Lipolysis in Elderly Postmenopausal Women

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The rate of fat oxidation at rest decreases with age in women. The mechanisms for this decrease are not clear. Theoretically, a decrease in the availability of fatty acids could explain the decline in fat oxidation. In consequence, the in vivo rate of production of fatty acids as a proxy for lipolysis was measured in 21 healthy women. Eleven of the volunteers were elderly (>65 years) and 10 were young (<24 years), and all were characterized for body composition. The nonadjusted rate of delivery of fatty acids into the systemic circulation was similar among elderly and young individuals (609 ± 80.3 *v* 597 ± 69.9 μmol/min, respectively, $P > .1$). When lipolysis was adjusted for the differences in fat-free mass using analysis of covariance (ANCOVA), rates were slightly increased in the elderly group (626 ± 80 μmol/min) and decreased in the young group (578 ± 84 μmol/min), but remained nonstatistically significant. It is concluded that mechanisms other than lipolysis must explain the decrease of fat oxidation in aging women, ie, a decrease in the capacity of muscle to oxidize fat and/or a decrease in its capacity for transport of long-chain fatty acids.

IT IS PRESENTLY UNCLEAR whether menopause increases cardiovascular risk independently of the effects of aging.[1,2] Recently, we showed that fat oxidation at rest decreases as a function of age in women,[3] and advanced the hypothesis that it may explain, at least partially, the tendency toward fat accumulation associated with aging in women. The mechanism of the decline in fat oxidation at rest is not clear, although it is evidently related to a decrease in fat-free mass and a consequent decline in aerobic fitness.[3]

Fatty acids, released from adipose tissue into the bloodstream via lipolysis,[4] may undergo terminal oxidative disposal ($CO_2 + H_2O$) or be recycled through nonoxidative pathways.[5] An age-related decrease in the rate of free fatty acid (FFA) release from adipose cells has been found[6] that could theoretically explain the decrease in fat oxidation at rest in elderly women by decreasing the availability of fatty acids. However, this study was undertaken with isolated cells in vitro, and the rates of lipolysis measured in this manner do not necessarily correlate with in vivo estimates of lipolysis.[7,8] Furthermore, we[9-14] and others[15-19] have found that in vivo the rate of fatty acid production exceeds the rate of fat oxidation, suggesting that regulation of fat oxidation is not simply a function of FFA availability. For example, we found that exercise itself, independent of changes in energy intake, body composition, or lipolysis, induced a shift in fuel utilization at rest toward fat oxidation.[12] Rates of lipolysis at rest in aging men have been reported to be either similar to or slightly higher than the rates in younger males by our group and others.[9,13,18] However, a paucity of data regarding lipolysis and aging/menopause exists in the female population, and to our knowledge, no direct comparison of in vivo rates of lipolysis in young and elderly women has been reported. Consequently, this study was designed to investigate whether the decrease in fat oxidation at rest associated with aging in women could be explained by low rates of lipolysis in vivo.

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SUBJECTS AND METHODS

Twenty-one nonsmoking women agreed to participate in the study. This group is a subset of a larger set of women who participated in a previously reported study.[3] They were selected for the present study to represent the extremes of age among women of the larger group. Consequently, 11 of the participants were elderly (age >60 years) and 10 were young (age <24 years). All had been weight-stable for at least 12 months (within 2 kg) and currently were not included in any weight-loss program. None of the volunteers were taking any medications. The screening and metabolic testing were all performed at the Clinical Research Center (CRC) of the University of Vermont.

Screening

A physical examination, resting electrocardiogram, and laboratory screening tests (routine SMAC-21, thyroid hormones, and thyrotropin), as well as an oral glucose tolerance test according to the National Diabetes Data Group,[20] were normal in all volunteers. All young subjects had a normal menstrual cycle and were not taking birth control pills or any other medication when examined. All of them were studied within 3 to 4 days after initiation of menstrual bleeding (early follicular phase). The elderly women were all healthy and were not taking estrogen-replacement therapy or any other medication at the time of study or within the previous 3 months. All volunteers received a verbal explanation of the study and signed a consent form approved by the Institutional Review Board of the University of Vermont.

Within 3 to 4 days after the subjects were screened, an estimation of body composition by underwater weighing with simultaneous measurement of residual lung volume[21] was undertaken.

Measurements of FFA kinetics and fat oxidation were scheduled 3 days after measurement of body composition. The volunteers were instructed not to exercise for at least 72 hours before this test. This test design was used to avoid the carryover effects of the last bout of exercise on fat metabolism. Although no formal effort was made to ensure compliance, all subjects were advised to maintain their regular dietary intake throughout the study period. Dietary practices were assessed by interview. None of the volunteers were in weight-loss programs. None of the subjects gained or lost more than 300 g during the test period.

The volunteers were admitted to the CRC the night before the test and were served supper at a fixed time (4:30 to 5:00 PM, a 15-cal/kg fat-free mass mixed meal with carbohydrate, protein, and fat proportions of 55:20:25, similar to their usual dietary practices) to standardize the

relative caloric load and the length of fasting prior to testing (approximately 14 hours).

The subjects were awakened at 6:30 AM, and an intravenous line was placed in a forearm vein for infusion of the tracer. A second intravenous line was placed in a dorsal vein of the opposite hand for blood sampling using the "hot box" technique.[22] After baseline blood samples were taken, a nonprimed constant infusion of ^{14}C-palmitic acid (0.20 μCi/min) was started and lasted 120 minutes. Samples for determination of hormones, substrates, and plasma enrichment of palmitic acid were taken every 10 minutes during the last 30 minutes of infusion. Fat oxidation was measured with indirect calorimetry as previously described.[3]

Analytical Methods and Calculations

Plasma glucose was determined using the glucose oxidase method on an automated glucose analyzer (YSI Instruments, Yellow Springs, OH). The serum insulin level was measured by radioimmunoassay at the Core Laboratory of the CRC.[23] The lipid profile (total cholesterol, triglycerides, and low-density lipoprotein [LDL] and high-density lipoprotein [HDL] cholesterol) was measured with standard methods at the Clinical Laboratory of the Medical Center Hospital of Vermont (MCHV) Campus of Fletcher Allen Health Care.

The plasma enrichment of palmitic acid was measured in an automated high-performance liquid chromatography system with minor modifications of the method originally reported by Miles et al,[24] as previously described in detail.[10,12,13] The rate of appearance of palmitic acid was calculated using the isotopic dilution principle[5] after verifying steady-state conditions for both the tracer and the tracee in plasma using a linear model of ANOVA (no time effects) and regression analysis (slopes of the concentrations regressed on time equal to zero). The serum concentration of fatty acids was measured with an enzymatic technique (Wako Chemicals, Richmond, VA). The total rate of appearance of fatty acids was calculated as described previously.[9-14]

Statistical Analysis

The data were reduced and analyzed on a personal computer and are presented as the mean ± SEM unless otherwise specified. Taking into consideration that there are differences among the two groups in body composition and energy expenditure, the mean of the variable of interest (FFA rate of appearance) was compared between elderly and young women with analysis of covariance (ANCOVA) in the BMDP software package (BMDP, Fullerton, CA). This statistical method adjusts the variable of interest for differences in the covariate. The covariate used was the fat-free mass, as this has been previously shown by us to be the best predictor of rate of appearance of FFA.[11] A P value less than .05 was set a priori as indicative of statistical significance. Student's t test (unpaired samples) was used for comparison of other variables as indicated.

RESULTS

Anthropometric and laboratory parameters for the two groups are depicted in Table 1. The elderly volunteers did not differ from the young group in body weight, but had a greater fat mass and a smaller lean body mass. Serum lipids displayed a nonsignificant tendency for higher levels of triglycerides and total and LDL cholesterol in the elderly group. Blood levels of glucose, insulin, and HDL cholesterol were similar. Whole-body rates of fat oxidation were lower in the elderly women (140 ± 18 μmol FFA/min) compared with the younger women (211 ± 27 μmol FFA/min, $P < .05$).

The kinetics of serum FFA is shown in Fig 1. There was no difference in the nonadjusted rate of appearance of FFA between the two groups (609 ± 80 *v* 597 ± 70 μmol/min). When lipolysis was adjusted using ANCOVA (Fig 1), the rates were increased in the elderly group (626 ± 80 μmol/min) and decreased in the young group (578 ± 84 μmol/min), but the differences still were not statistically different ($P > .5$).

Table 1. Anthropometric and Laboratory Characteristics of the Elderly and Young Subjects (mean ± SEM)

Characteristic	Elderly (n = 11)	Young (n = 10)	P
Age (yr)	67 ± 1.1	21 ± 0.6	<.001
Height (cm)	161 ± 3.2	166 ± 2.1	<.05
Weight (kg)	63 ± 2.3	67 ± 6.2	NS
Fat-free mass (%)	68 ± 1.1	80 ± 2.5	<.001
Fat mass (%)	32 ± 1.2	20 ± 2.5	<.001
Cholesterol (mg/dL)	202 ± 8.1	161 ± 9.5	NS
HDL Cholesterol (mg/dL)	49 ± 2.5	47 ± 3.3	NS
LDL Cholesterol (mg/dL)	125 ± 7.8	98 ± 11.1	NS
Triglycerides (mg/dL)	142 ± 17.3	82 ± 7.4	.08
Fatty acids (μmol)	612 ± 94.7	560 ± 60.7	NS
Glucose (mmol/dL)	5.1 ± 0.1	5.0 ± 0.2	NS
Insulin (pmol/L)	40 ± 12	32 ± 8	NS

DISCUSSION

The main finding of this study is that the rate of appearance of FFA (also called the effective systemic delivery of FFA) is not different in elderly postmenopausal, non–estrogen-replaced women versus young women (even after adjustment for fat-free mass) and therefore does not explain the decrease in fat oxidation as a function of age in women.[3] Thus, availability of FFA is not rate-limiting for fat oxidation in elderly postmenopausal women.

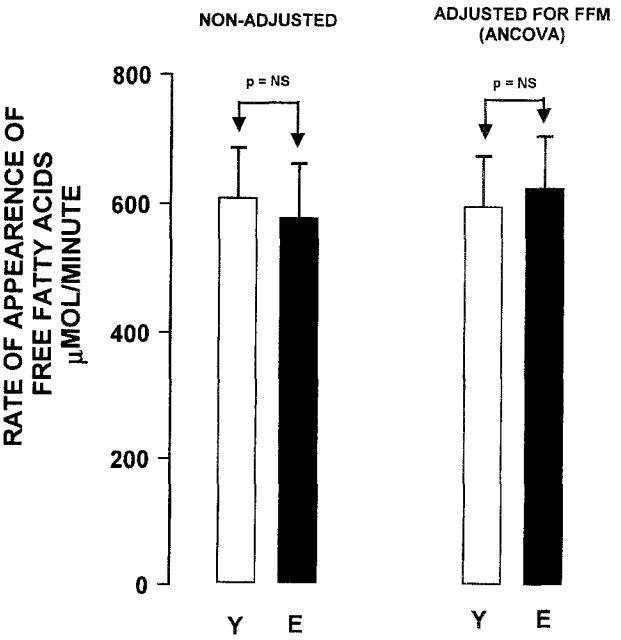

Fig 1. Rates of FFA production. Y, young subjects; E, elderly subjects.

Our data might be interpreted to be at odds with previous studies showing that there is an age-related decrement in hormone-stimulated lipolysis,[6,25] but the apparent discrepancy is not surprising. Lipolysis estimates in isolated cells are generally not well correlated with in vivo FFA production,[7,8] since the process of cell isolation deprives the tissue of its normal innervation, blood flow, and multiple hormone exposure. Our study was performed at the whole-body level in two groups of women who differ in body composition. If the rates of lipolysis are adjusted for the quantity of fat mass, then lipolysis may be interpreted as being lower in the elderly individuals. In a previous publication, we examined in detail the problem of normalization of lipolysis[11] and concluded that the fat-free mass was a better predictor of lipolysis than the fat mass. Using ANCOVA adjustment for the fat-free mass, we found that lipolysis was not different between the two groups.

Since lipolysis does not account for the decrease in fat oxidation, other mechanisms must be operative. Fat oxidation increases in response to exercise, even when energy intake is increased to match the negative caloric balance induced by the exercise sessions.[12] It was recently shown that a short bout of exercise did not increase fat oxidation to the same extent in elderly men versus younger individuals despite a similar increase in the rate of lipolysis,[26] suggesting that muscle tissue of elderly men has a lower capacity for fat oxidation. This defect is amenable to correction. We found that an 8-week trial of endurance training increased fat oxidation in elderly men despite unchanged body composition.[9] An adaptation of muscle to exercise training is consonant with an increase in the number of mitochondria and in the enzymatic capacity for fat oxidation of the trained muscle.[27] Thus, a possible mechanism to explain the decrease in fat oxidation in elderly women may be a decreased capacity of muscle to oxidize fat, possibly related to a decreased fitness level.[3]

Recently, evidence has accumulated to support the existence of fatty acid transporters.[28-30] We have found one of the proposed proteins in the skeletal muscle membrane of normal humans[31] and recently characterized the human tissue distribution of the mRNA[32] of the protein proposed by Schaeffer as a long-chain fatty acid transporter.[30] Thus, a complementary mechanism to the mitochondrial and enzymatic adaptations to exercise to explain the decrease in fat oxidation in elderly women is a decreased capacity of muscle to transport long-chain fatty acids, with a consequent decrease in intracellular availability of fatty acids for oxidation. This hypothesis is actively being studied in our laboratory.

Some limitations of this study should be noted. First, the two groups of women had differences in body composition, and thus, the effects of aging versus those attributable to body composition are not possible to separate. Second, lipolysis and fat oxidation were measured at the whole-body level. Our approach cannot separate the contributions to fat oxidation of blood-borne fatty acids versus intracellular depots. Under defined circumstances (ie, exercise), intracellular pools of triglyceride may contribute substantially to fatty acid oxidation.[33] Hence, a defect in intracellular lipolysis or in β-oxidation may explain the decrease in fat oxidation of elderly women. Results from this study and from previous reports[9,13,18] suggest that a gender difference may exist in age-associated changes in lipolysis: elderly men have a clearly higher rate of lipolysis than young men, which is seemingly not the case in women. The explanation for this apparent gender dimorphism is not clear. Interestingly, differences in the rate of energy expenditure between the genders have been found.[34,35] Finally, our measurement of metabolic variables was performed during a brief postabsorptive period; in reality, the process operates around the clock. In consequence, further research will be needed to examine if fat oxidation and/or lipolysis are altered by aging during the postprandial state.

In summary, in vivo lipolysis is similar between elderly and young women even when rates are adjusted statistically for differences in fat-free mass, and thus does not explain the decrease of fat oxidation in elderly women. Other mechanisms that could explain this decrease in fat oxidation are a decreased capacity of the muscle to oxidize fat (related to a decreased fitness level) coupled with decreased transport of fatty acids into muscle. A putative role for estrogens in fuel utilization remains to be established.

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