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Long-term effects of high intensity resistance and endurance exercise on plasma leptin and ghrelin in overweight individuals: the RESOLVE Study

Angelo Tremblay[1,2], Frédéric Dutheil[3,4], Vicky Drapeau[1,5], Lore Metz[6,7], Bruno Lesourd[8], Robert Chapier[9], Bruno Pereira[10], Julien Verney[6,7], Julien S Baker[11], Agnes Vinet[12], Guillaume Walther[12], Philippe Obert[12], Daniel Courteix[6,7], David Thivel[6,7]

[1]*Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec City, Canada*
[2]*Department of Kinesiology, Université Laval, Quebec City, Canada*
[3]*CNRS, LaPSCo, Physiological and Psychosocial Stress, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Preventive and Occupational Medicine, WittyFit, Université Clermont Auvergne, Clermont-Ferrand, France.*
[4]*Faculty of Health, School of Exercise Science, Australian Catholic University, Melbourne, VIC, Australia.*
[5]*Department of Physical Education, Université Laval, Quebec City, Canada*
[6]*Clermont Auvergne University, EA 3533, Laboratory of the Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P), Clermont-Ferrand, France.*
[7]*CRNH-Auvergne, Clermont-Ferrand, France.*
[8]*CHU G. Montpied, F-63000 Clermont-Ferrand, France*
[9]*Thermalia Center, F-63140 Châtelguyon, France*
[10]*Clermont-Ferrand University hospital, Biostatistics unit (DRCI), Clermont-Ferrand, France*
[11]*Institute of Clinical Exercise and Health Sciences, School of Science and Sport, University of the West of Scotland, Hamilton, Lanarkshire, Scotland, United Kingdom*
[12]*Avignon University LAPEC EA4278, F-84000 Avignon, France*

Running head: Hormonal response to exercise

Corresponding author
Angelo Tremblay, Ph.D.
Department of Kinesiology, PEPS
2300, rue de la Terrasse, Université Laval
Québec, Canada, G1V 0A6
Phone: 418-656-7294
E-mail: angelo.tremblay@kin.ulaval.ca

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Authors' implication: all authors significantly took part in the study from its conception to the analyses of the data and writing of this paper.

Abstract

Objective: To evaluate the effects of high intensity resistance and endurance exercise on body composition and plasma leptin and ghrelin concentrations in overweight individuals.

Methods: 100 participants were randomly assigned to three exercise interventions: high resistance-low aerobic (Re), low resistance-high aerobic (rE), and low resistance-low aerobic (re). Interventions began with 3 weeks of residential supervision (Phase1) after which participants had to manage the physical activity programs individually (Phase2). Body composition and plasma variables were measured at baseline and after Phase 1 as well as after 3, 6, and 12 months.

Results: Significant decreases in weight and body fat were observed after Phase 1 ($p<0.001$), and continued at a lower rate for up to 3 months, and then remained stable for the rest of the protocol. Once a body weight plateau was reached, body fat loss after the Re and rE conditions exceeded by 1.5-2 kg the fat loss observed in the re condition ($p<0.05$). Leptin was significantly decreased after Day 21 and Month 3 ($p<0.001$) and remained stable for the rest of the study. Ghrelin was significantly increased after Day 21 and Month 3 ($p<0.001$) and returned to a level comparable to baseline between Month 6 and 12 when body weight and fat had reached a plateau.

Conclusions: This study reinforces the idea that an increase in exercise intensity may accentuate body fat loss before the occurrence of a body weight plateau. Resistance to further fat loss was accompanied by a decrease in plasma leptin and an increase in plasma ghrelin.

Key words: obesity, physical activity, energy, appetite, hormones, fat

Introduction

Physical activity has been traditionally studied in the etiology and management of obesity because of its potential to increase energy expenditure. Specifically, research has aimed to determine if there is a deficit in exercise-induced thermogenesis in obese individuals whereas numerous clinical trials have tested physical activity as a calorie-burning agent in weight loss interventions. However, we have also shown that calorie for calorie, high intensity exercise is more susceptible to induce a negative energy balance than a low to moderate intensity physical activity (27, 29). This effect seems to be explained by post exercise adaptations such as an increase in resting metabolic rate (32) and an incomplete compensation in energy intake (14). From a mechanistic standpoint, an increase in beta-adrenergic stimulation was found to be involved in these post exercise effects (25, 32). Furthermore, the discovery of hormonal messengers such as leptin and ghrelin has enriched the study of mechanisms that may underlie the impact of exercise training on energy balance.

Following its discovery in 1994 (34), leptin was shown to promote a negative energy balance via anorectic and thermogenic effects (11, 18). Leptin was also found to be reduced in exercise-trained individuals (12) as well as following exercise protocols (13). This is concordant with the study of Pasman et al. (22) who reported a significant association between the number of hours of exercise and plasma leptin following a 16-month protocol combining diet and exercise training (22). Leptin has been found to increase with aging, altogether with increased leptin resistance (Rigamonti et al., 2002). In a recent meta-analysis, Rostas and collaborators found that exercise training favors decreased leptin concentrations in middle aged and older overweight and obese individuals, resistance training inducing a more pronounced leptin reduction than aerobic training alone. This suggests a role for exercise modality on plasma leptin which may be attributable to the different stimulus provided by resistance and aerobic exercise (Rostas et al., 2017).

Commented [D1]: Rigamonti AE, Pincelli AI, Corrà B, et al. Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. J Endocrinol. 2002;175:R1–R5.

Commented [D2]: PLoS One. 2017 Aug 15;12(8):e0182801. doi: 10.1371/journal.pone.0182801. eCollection 2017.
In middle-aged and old obese patients, training intervention reduces leptin level: A meta-analysis.
Rostás I[1], Pótó L[1,2], Mátrai P[1,2], Hegyi P[1,3,4], Tenk J[1], Garami A[1], Illés A[4], Solymár M[1], Pétervári E[1], Szűcs Á[5], Párniczky A[6], Pécsi D[1], Rumbus Z[1], Zsiborás C[1], Füredi N[1], Balaskó M[1].

The study of variations in plasma ghrelin is also worth consideration to understand the effects of exercise training in obese individuals. Ghrelin is an orexigenic hormonal messenger that increases in blood before eating and immediately decreases after food consumption (6). Accordingly, Cummings et al. (7) reported an increase in plasma ghrelin in obese individuals subjected to diet-induced weight loss. In response to exercise training, ghrelin was found to be increased when the intensity of the exercise stimulus was low to moderate (24). On the other hand, some evidence indicates that high-intensity exercise can reduce plasma ghrelin (2, 30). With age, ghrelin concentration has been found to decrease as well as the ghrelin signaling pathways (Rigamonti et al., 2002). Markofski et al. found that a 12-week aerobic + resistance training was able to increase fasting ghrelin concentrations by 47% in 70 years old individuals (Markofski et al., 2014). Interestingly recent results suggest that the effect of exercise training on ghrelin concentration might depend on the volume of exercise, with 4 months of moderate dose of aerobic exercise favoring reduced ghrelin while it remained unchanged in response to a low dose training program in old women (Bowyer et al., 2018).

Certain studies have examined the impact of exercise on plasma ghrelin in a context where the opposite effect of body weight loss was expected to be significant. For instance, Kim et al. (15) observed that body weight and percent body fat decreased in obese children after a 12-week aerobic and resistance exercise training while total ghrelin increased by 30.4% and acyl ghrelin did not change. Martins et al. (19) found that body weight decreased while plasma acyl ghrelin and appetite increased after 12 weeks of exercise training in sedentary obese women. This is concordant with results reported by Santosa et al. (23) and Zahorska Markiewicz et al. (33).

Taken together, these observations show that both leptin and ghrelin contribute to the metabolic regulation underlying the effects of exercise training. In addition, available literature reveals that this regulation can be modified by time, modalities of exercise practice and variations in body fat. From

Commented [D3]: Exercise training modifies **ghrelin** and adiponectin concentrations and is related to inflammation in older adults.
Markofski MM, Carrillo AE, Timmerman KL, Jennings K, Coen PM, Pence BD, Flynn MG.
J Gerontol A Biol Sci Med Sci. 2014 Jun;69(6):675-81.

Commented [D4]: J Behav Med. 2018 Nov 17. doi: 10.1007/s10865-018-9990-z. [Epub ahead of print]
The influence of exercise training dose on fasting acylated ghrelin concentration in older women.
Bowyer KP[1,2], Carson JA[1], Davis JM[1], Wang X[3].

a clinical standpoint, this observation has significant potential implications for obesity management that deserve further investigations. In the present study, we report relevant data collected in the RESOLVE Study (9) to document the impact of high-intensity resistance and endurance training combined with dietary guidelines on plasma leptin and ghrelin in overweight individuals tested at different time intervals over a 12-month intervention.

Methods

Subjects

A sample of 100 individuals (44 men, 56 women) were recruited to participate in this study via advertisement. As previously described (9), the following inclusion criteria had to be respected to permit eligibility: aged between 50 and 70 years, having a diagnosis of metabolic syndrome (METs) (1), being overweight and sedentary, having maintained a stable body weight and medical treatment over the last 6 months, to be post-menopausal for women, not to have restricted diet over the previous year and to have completed a satisfactory VO_2max test. Additionally, the participants had to be exempt from some diseases having the potential to interfere with the metabolic outcome of this study (9). All subjects gave their written consent to participate in the protocol.

Design

This study is part of the larger RESOLVE project that is a clinical trial designed to investigate the effects of a lifestyle intervention combining exercise and nutritional diet in individuals with metabolic syndrome. The full experimental design, population recruitment procedure, eligibility criteria, measurements as well as compliance and drop-out rates have been previously reported (5, 9, 31). Briefly, all participants underwent a comprehensive medical screening procedure to ensure their ability to complete the entire protocol. Eligible subjects were free from clinical signs of heart failure, coronary artery disease, previous cerebrovascular events, atrial fibrillation and congenital heart disease and were not using medication altering body weight or had not been on any restrictive diets in the previous year. The participants were randomly assigned to one of the three exercise interventions differing from each other by the relative intensity of resistance (R) and endurance (E) sessions (with stratification according to age, sex and body mass index), for 3 weeks: i) Condition Re was a high resistance-moderate endurance exercise whose modalities imposed 10 repetitions at 70% of 1 maximal repetition and 30% VO_2 peak for endurance exercise; ii) Condition rE was performed at moderate resistance (30%) and high endurance (70%) intensity; iii) Condition re was the reference condition with both resistance and endurance exercise being performed at 30% maximal reference values. It is important to note that evaluators were blinded relative to the condition being assigned to each subject. For the following 12 months, the participants were all requested to maintain the same training program individually while relying on guidelines and exercise prescription that they had received in Phase 1.

Anthropometric measurements, body composition (DXA), blood samples, clinical and physical assessments, daily food intake (3-day food diary) and various health-related questionnaires, were performed at baseline (D0), after the 3-week intervention (D21), 3, 6 and 12 months after (M3, M6 and M12). The study was approved by the human ethics committee from the University Hospital of Saint-Etienne, France. The intervention was registered with the American National Institutes of Health database: No. NCT00917917.

Measurements

Anthropometric measurements and body composition

The participants weight and height was recorded while wearing light clothes and standing bare-footed, using a digital scale and a standard wall-mounted stadiometer respectively. BMI was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured at midpoint between sub-costal and supra-iliac landmarks (21). Fat mass (FM) and fat-free mass (FFM) were assessed by dual-energy X-ray absorptiometry (DXA) following standardized procedures (QDR4500A scanner, Hologic, Waltham, MA, USA).

Daily energy intake.

Participants were asked to complete a 3-day dietary recall that was explained and detailed to them by a member of the investigation team (including 2 week-days and 1 weekend day). The participants were asked to indicate as precisely as possible all the details regarding the food ingested at each meal and in-between meals. During their first visit, a specialized dietitian detailed the diary and the methodology used to fill it in to the participants and the diaries were reviewed afterward with the participants and the dietitian during a 45 minutes interview. The records have been analyzed by a trained dietitian using the NutriLog software (Nutrilog SAS, Paris, France).

Blood samples

Fasting blood samples were drawn between 7.00 and 7.30 a.m. by an experienced nurse, aliquoted and stored at -80°C until analysis. Basic biological assays were performed in the biochemistry laboratory of the University Hospital of Clermont-Ferrand, France. Total ghrelin and leptin were assayed by ELISA using commercial kits (Millipore, Billerica, MA, USA). Sensitivity, intra- and interassay coefficients of variation were respectively 30 pg/ml, 1.1% and 6.9% for total ghrelin and 0.16 ng/ml, 5.1% and 7.4% for leptin.

Detailed lifestyle intervention

As previously described by Dutheil et al. (9), the protocol for each condition was divided in two phases:

Phase 1: This phase elapsed over 3 weeks during which participants stayed in a residential establishment where their exercise program and food intake were supervised. In each condition, participants had to perform 15-20 hours of exercise per week that included 90 minutes of daily aerobic exercise plus four 90 minute weekly resistance exercise sessions. As indicated above, the conditions differed by the relative intensity of either resistance or endurance exercise. A Polar S810 system was used to record and store heart rate values. Endurance training included aquagym, cycling and walking whereas resistance training was based on 8 exercises with free weights and traditional muscular development equipment. For each exercise, participants had to perform 3 series of 10 repetitions. Maximal test were realized at baseline to determine the individual acapcities of each participants. Regarding the resistance intervention, tests were realized for each of the selected exercises in order to determine the participants 10RM (maximal 10 repetitions). The training intensity increased from 65% to 85% of 10 maximal repetitions for Re, whereas rE and re remained at 30% of 10 maximal repetitions. Resistance training was done 4 times a week and consisted of 15 min warm-up followed by height exercises with free weights and traditional muscle building equipment. Exercises were high pulley machine (lower back), seated row (upper back and trapezius), bench press (chest), chest fly (chest), squat press (legs), leg extension machine (quadriceps), dumbbell curl (biceps brachial), triceps pushdown on high pulley (triceps brachial). Each exercise was performed for three sets of 10 repetitions with 1 min rest interval A VO2peak test was also realized by each participants at baseline. Intensity of the endurance sessions increased gradually from 40 to 75% of VO2max from week 1 to week 3 for rE, whereas Re and re remained at 30% of VO2max. Throughout the residential program, participants received both standard and personalized meals prescribed by dietitians. Protein intake was set at 1.2 g/kg body weight/d and accounted for 15-20% daily energy intake. Lipid and carbohydrate intake provided 30-35 and 45-55% daily energy intake, respectively. Total daily energy intake was calculated to promote a 500 kcal daily negative energy balance.

Phase 2: This phase covered the remaining part of the one-year intervention, i.e. between Day 21 (D21) and the end of Month 12. During this period, participants were requested to maintain the same training program individually while relying on guidelines and exercise prescription that they had received in Phase 1. They were met by the exercise coach and the dietitian at months 3, 6, and 12 (M3, M6, M12). As previously described (9), a compliance score was determined on the basis of the number of food questionnaires returned (score from 0 to 12 i.e. 12 = 100%) and the number of training sessions undertaken per week (score from 0 to 4, i.e. 4 = 100%). The overall compliance score was the mean of these two scores (nutrition and physical activity).

Statistical analysis

Statistical analyses were carried out using the statistical software Stata (version 13, StataCorp, College Station, US). All statistical tests were conducted for a two-sided type I error at 0.05. Continuous variables were described as mean and standard-deviation, according to statistical distribution (assumption of normality studied using Shapiro-Wilk test). Repeated correlated data were analyzed using random-effects models to study fixed effects group (Re, rE, re), time-point evaluation (baseline, D21, M3, M6, M12) and their interactions taking into account between and within subject variability (as random-effect). A Sidak's type I error correction was applied to take into account the multiple comparisons. Where appropriate, the normality of residuals was studied using Shapiro-Wilk test. If necessary, a logarithmic transformation was proposed to achieve the normality of dependent outcome. Furthermore, to determine if the treatment effects on plasma leptin and ghrelin were independent from variations in BMI, lean and fat mass, multivariable random-effects models were performed with these variations as covariates. Concerning non-repeated data, the following statistical tests were performed: Student t-test or Mann-Whitney test if conditions of t-test were not met (normality studied using Shapiro-Wilk and assumption of homoscedasticity verified by Fisher-

Snedecor test). All enrolled participants were included in the analysis. However, a sensitivity analyses provided similar results with completers only analysis.

Results

On the initially 100 recruited participants, 91 completed the first phase of the protocol (n=30Re; n=28rE; n=33re) and 78 completed the entire study, phase 1 and 2 (n=24Re; n=24rE; n=30re). This Figure 1 presents the flow-chart of the entire study. During phase 2, the mean compliance scores were 54.6 ± 22.1% for Re, 52.7 ± 26.1% for rE, and 52.1 ± 18.1% for re, and did not differ between MetS groups.

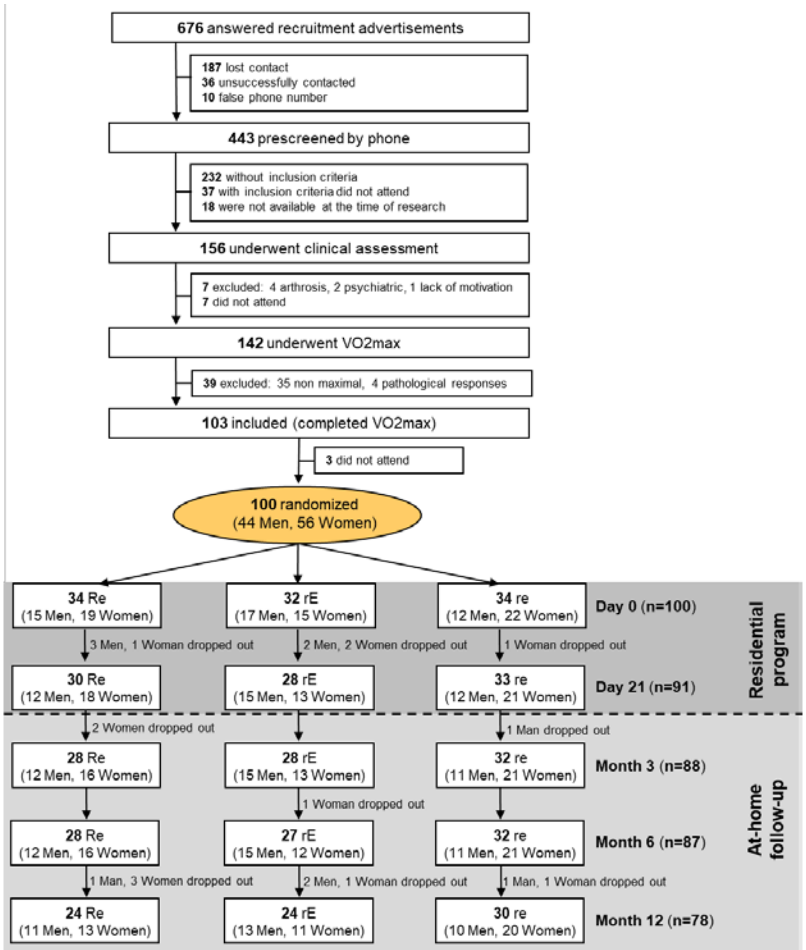

Figure 1. Flow-chart of the entire study. **D0: Day 0; D21: Day 21; M3: Month 3; M6: Month 6; M12: Month 12; Re: Resistance+endurance; rE: resistance+Endurance; re: resistance+endurance.**

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Variations in body weight and composition throughout the protocol are presented in Table 1. As expected, there was a significant decrease in body weight, fat mass and fat-free mass during Phase 1 while physical activity and food intake were closely supervised ($p<0.001$). From a quantitative standpoint, the mean fat mass loss during this period was about 3 kg, 3 kg and 2 kg in response to the

Re, rE and re conditions, respectively. Thus, its energy equivalent (9,300 kcal/kg) means that the negative energy balance during Phase 1 largely exceeded the 500 kcal daily energy deficit that was targeted at baseline.

Table 1 also shows that body fat loss continued in Phase 2 up to M3 ($p<0.001$). Specifically, the fat loss of 2-3 kg that was achieved over the 70 days elapsing between the end of Phase 1 (D21) and Month 3 was equivalent to a mean daily energy deficit of about 300 kcal/day. Beyond M3, fluctuations of fat mass were small and no net noticeable additional fat loss was observed up to the end of the protocol at M12.

As indicated, fat-free mass also decreased during the protocol (Table 1) ($p<0.001$). However, it is noteworthy to emphasize that fat-free mass preservation was almost entirely achieved during the whole protocol in the Re condition.

Table 1. Body weight and composition at different times during the protocol.

Variables	Time	Condition			Mixed Model		
		Re	**rE**	**re**	Group	Time	Interaction
Body weight (kg)	D0	85.4 ± 12.4	94.0 ± 13.7	89.0 ± 12.7	<0.05 Re < rE *	<0.001 D0 vs D21 *** D0 vs M3 *** D0 vs M6 *** D0 vs M12 *** D21 vs M3 *** D21 vs M6 *** D21 vs M12**	0.02
	D21	81.9 ± 11.7	87.1 ± 13.1	85.7 ± 12.1			
	M3	79.1 ± 11.3	86.1 ± 12.3	82.6 ± 12.2			
	M6	80.4 ± 12.6	86.1 ± 13.6	82.8 ± 12.5			
	M12	79.2 ± 11.9	84.9 ± 12.9	82.5 ± 12.7			
BMI (kg/m²)	D0	32.1 ± 3.9	34.4 ± 4.2	33.9 ± 4.0	0.04 Re < rE* Re < re 0*	<0.001 D0vs D21 *** D0vs M3 *** D0vs M6 *** D0vs M12*** D21 vs M3 *** D21 vs M6 *** D21 vs M12 *** M3 vs M12 * M6 vs M12 **	0.02
	D21	30.8 ± 3.8	33.0 ± 3.9	32.7 ± 3.8			
	M3	29.6 ± 3.7	31.5 ± 3.6	31.6 ± 3.9			
	M6	30.2 ± 4.1	31.5 ± 4.0	31.7 ± 3.9			
	M12	29.9 ± 3.9	31.3 ± 4.0	31.8 ± 4.0			
Fat mass (kg)	D0	27.7 ± 7.6	32.2 ± 7.7	32.3 ± 7.5	<0.001 Re < rE ** Re < re*** *	<0.001 D0vs D21 *** D0vs M3 *** D0vs M6 *** D0vs M12 *** D21vs M3 *** D21vs M6 *** D21vs M12 *** M3vs M12* M6vs M12 *	0.03
	D21	24.9 ± 7.1	29.3 ± 7.3	30.1 ± 7.3			
	M3	22.1 ± 6.9	26.3 ± 6,8	28.3 ± 6.8			
	M6	23.1 ± 8.3	25.8 ± 7.6	28.0 ± 6.6			
	M12	22.7 ± 7.0	26.7 ± 8.1	28.5 ± 7.3			
FFM (kg)	D0	57.5 ± 10.8	61.8 ± 11.4	56.5 ± 10.7	ns	<0.001 D0vs D21 *** D0vs M3*** D0vs M6 *** D0vs M12 *** D21vs M3 *** D21vs M6*** D21vs M12** M3vs M12 * M6vsM12**	<0.05
	D21	56.9 ± 10.2	60.7 ± 10.8	55.6 ± 10.2			
	M3	56.5 ± 10.6	59.5 ± 10.9	54.5 ± 10.8			
	M6	57.1 ± 10.4	60.3 ± 10.7	54.8 ± 10.6			
	M12	56.8 ± 11.1	58.1 ± 10.3	54.2 ± 10.8			

Values are means ± SD, D0, D21, M3, M6, and M12 refer to Day 0, Day 21, Month 3, Month 6, and Month 12, respectively; Re: Resistance+endurance; rE: resistance+Endurance; re: resistance+endurance. *p<0.05; **p<0.01; ***p<0.001.

Our analysis revealed a significant time effect (p<0.001) for daily energy intake with EI being significantly higher at D0 compared with the other time points (D21 to M12) without any difference between the other time points (D21 to M12). Although the analysis also shows a group effect with EI being significantly higher in the re group compared with both Re and rE ($p<0.01$), there was no group x tome interaction. Variations between time points were not significantly different between groups.

Figure 2 illustrates variations in plasma leptin during the protocol. As expected, there was a considerable decrease in leptinemia during Phase 1 ($p<0.001$). This decrease continued between Day 21 and Month 3 ($p<0.001$). As for fat mass, there was no apparent clinically significant change in leptinemia between M3 and M12. Variations in plasma ghrelin were also concordant with those of energy balance up to M3 (Figure 2). Indeed, according to the literature cited above, the negative energy balance that was imposed at the beginning of the protocol resulted in a significant increase in plasma ghrelin at D21 and M3 compared to baseline values ($p<0.001$). The Leptin/ghrelin ration was found significantly decreased at D21, M3, M6 and M12 compared with D0 in the rE and Re groups (with no difference between D21, M3, M6 and M12) ($p<0.01$) but remained unchanged in the re group.

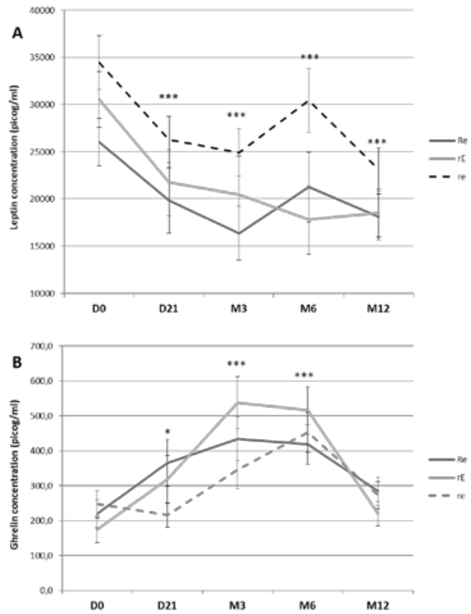

Figure 2. Plasma leptin (A) and ghrelin (B) concentrations before (D0) and after Phase 1 (D21) and after 3 (M3), 6 (M6) and 12 (M12) months (Phase 2) for the three treatment conditions: Resistance + endurance (Re), resistance + Endurance (rE), resistance + endurance (re)
Values are means ± SEM; *** p<0.001 compared with D0; * p<0.05 compared with D0.

However, contrary to other variables documented in this paper which reached a plateau at M3, there was a substantial decrease in ghrelinemia between M6 and M12 in each condition (Figure 1) to a level comparable to baseline values.

Discussion

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The main objective of this study was to investigate the impact of different modalities of physical activity practice differing by the intensity of the exercise in combination with diet guidelines stimulus on body composition and some appetite-related hormones in overweight individuals. A particularity of the protocol was its implementation during 3 weeks of close in-house exercise and diet supervision that were followed by a second phase up to 12 months during which participants had to manage the program individually. The beginning of the intervention in a controlled residential context promoted a greater than initially expected energy deficit that was slightly more pronounced in response to high intensity resistance or endurance exercise. After 3 weeks, once participants had the responsibility to manage their exercise practice by themselves, daily energy balance and fat loss were reduced up to 3 months from which no further clinically significant morphological changes were observed up to the end of the program. This apparent inability to further lose body fat after 3 months was accompanied by substantial opposite changes in leptinemia which decreased, and ghrelinemia which increased in response to fat loss. However, as further discussed in this section, a considerable decrease in plasma ghrelin was observed between 6 and 12 months of follow-up when body weight and fat were relatively stable.

Cross-sectional observations showed that vigorous physical activity is associated with reduced body fatness, independently of the energy cost of activities (27). This has been corroborated by intervention studies demonstrating that high intensity exercise accentuates body fat loss while increasing skeletal muscle oxidative potential (3, 29). These observations are also concordant with results obtained in standardized laboratory experiments indicating that calorie for calorie, high intensity exercise influences global energy balance via postexercise adaptations in energy intake, appetite and resting metabolic rate (14, 16, 32). From a clinical standpoint, these findings have contributed to the dissemination of guidelines to exercise specialists focussing on the relevance to prescribe vigorous physical activities as part of fitness programs. However, with respect to the management of excess weight, these studies have not documented the issue as to "how much additional body fat loss" could be achieved with high intensity physical activity in weight-reduced overweight individuals before the occurrence of resistance to further lose body fat. In this regard, the methodology of the present study contributed to answer this question by comparing the response of body fat over time in overweight people subjected to different modalities of exercise practice. The results showed that when high intensity exercise was included in the program, be it focussed on resistance or aerobic exercise, mean body fat loss was accentuated by 1.5 to 2.0 kg before the achievement of a body weight plateau. This reinforces the relevance to include vigorous physical activity in fitness programs provided that the exercise stimulus is compatible with the health status of individuals. Our results moreover reinforce the effect of high intensity exercise since our three groups responded similarly to the interventions in terms of energy intake.

The findings outlined in the present study also reveal that irrespective to modalities of physical activity practice, a body weight plateau is ultimately reached after some months of participation in a program based on exercise and healthy eating, and as indicated above, this happened after 3 months of intervention in our subjects. Interestingly, this was accompanied by a statistically significant and quantitatively important decrease in plasma leptin, which is concordant with previously reported variations in leptin (4). This is in agreement with many studies having demonstrated that a weight-reducing program favors a decrease in plasma leptin, which is related to decreased thermogenesis (8, 28) as well as an increase in hunger sensations (17). This is also concordant with the demonstration that leptin administration in weight-reduced obese individuals reverses these leptin-related changes in thermogenesis and appetite (17).

The orexigenic hormone ghrelin has been shown to increase previously with weight loss in the participants in other studies (7, 15, 19). This change represents a normal response which, together with the decrease in plasma leptin, promotes body energy preservation in a context of energy restriction. However, contrary to leptin which remained relatively stable when body weight had stabilized after 3 months during the experimental protocol, a pronounced decrease in plasma ghrelin was noted in each condition at the end of the study. Indeed, as depicted in Figure 2, plasma ghrelin had then returned to values comparable to baseline levels when body weight and fat remained much lower than their initial level. This unexpected finding may suggest that long-term physical activity practice results in hormonal adaptations that facilitate over time the maintenance of reduced body weight. Obviously, this hypothesis proposing that appetite control in the active person might be facilitated on the long-term because a decrease in ghrelin deserves experimental confirmation. If confirmed, this effect on ghrelin could provide a mechanistic explanation of the recognized benefit of exercise to facilitate body weight/fat maintenance in weight-reduced obese individuals (10, 20, 26). Interestingly, our results also show a reduced Leptin/Ghrelin ration in response to the two intensive interventions (Re and rE) but not in response to the re one. Although further research is needed regarding the effect of exercise on this ratio, this is of importance since it might suggest that intensive exercise might prevent patient for future weight regain compare to ow intensity interventions. Indeed, as previously showed, a higher fasting L/G ratio has been found associated with post-weight loss weight regain in overweight and patients with obesity (Crujeiras et al., 2014).

Commented [D7]: Pre-treatment circulating **leptin/ghrelin** ratio as a non-invasive marker to identify patients likely to regain the lost weight after an energy restriction treatment.
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The present study has some strengths and limitations that are worthy of consideration. Among the strengths, it is relevant to emphasize the duration of the protocol that was sufficiently long to permit the occurrence of resistance to further lose body fat and to examine its related hormonal changes. The high volume of training on Phase 1 composes an originality of the intervention made possible by the residential nature of the program and the continuous presence of professionals. It remains however hardly transferable in free-living condition as illustrated by the compliance results observed during phase 2. This self-management of exercise guideline in Phase 2 might also represent a strength of this study because of a better representativeness of what would happen under free-living conditions. With respect to hormonal determinations, it is possible that, as described in the introduction of this paper, the measurement of the active form of ghrelin would have contributed to a more thorough documentation of the hormonal impact of our exercise intervention. However, it is unlikely that the pronounced decrease in ghrelin that was found at the end of the study would not have been also seen for acyl ghrelin. Another limitation is the use of self-reported dietary recall that might have led to some underreported results which must be considered when interpreting the present results.

In summary, this study showed that increasing exercise intensity in an intervention combining physical activity and diet guidelines promotes an accentuation of fat mass loss before body weight reaches a new plateau in a reduced obese state. This occurrence of resistance exercise to lose fat was associated with a decrease in plasma leptin and an increase in plasma ghrelin. Unexpectedly, ghrelin almost returned to baseline values after several months of body weight stabilization. Further research is needed to determine if this hormonal adaptation represents a long-term benefit of exercise facilitating appetite control in active weight-reduced obese individuals.

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