Body composition changes affect energy cost of running during 12 months of specific diet and training in amateur athletes.

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Complete List of Authors:	Ghiani, Giovanna; University of Cagliari, Department of Medical Sciences Marongiu, Elisabetta; University of Cagliari, Department of Medical Sciences, Sports Physiology Lab. Melis, Franco; University of Cagliari, Department of Medical Sciences Angioni, Giuseppina; University of Cagliari, Department of Medical Sciences Sanna, Irene; University of Cagliari, Department of Medical Sciences Loi, Andrea; University of Cagliari, Department of Medical Sciences, Sports Physiology Lab. Pusceddu, Matteo; university of cagliari, department of medical science, sport physiology Lab. Pinna, Virginia; University of Cagliari, Department of Medical Sciences Crisafulli, Antonio; University of Cagliari, Department of Medical Sciences Tocco, Filippo; University of Cagliari, Department of Medical Sciences
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2 **diet and training in amateur athletes.**

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4 Giovanna Ghiani,¹ Elisabetta Marongiu,¹ Franco Melis,¹ Giuseppina Angioni,¹ Irene
5 Sanna,¹ Andrea Loi,¹ Matteo Pusceddu,¹ Virginia Pinna,¹ Antonio Crisafulli,¹ and
6 Filippo Tocco.¹

7

8 *¹Department of Medical Sciences, School of Sport Medicine, Sport Physiology Lab.,*
9 *University of Cagliari, Italy*

10

11 Correspondence to:

12 Giovanna Ghiani

13 Department of Medical Sciences,

14 School of Sport Medicine, Sport Physiology Lab.,

15 University of Cagliari, Italy

16 Via Porcell 4, 09124 Cagliari (Italy)

17 Tel +390706758927

18 Fax +390706758917

19 E-mail: giovanna.ghiani@me.com

Abstract

Considering the relation between body weight-composition and energy cost of running, we tested the hypothesis that modifying body composition by means of a combined protocol of specific diet and training, the energy cost of motion (C_r) may be reduced. 45 healthy and normal-weight subjects were divided into three groups that performed a different treatment: the first group attended only a dietary protocol (D), the second only a running program (R) and the third both (R&D). Each subject underwent three anthropometric and exercise evaluation tests during one year (at T0, T6, T12) to assess body composition and C_r adjustments. The mean fat mass (FM) values were reduced in R&D from 12.0 ± 4.0 to 10.4 ± 3.0 kg ($p < 0.05$ T0 vs. T12) and in the D group from 14.2 ± 5.8 to 11.6 ± 4.7 kg ($p < 0.05$ T0 vs. T12). Conversely, the mean fat free mass values increased in R&D (from 56.3 ± 8.8 to 58.3 ± 9.8 kg, $p < 0.05$ T0 vs. T12) and in the D group (from 50.6 ± 13.2 to 52.9 ± 13.6 kg, $p < 0.05$ T0 vs. T12). The mean C_r values of the 2 groups were significantly modified throughout the one year protocol (1.48 ± 0.16 and 1.40 ± 0.15 kcal·kg^{-b}·km⁻¹ in the R&D group at T0 and T12 respectively; 1.83 ± 0.17 and 1.76 ± 0.23 kcal·kg^{-b}·km⁻¹ in D group at T0 to T12 respectively). The R&D and D groups that underwent the diet protocol had a positive change in body composition during the year (FM-fat free mass ratio decline) which determined a C_r reduction.

Keywords: Diet; body composition; energy cost of motion; fat mass; free fat mass; running program.

Introduction

It is well known that in endurance running, athletes with the lowest body mass (BM) generally achieve the best results (Sedeaud 2014). This is probably due to the fact that generating the force to support body weight is the primary determinant of the metabolic cost of running (Farley and McMahon 1992; Lacour and Bourdin 2015). Especially a low percentage of fat mass (FM) characterizes middle and long-distance runners and probably plays a pivotal role in their performances (Kong and de Heer 2008). Indeed, the gender differences of body fat content appears to be one of the more important factors related to the better results of male runners (Wilmore et al. 1977), and if male and female runners are matched on ability in the same distance, they show a similar percentage of FM (Pate et al. 1995). A recent study shows that in obese subjects Cr appears to be constant rather than rising with increasing BM, probably because elastic tissue can adapt to this increment, thus maintaining its ability to store elastic energy (Taboga et al. 2012). On the other hand, Teunissen and coll. (2007) found that body mass reduction caused a decrease in net metabolic rate although not proportionally. Nevertheless, the authors of the aforementioned study reduced body mass by means of a harness system while not modifying body composition. Theoretically, inducing a shift between FM versus fat free mass, because of augmented body ability to store and release elastic energy, cost of running (Cr) should be reduced.

Metabolic measurement is still the criterion for analysis of endurance performance in running (Lacour and Bourdin 2015). Besides, the way in which different body size and anthropometrical characteristics impact performance has been recently investigated in runners, highlighting the need to taking into account the role of allometric scaling in the relationship between metabolic Cr and the running performance (Tartaruga et al. 2010;

66 Tartaruga et al. 2013a). In the light of the information reported above and considering that
67 knowledge of relations between body mass-composition changes and the energy cost of
68 running is rather scanty, we tested the hypothesis that Cr could be reduced by modifying
69 body composition with a specific diet and running program. To do this, the running
70 efficiency of 45 subjects (divided in 3 groups that performed a different treatment) was
71 monitored through one year by means of metabolic measurements, also taking into account
72 the influence of allometric scaling (Tartaruga et al. 2013b).

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Materials and Methods

Subjects

45 healthy and normal weight subjects (average BM index was 23.6 ± 2.3 SD), 29 males and 16 females, were enrolled. They were recruited with an advice on facebook. Inclusion criteria were: a) to have an active lifestyle and practice running or other sport activities at recreational level for at least three days a week, since at least five years; b) to have no specific dietary regimen; c) to have no metabolic diseases.

Anthropometric and metabolic characteristics of participants at the start of the study are shown in Table 1. All anthropometric measurements were taken using the Anthropometric Standardization Reference Manual by Lohman et al. (1988).

All participants were interviewed about their clinical history, so as to exclude any medication or drug treatment that could influence their metabolism. They were informed about the protocol and written consent was obtained. The study was approved by the local ethics committee and was carried out in accordance with the Helsinki Declaration.

Experimental design

Participants were divided in three groups: a group (no runners) attended only a dietary protocol (D), a group attended only a running program (R) and the last one (R&D), both. Each subject was measured anthropometrically and performed a treadmill test to assess body composition and Cr changes at entry (T0), after six months (T6) and finally after one year from entry (T12).

Anthropometric and diet approach.

For each subject basic anthropometric measurements including body mass (BM, kg), height, and body composition were taken. Body surface area (BSA) was also calculated by means of the Mosteller's formula (Mosteller 1987). In detail, body composition was

assessed by means of the plicometry method (triceps, biceps, sub scapular, and supra iliac skin folds). The skin fold thickness was measured with a Lange skin fold caliper (Beta technology) and FM % was predicted by using the Durnin and Womersley (1974) skin fold thickness equation. From the difference between BM and FM expressed in kg, fat free mass (kg) was calculated. As intertester variability is the major source of error in skin fold measurements all skin folds were taken on the right side of the body by the same experienced technician each time. Intratester reliability check was conducted on 16 subjects (8 females and 8 males) prior to testing and no significant differences were observed between the 2 trials for the sum of 4 skin folds. Moreover all measurements were always taken at the same time of the day and the participants were instructed to abstain from exercise prior to their test and avoid alcohol and caffeinated beverages 24 h prior to the test. In addition, participants were asked not to drink any fluid 4 h prior to their test and urinate within 30 min of test. It was checked that all female participants were in the same stage of their menstrual cycle during days of data collection. Skin fold thickness was taken three times and the average value was used in the equation.

Food frequency questionnaire.

Each subject was asked to fill in a semi-quantitative food-frequency questionnaire (Fidanza et al. 1995) to assess their usual intake, both in calories and nutrients. The questionnaire consists of 16 printed forms and 16 pages with colored photos of the most common foods and courses in the Italian diet. Instructions are included and the questionnaire was repeated at each control (at T6 and T12) to check the adherence and the compliance to the given diet (see Table 2).

Energy Expenditure.

A multisensory accelerometer (SenseWear Pro armband, Bodymedia, Inc., Pittsburgh, PA, USA) which was worn on the upper right arm was used to assess subjects' daily energy expenditure (EE). The device estimates EE using measurements from a biaxial accelerometer and sensors that quantify galvanic skin response, heat flux and skin temperature. The biaxial accelerometer records the number of steps and the duration of physical activity (Drenowatz and Eisenmann 2011). The physical activity level (PAL) of each participant was calculated by dividing the total daily energy expenditure by energy expenditure during sleep (Sijtsma et al. 2013).

Dietary protocol.

A personalized diet providing all the essential energy and nutrients required was given to R&D and D groups. The diet provided a personalized number of calories amounting to about 20% proteins, 50% carbohydrates and 30% fats. Total daily intake was divided into three main meals: breakfast (30%), lunch (45%) and dinner (25%). The choice of giving athletes a breakfast richer than their usual one was made because recent studies show a close link of daily meal distribution, obesity, mass and fat loss (Jakubowicz et al. 2013; Wang et al. 2013).

Training program.

Groups R and R&D attended a running program of at least 40 km per week in outdoor conditions (no treadmill) to attain an adequate energy expenditure (Slentz et al. 2005). In detail, participants were asked to perform 4 running sessions (about 10 km per session at the same speed on which the Cr was calculated, see below) per week spaced by 1 day of rest. The goal was to reach a total energy expenditure of about 400-600 kcal per day in order to evoke an adequate increase of metabolism and fat free mass (Zuti and Goldin

1976). To check the adherence and the compliance to the training program participants recorded the distance and the speed of training sessions by a standard GPS chronometer.

Treadmill tests.

Each subject underwent a preliminary incremental treadmill running test to volitional exhaustion (RunRace, Tecnogym, Forlì, Italy) to assess his anaerobic threshold (AT). After 3 min of rest in the standing position on the treadmill, the subject started to run at 6 km h⁻¹ and speed was increased by 1 km·h⁻¹ every 3 minutes, until exhaustion. The treadmill gradient was maintained at 1% throughout steps to reflect the energy cost of outdoor running (Jones and Doust 1996). Achievement of maximal oxygen uptake (VO₂max) was considered as the attainment of at least two of the following criteria: 1) a plateau in VO₂, despite increasing speed; 2) a respiratory exchange ratio >1.10; and 3) a heart rate ± 10 beats·min⁻¹ of predicted maximum heart rate (HR) calculated as 220-age (Howley et al. 1995). The ventilatory equivalent method was used to determine AT (Davis et al. 1979). Briefly, AT was assessed as an increase in ventilatory equivalent for oxygen, [i.e. pulmonary ventilation (V_E) and oxygen consumption (VO₂) ratio, (V_E/VO₂)] with no associated increase in the ventilatory equivalent for carbon dioxide [i.e. pulmonary ventilation (V_E) and carbon dioxide output (VCO₂) ratio, (V_E/VCO₂)].

After at least one week each participant performed a specific treadmill test to calculate the energy cost of running. After a warm-up, participants ran at a speed 1 km·h⁻¹ below the AT for 5 minutes to obtain a steady-state condition to calculate Cr. The fixed speed was unchanged in T0, T6 and T12 (values ranged from 8 to 13 km·h⁻¹ among subjects). In our purpose, since within this range the step frequency remains about constant (Cavagna 2010), an high running efficiency (because of our treatment) would occur probably indicating an

augment of elastic recoil energy from the stretched contracted muscle-tendon units of subjects. In this case the Cr should be reduced.

Assessment of respiratory variables. Throughout tests athletes wore a portable metabolic system (MedGraphics VO2000, St. Paul, Minneapolis, USA) for pulmonary gas exchange. This system is able to provide a 3-breath average of variables through telemetric transmission and it has been demonstrated to be reliable and have a good agreement compared to a standard metabolic cart for laboratory use (Crouter et al. 2006). Prior to testing, the VO2000 was calibrated according to the manufacturer's instructions. By means of the VO2000 the following parameters were assessed: oxygen uptake (VO_2), carbon dioxide production (VCO_2), pulmonary ventilation (V_E).

Measurement of aerobic energy expenditure. The aerobic Cr ($\text{kcal}\cdot\text{kg}^{-1}\cdot\text{km}^{-1}$) was calculated by dividing the average oxygen uptake (indexed by BM) by the distance covered in that time (Fletcher et al. 2009). By utilizing the Weir equation (Weir 1949; Mansell et al. 1990), this quantity was multiplied using the following equation: $\text{Cr} = 3.941\cdot\text{VO}_2 + 1.106\cdot\text{VCO}_2$ to obtain kcal from oxygen uptake. This equation was used when the respiratory exchange ratio (RER) was < 1 i.e. it was assumed that all aerobic energy derived from carbohydrate and fat oxidation. As reported above, Cr was measured during the last minute of five performed at a speed of $1\text{ km}\cdot\text{h}^{-1}$ slower than AT speed. Moreover, since previous researches have underlined that an arbitrary use of the $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ is inappropriate for the purpose of comparing Cr between subjects with different body characteristics (Markovic et al. 2007; Tartaruga et al. 2013b), the effect of allometric scaling on Cr was also investigated. The relationship of one variable (VO_2) with respect to another (BM) can be summarized by the allometric coefficient of the equation of the relationship. To obtain this allometric coefficient the first variable could be expressed as a

function of the second using the general exponential equation (1): $y = ax^b$ where y is the absolute VO_2 , x is the BM, a is a constant characteristic for the organism and b is the allometric or scaling exponent. This exponential function can be changed into a linear function (equation 2): $\log y = \log a + b \log x$, where b , the slope of the line, is the allometric coefficient. Thus, the log-log plot between subjects' VO_2 and BM at T0-T6 and T12 was used to determine the allometric exponent for scaling the subjects' Cr (Fig. 5).

Statistical analysis

The normality assumption was checked using the Kolmogorov-Smirnov test. The α level was set at $p < 0.05$. Comparisons between tests were performed using the repeated measures analysis of variance (ANOVA) followed by Neuman-Keuls post hoc when appropriate. Differences between groups were analyzed by means of the two-way ANOVA for repeated measures followed by Tukey post-hoc when appropriate. Inter-group time related differences (mean $\pm$ SD) were shown as delta (Δ) values between T0 and T6 and between T0 and T12 and assessed by means of one-way ANOVA followed by Tukey post-hoc when appropriate. Statistics were produced utilizing commercially available software (Graph-Pad, Prism). The α level was set at $p < 0.05$ in all cases.

Results

Absolute Cr, BM, FM and fat free mass values of each group, measured at the beginning of the study (T0), after six months (T6) and one year later (T12) are shown in Table 3.

All 3 groups displayed significant reduction in Cr over the entire year without no significant loss or gain in BM over the entire year. R&D body composition significantly changed after 6 and 12 months. In fact, while their FM was reduced by 15% from T0 to T6 and T12 ($p<0.05$), the fat free mass increased by 3% at T6 and T12 ($p<0.05$).

The R group body composition did not substantially change during the year. However, their FM slightly decreased at T6 but increased at T12 ($p<0.05$ vs. T6), changing back to T0 values. In contrast, fat free mass slightly increased at T6 and declined at T12 ($p<0.05$ vs. T6), going back to T0 values.

The D group body composition was significantly modified both in FM and fat free mass because the former decreased by 15% at T6 and 9% at T12 ($p<0.05$ vs. T0) and the latter increased by 3.4% at T6 and 4% at T12 ($p<0.05$ vs. T0).

Figures from 1 to 4 show Δ values for each parameter of T0, T6 and T12 to evaluate differences among the three groups following the three different regimens.

In detail, no significant difference in Δ Cr was found after 6 months (Fig. 1, top panel) while after 12 months Δ Cr was reduced in the R&D and D groups (Figure 1, bottom panel). Figure 2 did not display any difference in Δ FM after 6 months (top panel) while at T12 the R group FM increased by 0.48 ± 1.18 Kg ($p<0.05$ vs. R&D and D groups, which showed a decreasing trend). On the contrary, the Δ values of fat free mass increased in the R&D and D groups both after 6 and 12 months, while after one year the R group showed a fat free mass decrement (-0.37 ± 0.65 kg, $p<0.05$ vs. R&D and D groups) as shown in Fig. 3.

230 Consequent to FM and fat free mass opposite changes, the Δ BM values were unmodified
231 both after 6 and 12 months in all three groups (Fig. 4, top and bottom panels).

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Discussion

The aim of the study was to verify whether a one-year combined protocol of running and an isocaloric high-intake breakfast diet could modify body composition and this, in turn, evoking a Cr reduction. Our hypothesis was based on a presumable higher muscular tissue capacity to store elastic energy than adipose tissue. Athletes with an augmented fat free mass can potentially store more elastic strain energy because their stronger muscles can impose higher tendon stresses. To the best of our knowledge, this is the first study that analyzed the relationship between the fat free mass-FM ratio and the energy cost of running on a treadmill.

As expected, the dietary intervention modified the body composition in the R&D and D groups: the isocaloric high-intake breakfast diet decreased the FM and increased the fat free mass although without significant changes in BM. A recent analysis by Garaulet and Gómez-Abellán (2014) highlighted that much evidence exists about the importance of the timing of food and macronutrient distribution. Besides, subjects assigned to a high caloric intake during breakfast lost significantly more mass than ones assigned to high caloric intake during dinner (Jakubowics et al. 2013). The latter authors and others (Wang et al. 2013) described the presence of an active circadian clock in different organs related to food intake, which influences the changes in BM and body composition. During our one-year study we detected FM and fat free mass adjustments only, without any change in BM. In any case, the two dieting groups showed a fat free mass gain and a FM loss while the R group displayed opposite changes. This outcome means that the specific diet successfully modified body composition while exercise only did not. It is important to note that our results are quite different from those of the aforementioned studies by Jakubowicz et coll. (2013) where the isocaloric diet caused a mass loss. On the other hand, it is to be

considered that our sample was represented by normal-mass amateur athletes and not by obese subjects.

In agreement to the initial hypothesis, the Cr after 12 months had diminished with respect to T0 and difference among the three groups was found. In fact the two groups that followed a dietary regimen showed a larger Cr decrease (Fig. 1, bottom panel). As expected, the shift between FM and fat free mass was able to increase the total ability to store and release elastic energy during running and decrease Cr at a given speed. Noteworthy, when Cr was calculated without groups' specific allometric scaling (data not shown) this reduction was not found and in that case the results suggested that Cr is not dependent on body composition changes evoked by our protocol. Hence the need to taking into account the role of allometric scaling in the relationship between metabolic Cr and the performance in this kind of studies is strongly recommended (Tartaruga et al. 2010; Tartaruga et al. 2013a).

Previous past and recent studies (Margaria et al. 1963; Ferretti et al. 2011), have shown that the energy cost of running does not appear to be easily modified. The reference model by Margaria et al. (1963) pointed out that the mechanical efficiency of running is quite stable in athletes, that is, training does not lead to great improvement. Therefore, Cr values are only 5-7% lower in athletes than in untrained subjects. The analysis by Ferretti et al. (2011) reiterated that Cr is "*barely*" modifiable in running as concerns intrinsic human characteristics, while the evolution of equipment (track and shoe elasticity) has progressively contributed to improving performance. However, the above-mentioned points of views arise with the classical assessment of Cr, i.e. without considering the impact of allometric scaling. In our study, taking into account the different anthropometric factors between groups a different outcome was found.

In our setting, no significant changes in BM was found in any of the 3 groups without that, however, this would change the expected results. Considering the role of BM changes, Farley and McMahon (1992) suggested that the metabolic cost of running is proportional to body mass, while other authors recently found that the reduction of body mass with a harness system decreased the Cr even though not in a proportional way (Teunissen et al. 2007). In any case, it appears that Cr primarily depends on BM. On the contrary a recent review by Lacour and Bourdin (2015) pointed out on the role of others factors, like leg architecture and elastic energy storage and reuse, as pivotal in the running performance.

Conclusions. On the basis of our findings, the induced shift between FM and fat free mass with an augmented of the latter (and with BM unchanged) in R&D and D groups led to a Cr reduction during running. Thus the Cr appears to be strongly related to body composition at least within the range of changes evoked by our protocol.

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Disclosures

The Authors have no conflicts of interest to disclose with regards this manuscript.

Author contributions

G.G., F.M., A.C. and F.T. participated in the design of the experimental protocol and in the discussion of results. G.G., E.M., G.A., I.S., A.L., M.P., and V.P. performed most of the measurements and data analyses. G.G. and A.C. also drafted and revised the manuscript. G.G. and G.A. recruited volunteers. E.M. and F.M. carried out medical supervision. F.T. was responsible for all stages of the study and revised the manuscript.

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- 393

394 **Table 1.** Anthropometric and metabolic parameters of the three groups at the beginning of
395 the study

	RUNNING AND DIET	RUNNING GROUP	DIET GROUP	WHOLE SAMPLE
AGE (years)	43.7±4.3	43.9±3.0	41.5±9.7	41.1±7.5
BM (kg)	68.3±7.8	64.8±9.4	64.8±15.5	66.5±10.3
HEIGHT (cm)	168.8±8.4	167.3±7.7	164.2±8.0	167.3±8.2
BSA (m²)	1.6±0.01	1.51±0.01	1.48±0.01	1.54±0.01
FM (kg)	12.0±4.0	12.3±3.2	14.2±5.8	12.5±4.2
FAT FREE MASS (kg)	56.3±8.75	52.5±10.3	50.6±13.2	53.9±10.4
P (%)	15.4±2.7	15.8±2.5	15.5±2.0	15.5±2.5
L (%)	37.2±5.7	37.1±6.3	35.5±4.2	36.8±5.5
C (%)	47.4±7.1	47.2±7.1	49.0±4.8	47.7±6.6
TEE (kcal/24h)	2733±443	2656±386	2747±503	2653±443

PAL	1.7±0.2	1.7±0.2	1.6±0.1	1.7±0.2
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397 Values are Mean ± SD. **BM**: body mass; **BSA**: body surface area; **FM**: fat mass; **FFM**: fat
 398 free mass; **FFQ**: food frequency questionnaire; **P**: daily protein amount; **L**: daily lipid
 399 amount; **C**: daily carbohydrate amount; **TEE**: total daily energy expenditure; **PAL**:
 400 physical activity level.

401

402 **Table 2.** Compliance to the given diet: FFQ results at the beginning of the study and at T6
 403 and T12.

	RUNNING AND DIET	RUNNING GROUP	DIET GROUP
FFQ (T0) (kcal/24h)	2299±753	2393±1019	2530±771
FFQ (T6) (kcal/24h)	2352±635	2424±852	2512±546
FFQ (T12) (kcal/24h)	2235±563	2476±987	2523±611

404

405 Values are mean ± SD; **FFQ**: food frequency questionnaire.

406

Table 3. Groups’ data measured at the beginning of the study (T0), after six months (T6) and one year later (T12)

	TIME	RUNNING & DIET	RUNNING	DIET
Cr (kcal· kg ^{-b} · km ⁻¹)	T0	1.48±0.16	1.16±0.10	1.83±0.17
	T6	1.34±0.16*	1.05±0.11*	1.72±0.22*
	T12	1.40±0.15*†	1.14±0.12*†	1.76±0.23*†
BM (kg)	T0	68.3±7.8	64.8±9.4	64.8±15.5
	T6	68.0±8.7	64.6±9.6	64.1±15.1
	T12	68.5±9.0	64.9±9.5	65.5±15.7
FM (kg)	T0	12.0±4.0	12.3±3.2	14.2±5.8
	T6	10.1±2.8*	11.4±2.4	11.9±5.0*
	T12	10.4±3.0*	12.7±3.0†	11.6±4.7*
FAT FREE MASS (kg)	T0	56.3±8.75	52.5±10.3	50.6±13.2
	T6	58.2±9.2*	53.0±10.0	52.2±13.4*
	T12	58.3±9.8*	52.1±10.2†	52.9±13.6*

Cr: cost of running; **BM:** body mass; **FM:** fat mass; **FAT FREE MASS:** free fat mass.

Values are mean ± SD. *=*p*<0.05 vs. T0; †=*p*<0.05 vs. T6

Figure Captions

Figure 1. Cost of running (Cr) variation (Δ) after 6 months (T_0 - T_6 , top panel) and 12 months (T_0 - T_{12} , bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group. $*=p<0,05$ vs. running group.

Figure 2. Fat Mass (FM) variation (Δ) after 6 months (T_0 - T_6 , top panel) and 12 months (T_0 - T_{12} , bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group. $*=p<0,05$ vs. running group

Figure 3. Fat Free Mass variation (Δ) after 6 months (T_0 - T_6 , top panel) and 12 months (T_0 - T_{12} , bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group. $*=p<0,05$ vs. running Group

Figure 4. Body Mass (BM) variation (Δ) after 6 months (T_0 - T_6 , top panel) and 12 months (T_0 - T_{12} , bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group

Figure 5. Relationship between oxygen uptake expressed in absolute terms (VO_2) and body mass by using allometric log-linear for 3 groups (R&D, top panel; R, middle panel; D, bottom panel). Linear regression is shown with a 95% confidence interval.

Figure 1. Cost of running (Cr) variation (Δ) after 6 months (T0-T6, top panel) and 12 months (T0-T12 , bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group.
*=p<0,05 vs. running group.
156x267mm (300 x 300 DPI)

Figure 2. Fat Mass (FM) variation (Δ) after 6 months (T0-T6, top panel) and 12 months (T0-T12, bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group. *= $p < 0.05$ vs. running group

154x267mm (300 x 300 DPI)

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Figure 4. Body Mass (BM) variation (Δ) after 6 months (T0-T6, top panel) and 12 months (T0-T12, bottom panel) in the three groups. R&D: running and diet group; R: running group; D: diet group
159x267mm (300 x 300 DPI)

Figure 5. Relationship between oxygen uptake expressed in absolute terms (VO₂) and body mass by using allometric log-linear for 3 groups (R&D, top panel; R, middle panel; D, bottom panel). Linear regression is shown with a 95% confidence interval.
117x268mm (300 x 300 DPI)