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

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

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

Running head: Hormonal response to exercise

Corresponding author
THIVEL David (PhD)
Clermont Auvergne University, EA 3533, Laboratory of the Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P), BP 80026, F-63171 Aubière cedex, France
david.thivel@uca.fr
Phone and fax: 0033 4 73 40 76 79
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), 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 body weight and 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 the fat loss observed in the re condition by 1.5-2 kg (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 individuals with obesity whereas numerous clinical trials have tested physical activity as a calorie-burning agent in weight loss interventions (Doucet et al. 2000; Doucet et al. 1999). 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 (Tremblay et al. 1990; Tremblay et al. 1994; Donnelly et al. 2014). This effect seems to be explained by post exercise adaptations such as an increase in resting metabolic rate (Yoshioka et al. 2001) and an incomplete compensation in energy intake (Imbeault et al. 1997, Donnelly et al. 2014). From a mechanistic standpoint, an increase in beta-adrenergic stimulation was found to be involved in these post exercise effects (Yoshioka et al. 2001; Tremblay et al. 1992). 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 (Zhang et al. 1994), leptin was shown to promote a negative energy balance via anorectic and thermogenic effects (Halaas et al. 1995; Maffei et al. 1995). Leptin was also found to be reduced in exercise-trained individuals as well as following exercise protocols (Hickey et al. 1996; Hickey et al. 1997; Fedewa et al. 2018). This is concordant with the study of Pasman et al. 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 (Pasman et al. 1998). 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 individuals with obesity, resistance training inducing a more pronounced leptin reduction than aerobic training.
alone. These results suggest that exercise could increase leptin sensibility and that the different stimulus provided by resistance and aerobic exercise may affect plasma leptin differently (Rostas et al. 2017).

The study of variations in plasma ghrelin is also worth consideration to understand the effects of exercise training in individuals with obesity. Ghrelin is an orexigenic hormonal messenger that increases in blood before eating and immediately decreases after food consumption (Cummings et al. 2001). Accordingly, Cummings et al. reported an increase in plasma ghrelin in individuals with obesity subjected to diet-induced weight loss (Cummings et al. 2002). In response to exercise training, ghrelin was found to be increased when the intensity of the exercise stimulus was low to moderate (Tiryaki-Sonmez et al. 2015). On the other hand, some evidence indicates that high-intensity exercise can reduce plasma ghrelin (Broom et al. 2007; Vata,sever-Ozen et al. 2011). 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 year 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).

Other studies have found opposite changes of ghrelin in response to exercise even in the presence of body weight loss. For instance, Kim et al. (Kim et al. 1994) observed that body weight and percent body fat decreased in children with obesity 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. found that body weight decreased while plasma acyl ghrelin (as the active form of ghrelin that is mainly recognised
for its effects on energy intake) and appetite increased after 12 weeks of exercise training in sedentary women with obesity (Martins et al. 2007). This is concordant with results reported by Santosa et al. and Markiewicz et al. (Santosa et al. 2007; Zahorska-Markiewicz et al. 2004).

Taken together, these observations show that both leptin and ghrelin contribute to the metabolic regulation underlying the effects of exercise training on energy balance. In addition, available literature reveals that this regulation can be modified by time, modalities of exercise practice and variations in body fat. From 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 (Dutheil et al. 2013) to document the impact of high-intensity resistance and endurance training combined with dietary guidelines on, body composition, 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 (Cummings et al., 2002), the following inclusion criteria had to be respected to permit eligibility: aged between 50 and 70 years, having a diagnosis of metabolic syndrome (METs) (Alberti et al. 2005), 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$_2$max test. Additionally, the participants had to be exempt from some diseases having the potential to interfere with the metabolic outcome of this study and had to be free of any previous medical surgery that
could have impact on the studied metabolism, such as bariatric surgery (Dutheil et al. 2013). All subjects who have participated in the study 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 (Crendel et al. 2013; Dutheil et al. 2013; Vinet et al. 2015). 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 the following 12 months, the participants were all requested to maintain their assigned 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 and daily food intake (3-day food diary) 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 were 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 (m²). Waist circumference was measured at midpoint between sub-costal and supra-iliac landmarks (WHO, 2000). Fat mass (FM) and Lean mass 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 (Dutheil et al. 2013), 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 capacities of each participant. 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 (as requested by the national nutrition guideline, French Nutrition and Health National Plan, PNNS). 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 (Cummings et al., 2008), the participants’ adherence was monitored using a compliance score 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%). 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 groups.

**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 considering between and within subject
variability (as random-effect). A Sidak’s type I error correction was applied to consider 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 the 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).

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). Figure
1 presents the flow-chart of the entire study.

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 lean mass during Phase 1
while physical activity and food intake were 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, lean mass also decreased during the protocol (Table 1) (p<0.001). However, it is noteworthy to emphasize that lean mass preservation was almost entirely achieved during the whole protocol in the Re condition.

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

**Discussion**

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 stimulus in combination with diet guidelines 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 changes in plasma leptin that decreased, and ghrelin that increased in response to fat loss. However, as further discussed in this section, plasma ghrelin returned to baseline values 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 (Tremblay et al. 1990). This has been corroborated by intervention studies demonstrating that high intensity exercise accentuates body fat loss while increasing skeletal muscle oxidative potential (Bryner et al. 1997; Tremblay et al. 1994; Viana et al. 2019). 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 post-exercise adaptations in energy intake, appetite and resting metabolic rate (Imbeault et al. 1997; King et al. 1994; Yoshioka et al. 2001). 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 is concordant with the fact that EI was found to be greater in the re group. 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.

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 decrease in plasma leptin, which is concordant with previously reported variations in leptin (Considine et al. 1996). 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 (Doucet et al. 2000; Tremblay et al. 2004) as well as improved appetite control (Kissileff et al. 2012). This is also concordant with the demonstration that leptin administration in weight-reduced
individuals with obesity reverses these leptin-related changes in thermogenesis and appetite (Kissilef et al. 2012).

The orexigenic hormone ghrelin has been shown to increase previously with weight loss in the participants in other studies (Cummings et al. 2002; Kim et al. 2008; Martins et al. 2007). 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 of a more optimal regulation of 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 individuals with obesity (Ewbank et al. 1995; McGuire et al. 1998; Tremblay et al. 1990).

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. 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. 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 conditions. 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. Finally, physical evaluations could have been done at regular interval to adjust for the exercises’ intensities, which has not been possible for practical reasons.

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 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 individuals with obesity.

**Acknowledgements**

This work was supported by PRES Blaise Pascal University – Clermont II – Laboratory AME2P Metabolic Adaptations to Exercise in Physiological and Pathological conditions, and by the thermal baths of Chatel-Guyon and Omental association. We would like to thank Sarah de Saint Vincent from the Institut de Medecine du Travail (Institute of Occupational Medicine, Faculty of Medicine, Clermont-Ferrand, France) for her help to assess biomarkers using ELISA technology. We also would like to thank the dietitians of the RESOLVE trial: Carole Gravière and Aurélie Moreira. WE also want to thank Sarah DE SAINT VINCENT who performed the biological analysis.
**Fundings**

This work was funded by the Fondation Coeur et Arteres 59200 Loos, France; www.fondacoeur.com.

The funding source had no role in the design, conduct, or reporting of the study.

**Competing interest statement:** The authors have no conflict of interest to disclose.

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


## Tables

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Condition</th>
<th>Mixed Model</th>
<th>Group</th>
<th>Time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>Re</td>
<td>85.4 ± 12.4</td>
<td>94.0 ± 13.7</td>
<td>89.0 ± 12.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D21</td>
<td>rE</td>
<td>81.9 ± 11.7</td>
<td>87.1 ± 13.1</td>
<td>85.7 ± 12.1</td>
<td>D0 vs D21 ***</td>
</tr>
<tr>
<td>M3</td>
<td>re</td>
<td>79.1 ± 11.3</td>
<td>86.1 ± 12.3</td>
<td>82.6 ± 12.2</td>
<td>D0 vs M6 ***</td>
</tr>
<tr>
<td>M6</td>
<td></td>
<td>80.4 ± 12.6</td>
<td>86.1 ± 13.6</td>
<td>82.8 ± 12.5</td>
<td>D0 vs M12 ***</td>
</tr>
<tr>
<td>M12</td>
<td></td>
<td>79.2 ± 11.9</td>
<td>84.9 ± 12.9</td>
<td>82.5 ± 12.7</td>
<td>D21 vs M6 ***</td>
</tr>
</tbody>
</table>

| **BMI (kg/m²)** |           |             |       |      |             |
| D0              | Re        | 32.1 ± 3.9  | 34.4 ± 4.2  | 33.9 ± 4.0  | <0.001     |
| D21             | rE        | 30.8 ± 3.8c | 33.0 ± 3.9c | 32.7 ± 3.8c | D0 vs D21 ***|
| M3              | re        | 29.6 ± 3.7c | 31.5 ± 3.6c | 31.6 ± 3.9c | D0 vs M6 ***|
| M6              |           | 30.2 ± 4.1c | 31.5 ± 4.0c | 31.7 ± 3.9c | D0 vs M12 ***|
| M12             |           | 29.9 ± 3.9c | 31.3 ± 4.0c | 31.8 ± 4.0c | D21 vs M6 ***|

| **Fat mass (kg)** |           |             |       |      |             |
| D0              | Re        | 27.7 ± 7.6  | 32.2 ± 7.7  | 32.3 ± 7.5  | <0.001     |
| D21             | rE        | 24.9 ± 7.1c | 29.3 ± 7.3c | 30.1 ± 7.3c | D0 vs D21 ***|
| M3              | re        | 22.1 ± 6.9c | 26.3 ± 6.8c | 28.3 ± 6.8c | D0 vs M6 ***|
| M6              |           | 23.1 ± 8.3c | 25.8 ± 7.6c | 28.0 ± 6.6c | D0 vs M12 ***|
| M12             |           | 22.7 ± 7.0c | 26.7 ± 8.1c | 28.5 ± 7.3c | D21 vs M12 ***|

| **Lean Mass (kg)** |           |             |       |      |             |
| D0              | Re        | 57.5 ± 10.8 | 61.8 ± 11.4 | 56.5 ± 10.7 | <0.001     |
| D21             | rE        | 56.9 ± 10.2a| 60.7 ± 10.8b| 55.6 ± 10.2b| D0 vs D21 ***|
| M3              | re        | 56.5 ± 10.6c| 59.5 ± 10.9c| 54.5 ± 10.8c| D0 vs M6 ***|
| M6              |           | 57.1 ± 10.4c| 60.3 ± 10.7c| 54.8 ± 10.6c| D0 vs M12 ***|
| M12             |           | 56.8 ± 11.1c| 58.1 ± 10.3c| 54.2 ± 10.8c| D21 vs M12 ***|

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. a: p<0.05 for within group analysis compare to D0; b: p<0.01 for within group analysis compare to D0; c: p<0.001 for within group analysis compare to D0.

### Figures’ legends
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.

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.