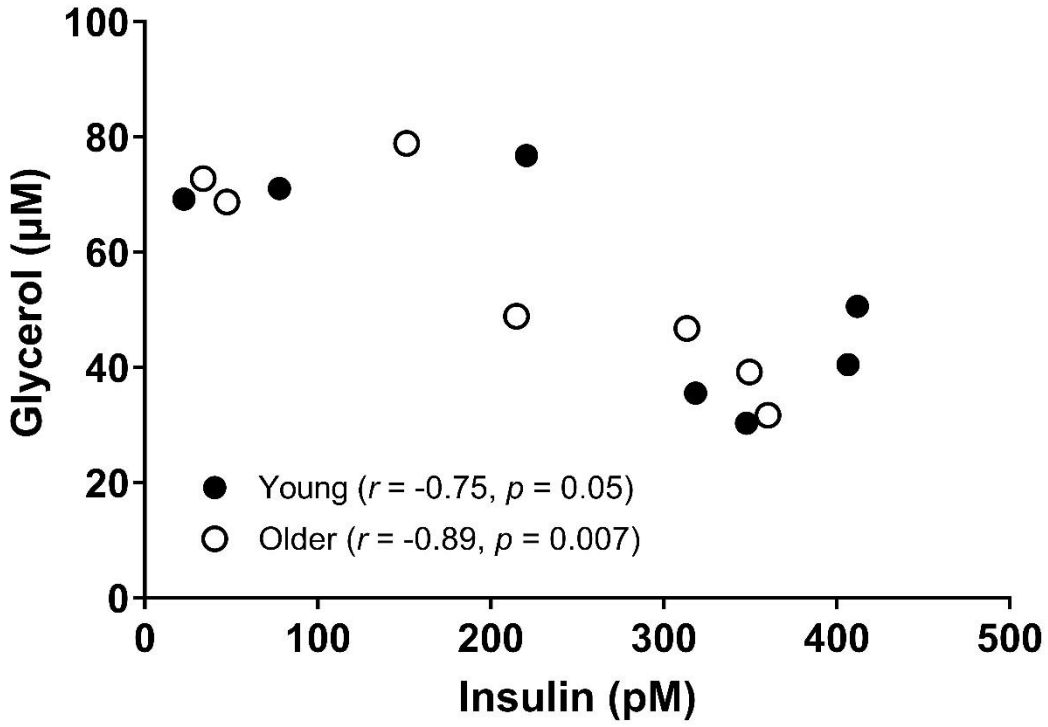

Figure 12. Relationship between plasma insulin concentrations and plasma glycerol concentrations in young and older participants.

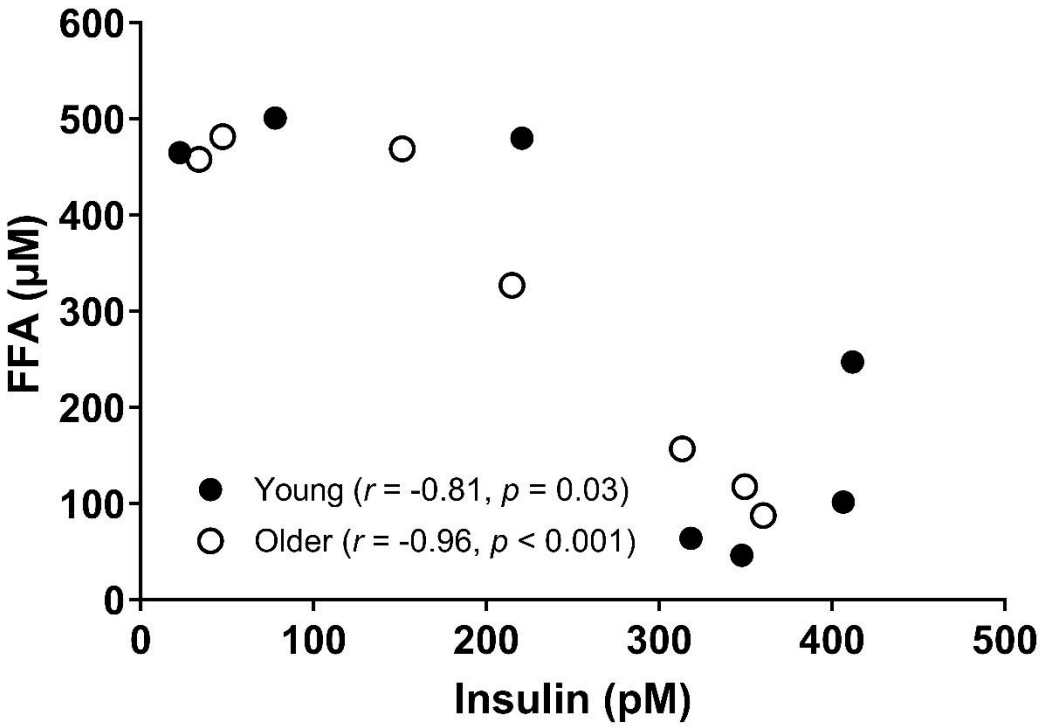

Figure 13. Relationship between plasma insulin concentrations and plasma free fatty acid (FFA) concentrations in young and older participants.

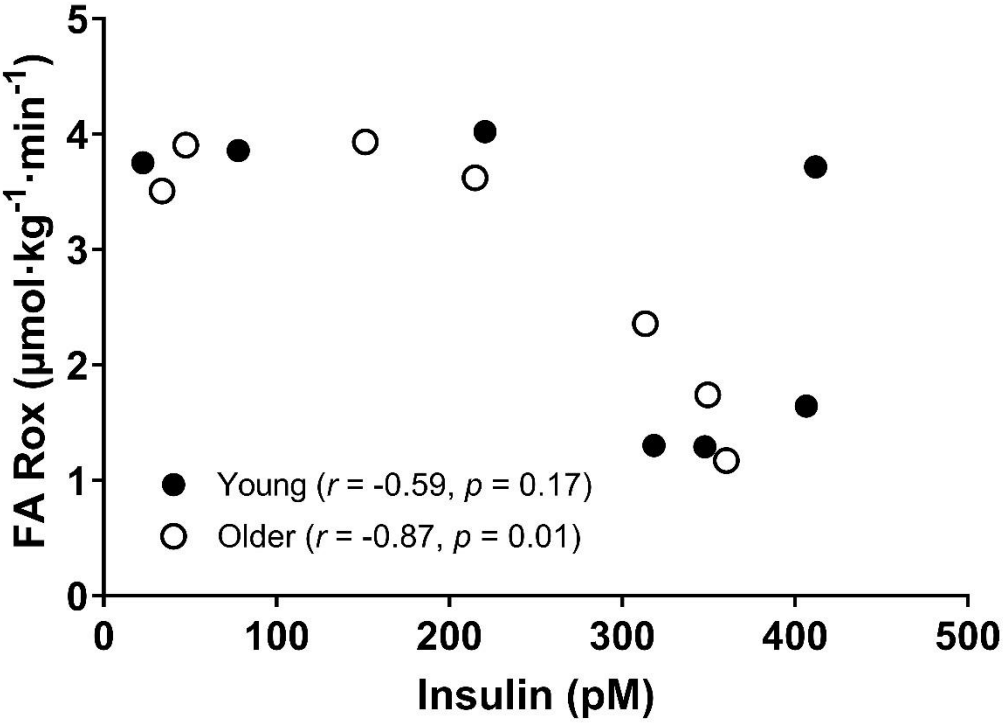

Figure 14. Relationship between plasma insulin concentrations and rate of total fatty acid (FA) oxidation (Rox) per unit of body mass in young and older participants.

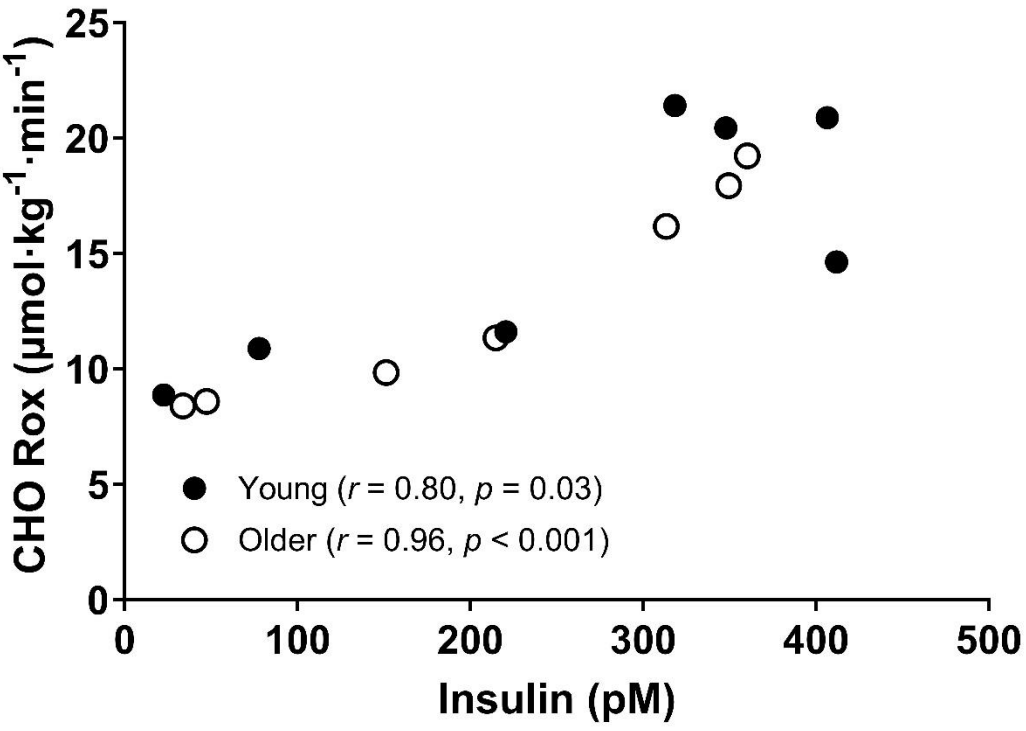

Figure 15. Relationship between plasma insulin concentrations and rate of total carbohydrate (CHO) oxidation (Rox) per unit of body mass in young and older participants.

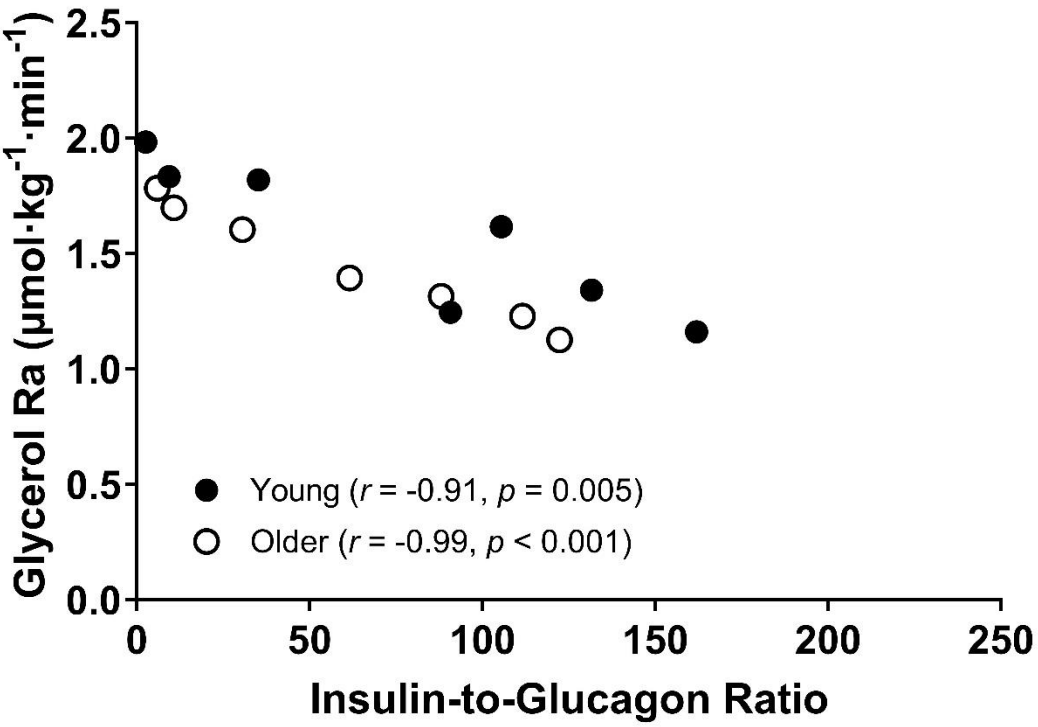

Figure 16. Relationship between plasma insulin-to-glucagon ratio and glycerol rate of appearance (Ra) per unit of body mass in young and older participants.

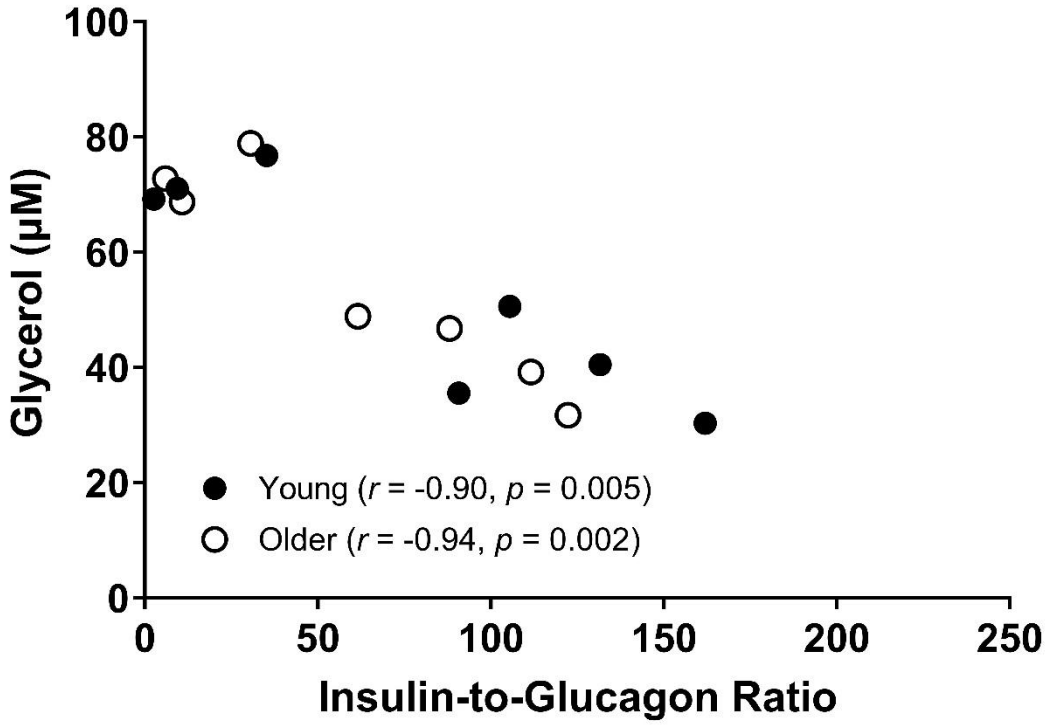

Figure 17. Relationship between plasma insulin-to-glucagon ratio and plasma glycerol concentrations in young and older participants.

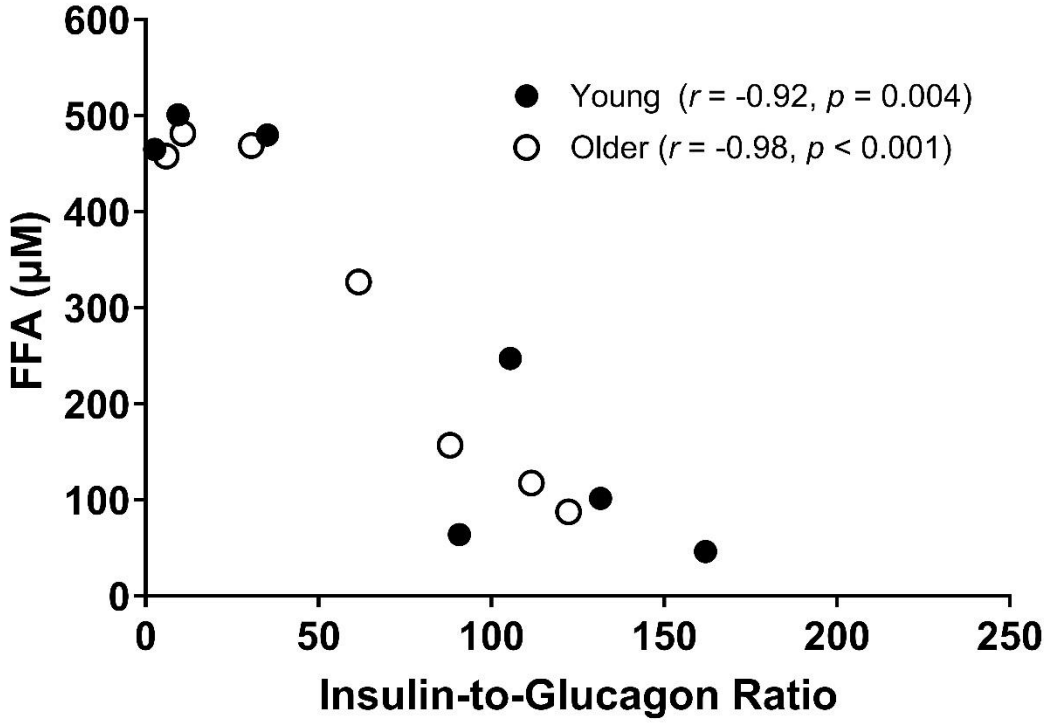

Figure 18. Relationship between plasma insulin to glucagon ratio and plasma free fatty acid (FFA) concentrations in young and older participants.

CHAPTER 5

Future Directions and Conclusions

LIMITATIONS AND FUTURE DIRECTIONS

In the present study, we sought to determine changes in the rate of lipolysis as estimated from the glycerol rate of appearance (Ra) during an oral glucose tolerance test (OGTT). Additionally, we sought to determine lactate oxidation rates following oral glucose consumption in young and older participants. To this end, we utilized [1,1,2,3,3-^{2}H]glycerol (or D_5-glycerol) and [3-^{13}C]lactate stable isotope tracers. An unfortunate consequence of the COVID-19 pandemic was our inability to conduct a repeated trial for determining free fatty acid (FFA) flux with [1-^{13}C]palmitate as we have done previously (23, 92, 108, 109). Although we provide evidence of the changes in the rates of lipolysis, total fatty acid (FA) oxidation, and total FA reesterification, we were unable to identify rates of appearance (mobilization), disposal, oxidation, and reesterification of plasma FFA. Data on postprandial plasma FFA kinetics in aging would be useful due to the purported roles of plasma FFA in determining substrate oxidation rates (14, 26, 41) and the known effects of plasma-derived FA on insulin action in skeletal muscle (122, 123). From our present data, we cumulatively suspect the following in older participants: higher rates of FFA appearance (43) due to lower rates of local intracellular FA reesterification (69); greater rates of plasma FFA uptake (114); greater rates of plasma FFA oxidation; and lower rates of plasma FFA reesterification. Future similar investigations might utilize D_5-glycerol in conjunction with [1-^{13}C]palmitate to explore these hypotheses. Our present data nonetheless identifies immediate changes in parameters of lipid substrate partitioning following oral glucose consumption and importantly, identifies aging-related differences.

As described in previous chapters of this dissertation, glycerol Ra ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) continuously declined in young ($p \leq 0.05$) but not older ($0.18 \geq p \geq 0.13$) participants between 60-120 min, and FA Rox ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) remained steady in young ($p \geq 0.65$) but not older ($p \geq 0.001$) participants between 60-120 min. Hence, we were unable to determine minimal rates of lipolysis and FA oxidation in young and older participants, respectively. The impact of this distinction is that we were also unable to determine aging-related differences in the restoration of baseline conditions. Because we sought to collect clinically relevant data, we utilized a 120-min observation period as is standard during a 75-g OGTT; therefore, a 2-hour endpoint was deemed appropriate. Leclerc et al. (6) utilized a 360-min observation period in which FA and carbohydrate (CHO) oxidation rates apparently returned to baseline rates at 240 min. Because the responses were delayed in older participants, future investigations might utilize a similar 360-min observation period or longer to determine aging-related differences in the restoration of baseline conditions.

As described in Chapter 4 of this dissertation, we were able to determine changes in plasma glucagon concentrations. However, we were unable to determine changes in the concentrations of the other counterregulatory hormones epinephrine, growth hormone (GH), and cortisol. Gerich and colleagues demonstrated that an increase in the concentrations of these hormones are responsible for glucose counter regulation during hypoglycemia by increasing hepatic glucose production (222) and increasing the rate of lipolysis (223, 224) to increase plasma FFA availability and spare glucose availability. Accordingly, we suspect that the concentrations of these hormones would have decreased during the OGTT in both groups. We were unable to determine changes in epinephrine or norepinephrine due to the excessive plasma volumes (≥1950 μL) required to exceed the lower limit of detection of the enzyme-linked immunosorbent assay (ELISA) kit (0.5 and 0.2

$ng \cdot mL^{-1}$, respectively) (#17-BCTHU-E02.1, ALPCO Diagnostics, Salem, NH). We attempted to determine changes in epinephrine and norepinephrine using high-performance liquid chromatography (HPLC) with electrochemical detection (Electrochemistry Separations Analysis, ESA, Coulochem II Model No. 5200A, Clemsford, MA), but we were unable to detect concentrations <500 $pg \cdot mL^{-1}$. Similarly, we were unable to determine changes in GH due to the excessive plasma volumes (>400 μL) required to exceed the lower limit of detection of the ELISA kit (0.05 $ng \cdot mL^{-1}$) (#22-HGHHUU-E01, ALPCO Diagnostics). Finally, we chose not to report changes in cortisol due to the unexpected change in the components of the ELISA kit used (#11-CRLHU-E01, ALPCO Diagnostics) for our initial determinations (10 participants); combination of the data from both kits might have introduced technical variability and confounded interpretation of the results.

Extant data on changes in the plasma concentrations of the counterregulatory hormones during an OGTT are informative, but incomplete. Young et al. (225) reported that plasma norepinephrine concentrations increased during an OGTT in young (25.7 ± 1.2 years) and older (72.9 ± 1.5 years) individuals. Welle et al. (226) and Tse et al. (227) similarly reported that norepinephrine concentrations increased 15 min after consumption of a glucose load. However, Hamburg et al. (228) and Welle et al. (226) reported that plasma epinephrine concentrations were unaffected. Application of their results to our present data is challenging due to their use of a larger, 100-g glucose load and their sampling of venous blood, which reportedly yields higher plasma concentrations (229) and clearance rates (230) of norepinephrine. Arafat et al. (231) reported that plasma GH concentrations progressively decreased during an OGTT until 120 min. Similarly, Fernandez-Real et al. (232) and Cakir et al. (233) reported that serum cortisol concentrations progressively decreased until 120 min. Unfortunately, these investigations were unable to identify immediate (i.e., 5 min and 15 min) changes in the circulating concentrations of these hormones or relate these changes to rates of lipolysis, FA oxidation, or CHO oxidation. Because we observed aging-related differences in the suppression of lipolysis that were linked to differences in the insulin response, future investigations should determine changes in the concentrations of the counterregulatory hormones that are known to increase the rate of lipolysis (131, 223, 224), suppress insulin secretion (234, 235), and reduce insulin sensitivity (236).

We were also unable to report changes in plasma GLP-1 and GIP concentrations. The incretins are subjected to rapid degradation by the proteolytic enzyme dipeptidyl peptidase-4 (DPP-4) expressed in multiple cell types, including the endothelial cells of blood vessels. Consequently, a large portion of the GLP-1 and GIP released in response to nutrient consumption exist in an inactive form in the systemic circulation (176, 177). Previous investigations have nonetheless reported changes in plasma GLP-1 and GIP concentrations from arterialized blood samples following nutrient consumption (237, 238). We chose not to determine changes in plasma GLP-1 and GIP concentrations due to the variability in the amount of time between blood sample collection and allocation to collection tubes containing a DPP-4 inhibitor. Such determinations would likely have misrepresented the amount of active GLP-1 and GIP in circulation. However, based on extant data, we expect that there would have been higher concentrations of incretin hormones in our older participants (150, 180) and potentially, an earlier increase in incretin hormone concentrations.

As described in Chapter 2 of this dissertation, higher levels of physical fitness (i.e., $\dot{V}O_2peak$) may reduce the need for elevated rates of lipolysis and consequently, FA availability in the postprandial period to support energy expenditure. However, another unfortunate consequence of the COVID-19 pandemic was our inability to determine the effects of endurance exercise training on parameters of metabolic flexibility. Previous investigations have reported that exercise training decreased postabsorptive plasma triglyceride concentrations (239) and plasma FFA concentrations during an OGTT (47) in healthy older individuals. These results in conjunction with the "Randle Cycle" theory (14) allow us to hypothesize that endurance training can improve the responses of total FA and CHO oxidation rates in the postprandial period. Thus, future investigations might investigate the beneficial effects of endurance training or even a single bout of exercise (45) on similar parameters of metabolic flexibility in aging.

Finally, although we successfully completed trials on 15 young and 14 older individuals, our data represent results obtained on healthy older volunteers free of comorbidities, unlike most typically studied in studies of older individuals. Thus, future investigations should investigate similar parameters of metabolic flexibility in "unhealthy" aging to interrogate how these responses contribute to the progression of aging-related metabolic disease states.

CONCLUSIONS

Metabolic flexibility describes the ability to switch back and forth between CHO and FA utilization in response to variations in energy substrate availability. In contrast, metabolic inflexibility refers to the inability to alter the pattern of substrate utilization in response to changing physiological conditions. The loss of metabolic flexibility has previously been documented in metabolic disease states as well as in "healthy" aging (10-13). Presently, we provide evidence of metabolic inflexibility in "healthy" aging following oral glucose consumption, i.e., in the postprandial period. In older compared to young participants, we report the following:

1. The suppression of lipolysis was delayed (30 min vs. 5 min in young)
2. The suppression of FA oxidation was delayed (higher at 60 min vs. young)
3. The rise in CHO oxidation was delayed (30 min vs. 5 min in young)
4. The suppression of FA oxidation was attenuated such that FA oxidation is favored over FA reesterification
5. The rise in plasma insulin concentrations was delayed (15 min vs. 5 min in young)

We also provide evidence of lactate control of energy substrate partitioning (30, 31) in the postprandial period. In both young and older participants, blood lactate concentrations were inversely correlated with lipolytic and FA oxidation rates. However, our results are interpreted to indicate that the effects of lactate signaling are exaggerated in aging. Coincident with an attenuated insulin response in older participants, lactate is therefore permissive to insulin in correcting postprandial lipid substrate partitioning in aging. From this perspective, lactate is not a hinderance, a role that is notably in contrast to its restrictive effects on metabolic flexibility during exercise in individuals with poor lactate clearance capacities (38).

Overall, metabolic inflexibility in the postprandial period in “healthy” aging is characterized by delayed, but not restricted changes in energy substrate partitioning.

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APPENDICES

Appendix A
Notice of Approval for Human Research

Berkeley
UNIVERSITY OF CALIFORNIA
Committee for Protection of Human Subjects (CPHS)
Office for Protection of Human Subjects (OPHS)

1608 Fourth Street, Suite 220
Berkeley, CA 94710-5940
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NOTICE OF APPROVAL FOR HUMAN RESEARCH

DATE:	*November 29, 2023*
TO:	George Brooks Justin Duong, Integ Biol
CPHS PROTOCOL NUMBER:	*2018-08-11312*
CPHS PROTOCOL TITLE:	*Aging Mitochondrial Fragmentation and Metabolic Inflexibility*
FUNDING SOURCE(S):	*SPO ID: 045421-001*

A(n) *continuing review* application was submitted for the above-referenced protocol. The Committee for Protection of Human Subjects (CPHS) has reviewed and approved the application by *Full Review* review procedures.

Effective Date: *December 02, 2023*

Expiration Date: *December 01, 2024*

Continuation/Renewal: Applications for continuation review should be submitted no later than 6 weeks prior to the expiration date of the current approval. *Note: It is the responsibility of the Principal Investigator to submit for renewed approval in a timely manner. If approval expires, all research activity (including data analysis) must cease until re-approval from CPHS has been received.* See Renew (Continue) an Approved Protocol.

Amendments/Modifications: Any change in the design, conduct, or key personnel of this research must be approved by the CPHS *prior* to implementation. For more information, see Amend/Modify an Approved Protocol.

For protocols that have been granted approval for more than one year: Certain modifications that increase the level of risk or add FDA oversight may require a continuing review application to be submitted and approved in order for the protocol to continue. If one or more of these changes occur, a Continuing Review application must be submitted and approved in order for the protocol to continue.

Unanticipated Problems and Adverse Events: If any study subject experiences an unanticipated problem involving risks to subjects or others, and/or a serious adverse event, the CPHS must be informed *promptly*. For more information on definitions and reporting requirements related to this topic, see Adverse Event and Unanticipated Problem Reporting.

This approval is issued under University of California, Berkeley Federalwide Assurance #00006252.

If you have any questions about this matter, please contact the OPHS staff at 642-7461 or email ophs@berkeley.edu .

Sincerely,

Committee for Protection of Human Subjects (CPHS)

UC Berkeley

Appendix B
Consent to Participate in Research

UNIVERSITY OF CALIFORNIA AT BERKELEY

BERKELEY DAVIS IRVINE LOS ANGELES MERCED RIVERSIDE SAN DIEGO SAN FRANCISCO SANTA BARBARA SANTA CRUZ

CONSENT TO PARTICIPATE IN RESEARCH
***Title of Study*: "Aging Mitochondrial Fragmentation and Metabolic Inflexibility"**

Key Information

You are being invited to participate in a research study. Participation in research is completely voluntary.

The purpose of the study is to determine the effects of age, sex, and physical fitness on the body's metabolic flexibility that is the ability to switch between carbohydrate and fat energy stores.

The study will take a total of 6 to 8 hours over the course of 3 days and you will be asked to undergo procedures such as physical fitness level and body measurements, physical exam, basic blood test including HIV test, review of your health history, Oral Glucose Tolerance Test, blood draws, tissue sampling, and genetic testing.

Risks and/or discomforts during screening may include: pain during hard exercise, shortness of breath, dizziness, pain or bruising from blood sampling, skin irritation from electrocardiography (ECG) electrodes, mouth irritation from breathing through a valve, saddle soreness from the bicycle seat.

Risks and/or discomfort during oral glucose tolerance testing may include: yucky sweet taste, a sugar high followed by dizziness, skin irritation from ECG electrodes, mouth irritation from breathing through a valve, pain from blood sampling, pain from muscle and fat sampling (biopsies), and possibility of a thin - 1cm long skin scar at the biopsy site.

As well, there are risks associated with genomic testing.

There is no direct benefit to you. The results from the study will allow us to learn more about the effects of aging on metabolic flexibility. You will be provided with your individual measurements of body composition, physical fitness, and lung capacity.

Introduction

My name is Professor George A. Brooks, Ph.D. I am a faculty member in Integrative Biology at the University of California, Berkeley, working with my colleague Umesh Masharani, M.D. from the University of California, San Francisco. We are planning to conduct a medical research study, which I invite you to take part in.

You are being invited to participate in this study because you are of the age and sex to participate and have no medical or other condition to exclude you from the study.

Purpose

The purpose of this study is to determine the effects of age, sex, and physical fitness on the body's metabolic flexibility, that is the ability to switch between carbohydrate and fat energy stores. Therefore, we need to recruit healthy, younger and older, physically fit and sedentary males and females.

If you are eligible and decide to join the study, you will be one of a group of 80 younger and older, healthy male and female volunteers. By younger adult we mean someone between 21 and 35 years of age. By older adult we mean someone between 60 and 80 years of age. If you fit into either of those age groups, you are eligible to participate in the study.

A key part of the study will involve an Oral Glucose Tolerance Test (OGTT). After an overnight fast, you will arrive in the testing laboratory the next morning. You will sit and rest, drink a sugary solution, and wait 2 hours while being tested. This OGTT is a standard biomedical test involving commercially made bottled sugar solutions. However, we will modify the procedure to infuse into your blood small quantities of non-radioactive isotope "tracer cocktails" to measure how your body uses metabolites during the oral glucose challenge. These "tracer cocktails" will be formulated in the Mariner Advanced Pharmacy and tested for purity and sterility before they will be administered to you.

Stable, Non-Radioactive Tracers:
We would like you to be aware of the stable and benign nature of the non-radioactive tracers that we are using in this study. In nature, elements can have heavy forms. We can use those forms to trace the paths of metabolism of important body energy sources (glucose, lactate, fatty acids, and glycerol). For our studies, we will substitute one or several heavy atoms for lighter, more common atoms in molecules of body energy sources. The substituted tracer atoms of hydrogen and carbon are not radioactive and because they appear in our food sources, we normally carry small percentages of those atoms. In point of fact, because we only substitute one or two heavy atoms in the energy sources of interest, the tracer molecules are hardly heavy at all gaining only one or several atomic mass units. Still, by using a very sensitive and sophisticated device called a mass spectrometer, we can detect and trace the molecules of interest as they course through the body.

Procedures

If you agree to be in this study, you will be asked to do the following: (1) undergo screening procedures to test your eligibility, and (2) if you are eligible, an OGTT (as described above). All study participants will undergo one OGTT with isotope tracers aboard.

In addition, your blood will be taken and small tissue samples (called "biopsies") of your leg thigh muscle and abdominal subcutaneous (under the skin) fat will be taken. We will isolate DNA and RNA from those three body tissues to learn about how certain genes are modified by physical activity, aging and gender to affect, or not, the ability to metabolize fats and carbohydrates.

Before you begin the main part of the study...

(1) Screening: You will need to have the following screening exams/ tests/ procedures to find out if you can safely participate be in the main part of the study.

Food record

1. Record all food and liquid consumed over a 3-day period. If you are entered into the study, we will again ask you to record a 1-day food record before the oral glucose tolerance test.

Health History

2. Fill out a health history form. We need to make sure that you do not have a heart or other condition such as diabetes that would compromise the results or possibly injure you.

Physical fitness Level and Body Measures

3. Determine fitness level (maximum oxygen consumption, VO_2max and Ventilatory Threshold, VT) by riding a stationary bicycle (i.e., a leg cycle ergometer) at progressively increasing power outputs until you can no longer continue because your legs become tired, your breathing becomes labored, or some combination of discomforts so that you just want to stop exercising and recover (1 time). The bicycle ergometer test will involve a series of graded, from mild to voluntary maximum effort stages. At the end of each stage, we will record your heart rate and wave forms by electrocardiography (ECG), and blood pressure by auscultation (listening with a stethoscope to your arm pulse sounds as pressure is relieved from a cuff).
4. Measure your lung function by having you wear a nose clip and blowing into a device called a spirometer (1 time).
5. Determine the amount of fat and muscle tissue you have by using skin calipers to gently pinch folds of skin in several locations on your body on screening and after the last OGTT (2 times).
6. Measure your standing height (1 time).
7. Measure your body weight by a scale on screening and each trial day (3-4 times).

Pregnancy Testing:

Because the drugs/procedures in this study can affect a fetus, pregnant women may not participate in this study. If you are a female of childbearing potential (i.e., you have had your first menstrual period and have not yet reached menopause), a urine test will be done at the initial visit to make sure you are not pregnant.

Physical Examination, Health History and Dietary Review

You will have a physical examination, similar to those done for regular medical care, again to ensure that you are healthy and low risk for injury for this study. This will involve observations of skin appearance, heart and chest sounds, peripheral pulse similar to those done for regular medical care. Additionally, your food records will be evaluated by members of the research team, and your Health History will be reviewed by the study physicians (medical doctors).

Basic Blood and HIV Testing

As part of initial screening, you will be asked to give a small blood sample (< 5 ml) for analysis at the Tang (Student Health) Center or similar facility. Two tests will be run: a "Basic Metabolic Panel" for blood metabolites and electrolytes, and a test for HIV (human immunodeficiency virus). In the event of a positive HIV test, a study staff research assistant will contact you for an in-person meeting at which time test results will be disclosed. The staff person will explain the results to you and refer you to the Tang Center, if you are a student, or to your personal physician for counseling and treatment. If you are HIV positive, you will be excluded from the study.

(2) During the main part of the study...

If the screening exams, tests, and procedures show that you can continue to be in the study and you choose to continue, this is what will happen next:

Oral Glucose Tolerance Test (OGTT):

You will do this test 1 time. The OGTT is described as follows:

1. Rest and record your food and fluid intake the day before.

2. Fast overnight (12 hours) and come to the laboratory at 6:00 AM.

3. Have a fluid infusion tube (catheter) placed in an arm vein and blood sampling catheter placed in a hand vein. Catheters will be placed by licensed health care professionals such as Dr. Masharani, another physician, physician assistant, or registered nurse.

4. Drink a 10 fluid ounce sugar solution that contains 75 grams of glucose (sugar).

5. Have infused into your arm vein approximately 1.0 gram of stable, non-radioactive tracer IV solutions.

- For measuring carbohydrate (CHO) metabolism, the IV solution will contain non-radioactive tracers [6,6-^{2}H]-(D_2)-glucose (a tracer for blood sugar), [3-^{13}C]lactate (the product of sugar breakdown), and [1,1,2,3,3-^{2}H]-(D_5)-glycerol (a substance your body produces when it breaks down fat tissue).

6. Have blood samples drawn through the hand vein catheter sampling tube before drinking the sugar solution, and every 15 min after (i.e., at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min) for a total of 9 sampling time points. At each of these blood sampling time points, 5 ml will be taken from a hand vein. Thus, a total of 45 ml (≈ 9 teaspoons) of blood will be taken during the OGTT. You should not experience any sensation due to blood loss especially as we will give fluids (sterile physiological saline) during trials to compensate for volume loss. Estimated saline delivery during each OGTT is estimated at 120 ml (about 1 cup) which should be sufficient to cover blood loss and maintain hydration without diluting the study participant's total blood volume of 4-6 liters.

7. Be retested for body composition by skinfold determination after the last OGTT.

<u>Tissue Biopsies</u>

Biopsies will be taken to assess the effects of aging, physical activity, and gender on tissue biochemistry, physiology, gene and protein expression. On a separate occasion (not a screening or OGTT day), muscle and fat biopsies will be conducted by Dr. Masharani in the Exercise Physiology Laboratory (Rooms 93 and 95, Maxwell Center, Haas Arena). While you rest quietly on a padded table, the skin on two small body sites will be numbed by use of an anesthetic ointment or injection. The sites will be on your abdomen (stomach) near the navel and on your lateral thigh. To prevent infection at the biopsy site, skin at the sampling site will be scrubbed with Betadine antiseptic solution and allowed to dry. Then, using a very fine needle, a small amount of numbing medicine will be given under the skin. After the numbing medicine takes effect (1-3 minutes), a small incision (< 1 cm) will be made in your skin, and a small needle will be inserted at the anesthetized site.

To sample adipose (body fat) at the abdominal site, the needle will not go deep, as adipose is immediately below the skin. Compared to muscle, white adipose (fat) tissue is "squishy" so to obtain a sample several (4-7) needle passes may be necessary to obtain a sample large enough to measure. Many people don't feel this procedure at all.

To sample muscle, the needle will need to go deeper, past the subcutaneous adipose and into the lateral thigh muscle. Because the numbing medicine can damage muscle, the muscle biopsy procedure can be felt. Because pain receptors are diffusely placed in muscle, some people don't feel the procedure whereas others feel it more. The sensation is very much like bumping the corner of a table when you are in a rush. The feeling is a sharp sting, but the pain subsides rapidly.

After the biopsy procedures, skin at the wound sites are pulled together and covered by a strong band aid called a Steri-Strip. Thereafter, no special treatment is necessary. However, we ask that you keep the water stream off the wound sites while showering. The Steri-Strip will fall off after a few days. When healed a small scar will remain, but it should be difficult to find the biopsy site.

Study Sites and Conditions:

The Exercise Physiology Laboratory, Rooms 93 and 95, Maxwell Center, Haas Arena at the University of California is equipped to do most of the screening physiology tests and most of the analyses required for this study. Professor Brooks' office and analytical facilities are located in 5101 VLSB. Due to sensitivity of the measurements, participants will only be allowed to read, study, watch non-stressful movies or hear easy listening music during OGTTs.

Study time:

Your study participation will take a total of approximately 6 to 8 hours, over the course of 3 days. Filling out the forms for initial screening should take an hour. Laboratory interview and screening should take an hour. Each OGTT, including setup and recovery, should take 4 to 6 hours. Biopsies should take 30 minutes per tissue.

Benefits

There will be no direct benefit to you from participation in the study. However, this study will allow us to learn more about the effects of aging, physical activity and sex (being male or female) on metabolic flexibility. Potential benefits may also accrue broadly to the public through the advancement of science and understanding of health and disease. You will be provided with your individual measurements of your body composition, physical fitness, lung capacity, results of metabolic panel testing in screening, and metabolism during OGTTs. However, results of genomic (DNA and RNA) analyses from tissue biopsies **will not** be shared with you. In the unlikely event that you are HIV positive, you will be informed of that result, so that you can be counseled and receive treatment.

Risks/Discomforts

1. Exercise:

   a. Exercise stress testing can cause dyspnea (shortness of breath) and continuous exercise testing can cause a degree of foot soreness.

   b. There is a slight risk anytime someone exercises, even if the task is of lesser intensity and duration than regularly practiced. The acute risk of exercise is apparent even if regular exercise decreases the risk of heart or other chronic diseases. Accordingly, lab personnel will be trained in lab safety procedures including means to contact emergency services.

   c. Exercise testing in an over-night fasted condition can result in sensations of fatigue or malaise (tiredness or weakness).

   d. Physical assessments can cause embarrassment for anyone, depending on experience, perception, and cultural norms. However, the physical examination will be conducted in a closed room, out of public view by a licensed and highly experienced physician. All staff, including Principal Investigator Brooks, Dr. Masharani from UCSF, the staff research associate, and the laboratory personnel will be highly trained in their specific tasks and educated in the importance of respect for study subject's privacy and sensitivities.

2. Catheterization:

a. Clotting. The presence of the catheter could cause clotting of blood within the blood vessel (estimated incidence = 2:1000) but has never happened in our experience of 40 years. A clot could cause the temporary or permanent blockage of that segment of the vein. Blockage of a superficial vein, while not desirable, does not significantly impair blood flow because there are many veins present. The clot should resolve within a few weeks.

b. Fainting. Fainting occurs in some persons when they sit or stand immediately following invasive procedures or the drawing of blood (incidence = less than 2%). Again, this has never happened in our experience of 40 years, as you will be laying down when catheters are placed.

c. Bleeding. During the catheterization procedure, some bruising or bleeding may occur from the vessels, but this will be kept to a minimum with direct pressure. Bleeding can also occur following the withdrawal of catheters. In the case of the arm vein, such bleeding may result in a "black and blue" bruised appearance, which may take several days to disappear.

d. Infection. When the skin is broken there will always be a chance of infection. However, sterile procedures and disposable supplies and solutions will be used throughout. The possibility of infection is mitigated by obtaining the tracer from Mariner Advanced Pharmacy that certifies solution sterility. In several hundred experiments since 1988, we have not had a single infection result from vascular catheterization.

e. Discomfort. Local discomfort from venous catheters will last approximately 1-2 days. If necessary, acetaminophen (Tylenol), not aspirin, can be recommended.

3. Biopsies

a. Fainting. Fainting occurs in some persons when they sit or stand following invasive procedures. For biopsies subjects will lay supine (facing up).

b. Bleeding. During the biopsy procedure, some bleeding may occur from small surface blood vessels, but this will be kept to a minimum with direct pressure.

c. Bruising. During the biopsy procedure, some bleeding may occur from small surface blood vessels and muscle tissue itself, but this will be kept to a minimum with direct pressure. A “black and blue”, bruise mark is rare following biopsies, but can occur.

d. Infection. When the skin is broken there will always be a chance of infection. However, sterile procedures and disposable supplies and solutions will be used throughout.

e. Discomfort. Local discomfort from biopsies will last approximately 1-2 days. If necessary, acetaminophen (Tylenol), not aspirin, can be recommended.

f. Scarring. The biopsy sites may develop a small (1 cm) scar that over time should become invisible.

4. Blood Test/HIV testing risks: Being tested for HIV may cause anxiety regardless of the test results. A positive test indicates that you have been infected with the HIV virus, but no one knows for certain when, if ever, you will become sick with AIDS or a related condition. Receiving positive results may make you very upset. If other people learned about your positive test results, there is a risk that you could be treated unfairly or badly, and even have trouble obtaining insurance or employment. To the extent permitted by law, we will keep your test results confidential and will not release them to anyone without your written permission. (*Note*: In California, the testing lab is required to report identified positive results to public

health authorities.)

5. Metabolic Panel: The blood test may reveal a previously unknown medical condition such as diabetes.

6. Risks of Participating in Genetic Research: (Genomic Data from DNA and RNA Analyses)

As part of this study, DNA and RNA will be isolated from tissue samples. The DNA will be used to study the genome and associated with findings from other tests. The research will include whole genome DNA and RNA sequencing.

Modern techniques of genotyping have the potential to reveal much about our susceptibility to various diseases and risks to the stresses of modern living. At present, current technology can be used to describe our ancestry. Such information is immensely personal, and its revelation could be embarrassing to you or used by others to discriminate against you in employment or when seeking insurance or loans.

Because it may be possible to re-identify de-identified genomic data, even if access to data is controlled and data security standards are met, confidentiality cannot be guaranteed, and re-identified data could potentially be used to discriminate against or stigmatize participants, their families, or groups. In addition, there may be unknown risks due to computational methods, analytic technologies, or techniques (e.g., generation of information that could allow participants' identities to be readily ascertained).

Genetic information that results from this study does not have known medical or treatment importance at this time. However, there is risk that information from a genetic study may influence insurance companies and/or employees regarding your health. Taking part in a genetic study may also have a negative impact or unintended consequences on family or other relationships. To safeguard your privacy, genetic information obtained in this study will not be placed in your medical record. If you do not share information about taking part in this study, you will reduce your risk.

Breach of confidentiality: As with all research, there is a chance that confidentiality could be compromised; however, we will take precautions to minimize this risk.

Confidentiality
Your study data will be handled as confidentially as possible. If results of this study are published or presented, individual names and other personally identifiable information will not be used.

If screening results in being ineligible to participate in the main study, your data will be discarded immediately.

To minimize the risks to confidentiality, we will take the following measures:

- All subjects' data will be assigned with alpha-numeric code.
- Encrypted, digital records will be stored in password-protected computers accessible only to study investigators. Paper records will be stored in a locked file cabinet in the Principal Investigator's office.
- The list of codes associated with identifiers will be stored separately in a locked cabinet accessible only to the Principal Investigator.
- All samples will be removed of identifiers immediately upon collection and stored in a secure freezer in the Principal Investigator's laboratory at UC Berkeley.
- All deidentified genomic and proteomic human data will be stored on UC Berkeley's Savio P2, or equivalent UCB secure storage system. P2-level data security is considered the standard for de-identified human subject data storage.
- You may withdraw consent for research use of genomic or phenotypic data at any time without penalty or loss of benefits to which you are otherwise entitled. In this event, your data will be

withdrawn from any repository, if possible, and your data will not be used for future research. However, data already distributed for research use after your withdrawal will not be retrieved.

- Your personal information may be given out if required by law. Authorized representatives from the following organizations may review your research data for purposes such as monitoring or managing the conduct of this study:
  - Representatives of The National Institutes of Health (NIH),
  - Representatives of the University of California,
  - The Food and Drug Administration (FDA), and other government agencies involved in keeping research safe for people

We will keep your study data as confidential as possible, unless it is certain information that we must report for legal or ethical reasons. To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health (NIH). With this Certificate, researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings.

Exceptions: A Certificate of Confidentiality does not prevent researchers from voluntarily disclosing certain information about you for legal or ethical reasons. For example, we will report information about child abuse, elder abuse, or intent to hurt yourself or others. If an insurer, employer, or other person obtains your written consent to receive research information, we cannot use the Certificate to withhold that information. In addition, the Certificate may not be used to withhold information from the federal government needed for auditing or evaluating federally funded projects or information needed by the FDA, e.g., for quality assurance or data analysis.

Confidentiality of Data in Genetics Studies: Genetic information generated from the tissue samples and associated phenotypic data will be submitted to a National Institutes of Health (NIH) designated repository, such as the database of Genotypes and Phenotypes (dbGaP). dbGaP is a large database where information from many studies is stored, managed, and made available to other scientists and clinicians for future research.

Prior to submitting the data to the NIH repository, data will be stripped of identifiers such as your name, address, and any other identification numbers that would identify you. In terms of genomic data obtained from your body tissues this means that:

- Access to de-identified, individual-level participant data will be controlled.
- Aggregate study information (including genomic summary results) and study analyses may be shared in the scientific literature or through other public scientific resources, such as data repositories or other data sharing resources that provide broad or unrestricted access to the information, and
- The privacy protections, and limitations of those protections, afforded by a Certificate of Confidentiality to individual-level data do not apply to summary results.

Retaining research records: When this research is complete, all study records will be maintained indefinitely. De-identified collected samples will be stored indefinitely after the study complete. We will retain the signed consent forms for 10 years after the study is complete. Biospecimen (such as blood, tissue DNA and RNA) collected from you for this research study and/or information obtained from your biospecimens may be used in this research or other research and shared with other organizations. You will not share in any commercial value or profit derived from the use of your biospecimens and/or information obtained from them.

This study is not designed to assess one's health or make predictions of one's health in the future, therefore no such interpretations will be made from genetic analyses of this study. Clinically relevant research results, including genetic information, will not be disclosed to subjects.

Identifiers might be removed from the identifiable private information or identifiable biospecimens. After such removal, the information or biospecimens could be used for future research studies or distributed to other investigators for future research studies without additional informed consent from the subject or legally authorized representative.

Compensation/Payment
In return for your time and effort, if you are entered into the study, you will be paid $15/hour. As well, a $100 bonus will be paid for completion of the study. Payment will be in the form of a check from a UCB account. Anticipate that the check will be mailed to you about 6 weeks after your participation in the study has ended.

Costs of Study Participation
You will not be charged for any of the study activities.

Treatment and compensation for injury

It is important that you promptly tell the researcher, Professor Brooks, if you believe that you have been injured because of taking part in this study. You can tell the researcher in person or call him/her at 510 642 2861.

If you are injured as a result of taking part in this study, University of California will provide necessary medical treatment. The costs of the treatment may be billed to your insurer just like other medical costs or covered by the University of California or the study sponsor National Institutes of Health, depending upon a number of factors. The University and the study sponsor do not normally provide any other form of compensation for injury. For more information, call the Office of UC Berkeley's Committee for the Protection of Human Subjects (OPHS at (510) 642-7461.

Rights

Participation in research is completely voluntary. You have the right to decline to participate or to withdraw *at any point* in this study without penalty or loss of benefits to which you are otherwise entitled.

If you decide to withdraw from the study, the data collected about you up to the point of withdrawal will remain part of the study and may not be removed from the study database, per FDA regulations.

Questions
If you have any questions or concerns about this study, you may contact Professor Brooks at (510) 642-2861 (office) or [redacted] (mobile); or the labs (510) 642-9560 [VLSB], (510) 643-3175 [Haas Arena].

If you have any questions or concerns about your rights and treatment as a research subject, you may contact the office of UC Berkeley's Committee for the Protection of Human Subjects, at 510-642-7461 or subjects@berkeley.edu.

CONSENT

You will be given a copy of this consent form and of the Medical Research Subject's Bill of Rights to keep.

If you wish to participate in this study, please check the boxes and sign and date below.

My specimens and associated data may be kept for use in genetic research or analysis. __ Yes or __ No

My genetic data may be kept for broad sharing for future research. __ Yes or __ No

My genetic data may be kept for submission to NIH/federal repository or database. __Yes or __ No

_____	_____
Participant's Name *(please print)*	Date
_____	_____
Participant's Signature	Date
_____	_____
Person Obtaining Consent	Date

Appendix C
Glycerol Assay Protocol

Glycerol Assay

Weiland/Bergmeyer Method

Principle (1)

1. Glycerol is phosphorylated by glycerokinase (GK) and ATP to yield glycerol-3-phosphate (G3P):

$$\text{Glycerol} + \text{ATP} \rightarrow \text{G3P} + \text{ADP}$$

2. Glycerol-3-phosphate is oxidized by glycerol-3-phosphate dehydrogenase (GDH) and NAD^+ to yield dihydroxyacetone phosphate (DHAP):

$$\text{G3P} + NAD^+ \leftrightarrow \text{DHAP} + \text{NADH} + H^+$$

3. The equilibrium of the GDH reaction lies to the left. Working at pH 9.80 and trapping the DHAP with hydrazine allows for quantification of glycerol.
4. The amount of NADH formed is equivalent to the amount of glycerol present.

Preparation of the Glycine-Hydrazine Buffer (100 mL)

1. 1.500 g glycine (0.20 M)	Fisher BP381
2. 4.850 mL hydrazine (1.00 M)	Sigma-Aldrich 207942
3. 44.950 mL H_2O	
4. 200 µL of 1.0 M $MgCl_2$	ThermoFisher AM9530G
5. 4.500 mL of 2 N NaOH (pH = 9.80)	Fisher SS264
6. 45.500 mL H_2O	

Store in an amber bottle for ≤8 days.

Preparation of the Working Reagent (20 mL)

1. 0.018 g ATP	Sigma-Aldrich A5394 or A2383
2. 0.009 g NAD^+	Sigma-Aldrich N7004
3. 19.800 mL glycine-hydrazine buffer	
4. 35 µL GK	Sigma-Aldrich G6278
5. 185 µL GDH	Roche GDH-RO

Deproteinization

1. Transfer one volume of whole blood or plasma into 2 volumes of 7% perchloric acid (PCA).
2. Centrifuge the deproteinized samples at 3,000–10,000 g for 10 min at 4°C.
3. Transfer one volume of standards into 2 volumes of 7% PCA.

Procedure

1. Add 16 µL of 1 N NaOH to each well.
2. Add 40 µL of each standard or sample (clear supernatant). Use H_2O in 7% PCA as the blank.
3. Add 200 µL of the working reagent to each well.
4. Incubate at 37 °C for 15 min.
5. Read at 340 nm.
6. Read at 366 nm Report the average.

Range

1000 µM – 100 µM

References

1. **Weiland O**. Glycerol. In: *Methods of Enzymatic Analysis*, edited by Bergmeyer H-U. New York: Academic Press, 1971, p. 211-214.

Appendix D
Glycerol Assay Protocol

Glycerol Assay
Wako Method

Principle

1. Glycerol is phosphorylated by glycerokinase (GK) and ATP to yield glycerol-3-phosphate (G3P):

$$\text{Glycerol} + \text{ATP} \rightarrow \text{G3P} + \text{ADP}$$

2. Glycerol-3-phosphate is oxidized by glycerol-3-phosphate oxidase (GPO) to yield dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2):

$$\text{G3P} + O_2 \rightarrow \text{DHAP} + H_2O_2$$

3. The hydrogen peroxide produced causes HMMPS and 4-aminoantipyrine to undergo a quantitative oxidative condensation catalyzed by peroxidase (POD), producing a blue color. The amount of glycerol contained is determined by measuring the absorbance of the blue color.

Reagents

1. L-Type Triglyceride M Enzyme Color A, 60 mL — Fujifilm 994-02891
   L-Type Triglyceride M Enzyme Color A, 35 mL — Fujifilm 992-02892
2. HR Series NEFA-HR(2) Solvent A — Fujifilm 995-34791

Preparation of the Working Reagent

1. To 60 mL of L-Type Triglyceride M Enzyme Color A, add
   a. 0.010 g peroxidase (25 $U \cdot mL^{-1}$) — Sigma-Aldrich 77332
   b. 0.056 g 4-aminoantipyrine (4.6 mM) — Sigma-Aldrich A4382
2. To 35 mL of L-Type Triglyceride M Enzyme Color A, add
   a. 0.006 g peroxidase (25 $U \cdot mL^{-1}$)
   b. 0.033 g 4-aminoantipyrine (4.6 mM)

Procedure

1. Add 20 µL of each standard or sample. Use H_2O as the blank.
2. Add 90 µL of the working reagent to each well.
3. Incubate at 37 °C for 5 min.
4. Read at 600 nm.
5. Add 100 µL of Solvent A to each well.
6. Incubate at 37 °C for 5 min.
7. Read at 600 nm. Report the average.

Range

1000 µM – 25 µM

Appendix E
Free Fatty Acid Assay Protocol

Non-Esterified Fatty Acid Assay

Wako Method

Reagents

1. HR Series NEFA-HR(2) Color Reagent A	Fujifilm 999-34691
2. HR Series NEFA-HR(2) Solvent A	Fujifilm 995-34791
3. HR Series NEFA-HR(2) Color Reagent B	Fujifilm 991-34891
4. HR Series NEFA-HR(2) Solvent B	Fujifilm 993-35191
5. NEFA Standard Solution	Fujifilm 276-76491

Preparation of the Working Reagents

1. Reconstitute Color Reagent A in Solvent A.
2. Reconstitute Color Reagent B in Solvent B.

Procedure

1. Add 20 µL of each standard or sample. Use H_2O as the blank.
2. Add 100 µL of Color Reagent A to each well.
3. Incubate at 37 °C for 10 min.
4. Add 200 µL of Color Reagent B to each well.
5. Incubate at 37 °C for 10 min.
6. Read at 550 nm.

Range

1000 µM – 25 µM

Appendix F
Triglyceride Assay Protocol

Triglyceride Assay

Wako Method

Principle

1. Triglycerides are hydrolyzed to glycerol and free fatty acids (FA) in a reaction catalyzed by lipoprotein lipase (LPL):

$$\text{Triglyceride} + 3\ H_2O \rightarrow \text{Glycerol} + 3\ \text{FA}$$

2. Glycerol is phosphorylated by glycerokinase (GK) and ATP to yield glycerol-3-phosphate (G3P):

$$\text{Glycerol} + \text{ATP} \rightarrow \text{G3P} + \text{ADP}$$

3. Glycerol-3-phosphate is oxidized by glycerol-3-phosphate oxidase (GPO) to yield dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2):

$$\text{G3P} + O_2 \rightarrow \text{DHAP} + H_2O_2$$

4. The hydrogen peroxide produced causes HMMPS and 4-aminoantipyrine to undergo a quantitative oxidative condensation catalyzed by peroxidase (POD), producing a blue color. The amount of glycerol contained is determined by measuring the absorbance of the blue color.
5. The amount of triglycerides contained is determined by subtracting the concentration of glycerol determined before the addition of LPL from the final glycerol concentration.

Reagents

1.	L-Type Triglyceride M Enzyme Color A, 60 mL	Fujifilm 994-02891
	L-Type Triglyceride M Enzyme Color A, 35 mL	Fujifilm 992-02892
2.	L-Type Triglyceride M Enzyme Color B, 20 mL	Fujifilm 990-02991
	L-Type Triglyceride M Enzyme Color B, 13 mL	Fujifilm 998-02992

Procedure

1. Add 20 µL of each standard or sample. Use H_2O as the blank.
2. Add 30 µL of Enzyme Color B to each well.
3. Incubate at 37 °C for 5 min.
4. Add 90 µL of Enzyme Color A to each well.
5. Incubate at 37 °C for 5 min.
6. Read at 600 nm.

Range

2000 µM – 25 µM

Appendix G
Lactate Assay Protocol

Lactate Assay

Brooks/Hohorst/Bergmeyer Method

Principle (1)

1. Lactate is oxidized by lactate dehydrogenase (LDH) and NAD^+ to yield pyruvate:

$$\text{Lactate} + NAD^+ \leftrightarrow \text{Pyruvate} + NADH + H^+$$

2. The equilibrium of the LDH reaction lies far to the left. Working at pH 9.00, using excessive amounts of NAD^+, and trapping pyruvate with hydrazine allows for quantification of lactate.

$$\text{Lactate} + NAD^+ + \text{Hydrazine} \rightarrow \text{Pyruvate hydrazone} + NADH + H_3O^+$$

3. The amount of NADH formed is equivalent to the amount of lactate present.

Preparation of the Glycine-Hydrazine Buffer (300 mL)

1. 11.400 g glycine (0.51 M) — Fisher BP381
2. 6.000 mL hydrazine (0.41 M) — Sigma-Aldrich 207942
3. 280 mL H_2O
4. 1.500 mL of 2 N NaOH (pH = 9.00) — Fisher SS264
5. 12.500 mL H_2O

Store in a glass bottle for ≤8 days.

Preparation of the Working Reagent (25 mL)

1. 0.050 g NAD^+ — Sigma-Aldrich N7004
2. 25.0 mL glycine-hydrazine buffer
3. 25 µL LDH — Sigma-Aldrich L2500

Deproteinization

1. Transfer one volume of whole blood into 2 volumes of 7% perchloric acid (PCA).
2. Centrifuge the deproteinized samples at 3,000–10,000 g for 10 min at 4°C.
3. Transfer one volume of standards into 2 volumes of 7% PCA.

Procedure

1. Add 8 µL of 2 N NaOH to each well.
2. Add 20 µL of each standard or sample (clear supernatant). Use H_2O in 7% PCA as the blank.
3. Add 250 µL of the working reagent to each well.
4. Incubate at 37 °C for 40 min.
5. Allow the plate to cool at room temperature for 20 min.
6. Read at 340 nm.

Range

10.00 mM – 0.35 mM

References

1. **Hohorst H-J**. L-(+)-Lactate Determination with Lactic Dehydrogenase and DPN. In: *Methods of Enzymatic Analysis*, edited by Bergmeyer H-U. New York: Academic Press, 1971, p. 266-270.