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RESEARCH ARTICLE

Aging delays the suppression of lipolysis and fatty acid oxidation in the postprandial period

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Abstract

Plasma glycerol and free fatty acid concentrations decrease following oral glucose consumption, but changes in the rate of lipolysis during an oral glucose tolerance test (OGTT) have not been documented in conjunction with changes in fatty acid (FA) oxidation or reesterification rates in healthy individuals. After a 12-h 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 hand vein was catheterized for arterialized blood sampling. Indirect calorimetry was performed simultaneously to determine total FA and carbohydrate (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 OGTT. Glycerol rate of appearance (Ra), an index of lipolysis, decreased significantly from baseline 5 min postchallenge in young participants and 30 min in older participants. At 60 min, FA Rox decreased in both groups, but was significantly higher in older participants. Between 5 and 90 min, CHO Rox was significantly lower in older participants. In addition, FA Rs was significantly lower in older participants at 60 and 90 min. The area under the curve (AUC) for FA Rox was greater than that for FA Rs in older, but not in young participants. Our results indicate that, in aging, the postprandial suppression of lipolysis and FA oxidation are delayed such that FA oxidation is favored over CHO oxidation and FA reesterification.

NEW & NOTEWORTHY To our knowledge, our investigation is the first to demonstrate changes in lipolysis during an oral glucose tolerance test (OGTT) in healthy young and older individuals. Plasma glycerol and free fatty acid concentrations changed after glycerol rate of appearance (Ra), indicating that plasma concentrations are incomplete surrogates of the lipolytic rate. Moreover, simultaneous determinations of substrate oxidation rates are interpreted to indicate that metabolic inflexibility in aging is characterized by delayed changes in postprandial substrate utilization related to the lipolytic rate.

aging; lactate; metabolic flexibility; OGTT; reesterification

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. 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, 4) and exogenous fuel administration in humans. Following oral CHO consumption in resting (5, 6) and exercising (7, 8) 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 used an oral glucose tolerance test (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 (5, 6, 9). Other previous investigations also documented simultaneous reductions in FA oxidation rates and plasma free fatty acid (FFA) concentrations (10–12). Because FAs serve as the primary energy substrate during postabsorptive rest (13, 14), metabolic flexibility to oral CHO is also illustrated by the concurrent suppression of whole body FA oxidation. 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 and in "healthy" aging (15–17). Central to the "Randle Cycle" theory (18) is that an abundance of FA determines energy substrate partitioning. From there, it follows that metabolic inflexibility is induced

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by an excess availability of FFA in plasma during hyperinsulinemia and the subsequent elevation of FA oxidation that occurs at the expense of CHO oxidation (19, 20). Several previous investigations have used combined infusions of insulin, lipid, and heparin to simulate intravascular lipolysis and observe the effects of elevated FFA on substrate oxidation rates. As predicted by the "Randle Cycle" theory (18), FA oxidation increased and CHO oxidation decreased when FFA availability was elevated (21–24). Physiologically, however, the persistence of FFA availability during hyperinsulinemia would suggest a defect in the insulin-mediated suppression of lipolysis (25). Bonadonna et al. (26) reported that FFA mobilization during insulin infusion was higher in older compared with younger individuals and resulted in greater total lipid oxidation. Although higher rates of lipolysis were suspected to have resulted in increased FFA availability, it was unclear whether the lipolytic rate was different or altered by their experimental conditions. To our knowledge, the lipolytic response during physiological hyperinsulinemia has not yet been investigated in aging. Consequently, at present, it is unclear whether there are aging-related modifications to the lipolytic rate in the postprandial period, and whether such modifications promote metabolic inflexibility in aging through the induction of substrate competition by increasing FFA availability.

The OGTT is a standard physiological method for assessing the influence of a glucose load on parameters of metabolic flexibility in the postprandial period. As described, after oral glucose consumption, the suppression of whole body FA oxidation occurs as CHO oxidation rises (5, 6, 9–12). Reductions in the plasma concentrations of glycerol alone (27) or concurrently with FFA (28, 29) 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 (30), they do not provide information on the turnover of those metabolites. To our knowledge, only one investigation has used a glycerol isotope tracer to determine changes in the lipolytic rate during an OGTT. Diniz Behn et al. (31) reported a reduction in the glycerol rate of appearance (Ra), a marker of lipolysis, during an OGTT in very young (12–21 yr old) overweight or women with obesity. 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. Fatty acids released from lipolysis are either oxidized in different tissues or reesterified into triglycerides (TG) in the liver, adipose tissue (32), or skeletal muscle (33, 34). Because FAs taken up by skeletal muscle have the potential to alter insulin signaling if not oxidized (35), growing our understanding of FA metabolism during the postprandial period is imperative. At present, it is unclear whether there are aging-related modifications to lipolytic, FA oxidation, or FA reesterification rates in the postprandial period, and whether such modifications promote metabolic inflexibility in aging.

A rise in blood lactate concentrations above baseline (i.e., lactatemia) during an OGTT has also been documented (36–38). We have recently reviewed the role of lactate in signaling and its effects on energy substrate partitioning (39). Briefly, lactate binding to hydroxycarboxylic acid receptor 1 (HCAR-1) on adipose cells (40, 41) mediates the insulin-induced inhibition of lipolysis via autocrine signaling (42). Evidence of the antilipolytic effect of lactate in humans was first provided by Boyd et al. (43), who demonstrated that lactate infusion during mild exercise prevented an increase in plasma glycerol and FFA concentrations. Similar results were obtained earlier in resting (44) and exercising (45) dogs receiving lactate infusions. In humans, lactate production by adipose cells following oral glucose consumption (46, 47) provides the foundation for an autocrine mechanism by which lactate exerts its antilipolytic effects. Just as our understanding of the changes in the lipolytic rate during an OGTT is incomplete, it is not known whether physiological lactatemia induced by oral glucose consumption has any relationship with lipolysis.

The purpose of this investigation was to assess metabolic flexibility by determining concurrent changes in the rates of lipolysis as estimated from the glycerol Ra, total FA, and CHO oxidation, and total FA reesterification during an OGTT in healthy young and older individuals. We hypothesized that lipolysis would decrease in response to oral glucose consumption. We hypothesized that FA oxidation and reesterification rates would decrease, and CHO oxidation rates would increase in response to oral glucose consumption. Furthermore, we hypothesized that the changes in substrate oxidation rates would be delayed in aging.

MATERIALS AND METHODS

Study Participants

The overall study design has been reported separately (48, 49), but is summarized here for readers' convenience. 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 young (21–35 yr; 7 men and 8 women) and 14 older (60–80 yr; 7 men and 7 women) participants 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 (50). 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). The exercise testing protocol has been reported separately (51). For dietary controls, participants provided 3-day dietary records that

were analyzed for caloric intake and macronutrient consumption (Diet Analysis Plus v.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 nonsmokers; to have normal pulmonary function (vital capacity 1-s forced expiratory volume of $>$70%); to have a fasting blood glucose concentration of $<$100 mg·dL^{-1}; to have a hemoglobin A1c percentage of $<$5.7% of total hemoglobin; to have a fasting blood total cholesterol concentration of $<$200 mg·dL^{-1}, LDL cholesterol concentration of $<$100 mg·dL^{-1}, HDL cholesterol concentration of $\geq$40 mg·dL^{-1}, and total TG concentration of $<$150 mg·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 1 wk. 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.

Experimental Procedures

Participants underwent a 120-min OGTT with primed, continuous infusion of [1,1,2,3,3-^{2}H]glycerol (D_5-glycerol) and [3-^{13}C]lactate (Cambridge Isotope Laboratories, Inc., Andover, MA). Data on lactate kinetics in young (52) and older (49) participants are reported separately, but the use of [3-^{13}C]lactate is mentioned in this report for its relevancy to the present data.

Participants reported to the laboratory in the morning following a 12-h overnight fast. Women who participated did so during the mid-follicular phase of their menstrual cycle. For the 24 h 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 15-mL priming bolus containing 40 mg of 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·min^{-1}. Arterialized blood sampling was repeated at 75 and 90 min after the start of continuous infusion.

After 90 min of continuous infusion, participants drank a solution containing 75 g of D-glucose in 296 mL (No. 10-O-075, Azer Scientific Inc., Morgantown, PA). Participants consumed the solution in $\leq$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

Samples for the determination of arterialized blood lactate and glucose concentrations and glycerol IE were immediately deproteinized in two volumes of cold 7% perchloric acid (PCA) after collection. Samples for the determination of plasma glycerol, FFA, TG, insulin, C-peptide, glucagon, epinephrine, and norepinephrine concentrations were collected in tubes containing K_3EDTA (Vacuette, Greiner Bio-One, Monroe, NC). In addition, per mL of whole blood, 8 TIU (trypsin inhibitory units) of aprotinin and 10 μL of DPP IV inhibitor (No. DPP4-M, Sigma-Aldrich, St. Louis, MO) were added prior to collection to prevent the degradation of insulin, C-peptide, and glucagon. All samples were placed on ice immediately 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.

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 nonrebreathing 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 were 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.

Metabolite Analyses

Arterialized plasma glycerol concentrations were determined enzymatically (53) and simultaneously with TG concentrations using a prepared set of reagents (Wako L-Type Triglyceride M, Fujifilm Healthcare Solutions, Lexington, MA). 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]. Arterialized blood lactate concentrations were determined enzymatically (54) from PCA extracts of whole blood. Similarly, arterialized blood glucose concentrations were determined from PCA extracts using a prepared reagent [Pointe Scientific Glucose (Hexokinase), Fisher Scientific, Hampton, NH].

Hormone Analyses

Arterial plasma concentrations of insulin (No. 80-INSHU-E01.1), C-peptide (No. 80-CPTHU-E01.1), and glucagon (No. 48-GLUHUU-E01) were determined using enzyme-linked immunosorbent assay (ELISA) kits purchased from ALPCO Diagnostics (Salem, NH). Plasma concentrations of epinephrine (No. ab287788) and norepinephrine (No. ab287789) were determined using ELISA kits purchased from Abcam (Boston, MA). 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.

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 (30). Perchloric acid 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 50 W-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 min. 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.

Determination of Glycerol Kinetics

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 (55):

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

$$\mathrm{Ra}\left(\mu\mathrm{mol}\cdot\mathrm{min}^{-1}\right) = \left[F - V\left[(C_1 + C_2)/2\right] \times \left[(\mathrm{IE}_2 - \mathrm{IE}_1)/(t_2 - t_1)\right]\right]/\left[(\mathrm{IE}_2 + \mathrm{IE}_1)/2\right],$$

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

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

where *F* represents the tracer infusion rate (0.32 mg·min^{-1}), *V* is the estimated volume distribution for glycerol (270 mL·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.

Determination of Substrate Oxidation and Fatty Acid Reesterification Rates

Respiratory data were exported from the Parvo Medics TrueOne program using 5-s averaging display. Subsequently, respiratory data were 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 (56, 57):

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

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

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

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

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

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·min^{-1}. Total CHO Rox was converted to units of mg·min^{-1} using the caloric equivalent of CHO (4.2 kcal·g^{-1}), and then to units of μmol·min^{-1} using the molecular weight of glucose (180.16 g·mol^{-1}). Total Lipid Rox was converted to units of mg·min^{-1} using the caloric equivalent of lipid (9.5 kcal·g^{-1}), and then to units of μmol·min^{-1} using the molecular weight of a representative TG (860 g·mol^{-1}). Furthermore, 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 (56, 58):

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

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}.

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. Scores were calculated using the equation described by Matthews et al. (59):

$$\mathrm{HOMA-IR} = (\mathrm{BPI} \times \mathrm{BPG})/22.5,$$

where BPI is the baseline plasma insulin concentration in μIU·mL^{-1} and BPG is the baseline blood glucose concentration in mM. A HOMA-IR score of 1 indicates "normal" insulin sensitivity (60), and higher scores indicate greater degrees of insulin resistance in nondiabetic individuals (61). In addition, the composite insulin sensitivity index (CISI) was used to estimate whole body insulin sensitivity during the OGTT. Scores were calculated using the equation described by Matsuda and DeFronzo (62):

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

where BPI is the baseline plasma insulin concentration in μIU·mL^{-1}, BPG is the baseline blood glucose concentration in mg·dL^{-1}, MPI is the mean plasma insulin concentration from 30 to 120 min in μIU·mL^{-1}, and MBG is the mean blood glucose concentration from 30 to 120 min in mg·dL^{-1}. Plasma insulin concentrations were converted from units of pM to μIU·mL^{-1} by dividing by 6 (63). A CISI score ≤2.5

Table 1. *Summary of ANOVA results describing time × age interactions and main effects for time and age for variables studied*

Variable	Time × Age		Time		Age	
	F	*P* Value	*F*	*P* Value	*F*	*P* Value
[Glycerol]	0.87	0.52	126.0	<0.001	0.16	0.69
[FFA]	2.15	0.05	203.9	<0.001	0.63	0.43
[TG]	2.91	0.01	25.97	<0.001	1.00	0.33
[Lactate]	2.14	0.05	32.16	<0.001	0.26	0.61
[Insulin]	3.33	0.004	41.84	<0.001	1.74	0.20
[C-Peptide]	4.96	0.01	150.8	<0.001	6.73	0.02
[Glucagon]	1.48	0.19	61.60	<0.001	1.03	0.32
[Epinephrine]	0.42	0.86	2.02	0.12	2.48	0.13
[Norepinephrine]	2.48	0.03	1.33	0.27	0.59	0.45
Glycerol Ra	2.50	0.02	92.17	<0.001	0.82	0.37
Glycerol Rd	2.48	0.03	91.96	<0.001	0.823	0.37
Glycerol MCR	1.10	0.36	38.04	<0.001	0.01	0.93
FA Rox	2.81	0.02	133.8	<0.001	0.29	0.59
FA Rs	3.29	0.004	77.79	<0.001	3.41	0.08
CHO Rox	3.11	0.007	140.4	<0.001	14.15	<0.001

Brackets around variables indicate arterial plasma or whole blood (Lactate only) concentrations. Data was analyzed by repeated-measures two-way ANOVA with Tukey's multiple comparisons test. CHO, carbohydrate; FA, fatty acid; FFA, free fatty acid; MCR, metabolic clearance rate; Ra, rate of appearance; Rd, rate of disappearance; Rox, rate of oxidation; Rs, rate of reesterification; TG, triglyceride.

is associated with insulin resistance (64), whereas higher scores suggest greater degrees of insulin sensitivity.

Estimations of Pancreatic β-Cell Function

The HOMA method was also used to estimate β-cell function (%β) from baseline, fasting blood glucose, and plasma insulin concentrations. Scores were calculated using the equation described by Matthews et al. (59):

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

A HOMA-%β score of 100% indicates "normal" β-cell function (60), and lower scores indicate lesser β-cell function.

The insulinogenic index (IGI) and oral disposition index (DI) methods were also used to estimate β-cell function during the "early phase" of insulin secretion (i.e., 0–30 min postchallenge). Scores were calculated using the equations described by DeFronzo et al. (65, 66):

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

$$\text{DI} = \text{IGI} \times \text{CISI},$$

where $\Delta I_{30\text{-}0}$ is the change in plasma insulin concentration from baseline at 30 min in pM and $\Delta G_{30\text{-}0}$ is the change in blood glucose concentration from baseline at 30 min in mM. Lower IGI scores have been reported in individuals with impaired glucose tolerance (67), and lower DI scores have been reported in individuals at risk for developing diabetes (68). Accordingly, higher IGI and DI scores are interpreted to reflect greater insulin secretory capacity.

Statistical Analyses

Data were analyzed using GraphPad Prism 10 (v.10.1.2 for Windows, GraphPad Software, Boston, MA). Statistical significance was set at $\alpha \leq 0.05$. An unpaired *t* test was used to assess the significance of mean differences between participant characteristics. Repeated-measures two-way ANOVA with Tukey's multiple comparisons test was used to assess the significance of mean differences between groups and across time points. Data are presented as means ± standard error of the mean (SE). Time × age interactions and main effects for time and age are summarized in Table 1. For brevity, significance for time × age interactions and main effects for time and age are described in-text only for select variables studied. Otherwise, only results of multiple comparisons are described. 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 also using GraphPad Prism 10. An unpaired *t* test was used to assess the significance of differences in AUC. Data are presented as means ± SE. Pearson correlation coefficients were used to assess the significance of relationships among glycerol Ra and select variables studied.

RESULTS

Participant Characteristics

Anthropometric, physical performance, and dietary characteristics of young and older participants have been reported elsewhere (48, 49), but results are summarized here for readers' convenience. Older participants' age, BMI, and body fat percentage were significantly higher than young participants ($P < 0.05$). In contrast, young participants' absolute and relative $\dot{V}O_{2peak}$ and VT were significantly higher than older participants ($P < 0.05$). There were no significant differences in body mass, pulmonary function (FEV_1/FVC), daily caloric intake, or macronutrient intake between groups.

Indexes of insulin sensitivity and insulin secretion are presented in Table 2. Older participants' HOMA-IR score was significantly higher than young participants ($P = 0.01$). In contrast, young participants' IGI and DI scores were significantly higher than older participants ($P \leq 0.007$). There was no difference in the CISI or HOMA-%β scores between groups ($P \geq 0.13$).

Glycerol, FFA, and TG Concentrations during an OGTT

Arterial plasma glycerol concentrations decreased significantly from baseline 30 min postchallenge in both young (50.58 ± 3.01 vs. 69.19 ± 2.50 μM, $P < 0.001$) and older (48.84 ± 4.19 vs. 72.76 ± 5.12 μM, $P < 0.001$) participants, and then continuously declined until 120 min ($P < 0.001$).

Table 2. *Indexes of insulin sensitivity and insulin secretion in young and older participants*

Variable	Young	Older	*P* Value
HOMA-IR	0.80 ± 0.1	1.27 ± 0.2	0.02
CISI	7.45 ± 0.9	6.00 ± 0.6	0.19
HOMA-%β, %	66.71 ± 7.3	100.99 ± 19.6	0.13
IGI, pM·mM^{-1}	134.31 ± 23.5	55.66 ± 6.8	0.007
DI	803.86 ± 111.1	305.67 ± 35.4	0.001

Values are means ± SE. Young ($n = 15$) and older ($n = 14$) participants. CISI, composite insulin sensitivity index; DI, oral disposition index; HOMA-%β, homeostatic model assessment method of pancreatic β-cell function; HOMA-IR, homeostatic model assessment method of insulin resistance; IGI, insulinogenic index. Comparisons were made by unpaired *t* test.

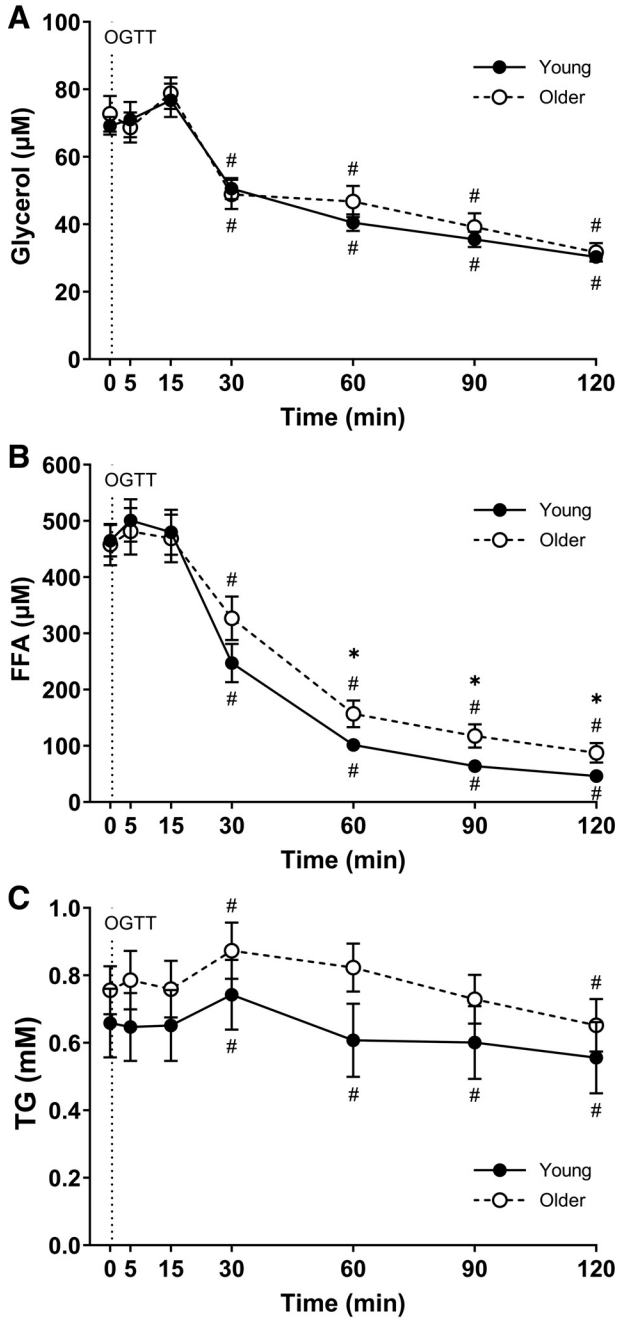

Figure 1. Arterial plasma concentrations of glycerol (*A*), free fatty acids (FFA) (*B*), and triglycerides (TG) (*C*) before and during an oral glucose tolerance test (OGTT) in young ($n = 15$) and older ($n = 14$) participants. Values are means ± SE. #Significantly different from 0 min, $P < 0.05$. *Significantly different between groups, $P < 0.05$.

There were no significant differences in glycerol concentrations between groups at any time point ($P \geq 0.24$) (Fig. 1*A*). Plasma FFA concentrations decreased significantly from baseline 30 min postchallenge in both young (247.14 ± 32.78 vs. 464.71 ± 27.01 μM, $P < 0.001$) and older (326.87 ± 37.75 vs. 457.93 ± 35.52 μM, $P < 0.001$) participants, and then continuously declined until 120 min ($P < 0.001$). In addition, FFA concentrations were significantly higher in older participants between 60 and 120 min ($P \leq 0.05$) (Fig. 1*B*).

Arterial plasma TG concentrations increased significantly from baseline 30 min postchallenge in both young (0.74 ± 0.10 vs. 0.66 ± 0.10 mM, $P < 0.001$) and older (0.87 ± 0.08 vs. 0.75 ± 0.07 mM, $P = 0.01$) participants. However, in young participants, TG concentrations decreased below baseline at 60 min (0.61 ± 0.10 mM, $P = 0.03$) and then continuously declined until 120 min ($P \leq 0.01$). In contrast, in older participants, TG concentrations decreased to baseline values at 60 min and 90 min, and then decreased below baseline at 120 min (0.65 ± 0.08 mM, $P = 0.03$). There were no significant differences in TG concentrations between groups at any time point ($P \geq 0.11$) (Fig. 1*C*).

Lactate Concentrations during an OGTT

Arterial blood lactate concentrations are presented in Table 3, but they have been graphically reported separately (49). We observed a significant time × age interaction ($F = 2.14$, $P = 0.05$) and a significant main effect for time ($F = 32.16$, $P < 0.001$), but not age ($F = 0.26$, $P = 0.61$). Blood lactate concentrations increased significantly from baseline 5 min postchallenge in young participants ($P = 0.04$) and at 15 min in older participants ($P = 0.02$), and then remained elevated above baseline until 120 min in both groups ($P \leq 0.04$). There were no significant differences in lactate concentrations between groups at any time point ($P \geq 0.08$).

Insulin and C-Peptide Concentrations during an OGTT

We observed a significant time × age interaction for arterial plasma insulin concentrations ($F = 3.33$, $P = 0.004$) and a significant main effect for time ($F = 41.84$, $P < 0.001$) but not age ($F = 1.74$, $P = 0.20$). Insulin concentrations increased significantly from baseline 5 min postchallenge in young participants (77.82 ± 12.62 vs. 22.71 ± 2.50 pM, $P = 0.01$) and at 15 min in older participants (151.40 ± 17.49 vs. 33.85 ± 3.60 pM, $P < 0.001$), and then remained elevated above baseline until 120 min in both groups ($P < 0.001$). In addition, insulin concentrations were significantly higher in older participants at baseline ($P = 0.02$) but higher in young participants at 5 min and 30 min ($P \leq 0.05$) (Fig. 2*A*). C-peptide concentrations were increased significantly from baseline 5 min and 60 min postchallenge in young participants ($P < 0.001$) but only at 60 min in older participants ($P < 0.001$). In addition, C-peptide concentrations were significantly higher in older participants at baseline ($P = 0.001$) but higher in young participants at 5 min and 60 min ($P \leq 0.04$) (Fig. 2*B*).

Glucagon, Epinephrine, and Norepinephrine Concentrations during an OGTT

Arterial plasma glucagon concentrations decreased significantly from baseline 30 min postchallenge in both young (5.87 ± 0.75 vs. 9.78 ± 0.80 pM, $P = 0.001$) and older (5.24 ± 0.65 vs. 7.49 ± 0.79 pM, $P = 0.002$) participants, and then remained low until 120 min ($P \leq 0.002$). There were no significant differences in glucagon concentrations between groups at any time point ($P \geq 0.06$) (Fig. 2*C*).

Arterial plasma epinephrine concentrations remained at baseline levels in both young (21.78 ± 0.64 pg·mL^{-1}, $P \geq 0.31$) and older (23.45 ± 0.59 pg·mL^{-1}, $P \geq 0.74$) participants throughout observation (Fig. 2*D*). Similarly, norepinephrine concentrations remained at baseline levels in both young

Table 3. *Arterial blood lactate concentrations before and during an OGTT in young and older participants*

Time, min	Young	*P* Value vs. 0 min	Older	*P* Value vs. 0 min	*P* ValueYoung vs. Older
0	0.60 ± 0.05		0.65 ± 0.02		0.34
5	0.71 ± 0.05	0.04	0.62 ± 0.04	0.96	0.21
15	1.09 ± 0.09	<0.001	0.90 ± 0.06	0.02	0.08
30	0.87 ± 0.09	0.009	0.84 ± 0.05	0.04	0.77
60	1.19 ± 0.09	<0.001	1.05 ± 0.06	<0.001	0.23
90	1.08 ± 0.09	<0.001	1.10 ± 0.07	<0.001	0.88
120	1.02 ± 0.10	0.002	1.11 ± 0.07	0.001	0.46

Values are means ± SE. Young ($n = 15$) and older ($n = 14$) participants. Data were analyzed by repeated-measures two-way ANOVA with Tukey's multiple comparisons test. OGTT, oral glucose tolerance test.

(257.67 ± 1.63 pg·mL^{-1}, $P \geq 0.09$) and older (266.34 ± 9.74 pg·mL^{-1}, $P \geq 0.58$) participants throughout observation (Fig. 2*E*). There were no significant differences in epinephrine ($P \geq 0.06$) or norepinephrine ($P \geq 0.09$) concentrations between groups at any time point.

Glycerol Kinetics during an OGTT

We observed a significant time × age interaction for arterial glycerol Ra ($F = 2.50$, $P = 0.02$) and a significant main effect for time ($F = 92.17$, $P < 0.001$) but not age ($F = 0.82$, $P = 0.37$). Glycerol Ra decreased significantly from baseline 5 min postchallenge in young participants (1.83 ± 0.10 vs. 1.98 ± 0.11 μmol·kg^{-1}·min^{-1}, $P = 0.03$) and at 30 min in older participants (1.39 ± 0.10 vs. 1.78 ± 0.12 μmol·kg^{-1}·min^{-1}, $P = 0.002$), and then remained low between 30 and 120 min in both groups ($P \leq 0.003$). Conspicuously, in young participants, glycerol Ra was not significantly different from baseline at 15 min ($P = 0.22$). There were no significant differences in glycerol Ra between groups at any time point ($P \geq 0.14$) (Fig. 3*A*). In addition, there was no significant difference in the AUC for three times the absolute glycerol Ra between groups ($P = 0.67$) (Fig. 3*B*).

The pattern of glycerol Rd was similar to that of Ra. We observed a significant time × age interaction for arterial glycerol Rd ($F = 2.48$, $P = 0.03$) and a significant main effect for time ($F = 91.96$, $P < 0.001$) but not age ($F = 0.82$, $P = 0.37$). Glycerol Rd decreased significantly from baseline 5 min postchallenge in young participants (1.83 ± 0.10 vs. 1.98 ± 0.11 μmol·kg^{-1}·min^{-1}, $P = 0.03$) and at 30 min in older participants (1.10 ± 0.10 vs. 1.78 ± 0.12 μmol·kg^{-1}·min^{-1}, $P = 0.002$), and then remained low between 30 and 120 min in both groups ($P \leq 0.003$). As well, in young participants, glycerol Rd was not significantly different from baseline at 15 min ($P = 0.19$). There were no significant differences in glycerol Rd between groups at any time point ($P \geq 0.13$) (Fig. 3*C*).

We did not observe a significant time × age interaction for arterial glycerol MCR ($F = 1.10$, $P = 0.36$) or a significant main effect for age ($F = 0.01$, $P = 0.93$). However, we observed a significant main effect for time ($F = 38.04$, $P < 0.001$). Glycerol MCR increased significantly from baseline 120 min postchallenge in young participants (35.98 ± 2.37 vs. 27.96 ± 1.91 mL·kg^{-1}·min^{-1}, $P < 0.001$). However, in older participants, glycerol MCR increased significantly 60 min postchallenge (32.74 ± 1.94 vs. 26.36 ± 1.57 mL·kg^{-1}·min^{-1}, $P = 0.01$) and then continuously rose until 120 min ($P \leq 0.004$). There were no significant differences in glycerol MCR between groups at any time point ($P \geq 0.46$) (Fig. 3*D*).

Total Energy Expenditure Rates during an OGTT

Total EE increased significantly from baseline 5 min postchallenge 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. There were no significant differences in EE between groups at any time point ($P \geq 0.45$) (Supplemental Fig. S1*A*). However, when expressed relative to total body mass (TBM), EE increased significantly from baseline 5 min postchallenge in young (12.19 ± 0.39 vs. 10.15 ± 0.28 μmol·kg^{-1}·min^{-1}, $P = 0.01$) and at 30 min in older participants (12.53 ± 0.58 vs. 9.57 ± 0.47 μmol·kg^{-1}·min^{-1}, $P = 0.01$), and EE was significantly higher in young participants between 5 and 90 min ($P \leq 0.01$) (Supplemental Fig. S1*B*).

Rates of Total FA Oxidation and Reesterification during an OGTT

We observed a significant time × age interaction for total FA Rox ($F = 2.81$, $P = 0.01$) and a significant main effect for time ($F = 133.8$, $P < 0.001$) but not age ($F = .29$, $P = 0.59$). FA Rox decreased significantly from baseline 60 min postchallenge in both young (1.64 ± 0.24 vs. 3.75 ± 0.10 μmol·kg^{-1}·min^{-1}, $P < 0.001$) and older (2.36 ± 0.22 vs. 3.51 ± 0.12 μmol·kg^{-1}·min^{-1}, $P < 0.001$) participants, and then remained below baseline until 120 min ($P < 0.001$). In addition, FA Rox was significantly higher in older participants at 60 min ($P = 0.04$) (Fig. 4*A*). Results were similar when FA Rox was expressed in absolute terms (Supplemental Fig. S2*A*).

We observed a significant time × age interaction for total FA Rs ($F = 3.29$, $P = 0.004$) and a significant main effect for time ($F = 77.79$, $P < 0.001$) but not age ($F = 3.41$, $P = 0.08$). FA Rs decreased significantly from baseline 5 min postchallenge in both young (1.74 ± 0.23 vs. 2.28 ± 0.14 μmol·kg^{-1}·min^{-1}, $P = 0.002$) and older (1.28 ± 0.16 vs. 1.93 ± 0.17 μmol·kg^{-1}·min^{-1}, $P < 0.001$) participants, and then continuously declined until 30 min ($P < 0.001$). In young participants, FA Rs increased to baseline rates at 60 min ($P = 0.98$), increased significantly above baseline at 90 min (2.49 ± 0.11 μmol·kg^{-1}·min^{-1}, $P = 0.003$), and then returned to baseline rates at 120 min ($P > 0.99$). In contrast, in older participants, FA Rs increased continuously between 60 and 120 min and increased significantly above baseline at 120 min (2.21 ± 0.17 μmol·kg^{-1}·min^{-1}, $P < 0.001$). In addition, FA Rs was higher in young participants at 60 min ($P = 0.02$) and 90 min ($P = 0.03$) (Fig. 4*B*).

There were no significant differences in the AUC for total FA Rox ($P = 0.50$) or FA Rs ($P = 0.12$) between groups. In

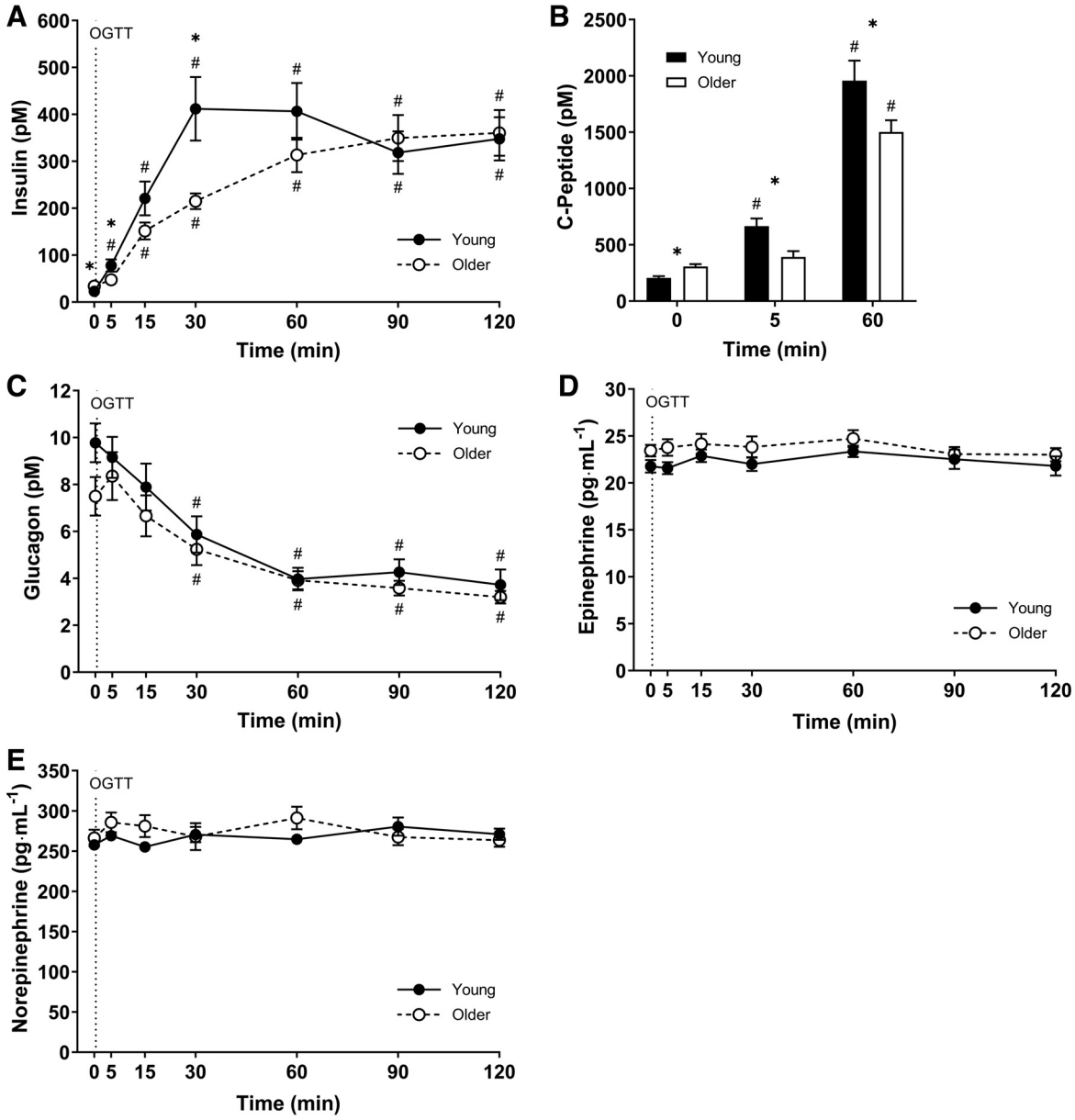

Figure 2. Arterial plasma concentrations of insulin (*A*), C-peptide (*B*), glucagon (*C*), epinephrine (*D*), and norepinephrine (*E*) before and during an oral glucose tolerance test (OGTT) in young ($n = 15$) and older ($n = 14$) participants. Values are means ± SE. #Significantly different from 0 min, $P < 0.05$. *Significantly different between groups, $P < 0.05$.

young participants, there were no significant differences in the AUC for FA Rox and FA Rs ($P = 0.34$). However, in older participants, the AUC for FA Rox was significantly greater than the AUC for FA Rs ($P = 0.008$) (Fig. 5*A*).

Total CHO Oxidation Rates during an OGTT

We observed a significant time × age interaction for total CHO Rox ($F = 3.11$, $P = 0.007$) and significant main effects for time ($F = 140.4$, $P < 0.001$) and age ($F = 14.15$, $P < 0.001$). Total CHO Rox increased significantly from baseline 5 min postchallenge in young (10.89 ± 0.40 vs. 8.86 ± 0.30 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.02$) and at 30 min in older participants (11.34 ± 0.60 vs. 8.40 ± 0.51 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.02$), and then remained elevated until 120 min ($P \leq 0.01$). In addition, CHO Rox was significantly higher in young participants between 5 and 90 min ($P \leq 0.03$) (Fig. 4*C*), and the AUC for CHO Rox was significantly greater for young participants ($P = 0.04$) (Fig. 5*B*). However, when expressed in absolute terms, CHO Rox was significantly higher in young participants at 60 min only (1.07 ± 0.06 $kcal \cdot min^{-1}$ vs. 0.85 ± 0.05 $kcal \cdot min^{-1}$, $P = 0.01$) (Supplemental Fig. S2*B*).

Correlations between Glycerol Ra and Glycerol, FFA, Lactate, and Insulin Concentrations

Glycerol Ra was positively correlated with plasma glycerol concentrations in young ($r = 0.96$) and older ($r = 0.94$) participants (Supplemental Fig. S3*A*). Similarly, glycerol Ra was positively correlated with FFA concentrations in young ($r = 0.97$) and older ($r = 0.95$) participants (Supplemental Fig. S3*B*). The correlations were significant for both groups ($P \leq 0.002$).

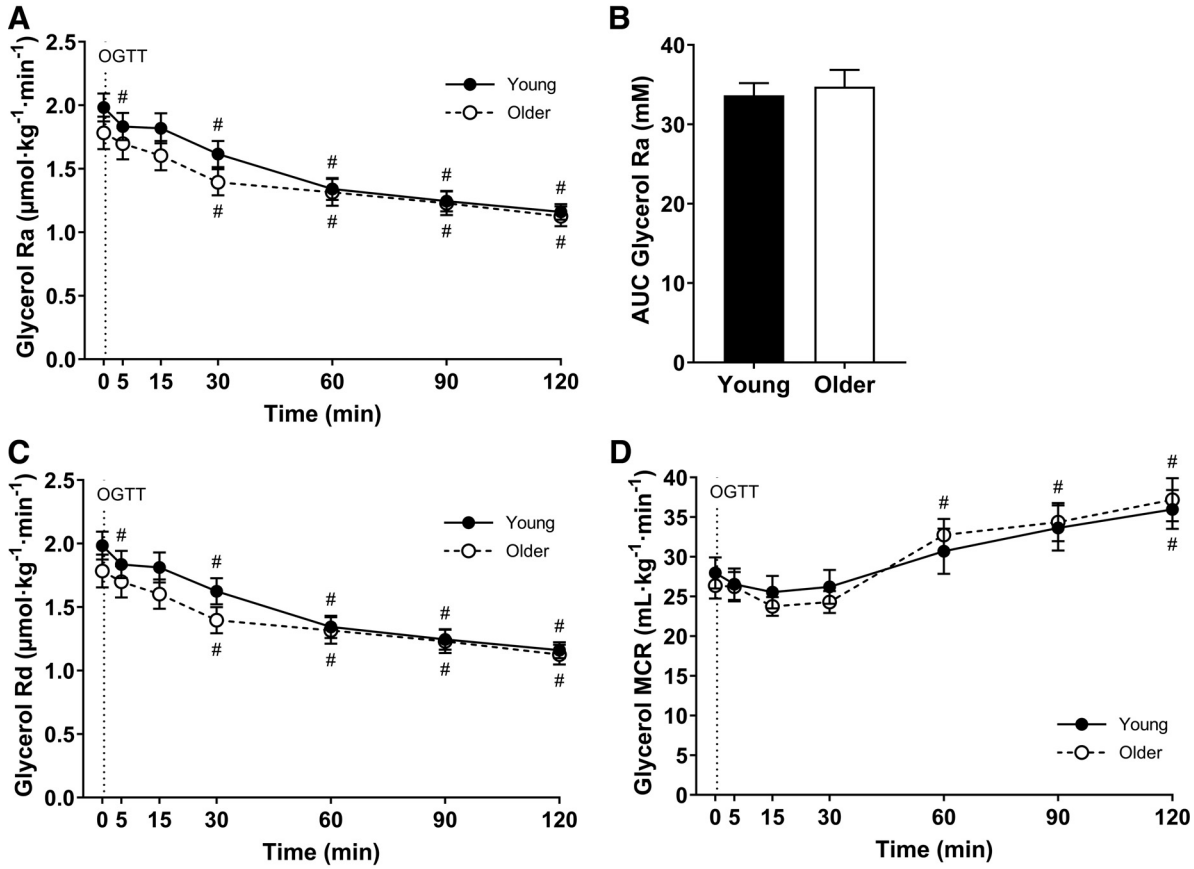

Figure 3. Arterial glycerol rate of appearance (Ra) (*A*), area under the curve (AUC) for three times the absolute glycerol Ra (*B*), glycerol rate of disappearance (Rd) (*C*), and glycerol metabolic clearance rate (MCR) (*D*) before and during an oral glucose tolerance test (OGTT) in young ($n = 15$) and older ($n = 14$) participants. Values are means ± SE. #Significantly different from 0 min, $P < 0.05$.

Glycerol Ra was inversely correlated with lactate concentrations in both groups, but the correlation was higher ($r = -0.93$ vs. $r = -0.71$) and more significant ($P = 0.003$ vs. $P = 0.07$) in older participants (Fig. 6*A*) (Table 4). Similarly, glycerol Ra was inversely correlated with plasma insulin concentrations in both groups, but the correlation was higher ($r = -0.98$ vs. $r = -0.93$) and more significant ($P < 0.001$ vs. $P = 0.003$) in older participants (Fig. 6*B*).

Correlations of Substrate Oxidation Rates to Markers of Lipolysis

Glycerol Ra was positively correlated with total FA Rox ($r \geq 0.87$) but inversely correlated with total CHO Rox ($r \geq -0.96$) in both groups (Fig. 6, *C* and *D*). Similarly, plasma FFA concentrations were positively correlated with FA Rox ($r \geq 0.93$) but inversely correlated with CHO Rox ($r \geq -0.97$) in both groups (Supplemental Fig. S4, *A* and *B*). All correlations were significant for both groups ($P \leq 0.01$).

DISCUSSION

The purpose of this investigation was to determine concurrent changes in the rates of lipolysis as estimated from the glycerol Ra, total FA and CHO oxidation, and total FA reesterification following oral glucose consumption in healthy young and older individuals. Although the lipolytic rates were comparable between groups, we observed notable differences in other parameters of energy substrate partitioning, particularly in the immediate response to oral glucose consumption. In brief, following oral glucose consumption, we report the following: *1*) the suppression of lipolysis is delayed in aging, *2*) blood lactate concentrations are inversely related to the lipolytic rate, *3*) the suppression of FA oxidation is delayed in aging, *4*) the rise in CHO oxidation is delayed in aging, *5*) FA reesterification is not the primary fate of FA released from lipolysis, and *6*) the lipolytic rate informs aging-related differences in postprandial energy substrate partitioning. Our results are discussed sequentially.

Glycerol Ra Provides a Complete Assessment of Lipolysis during an OGTT

In postabsorptive or exercising humans, glycerol is produced from lipolysis, but "free" glycerol is not known to be recycled to TG within adipose. Consequently, the glycerol Ra is representative of the rate of lipolysis (69, 70). To our knowledge, only one other investigation has used a glycerol isotope tracer to determine changes in the rate of lipolysis during an OGTT. Diniz Behn et al. (31) reported a reduction in lipolysis during an OGTT in very young (12–21 yr old), overweight, or women with obesity. Similarly using a glycerol isotope tracer and paired with arterialized blood sampling, we expand on their data by demonstrating that the

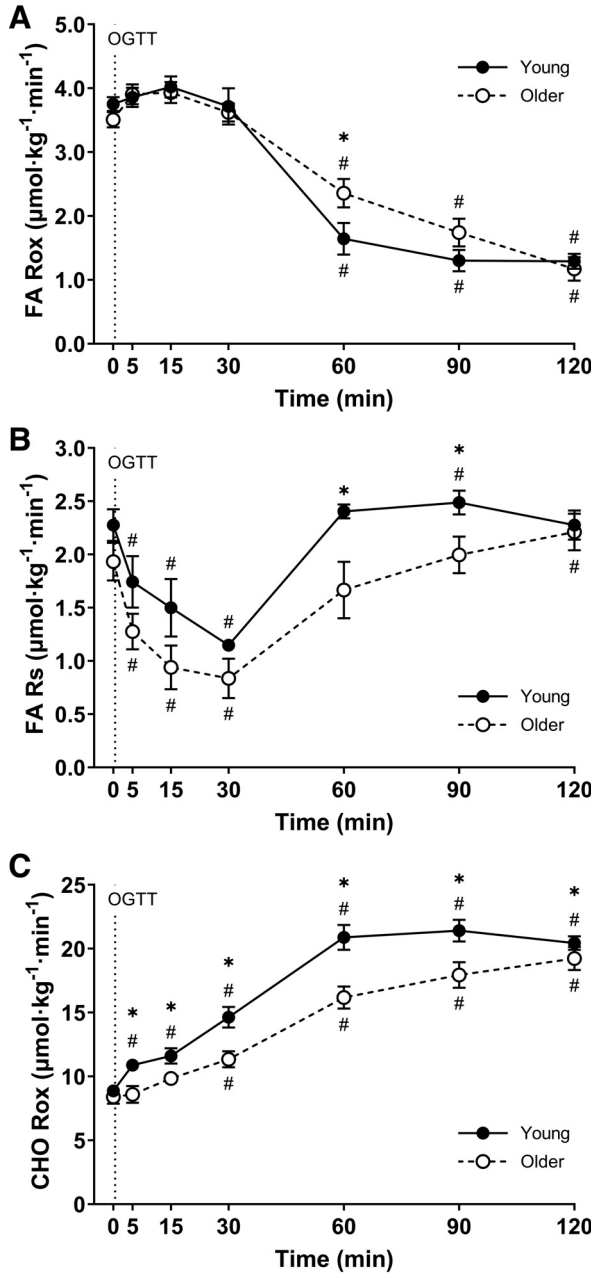

Figure 4. Rates of total fatty acid oxidation (FA Rox) (*A*), fatty acid reesterification (FA Rs) (*B*), and carbohydrate oxidation (CHO Rox) (*C*) before and during an oral glucose tolerance test (OGTT) in young ($n = 15$) and older ($n = 14$) participants. Values are means ± SE. #Significantly different from 0 min, $P < 0.05$. *Significantly different between groups, $P < 0.05$.

rate of lipolysis also decreases in healthy young and older individuals during an OGTT.

Changes in arterial plasma glycerol and FFA concentrations were closely related in young and older groups. 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 remained below baseline between 30 and 120 min. Thus, our results support previous investigations that have interpreted the reduction in glycerol concentrations alone (27), or concurrently with FFA concentrations (28, 29), as the suppression of lipolysis at 30 min postchallenge.

The blood sampling protocol used 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 reveal that the rate of lipolysis decreased immediately (i.e., 5 min) after glucose consumption in our young group. Thus, because they remained relatively unchanged at 5 min and 15 min ($P \geq 0.58$), changes in [Glycerol] and [FFA] are incomplete surrogates of the lipolytic rate. Importantly, they are presently unable to identify aging-related differences. Both [Glycerol] and [FFA] remained unchanged from baseline at 5 min in both groups ($P \geq 0.60$). However, as described, the reduction in Ra at 5 min was only significant in our young group ($P = 0.03$ vs. $P = 0.25$), and we observed a significant time × age interaction for Ra. Hence, our results demonstrate that the control of lipolysis in the postprandial period is negatively affected in aging.

Notably, in our young group, glycerol Ra at 15 min was not different from baseline ($P = 0.22$) despite being similar to Ra at 5 min ($P > 0.99$), presumably due to a higher variation in Ra at 15 min. A paired *t* test comparing the mean difference in young participants' Ra from 0 min to 15 min yielded a

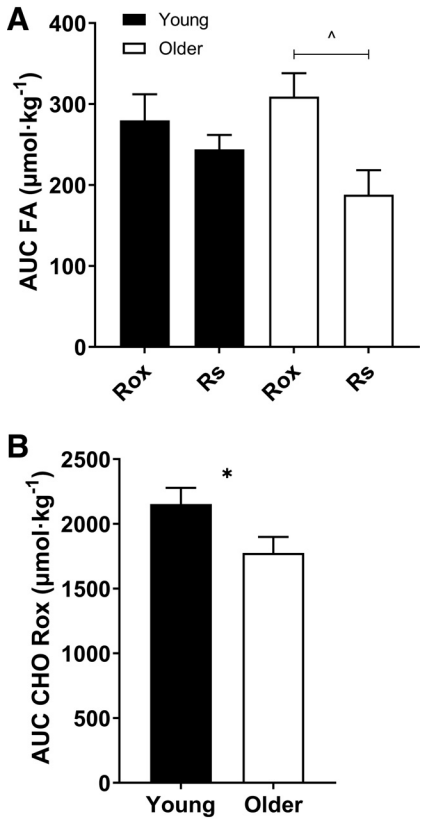

Figure 5. Area under the curve (AUC) for rates of total fatty acid oxidation (FA Rox) and reesterification (FA Rs) (*A*) and total carbohydrate oxidation (CHO Rox) (*B*) during an oral glucose tolerance test (OGTT) in young ($n = 15$) and older ($n = 14$) participants. Values are means ± SE. *Significantly different between groups, $P < 0.05$. ^Significantly different between FA Rox and FA Rs, $P < 0.05$.

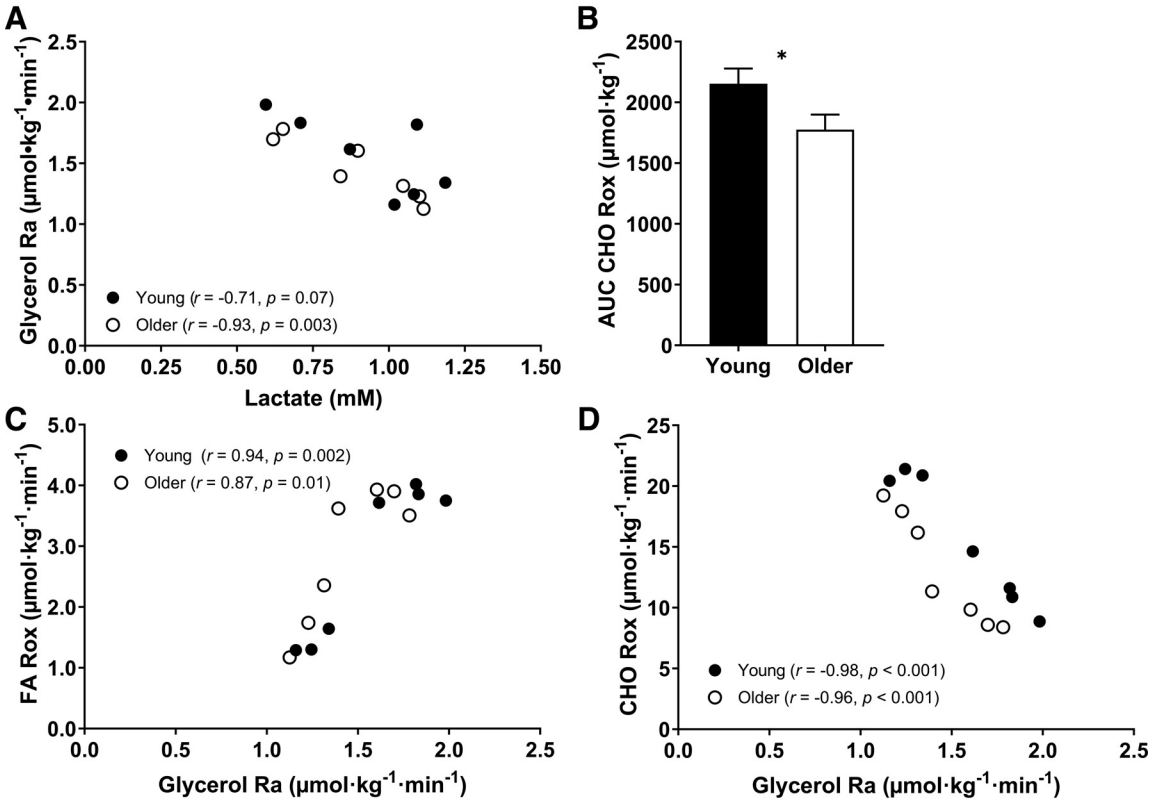

Figure 6. Relationship between arterial glycerol rate of appearance (Ra) and blood lactate concentrations (*A*), plasma insulin concentrations (*B*), total fatty acid oxidation rates (FA Rox) (*C*), and total carbohydrate oxidation rates (CHO Rox) (*D*) before and during an oral glucose tolerance test (OGTT) in young ($n = 15$) and older ($n = 14$) participants. Values are means. *r*, Pearson correlation coefficient. *Significantly different between groups, $P < 0.05$.

P value of 0.02. Consequently, the present data are interpreted to indicate a rapid and sustained reduction in the rate of lipolysis in our young, but not older group.

Insulin has an essential role in the suppression of lipolysis (71). Previous investigations using stable isotope tracers reported a reduction in the Ra of glycerol (72) and FFA (58) during variable insulin infusion rates to demonstrate the insulin-mediated suppression of lipolysis. Presently, the significant inverse correlations between [Insulin] and glycerol Ra in both groups support the role of insulin in the inhibition of lipolysis.

A prominent finding of the present investigation is the immediate increase in [Insulin] 5 min after glucose consumption in our young, but not older ($P = 0.62$) group. C-peptide concentrations were also increased in our young, but not older ($P = 0.32$) group at 5 min. Because C-peptide concentrations are used as a measure of insulin secretion (60), we report a significant increase in insulin secretion in our young group. Hence, the immediate suppression of lipolysis can be attributed, in part, to the immediate increase in insulin secretion.

Table 4. *Summary of correlations between arterial glycerol Ra and select variables studied before and during an OGTT in young and older participants*

	Young			Older		
Variable	Pearson *r*	R^2	*P* Value	Pearson *r*	R^2	*P* Value
[Lactate]	−0.71	0.51	0.07	−0.93	0.86	0.003
[Insulin]	−0.78	0.61	0.04	−0.98	0.96	<0.001
FA Rox	0.94	0.89	0.002	0.87	0.76	0.01
CHO Rox	−0.98	0.96	<0.001	−0.96	0.91	<0.001

Young ($n = 15$) and older ($n = 14$) participants. Brackets around variables indicate arterial plasma (Insulin) or whole blood (Lactate) concentrations. Pearson correlation coefficients were used to assess the significance of relationships. Pearson *r*, correlation coefficient; FA, fatty acid; CHO, carbohydrate; OGTT, oral glucose tolerance test; Ra, rate of appearance; Rox, rate of oxidation.

Despite a higher HOMA-IR score in our older group, we did not observe any other indication of insulin "resistance" as observed in metabolic disease states. The mean HOMA-IR score of our older group was lower than HOMA-IR scores previously reported in metabolic disease states (73–75), indicating greater insulin sensitivity in our healthy older group. Moreover, the CISI scores of our young and older groups were similar and were greater than the scores previously reported in insulin-resistant individuals (67, 76). Thus, the delayed suppression of lipolysis in our older group can be attributed, in part, to lower circulating insulin associated with lower pancreatic β-cell function, but not insulin "resistance". Further support of this interpretation is provided in [Insulin] and [C-Peptide], which were higher in our young group at 30 min and 60 min, respectively. In addition, our estimates of β-cell function (IGI and DI) during the initial 30 min of observation, and higher [C-Peptide] in our young group at 5 min, collectively indicate that our young group exhibited a greater insulin secretory capacity. Our observations are consistent with some (77, 78), but not all (66) previous investigations that have similarly reported a diminished insulin response following oral glucose consumption in older individuals resulting from aging-related β-cell dysfunction (68).

In healthy humans, the antilipolytic effect of insulin is counterregulated by catecholamines, epinephrine and norepinephrine, but not glucagon (79, 80). Previous investigations using graded infusions of catecholamines reported an increase in markers of lipolysis in resting (81, 82) and exercising (83) humans to demonstrate the promotion of lipolysis. Presently, [epinephrine] and [norepinephrine] did not change from baseline in either young or older groups during observation, and concentrations were not different between groups at any time point. Thus, despite a significant time × age interaction for [norepinephrine], we do not attribute the delayed reduction in glycerol Ra and higher [FFA] observed in our older group to differences in circulating catecholamines. These results are interpreted to indicate that the postprandial lipolytic response is controlled primarily by an increase in antilipolytic signals as opposed to a reduction in signals that promote lipolysis.

Our finding that glycerol Ra was similar between groups at all time points is consistent with previous reports of comparable lipolytic rates between young and older individuals during physiological conditions, including prolonged fasting (84) and during exercise at a given power output before (85) and after (86) endurance training. Moreover, because the CISI estimates insulin sensitivity during an OGTT (62), the absence of any significant difference in glycerol Ra between groups is consistent with the similar CISI scores ($P = 0.19$). However, the absence of significant differences in glycerol Ra is inconsistent with previous investigations that reported a dose-response relationship between plasma insulin concentrations and FFA mobilization rates (as a surrogate for the lipolytic rate) (87, 88). Notably, Bonadonna et al. (26) reported that FFA mobilization rates were greater in elderly compared with younger individuals during euglycemic-insulin clamp experiments yielding comparable plasma insulin concentrations. Because glycerol Ra is a more appropriate measure of lipolysis than FFA mobilization (70), results of Bonadonna et al. (26) are difficult to interpret in the context of the lipolytic rate. Greater rates of FFA mobilization might reflect lower rates of local FA reesterification within adipocytes (58) as opposed to greater rates of lipolysis. Alternatively, our results of glycerol Ra can be interpreted to indicate that insulin sensitivity and/or responsiveness of adipose tissue is preserved in aging. Still, because we observed a significant time × age interaction for Ra, our results demonstrate 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 group in their ability to rapidly suppress lipolysis in response to oral glucose consumption. Presently, it is unclear why [FFA] was significantly higher in our older group between 60 and 120 min. We determined that there was no difference in the extent to which lipolysis was reduced (i.e., % decrease) between groups at any time point (data not shown). Moreover, because the AUC for three times the absolute glycerol Ra was not different between groups ($P = 0.67$), we do not suspect that FFA mobilization was greater in older group. Potentially, our older group exhibited quantitatively less, or slower peripheral uptake of plasma FFA to maintain higher [FFA], or our young group exhibited greater FA reesterification within adipose (58, 89) to yield lower [FFA]. Future similar investigations might use D_5-glycerol in conjunction with a FA isotope tracer (e.g., [1-^{13}C]palmitate) to explore these hypotheses.

Blood Lactate Concentrations Are Inversely Related to the Lipolytic Rate

Ahmed et al. (42) described an autocrine mechanism in mouse adipocytes in which lactate signaling has a permissive effect on the inhibitory effects of insulin on lipolysis. In humans, lactate production by adipocytes following oral glucose consumption (46, 47) provides the foundation for this autocrine mechanism. Presently, the inverse correlations between glycerol Ra and [Lactate] in both groups support the role of lactate signaling in the inhibition of lipolysis. Our results are consistent with other reports describing lactate inhibition of lipolysis in isolated mouse (40) and human (41) adipocytes incubated in lactate, resting (44) and exercising (45) dogs receiving lactate infusions, and exercising humans receiving lactate infusions (43). However, our results are inconsistent with those of Ferrannini et al. (90), who reported that lipolysis was unaffected by lactate infusion in resting humans during euglycemic-insulin clamp. As noted by the authors, lactate inhibition of lipolysis might warrant "different metabolic circumstances" (90). Potentially, the suppression of insulin secretion by catecholamine stimulation during exercise (43, 45) or by pancreatectomy (44) might improve the sensitivity of adipocytes to lactate signaling. From extant data (42, 46, 47), our results are interpreted to indicate that autocrine lactate signaling has a permissive effect on the insulin-mediated suppression of adipocyte lipolysis specifically in the postprandial period. Rephrased, we speculate that lactate contributes to, but is not solely responsible for, the inhibition of adipocyte lipolysis. Further support of this interpretation is provided in the magnitude and significance of the inverse correlations between glycerol Ra and [Insulin], which were marginally greater than those between glycerol Ra and [Lactate] within groups.

Although correlations do not provide proof of causation, we contend that our correlations support the role of lactate signaling in the inhibition of lipolysis. Particularly, the mechanism is supported by other investigators (40–45), whereas inverse relationships with other variables are not. Although arterial blood glucose concentrations (48) are most likely also inversely related to glycerol Ra in the present study, hyperglycemia by itself is not known to have a role in the suppression of lipolysis in humans (91, 92). Notably, in our young group, we observed a concurrent increase in [Lactate] and [Insulin] and reduction in glycerol Ra at 5 min, whereas in our older group, these responses were collectively delayed. The coincidental nature of these observations is bolstered by the significance of the correlations (young, $P = 0.07$ and older, $P = 0.003$) that affirm the potential permissive effects of lactatemia on the insulin-mediated suppression of lipolysis. However, further investigation is required to confirm the role of lactate signaling in the control of lipolysis, in the postprandial period or otherwise.

The Postprandial Fall in FA Oxidation Is Delayed in Aging

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 (25), FA availability, and FA oxidation (93). Our results in our young group agree with previous investigations

that reported that the reduction in FA oxidation occurs beyond the first 30 min after glucose consumption (9–12). However, unlike previous investigations, we observed a stable and minimal FA Rox between 60 and 120 min. These investigations observed an apparent continuous decline in FA oxidation rates between 60 and 120 min. It is unclear why this discrepancy exists, as these investigations used a similar 75-g (9) or larger 100-g (10, 11) glucose load that should have, theoretically, resulted in greater suppression of FA oxidation (94, 95). At minimum, we confirm extant data on the changes in FA oxidation immediately following oral glucose consumption in healthy young individuals.

Total FA Rox fell below baseline rates at 60 min in both groups. However, at 60 min, FA Rox was higher in our older group. When expressed as an absolute rate, FA Rox also trended to be higher in our older group at 90 min ($P = 0.07$). In contrast, between 60 and 120 min, FA Rox was relatively unchanged in our young group, suggesting that a minimal rate had been reached by 60 min. As well, we observed a significant time × age interaction for FA Rox. Thus, our results demonstrate that the postprandial suppression of FA oxidation is delayed in aging. Because FAs serve as the primary energy substrate during postabsorptive rest (13, 14), 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 later). However, worth noting first is the reciprocal changes in CHO oxidation rates.

The Postprandial Rise in CHO Oxidation Is Delayed in Aging

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 (93). In our young group, total CHO Rox rapidly increased from baseline until 60 min. These early changes in CHO Rox mirrored the changes reported in previous investigations that similarly observed healthy young men and women (5, 10, 11). Moreover, our results agree with previous investigations that reported stable and maximal CHO oxidation rates between 60 and 120 min after glucose consumption in young individuals (6, 9). However, we observed a significant increase in CHO Rox 15 min and 30 min after glucose consumption, which disagrees with the results of others (6, 11). Anthropometric differences in the populations studied (e.g., body fat content) might have contributed to this difference. 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.

Our results demonstrate that the postprandial rise in CHO oxidation is delayed in aging. As described, we observed a significant time × age interaction for CHO Rox. Furthermore, CHO Rox increased above baseline 5 min after glucose consumption in our young, but not in older group. An increase in CHO Rox above baseline was not observed until 30 min in our older group. Moreover, between 60 and 120 min, CHO Rox remained relatively steady in our young group, but steadily increased in our older group. Because these changes were also observed when CHO Rox was expressed as an absolute rate, the divergent responses of CHO Rox between groups cannot be completely explained by the minor difference in TBM ($P = 0.22$) or in the relative size of the glucose load ($P = 0.36$, data not shown).

The ability of skeletal muscle to suppress lipid oxidation during hyperglycemia is influenced by insulin sensitivity, percentage body fat, and aerobic fitness (96, 97). Because the total energy expenditure 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, 5). Because peripheral insulin sensitivity (98) and skeletal muscle mass (99) 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 indicate that our older group did not exhibit these aging-related decrements, as there were no differences in HOMA-IR, CISI, or fat-free mass ($P = 0.79$, data not shown). Basu et al. (100) reported that insulin action was lower in their elderly than young participants due to greater body fat content. Presently, we similarly report a higher body fat percentage and greater body fat mass in our older group ($P = 0.005$, data not shown). Coincident with higher [Insulin], CHO Rox was higher in our young group 30 min after glucose consumption. These results allow us to speculate greater glucose uptake and oxidation (94). The delayed rise in CHO Rox observed in our older group 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 fat-free mass for glucose uptake. The impact of this distinction is extended to explain the observed differences in lipid metabolism. Notably, at 60-min postchallenge, [FFA] and FA Rox were greater in our older group despite similar [Insulin] ($P = 0.20$). Our results are consistent with those of Bonadonna et al. (26), who reported that plasma FFA concentrations and FFA oxidation rates were greater in elderly compared with younger individuals during euglycemic-insulin clamp. Hence, the delayed suppression of FA oxidation and greater [FFA] observed in our older group is attributed to lower insulin action in aging (98, 101).

In addition to the delayed increase from baseline, we report that CHO Rox was significantly lower in our older group between 5 and 90 min. Resting metabolic rate (102) and the metabolic scope reportedly decrease with advancing age. Thus, our finding that postchallenge CHO Rox was lower in our older group is potentially unsurprising. However, we did not observe any significant differences in resting, postabsorptive EE whether expressed as an absolute rate ($P = 0.74$) or relative to TBM ($P = 0.32$). Consequently, the ability to increase EE and alter the balance of CHO and FA utilization in our older group is attributed to other factors. As previously described, greater body fat mass (103) and its associated effects on insulin action (100) is likely responsible for lower postchallenge CHO Rox. Consistently, when expressed as absolute instead of relative rates, there were less differences in CHO Rox between young and older groups postchallenge, indicating that differences in body composition also contributed to lower postchallenge CHO Rox in our older group.

Collectively, results of postprandial glycerol Ra, FA Rox, and CHO Rox demonstrate that responses were delayed, but not restricted in our older group. By the end of observation, FA Rox ($P = 0.59$) and CHO Rox ($P = 0.26$) were not different between groups. These are consistent with a companion report (48) in which we described the inability of the difference in peak and baseline RER to characterize metabolic flexibility in our healthy groups. Hence, postprandial metabolic inflexibility in "healthy" aging is characterized by delayed changes in energy substrate partitioning.

Reesterification Is Not the Primary Postprandial Fate of FA

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 (104). Because the only fates of FA released from lipolysis are oxidation and reesterification, results of FA Rox and FA Rs will be discussed concurrently.

Fatty acid reesterification rates over time.

In both young ($P \geq 0.72$) and older ($P \geq 0.16$) groups, FA Rox increased nonsignificantly from baseline between 5 and 15 min. Although not significant, the increases in FA Rox were substantial enough to cause a simultaneous, significant reduction in FA Rs below baseline. Therefore, our data indicate that FAs released from lipolysis between 5 and 30 min were used to support total EE rather than being recycled.

During the second hour of observation, FA Rs increased transiently above baseline rates at 90 min and 120 min in our young and older groups, respectively. Fatty acid Rs also remained at baseline rates at 60 min and 120 min in our young group. Notably, [Insulin] was also elevated above baseline at these time points. Insulin has an essential role in the regulation of FA reesterification (58, 105). Because greater circulating insulin concentrations result in greater suppression of FA reesterification (95), this was an unexpected result. We initially hypothesized that FA Rs would be suppressed throughout the OGTT primarily due to the suppression of lipolysis (58), and we demonstrated that the rate of lipolysis decreased in both groups. Alongside the lower arterial plasma concentrations of glycerol and FFA at those time points, it is therefore unclear whether these brief increases in FA Rs resulted in substantial TG synthesis. Unfortunately, we are not able to determine the rates of oxidation and reesterification of plasma FFA. As previously described, Bonadonna et al. (26) reported greater rates of plasma FFA mobilization and oxidation in their elderly participants during euglycemic-insulin clamp. We predict that plasma FFA reesterification rates were reduced in their elderly participants as a result. Accordingly, we suspect that reesterification of circulating plasma FFA was reduced in our older group. This could at least partially explain the higher [FFA] between 60 and 120 min. Thus, our data and those of others allow us to speculate that reesterification was not the primary fate of FA released from lipolysis. Future similar investigations might use D_5-glycerol in conjunction with a FA isotope tracer (e.g., [1-^{13}C]palmitate) to explore this hypothesis.

Aging-related differences in fatty acid reesterification.

In contrast to FA Rox, FA Rs were significantly lower at 60 min and 90 min in our older group. Empirically, this resulted from greater FA Rox at 60 min and 90 min ($P = 0.07$). We therefore estimated the division of FA for each pathway by comparing the AUC for FA Rox to the AUC for FA Rs. There were no differences observed in our young group, which we interpret to indicate that similar amounts of FAs were allotted to oxidation and reesterification. However, the AUC for FA Rox was greater than the AUC for FA Rs in our older group. With a significant time × age interaction for FA Rs, our results therefore also indicate that postprandial lipid substrate partitioning was altered in our older group such that FA reesterification was diminished in favor of FA oxidation.

Aging-related differences in FA reesterification have not been extensively studied. Notably, Bonadonna et al. (26) reported rates of plasma FFA turnover and oxidation, but not rates of plasma FFA reesterification. Similarly, Sial et al. (85) reported rates of glycerol tracer-measured lipolysis and lipid oxidation rates in young and elderly individuals during exercise, but not rates of total FA reesterification. Our estimations of FA reesterification from the data of Sial et al. (85) ([3 × glycerol Ra] − [3 × fat oxidation]) reveal that the average FA reesterification rate during exercise was higher in their elderly population than in their young population exercising at the same absolute intensity due to lower rates of FA oxidation. Our results agree in the broad sense that FA partitioning is altered in aging, and that aging-related differences in FA reesterification result primarily from differences in FA oxidation. Also in agreement with our data, Sial et al. (85) reported no differences in lipolytic and lipid oxidation rates, and thus no differences in FA reesterification rates, during postabsorptive rest.

Skeletal muscle takes up circulating FFA during hyperglycemia to serve as substrates for TG synthesis via reesterification (89). Importantly, FAs taken up but not oxidized have the potential to alter insulin signaling (35). Reesterification in skeletal muscle therefore serves as a mechanism to protect against insulin resistance (33). 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 group. However, interpretation of our reesterification data is greatly limited by the absence of FA isotope tracer data to describe the reesterification of plasma FFA. Although the data of Bonadonna et al. (26) allows us to speculate that plasma FFA oxidation rates were elevated in favor of FFA reesterification in our older group, the higher [FFA] between 60 and 120 min challenges our hypotheses since FFA mobilization was most likely not different between groups (no differences in the AUC for three times the glycerol Ra). Potentially, lower plasma FFA oxidation and reesterification yielded higher [FFA]. Such results have important implications for aging-related metabolic disease states in which elevated plasma FFA concentrations give rise to greater rates of FA uptake (106) into skeletal muscle with lower capacities for FA oxidation (107, 108). Because glycerol MCR was not different between groups at any time point, and because there were no differences in the AUC for total 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 plasma FFA in aging warrants further investigation.

Lipolysis and FFA Availability Influence Postprandial Energy Substrate Partitioning

As described, the suppression of lipolysis was delayed in our older group (30 min vs. 5 min). Bonadonna et al. (26) reported that FFA availability and FA oxidation during hyperinsulinemia were higher in older compared with younger individuals. Although higher rates of lipolysis were suspected to have increased FFA availability, it was unclear whether the lipolytic rate was altered by their experimental conditions. Presently, we observed no differences in glycerol Ra between groups, but [FFA] was higher in older participants between 60 and 120 min. The higher [FFA] at 60 min and 90 min importantly coincided with higher FA Rox and lower CHO Rox.

The correlations between glycerol Ra, [FFA], FA Rox, and CHO Rox were comparable between groups in magnitude 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 group. The correlations also suggest that the delayed suppression of glycerol Ra and coincident higher [FFA] could have enabled higher FA Rox, thereby delaying the rise in CHO Rox and reducing FA Rs. Consistently, in our older group, correlations of [FFA] to substrate oxidation rates were higher, suggesting that [FFA] had a larger influence than glycerol Ra on substrate oxidation rates, as predicted by the "Randle Cycle" theory (18). Previous investigations have also demonstrated greater rates of FA oxidation and lower rates of CHO oxidation when plasma FFA availability was elevated during hyperinsulinemia by lipid and heparin infusion (19, 21, 22). Kruszynska et al. (12) 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 by Bonadonna et al. (26), but importantly, we observed similar results during physiological hyperinsulinemia in which lipolysis and plasma FFA availability were allowed to fall below baseline. Other previous investigations determined that elevated plasma FFA availability induces transient changes in insulin action (109) to affect substrate oxidation rates (19, 21, 22). Although we are unable to determine whether plasma FFA were elevated at a concentration high enough and for long enough (110) to have transiently affected insulin action in our older group, 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 indicate that the delayed suppression of lipolysis and greater FFA availability contributed to the delayed suppression of FA Rox at the expense of CHO Rox and FA Rs.

Limitations

In the present study, we sought to determine changes in the rate of lipolysis as estimated from the tracer-measured glycerol Ra during an OGTT. In addition, we sought to determine lactate oxidation rates following oral glucose consumption in young and older participants (49). To this end, we used [1,1,2,3,3-^{2}H]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 FFA flux with [1-^{13}C]palmitate as we have done previously (30, 56, 111, 112). Although we provide evidence of the changes in total FA substrate partitioning during an OGTT, we were unable to precisely identify the rates of 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 (26) and the known effects of plasma-derived FA on insulin action in skeletal muscle (113, 114). Cumulatively, during the OGTT, we suspect greater local (i.e., within adipocyte) reesterification in our young group, minor plasma FFA reesterification in both groups, greater rates of plasma FFA uptake in our young group, and greater oxidation of plasma FFA at the expense of reesterification in our older group. Future similar investigations might use D_5-glycerol in conjunction with [1-^{13}C]palmitate to explore these hypotheses. Our present data nonetheless identifies immediate changes in parameters of energy substrate partitioning following oral glucose consumption and importantly, identifies aging-related differences.

As described previously, because we sought to determine lactate kinetics (49), we used an OGTT as opposed to a mixed-meal tolerance test (MMTT) to promote a large rise in blood glucose concentrations (48, 115) and subsequently, to maximize the potential for lactate production and oxidation. Worth noting is that the glucose and incretin hormone responses vary depending on the macronutrient composition of an oral challenge (115–117). Hence, the insulin and lactate responses would also vary depending on the macronutrient composition of an oral challenge. Because a MMTT more appropriately represents daily oral challenges, the omission of dietary lipid and/or protein from the oral challenge of the present study therefore questions whether lactate signaling has a physiologically relevant role in the suppression of lipolysis. As previously described, the autocrine mechanism by which lactate exerts a permissive effect with insulin (42) is predicated on lactate production by adipocytes following CHO consumption (46, 47). Rephrased, a MMTT containing dietary CHO is likely to result in adipocyte lactate production and the inhibition of lipolysis. This can be inferred from the data of Woerle et al. (118) that reported a rise in plasma lactate and fall in plasma FFA concentrations following meal ingestion (50% carbohydrates) in humans. Thus, we maintain that lactate signaling has a physiologically relevant role in the suppression of lipolysis. Still, further investigation of adipocyte lactate production and lipolysis

during a MMTT is warranted to more completely represent the postprandial period.

Although we attribute the delayed responses of glycerol Ra, FA Rox, and CHO Rox as reflections of lower circulating insulin in our older group, there are multiple factors outside the scope of our methodology that could assist the interpretation of our data. Among these include, but are not limited to: gastric emptying (119), intestinal CHO absorption (120), incretin secretion (121), and pancreatic β-cell sensitivity to incretin stimulation (66, 68). All factors are purportedly reduced in aging. Because they influence the insulin response, these factors might also be used to explain the delayed suppression of lipolysis. Notably, extant data on the incretin response to oral challenges are inconsistent, with some reporting comparatively higher incretin secretion in aging (66) and others reporting lower (121). Considering the role of incretin stimulation in the secretion of insulin in the postprandial period (122), we acknowledge our inability to account for arterial plasma incretin concentrations in our young and older groups as a limitation. In addition, due to limitations in sample volumes, we were only able to determine changes in [C-Peptide] at 2 of the 6 postchallenge time points. Although this data allowed us to speculate greater rates of insulin secretion in our young group, we were unable to calculate insulin secretion rates during the OGTT (66).

We (30, 56, 111, 112) and others (89, 123) have interpreted the arterial glycerol Ra as a marker of whole body lipolysis under the assumption that all glycerol released from lipolysis in adipose and skeletal muscle appears in circulation (69). However, the contribution of intravascular lipolysis to the circulating glycerol pool must also be considered. This is particularly important during the postprandial period, as lipolysis within blood vessels is purported to precede FA uptake and storage in peripheral tissue (124). From extant data that suggest muscle does not always release glycerol as does adipose during lipolysis (27, 125, 126) and the currently unknown contribution from intravascular lipolysis, we interpret our results of glycerol Ra to represent predominantly adipocyte lipolysis. Still, we recognize that our inability to determine the contribution of other sites of lipolysis to the glycerol Ra is a limitation.

As previously described, between 60 and 120 min, glycerol Ra continuously declined in our young but not in older group, and FA Rox and CHO Rox remained steady in our young but not in older group. Hence, we were unable to identify minimal rates of lipolysis in our young group, minimal FA oxidation rates in our older group, and maximal CHO oxidation rates in our older group. We were also unable to observe these variables return to baseline rates. Because we sought to collect clinically relevant data, we used a 120-min observation period as is standard during a 75-g OGTT; therefore, a 2-h end point was deemed appropriate. Leclerc et al. (9) used a 360-min observation period in which CHO and FA oxidation rates apparently returned to baseline rates at 240 min. Because the responses were delayed in our older group, future investigations might use a similar 360-min observation period or longer to determine aging-related differences in the restoration of baseline conditions.

Importantly, 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.

And finally, while not a limitation, but rather a distinction is that in this investigation, arterialized blood was sampled shortly and repeatedly after an oral glucose challenge. Thus, we measured metabolites and hormones that had passed through the lung parenchyma and blood compartments, but not muscle, adipose, or the integument as results from arm vein blood sampling. Hence, in addition to a healthy population, the site of blood sampling may be a cause of apparent differences between present and previous results following oral glucose challenges. Notably, unlike other investigations (127–129), we report no changes in plasma norepinephrine concentrations following oral glucose consumption. This discrepancy is most likely due to our sampling of arterialized blood, as venous blood reportedly yields higher plasma concentrations (130) and clearance rates (131) of norepinephrine.

Conclusions

Our results demonstrate that rates of lipolysis (as indicated from the glycerol Ra) and FA oxidation decreased whereas rates of CHO oxidation increased in response to oral glucose consumption in healthy, young and older individuals. Because these responses were delayed in our older group, we also demonstrate that postprandial lipid substrate partitioning is negatively affected in aging such that lipolysis and FA oxidation are favored over CHO oxidation and FA reesterification. Observations are consistent with the "Randle Cycle" theory of energy substrate partitioning (18). However, divergent responses between groups are attributed to higher circulating insulin, but not superior insulin sensitivity in our young group. Overall, postprandial metabolic inflexibility in "healthy" aging is characterized by delayed changes in energy substrate partitioning. Finally, because glycerol Ra was inversely correlated with [Lactate] to a similar magnitude as [Insulin], we provide support for the permissive effects of lactate signaling on the inhibition of lipolysis (42). Further investigation using FA isotope tracers is encouraged to further elucidate aging-related changes in postprandial lipid substrate partitioning.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental Figs. S1–S4: https://doi.org/10.6084/m9.figshare.26253590.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.A.B. conceived and designed research; A.D.O., R.G.L., J.A.A., C.C.C., J.J.D., M.J.H., U.M., and G.A.B. performed experiments; A.D.O., R.G.L., and G.A.B. analyzed data; A.D.O., R.G.L., J.A.A., U.M., and G.A.B. interpreted results of experiments; A.D.O. prepared figures; A.D.O. and G.A.B. drafted manuscript; A.D.O., R.G.L., J.A.A., C.C.C., J.J.D., M.J.H., and G.A.B. edited and revised manuscript; A.D.O., R.G.L., J.A.A., C.C.C., J.J.D., M.J.H., U.M., and G.A.B. approved final version of manuscript.

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