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ORIGINAL ARTICLE

Effect of three different protocols of aerobic interval exercise over fat oxidation

Influence de trois protocoles différents d'exercice d'aérobie sur l'oxydation des graisses

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KEYWORDS
Fat oxidation;
Interval exercise;
Exercise intensity;
Training;
Aerobic exercise.

Summary
Objectives. — The objective of this research is to explore the influence of different exercise protocols on fat oxidation.
Design. — Uncontrolled experimental study.
Methods. — For this purpose, 10 sedentary male subjects were recruited, 30 to 39 years old, with elevated body mass index (BMI). Each participant was evaluated in four separate sessions. The first session consisted in determining peak aerobic power (PAP). The following sessions participants performed three equivalent exercise protocols, consisting of each one in three bouts of 15-minute exercise separated by 5 minutes of rest in between. The constant intensity protocol included exercise periods at 55% of PAP, while the other two (increasing and decreasing intensity protocols) consisted in exercise periods at 40, 55 and 70% of PAP in an increasing or decreasing order respectively.
Results. — There were no significant differences ($P > 0.05$) in total caloric expenditure among the three protocols (range: 5.9 ± 0.2 to 6.1 ± 0.2 kcal·kg^{-1}). DIP causes a progressive increase in fat oxidation comparative to IIP and CIP protocols ($P < 0.01$). In period third of the protocols, DIP oxidized significantly more fat than the other two. The perception of effort was significantly lower in the DIP compared to the other two protocols ($P < 0.01$).

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Conclusions. — Fat oxidation in exercises at intervals of different intensity depends on the order of these periods, being greater when performed decreasingly, as well as the perception of effort, being more useful for sedentary subjects with high BMI.

MOTS CLÉS
Oxydation des graisses ;
Exercice d'intervalle ;
Intensité de l'exercice ;
Entraînement ;
Exercice aérobie.

Résumé
Objectifs. — L'objectif de cette recherche est d'explorer l'influence de différents protocoles d'exercice sur l'oxydation des graisses.
Conception. — Étude expérimentale non contrôlée.
Méthodes. — Pour cela, dix sujets masculins sédentaires ont été recrutés, âgés de 30 à 39 ans, avec un indice de masse corporelle (IMC) élevé. Chaque participant a été évalué en quatre sessions distinctes. La première séance consistait à déterminer la puissance aérobie maximale (PAP). Les participants aux sessions suivantes ont exécuté trois protocoles d'exercices équivalents, consistant chacun en trois séances d'exercice de 15 minutes séparées par 5 minutes de repos entre les deux. Le protocole à intensité constante comprenait des périodes d'exercice à 55 % de PAP, tandis que les deux autres (protocoles à intensité croissante et décroissante) consistaient en des périodes d'exercice à 40, 55 et 70 % de PAP dans un ordre croissant ou décroissant respectivement.
Résultats. — Il n'y avait pas de différences significatives ($p > 0,05$) dans la dépense calorique totale entre les trois protocoles (gamme : $5,9 \pm 0,2$ à $6,1 \pm 0,2$ kcal·kg^{-1}). Le DIP provoque une augmentation progressive de l'oxydation des graisses par rapport aux protocoles IIP et CIP ($p < 0,01$). Dans la troisième période des protocoles, le DIP a oxydé significativement plus de graisse que les deux autres. La perception de l'effort était significativement plus faible dans le DIP par rapport aux deux autres protocoles ($p < 0,01$).
Conclusions. — L'oxydation des graisses dans les exercices à des intervalles d'intensité différente dépend de l'ordre de ces périodes, étant plus grande lorsqu'elles sont effectuées de manière décroissante, ainsi que de la perception de l'effort, étant plus utile pour les sujets sédentaires avec un IMC élevé.

Practical implications

- Prescribe exercise of decreasing intensity for people who need to use more fat as an energy source.
- To achieve better adherence to physical exercise programs, prescribe decreasing exercise given that a lower assessment is shown with the Borg scale.
- Future research is needed to determine if lower durations and intensities achieve the same metabolic benefits.

Introduction

Obesity is considered a pandemic [1]. According to the World Health Organization (WHO), in 2016 more than 1900 million adults over 18 years old were overweight, of which 650 million were obese [2]. Besides, obesity and overweight determine a high risk of chronic non-communicable diseases such as type-2 diabetes mellitus, hypertension, cardiovascular disease, and some types of cancers [3,4].

The Chilean National Health Survey 2016—2017 [5] showed that 74.2% of Chilean population has a BMI ≥ 25. These numbers have induced the development of many initiatives to stop the progression of obesity, due to the high burden of the illness and its comorbidities. According to the WHO, the treatment of obesity should be focused on managing risk factors and long-term weight maintenance, since a decrease of 5—10% body weight has a significant impact on associated diseases [3].

Being physically active is considered a protective factor in obese patients independently of BMI [6]. The American College of Sports Medicine (ACSM) determined that the recommendation for this population at risk is aerobic exercise from moderate to high intensity (40—60% of maximum oxygen consumption), with a duration of at least 20 minutes for five days a week [7]. Moreover, evidence suggests that mild to moderate exercise increases the use of free fatty acids as an energy alternative to carbohydrates [8,9].

Various exercise referral schemes have been planned for overweight and obese individuals to achieve maximum fat oxidation. Within these programs, bouts of aerobic exercise alternate with periods of rest have been widely studied [10—15]. Stich et al. [10] and Goto et al. [11,12] observed a higher rate of fatty acid oxidation in subjects who went through exercise at intervals compared to those who performed it continuously. According to other similar studies [13—15], there is evidence that bouts of aerobic exercise are more efficient than continuous exercise with equivalent intensity and duration. However, it is necessary to determine the design of a protocol that could allow the optimization of fat oxidation and the applicability in general population.

Table 1 Characteristics of the study subjects and glycaemia, insulinaemia and capillary lactate concentrations pre and post-exercise.

Anthropometric measurements, peak oxygen consumption and biochemical findings of participating subjects in the study		
	Mean	Ds
Age (year)	33.6	3.03
Weight (kg)	92.05	16.47
Height (cm)	174.3	9.72
BMI	30.08	0.8
VO_2 peak ($mL·kg^{-1}·min^{-1}$)	32.79	7.02
Total Cholesterol	210.4	40
HDL (mg/dL)	45.06	14.5
LDL (mg/dL)	127.21	37.67
VLDL (mg/dL)	38.05	14.8
Triglicerides (mg/dL)	190.24	74.1

Glycaemia, insulinaemia and capillary lactate concentrations pre and post-exercise			
Protocol	Pre-exercise	Post-exercise	*P*-value
Glycemia (mg/dL)			
IIP	83.7 ± 3.7	90.5 ± 3.4	> 0.05
CIP	86.8 ± 3.5	85.9 ± 4.3	> 0.05
DIP	88.4 ± 2.9	85.6 ± 2.9	> 0.05
Insulinaemia (μUI/mL)			
IIP	8.2 ± 0.8	8.1 ± 1.5	> 0.05
CIP	9.2 ± 1.2	8.9 ± 1.6	> 0.05
DIP	13.1 ± 1.3	7.1 ± 1.1	< 0.01
Lactate (mM)			
IIP	2.1 ± 0.1	15.7 ± 1.5	< 0.01
CIP	2.3 ± 0.1	6.0 ± 0.5	< 0.01
IIP	2.1 ± 0.1	4.2 ± 0.3	< 0.01

Values expressed as mean ± standard deviation (DS).

Therefore, this study aims to compare three different protocols of interval exercise performed at constant, increasing and decreasing intensity respectively: in a apparently healthy, sedentary population of individuals with elevated BMI. Our hypothesis suggests that bouts of exercise of decreasing intensity is the most effective protocol over fat oxidation compared to the other protocols, with equal duration and average intensity.

Methods

Ten male volunteers were recruited by telephone, ages between 30 and 39 years, sedentary and with BMI ≥ 25. Their main features were (values expressed in arithmetic mean ± standard error of the mean) age 33.9 ± 0.9 years; height 174.2 ± 3.1 cm; weight 92.1 ± 5.2 kg; body fat (determined by bioelectrical impedance with body composition analyzer TBF-300A, Tanita, Tokyo, Japan), 29.1 ± 1.4% and BMI 30.1 ± 0.8 kg/m^2.

The level of physical activity was assessed by the International Physical Activity Questionnaire (IPAQ) [16], resulting in six minimally active subjects and four inactive subjects according to the WHO classification [17]. Their sedentary condition was later confirmed by the volunteers who reported no practice of physical activity at least for 30 minutes three times a week. The subjects had no concomitant diseases or were under drug treatment that was relevant for the purposes of this investigation.

They all underwent a general medical evaluation before the study, including blood glycaemia (85 ± 3 mg/dL); lipid profile (total cholesterol 210 ± 13 mg/dL, HDL 45 ± 5 mg/dL, VLDL 38 ± 5 mg/dL, LDL 127 ± 12 mg/dL and triglycerides 190 ± 23 mg/dL) and insulin resistance index (HOMA-IR [18] 2,44 ± 0.28). They also underwent a cardiac stress test (ECG) according to standard protocol with normal results for all subjects. The results of these measurements per individual as well as peak oxygen consumption (peak VO_2) are presented in Table 1.

All subjects signed an informed consent before the beginning of the study. The research was previously approved by the Bioethics Committee for Human Research of the Faculty of Medicine, University of Chile.

The subjects were assessed in four opportunities, separated by a minimum of three days and a maximum of seven days [19]. In the first instance, peak aerobic power (PAP) measured by magnetic cyclo-ergometer (Jaeger, Würzburg, Germany) was assessed with a protocol of increased load of 25 W every 2 minutes, as used by Goto et al. [12]. The subjects had a VO_2 peak of 31.41 ± 2.22 $mL·kg^{-1}$ min^{-1} (mean ± SEM), and all achieved two of the four criteria established by the ACSM for determining PAP [7]. In a second instance, subjects were distributed randomly into one

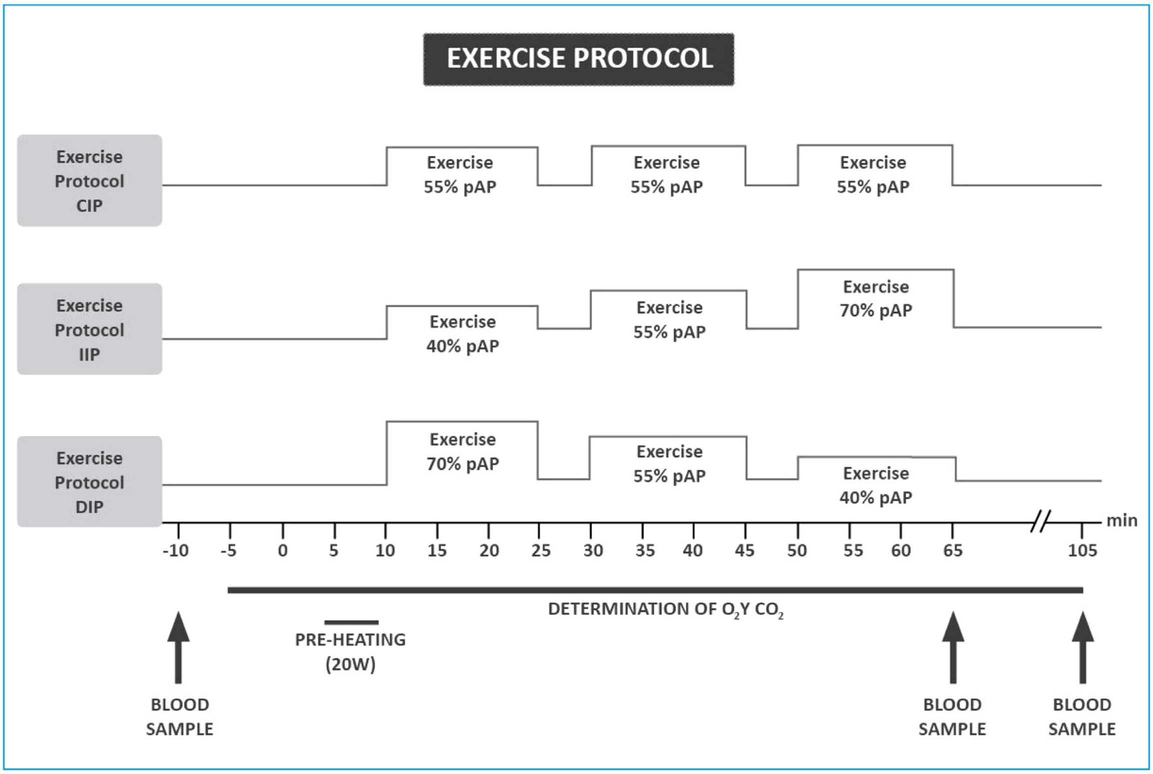

Figure 1 Exercise Protocols. W: watts; O_2: oxygen; CO_2: carbon dioxide; CIP: constant intensity protocol; IIP: increasing intensity protocol; DIP: decreasing intensity protocol; PAP: peak aerobic power.

exercise protocol: constant, decreasing or increasing intensity exercise. Subjects were again randomly distributed into the two remaining protocols, in a third and fourth instance, until completion of all established protocols.

The protocols consisted of three bouts of 15 minutes exercise, separated by periods of five minutes of rest in between. The constant intensity protocol (CIP) worked with 55% of the PAP, the increasing intensity protocol (IIP) with 40, 55 and 70% of the PAP, and the decreasing intensity protocol (DIP) with 70, 55 and 40% of the PAP, as shown in Fig. 1.

Each subject performed the exercise protocols after twelve hours of fasting. The day before the test, subjects did not practice high intensity exercise nor ingested food that could affect their physical work capacity. Prior to the beginning of any protocol, each subject had to rest in sitting position for 5 minutes to take the first blood sample from the right antecubital vein. Afterwards, analysis of the respiratory quotient (RQ) was conducted for 5 minutes with the subjects at rest. At the end of this period, subjects began pedaling (20 W) for 5 minutes warm up on the cycle ergometer, in order to prevent injuries.

Notably, RQ was recorded continuously throughout the workout and lasted until 50 minutes after completion. Additional blood samples were collected after the end of the exercise and 50 minutes later. Additionally, the volunteers were questioned about their perception of exertion at the end of each exercise session using the Borg scale [20].

All protocols were conducted at ambient temperature controlled between 21 and 23° C.

Respiratory gases were evaluated by ergo spirometer (Oxycon Pro, Jaeger, Würzburg, Germany) calibrated prior to each evaluation. With this instrument, average oxygen consumption and carbon dioxide production were obtained every 30 seconds by the analysis method of breath by breath.

Blood samples collected were immediately refrigerated and derived to the Bioanalysis Laboratory Ltda. Chile (Av Providencia 2392; Providencia, Santiago, Chile). Hence, plasma glucose concentration was determined by the glucose-peroxidase enzyme method yielding a coefficient of variation intra-assay of 0.6% and inter-assay of 1.3%. Also, insulin was determined with immune chemiluminescence method resulting in a sensitivity of 6.0 pM and a coefficient of variation intra-assay of 2.0% and inter-assay of 5.0%.

Furthermore, capillary lactate was determined in situ by an automatic analyzer withdrawing blood from the fingertips (Sirius, h/p/cosmos, Nussdorf-Traunstein, Germany).

Calculation of the rate of fat and carbohydrate oxidation was conducted with stoichiometric equations described by Frayn [21], using as reference the caloric equivalent of palmitoyl-stearoyl-oleoyl-glycerol and glycogen, respectively [22]. Protein intake input to caloric consumption was estimated assuming a rate of 0.135 nitrogen excretion $mg \cdot kg^{-1} \cdot min^{-1}$, an average value used consensually in these studies [23].

For statistical analysis, data are expressed as arithmetic means ± standard error of the mean (SEM). For statistical comparisons, when the variable in question showed normality with Shapiro-Wilks test, paired *t*- Student test was used. In cases where a normal distribution was not found, Wilcoxon and were used. Differences were considered statistically significant with a $P < 0.05$ (95% CI). All calculations were performed using SPSS statistical software, v15.0 (IBM

Corporation, Somers, USA). Graphics were made with Graphpad Prism 6.

Results

Table 1 shows the values of glucose, insulin and capillary lactate concentrations observed immediately previous and after the exercise periods, for each protocol. Regarding glycaemia, no significant differences were observed in any of the three protocols ($P > 0.05$). Insulinaemia only showed a significant decrease in the DIP ($P < 0.01$). Capillary lactate values, however, showed significant differences between the pre-exercise sample and the immediately after exercise samples in all three protocols ($P < 0.01$). Furthermore, the values of capillary lactate post-exercise are significantly different between the three protocols ($P < 0.01$), being smaller for the DIP.

Regarding caloric expenditure, there were no significant differences ($P > 0.05$) in total caloric expenditure among the three protocols (Range: 5.9 ± 0.2 to 6.1 ± 0.2 kcal·kg^{-1}). Significant differences ($P < 0.05$) between the IIP and other protocols were observed in the post-exercise period and in the total sum of rest periods in between exercise intervals.

The proportion of oxidized substrates (carbohydrate and fat) is estimated according to RQ for each period. Calories attributable to carbohydrates (expressed as caloric equivalents of glycogen) and fat (expressed as caloric equivalent of a representative triglyceride: palmitoyl-stearoyl-oleoyl-glycerol). The values of the first three minutes of each exercise interval are not shown due to variability of RQ in that period.

There are significant differences in the oxidized substrate, comparing IIP and DIP. In the first period of IIP (40% intensity PAP), fat oxidation is significantly lower than that of carbohydrates. By contrast, at the same intensity (40% PAP) in DIP (third period), the contribution of both types of substrates is similar. CIP causes a progressive increase in fat oxidation at the expense of carbohydrate oxidation. A progressive fall in the consumption of carbohydrates stands out during the first exercise interval, coinciding with an increased use of fats, in the three protocols. DIP causes a progressive increase in fat oxidation comparative to IIP and CIP protocols. In period third of the protocols, DIP oxidized significantly more fat than the other two.

Calories attributable to carbohydrates are significantly higher ($P < 0.01$) in all periods of IIP except for the sum of the two periods of rest in between exercise intervals, where it is significantly lower ($P < 0.01$).

Perception of exertion; average ($\pm$ SEM) of 16.4 ± 0.7 (hard - very hard) were obtained as results for the IIP; 14.1 ± 0.4 (something hard - hard) for CIP; and 12.2 ± 0.3 (moderate - something hard) for DIP, being all of these differences statistically significant ($P < 0.01$) (Fig. 2).

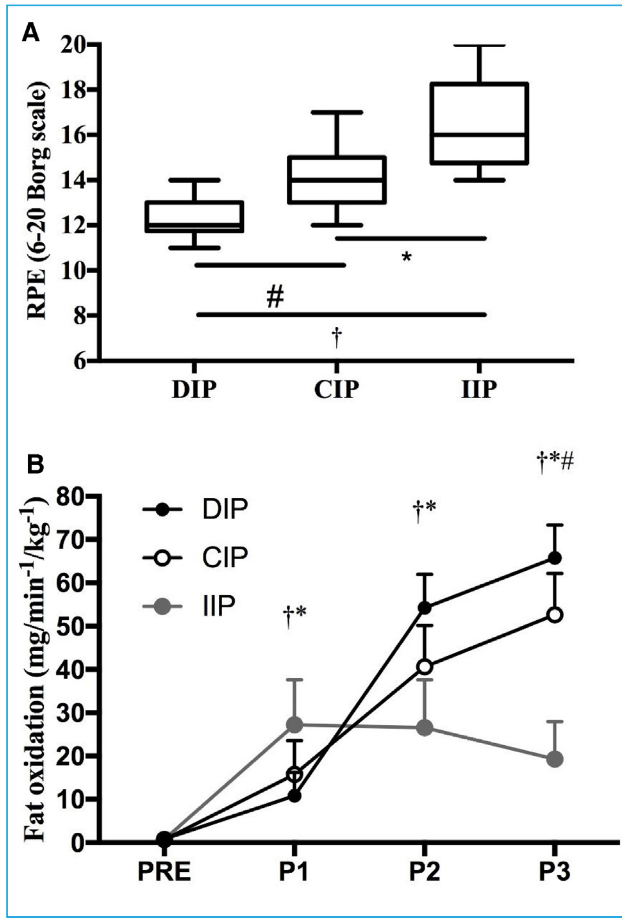

Figure 2 Perception of exertion and fat consumption during the total experimental protocol and in between the different interval periods of the same. A: † Significant difference between DIP in comparison to IIP group. * Significant difference between CIP in comparison to IIP group. # Significant difference between DIP in comparison to CIP group. B: † Significant difference between DIP in comparison to IIP group. * Significant difference between CIP in comparison to IIP group. # Significant difference between DIP in comparison to CIP group.

Discussion

This study supports the idea that the decrease of glycogen reserve causes an increase in the use of triglycerides, not only between the exercise periods, but also within the same exercise interval (with the condition that carbohydrate reserves are high at that time, such as it occurs in the first interval of each exercise protocol).

The results obtained confirm the hypothesis, which in equally average duration and intensity conditions, exercise in DIP and CIP are more effective over fat oxidation than the exercise performed in IIP in healthy, sedentary individuals with elevated BMI. The available evidence sustains a plausible biological explanation in which both systemic neuroendocrine and biochemical mechanisms take place at the level of skeletal muscle cells supporting the results obtained.

Several observations demonstrate an enhanced neuroendocrinological response (mainly sympathetic/adrenergic) in exercise performed at intervals compared with continuous periods, which would favor a lipolytic state [12]. It has been shown in several studies that catecholamine release is significantly greater in the second exercise interval periods compared to the first, which promotes lipolysis [10—15]. Additionally, it is expected that episodic release of catecholamine causes a less continuous desensitization, given the great down regulation that adrenergic receptors have against prolonged exposure to agonist [24]. This would obviously increase the metabolic response in both skeletal muscle and adipose tissue. It has also been shown significantly greater decreases of insulin, a powerful anti-lipolytic agent, in intervals subsequent to the first, favoring the use of free fatty acids [10—15]. This effect may be due, in part or totally, to increased adrenergic activity, discussed before, since it is known that activation of alpha-adrenergic receptors in the pancreatic beta cell causes a potent inhibition of insulin secretion [25]. Besides the above-mentioned hormones, increased secretion of cortisol [10] and of atrial natriuretic peptide [26], as well as an increase in production of interleukins (IL), particularly IL-6 [15], seen in these protocols of exercise at intervals - compared to continuous exercise would contribute to greater use of fats, since all of these substances also have a marked lipolytic effect [27].

The result of this neuro-endocrinologic enhanced response, particularly the greater proportion of epinephrine/insulin in plasma, associated with further lowering of glycaemia recorded in the exercise interval periods compared to continuous exercise [11,13], create a markedly lypolitic environment supporting the effectiveness of these types of exercise over fat oxidation.

Moreover, it has been shown that glycogen levels of skeletal muscle fiber affect the activity of AMP kinase (AMPK), promoting the uptake and utilization of glucose and free plasma fatty acids by muscle [28]. Since the AMPK is activated by AMP, it is considered that this enzyme plays a pivotal role in the regulation of metabolic fluxes within the muscle, acting as a true ''energy sensor'' and activating the oxidation of fatty acids and carbohydrates [28,29] In the study of Steinberg G. (2009) [29] it is shown that this enzyme has a subunit that binds glycogen and this binding leads to its inhibition, supporting the idea that glycogen, apart from its condition of energy status reserve, is an active regulator of carbohydrate oxidation and, in greater extent, of fatty acids [29]. Thus, decreasing of intramuscular glycogen (typical of a high intensity exercise) causes the activation of AMPK, promoting a metabolic switch that leads to increased fatty acid oxidation [28,29]. This is why performing a workout session preceded by another session, such as during interval exercise, the subject has decreased glycogen levels in the second session with respect to the first, being this condition capable of a significant activation of the AMPK [28,29]. Furthermore, the greater the intensity of the previous period of exercise, a further decrease in glycogen levels in the subsequent period and a higher fat consumption occur, not only by the lack of alternative substrate, but also because of the stimulation of lipid catabolism due to activation of AMPK. Hormonal and metabolic mechanisms previously described are potentially synergistic, and due to an initial increase in glycogen decline, could all account for the increased fat oxidation observed in this study with the DIP.

Another factor that could potentially contribute to increased lipolysis observed in the DIP was the lower production of lactate at the end of this protocol. Evidence shows that lactate acts on adipose tissue as an anti-lipolytic signal at the level of GPR81 receptor associated with inhibition of adenylate cyclase in adipocytes [30]. In fact, the inverse relationship between the concentration of plasma lactate and fat oxidation has been well documented [31].

Beyond the potential physiological and biochemical mechanisms that may be accounting for the observed effects, it is relevant to point out that this study is the first to test this type of exercise protocols at short intervals in subjects with elevated BMI. It should be noted that the group of individuals recruited had no metabolic diseases, therefore caution should be taken when projecting the findings of this study to the general population of individuals with elevated BMI, where they may have associated metabolic pathologies.

The results obtained, along with the fact that the exercise protocols used in this study were of short duration, and that the most effective protocol over fat oxidation was also the one that experimental subjects perceived as the less strenuous (DIP), are particularly promising in the practical application of these findings.

In this regard, it should be noted that the design of the protocols used related to the total exercise duration (55 minutes from the beginning of the first period and the completion of the latter) and average intensity (55% of the PAP) was ideated purely based on practical considerations, taking into account the potentiality of this exercise as a routine activity in the study population (middle-aged individuals, occupationally active and not used to performing regular exercise).

The acquisition and maintenance of exercise habits have important factors such as the perception of exhaustion at the end of the exercise and effectiveness, which is why we estimate that DIP provides a valuable combination to optimize the effects of physical activity over fat mass reduction and to contribute promoting exercise habits of simple application, limited risk and easy compatibility with current lifestyle.

Conclusions

That fat oxidation in exercises at intervals of varying intensity depends on the order of the periods of exercise, being higher when they are executed in a decreasing order. In addition, the perception of exertion in the decreasing protocol is lower, making this more useful and feasible for sedentary subjects with elevated BMI. We hope this study will be the prelude to more research on this subject, thus allowing the implementation of more efficient exercise protocols in users with obesity.

Disclosure of interest

The authors declare that they have no competing interest.

Confirmation of ethical compliance

This study complied with the standards established by the Declaration of Helsinki and the Law on Human Studies and was approved by the ethics committee of the Faculty of Medicine of the University of Chile. All participants gave their informed consent in writing before participate in the study.

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