

Effects of aging on basal fat oxidation in obese humans

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Abstract

Basal fat oxidation decreases with age. In obesity, it is not known whether this age-related process occurs independently of changes in body composition and insulin sensitivity. Therefore, body composition, resting energy expenditure, basal substrate oxidation, and maximal oxygen consumption (VO_2max) were measured in 10 older (age, 60 ± 4 years; mean \pm SEM) and 10 younger (age, 35 ± 4 years) body mass index-matched, obese, normal glucose-tolerant individuals. Fasting blood samples were also collected. Older subjects had slightly elevated fat mass (32.2 ± 7.1 vs 36.5 ± 6.7 kg, $P = .16$); however, waist circumference was not different between groups (104.3 ± 10.3 vs 102.1 ± 12.6 cm, $P = .65$). Basal fat oxidation was 22% lower (1.42 ± 0.14 vs 1.17 ± 0.22 mg/kg fat-free mass per minute, $P = .03$) in older subjects. The VO_2max was also decreased in older individuals (44.6 ± 7.1 vs 38.3 ± 6.0 mL/kg fat-free mass per minute, $P = .03$); but insulin sensitivity, lipemia, and leptinemia were not different between groups ($P > .05$). Fat oxidation was most related to age ($r = -0.61$, $P = .003$) and VO_2max ($r = 0.52$, $P = .01$). These data suggest that aging per se is responsible for reduced basal fat oxidation and maximal oxidative capacity in older obese individuals, independent of changes in insulin resistance, body mass, and abdominal fat. This indicates that age, in addition to obesity, is an independent risk factor for weight gain and for the metabolic complications of elevated body fat.

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1. Introduction

Aging and obesity are both associated with increased risk of developing type 2 diabetes mellitus and cardiovascular disease [1,2]. Obesity also tends to increase with advancing age [3], which may explain the association between age and such diseases. Impaired glucose tolerance and insulin resistance, which arise in obesity, are due in part to increased appearance of free fatty acids and reduced rates of skeletal muscle fat utilization, leading to accumulation of intracellular fatty acid metabolites that interfere with insulin-signaling pathways [4]. These metabolic defects are also more prevalent in older populations [3]. In addition, basal fat oxidation (F_{ox}) is decreased in obese and insulin-resistant individuals [5] and has been shown to deteriorate with age in

cross-sectional studies of lean healthy individuals as a result of changes in body composition (increased fat mass and decreased fat-free mass [FFM]) [6–8]. These observations indicate that the impairments in substrate utilization that occur in obesity may be greater in older obese individuals; and therefore, they may be more susceptible to the metabolic abnormalities associated with increased body fat. Skeletal muscle mitochondrial function and protein synthesis is impaired in older individuals [9,10] and in obesity and insulin resistance [11,12], suggesting that, in older groups, age per se may be responsible for alterations in substrate utilization at the mitochondrial level. Understanding age-related changes in oxidative capacity may be important to formulating optimal therapeutic strategies for older populations affected by abnormalities in substrate utilization. However, examination of the direct effects of aging alone requires control of several variables (eg, physical activity, body mass, FFM, and sex). This has not been done effectively in previous studies. To our knowledge, the effects of aging per se on substrate utilization have

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not been fully examined in obese individuals. Therefore, this study fills a gap in the literature by investigating the hypothesis that basal F_{ox} is decreased in older obese individuals as a function of age alone. To examine the effects of age per se, we chose sedentary, normal glucose-tolerant (NGT), and body mass index (BMI)- and sex-matched individuals to minimize the effects of physical activity, insulin resistance, body composition, and sex on substrate utilization measures.

2. Subjects and methods

2.1. Subjects

Twenty obese (BMI >30 kg/m²), NGT (fasting plasma glucose <100 mg/dL), male and female volunteers were recruited from the general population. Volunteers were divided into 2 sex- and BMI-matched groups of 10 younger (5 male, 5 female; age, 35 ± 4 years; BMI, 32.1 ± 1.4 kg/m²; mean ± SD) and 10 older (5 male, 5 female; age, 60 ± 4 years; BMI, 31.0 ± 5.0 kg/m²) individuals. A medical screening was performed to exclude subjects with heart, kidney, liver, intestinal, and pulmonary diseases, or those taking medications for hypertension, diabetes, or other obesity-related conditions. During this screening, volunteers also underwent basal metabolic rate measures via indirect calorimetry (as described in Section 2.3) to calculate their individual caloric requirements (using the Weir [13] equation multiplied by a factor of 1.25 for sedentary lifestyle correction [14]). All volunteers were sedentary (as assessed by the Physical Activity Readiness Questionnaire and the Minnesota Leisure Time Physical Activity Questionnaire [15]) and had been weight stable for 6 months before the study. All young, female, premenopausal volunteers were studied during the early-follicular phase (days 1–5) of their menses to minimize the effects of reproductive hormones on metabolism. Dietary records were collected for 3 days before the study to monitor adequate nutrient intake, during which time subjects were requested to refrain from nonhabitual exercise and from caffeinated and alcoholic beverages. The study was approved by the Institutional Review Board, and signed informed consent was obtained from all volunteers before commencing the study.

2.2. Body composition

Anthropometric data were measured using standard techniques. Height was measured using a stadiometer to the nearest 1.0 cm without shoes. Body weight was measured to the nearest 0.1 kg with the subjects wearing their underclothing and a hospital gown. Waist circumference (WC) was measured midway between the lower rib margin and iliac crest in a horizontal position to the nearest 1.0 cm. Body density was determined by hydrostatic weighing after an overnight fast as previously described [16]. Underwater weight was determined using electronic load cells, and residual lung volume was determined by open-circuit

nitrogen washout. Percentage body fat was calculated using Siri equations as previously described [16].

2.3. Metabolic measurements

Study subjects were admitted to the inpatient ward of the General Clinical Research Center the day before testing and fed a standardized diet (55% carbohydrate, 35% fat, 10% protein), the caloric load of which was calculated from each volunteer's basal metabolic rate measured during medical screening. After a 12-hour overnight fast, subjects were awakened at 6:00 AM and taken by wheelchair to void and to be weighed, and then reclined in a semidarkened, thermo-neutral (22°C ± 1°C) environment under a clear plastic hood (Brooks Instruments, Hatfield, PA) for 30 minutes for indirect calorimetry measurements [17]. Air was pulled through the hood at a rate of 50 L/min to maintain a slight negative pressure, allowing fresh air movement through the hood at all times. Continuous open-circuit exhaled gas was analyzed using Hartmann-Braun (Frankfurt, Germany) differential paramagnetic O₂ (Magnos 4 G) and nondispersive infrared CO₂ (Uras 4) analyzers. The analyzers were calibrated before the collection with known gas concentrations. The molar ratio of O₂ consumed to CO₂ produced was used to derive a measure (respiratory quotient [RQ]) of the relative amounts of substrate that were being oxidized. Energy expenditure was calculated using equations of Weir [13], and substrate oxidation rates were calculated according to Frayn [18]. Timed urinary nitrogen excretion measurements (Roche Modular Diagnostics, Indianapolis, IN) were also made for estimates of protein oxidation (P_{ox}) rates [18].

2.4. Exercise capacity

After the metabolic measurements, each subject performed an incremental treadmill exercise test to determine their maximal oxygen consumption (VO₂max), a marker of maximal aerobic capacity. The test was performed at speeds between 2 and 4 mph, and the incline of the treadmill was increased 2.5% every 2 minutes until fatigue. Exhaled air was collected through a 2-way Hans-Rudolph (Shawnee, KS) mouthpiece into a FITGO mixing chamber (Shawnee, KS). Concentrations of O₂ and CO₂ were measured using an electrochemical O₂ analyzer (S-3A, Applied Electrochemistry, AEI Technologies, Naperville, IL) and infrared CO₂ analyzer (LB-2; Beckman, Fullerton, CA), respectively. Subjects were considered to have reached their maximum aerobic capacity when at least 2 of the following 3 criteria were achieved: (1) a plateau in VO₂, (2) a heart rate within 10 beats per minute of age-predicted maximum, and/or (3) a respiratory exchange ratio >1.0.

2.5. Blood measurements

During the inpatient stay, a fasting blood sample was drawn from an antecubital vein for the measurement of triglycerides (TG), total cholesterol (T-Chol), glucose, insulin, and leptin. Two further samples were collected for

glucose and insulin at 10-minute intervals to calculate an insulin resistance index according to Matthews et al [19].

$$\text{HOMA-IR} = \frac{\text{FPG} \times \text{FSI}}{22.5 \times 18}, \quad (1)$$

where FPG represents fasting plasma glucose in milligrams per deciliter and FSI is fasting serum insulin in microunits per milliliter.

2.6. Statistical analysis

Statistical analyses were carried out using Statview (SAS Institute, Cary, NC), and all data are expressed as mean ± SD. All data comparisons in parentheses represent values for young vs old. Between-group (young vs old, and male vs female) comparisons for all variables were analyzed using 1-way analysis of variance and Fisher least significant difference post hoc test. No sex differences were found in any of the dependent variables. Bivariate correlations were used to examine the relationships between age and other measured variables, and between F_{ox} and other variables. In addition, bivariate analyses were also applied to F_{ox} and fat mass in both young and old groups to assess fat balance. To examine the direct relationships of age on energy expenditure, F_{ox} , and maximal oxygen uptake, partial correlations were performed while controlling for FFM. Statistical significance was accepted at $P < .05$.

3. Results

3.1. Dietary control

Analysis of the 3-day diet records showed no differences in caloric intake or macronutrient composition between groups before metabolic testing. In the younger vs older groups, total caloric intake was 1862 ± 610 vs 1933 ± 169 kcal ($P = .83$), percentage of calories from carbohydrate was $47\% \pm 10\%$ vs $43\% \pm 11\%$ ($P = .45$), percentage of calories from fat was $35\% \pm 6\%$ vs $36\% \pm 9\%$ ($P = .90$), and percentage of calories from protein was $17\% \pm 6\%$ vs $19\% \pm 4\%$ ($P = .56$).

3.2. Body composition

Demographic and body composition information is presented in Table 1. The age groups displayed no differences in BMI, body mass, fat mass, FFM, or WC

Table 1
Subject characteristics

Variable		Younger	Older	P
Age	y	35 ± 4	60 ± 4	<.001 ***
Height	cm	171 ± 9	172 ± 10	.69
Weight	kg	93.9 ± 11.5	91.4 ± 9.3	.57
BMI	kg/m ²	32.1 ± 1.4	31.0 ± 5.0	.47
FM	kg	32.2 ± 7.1	36.5 ± 6.7	.16
FFM	kg	61.8 ± 10.0	53.1 ± 11.0	.07
WC	cm	104.3 ± 10.3	102.1 ± 12.6	.65

Data are mean ± SD. FM indicates fat mass.

*** $P < .001$.

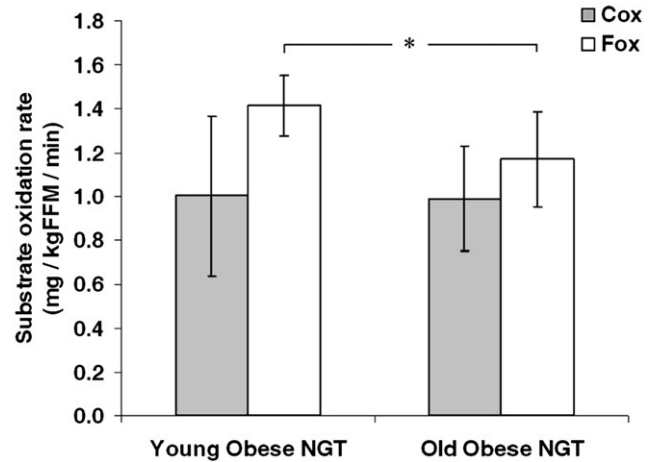


Fig. 1. Energy metabolism. Substrate oxidation rates were estimated by indirect calorimetry in 2 BMI- and sex-matched obese NGT age groups. Carbohydrate oxidation was not different between younger and older individuals; however, F_{ox} was significantly reduced in the older cohort ($*P < .05$). Error bars represent mean ± SD.

(all $P > .05$). Percentage fat mass, however, was elevated in older individuals (young vs old: $34.3\% \pm 6.4\%$ vs $40.3\% \pm 7.4\%$, $P = .04$), whereas percentage FFM was reduced in the older group ($65.7\% \pm 6.4\%$ vs $58.0\% \pm 9.0\%$, $P = .05$).

3.3. Indirect calorimetry

Fig. 1 and Table 2 illustrate data from the indirect calorimetry measurements taken at rest after an overnight fast, plus data from the subjects' maximal exercise test. No differences in resting RQ ($P = .12$), carbohydrate oxidation (C_{ox} , $P = .96$), P_{ox} ($P = .39$), or energy expenditure (resting [REE]) ($P = .15$) were noted between age groups. Basal F_{ox} , expressed per unit of FFM, was significantly decreased in the older age group ($-22.0\% \pm 7.8\%$, $P = .03$), as was VO_{2max} ($-16.0\% \pm 4.7\%$, $P = .03$) and maximal heart rate (HRmax) ($-12\% \pm 1.4\%$, $P = .0007$) recorded during exhaustive exercise.

3.4. Blood measurements

Differences in blood chemistry between age groups are shown in Table 3. Measures of FPG confirmed the subjects'

Table 2
Metabolic data

Variable		Younger	Older	P
RQ		0.77 ± 0.02	0.79 ± 0.02	.12
REE	kcal/kg FFM per min	0.021 ± 0.002	0.019 ± 0.002	.15
C_{ox}	mg/kg FFM per min	1.00 ± 0.36	0.99 ± 0.24	.96
F_{ox}	mg/kg FFM per min	1.42 ± 0.14	1.17 ± 0.22	.03 *
P_{ox}	mg/kg FFM per min	0.76 ± 0.22	0.66 ± 0.29	.39
VO_{2max}	mg/kg FFM per min	44.6 ± 7.1	38.3 ± 6.0	.03 *
HRmax	beats per min	182 ± 10	163 ± 14	<.001 ***

Data are mean ± SD. Younger, n = 10; older, n = 10.

* $P < .05$.

*** $P < .001$.

Table 3
Blood chemistry

Variable		Younger	Older	<i>P</i>
FPG	mg/dL	94.0 ± 17.8	95.5 ± 5.8	.70
FSI	μU/mL	12.8 ± 5.9	15.2 ± 4.3	.21
HOMA-IR		3.03 ± 1.88	3.61 ± 1.10	.33
TG	mg/dL	139.2 ± 56.4	145.2 ± 69.5	.49
T-Chol	mg/dL	200.8 ± 46.2	183.6 ± 41.6	.76
Leptin	mg/dL	23.0 ± 15.2	24.5 ± 15.5	.98

Data are mean ± SD. FPG: divide milligrams per deciliter by 18 to convert to millimoles per liter. HOMA-IR is insulin resistance according to Matthews et al [19].

normal fasting glucose tolerance (<100 mg/dL). No differences in insulin resistance, TG, T-Chol, or leptin were seen between younger and older individuals (all comparisons $P > .05$).

3.5. Correlation analyses

To investigate the association between age and other measured variables, bivariate correlations were performed. Table 4 shows that basal F_{ox} ($P < .01$), VO_2max ($P < .01$), and HRmax ($P < .001$) each showed a significant inverse association with age. The significant relationships between age and basal F_{ox} , and age and VO_2max persisted when the effect of FFM was controlled for (partial correlation analysis): age vs basal F_{ox} , $r = -0.58$, $P = .007$; age vs VO_2max , $r = -0.44$, $P = .04$. Table 5 demonstrates that, of the variables previously documented to affect fat utilization, age and VO_2max were the best predictors of basal F_{ox} in our obese cohorts ($r = -0.61$, $P = .003$ and $r = 0.52$, $P = .01$, respectively). A bivariate analysis was also performed to assess the relationship of fat mass and basal F_{ox} . No association between fat mass and basal F_{ox} was

Table 4
Bivariate correlations between age and other variables

Age vs	<i>r</i>	<i>P</i>
Weight	-0.11	.61
BMI	-0.06	.80
FFM	-0.41	.06
FM	0.32	.14
WC	-0.07	.75
REE	-0.40	.07
RQ	0.38	.09
C_{ox}	-0.05	.92
F_{ox}	-0.61	.003 **
P_{ox}	-0.27	.260
VO_2max	-0.57	.004 **
HRmax	-0.73	.0001 ***
FPG	0.01	.98
FSI	-0.02	.47
HOMA-IR	-0.05	.38
TG	-0.15	.50
T-Chol	-0.18	.42
Leptin	-0.01	.90

Units of measurement are the same as for previous tables.

** $P < .01$.

*** $P < .001$.

Table 5
Bivariate correlations between F_{ox} and other variables

F_{ox} vs	<i>r</i>	<i>P</i>
Age	-0.61	.003 **
Weight	-0.31	.17
BMI	-0.37	.10
FFM	-0.14	.57
FM	-0.22	.34
WC	-0.41	.07
REE	.65	.001 **
VO_2max	0.52	.01 *
FPG	-0.25	.28
FSI	-0.04	.89
HOMA-IR	-0.06	.84
TG	-0.04	.88
T-Chol	0.07	.75
Leptin	0.19	.42

Units of measurement are the same as for previous tables.

* $P < .05$.

** $P < .01$.

found in either group: younger, $r = 0.40$, $P = .20$; older, $r = -0.29$, $P = .45$.

4. Discussion

This study provides preliminary evidence that basal F_{ox} is reduced in older obese NGT individuals as a function of age per se. In addition, it was shown that aerobic fitness, as indicated by reduced maximal oxygen uptake, also declines with age. These metabolic alterations were independent of absolute differences in body composition, insulin sensitivity, fasting lipemia, and leptinemia, physiological components that are related to substrate oxidation. It was also demonstrated that, in obese NGT humans, basal F_{ox} is not related to body fat mass in either younger or older individuals.

Obesity is a determinant of decreased fat oxidative capacity in the basal state [5]; however, increasing age may also be an independent factor associated with impairment in substrate oxidation. Fat-free mass, which represents numerous metabolically active tissues (skin, bone, muscle) and organs (heart, liver, kidney, brain), is thought to be a major determinant of energy expenditure and fat utilization in lean healthy humans [20]. Several studies have indicated that FFM and, indeed, muscle mass decline with age [21]; therefore, it is reasonable to assume that this may be responsible for the reduction in REE and F_{ox} seen in older populations. Previous studies investigating the effects of age on energy expenditure and fat utilization have focused on differences in lean healthy individuals [22–24]; to our knowledge, no studies have investigated age-related variation in obesity while ruling out the effects of physical activity, body composition, insulin sensitivity, lipemia, and leptinemia, factors independently associated with substrate oxidation. To assess the effects of age per se, it is imperative that factors associated with substrate utilization be controlled. In this study, we chose 2 BMI-matched, sedentary, NGT groups, representative of a younger and an older obese

population, who were similar with respect to body composition (including WC, a marker of abdominal adiposity), insulin resistance (homeostasis model assessment of insulin resistance [HOMA-IR]), leptinemia, and lipemia (TG and T-Chol). Under such conditions, despite a trend toward lower FFM (Table 1, $P = .07$), we have demonstrated that basal F_{ox} is significantly lower in older obese individuals when expressed as a function of FFM, demonstrating a direct impact of aging on substrate utilization. Interpreting this finding with the knowledge that fat utilization is impaired in obesity indicates that increasing age plus elevated fat mass may have an additive detrimental effect on metabolic substrate use. Changes in substrate utilization may be explained by substrate availability; yet previous publications have failed to show a difference in serum FFA concentrations between young and old BMI-matched groups [22,25], reducing the likelihood that this theory is correct. In addition, reduced tissue perfusion may also explain reduced substrate utilization, as obesity and insulin resistance are related to impaired capillary perfusion [26,27]. Yet, in this study, no differences in C_{ox} were found, suggesting that the cause of the impairment in F_{ox} may lie downstream of tissue substrate availability and uptake. An age-related impairment in substrate oxidative capacity, as a result of reduced mitochondrial fatty acid uptake or respiratory chain dysfunction, is perhaps more likely. Rimbart et al [24] demonstrated that skeletal muscle carnitine palmitoyltransferase 1 activity is unchanged in older lean individuals, illustrating that long-chain fatty acid uptake into the mitochondria is not age related; however, the same group also noted that citrate synthase activity was decreased in their older cohort, indicating reduced mitochondrial density and thus reduced total oxidative capacity. No similar data exist in obese older groups. Further to this, Conley et al [28], Boffoli et al [29], Short et al [30], and Troncone et al [31] have shown that mitochondrial complex activity and oxidative capacity are reduced in skeletal muscle of elderly individuals. In addition, reductions in skeletal muscle mitochondrial protein synthesis have also been documented in older groups [32]. These alterations in mitochondrial function in older individuals are likely attributed to accumulative oxidative damage by reactive oxygen species [30,33,34] and indicate a probable cause of age-related impairment in substrate oxidation. Such explanations of the current data set are, however, speculative; and further work is required. In addition, age- and sex-related differences in F_{ox} , although not significant here (1-way analysis of variance: age \times sex interaction, $P = .40$), should be investigated in future studies of larger cohorts. The absence of an effect of sex was also true for all other variables (all $P > .05$).

Maximal oxygen consumption (or aerobic fitness; VO_{2max}) is an additional marker of mitochondrial function. Aerobic fitness has been shown to decline with age [24], but this is most likely attributed to the general decrease in physical activity levels and maximal cardiac output seen in the older population [20]. In our study, to minimize the

effects of physical activity, we chose only sedentary subjects; and yet VO_{2max} was reduced in the older cohort when expressed as a function of FFM. Maximal oxygen uptake is largely related to oxygen exchange capacity, tissue perfusion capacity, and mitochondrial capacity [35]; and improvements in VO_{2max} are associated with increased skeletal muscle mitochondrial concentration and capillarity [36]. Our finding that VO_{2max} is impaired in older individuals adds to the evidence that mitochondrial dysfunction may be a key component in the aging-related dysregulation of substrate oxidation.

Leptin, an adipocytokine that circulates in the blood in relation to fat mass, is also a marker of fat balance and energy metabolism [37]. Previous studies demonstrate that leptin directly stimulates fatty acid oxidation in skeletal muscle via an adenosine monophosphate-activated protein kinase pathway, activating acetyl coenzyme A carboxylase, an enzyme directly involved in mitochondrial long-chain fatty acid uptake [38]. However, although these findings are prominent in lean individuals, it appears that, in obesity, where high circulating concentrations are found, a state of leptin resistance occurs [39]. In this study, we demonstrated elevated leptin concentrations in our obese cohort similar to those documented in previous reports [39]; yet we found no differences between BMI-matched younger and older individuals. This suggests that, with age, either leptin production or leptin sensitivity is unchanged; and therefore, leptin's direct effects on fat oxidative capacity via cytosolic enzymes in skeletal muscle are also not altered. This adds to the hypothesis that changes in substrate utilization in older individuals are probably dictated by factors downstream of fatty acid uptake and availability, that is, mitochondrial dysfunction.

Our correlation analyses indicate that age is related to basal F_{ox} ($P = .003$) and maximal exercise capacity (VO_{2max} ; $P = .004$) in obese individuals. These relationships persisted when the effect of FFM was controlled, suggesting a direct involvement of aging on differences in substrate oxidative capacity. Our data also demonstrate that basal F_{ox} is best predicted by age, maximal exercise capacity, and REE in obese individuals, emphasizing the effects of age per se and highlighting the probable implication of mitochondrial function. Investigations of energy expenditure and F_{ox} in lean healthy groups indicate that older individuals are more susceptible to weight gain [6-8,40], but that F_{ox} appears to be related to the level of body fat mass, indicating an adaptive oxidative response to fat intake in lean individuals [8,41]. In this study, no relationships were demonstrated between F_{ox} and fat mass, indicating that, in obesity, there may be a loss of lipolytic homeostasis in relation to body composition. Further to this, F_{ox} and fat mass appear to be less related in our older cohort, again demonstrating an effect of age per se on the loss of adaptive F_{ox} with increases in fat mass.

Obesity is associated with several metabolic disturbances (eg, dyslipidemia, glucose intolerance, and hypertension)

that increase the risk of developing macrovascular complications. It is known that obesity is related to decreased fat oxidative capacity; and our current data indicate that basal F_{ox} is reduced in older obese individuals as a function of age per se while controlling for physical activity, glucose tolerance, and body composition. This indicates that older obese individuals may be more susceptible to the metabolic disturbances caused by increased body fat. Given the small size of the study groups in this investigation, the findings should be treated as preliminary data until further work using large cohorts and greater statistical power has been carried out. Nonetheless, this work creates an interesting direction for metabolic research. Future work should attempt to identify the mechanisms at play—and should focus on age-related differences in tissue perfusion, fatty acid transport, and mitochondrial function in particular—to improve the understanding of disease onset in the aged, and to identify therapeutic targets for “at risk” individuals.

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