



PAPER

Regulation of macronutrient balance in healthy young and older men

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OBJECTIVE: To determine the influence of age on the ability to adjust macronutrient oxidation to changes in diet composition. Our hypothesis was that the ability to adjust macronutrient oxidation to changes in diet composition would be impaired with age.

DESIGN: Cross-sectional, randomized to three different isocaloric diets containing a constant percentage protein but varying in percentage fat and percentage carbohydrate: mixed diet (M; 15/30/55); high-fat diet (HF; 15/60/25), and high-carbohydrate (HC; 15/15/70).

SUBJECTS: Six young (YM; age = 25 ± 1 y) and five middle-aged and older men (OM; age = 63 ± 3 y).

MEASUREMENTS: Each subject underwent 24 h whole-room calorimetry on day 4 of each diet to determine 24 h macronutrient oxidation rates. Macronutrient balance was calculated from the individual macronutrient oxidation rates and the corresponding macronutrient intake.

RESULTS: Body mass, percentage fat, and fat-free mass were similar in the two groups. Twenty-four-hour energy expenditure (EE) and energy balance did not differ across diets or between groups; 24 h EE was ~7% lower (NS) in the OM. Macronutrient oxidation rates were not significantly different in YM vs OM during M. Protein oxidation was similar across diets, but higher ($P < 0.05$) in OM. Fat oxidation contributed 28.8 ± 7.0% vs 37.8 ± 4.7% to 24 h EE on M (NS) in the OM vs YM, respectively. This increased to 58.4 ± 6.7 vs 51.9 ± 5.3% of 24 h EE (NS) in the OM vs YM, respectively, during HF and decreased to 25.4 ± 9.7 vs 20.2 ± 14.3% (NS) during HC (diet effect, both $P < 0.05$). Carbohydrate oxidation contributed 54.3 ± 10.5% vs 56.6 ± 2.4% of 24 h EE (NS) on M in the OM vs YM, respectively. This decreased to 19.5 ± 10.6 vs 29.9 ± 12.6% (NS) during HF and increased to 53.6 ± 12.3 vs 64.7 ± 14.3% (NS) in the OM vs YM, respectively during HC (diet effect, $P < 0.05$).

CONCLUSION: Taken together, these results suggest that the ability to adjust macronutrient oxidation to changes in diet composition is maintained in OM and, thus, is unlikely to contribute to the increased susceptibility to weight gain and obesity development that accompanies aging.

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Introduction

In the US and other industrialized countries body fatness rises with advancing age.¹⁻³ Consequently, middle-aged and older adults demonstrate the highest prevalence of overweight and obesity than any other age group^{4,5} and are

considered a high-risk group for weight gain. There are both behavioral and metabolic factors that could contribute to the increased susceptibility to obesity in middle-aged and older adults. For example, increases in dietary energy and reductions in physical activity are behavioral factors that could contribute alone or in combination to produce a positive energy balance and weight gain. Similarly, alterations in obligatory energy expenditure or substrate oxidation also could contribute. For example, a reduction in fat oxidation has been associated with an increased risk of obesity in some populations.^{6,7} In this regard, a reduced ability to increase fat oxidation in response to an increase in dietary fat content

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may contribute to increased susceptibility to obesity in middle-aged and older adults.

In young never-obese adults fed at maintenance energy requirements, the respiratory quotient changes relatively quickly in response to alterations in the fat-to-carbohydrate ratio of the diet.^{8–10} Whether middle-aged and older adults maintain the ability to alter fuel metabolism in this way is unknown. Therefore, the purpose of the present study was to determine the influence of age on the ability to adjust macronutrient oxidation in response to changes in diet composition. We hypothesized that middle-aged and older adults would demonstrate an impaired ability to adjust fat oxidation in response to changes in dietary fat content. If observed, this may be one potential mechanism by which the susceptibility to obesity development is increased in middle-aged and older adults.

Methods

Subjects

Six young (YM) and five middle-aged and older men (OM) volunteered to participate in the present study. All subjects were screened for the presence of chronic diseases by physical examination and blood and urine chemistries. The OM were further screened for the presence of coronary heart disease with a maximal exercise electrocardiogram. All subjects were non-obese (body mass index (BMI) ≤ 30 kg/m²) and sedentary-to-recreationally physically active. None of the subjects participated in a program of regular physical activity. Subjects were weight stable (± 2.0 kg with previous 6 months) with habitual fat intakes of between 20 and 40% of total energy intake.

The nature, purpose and risks of the study were explained to each subject before written informed consent was obtained. The Colorado State University Human Research Committee and the Colorado Multiple Institutional Review Board approved the experimental protocol.

Protocol

Each subject underwent 24 h whole-room indirect calorimetry on four occasions. The initial whole-room calorimetry visit, which included a standardized activity protocol, was used to determine 24 h energy requirements. This initial whole-room calorimetry visit allowed us to target zero energy balance with greater precision. The subsequent whole-room calorimetry visits (order randomized) were used to measure 24 h macronutrient oxidation rates on day 4 of each of three different isocaloric diets. Twenty-four-hour macronutrient balances were calculated from the respective 24 h oxidation and intake of protein, fat and carbohydrate. On the 3 days preceding each calorimetry visit food was provided in an amount necessary to maintain weight stability (< 0.5 kg). All food was provided and prepared by the General Clinical Research Center's (GCRC) metabolic kitchen. Subjects were required to consume at least one

meal in the GCRC each day. Body weight stability was also verified during these visits. All other food was packaged to be taken away and eaten at home or at their place of work. Each experimental diet contained a constant percentage of protein but varied in the percentage of fat and carbohydrate. The specific macronutrient composition (protein, fat, carbohydrate) of each diet, as a percentage of total energy intake, was 15/30/55 for the mixed diet condition (M), 15/60/25 for the high-fat diet condition (HF), and 15/15/70 for the high-carbohydrate condition (HC). A 2 week wash out period separated each diet condition.

Procedures

Body weight was measured on a physician's balance scale to the nearest 0.1 kg prior to each whole-room calorimeter visit. The percentage of body fat and fat-free mass was measured in all subjects using dual-energy X-ray absorptiometry (Model DPX-IQ Lunar Corp., Madison, WI).

Twenty-four-hour energy expenditure (EE) and macronutrient oxidation was measured using whole-room indirect calorimetry. The whole-room calorimeter is similar to that described previously.^{8,11–14} Twenty-four-hour EE and macronutrient oxidation rates were calculated from oxygen consumption and carbon dioxide production.¹⁵ Protein oxidation was estimated from urinary nitrogen content. Subjects entered the whole-room calorimeter after a 12 h overnight fast at 08:00 and left the following day at 07:00. The results were extrapolated to 24 h. Upon entry in the calorimeter resting EE was measured after 30–45 min of lying quietly in bed. During this and subsequent calorimeter visits subjects were required to perform two 30 min bouts of exercise on a cycle ergometer at 40% of their individually determined peak oxygen consumption. Peak oxygen consumption was measured during incremental cycle ergometry (Monark, Varberg, Sweden) to exhaustion. Oxygen consumption, carbon dioxide production and ventilation were measured using a metabolic cart (SensorMedics 2900, Yorba Linda, CA). Each subject also performed two 30 min bouts of walking at a rate of 8 m/10 s. The rationale for having our subjects perform these activities was to standardize activity patterns across the dietary conditions. Each subject followed the same standardized activity pattern and were fed meals at the same time of the day during each whole-room calorimeter visit. The exercise in the calorimeter also gave an activity pattern that was more representative of a day when not confined to the room. Therefore, the calorimeter visit was not unusually sedentary.

Four-day diet records were obtained on all subjects to determine the average habitual total calorie and macronutrient intake. A research dietitian instructed subjects on the procedures for weighing and recording food intake. Nutritionist IV software (First DataBank, Inc., San Bruno, CA) was used to determine the self-reported total energy intake and macronutrient composition of each individual's diet. GCRC Dietplanner (version 2.11; developed by Doris Dare, MS, RD,

at the University of San Francisco GCRC) was used to design and plan the study diets for the present study.

Because insulin action may play a role in determining the relative oxidation vs storage of individual macronutrients, insulin sensitivity was estimated using Bergman's minimal model with a modified frequently sampled intravenous glucose tolerance test (IVGTT).¹⁶ Three baseline blood samples for the modified IVGTT were drawn. Glucose was then injected (0.3 g/kg) at time 0 and insulin (0.03 U/kg) was injected 20 min later. A total of 33 blood samples (6 ml) were drawn for subsequent determination of glucose and insulin concentration over the course of the 3 h protocol. Glucose was measured enzymatically using the hexokinase method on an automated Roche COBAS Mira Plus glucose analyzer. Insulin concentrations were measured by radioimmunoassay (Kabi Pharmacia, Piscataway, NJ).

Statistical analysis

Differences in subject characteristics were assessed by independent *t*-tests. Repeated measures analysis of variance was used to assess the diet, group and diet×group interaction effects for each dependent variable. No diet×group interaction effects were observed and, thus, no *post-hoc* comparisons were performed. A significance level of *P* < 0.05 was selected *a priori*. All values are presented as means ± s.e.

Results

Subjects characteristics

Subject characteristics are shown in Table 1. There was an ~40 y age difference between the YM and OM. Body mass, body fat percentage, fat-free mass, insulin sensitivity were similar in the two groups (all *P* > 0.05); however, as expected, peak oxygen consumption was significantly lower in the OM compared with YM. Habitual total energy intake as well as the percentage of protein, fat and carbohydrate was also similar in the two groups (Table 2, all *P* > 0.05).

Components of 24 h EE, energy intake and energy balance

There was no significant effect of diet on 24 h energy expenditure (24 hEE) (Figure 1, top panel), sleeping metabolic rate,

Table 1 Subject characteristics

Variable	Young (n = 6)	Older (n = 5)
Age (y)	25 ± 1	63 ± 3*
Body mass (kg)	79.0 ± 3.0	82.0 ± 4.0
Body fat (%)	21.9 ± 3.0	25.6 ± 1.0
Fat-free mass (kg)	61.0 ± 1.7	61.0 ± 3.1
VO ₂ peak (l/min)	3.1 ± 0.3	2.3 ± 0.1*
S _i (×10 ⁻⁴ min/μU/ml)	8.0 ± 1.7	8.6 ± 0.2

Values are means ± s.e. VO₂ peak = peak oxygen consumption; S_i = insulin sensitivity index. **P* < 0.05 vs young subjects.

Table 2 Habitual dietary intake of the subjects in the present study

Variable	Young (n = 6)	Older (n = 5)
Total energy (kcal/day)	2605 ± 158	2325 ± 148
Protein (%)	15.2 ± 1.2	16.4 ± 1.7
Fat (%)	31.6 ± 1.8	31.9 ± 3.5
Carbohydrate (%)	50.9 ± 1.6	50.0 ± 3.9

Values are means ± s.e. % = percentage of total energy intake.

or resting metabolic rate (data not shown). Twenty-four-hour EE was approximately 7% lower (*P* > 0.05) in the OM compared with YM both adjusted and unadjusted for fat-free mass (adjusted means not shown). By design, 24 h energy intake (EI) was similar across diet condition and not significantly different in the OM vs YM (Figure 1, middle panel).

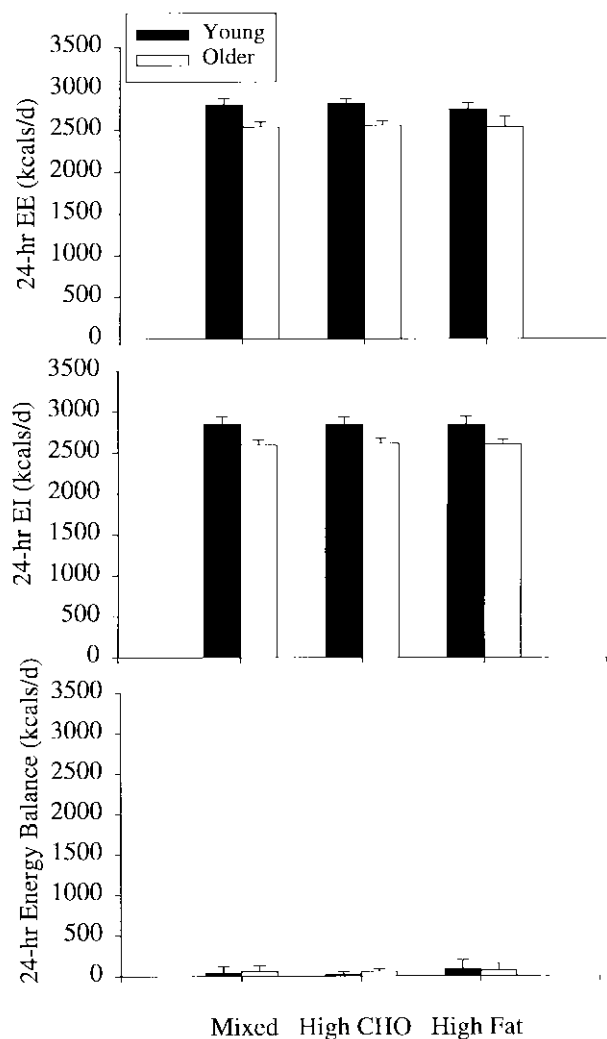


Figure 1 Twenty-four-hour energy expenditure (upper panel), energy intake (middle panel), and energy balance in the young and middle-aged and older men during each diet condition. Values are means ± s.e.

Energy balance was similar ($\sim +50-100$ kcal; $P > 0.05$) across diet conditions and did not differ between groups (Figure 1, bottom panel; $P > 0.05$). There was no significant group \times time interaction effects observed for any of these variables.

Twenty-four-hour non-protein respiratory quotient (NPRQ), macronutrient oxidation and macronutrient balance

The 24 h NPRQ was significantly influenced by diet composition (Figure 2). However, there was no significant difference in the 24h NPRQ between the YM and OM across diet conditions. As expected, there was no significant diet effect on protein oxidation. However, protein oxidation was higher ($P < 0.05$) in the OM compared with YM (Figure 3, top panel) across diet conditions. Fat and carbohydrate oxidation were significantly influenced by diet composition (Figure 2, middle and lower panels). There were no significant differences in fat or carbohydrate oxidation between the YM and OM across diet conditions. There were no significant group \times diet interaction effects observed for the 24 h RQ or protein, fat and carbohydrate oxidation.

Protein balance was not influenced by diet composition (Figure 4, top panel, $P > 0.05$). However, the OM demonstrated a significantly more negative protein balance compared to the YM across diet conditions. Both fat ($P = 0.057$) and carbohydrate ($P = 0.07$) balance were tended to be influenced by diet composition (Figure 3, middle and lower panels). There were no significant differences in fat or carbohydrate balance in the YM and OM across diet conditions. Furthermore, there was no significant diet \times group interaction effects observed for the individual macronutrient balances.

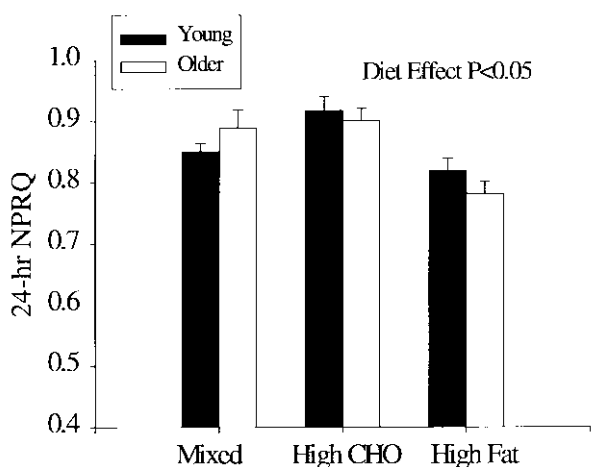


Figure 2 Twenty-four-hour non-protein respiratory quotient (NPRQ) in the young and middle-aged and older men during each diet condition. Values are means \pm s.e.

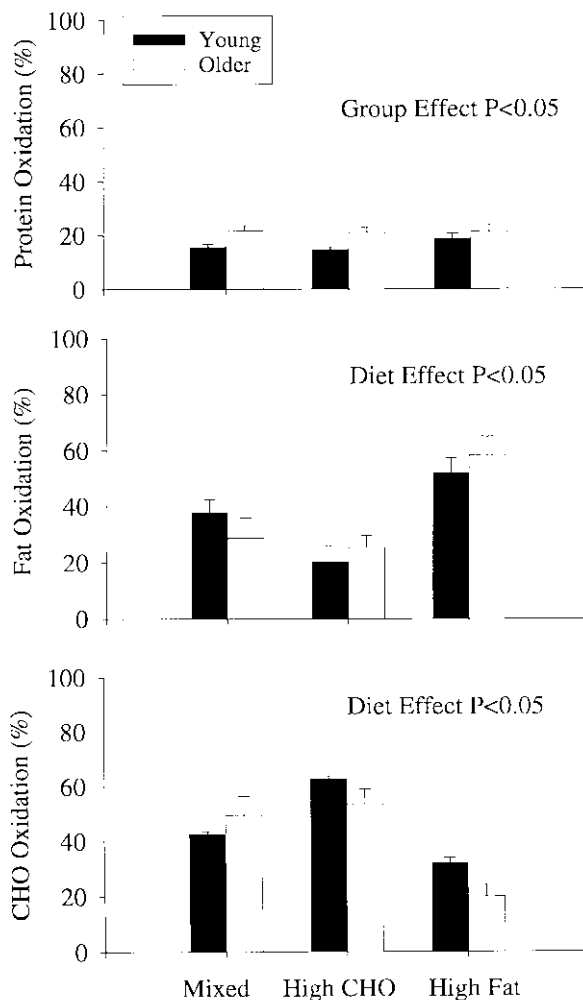


Figure 3 Twenty-four-hour protein, fat and carbohydrate oxidation rates in the young and middle-aged and older men during each diet condition. Values are means \pm s.e. CHO = carbohydrate.

Discussion

The major new finding of the present study was that the ability to adjust macronutrient oxidation in response to isocaloric changes in diet composition was not impaired in middle-aged and older adults. Although fat oxidation tended to be lower in the middle-aged and older men during the mixed dietary condition, this had no bearing on the ability to increase fat oxidation in response to increase in dietary fat content. Thus, these findings do not support the idea that middle-aged and older adults are metabolically susceptible to obesity development.

We did not observe an impaired ability to adjust macronutrient oxidation in response to isocaloric changes in diet composition in middle-aged and older adults. Taken together with the observation that many middle-aged and older adults are generally overweight or obese,^{4,5} our findings suggests that other factors must be considered as

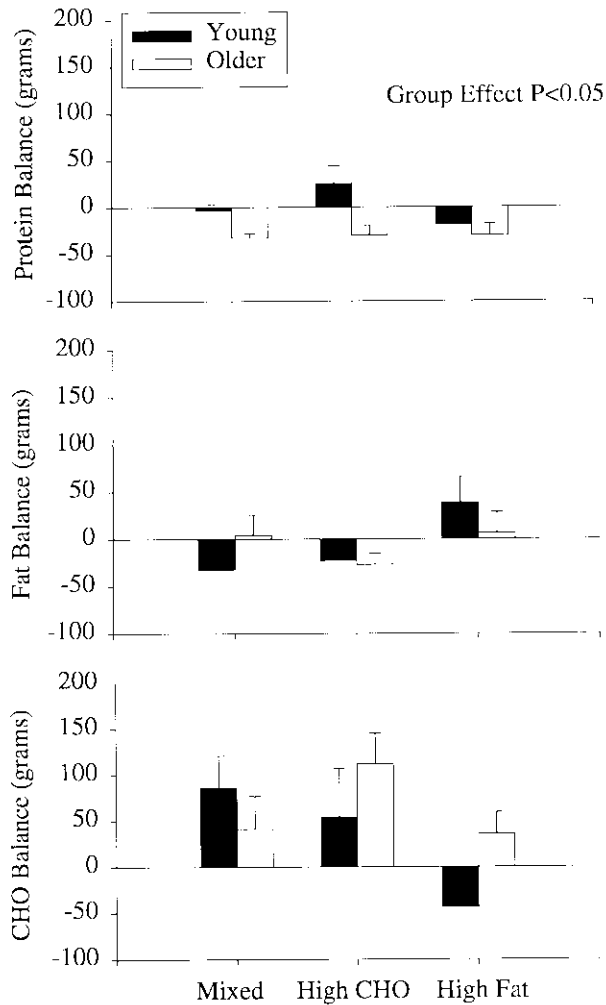


Figure 4 Twenty-four-hour protein, fat, and carbohydrate balances in the young and middle-aged and older men during each diet condition. Values are means \pm s.e. CHO = carbohydrate.

being responsible for the increased susceptibility to obesity that accompanies aging. In this regard, total energy requirements decline with age due largely to reductions in the amount of energy expended in physical activity.^{17,18} If energy intake is not reduced by a similar amount a positive energy balance and weight gain will occur. In addition, the regulation of energy intake also appears to be impaired with age.¹⁹ Thus, it is possible, even likely, that a progressive impairment in the control of food intake with age, together with a progressive reduction in physical activity, contributes to obesity development in middle-aged and older adults.

Fat oxidation tended to be lower in the older compared to young men during the mixed dietary condition, although this did not reach statistical significance. Indeed, lower levels of fat oxidation with age have been observed under resting conditions,^{20–22} in response to meal ingestion,²³ as well as during exercise.²⁴ However, it is important to emphasize that

this may have little bearing on the adjustments in fat oxidation that occur in response to changes in dietary composition. For example, despite lower levels of fat oxidation during the mixed diet condition, the older men from the present study demonstrated similar (even greater) increases in fat oxidation during the high-fat diet compared to the young men. Therefore, it would appear that alterations in substrate oxidation with age under one condition should not be generalized to a 24 h period.

Interestingly, protein oxidation was higher and protein balance was more negative in the middle-aged and older men compared with the young adults. The reason for this finding is unclear. We do not think this reflects methodological error as this was observed under each of the diet conditions. These findings are consistent with other studies suggesting that protein requirements are elevated in older men and women.^{25,26} However, this issue remains controversial.^{27,28} Nonetheless, our findings may provide insight into the loss of muscle mass observed with age, although our study was not designed to address this issue.

We would like to emphasize that a major strength of our study was the extraordinary achievement of energy balance in the calorimeter. Each subject underwent an initial whole-room calorimeter visit to determine 24 h energy requirements on a calorimetry day. In addition, we provided our subjects with a standardized activity protocol to perform during each whole-room calorimetry visit. This level of activity allowed us to avoid a calorimeter visit that was unusually sedentary. Together, this allowed us to test our stated hypothesis in the absence of a confounding positive or negative energy balance (either between or across conditions).

There are some potential limitations of the present study that should be addressed. First, the group of middle-aged and older men studies in the present investigation may not be representative of all middle-aged and older adults. It is possible that these individuals were more active than most in this age group. In addition, our middle-aged and older subjects were relatively lean. We recognize that it is possible that impairment in the ability to adjust macronutrient oxidation in response to isocaloric changes in diet composition might have been observed if obese older adults were studied. However, it is important to emphasize that, because we did not observe any impairment in lean middle-aged and older adults, our findings suggest that an impairment in the ability to adjust macronutrient oxidation in response to isocaloric changes in diet composition is not an inevitable consequence of aging *per se*.

Second, it is possible that middle-aged and older adults in the present study demonstrated an impaired ability to adjust macronutrient oxidation to changes in diet composition prior to the time during which macronutrient oxidation was measured (ie day 4). However, there was a non-significant trend suggesting the middle-aged and older adults may actually demonstrate a superior ability to adjust macronutrient oxidation to changes in diet composition. Thus, although we cannot exclude this as a possibility, the above suggestion appears unlikely.

Third, it is also possible that the middle-aged and older adults in the present study would have demonstrated an impaired ability to adjust macronutrient oxidation to changes in diet composition under conditions of energy imbalance. We believe this is unlikely based on previous observations. Roberts *et al*¹⁹ reported no effect of age on the increase in total energy expenditure, body weight, or body fat with experimental overfeeding in men. In contrast, the older subjects in this previous study failed to demonstrate a spontaneous hypophagia following the period of overfeeding. In turn, body weight also failed to return to baseline following overfeeding as occurred in the young subjects. Taken together with the well-documented reduction in total energy expenditure with age,^{17,18} these observations suggest that factors other than the ability to adjust macronutrient oxidation must be considered as being responsible for the obesity development that occurs in middle-aged and older adults.

Finally, the number of young and middle-aged and older men we studied was small. Therefore, we cannot exclude the possibility of inadequate power to detect significant differences between our groups. However, we should emphasize that although not significant, the directional differences in substrate oxidation between the young and older men in the present study (ie increase in fat oxidation during the high fat diet) would actually support a superior adjustment in substrate oxidation in response to changes in diet composition in the older men. Future studies will be necessary to confirm or refute our findings.

In summary, the results of this preliminary study suggest that the ability to adjust macronutrient oxidation in response to changes in diet composition is not impaired in healthy, non-obese middle-aged and older men. Future studies will be necessary to determine the factors that contribute to obesity development in this age group.

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