Fat Mass Localization Alters Fuel Oxidation during Exercise in Normal Weight Women

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ABSTRACT

ISACCO, L., P. DUCHE, D. THIVEL, A. MEDDAHI-PELLE, S. LEMOINE-MOREL, M. DUCLOS, and N. BOISSEAU. Fat Mass Localization Alters Fuel Oxidation during Exercise in Normal Weight Women. *Med. Sci. Sports Exerc.*, Vol. 45, No. 10, pp. 1887–1896, 2013. **Purpose**: Abdominal and lower body fat mass tissues exhibit particular metabolic profiles at rest and during exercise. However, data are missing in normal weight women during exercise. The purpose of this study was to investigate the effect of low (LA/LB) and high (HA/LB) abdominal to lower body (A/LB) fat mass ratio on metabolic and hormonal responses during exercise in premenopausal normal weight women. **Methods**: After preliminary testing ($\dot{V}O_{2max}$ and body composition assessment), substrate oxidation (RER, lipid, and carbohydrate oxidation rates), metabolic response (glycerol, free fatty acids, and glucose), and hormonal response (insulin, growth hormone, atrial natriuretic peptide, adrenaline, and noradrenaline) were determined during exercise (45 min at 65% of $\dot{V}O_{2max}$) in 21 premenopausal normal weight women (10 HA/LB women vs 11 LA/LB women). **Results**: Waist circumference was significantly higher in HA/LB women compared with LA/LB women ($P < 0.01$). No difference in other anthropometric characteristics, $\dot{V}O_{2max}$, and resting blood values was observed between the two groups. LA/LB subjects exhibited greater lipid oxidation rates compared with HA/LB women during exercise ($P < 0.01$). This occurred with lower plasma insulin ($P < 0.05$) and glucose ($P < 0.05$) concentrations and higher plasma free fatty acids ($P < 0.05$), glycerol ($P < 0.05$), growth hormone ($P < 0.05$), and atrial natriuretic peptide levels ($P < 0.01$) during exercise in the LA/LB group compared with the HA/LB group. **Conclusions**: The present study demonstrated that LA/LB women exhibited an increase in whole-body lipid mobilization and use during exercise compared with HA/LB counterparts. This greater reliance on lipid as fuel metabolism during exercise could be explained by substrate availability and metabolic and hormonal responses. It appeared that LA/LB women exhibited greater metabolic flexibility during an exercise bout of 45 min at 65% of $\dot{V}O_{2max}$ on cycle ergometer. **Key Words:** ADIPOSITY PHENOTYPE, ENERGY METABOLISM, HORMONES, FEMALE POPULATION

Physical activity is one of the primary factors supporting good health and weight management because it favors an increase in lipolysis and promotes fat oxidation (32). In healthy normal weight subjects, sexual dimorphism is observed concerning substrate oxidation during exercise. Compared with men, women exhibit greater reliance on fat oxidation at the same relative exercise intensity (34). Different factors such as specific estrogen levels (14,15), free fatty acids (FFA) availability (7), sex difference in catecholamines stimulated lipolysis (5), or total amount of fat mass (FM) and adipose tissue distribution (16,40) may explain these sex differences. In premenopausal women, a higher proportion of lower body FM (mainly subcutaneous tissue) is observed whereas abdominal FM (mainly visceral) is more developed in men (5,24). Abdominal fat depot is preferentially associated with metabolic disorders, such as insulin resistance, dyslipidemia, and metabolic syndrome, whereas lower body FM has been proposed as a protective factor against metabolic disruptions (9,30,33). From a metabolic point of view, there are some evidences showing that individuals with more abdominal FM than lower body FM display substrate mobilization and utilization impairments (5,18). Specifically, in the female population, from menopause, women exhibit greater abdominal FM (due to lower estrogen levels) and a decline in total lipid oxidation at rest and during exercise (35,36). Kanaley et al. (18) observed a greater FFA availability

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Submitted for publication September 2012.
Accepted for publication March 2013.

0195-9131/13/4510-1887/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e3182935fe3

during exercise in lower body obese women compared with abdominal obese women without any difference in total lipid oxidation rates. A decrease in abdominal to lower body FM distribution (waist to hip ratio) in obese women was associated with higher FFA use at rest (20) and after a low-intensity exercise training program (40% $\dot{V}O_{2max}$, three times per week for 12 wk) (38). If changes in insulin concentrations and insulin and adrenergic sensitivities may in part explain such differences (17), scientific evidences remain limited so far to explain these adaptations.

Several techniques have been used to determine body FM localization, such as selected anthropometric variables (e.g., waist to hip ratio), dual-energy x-ray absorptiometry (DXA), and other imaging techniques (e.g., magnetic resonance imaging) (4,8,18,20,33). Today, DXA is commonly used and offers a precise estimation of total and localized body composition as it is well correlated with magnetic resonance imaging results (4,8,29).

Fat mass distribution in postmenopausal or obese women may induce changes in substrate oxidation at rest or during exercise. However, to the best of our knowledge, there is no data available on how FM distribution affects substrate oxidation in normal weight premenopausal women. Thus, the purpose of the present study was to investigate the influence of abdominal to lower body (A/LB) FM ratio in normal weight premenopausal women on substrate oxidation at rest and during a moderate-intensity exercise. On the basis of sexual dimorphism and data obtained on postmenopausal and obese women, we hypothesized that normal weight premenopausal women, with lower A/LB FM ratio, would exhibit greater reliance on lipid as fuel metabolism during exercise compared with women with higher A/LB FM ratio.

METHODS

Participants

A total of 21 recreationally active women (mean ± SEM age = 22.0 ± 0.59 yr; <4 h of physical activity per week, determined by interviews) were recruited by posted notices and e-mail. None were pregnant, and all had been weight stable at least for 3 months before the start of the experimentation. All subjects were premenopausal with normal weight (body mass index [BMI] values within the healthy weight range ($19.5 < BMI < 25\ kg \cdot m^{-2}$) and waist circumference (WC ≤ 80 cm [2]). As no standard exists concerning A/LB FM ratio in premenopausal lean women, ratios were calculated for the whole population ($n = 21$, 0.80 ± 0.03, ranged from 0.56 to 1.06), and according to the median (0.78), women were divided in two groups: low A/LB FM ratio when the ratio was lower than 0.78 (LA/LB: $n = 11$, 0.68 ± 0.02, ranged from 0.56 to 0.77) and high A/LB FM ratio when the ratio was higher than 0.78 (HA/LB: $n = 10$, 0.90 ± 0.03, ranged from 0.82 to 1.06). As we had an odd number, the sample of both groups could not be equal. To assess the adequacy of the resulting classification, we performed a discriminant analysis introducing lipid oxidation rates during exercise as the main outcome. The analysis confirmed the two group classification.

Among the 21 participants, 11 were oral contraceptive users (low-dose monophasic combined oral contraception; ethinyl estradiol ≤ 30 μg). Ten women were eumenorrheic with regular menstruations (length of cycles = 28 ± 0.5 d for at least 1 yr). Five eumenorrheic women and six oral contraceptive users were part of the LA/LB group, and five eumenorrheic women and five oral contraceptive users were part of the HA/LB group. For better standardization, eumenorrheic women were all tested during their luteal phase, whereas oral contraceptive users participated during the active phase of pill consumption. On the basis of clinical and biochemical findings, none of them had hirsutism or polycystic ovarian syndrome. The characteristics of all participants are presented in Table 1. Informed consent form was obtained from each subject, and the study protocol was approved by the relevant ethical authorities (CPP Sud Est VI-AU818) and complied with the Declaration of Helsinki.

Experimental Design

All women attended the laboratory on three separate occasions. During the first visit, an initial screening interview and a physical examination including anthropometric measurements and body composition assessment were performed before including participants to the study. A second preliminary

TABLE 1. Subject characteristics.

Variables	LA/LB ($n = 11$)	HA/LB ($n = 10$)	P
Age (yr)	22.5 ± 1.16	21.6 ± 0.39	NS
Height (m)	1.65 ± 0.01	1.66 ± 0.01	NS
Weight (kg)	61.13 ± 1.50	63.59 ± 2.19	NS
BMI ($kg \cdot m^{-2}$)	21.31 ± 0.62	23.38 ± 0.74	NS
WC (cm)	70.36 ± 1.07	77.00 ± 2.09	**
%FM	25.61 ± 1.33	27.78 ± 0.10	NS
FFM (kg)	41.44 ± 0.64	42.46 ± 1.69	NS
$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$ FFM)	53.28 ± 1.80	49.79 ± 2.40	NS
A/LB FM ratio	0.68 ± 0.02	0.90 ± 0.03	***
A/LB FFM ratio	0.16 ± 0.02	0.17 ± 0.01	NS

Data are presented as mean ± SEM.
** $P < 0.01$ and *** $P < 0.001$.
NS, no significant difference between groups; A/LB, abdominal to lower body FM ratio; LA/LB, low abdominal to lower body FM ratio group; HA/LB, high abdominal to lower body FM ratio group; BMI, body mass index; WC, waist circumference; FM, fat mass; FFM, fat-free mass; $\dot{V}O_{2max}$, maximal oxygen consumption.

session was then arranged to familiarize individuals with the experimental procedures and to determine their maximal oxygen consumption ($\dot{V}O_{2max}$). In a third experimental session, participants were asked to complete a 45-min cycle test (from 12:00 p.m. to 12:45 p.m.) 3 h after consumption of individually standardized breakfast. On the third session, blood samples (15 mL) were drawn prior, during and at the end of exercise (8:00 a.m., 12:00 p.m., 12:30 p.m., and 12:45 p.m.).

Anthropometric and body composition measurements. A digital scale was used to measure body mass to the nearest 0.1 kg, and barefoot standing height was assessed to the nearest 0.1 cm by using a wall-mounted stadiometer. BMI was calculated as body mass (kg) divided by height squared (m^2). WC was measured in a standing position with a nonelastic tape that was applied horizontally midway between the costal arch and the iliac crest. Body composition (fat-free mass [FFM] and FM) was determined by DXA (fan beam DXA, QDR 4500 x-ray bone densitometer; Hologic, Bedford, MA).

Adipose and FFM tissue localization—abdominal to lower body (A/LB) FFM and FM ratios. From DXA analysis, abdominal FM (visceral and subcutaneous tissues) were determined manually by an experienced technician by drawing a rectangular box around the region of interest between vertebral bodies L1 and L4. The upper limit was set with the horizontal line going through the T12/L1 vertebral space, and the lowest limit was set with a horizontal line going through the L4 and L5 vertebral space (4,13). Data were analyzed with Hologic QDR software for Windows (version 12.6), which integrates whole-body measurements and standard body regions, such as the trunk, arms, and lower limbs delineated by specific anatomical landmarks. Lower body FM (subcutaneous tissue) were also assessed from lower limb measurement.

The A/LB FM ratio was calculated as follows:

$$\text{A/LB FM ratio} = \frac{\text{abdominal fat mass (g)}}{\text{lower body fat mass (g)}}$$

Similarly, A/LB FFM ratio was calculated from FFM located in the abdominal region of interest and lower body FFM. The A/LB FFM ratio was calculated to account for differences between groups for FFM localization.

Preliminary visit—maximal exercise testing. $\dot{V}O_{2max}$ was measured during a graded exhaustive exercise test on a cycle ergometer (Ergoline, Bitz, Germany). After a 4-min warm-up at 75 W, power output was increased by 25 W increments every 3 min until participant's exhaustion (test lasted between 10 and 15 min after warm-up). Participants were strongly encouraged by the experimenters throughout the test to perform a maximal effort. Respiratory gases ($\dot{V}O_2$ and $\dot{V}CO_2$) were measured breath-by-breath through a mask connected to O_2 and CO_2 analyzers (Oxycon pro-Delta, Jaeger, Hoechberg, Germany). $\dot{V}O_{2max}$ was determined as the highest oxygen uptake for a 15-s period. Ventilatory parameters were averaged every 30 s. ECG was monitored throughout the test. The criteria, which have been adopted to assess the achievement of $\dot{V}O_{2max}$, included the following: 1) maximal heart rate within 10% of age-predicted maximal values (220 − age ± 10 beats per minute); 2) RER values higher than 1.1; and 3) oxygen uptake reaching a plateau with increasing work rate.

Experimental session. Participants attended the laboratory at 7:30 a.m. They were asked to avoid alcohol and any food containing biogenic amines during 24 h preceding the test, which can alter the catecholamine analysis. Subjects were also asked to avoid any kind of strenuous exercise the day before the experimental session.

Individually standardized breakfast. Three hours before the start of the exercise testing, subjects received an individually standardized breakfast at 8:15 a.m., which had to be consumed within the next 45 min. Energy content represented 39.75 $kJ \cdot kg^{-1}$ body mass, and qualitative aspects were respected (55% CHO [21.86 $kJ \cdot kg^{-1}$; 1.31 $g \cdot kg^{-1}$], 30% lipid [11.93 $kJ \cdot kg^{-1}$; 0.32 $g \cdot kg^{-1}$], and 15% protein [5.96 $kJ \cdot kg^{-1}$; 0.36 $g \cdot kg^{-1}$]). Breakfast included milk, sugar, bread, butter, and fruit. Coffee and chocolate were not allowed.

In addition, during the week before experimental session, eating habit interviews were realized to control spontaneous subjects' energy intake. All women exhibited well-balanced energy consumption and no difference appeared between the two groups (mean: 7949 $kJ \cdot d^{-1}$; 53% CHO [67.95 $kJ \cdot kg^{-1}$; 4.06 $g \cdot kg^{-1}$], 32% lipid [41.05 $kJ \cdot kg^{-1}$; 1.09 $g \cdot kg^{-1}$], and 15% protein [19.25 $kJ \cdot kg^{-1}$; 1.15 $g \cdot kg^{-1}$]). They also confirmed that the investigated week was representative of their current diet.

Exercise test. Participants performed a 45-min exercise bout on a cycle ergometer at 65% of their $\dot{V}O_{2max}$. The test began 3 h after the end of breakfast, approximately at 12:00 p.m. For each individual, the workload during the session was adjusted throughout the exercise bout to keep $\dot{V}O_2$ constant at 65% of $\dot{V}O_{2max}$. Respiratory gases ($\dot{V}O_2$ and $\dot{V}CO_2$) were measured breath by breath through a mask connected to O_2 and CO_2 analyzers (Oxycon pro-Delta, Jaeger, Hoechberg, Germany). Ventilatory parameters were averaged every 30 s. ECG was monitored throughout the test. RER ($\dot{V}CO_2/\dot{V}O_2$) was calculated at rest (12:00 p.m.) then at the 30th (12:30 p.m.) and the 45th minute (end of exercise: 12:45 p.m.) of exercise. Fat and carbohydrate (CHO) oxidation rates were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements according to Peronnet and Massicotte (31) equations:

$$\text{CHO}\,(\text{mg}\cdot\text{min}^{-1}) = 4.585\dot{V}\text{CO}_2\,(\text{mL}\cdot\text{min}^{-1}) - 3.2255\dot{V}\text{O}_2\,(\text{mL}\cdot\text{min}^{-1})$$
$$\text{Fat}\,(\text{mg}\cdot\text{min}^{-1}) = 1.6946\dot{V}\text{O}_2\,(\text{mL}\cdot\text{min}^{-1}) - 1.7012\dot{V}\text{CO}_2\,(\text{mL}\cdot\text{min}^{-1})$$

Fat and CHO oxidation rates were expressed as milligrams per minute per kilogram of FFM and calculated at rest (12:00 p.m.), at the 30th minute (12:30 p.m.), at and the 45th minute (12:45 p.m.) of exercise. Energy expenditure (kJ) during exercise was calculated as follows: $\dot{V}O_2$ ($L \cdot min^{-1}$) × energy equivalent of oxygen × 45 (duration, min).

Blood samples. When arriving at the laboratory (7:30 a.m.), a venous catheter was inserted into an antebrachial vein. Participants had to sit quietly for 30 min, and then the first blood sample was collected (8:00 a.m.). Samples were first collected in fasting condition (8:00 a.m.) to determine subjects' fasting biologic profile, then right before exercise (12:00 p.m.), at the 30th minute of exercise (12:30 p.m.), and at the end of exercise (45th minute of exercise: 12:45 p.m.). Catecholamines were measured only before (12:00 p.m.) and at the end of exercise (12:45 p.m.).

At every blood collection, hematocrit was immediately measured in duplicates by microcentrifugation (Sigma 1–14). Samples were centrifuged (4000*g* for 10 min at 4°C), aliquoted, and stored at −80°C until analysis.

Biochemical Assays

Plasma triglycerides (TG), total cholesterol, HDL-C, LDL-C, glycerol, FFA, and glucose were assessed using an automated analyzer (Konelab 20; Thermo Electron, Waltham, MA). The biochemical assay kits were purchased from Randox Laboratories (Crumlin, UK). Plasma insulin, growth hormone (GH), testosterone, and atrial natriuretic peptide (ANP) were measured by enzyme-linked immunosorbent assay with Euromedex kits (Paris, France). Plasma adrenaline and noradrenaline were determined by high-performance liquid chromatography, following the method of Koubi et al. (19) and using Euromedex kits (Paris, France).

All blood analyses were performed at the same time. Detection limits of TG, total cholesterol, HDL-C, glycerol, FFA, glucose, insulin, ANP, GH, testosterone, and catecholamines were 0.02 $mmol \cdot L^{-1}$, 0.1 $mmol \cdot L^{-1}$, 0.04 $mmol \cdot L^{-1}$, 0.07 $g \cdot L^{-1}$, 0.140 $mEq \cdot L^{-1}$, 0.1 $mmol \cdot L^{-1}$, 28 $pg \cdot mL^{-1}$, 5.5 $pg \cdot mL^{-1}$, 0.06 $ng \cdot mL^{-1}$, 0.3 $nmol \cdot L^{-1}$, and 0.06 $nmol \cdot mL^{-1}$, respectively. The intra-assay coefficients of TG, total cholesterol, HDL-C, glycerol, FFA, glucose, insulin, ANP, GH, testosterone, and catecholamines were 1%, 1.1%, 0.8%, 3.9%, 1.5%, 1.8%, 2.3%, 2.7%, 2.3%, 4.9%, 5.3%, and 4.5%, respectively.

As plasma volume changes may occur during acute exercise, all metabolic and hormonal concentrations were corrected according to plasma volume fluctuations from hematocrit changes as proposed by Van Beaumont (39).

Calculations

Metabolic and hormonal responses during exercise were also expressed as the area under the response curve (AUC) calculated with trapezoid integration. Two indices of insulin resistance were calculated from glucose and insulin concentrations: glucose–insulin ratio (G/I) and Homeostatic Model Assessment-Insulin resistance (HOMA-IR) index: (fasting insulin level × fasting glucose level) / 22.5. From WC and TG values, we determined whether women exhibited hypertriglyceridemic waist (HTGW). HTGW is characterized by a WC ≥88 cm and TG concentrations ≥150 $mg \cdot dL^{-1}$ (22).

Statistical Analysis

All statistical analyses were carried out with Statistica software (version 8.00, USA). On the basis of previous results measuring CHO oxidation during exercise in women (37), sample size estimation was performed before the beginning of the protocol to ensure a statistical power >90% (considering alpha level = 0.05, SD = 1 $mg \cdot min^{-1} \cdot kg^{-1}$, and minimal difference between groups from 1.5 $mg \cdot min^{-1} \cdot kg^{-1}$).

Results are expressed as mean ± SEM. The normality of the distribution was tested with the Kolmogorov–Smirnov test, and the homogeneity of variance was tested with the *F*-test. To assess the adequacy of the group classification, we performed a discriminant analysis introducing lipid oxidation rates during exercise as the main outcome. The analysis confirmed the two group classification. Physiological and anthropometric characteristics of the subjects were compared between groups with unpaired *t*-tests. The effect of group and time for all other variables was assessed by using a one-way (factor: group) ANOVA with repeated measures (time). When a significant effect was found, *post hoc* multiple comparisons were made by Newman–Keuls. Pearson correlations were used to test relationships between variables. Statistical significance was set up at $P < 0.05$.

RESULTS

Participant Characteristics

Total body mass, BMI, %FM, FFM, and A/LB FFM ratio were not significantly different between groups ($P = 0.57$, 0.53, 0.21, 0.58, and 0.13, respectively), whereas significant A/LB FM ratio characterized each group ($P = 0.0005$) (Table 1). WC was higher in the HA/LB group compared with the LA/LB group ($P = 0.009$) (Table 1). Physical fitness level, assessed by $\dot{V}O_{2max}$ values, was not significantly different between the two groups ($P = 0.34$). Biological profiles in the fasting condition were similar between the LA/LB and the HA/LB groups, and no woman exhibited HTGW (Table 2).

Metabolic and Hormonal Responses to Exercise

There was no significant difference ($P = 0.59$) in energy expenditure during exercise between the LA/LB group (1236 ± 18 kJ) and the HA/LB group (1223 ± 21 kJ).

Substrate Use

RER. RER values were significantly greater in the HA/LB group compared with the LA/LB group during the exercise session ($P = 0.005$), but no time effect was observed ($P = 0.11$).

Substrate oxidation ($mg \cdot min^{-1} \cdot kg^{-1}$ FFM). At rest, no difference was observed in CHO and lipid oxidation rates between the two groups ($P = 0.10$ and 0.11, respectively). During exercise, CHO oxidation rates were lower ($P = 0.01$) and lipid oxidation rates were higher ($P = 0.002$) in the LA/LB group compared with the HA/LB group (Fig. 1). A

TABLE 2. Biological profiles in fasting condition in both groups.

Variables	LA/LB (n = 11)	HA/LB (n = 10)	P
HDL-C (mmol·L^{-1})	4.22 ± 0.22	3.73 ± 0.22	NS
LDL-C (mmol·L^{-1})	3.24 ± 0.26	3.52 ± 0.28	NS
Total cholesterol (mmol·L^{-1})	5.23 ± 0.28	5.10 ± 0.21	NS
TG (mmol·L^{-1})	1.11 ± 0.11	0.97 ± 0.10	NS
Glucose (mmol·L^{-1})	5.22 ± 0.11	5.38 ± 0.11	NS
Insulin (pmol·L^{-1})	24.10 ± 1.25	19.52 ± 1.25	NS
G/I	0.82 ± 0.18	0.68 ± 0.17	NS
HOMA-IR	0.82 ± 0.18	0.84 ± 0.31	NS
Testosterone (nmol·L^{-1})	6.11 ± 1.25	6.87 ± 1.49	NS
HTGW	No	No	

Data are presented as mean ± SEM.
NS, no significant difference between groups for all variables; LA/LB, low abdominal to lower body FM ratio group; HA/LB, high abdominal to lower body FM ratio group; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HTGW, hypertriglyceridemic waist.

time effect was also observed for CHO ($P < 10^{-6}$) and lipid ($P < 10^{-6}$). A group–time interaction indicated that the LA/LB group had higher lipid oxidation rates at 30 and 45 min of exercise ($P = 0.008$) compared with the HA/LB group (Fig. 1B). A significant correlation was observed between the lipid oxidation rates and the A/LB FM ratio ($r = -0.51$, $P = 0.01$), but no significant correlation was observed between the CHO oxidation rates and the ratio ($r = 0.24$, $P = 0.25$). Lipid provided 21.34% and 12.15% of the total energy expenditure during exercise in LA/LB and HA/LB women, respectively.

Glucose and insulin responses. Under resting conditions and in fed state (12:00 p.m., before exercise), plasma glucose concentrations were not different between the two groups ($P = 0.10$), whereas plasma insulin concentrations were lower in LA/LB compared with HA/LB ($P = 0.0009$). G/I at 12:00 p.m. demonstrated a significant group difference as LA/LB women exhibited greater values than HA/LB (0.19 ± 0.03 and 0.09 ± 0.03, respectively, $P = 0.04$), indicating a lower insulin sensitivity in HA/LB for the same glycemia.

Exercise induced a decrease in plasma glucose levels in both groups (time effect: $P = 0.02$). Plasma glucose values were lower in LA/LB compared with HA/LB (group effect: $P = 0.03$) (Fig. 2A). During exercise, glucose AUC was also lower in the LA/LB group than that in the HA/LB group ($P = 0.0006$) (Fig. 2B).

Insulin concentrations decreased during exercise in both groups ($P < 10^{-6}$). A significant difference was observed between groups ($P = 0.02$) with greater insulin values in HA/LB. A group–time interaction showed that the LA/LB group had lower insulin concentrations at rest and at 30 min of exercise ($P = 0.001$) compared with HA/LB (Fig. 2C). During exercise, AUC for insulin was higher in HA/LB than that in LA/LB women ($P = 0.0004$) (Fig. 2D). No significant correlation was observed for insulin ($r = 0.16$, $P = 0.49$), glucose ($r = 0.29$, $P = 0.20$) concentrations, and A/LB FM ratio.

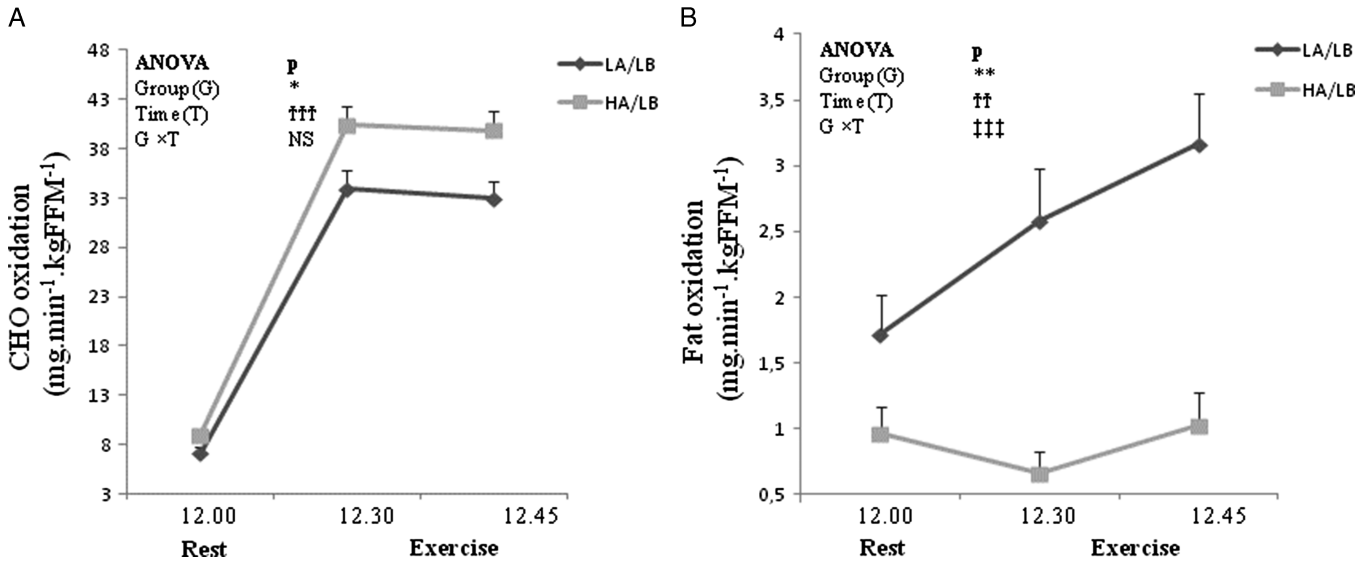

FIGURE 1—Substrate oxidation rates at rest and during exercise in both groups. A, CHO oxidation rates at rest and during exercise in both groups. B, Fat oxidation rates at rest and during exercise in both groups. Data are presented as mean ± SEM. NS, not statistically significant; group effect, *$P < 0.05$ and **$P < 0.01$; time effect, ††$P < 0.01$ and †††$P < 0.001$; interaction group × time, ‡‡‡$P < 0.001$. LA/LB, low abdominal to lower body FM ratio group; HA/LB, high abdominal to lower body FM ratio group; CHO, carbohydrates.

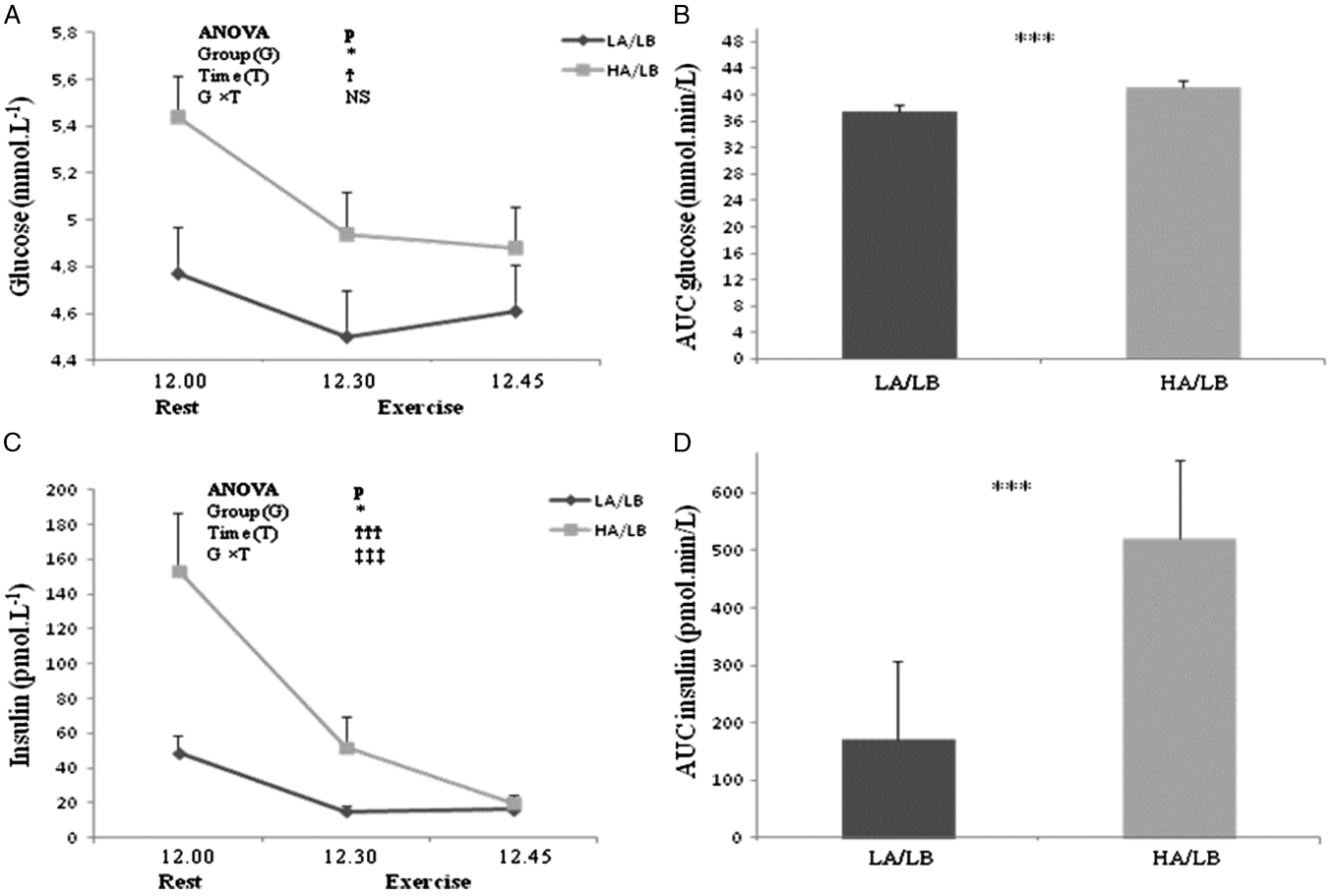

FIGURE 2—Glucose and insulin responses at rest and during exercise in both groups. A, Glucose responses at rest and during exercise in both groups. B, AUC for glucose in both groups. C, Insulin responses at rest and during exercise in both groups. D, AUC for insulin in both groups. Data are presented as mean ± SEM. NS, not statistically significant; group effect, *$P < 0.05$ and ***$P < 0.001$; time effect, †$P < 0.05$ and †††$P < 0.001$; interaction group × time, ‡‡‡$P < 0.001$. LA/LB, low abdominal to lower body FM ratio group; HA/LB, high abdominal to lower body FM ratio group.

FFA and glycerol responses. Plasma FFA and glycerol concentrations were not different between the two groups at 12:00 p.m. ($P = 0.79$ and 0.33, respectively). Exercise induced an increase in plasma FFA concentrations in all subjects (time effect: $P = 0.003$), with lower values in HA/LB compared with LA/LB (group effect: $P = 0.03$) (Fig. 3A). A group–time interaction demonstrated that LA/LB presented higher FFA levels at the 30th and 45th minute of exercise ($P = 0.006$). During exercise, AUC for FFA was higher in LA/LB compared with HA/LB women ($P = 0.0006$) (Fig. 3B). A significant correlation was found between FFA concentrations and A/LB FM ratio ($r = -0.47$, $P = 0.03$).

Glycerol concentrations increased during exercise (time effect: $P = 10^{-6}$) in both groups and were higher in LA/LB than that in HA/LB (group effect: $P = 0.03$) (Fig. 3C). Glycerol concentrations were significantly higher in LA/LB compared with HA/LB at the 30th and 45th minute of exercise (group–time interaction: $P = 0.02$). During exercise, AUC for glycerol was higher in LA/LB than that in HA/LB women ($P = 0.0005$) (Fig. 3D). No significant correlation was found between glycerol concentrations and A/LB FM ratio ($r = -0.25$, $P = 0.29$).

GH and ANP responses. Under resting conditions, plasma GH concentrations were not significantly different between the two groups ($P = 0.37$) (Fig. 4A). A group effect was observed for GH concentrations with greater plasma values in LA/LB when compared with HA/LB ($P = 0.03$) (Fig. 4A). The exercise induced an increase in plasma GH concentrations in all subjects (time effect: $P = 0.005$). During exercise, AUC for GH was lower in HA/LB than that in LA/LB women ($P = 0.003$) (Fig. 4B).

A group effect was observed for ANP concentrations with greater values in LA/LB than HA/LB women at rest ($P = 0.01$) and during exercise ($P = 0.009$) (Fig. 4C). During exercise, AUC also showed a group effect with greater ANP values in the LA/LB group ($P = 0.0007$) (Fig. 4D). No significant correlation was observed for GH ($r = -0.20$, $P = 0.40$) and ANP ($r = -0.27$, $P = 0.24$) concentrations and A/LB FM ratio.

Adrenaline and noradrenaline responses. Plasma adrenaline and noradrenaline concentrations were not different between groups at 12:00 p.m. ($P = 0.89$ and $P = 0.76$ for adrenaline and noradrenaline, respectively). Catecholamine responses did not differ between the two groups during exercise ($P = 0.91$ and $P = 0.88$ for adrenaline and

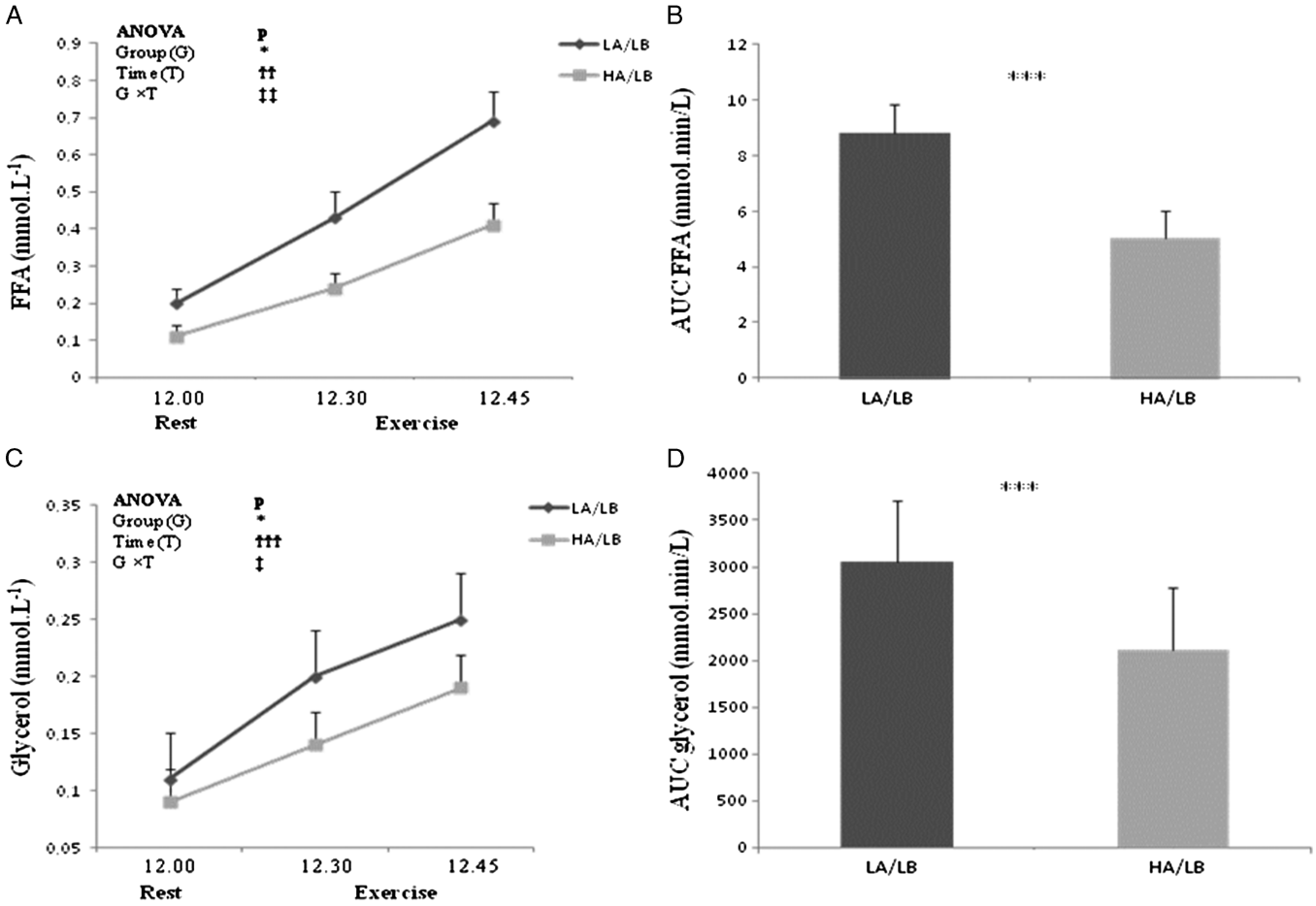

FIGURE 3—FFA and glycerol responses at rest and during exercise in both groups. A, FFA responses at rest and during exercise in both groups. B, AUC for FFA in both groups. C, Glycerol responses at rest and during exercise in both groups. D, AUC for glycerol in both groups. Data are presented as mean ± SEM. Group effect, *$P < 0.05$ and ***$P < 0.001$; time effect, ††$P < 0.01$ and †††$P < 0.001$; interaction group × time, ‡$P < 0.05$ and ‡‡$P < 0.01$. LA/LB, low abdominal to lower body FM ratio group; HA/LB, high abdominal to lower body FM ratio group.

noradrenaline, respectively). A time effect was observed for catecholamines with greater values at the end of exercise (12:45 p.m.) compared with resting values ($P = 0.0005$ and $P < 10^{-6}$ for adrenaline and noradrenaline, respectively). No significant difference was observed between groups for AUC during exercise ($P = 0.90$ and $P = 0.65$ for adrenaline and noradrenaline, respectively), and there was no significant correlation between catecholamine ($r = 0.11$, $P = 0.66$; $r = 0.06$, $P = 0.80$ for adrenaline and noradrenaline, respectively) concentrations and A/LB FM ratio.

DISCUSSION

The present data indicated that A/LB FM ratio alters fuel use and metabolic and hormonal responses during exercise in premenopausal women with normal weight and WC. In this population, a lower A/LB FM ratio was associated with higher lipid mobilization and oxidation and a greater metabolic flexibility during exercise.

General characteristics. In the present study, $\dot{V}O_{2max}$, total FM, total, and localized FFM values were not significantly different between the two groups. However, they differed in terms of WC and A/LB FM ratio. As in normal weight women (20), normal body and FM values were associated with healthy metabolic profiles. To date, most studies showing that abdominal FM expansion is associated with insulin resistance and dyslipidemia whereas lower body FM is presented as a protective factor have been performed in subjects with obesity or type 2 diabetes (1,25). The present results showed that in normal weight individuals without HTGW or insulin resistance, there are no significant differences between the HA/LB and the LA/LB groups for fasting glucose and insulin concentrations, G/I, and HOMA-IR. However, measurements performed 3 h after a standardized breakfast showed that if plasma glucose concentrations still did not differ between both groups, insulin levels were significantly higher in the HA/LB group ($P < 0.01$). This suggests lower insulin sensitivity in the postprandial state in normal weight women with greater A/LB FM ratio, similarly to what is observed in overweight and obese women.

Abdominal FM in women has been shown to be associated with high plasma androgen levels (6), especially in conditions such as polycystic ovarian syndrome where women often present hyperandrogenism combined with

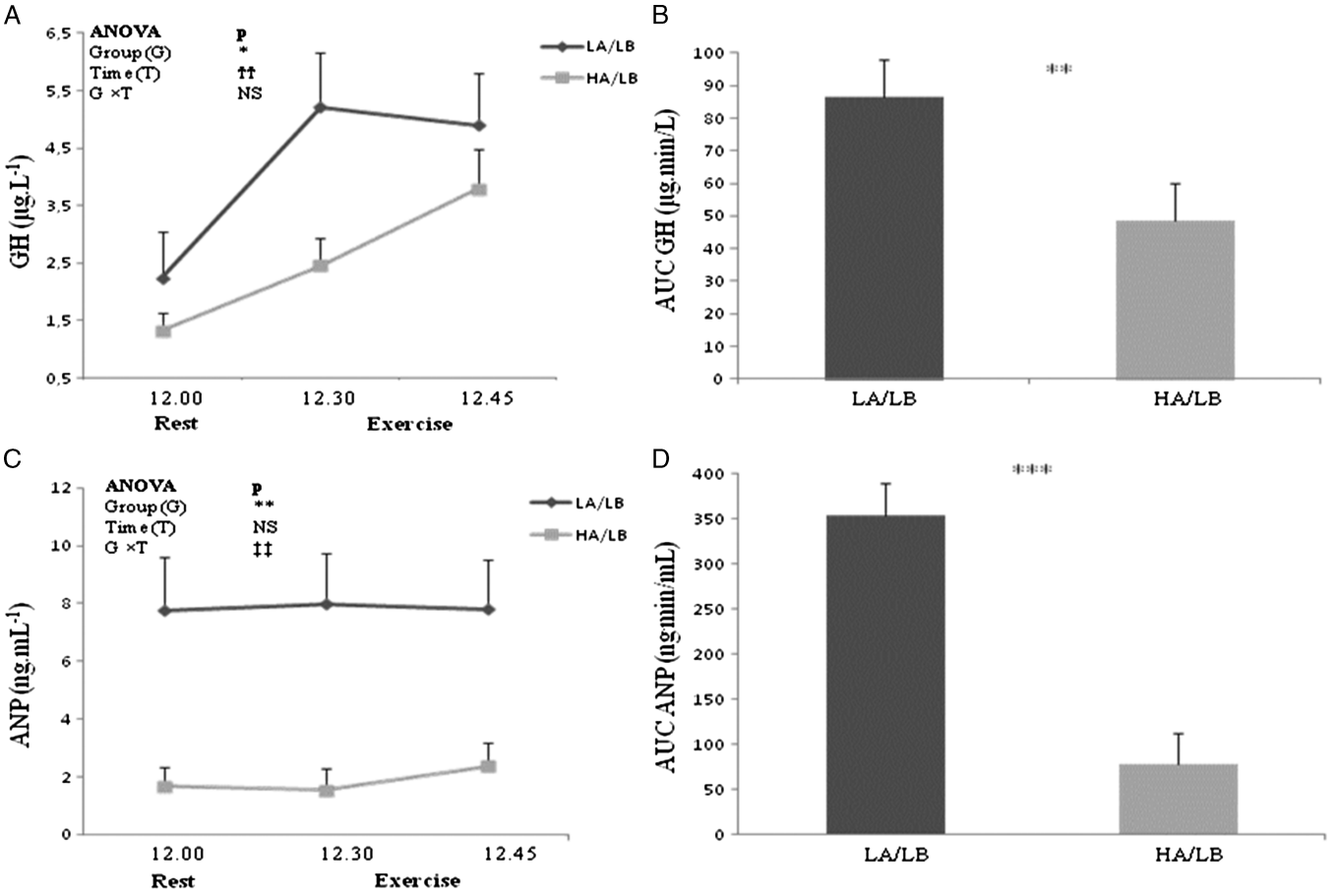

FIGURE 4—GH and ANP responses at rest and during exercise in both groups. A, GH responses at rest and during exercise in both groups. B, AUC for GH in both groups. C, ANP responses at rest and during exercise in both groups. D, AUC for ANP in both groups. Data are presented as mean ± SEM. NS, not statistically significant; group effect, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.01$; time effect, ††$P < 0.01$; interaction group × time, ‡‡$P < 0.01$. LA/LB, low abdominal to lower body FM ratio group; HA/LB, high abdominal to lower body FM ratio group.

abdominal obesity, hyperinsulinemia, and oligomenorrhea (11). In the present study, however, we did not observe any effect of a higher A/LB FM ratio on the androgenic profile of premenopausal women with normal weight and WC.

Metabolic and hormonal responses to exercise. Although there was no difference between the two groups at rest for substrate oxidation variables, women in the LA/LB group exhibited higher plasma FFA and glycerol levels indicating greater lipid mobilization as well as higher lipid use and contribution to energy expenditure during exercise. Although we did not assess the depletion of substrate pools such as glycogen and intramuscular TG, which contribute significantly to energy expenditure during exercise at 65% of $\dot{V}O_{2max}$ (32), we could establish that whole-body substrate oxidation is affected by the adipose tissue localization, independently of FFM localization. This suggests that women with greater A/LB FM ratio exhibited a reduced ability to switch efficiently to a greater reliance on lipid oxidation during prolonged exercise, that is, altered metabolic flexibility (10). The negative correlation between A/LB fat mat mass ratio and lipid oxidation rates ($P < 0.05$) and the specific metabolic and hormonal responses to exercise further confirm that increased abdominal fat storage altered lipid metabolism in premenopausal women with normal weight and WC. Thus, a higher A/LB FM ratio in normal weight women could be associated with low lipid turnover during exercise and may favor a risk of lipotoxicity and insulin resistance with age advancing.

There are, to our knowledge, little data available looking at the effect of preferential fat deposition at the abdominal or peripheral level on fuel metabolism in premenopausal normal weight women with normal WC. Toth et al. (36) observed lower lipid oxidation rate in postmenopausal compared with premenopausal women, which was in part explained by increased abdominal FM. Using the waist-to-hip ratio to assess adipose tissue localization, Kanaley et al. (18) observed that women with lower body obesity had greater plasma FFA responses but similar total lipid oxidation when compared with women with upper body obesity. In contrast, in our study, both plasma FFA and glycerol concentrations and lipid oxidation rates were increased in the LA/LB group compared with the HA/LB group. Furthermore, a negative correlation was found between A/LB FM ratio and FFA concentrations, indicating that, in addition to reduced ability for lipid oxidation, a high A/LB FM ratio in normal weight women is associated with low lipolytic ability.

However, this is inconsistent with previous studies showing that under resting condition, excess abdominal FM is rather characterized by greater lipolytic activity with lower glucose disposal and oxidation (23). At rest, Okura et al. (30) reported in overweight women that an excess in intra-abdominal FM resulted in an increase of lipolytic activity of intra-abdominal FM tissue and chronic excessive FFA release into the circulation leading to metabolic abnormalities. In our study, the transient increase in lipolytic activity induced by exercise, as opposed to chronically increased lipolysis with obesity, is likely to explain the lack of metabolic disturbance with high FFA plasma levels in our population. Considering the role of lipolytic and antilipolytic hormones (and adipocyte sensitivity) as factors determining lipid availability and use, we investigated hormonal responses to exercise (12).

Significantly higher postprandial plasma insulin levels in the HA/LB group, despite unaltered plasma glucose concentrations, indicated lower insulin sensitivity with preferential abdominal fat deposition. Insulin inhibits lipolysis and FFA flux and stimulates glucose uptake (21). Lipolysis inhibition and increased glucose uptake during exercise, caused by higher insulin levels in the HA/LB group, may explain lower lipid mobilization and contribution of lipid to energy expenditure compared with the LA/LB group. Difference in lipid metabolism between the two groups may in part be linked to differences in lipolytic stimulation by GH and ANP levels, which increased significantly more in response to exercise in the LA/LB group than that in the HA/LB. ANP is released from the heart and exerts its lipolytic effect through an increase in intracellular cGMP concentrations (21), a signaling pathway independent of insulin and catecholamines (mediated by cAMP). However, as GH response requires 2–3 h to increase lipid mobilization, its lipolytic effect was most likely negligible during the 45-min exercise performed in the present study (26,28). Adrenaline and noradrenaline are other potent lipolytic hormones acting through β1, β2, and β3 adrenoreceptors whereas they inhibit lipolysis through α2 adrenoreceptors (16,27). In resting conditions, intra-abdominal adipocytes are more sensitive to β-adrenergic stimulation and less sensitive to α-adrenergic receptors than subcutaneous lower body FM tissue (3). Contrary to ANP and GH, we did not observe any difference in catecholamine levels between the LA/LB and the HA/LB groups, although their level increased during exercise. Hence, this does not support a role for catecholamines in explaining differences for lipid metabolism between the LA/LB group and the HA/LB group. It can be first hypothesized that β-adrenergic sensitivity is increased in all adipose tissues during exercise (21) or that cycling only provides a lipolytic stimulus localized to the lower limbs with little effect on lipid mobilization in abdominal adipose tissue. Investigation during arm cranking exercise or running in the LA/LB and the HA/LB groups could allow determining the effects of different muscle groups on lipid metabolism.

CONCLUSIONS

Even if further studies with greater sample size are needed to corroborate the present results, our study showed that using the A/LB FM ratio to assess preferential fat deposition allowed detecting altered whole-body lipid metabolism and hormonal responses during exercise in premenopausal women with normal weight, WC, and similar A/LB FFM ratio. Increased plasma FFA and glycerol levels and higher lipid oxidation rate in the LA/LB group indicated a greater ability for lipid mobilization and use in women with preferential lower body fat deposition during a 45-min cycling exercise. Lower insulin levels and higher ANP concentrations in this group of women are the factors most likely to explain better lipid mobilization when compared with women with higher abdominal fat deposition. Moreover, a lower metabolic flexibility in HA/LB women during exercise may place them at greater metabolic risks with age advancing.

The effect of adipose tissue deposition should be taken into account when physical activity is prescribed with the purpose of increasing energy expenditure, fat use, and maintaining adequate level of body fat. Similar lipid oxidation and level of adipose tissue lipolysis may be reached with exercise differing in intensity, duration, and modalities in LA/LB and HA/LB women. This may be of particular interest both in rehabilitation programs for athlete women and in physical activity prescriptions in recreationally active women.

The authors are grateful to "Ministère Jeunesse et Sport" and "Fédération Française d'Education Physique et de Gymnastique Volontaire" for their financial support.

The authors thank the staff at the Clermont-Ferrand University Hospital of Clermont-Ferrand, the Biologic Pharmacologic Laboratory of Rennes, Chrystele Jouve from the Laboratory of Human Nutrition (INRA, Clermont-Ferrand), Fatima Medjahed-Hamidi, and Graciela Pavon-Djavid from the Inserm U698 (Paris) for their technical assistance during the experimental protocol and analyses. We also thank Professor Eddy Van Praagh for his help to improve the English language of the manuscript.

All authors have been involved in the design and conduct of the study and/or in the data analysis and manuscript writing.

There is no conflict of interest.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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