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PAPER

Reduced whole-body fat oxidation in women and in the elderly

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OBJECTIVE: To test the hypothesis that the increase in fat mass observed with aging might be related to a decrease in whole-body fat oxidation.
SUBJECTS AND MEASUREMENTS: Forty volunteers had measurements of sleeping and 24 h substrate oxidation in calorimetric chambers, body composition with the ^{18}O dilution technique, VO_{2max}, and fiber composition analysis from a biopsy of vastus lateralis. They were divided into 10 young women, 10 young men, 10 elderly women and 10 elderly men.
RESULTS: Sleeping fat oxidation and 24 h fat oxidation were lower in women than in men and in elderly than in young participants. Sleeping fat oxidation was correlated to fat-free mass and energy balance (multivariate analysis). Twenty four hour fat oxidation was correlated to total energy expenditure and energy balance (multivariate analysis). After adjustment for differences in these factors, sleeping and 24 h fat oxidation were no longer different between age and sex groups. None of the parameters of macronutrient metabolism was correlated with muscle fiber composition.
CONCLUSION: Our data suggest that fat oxidation is lower in elderly subjects. This difference could favour fat mass gain if fat intake is not adequately reduced. Differences in fat-free mass and in total energy expenditure appear to participate in the reduction in fat oxidation.
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Keywords: fat oxidation; indirect calorimetry; aging; sex

Introduction

Aging is associated with well-known changes in body composition; fat-free mass (FFM), mainly muscle tissue, decreases and fat mass increases (in absolute values and in percentage of body weight).[1,2] An increase in fat mass can result from a positive fat (and energy) balance, ie a situation where fat utilization is lower than fat intake. The excess fat is stored as triglycerides in adipose tissue and muscle. Although measurements are difficult and lack accuracy, fat intakes have been shown to be either reduced[3] or unchanged[4,5] in elderly persons. Positive fat balance could therefore result from a decreased fat utilization. Triglyceride hydrolysis releases non-esterified fatty acids (NEFA) which are oxidized in muscle and liver for producing energy, and fat oxidation is positively correlated with FFM.[6] Under normal circumstances digestive fat losses are trivial. Age-related changes in fat oxidation are debatable. In elderly persons, resting fat oxidation is reduced in some[7–9,10] but not in all studies.[10,11] This reduction appears to be related to the reduced FFM, as differences are cancelled out after accounting for age-related differences in FFM.[7,10] During an acute exercise bout, elderly volunteers rely more on glucose oxidation for producing energy than do their young pairs.[10] Usual physical activity modulates resting fat oxidation, as it is higher in very active young and elderly persons than in sedentary ones.[7,9,10] Furthermore, resting fat oxidation is reduced by bed-rest[12] and increased by endurance training in young[13–15] and elderly subjects.[16–18] Since their usual physical activity is reduced,[19] elderly persons are expected to show a lower fat oxidation than young subjects. These arguments, together with the reduced resting fat oxidation, argue in favour of a decline in 24 h fat oxidation. The 24 h fat oxidation is the only valid parameter for the calculation of fat balance, since resting fat oxidation only represents 24% of daily fat

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oxidation.[18] Twenty-four hour measurement requires an indirect room calorimeter. Apart from one study in Pima Indians,[20] where 24 h respiratory quotient was positively correlated with age, there are no other data confirming a reduced 24 h fat oxidation in the elderly.

Therefore, the aim of the present study was to compare 24 h and resting fat oxidation in young and elderly subjects with varying physical activity, to test the hypothesis that both are reduced in the elderly.

Subjects and methods

Subjects

Forty healthy volunteers were recruited after a full medical examination. They had been weight-stable (within 1 kg) for the last 2 months. Participants were chosen to vary in their usual physical activity between very sedentary and very active. None of them were suffering from diabetes, and none were taking drugs known to influence energy or macronutrient metabolism. They all had a normal plasma lipid profile. Subjects with a body mass index higher than 25 (young subjects) or 30 $kg \cdot m^{-2}$ (elderly subjects) or consuming more than two cups of tea or coffee per day (since caffeine influences fat oxidation[8]) were excluded from the study. All volunteers had a waist-to-hip ratio lower than 1.0. Subjects refrained from any exercice bout for 36 h before entering the room calorimeter and gave informed written consent for participating in the study. The study protocol was approved by the local ethics committee and by the French Ministry of Health.

Subjects were separated into young ($n=20$, <35 y) and elderly ($n=20$, >60 y). There was an equal number of men and women in each age group. The physical characteristics of the volunteers are given in Table 1.

Study protocol

The protocol was centered on the 24 h measurements in the room calorimeters. Before (within a week) entry in the calorimeter, subjects had physical fitness (VO_{2max}), and body composition determined in order to calculate energy requirements. On the morning volunteers left the calorimeters, while still fasted, they had a blood collection for biochemical analyses and a muscle biopsy of the vastus lateralis. Methods for those explorations are given below.

Calculation of energy requirements. Fat oxidation is increased in situations of negative energy balance.[21] It is therefore necessary to estimate energy requirements corresponding to the stay in the calorimeter. This was achieved with a model derived from our previous studies,[22] where energy expenditure (EE) is calculated from fat-free mass (FFM), the duration and intensity of physical activities and sex. In the room calorimeters, the activity schedule was fixed for all volunteers (see below), and consisted of four periods of 30 min walking on a treadmill, plus 20 min weight lifting. The walking speed was adjusted to each individual fitness.

FFM was calculated from total body water and the 73.2% hydration factor.[23] Total body water was estimated from the age specific equations derived for bioelectrical impedance analysis.[24] (Note that this estimate of body composition was only used for the prediction of energy requirements in the room calorimeters. Adjustments of fat oxidation with body composition presented in the results section were obtained by ^{18}O dilution. See below.)

Determination of VO_{2max}. Volunteers underwent a test to exhaustion on a cycle ergometer (Ergomeca) connected to a gas and volume analyzer (CPX/D Medical Graphics, St Paul, MN). A satisfactory medical history and examination and a negative maximal test were preconditions to starting the protocol. Starting at 30 W, 30 W increments were applied every 2 min 30 s to exhaustion, with blood pressure and electrocardiographic recordings and strong verbal encouragement throughout the test. Oxygen consumption (VO_2) was measured continuously by open-circuit spirometry and averaged every 30 s with the use of the automated one-line system. The test was considered maximal when three of

Table 1 Physical characteristics of the volunteers (mean ± s.e.)

	Young				*Elderly*						
	Women		*Men*		*Women*		*Men*		*Anova*		
	Mean	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Age (A)*	*Sex (S)*	*A×S*
Age (y)	25.0	1.5	24.4	1.0	64.8	1.0	64.3	1.4	<0.0001	0.66	0.97
Weight (kg)	59.7	2.5	69.7	2.1	62.0	1.8	74.6	3.0	0.14	<0.0001	0.60
Height (cm)	162.3	1.9	173.6	1.2	155.2	2.0	171.7	1.4	0.02	<0.0001	0.17
BMI ($kg\ m^{-2}$)	22.6	0.8	23.3	0.9	25.8	0.8	25.3	0.9	0.004	0.96	0.50
Percentage fat mass	23.6	2.0	15.2	2.1	33.9	1.6	22.5	1.5	<0.0001	<0.0001	0.42
FFM (kg)	45.8	1.1	64.0	1.6	38.6	0.8	54.7	1.7	<0.0001	<0.0001	0.45
VO_{2max} ($l\ min^{-1}$)	2.11	0.09	3.16	0.21	1.37	0.13	2.38	0.16	<0.0001	<0.0001	0.92
Plasma glucose ($mmol\ l^{-1}$)	4.43	0.08	4.46	0.13	4.70	0.15	4.81	0.20	<0.0001	0.07	0.02
Plasma triglycerides ($mmol\ l^{-1}$)	1.07	0.10	1.01	0.12	1.20	0.11	1.17	0.14	0.21	0.67	0.91
Plasma insulin ($\mu U\ ml^{-1}$)	9.65	0.87	8.33	0.71	9.48	0.75	8.47	0.45	0.98	0.11	0.84
Plasma NEFA ($\mu mol\ l^{-1}$)	523	51	499	47	494	82	451	38	0.50	0.56	0.87

NEFA: nor esterified fatty acids.

the following four conditions were fulfilled: constant VO_2 despite 30 W increment; maximal HR (HR_{max}) near theoretical HR_{max} ($HR_{max} = 220 - age$ (y)); respiratory quotient >1.1; and exhaustion of the subject.

Description of the stay in the calorimeter. Volunteers had been acclimated to the environment in the calorimeters by staying a few hours in the room on the day they came in for the initial physical examination. On the day of the measurements, subjects entered the room at 05:00 pm and stayed for 37 h (ie two nights and a complete day). They stayed in bed from 11:00 pm to 07:00 am, and ate at 08:00 am, 12:30 pm and 07:00 pm. They walked on the treadmill, at about 50% of VO_{2max}, for 30 min at 10:00 am; 11:30 am; 3:00 pm and 04:30 pm. They practised weight lifting for 20 min at 6:00 pm. In between these events, volunteers were free to engage in light activities (reading books, listening to the radio, watching television), but were not allowed to rest in bed or to have extra exercise.

Meals were served through an air-lock and were composed to suit volunteers' taste and to match their energy requirements (cf above). The macronutrient composition was 50% carbohydrates, 35% fat, and 15% protein. Volunteers were encouraged to eat all the food provided but had free access to water. Any food left was weighed, and actual energy intake was calculated from French food composition tables.[25] Energy balance achieved in the room calorimeters was $-3.0 \pm 1.2\%$ of energy intake and did not differ between age and gender groups.

During the stay in the calorimeter, subjects were asked to collect their urine samples in bottles: one bottle was for the collection until 7:00 am the first morning, one from 7:00 am until 12:30 pm, one from 12:30 pm until 18:30 pm and the last bottle was for the collection until 7:00 am the second morning. Urine volumes were measured and an aliquot was frozen for later nitrogen content analysis by pyrochemiluminescence (ANTEK 7000 elemental analyzer). Faeces samples were collected, weighed and frozen for later analysis of fat content.

Body composition was measured while the volunteers stayed in the room calorimeter with the ^{18}O dilution technique.[26] Briefly, subjects gave a baseline urine sample at 11:00 pm, drank a weighed amount of 2% ^{18}O-enriched water (~ 50 g) and went to bed. They were woken at 6:00 and 7:00 am the following morning to provide a urine sample. ^{18}O enrichments and total body water calculations were performed as described elsewhere.[26]

Calculation of substrate oxidation. Respiratory gas exchanges were measured continuously using two open-circuit whole-body calorimetric chambers as previously described.[27] Gas analysers were calibrated upon commencement, after 13 h (evening) and at the end of the 24 h measurement period using standard gas mixtures. Gas exchanges were computed from the minute-by-minute measurement of outlet air flow, differences in gas concentrations, atmospheric pressure, chamber air temperature and hygrometry and taking into account the gas analyser's drift and the variation of the volumes of CO_2 and O_2 in the chambers. The validity of gas exchange measurements was checked gravimetrically. The recovery was 100.6 ± 1.5 for O_2 and 100.7 ± 1.7 for CO_2. Total energy expenditure, fat and glucose oxidation were calculated with equations derived by Ferrannini.[28] Protein oxidation was calculated from nitrogen excretion. The equations are:

$$\text{REE (kcal min}^{-1}) = 3.91\ VO_2 + 1.10\ VCO_2 - 3.34\ N$$

$$\text{Glucose oxidation (g min}^{-1}) = 4.55 VCO_2 - 3.21\ VO_2 - 2.87\ N$$

$$\text{Fatty acid oxidation (g min}^{-1}) = 1.67\ (VO_2 - VCO_2) - 1.92\ N$$

$$\text{Respiratory quotient} = VCO_2/VO_2$$

Data from the first night were discarded (this period was considered as an acclimation time to the environment). Data were separated into those pertaining to the awake period (7:00 am the first morning until 2:00 am) and those pertaining to the sleeping period (from 2:00 am to 7:00 am in the second night). A close inspection of heart-rate recordings was used to remove periods of time (if any) when the subject was not sleeping.

Biochemical analyses. A 10 ml blood sample was drawn for measurements of plasma glucose, triglycerides and cholesterol (on a HITACHI 911 automatic analyser), NEFA and glycerol (on a spectrophotometer using an enzymatic method; Waco Chemicals, Neuss, Germany and Boehringer, Mannheim, France, respectively) and insulin (by specific radioimmunoassay; CIS biointernational, Gif Sur Yvette, France).

Muscle biopsy. A 60–120 mg sample of muscle was taken from the vastus lateralis after local anesthesia according to the technique described by Berthon *et al.*[29] Serial sections 10 μm thick were cut on a cryostat at $-25^{\circ}C$, perpendicular to the muscle fibres. The cells were stained with azorubine to evidence their outline. The contractile type of the fibres was determined by the method of Brooke and Kaiser.[30] The myofibrillar mATPase activity was revealed after acid preincubations at pH 4.3 and 4.55. According to this technique fibers were classified as I, IIA and IIB.

Statistical methods. Results are expressed as mean ± s.e. unless stated otherwise. Comparisons of means were performed with a general model for analysis of variance, with two categories: age and sex. Simple linear relationships between variables were sought by a correlation matrix. Forward stepwise multiple regression analyses were performed with the significant predictors obtained with simple regression.

All calculations were performed with Statview 4.0 statistical package (Abacus Concepts, California), except for the

adjustments, which were performed according to the method described in Ravussin and Bogardus[31] and analyses of covariance.[35] Significance was accepted at the 5% level.

Results

FFM and VO_2 max were higher in men than in women, and in young than in elderly persons (Table 1). Conversely, percentage fat was higher in women and in elderly participants.

Table 2 shows substrate oxidation rates. Energy expenditure, fat and protein oxidation rates were significantly lower, and glucose oxidation was higher in women than in men, both during the sleeping and the 24 h period. Fat oxidation was lower in elderly than in young participants, during sleeping ($P=0.0018$) and over the 24 h ($P=0.0601$). Sleeping glucose oxidation was higher in elderly than in young subjects. Protein oxidation and energy expenditure did not differ between age groups.

Table 3 shows the correlation coefficients of the relationship between fat oxidation and various parameters. Sleeping fat oxidation was positively correlated with VO_2 max, FFM and sleeping metabolic rate, but negatively with percentage fat mass and energy balance. Multiple regression showed that FFM (positively) and energy balance (negatively) determined the variance in sleeping fat oxidation. After adjustment for energy balance and FFM, there was no effect of age ($P=0.36$) and of sex ($P=0.39$) on sleeping fat oxidation.

Twenty-four hour fat oxidation was positively correlated with VO_2 max, FFM and total energy expenditure (TEE), and negatively with percentage fat mass and energy balance. Multiple regression analysis showed that energy balance (negatively) and TEE (positively) determined the variance in 24 h fat oxidation. After adjustment for differences in energy balance and TEE, 24 h fat oxidation did not differ between sexes ($P=0.52$) and age ($P=0.50$).

Muscle fibre composition was not affected either by sex or age (Table 4). None of the parameters of energy or macronutrient metabolism were correlated with muscle fibre composition.

Discussion

The present study suggests that fat oxidation, both during sleeping and over 24 h, is lower in elderly than in young subjects, and in women than in men. The age-related changes in resting fat oxidation are debated in the literature.

Table 2 Substrate oxidation rates and energy expenditure

	Young				*Elderly*						
	Women		*Men*		*Women*		*Men*		*Anova*		
	Mean	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Mean*	*s.e.*	*Age (A)*	*Sex (S)*	*A×S*
Sleeping period											
Fat oxidation (g h^{-1})	2.92	0.54	4.03	0.55	1.84	0.14	2.37	0.34	0.0495	0.0018	0.48
Glucose oxidation (g h^{-1})	4.12	0.92	4.34	0.79	5.28	0.31	7.75	0.55	0.0443	0.0012	0.09
Protein oxidation (g h^{-1})	3.90	0.22	5.3	0.36	3.97	0.25	5.98	0.46	<0.0001	0.24	0.34
SMR (kcal h^{-1})	54.2	2.4	69.6	1.9	48.6	1.2	68.6	1.8	<0.0001	0.08	0.23
24 hours											
Fat oxidation (g)	108	9	138	15	84	12	113	17	0.025	0.0601	0.97
Glucose oxidation (g)	217	16	292	30	256	15	316	28	0.040	0.16	0.71
Protein oxidation (g)	65	4	85	3	62	4	92	5	<0.0001	0.64	0.19
TEE (kcal)	2132	67	2797	70	2040	83	2677	67	<0.0001	0.13	0.88

SMR: sleeping metabolic rate; TEE: total energy expenditure.

Table 3 Regression analyses of fat oxidation and covariables

	Sleeping fat oxidation		*24 h fat oxidation*	
Variable	*Simple regression (R)*	*Multiple regression $R^2=0.53$*	*Simple regression (R)*	*Multiple regression $R^2=0.60$*
VO_{2max}	0.51***		0.51***	
Percentage fat	−0.43**		−0.37*	
FFM	0.37*	(0.43)[a]	0.45**	
Energy balance	−0.43**	(−0.57)[a]	−0.60***	(−0.4)[a]
SMR	0.62***			
TEE			0.71***	(0.6)[a]

FFM: fat-free mass; SMR: sleeping metabolic rate; NEFA: non-esterified fatty acid; TEE: total energy expenditure.
*$P<0.05$; **$P<0.01$; ***$P<0.001$. The values in parentheses are the standard coefficients. Only significant covariates are shown, both from the simple regression and after multiple regression.
[a]Significant determinant during the forward stepwise multiple regression analysis.

Table 4 Muscle fibre composition

	Young	*Elderly*
Type I	46.8±17.9	47.6±11.5
Type IIA	37.1±13.2	40.8±16.4
Type IIB	16.0±11.8	11.8±10.7

Results are mean±s.e. as percentage of the total area occupied by fibres.

Part of the controversy arises from the way in which results are expressed (in absolute or adjusted values), from a potential sexual dimorphism, and from the impact of physical activity. A lower fat oxidation (in absolute values) was shown in elderly women.[7] Resting fat oxidation is also lower in elderly than in young men.[8,9] In a retrospective study, Nagy *et al*[6] found that resting fat oxidation was negatively related to age in 720 subjects, both women and men. On the opposite, Poelhman *et al*[32] found no differences in resting respiratory quotient (RQ) between young and elderly men. Although RQ is not a direct measurement of fat oxidation, it is related to the relative contribution of fatty accids and glucose to energy production. Nagy *et al*[6] and Toth *et al*[33] have clearly demonstrated that FFM is a significant determinant of fat oxidation. When differences in FFM are taken into account, fat oxidation does not differ between young and elderly women.[7] In a study by Sial *et al*,[10] young and elderly men matched for FFM had similar resting fat oxidation. The same was true for young and elderly women.[10] However, elderly persons with a similar FFM to their young pairs cannot be fully representative of the elderly since they do not display the usual changes in body composition. In some[6,34,35] but not all studies,[7] fat oxidation is higher in men than in women. The present results show a lower sleeping fat oxidation in elderly volunteers when expressed in absolute values. We chose to calculate sleeping rates, since with room calorimeters it is possible to average respiratory gas exchange data on a larger time scale. Sleeping fat oxidation measurements are therefore more precise than resting fat oxidation estimated with a canopy.[27] Data presented here also confirm that FFM and energy balance[21,36] are significant predictors of sleeping fat oxidation. However, after adjustment for differences in energy balance and FFM, young and elderly persons do not differ in fat oxidation. In other words, the quantity of fat (in grams) oxidized during a given sleeping period is lower in elderly subjects, probably because of a lower FFM, but these data do not suggest that there is a genuine metabolic defect. The present study did show that women oxidize less fat than men, this also being related to the difference in FFM. Although VO_{2max} is positively related to sleeping fat oxidation, multivariate analysis suggests that it may be because of the correlation with FFM. Furthermore, since participants were chosen to have very different usual physical activity, this parameter (if estimated with VO_{2max}) does not seem to modulate sleeping fat oxidation to a large extent. More than in the resting conditions, it is the 24 h fat oxidation that influences fat balance. Indeed sleeping fat oxidation represents about 24% of daily fat oxidation.[18] The remaining 76% can be influenced by physical activity (since for low-intensity exercise fat is the main fuel[37]) and by meals (since carbohydrates decrease fat oxidation,[12,38]).

Sial *et al*[10] have shown that, when compared to young subjects, elderly men (or women) rely more on fat than on glucose for energy production during exercise. Furthermore, respiratory quotient measured during a 100 W exercise taken by middle-aged men 14 y apart was increased on the second occasion.[39] The present study is the first one to report comparison of 24 h fat oxidation in young and elderly subjects. Data support a reduced fat oxidation in elderly (*vs* young) participants, and in women (*vs* men). Results reported here suggest that fat oxidation is reduced in proportion of the decline in total energy expenditure. In other words, the body needs to oxidize less fuel because of a lower metabolic rate and the reduction concerns fat oxidation, not glucose oxidation. Since fat intake is not always found to decrease with age,[4,5] the reduced fat oxidation (in grams per day) can promote a positive fat and energy balance. Therefore, if the reduced fat oxidation observed in the carefully controlled conditions of this study also stands in free-living conditions, it can be suggested that this is a factor favouring the gain in fat mass. Further studies would be necessary to prove this point in free-living circumstances. It is also likely that in the free-living conditions the difference in usual physical activity is greater between age groups,[19] suggesting an even greater difference in 24 h fat oxidation. There is currently no recommendation about fat intake in elderly subjects. The present study brings arguments for an intervention study aiming at determining whether a reduced fat intake would be beneficial.

In the present study, none of the histological parameters measured in muscle correlated with sleeping or 24 h fat oxidation. Changes in fibre-type composition in muscles with age are debatable, with as many studies showing changes as studies failing to demonstrate an age-related difference (see review by Houmard *et al*[40]). However, at the subcellular level a lower muscle respiratory capacity[41] and hydroxyacyl-C_OA deshydrogenase[42] activities were shown in elderly subjects.

In conclusion, fat oxidation was reduced in elderly men and women during sleeping and over the 24 h; this decline is likely to promote fat mass gain and supports the recommendation that fat intake should be reduced in elderly persons. Factors likely to participate to the lower fat utilization were the reduced FFM and total energy expenditure. Neither sex nor VO_{2max} seemed to influence fat oxidation to a great extent.

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References

1 Cohn SH, Vartsky D, Yasumura S, Sawitsky A, Zanzi I, Vaswani A, Ellis KJ. Compartmental body composition based on total-body nitrogen, potassium and calcium. *Am J Physiol* 1980; **239**: E524–E530.

2 Flynn MA, Nolph GB, Baker AS, Martin WM, Krause G. Total body potassium in aging humans: a longitudinal study. *Am J Clin Nutr* 1989; **50**: 713–717.

3 Morley JE. Anorexia of aging: physiologic and pathologic. *Am J Clin Nutr* 1997; **66**: 760–773.

4 Euronut SENECA investigators. Intake of energy and nutrients. *Eur J Clin Nutr* 1991; **45**: 105–119.

5 Euronut SENECA investigators. Longitudinal changes in the intake of energy and macronutriments of elderly Europeans. *Eur J Clin Nutr* 1996; **50**(Suppl 2): S67–S76.

6 Nagy TR, Goran MI, Weinsier RL, Toth MJ, Schutz Y, Poehlman ET. Determinants of basal fat oxidation in healthy Caucasians. *J Appl Physiol* 1996; **80**: 1743–1748.

7 Calles-Escandon J, Driscoll P. Free fatty acid metabolism in aerobically fit individuals. *J Appl Physiol* 1994; **77**: 2374–2379.

8 Arciero PJ, Gardner AW, Calles-Escandon J, Benowitz NL, Poehlman ET. Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *Am J Physiol* 1995; **268**: E1192–E1198.

9 Horber FF, Kohler SA, Lippuner K, Jaeger P. Effect of regular physical training on age-associated alteration of body composition in men. *Eur J Clin Invest* 1996; **26**: 279–285.

10 Sial S, Coggan AR, Carroll R, Goodwin J, Klein S. Fat and carbohydrate metabolism during exercise in elderly and young subjects. *Am J Physiol*, 1996; **271**: E983–E989.

11 Melanson KJ, Saltzman E, Russell RR, Roberts SB. Fat oxidation in response to four graded energy challenges in younger and older women. *Am J Clin Nutr* 1997; **66**: 860–866.

12 Ritz P, Acheson KJ, Gachon P, Vico L, Bernard JJ, Alexandre C, Beaufrère B. Energy and substrate metabolism during a 42-day bed-rest in a head-down tilt position in humans. *Eur J Appl Physiol* 1998; **78**: 308–314.

13 Martin WH, Dalsky GP, Hurley BF, Matthews DE, Bier DM, Hagberg JM, Rogers MA, King DS, Holloszy JO. Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *Am J Physiol* 1993; **265**: E708–E714.

14 Treuth MS, Hunter GR, Williams M. Effects of exercise intensity on 24-h energy expenditure and substrate oxidation. *Med Sci Sports Exerc* 1996; **28**: 1138–1143.

15 Treuth MS, Hunter GR, Figueroa-Colon R, Goran MI. Effects of strength training on intra-abdominal adipose tissue in obese prepubertal girls. *Med Sci Sports Exerc* 1998; **30**: 1738–1743.

16 Treuth MS, Hunter GR, Weinsier RL, Kell SH. Energy expenditure and substrate utilization in older women after strength training: 24-h calorimeter results. *J Appl Physiol* 1995; **78**: 2140–2146.

17 Poehlman ET, Gardner AW, Arciero PJ, Goran MI, Calles-Escandon J. Effects of endurance training on total fat oxidation in elderly persons. *J Appl Physiol* 1994; **76**: 2281–2287.

18 Morio B, Montaurier C, Ritz P, Fellmann N, Coudert J, Beaufrère B, Vermorel M. Time–course effects of endurance training on fat oxidation in sedentary elderly people. *Int J Obes Relat Metab Disord* 1999; **23**: 1–9.

19 Prentice AM. Energy expenditure in the elderly. *Eur J Clin Nutr* 1992; **46**: 21–28.

20 Rising R, Tataranni PA, Snitker S, Ravussin E. Decreased ratio of fat to carbohydrate oxidation with increasing age in Pima Indians. *J Am Coll Nutr*. 1996; **15**: 309–312.

21 Abbott WG, Howard BV, Christin L, Freymond D, Lillioja S, Boyce VL, Anderson TE, Bogardus C, Ravussin E. Short-term energy balance: relationship with protein, carbohydrate, and fat balances. *Am J Physiol* 1988; **255**: E332–E337.

22 Morio B, Ritz P, Verdier E, Montaurier C, Beaufrère B, Vermorel M. Critical evaluation of the factorial and heart-rate recording methods for the determination of energy expenditure of free-living elderly people. *Br J Nutr* 1997; **78**: 709–722.

23 Visser M GD, Deurenberg P, Wang J, Pierson RN, Heymsfield SB. Density of fat-free body mass: relationship with race, age, and level of body fatness. *Am J Physiol* 1997; **272**: E781–E787.

24 Vaché C, Rousset P, Gachon P, Gachon AM, Morio B, Boulier A, Coudert J, Beaufrère B, Ritz P. Bioelectrical impedance analysis measurements of total body water and extracellular water in healthy elderly subjects. *Int J Obes Relat Metab Disord* 1998; **22**: 537–543.

25 Feinberg M, Favier JC, Ireland-Ripert J. *Répertoire Général des Aliments*. Lavoisier: Paris; 1991.

26 Vaché C, Gachon P, Ferry M, Beaufrère B, Ritz P. Low–cost measurement of body composition with ^{18}O-enriched water. *Diabetes Metab* 1995; **21**: 281–284.

27 Morio B, Beaufrère B, Montaurier C, Verdier E, Ritz P, Fellmann N, Boirie Y, Vermorel M. Gender differences in energy expended during activities and in daily energy expenditures of elderly people. *Am J Physiol* 1997; **273**: E321–E327.

28 Ferrannini E. The theoretical bases of indirect calorimetry. A review. *Metabolism* 1988; **37**: 287–301.

29 Berthon P, Freyssenet D, Chatard JC, Castells J, Mujika I, Geyssant A, Guezennec CY, Dennis C. Mitochondrial ATP production rate in 55 to 73-year-old men: effect of endurance training. *Acta Physiol Scand* 1995; **154**: 269–274.

30 Brooke MH, Kaiser KK. Muscle fiber types: how many and what kind? *Arch Neurol* 1970; **23**: 369–379.

31 Ravussin E, Bogardus C. Relationship of genetics, age, and physical fitness to daily energy expenditure and fuel utilization. *Am J Clin Nutr* 1989; **49**: 968–975.

32 Poehlman ET, McAuliffe TL, Van Houten DR, Danforth E. Influence of age and endurance training on metabolic rate and hormones in healthy men. *Am J Physiol* 1990; **259**: E66–E72.

33 Toth MJ, Arciero PJ, Gardner AW, Calles-Escandon J, Poehlman ET. Rates of free fatty acid appearance and fat oxidation in healthy younger and older men. *J Appl Physiol* 1996; **80**: 506–511.

34 Toth MJ, Gardner AW, Arciero PJ, Calles-Escandon J, Poehlman ET. Gender differences in fat oxidation and sympathetic nervous system activity at rest and during submaximal exercise in older individuals. *Clin Sci* 1998; **95**: 59–66.

35 Calles-Escandon J, Poehlman ET. Aging, fat oxidation and exercise. *Aging (Milano)* 1997; **9**: 57–63.

36 Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, Ravussin E. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol*, 1990; **259**: E650–E657.

37 Henriksson J. Muscle fuel selection: effect of exercise and training. *Proc Nutr Soc* 1995; **54**: 125–138.

38 Ritz P, Krempf M, Cloarec D, Champ M, Charbonnel B. Comparative continuous-indirect-calorimetry study of two carbohydrates with different glycemic indices. *Am J Clin Nutr* 1991; **54**: 1855–859.

39 Gardner AW, Poehlman ET, Sedlock DA, Corrigan DL, Siconolfi SA. A longitudinal study of energy expenditure in males during steady-state exercise. *J Gerontol* 1988; **43**: B22–B25.

40 Houmard JA, Weidner ML, Gavigan KE, Tyndall GL, Hickey MS, Alshami A. Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. *J Appl Physiol* 1998; **85**: 1337–1341.

41 Coggan A, Habash D, Mendenhall L, Swanson S, Kien C. Isotopic estimation of CO_2 production during exercise before and after endurance training. *J Appl Physiol* 1993; **75**: 70–75.

42 Saltin B, Kiens B, Savard G, Pedersen PK. Role of hemoglobin and capillarization for oxygen delivery and extraction in muscular exercise. *Acta Physiol Scand* 1986; **556**(Suppl): 21–32.