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## Authors

Arevalo, Jose A
Leija, Robert G
Osmond, Adam D
et al.

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RESEARCH ARTICLE

*Clinical Metabolism*

# Delayed and diminished postprandial lactate shuttling in healthy older men and women

**Jose A. Arevalo,[1] Robert G. Leija,[1] Adam D. Osmond,[1] Casey C. Curl,[1] Justin J. Duong,[1] Melvin J. Huie,[1] Umesh Masharani,[2] and George A. Brooks[1]**

[1]*Exercise Physiology Laboratory, Department of Integrative Biology, University of California, Berkeley, United States and* [2]*Department of Medicine, University of California, San Francisco, United States*

## Abstract

Lactate, a product of glycolysis, is formed under aerobic conditions. Extensive work has shown lactate flux in young and exercising humans; however, the effect of age is not known. We tested the hypothesis that postprandial lactate shuttling (PLS) would be diminished in older adults. We used [3-$^{13}$C]lactate and [6,6-$^{2}$H]glucose tracers, an oral glucose tolerance test (OGTT), and arterialized blood sampling to determine postprandial lactate rates of appearance (Ra), disappearance (Rd), and oxidation (Rox) in 15 young (28.1 ± 1.4 yr) and 13 older (70.6 ± 2.4 yr) healthy men and women. In young participants, fasting blood [lactate] (≈0.5 mM) rose after the glucose challenge, peaked at 15 min, dipped to a nadir at 30 min, and rose again peaking at 60 min (≈1.0 mM). Initial responses in lactate Ra of older participants were delayed and diminished until 90 min rising by 0.83 mg·kg$^{-1}$·min$^{-1}$. Lactate Rox was higher throughout the entire trial in young participants by a difference of ∼0.5 mg·kg$^{-1}$·min$^{-1}$. Initial peaks in lactate Ra and concentration in all volunteers demonstrated the presence of an enteric PLS following an OGTT. Notably, in the systemic, but not enteric, PLS phase, lactate Ra correlated highly with glucose Rd ($r^2$ = 0.92). Correspondence of second peaks in lactate Ra and concentration and glucose Rd shows dependence of lactate Ra on glucose Rd. Although results show both enteric and systemic PLS phases in young and older study cohorts, metabolic responses were delayed and diminished in healthy older individuals.

**NEW & NOTEWORTHY** We used isotope tracers, an oral glucose tolerance test, and arterialized blood sampling to determine postprandial lactate flux rates in healthy young and older men and women. Lactate rates of appearance and oxidation and the lactate-pyruvate exchange were delayed and diminished in both enteric and systemic postprandial lactate shuttle phases in older participants.

*aging; OGTT; isotopic tracers; metabolism*

## INTRODUCTION

The presence of a purported postprandial lactate shuttle (PLS) (1, 2) was recently demonstrated by Leija et al. (3), whose results indicated the presence of enteric and systemic postprandial lactate shuttle phases. Elements of the PLS were foreshadowed by results of studies showing hepatic portal vein lactate accumulation in rats given a glucose load by gavage (4). Results showing the presence of a systemic PLS phase included observations of postprandial lactate production in rat muscles following an oral glucose challenge (5, 6). As well, a variety of other studies have described the presence of a "glucose paradox," alternatively termed the "indirect pathway of hepatic glycogen synthesis" (7).

Isotopic tracer technology has made it possible to better understand metabolic flux in vivo (8). In particular, the use of $^{13}$C magnetic resonance spectroscopy (MRS) revealed that liver glycogen was detected 2 min after human subjects consumed [1-$^{13}$C]glucose (9). In the report by Stender et al., it is unclear whether venous or arterial blood was sampled. However, the incorporation of $^{13}$C-label into blood lactate was significant (Fig. 4 in their work). Independently, Schlicker et al. (10) administered uniformly labeled glucose in the form of a mixed meal or an oral glucose tolerance test (OGTT) and observed rapid systemic lactate appearance. Again, it is unclear whether venous or arterial blood was sampled, but major labeling of blood lactate occurred. The authors proposed that lactate served as a metabolic buffer normalizing blood [glucose] after an oral glucose challenge (10).

Much of what is known about the effects of aging on postprandial metabolism is derived from glucose tracer

Correspondence: George A. Brooks (gbrooks@berkeley.edu).
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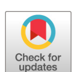


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measurements (11, 12). In contrast, although investigators have observed lactatemia in the aged population, little is known about age effects on lactate kinetics (13–17). Hence, we hypothesized that the PLS would be diminished in older adults. Therefore, utilizing [3-$^{13}$C]lactate,$^{13}$C-bicarbonate, and [6,6-$^{2}$H]glucose (D2-glucose) tracers, along with an OGTT and arterialized blood sampling, we sought to better understand the effect of age on lactate kinetics in healthy young and older humans.

## METHODS

### Experimental Design

The study was approved by the University of California, Berkeley Committee for the Protection of Human Subjects (CPHS 2018-08-11312), and abides by the standards set by the Declaration of Helsinki. Young individuals between the ages of 21 and 35 yr and older individuals aged 60–80 yr were recruited for this study. Verbal and written information detailing the study was provided to the participant, and informed written consent was obtained. Participants were screened for metabolic and cardiovascular diseases by means of a health history questionnaire, height and weight, physical activity frequency and 3-day food records, basic metabolic panel, electrocardiogram, pulmonary function assessment, assessments of body composition, and physical examination. A licensed physician cleared the participants to continue with the study. Once cleared to participate, physical fitness of volunteers was assessed by determinations of ventilatory threshold (VT) and peak oxygen consumption ($\dot{V}O_{2peak}$) using a continual, progressive leg cycle ergometer test and an open-circuit automated indirect calorimeter (Parvo Medics TrueOne 2400 Metabolic System, Salt Lake City, UT) that was calibrated using room air and a certified calibration gas (16% $O_2$ and 4% $CO_2$). The participants pedaled at a self-selected cadence until voluntary cessation, or in some cases with older volunteers, the ventilatory threshold was reached [respiratory exchange ratio (RER = R = $\dot{V}CO_2/\dot{V}O_2$) of 0.97–1.0]. In those cases, $\dot{V}O_{2peak}$ was predicted from equations in American College of Sports Medicine (ACSM) Guidelines (18). For participants who reached $\dot{V}O_{2peak}$ ACSM equations based on VT showed good agreement (2).

Participants were healthy and physically active but not athletes in training (19). Screenings were done at least 1 wk before an OGTT with isotope tracers aboard.

### Tracers

We administered primed-continuous infusions (CI) of glucose and lactate with a [$^{13}$C]bicarbonate bolus (20). Specifically: [6,6-$^{2}$H]glucose (D2-glucose, M + 2 signal, labels lost in glycolysis) was infused to determine total glucose turnover. The blood glucose pool was primed with a 250 mg D2-glucose bolus and continuously infused at a rate of 2 mg/min. [3-$^{13}$C]lactate (label lost in the TCA or recycled to glucose in the liver and kidneys) was infused to estimate gluconeogenesis (via Cori cycle, i.e., $^{13}$C-glucose from lactate yields an M + 1 signal in the glucose isotopomer from mass spectrometry). For lactate, the priming bolus was 57.5 mg of [3-$^{13}$C]lactate and the continuous infusion rate was 2.5 mg/min. As well, 136 mg of $^{13}$C-bicarbonate ($HCO_3^-$) was infused to prime the bicarbonate pool. $^{13}$C-tracers were given as $Na^+$-salts. Isotope tracer cocktails, including boluses and continuous infusates, were prepared by Mariner Advanced Pharmacy and Compounding Company, San Mateo, CA (2).

### Procedures

Details of experimental procedures have been previously reported (2, 3) but are abstracted here for the convenience of readers. The day prior to the OGTT, participants were asked to record and maintain their standard diet, refrain from strenuous physical activity, and fast 12 h prior to reporting to the laboratory. Women were scheduled during their mid-follicular menstrual cycle phase. Participants arrived at 0500 and rested for 90 min prior to the OGTT. During this time, a catheter for arterialized blood sampling was inserted into a warmed hand vein. As well, for tracer infusions, a second catheter was inserted in a contralateral arm vein. No adverse effects of the catheterization were reported. Background samples for $\dot{V}O_2$, $\dot{V}CO_2$, RER (= R = $\dot{V}CO_2/\dot{V}O_2$), expired $^{13}CO_2$, and arterialized blood were taken for determinations of endogenous isotopic enrichments as well as insulin and counter-regulatory hormone levels (insulin, glucagon, epi- and norepinephrine). Subsequently, priming boluses of D2-glucose, [3-$^{13}$C]lactate, and $H^{13}CO_3^-$ were administered, and continuous infusions of D2-glucose and [3-$^{13}$C]lactate commenced. A 90-min isotope equilibration period was allowed with simultaneous blood and breath samples taken at 75 and 90 min of CI prior to the OGTT (3).

### Oral Glucose Tolerance Testing

At 0700, participants drank (within 2 min) a 10 oz. (296 mL) solution containing 75 g of D-glucose (Azer Scientific, Cat.# 10-0-75). Once the drink was consumed, a 2-h timer was initiated and arterialized blood and expired air samples were taken at 5, 15, 30, 60, 90, and 120 min post consumption.

### Blood Lactate Concentrations

To guide experimentation, arterialized blood [lactate] ([lactate]$_a$) was measured by Lactate Plus meter (Nova Biomedical, Waltham, MA, USA) at every time point prior to (75 and 90 min) and after OGTT (5, 15, 30, 60, 90, and 120 min). Subsequently, blood [lactate] was determined enzymatically and by mass spectrometry.

### Blood Pyruvate Concentrations

Arterialized [pyruvate] ([pyruvate]$_a$) was analyzed as previously (3, 21).

Briefly, 300 μL of perchloric acid extracts were spiked with an internal standard of alpha-ketovalerate (α-ketovalerate) and mixed with a 4 N HCL + 4% ortho-phenylenediamine solution (1:1). The solution was heated for 60 min at 90°C, allowed to cool to room temperature and subsequently extracted using 2.4 mL of methylene chloride. The aqueous layer was removed and the remaining solution was dried under a gentle stream of $N_2$. Next, 75 μL of pyridine and 75 μL of *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA + 1%TMCS) were mixed and directly added to the dried residue.


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### Determination of Blood Isotopic Enrichments

Isotopic enrichments (IE) of blood metabolites for calculation of lactate flux rates were analyzed as previously (3, 22). Briefly, blood samples were immediately mixed and vortexed in 7% perchloric acid and centrifuged at 3000 *g* for 10 min at 4°C. The clear supernatants were collected, neutralized with 2 N KOH, and transferred to ion exchange columns previously washed with double distilled and deionized water (dd $H_2O$). Samples passed through a cation resin (Analytical Grade 50 W-8X, 50–100 mesh $H^+$ resin, Bio-Rad Laboratories, Hercules, CA), washed with dd $H_2O$, and then flowed through an anion resin (Analytical Grade 1-8X, 100–200 mesh Formate resin, Bio-Rad Laboratories, Hercules, CA), and eluted by adding 2 N formic acid. Samples were transferred to 2-mL gas chromatography vials and lyophilized.

For lactate aliquots, samples were resuspended in 200 μL of 2,2-dimethoxypropane and transferred to a vial to which 20 μL of 10% HCl in methanol was added. After 60 min at room temperature (RT), 50 μL of *N*-propylamine was added, heated for 30 min at 100°C, dried under a stream of $N_2$ gas, resuspended in 200 μL of ethyl acetate, transferred to a GC-MS vial, dried again, resuspended in 20 μL of heptafluorobutyric anhydride, left incubating at RT for 5 min to react, and dried once more. The derivatized lactate was resuspended in 50 μL of ethyl acetate, transferred to a GC-MS vial insert, and capped.

Pyruvate IE was determined by GC-MS (GC model 6890 series and MS model 5973N, Agilent Technologies) on a DB-1701 column 30 m × 0.25 μm × 0.25 m. Methane was used for chemical ionization with selected ion monitoring of mass-to-charge ratio ($m/z$) 233 (nonlabeled pyruvate), 234 (M + 1 isotopomer, [3-$^{13}$C]pyruvate), and 261 (α-ketovalerate).

Glucose IE determination is reported elsewhere (2).

Expired air samples were stored at room temperature until analyzed via isotope ratio mass spectrometry by The University of California, Davis Stable Isotope Facility (Davis, CA), and reported elsewhere (2).

### Calculations

Lactate flux rates [i.e., rate of appearance (Ra, mg·kg$^{-1}$·min$^{-1}$), rate of disposal (Rd, mg·kg$^{-1}$·min$^{-1}$), and metabolic clearance rate (MCR, mL·kg$^{-1}$·min$^{-1}$)] were calculated from the equations of Steele and modified for use with stable isotopes (23):

$$\mathrm{Ra} = \frac{F - V\left(\frac{C_1+C_2}{2}\right)\left(\frac{\mathrm{IE}_2-\mathrm{IE}_1}{t_2-t_1}\right)}{\left(\frac{\mathrm{IE}_1+\mathrm{IE}_2}{2}\right)} \tag{1}$$

$$\mathrm{Rd} = \mathrm{Ra} - V\left(\frac{C_2 - C_1}{t_2 - t_1}\right) \tag{2}$$

$$\mathrm{MCR} = \frac{\mathrm{Rd}}{\frac{(C_1+C_2)}{2}} \tag{3}$$

where $F$ represents [3-$^{13}$C]lactate infusion rate (mg·kg$^{-1}$·min$^{-1}$); $V$ is the volume of distribution (180 mL·kg$^{-1}$); $C_1$ and $C_2$ are concentrations (mg·L$^{-1}$) at sampling times $t_1$ and $t_2$, respectively; $\mathrm{IE}_1$ and $\mathrm{IE}_2$ are the excess isotopic enrichments of lactate at these sampling times.

Glucose kinetics, gluconeogenesis, and glycogenolysis calculations were reported elsewhere (2).

Total, direct, and indirect lactate rates of oxidation (Rox, mg·kg$^{-1}$·min$^{-1}$) were calculated as previously reported (24):

$$\%\mathrm{Lactate\ Rd\ Oxidized} = \frac{(\mathrm{IECO_2} \times \dot{V}\mathrm{CO_2} \times 90.08)}{F \times k \times 22.4} \times 100 \tag{4}$$

$$\mathrm{Total\ Lactate\ Rox} = \frac{(\mathrm{Lactate\ Rd} \times \%\mathrm{Lactate\ Rd\ Oxidized})}{100} \tag{5}$$

where $\mathrm{IECO_2}$ is the excess IE of expired $^{13}CO_2$; $\dot{V}CO_2$ (L/min); 90.08 is the molecular weight of [3-$^{13}$C]lactate, $F$ is the [3-$^{13}$C]lactate infusion rate (mg·kg$^{-1}$·min$^{-1}$); $k$ is the correction factor for the retention of $CO_2$ in body pools, as determined previously (25) to be 0.83 at rest; and 22.4 is the molar volume of $CO_2$:

$$\mathrm{Indirect\ Lactate\ Rox} = \mathrm{Rgng} \times c \tag{6}$$

$$\mathrm{Direct\ Lactate\ Rox} = \mathit{Total\ Lactate\ Rox} - \mathit{Indirect\ Lactate\ Rox} \tag{7}$$

where $c$ is the relative glucose oxidation as determined previously to be 0.25 at rest (26, 27).

### Statistical Analysis

Sampling time points 75 and 90 min pre-OGTT were averaged to represent time point "0," the time point immediately prior to the glucose challenge. Statistical analyses were performed using GraphPad Prism 10.0.3 software (GraphPad Software Inc., Boston, MA, USA, www.graphpad.com). Descriptive statistics are expressed as means ± SE. Comparisons between characteristics of 15 young and 13 older healthy men and women were done by the paired-samples $t$ test. Comparisons of mean differences in lactate kinetics between age groups, pre- and post-OGTT, were done by ANOVA with repeated measures. Statistical significance was set at $\alpha = 0.05$, and values are represented as means ± SE.

## RESULTS

### Participant Characteristics

Participant characteristics of 15 young (28.1 ± 1.4 yr) and 13 older (70.6 ± 2.4 yr) healthy men and women were previously presented (2). Specifically, body mass index and body fat percentage were significantly lower in young compared with older participants ($P < 0.01$). $\dot{V}O_{2peak}$, peak power, ventilatory threshold 1, and daily caloric (energy) intake were higher in young compared with older participants ($P \leq 0.05$).

### Lactate Concentrations following an OGTT

Fasting arterial lactate concentration ([lactate]$_a$) was not different between young and older participants (young: 0.595 ± 0.05 vs. older: 0.651 ± 0.02 mM; Fig. 1*A*). Arterial


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[lactate] in young participants was significantly elevated above baseline 5 min after the glucose challenge, peaked at 15 min ($P < 0.01$), declined at 30 min, and rose again remaining elevated. The blood lactate response was dampened in older participants not rising significantly until 15 min post glucose challenge, dipping slightly at 30 min, then continuing to rise throughout the trial as in the young participants; $P < 0.05$ (Fig. 1*A*).

### Lactate Ra following an OGTT

As with arterial $[lactate]_a$, lactate flux rates in young subjects followed a similar pattern of an initial rise, dip, and secondary rise. Responses in older individuals were dampened, but relative to changes in $[lactate]_a$, changes in lactate kinetics relatively were greater than in $[lactate]_a$.

Twelve-hour fasting values for lactate Ra was similar to those previously reported (young: 2.46 ± 0.12 vs. older: 2.27 ± 0.17 $mg \cdot kg^{-1} \cdot min^{-1}$; Fig. 1*B*) (20). In response to the glucose challenge, in young individuals, lactate Ra rose significantly at 5 min, peaked at 15 min, dipped slightly at 30 min, then rose and remained elevated; $P < 0.05$ (Fig. 1*B*). However, there was a delay in the rise of Ra in older individuals until 90 min post challenge; $P < 0.05$. Due to this delay, Ra was significantly higher in young compared with the older participants at 15, 30, and 60 min post challenge; $P < 0.05$ (Fig. 1*B*).

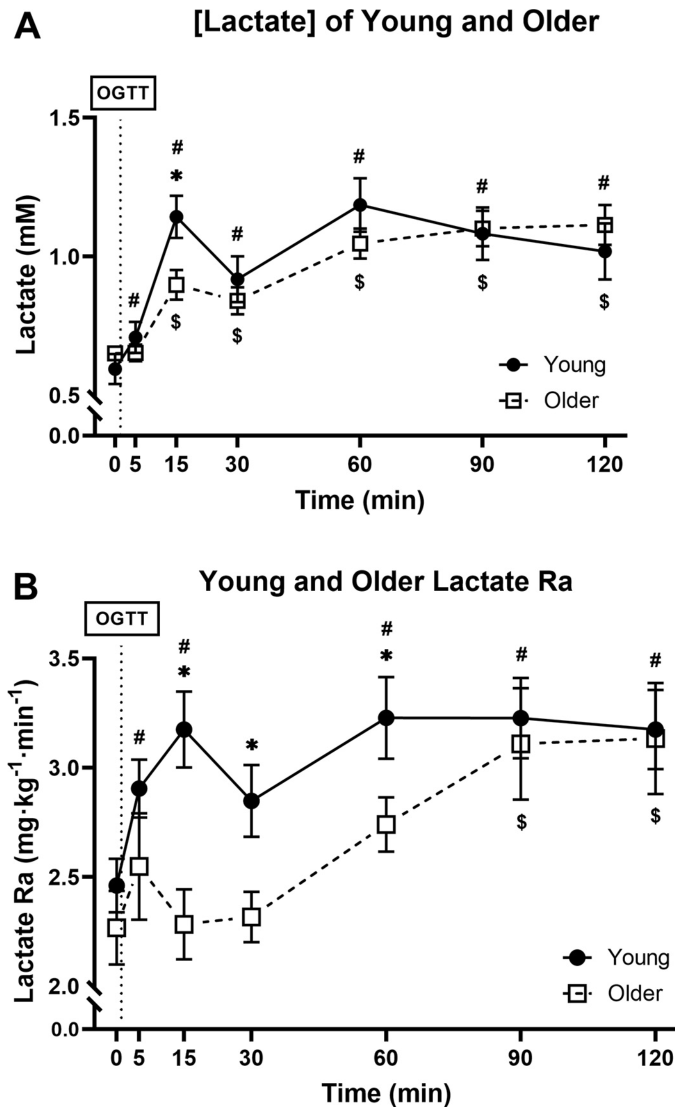


**Figure 1.** Arterial lactate concentrations (*A*) and rate of appearance (Ra, *B*) of [3-$^{13}$C]lactate before and after an OGTT in young and older healthy men and women. After an oral glucose challenge, blood [lactate] and Ra rise rapidly and before glucose. Enteric lactate production is indicated (3). Later on, in the systemic PLS phase, lactate production depends on glucose disposal (Fig. 5). Lactate responses are delayed and diminished in aging. OGTT, oral glucose tolerance test; PLS, postprandial lactate shuttle. *Significantly different between age groups; $P < 0.05$. #Significantly higher than pre-OGTT in young; $P < 0.05$. $Significantly higher than pre-OGTT in older; $P < 0.05$.

### Lactate Rd following an OGTT

In young individuals, lactate Rd was significantly higher at 15 min and 60 min post challenge compared with older individuals; $P < 0.05$ (Fig. 2*A*). In young individuals, there was a significant jump at 5 min post challenge; $P < 0.05$. Similar to Ra in the older individuals, we observed a delay in the rise of Rd until 90 min post challenge and remained elevated; $P < 0.01$ (Fig. 2*A*).

### Lactate MCR following an OGTT

No differences in lactate MCR were revealed between age groups (Fig. 2*B*). No changes from pre-OGTT were also observed.

### Lactate Rox following an OGTT

Lactate Rox was significantly higher in young compared with older individuals throughout the entire study ($P < 0.05$; Fig. 3*A* for time point 120 min, $P = 0.0551$), mostly coming from direct lactate Rox (Fig. 3*B*; for time point 120 min, $P = 0.0504$). Rox in both young and older participants rose significantly 5 min post challenge and remained steady until after 60 min post challenge.

### Pyruvate Concentrations following an OGTT

Fasting arterial ($[pyruvate]_a$) was not different between young and older participants (young: 66.70 ± 23.40 vs. older: 72.84 ± 15.71 μM; Fig. 4*A*). Arterial [pyruvate] was significantly different at 5 min after the glucose challenge between age groups ($P < 0.05$). The $[pyruvate]_a$ of older participants remained significantly higher throughout the trial ($P < 0.01$), then peaked at 60 min and remained elevated ($P < 0.0001$). The blood [pyruvate] response was dampened in young participants slightly dipping at 5 min post glucose challenge, then a steady rise until its peak at 60 min ($P < 0.01$), and dropping to almost to baseline levels at 120 min (Fig. 4*A*).

### Lactate and Pyruvate Isotopic Enrichments

Lactate and pyruvate isotopic enrichment (IE) was not significantly different between age groups and did not change post glucose challenge (Fig. 4*B*).

### Lactate-to-Pyruvate Concentration Ratios

The fasting lactate-to-pyruvate ratio (L/P) was not different between young and older participants (young: 10.10 ± 0.92 vs. older: 10.23 ± 2.20; Fig. 4*C*). Values for the L/P were


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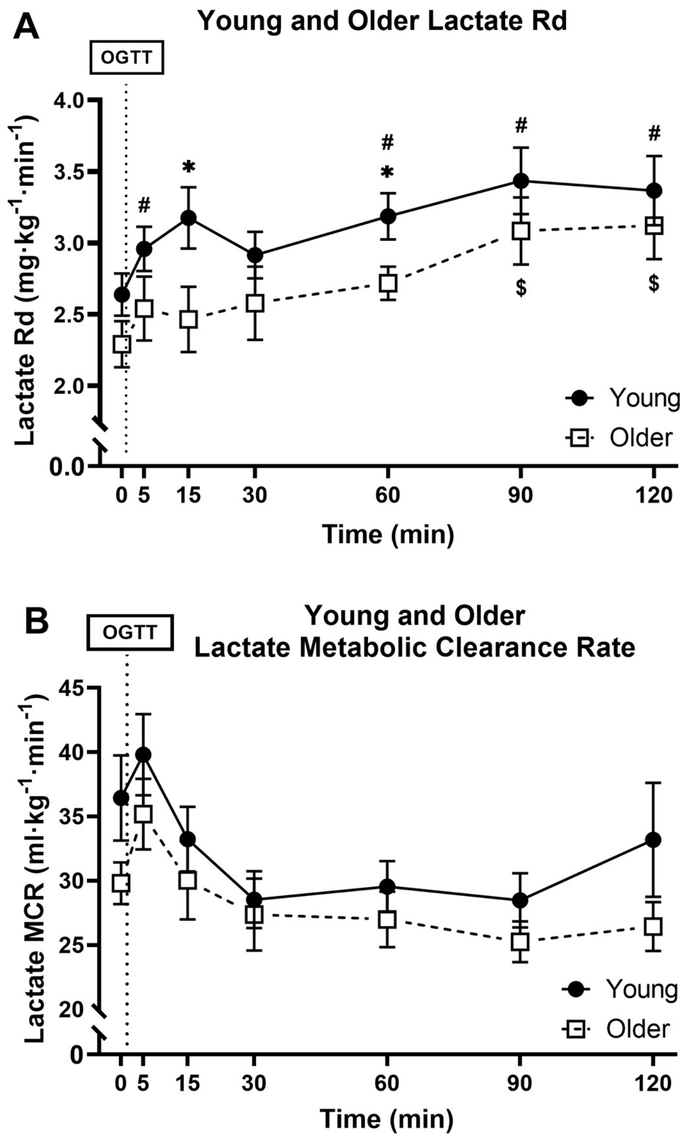


**Figure 2.** Rate of disappearance (Rd; *A*) and metabolic clearance rate (MCR; *B*) of [3-$^{13}$C]lactate before and after an OGTT in young and older healthy men and women. No differences in MCR were observed between age groups. OGTT, oral glucose tolerance test. *Significantly different between age groups; $P < 0.05$. #Significantly higher than pre-OGTT in young; $P < 0.05$. $Significantly higher than pre-OGTT in older; $P < 0.01$.

significantly higher in the young compared with older participants 5 min post glucose consumption and remained higher throughout the trial; $P \leq 0.001$ (Fig. 4*C*). In young participants, L/P rose 5 min after glucose consumption and peaked at 15 min dropping back to 5-min levels for the rest of the trial; $P < 0.05$ (Fig. 4*C*). L/P in the older participants did not change significantly from baseline as the result of an oral glucose challenge.

### Systemic Phase Dependence of Lactate Ra on Glucose Rd

For all study cohorts, lactate Ra and Glucose Rd were highly correlated over time; $R^2 = 0.85$, $P = 0.003$ (Fig. 5*A*). The relationship was strongest when considering only the systemic phase (i.e., after 30 min post glucose challenge); $R^2 = 0.92$, $P = 0.04$ (Fig. 5*B*).

## DISCUSSION

We undertook to test for the presence of a postprandial lactate shuttle and determine if aspects of the PLS changed with aging. For that purpose, we monitored lactate and glucose kinetics in young and older healthy men and women after an oral glucose challenge. We report *1*) the presence of postprandial lactate shuttling in all study participants and that *2*) young individuals showed an immediate rise in the rate of appearance (Ra) and disappearance (Rd) of lactate, *3*) lactate disposal via oxidation (Rox) was higher in young individuals, and 4) responses were delayed and diminished in older subjects, thereby revealing effects of age on the PLS. Results are discussed sequentially.

Postprandial lactate shuttling with enteric and systemic PLS phases was observed in all study participants. Changes in $[\text{lactate}]_a$ following the glucose challenge were due to increased Ra that preceded changes in arterial [glucose] but initial responses were delayed and diminished in older participants. Overall blood [lactate] and lactate Ra responses were similar in older compared with young participants, and total lactate Rox was lower in older participants. Noteworthy also was that during the systemic PLS phase, lactate Ra correlated highly with glucose Rd ($R^2 = 0.92$). Although results show both enteric and systemic PLS phases in all study cohorts, metabolic responses were delayed and diminished in healthy older individuals. Aspects of these results are discussed next.

### Participant Characteristics

We observed the expected outcomes due to aging, such as the decline in $\dot{V}O_{2peak}$, peak power output, and VT (13–15, 28–32). It is understood that training can improve $\dot{V}O_{2peak}$ in older individuals (16). However, we did not see an age-related decline in lean body mass (LBM) as determined by skinfold measurements. Our participants were healthy and physically active (as defined by >3 bouts of exercise per week), and thus, older participants appeared to maintain their LBM through physical activity (31, 32).

### Effect of Age on Fasting and Post-OGTT Blood Lactate Concentrations

In young individuals, blood [lactate] rose and peaked at 15 min after the glucose challenge (Fig. 1*A*). These results in young have been reported previously (3), and the rise in [lactate] after a glucose challenge is consistent with the literature (33). We did not observe an effect of age on $[\text{lactate}]_a$ in either the fasted state or post glucose challenge (Fig. 1*A*). Hence, those results are consistent with previous determinations of [lactate] in individuals fasting or after a hyperinsulinemic-euglycemic clamp (14, 17).

### Effect of Age on Fasting and Post Glucose Challenge on Blood Pyruvate Concentrations

Fasting $[\text{pyruvate}]_a$ was similar in young and older participants. In young sedentary men, $[\text{pyruvate}]_a$ did not change significantly at the onset of exercise (21). In our young participants, we did not see a change until after 30 min post glucose consumption where at 60 min, $[\text{pyruvate}]_a$ rose by 25 μM. Fasting $[\text{pyruvate}]_a$ was similar in our older participants to


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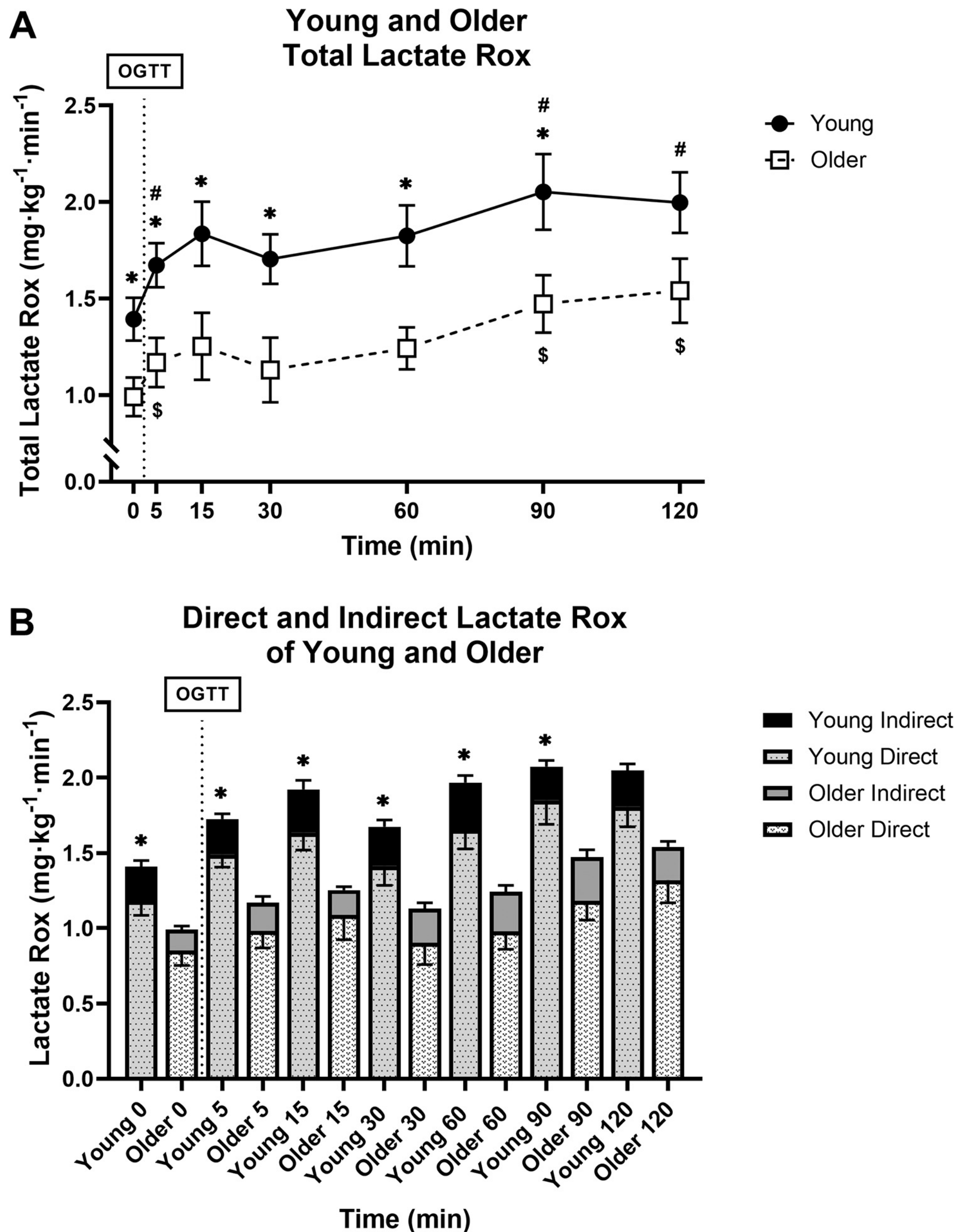


**Figure 3.** Total rate of oxidation (Rox, *A*) and rate of direct and indirect (from gluconeogenesis and glucose oxidation) oxidation (Rox, *B*) of [3-$^{13}$C]lactate before and after an OGTT in young and older healthy men and women. Lactate disposal via oxidation is delayed and diminished in aging. OGTT, oral glucose tolerance test. *Significantly different between age groups; $P < 0.05$. #Significantly higher than pre-OGTT in young; $P < 0.05$. $Significantly higher than pre-OGTT in older; $P < 0.05$.

lean older participants (34), and the peak at 60 min is a novel finding. When compared with [lactate]$_a$, [pyruvate]$_a$ remained elevated after the peak at 60 min, whereas [lactate]$_a$ continued to rise. Thus, after glucose consumption, there is an age effect on the net release of pyruvate prior to lactate.

### Effect of Age on Lactate Kinetics

In young individuals, lactate Ra rose significantly by 0.44 mg·kg$^{-1}$·min$^{-1}$ following the consumption of glucose, peaking at 15 min, as observed previously (3). However, lactate Ra in older individuals began to rise at 60 min post glucose challenge and peaked at 90 min by 0.83 mg·kg$^{-1}$·min$^{-1}$ (Fig. 1*B*). The initial spike in Ra in young individuals supports the hypothesis of an enteral phase of the postprandial lactate shuttle (PLS). In data reported elsewhere (3), following an OGTT, [lactate]$_a$ and Ra rise before corresponding changes in [glucose]$_a$ or Ra. Similarly, following an OGTT, the rise in lactate Rd in older individuals was delayed until 120 min post challenge. No age effect was seen in the lactate MCR post glucose challenge. Lactate concentrations change with exercise and is not dependent on insulin, thus since we did not measure changes to lactate during exercise our results on MCR are expected with resting metabolic flux measurements (20, 22, 35, 36). Lactate Rox was higher throughout the entire trial in young participants, revealing an effect of age on whole body lactate oxidation (Fig. 3, *A* and *B*). Previously, Emhoff et al. (24) showed that trained young men had a higher resting direct lactate Rox compared with untrained healthy young men. We are the first to reveal how age affects lactate Rox in human subjects at rest and subsequent to a carbohydrate load.

Of note in results of the present investigation are data, presented in Fig. 5, *A* and *B,* it appears that during the


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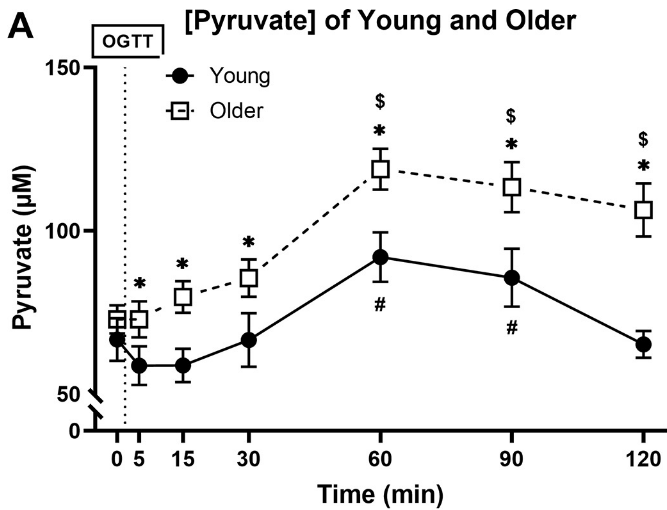
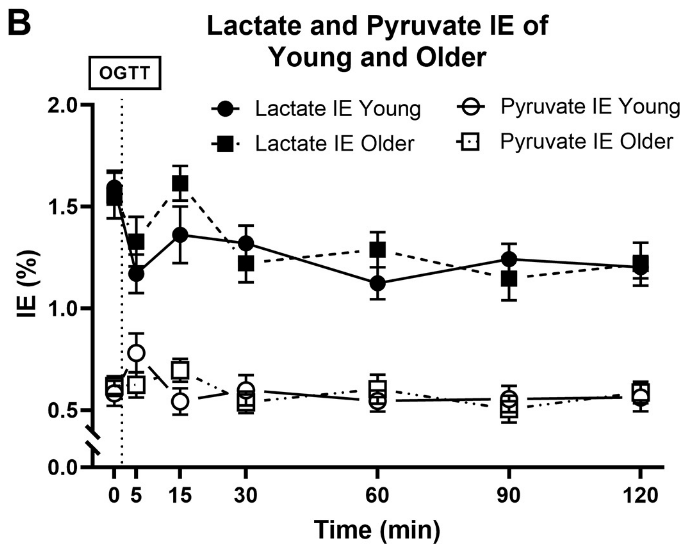
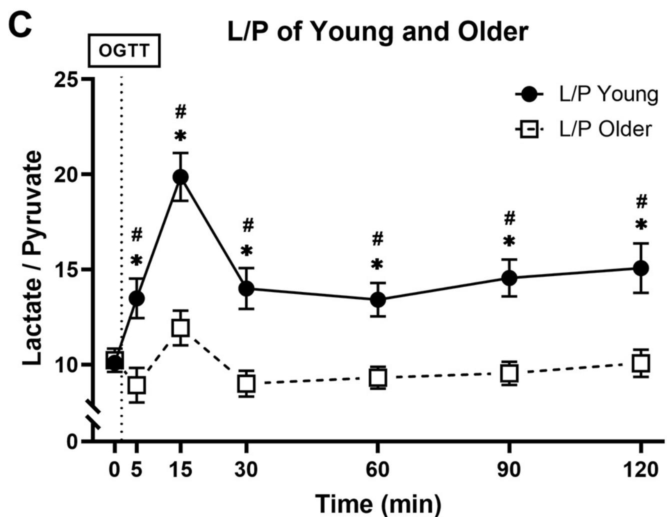
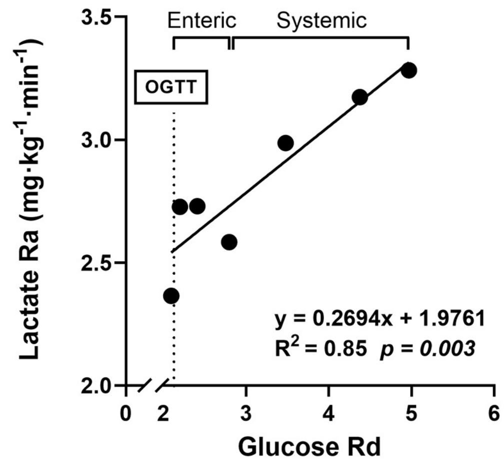
systemic PLS phase, lactate Ra depends on glucose Rd. As described in the introduction, a variety of previous studies describing the presence of a "glucose paradox," alternatively the "indirect pathway of hepatic glycogen synthesis" (7) can reasonably be interpreted to predict the presence of a systemic PLS phase (3).



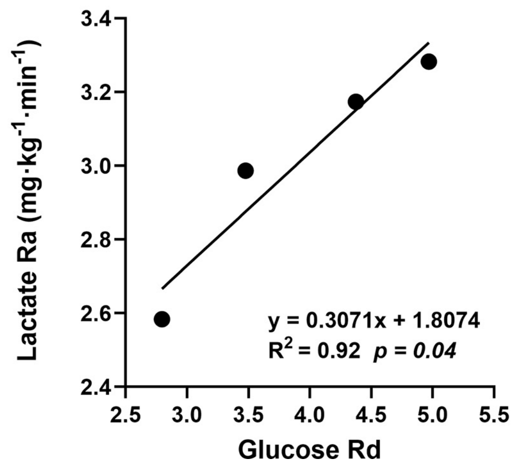




**Figure 5.** Correlation between glucose Rd and lactate Ra in young and older healthy men and women throughout the trial ($P = 0.003$ *A*) and the systemic phase ($P = 0.04$, *B*). In the systemic PLS phase lactate Ra depends on glucose Rd. PLS, postprandial lactate shuttle; Ra, rate of appearance, Rd, rate of disappearance.

**Figure 4.** Blood pyruvate concentrations (*A*), lactate and pyruvate isotopic enrichments (IE, *B*), and lactate-to-pyruvate ratio (L/P, *C*) before and after an OGTT in young and older healthy men and women. Following an oral glucose change, glycolytic carbon flow is primarily through lactate, not pyruvate. OGTT, oral glucose tolerance test. *Significantly different between age groups; $P < 0.05$. #Significantly higher than pre-OGTT in young; $P < 0.05$. $Significantly higher than pre-OGTT in older; $P < 0.0001$.

### Effect of Age on Lactate-to-Pyruvate Concentration Ratios

In this study we determined pyruvate as well as lactate levels and isotopic enrichments in individuals during and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after OGTT. Given the history of literature on the subject (37, 38), we could reasonably assume that readers would want to see those values, especially because historically, increments in blood [lactate] and the lactate/pyruvate (L/P) ratio have mistakenly been taken to indicate the presence of oxygen-limited metabolism (39) that was clearly not the case in resting healthy participants at sea level altitude.

The lack of an effect of age on pyruvate isotopic enrichments (IE) was not surprising, given the rapid interconversion between lactate and pyruvate in blood (21, 40, 41). Fasting L/P ratios were similar in young and older participants and were similar to those previously reported (21). In this study, arterial lactate levels were marginally higher in young compared with older individuals (Fig. 1*A*). In contrast, arterial pyruvate levels were significantly higher in older individuals (Fig. 4*A*). Consequently, the nominal L/P of 10 in all subjects before the oral glucose challenge rose twice as high in young compared with older volunteers (Fig. 4*C*). These results indicate diminished glycolysis and lactate shuttling in older compared with younger subjects. Overall, the L/P ratios for concentration and isotopic enrichments indicate that glycolytic carbon flow is to lactate.

### Effect of Age on the Postprandial Lactate Shuttle

Our results of young men and women revealed an immediate rise in lactate Ra following an oral glucose challenge is supported by the results of Schlicker et al. (10), who measured the conversion of glucose to lactate in participants (ages of 20 and 50 yr) after consuming a uniformly labeled ([U-$^{13}$C]glucose) OGTT. In our older individuals, however, a delay in lactate Ra was evident and this coincides with the steady rise in glucose Ra in the same group (2), suggesting the PLS is delayed with age progression.

In addition, Curl et al. (2) showed an age effect decreasing glucose Rd post challenge. When compared with the delay in lactate Ra (Fig. 1*B*), we see a strong positive correlation revealing an age effect on glucose conversion to lactate (Fig. 5*A*). The relationship remains strong during the later systemic PLS phase (Fig. 5*B*) and is in line with results of Woerle et al. (42), who measured glucose Rd after a meal containing 78 g of glucose. Thus, these present results lead us to conclude the systemic PLS is delayed with age progression.

### Mechanisms of Lesser PLS Activity and Lactate Oxidation in Aging

In skeletal muscle, the respiratory apparatus exists as a network, or reticulum, spread throughout the muscle fibers (43, 44). In normal circumstances, the mitochondrial reticulum undergo a controlled continuous cycle of fission (fragmentation) and fusion (connection) (45). In particular, dynamin-related protein 1 is the main regulator of fission and has a role in maintaining mitochondrial health by signaling the death of the damaged mitochondria, known as mitophagy (46). With age progression, the ability to signal mitophagy is diminished, which further exacerbates mitochondrial fragmentation (47), and the mass of the mitochondrial reticulum is reduced (48).

For all metabolites, including lactate, flux depends on concentration gradients between cells, tissues, and organs of production and disposal. Due to the relatively high proportion of muscle to whole body mass in the individuals we studied, the muscle mitochondrial reticulum was likely the major site of lactate oxidative disposal. Previously, Conley et al. (49) used MRS to estimate maximal muscle ATP flux and concluded that muscle oxidative capacity was reduced by 50% in older compared with young counterparts. In addition, Short et al. (50) measured blood glucose levels before and after consumption of a meal revealing postmeal blood glucose levels were negatively correlated with muscle mitochondrial function suggesting a link to insulin sensitivity. Thus, although it is tempting that reduced capacity for lactate clearance via oxidation was attributed to lesser muscle mitochondrial capacity in aging, it is unclear that reduced mitochondrial capacity in healthy aging was responsible for lesser lactate oxidative disposal. In this regard, not the lack of age-related difference in lactate clearance following an oral glucose challenge (Fig. 2), rather, a shift to increased lactate disposal via gluconeogenesis may also be involved (2). Therefore, this retention of carbohydrate carbon in older individuals may be considered to be a more conservative and advantageous age-related compensation.

### Effect of Age on Gastric Emptying

The gut has a major role to play, and the expression of transporters is important in glucose homeostasis after meal consumption (51). In addition, gastric emptying plays a role post consumption and is affected by age. For instance, Moore et al. (52) injected radio-labeled tracers in a meal of pork liver (solid) and mixed in orange juice (liquid) and were consumed by a group of young and older individuals. Gastric emptying of the solid meal did not differ with age; however, emptying of the liquid drink was delayed in the older participants. In contrast, the appearances of $^{13}$C-labeled lactate after consumption of $^{13}$C-glucose in meal or drink forms were similar in Schlicker et al. (10). Still, it remains that part of the delayed responses in older study participants may be attributable to slower gastric emptying in older compared with young participants (11, 53).

### Implications for the Effects of Age on the Lactate Transporter and Dehydrogenase Expression

In this investigation, tissue lactate transporter (MCT) levels were not determined, but the delay in lactate kinetics observed in our older participants may be a result of changes in MCT1 expression in enterocytes (54), muscles (55, 56), and other tissues, including brain (57). Similarly, tissue lactate dehydrogenase (LDH) levels were not determined, but the lower lactate-to-pyruvate conversion can be interpreted to be consistent with prior results showing that LDH activity is less in aging in humans and rodents (58–60). Therefore, our results revealing a decline in the enteric and systemic postprandial lactate shuttle are in line with the aging effect on MCT and LDH.

### Effects of Lean Body Mass

As noted earlier, changes in LBM in aging affect numerous physiological parameters (30, 31). However, the participants we studied maintained LBM in aging. Hence, it might be argued that we studied a rather unique aging population. The counterargument is that the age-related changes we observed were qualitative changes, probably more related to


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the mass of the muscle mitochondrial reticulum than total body mass (61). Still, in our attempts to identify an age-related LBM effect on metabolism, parameters of lactate kinetics were plotted in terms of kg LBM and statistically evaluated. No differences in data interpretation emerged.

### Clinical Relevance of Diminished PLS

The development of insulin resistance (IR) is common in aging (62), and lactatemia is often associated with IR (63). Endurance exercise training greatly enhances lactate clearance by oxidation (64) and gluconeogenesis (65). With such an understanding of lactate shuttling, the observation was to be expected that endurance training lowered fasting [lactate] in obese individuals (66).

Given the presence of lactatemia in IR states, at a higher level, two explanations need to be considered. The first is that lactatemia is the result of reduced clearance. An alternative is that enhanced lactate production is a means to bypass or shuttle around reduced glucose clearance capacity. Lactate shuttling during exercise has long been known (37, 67, 68), and now results of recent glucose feeding studies indicate the presence of a postprandial lactate shuttle (3, 10) indicating that lactate is a major vehicle for carbohydrate carbon distribution and metabolism, not just during exercise, but after CHO nutrition, and perhaps always. For the present, it may be that fasting blood [lactate] can be utilized as a marker for metabolic inflexibility (69). We did not observe an age-related rise in arterial [lactate]; on the contrary, we did observe an age-related decrease in the arterial lactate response to an OGTT. Clinical relevance of the postprandial lactate shuttle needs to be explored. A first clue may be to interrogate the rise in blood [lactate] following metformin treatment.

### Conclusions

Although no age effect was observed in blood [lactate] after an OGTT, the lactate rate of appearance was delayed and diminished in older healthy individuals. The lactate rate of oxidation was lower in older individuals both prior to and following the glucose challenge. In addition, the lactate-to-pyruvate exchange was impeded in the older participants. Therefore, our findings reveal age effects on both enteric and systemic postprandial lactate shuttle phases.

## DATA AVAILABILITY

Data will be made available upon reasonable request.

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## DISCLOSURES

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

## AUTHOR CONTRIBUTIONS

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

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