Effect of High-Intensity and Concurrent Training in Body Composition in Costa Rican Overweight and Obese Women

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Abstract
This study was designed to determine the changes in body composition, Body Weight (BW), Fat Mass (FM), Body Fat Percentage (BF%) and, Skeletal Muscle Mass (SMM) of High Intensity Interval Training (HIIT) and Concurrent Training (CT), comparing them to a Control Group (CG) for four months of intervention in overweight and obese women.

Methodology: 67 obese and overweight women (37.54 ± 8.8 years, BMI = 28.49 ± 4.9, BF% = 43.63 ± 5.6%) were randomly assigned to three groups, HIIT (n = 26, mean age = 37.9 ± 7.3 years, BF% = 42.36 ± 4.3%), CT (n = 20, mean age = 38.0 ± 8.5 years, BF% = 45.98 ± 5.4%) and CG (n = 21, mean age = 36.6 ± 11.0 years, BF% = 42.97 ± 6.8%). Before and after 16 weeks of HIIT (intervals at ≥ 80% Maximal Heart Rate) and CT (strength training, 65%-75% 1RM + endurance training, 60%-80% Maximal Heart Rate), participants underwent Dual-Energy X-Ray Densitometry (DEXA). The CG did not exercise at all.

Results: There were no changes in BW among the experimental groups (p > 0.05). Compared to CG, the HIIT and CT groups decreased FM, BF% and increased SMM (p < 0.05). CT was better than HIIT on FM (relative change, Δ%, HIIT = -3.4% vs. CT = -4.5%), BF% (HIIT = -2.6% vs. CT = -4.3%) and SMM (HIIT = +1.7% Vs. CT = +5.4%).

Conclusion: Findings show improvements of body composition in overweight and obese women. In this context, although the results were not highly marked, CT seems to be a better approach for the prevention and management of the women overweight and obesity than HIIT.

Keywords
Obese, Overweight, Women, Exercise, High intensity, Concurrent training

Abbreviations
ET: Endurance Training; ANOVA: Analysis of Variance; BF%: Body Fat Percentage (BF%); BW: Body Weight; CT: Concurrent Training; CG: Control Group; DEXA: Dual-Energy X-Ray Densitometry; EG1: Experimental Group 1; EG2: Experimental Group 2; FM: Fat Mass; SMM: Skeletal Muscle Mass; HR: Heart Rate; HIIT: High Intensity Interval Training; HRmax: Maximum Heart Rate; VO2max: Maximum Oxygen Consumption; MET: Metabolic Equivalent of Task; MS: Muscular Strength; R: Randomization

Introduction
The high prevalence of overweight and obesity in developing countries are a result of continued changes to lifestyle patterns, such as diet and physical activity, leading to an accelerated increase in overweight, obesity, and related chronic diseases in many countries [1]. Today, overweight and obesity remain a major public health concern, and exercise interventions have been pointed out as an important target in the prevention of weight gain and overweight/obesity development [2].

It has showed that 8 months of Strength Training (ST), (3 times per week, 3 sets, 8-12 repetitions), Endurance Training (ET), (12 miles per week at 65-80% peak VO2) performed on a treadmill, elliptical trainers or cycle-ergometer) or their combination can improve the body composition variables of overweight/obese adults [3]. To perform ST 2-3 times a week, in combination with ET is recommended for these individuals to maintain or improve their health [4,5].
An excess of body fat has been identified as one of the most important risk factors for chronic disease morbidity and mortality in men and women. Most women and men, gain fat through middle adulthood and adult weight gain has many adverse health implications: the risk of heart disease, diabetes, and certain cancers is substantially elevated with higher weight [6]. Women experience a reduction in basal metabolic rate and loss of Skeletal Muscle Mass (SMM) as they transition to menopause; thereby increasing their risk for body weight gain and obesity [7].

High Intensity Interval Training (HIIT) promotes positive adaptations that attenuate the effects of obesity [8] and could be used as a time-effective therapy for the management of body fat levels in overweight and obese women [9]. HIIT has demonstrated significant reductions (p < 0.05) in body composition variables such as Body Weight (BW), Fat Mass (FM) and Body Fat percentage (BF%) in women [10-12]. For example, Trapp, et al. [10] carried out a 15 weeks of HIIT intervention (20 min per session, 60 repetitions, 8 s maximal effort intervals with 12 s rest, 3 times per week performed on a cycle-ergometer) in obese young women (20.2 ± 2.0 years old) and their results shows significant reductions in FM (Δ% = -11.11%) and BF% (Δ% = -7.69%) without significant changes in SMM (p > 0.05). The key of HIIT prescription is training intensity; it is characterized by a physical effort of 80% to 100% of maximum Heart Rate (HR_max) in intermittent interval training sessions of up to 30 minutes [13].

On the other hand, over recent years, a growing body of scientific knowledge strongly suggests that regular performance of ST and ET is a major factor in the prevention and treatment of cardiovascular disease including risk factors such as obesity [14]. Concurrent training (CT, ST+ET) has demonstrated results in loss of fat mass percentage [15-17] and important increments in SMM [18].

Previous studies have combined HIIT and ST, showing improvements in health parameters like body composition and it has previously been shown that, during CT, body fat decreased more with increasing ET intensities [19]. Including exercise modes into the same training regimen may be even more effective than adhering to only either training mode alone for this target [20].

However, there is currently a dearth of knowledge about the independent effects of HIIT and CT on body composition, relative to the effects of others exercise modes [21]. For example, Trilk, et al. [22] applied a HIIT protocol in a period of 4 weeks (4-7 intervals of 30 s with 4 min rest, 3 times per week performed on a cycle-ergometer) and they did not meet significant changes in BW, FM, BF% (p > 0.05) of overweight women (30.1 ± 6.8 years old) tested by DXA, and Ali-Mohamadi, et al. [23] carried out a CT intervention for 8 weeks in overweight women (23.2 ± 2.4 years old) who performed a ST program (6 exercises, 3 sets of 8 repetitions at 80% 1RM, 2-3 min rest between sets and 3-5 min between each exercise) and ET program (10 min + 30 s per session at 75-80% HR_max) 3 times per week. They obtained significant reductions (p < 0.05) in FM (Δ = -26.95%) and BF% (Δ = -23.23%), but not in SMM (p > 0.05).

Due to the difference in procedures among them, most of the training protocols show dissimilarities in variables such as frequency, intensity, duration and type of exercise, which make it more difficult to understand which methods are the most beneficial to overweight and obese women.

HIIT clearly provides a potent physiologic stimulus for improving body composition, but, there are many questions that remain. It has been widely accepted that SMM is important for regulating multiple metabolic processes, therefore it is critical to identify modes of exercise training that can result in loss of fat mass while also preserving SMM or even building SMM in a more effective way and HIIT could provide a potent anabolic response for overweight and obese women [24].

Therefore, this study aimed to determine the changes in body composition based on two training programs, HIIT and CT, by comparing them with a Control Group (CG) over the course of four months in overweight and obese women. Session duration in both training programs was compared by taking into account the caloric expenditure (METs) so as to minimize any imbalance in physical effort protocols.

Methods

The study was performed over a period of 16 weeks. Participants were randomly separated into 3 groups. Group 1 performed 16 weeks of HIIT, group 2 performed CT and group 3 was asked to continue with their routines and not to engage in any physical activity during the research period.

Subjects

Participants were recruited through Facebook and poster advertisements. All participants completed health history and physical activity questionnaires and met the following inclusion criteria: 18-years-old or older, physically independent, body fat percentage ≥ 26% as overweight and obesity indicator [25], free from cardiac or orthopedic problems, not receiving nutritional therapy, not smokers, and not be pregnant before start of the study. One hundred four (n = 104) women were accessed for eligibility. After individual interviews, 28 women who did not meet the inclusion criteria were excluded. The remaining 89 women were selected for par-
assessed for eligibility (n = 104)

No meeting for inclusion criteria (n = 15)

Randomized (n = 89)

Group 1 (n = 31) Allocated to HIIT intervention 3 × a week 16 weeks of HIIT training

Lost in follow-up (n = 5)

Analyzed (n = 26) Exclude from analysis (n = 0)

Group 2 (n = 29) Allocated to CT intervention 3 × a week 16 weeks of CT training

Lost in follow-up (n = 9)

Analyzed (n = 20) Exclude from analysis (n = 0)

Group 3 (n = 29) Allocated to Control Continue daily routine No physical activity

Lost in follow-up (n = 8)

Analyzed (n = 21) Exclude from analysis (n = 0)

Figure 1: Experimental design.
Procedures

At the beginning and the end of the experiment, 2 weeks were allocated for evaluations (weeks 1-16), consisting of body composition analysis by Dual-Energy X-Ray Absorptiometry (DXA). This was done at the Body Composition Laboratory at Research Center in Sciences of Human Movement (CIMOHU, for its acronym in Spanish), University of Costa Rica. The experimental design is displayed in Figure 1.

Body composition

Changes in body composition variables BW, FM, BF% and SMM were measured by Dual-Energy X-Ray Densitometry (DEXA) with a densitometry model Lunar Prodigy Advanced PA+350411 of GE Healthcare with a 2% margin of error. A complete body exam was applied to every subject at the beginning and end of study. Before the test, participants were instructed to lie on a flat table face up and remain still during the scan.

SMM was estimated by the predictive equation proposed by Kim, et al. [26] and it was determinate as Fat-Free Muscle Mass (FFMM).

Muscle strength

One repetition maximal was evaluated for maximal dynamic strength in exercises chest press, leg press and biceps curl only for Strength Training (ST) exercise prescription. A warm-up set (6-10 repetitions) with light load to familiarize the subjects with the testing equipment and lifting technique was preceded for each exercise testing. Procedure was initiated 2 minutes after the warm-up set and subjects were instructed to try to accomplish 2 repetitions with the load in 3 attempts in all exercises tested. A period of 3-5 minutes rest was given between each attempt and 5 minutes between exercises. The last resistance lifted in which the subject was able to complete only 1 repetition was recorded. Each exercise technique was standardized and continuously monitored to ensure reliability. 1RM testing sessions were supervised by one experienced researcher to maximize safety and test reliability. Verbal encouragement was given throughout each test [27].

Training Programs

Concurrent training

Supervised CT was performed in the Fitness Gym facilities in Cartago, Turrialba. (Cartago is a Costa Rican province and Turrialba is a city in that province). The protocol was based on recommendations for CT (ST+ET) in overweight and obese women population to improve body composition [4] and, it was made in a novel way. All participants were personally supervised by a physical education professional to help ensure consistent and safe exercise performance.

Strength training program

Subjects performed ST using a combination of free weights and machines. The sessions were performed 3 times per week in non-consecutive days. The ST program was a whole-body program of 8-9 exercises (Table 1) with 4-5 exercises performed with free weights and 4 with machines. Exercises were performed in the following order: Upper-limb, lower limb and, core. Some exercises were changed every 4 weeks to maintain the neuromuscular stimulus of the participants.

Participants performed 2-4 sets of 15-8 repetitions with a load of 65-75% of 1RM. Participants were instructed to inhale during the eccentric phase and exhale during the concentric phase of each repetition while maintaining a constant velocity of movement at a ratio of approximately 2:2 (concentric and eccentric phases, respectively). Participants were allowed 1-2 minutes of rest between sets of an exercise and 2-3 minutes between exercises.

The loads of each exercise were adjusted according to the improvements in exercise capacity for 1RM each 4 weeks. The subjects were exercising with as much resistance as possible while maintaining proper exercise technique throughout the study. Progression for the ST was accomplished by increasing the load for 5% of 1RM each 4 weeks.

Endurance training program

In the ET sessions the subjects underwent a heart rate-controlled running training program with cardiac monitors for beginners outdoors. During the first 2 weeks, participants alternate between walking and running in order to allow them to get used to the training load; after 3 weeks, the subjects ran, if possible, continuously at an intensity of 70-80% of their maximal heart rate as determined by the formula 220-age [4] for 20-30 min through the study.

CT participants did 20 minutes of ST and 20 minutes of ET during the first weeks of the research period and 30 minutes of ST and 30 minutes of ET during the last weeks of the research period. All of the participants did 5 minutes of warm-up and 5 minutes of stretching before each session and 5 additional minutes of stretching at the end of each session. The caloric expenditure was approximately 3.62 METs in the first sessions and, 4.54 METs for last sessions [28]. The session’s duration was 40 min in weeks 1-8 and 60 min in weeks 9-16. Protocol of CT prescription is displayed in Table 1.

High intensity interval training program

Subjects performed HIIT using their body weight. The sessions were performed 3 times per week in non-con-
secutive days. The HIIT program was a whole-body program of 10 body exercises in the first 8 weeks with a HIIT commercial video called Insanity Max30 Program® in the last 8 weeks of the study. Exercises were performed in the following order: Jogging, jumping jacks, high knees, 1-2-3 drills, push-ups, frog jumps, squats, plank touches, jump rope, 1-2-3 knees up. Some exercises were changed every 3 weeks to maintain the neuromuscular stimulus of the participants.

Participants performed 10-12 intervals of 30-45 seconds with their body weight load for 8 weeks and the last 8 weeks performed 6 intervals of 5 min with Insanity Max30 Program®. Participants were instructed to maintain a constant velocity of movement according to their physical capacity. Participants were allowed 1 min 30 s of rest between intervals at the beginning of the study and 30 s at the end.

Progression for HIIT was adjusted by raising the number of intervals and time of exercise and, reducing time of rest. The subjects were exercising with as much resistance as possible while maintaining proper exercise technique throughout the study. All of the participants did 5 minutes of warm-up and 5 minutes of stretching before each session and 5 additional minutes of stretching at the end of each session. The duration of the HIIT sessions was 35-45 minutes long with a caloric expenditure similar of CT [28]. All participants were personally supervised by a physical education professional to help ensure consistent and safe exercise performance. Protocol of HIIT prescription is displayed in Table 2.

### Statistical Analyses

Analyses of Variance (ANOVA) in two-factor mixed-design ANOVA with repetitive measures in one factor (3 groups × 2 measures) was applied with SPSS statistical program version 21.0. In addition, statistical monitoring was applied to the significant interactions using a post hoc analysis of simple effects to determine the differences between groups and measurements when needed. The
significance level of 95% ($p < 0.05$) was useful for every case. Both Hedges’s effect size [29] and the corresponding percentage changes were applied. To calculate Hedges’ $g$ size effect size [29], the following formula was used:

$$g = \frac{\text{Posttest} - \text{Pretest}}{DS}$$

$$DS = \sqrt{\frac{(n_{\text{post}} - 1)DS_{\text{post}}^2 + (n_{\text{pre}} - 1)DS_{\text{pre}}^2}{n_{\text{post}} + n_{\text{pre}} - 2}}$$

$n =$ number of subjects.

Based on Thomas, Salazar & Landers [30]. Size Effect (ES) is interpreted as $< 0.40$ small, $> 0.40$ a $< 0.70$ moderate, $> 0.70$ big. Changing percentage was calculated using the following formula:

$$\Delta\% = \frac{\text{Posttest} - \text{Pretest}}{\text{Pretest}} \times 100$$

Results

Forty-six ($n = 46$) overweight and obese women completed a total of 48 training sessions and 67 of them applied both pre-tests and post-tests. The sample was composed for 44% housewives, 40% workers and, 14% university students. The physical and physiological variables of the three groups at the beginning and at the end of the 16-week period are summarized in Table 3. The probability of a Type II Error was 78% for FM, 94% for BF% and 99% for SMM. Comparisons of the three groups (HIIT, CT and CG) showed that they were homogenous for body composition parameters, BW ($p = 0.256$), FM ($p = 0.970$), BF% ($p = 0.820$) and, SMM ($p = 0.863$) before the commencement of the training program. At the post-intervention, a not significant change ($p > 0.05$) in the BW of all groups was obtained. On the contrary, FM decreased significantly in HIIT group ($p < 0.05$, ES = -0.14) and in CT group ($p < 0.05$, ES = -0.13). In the between groups’ comparison, FM in CT group were significantly different ($p < 0.05$) from the two other groups (HIIT and CG). Similarly, in post-intervention, the HIIT participants’ BF% decreased significantly ($p < 0.05$, ES = -0.24). In CT group, the BF% was also significantly decreased ($p < 0.05$, ES = -0.36). In the between groups’ comparison, BF% in CT group were significantly different ($p < 0.05$) from the two other groups (HIIT and CG). At the end of the training period, a significant increase was observed in SMM of both trained groups ($p < 0.05$, ES = 0.10 and 0.29, for HIIT and CT, respectively), but a higher increase was noted in CT group when compared to HIIT or control groups ($p < 0.05$).

Discussion

The main focus of this study was to compare the effects of HIIT versus CT on body composition parameters in overweight and obese women. The present study
results demonstrated for the first time that both types of training were effective in improving body composition in this sample. A major finding from our study was the trainings groups participants’ relatively improvement in BF%, which was attained through concurrent FM loss and SMM gain (Figure 2). No changes were observed in BW for both training groups and for all variables in CG.

Exercise alone is not an effective method for achieving initial weight loss. Without calorie restriction, weight loss through exercise alone is quite small, about 0.1 kg/week after 12 months, but, even in the absence of significant weight loss, regular aerobic and resistance exercise improves cardiovascular fitness and obesity-related comorbidities [31]. According to the American College of Sports Medicine’s position, ST and ET combined are superior to either exercise modality alone with respect to

Figure 2: Fat mass, body fat percentage, and skeletal muscle mass at pre- and post-training. *p < 0.05 pre-training vs. post-training, £p < 0.05 training groups vs. control, ¥p < 0.05 HIIT vs. CT. Data are expressed as mean and SD.
concurrent FM loss and SMM gain. Based on our participants’ significant improvements in body composition, the combined training approach appears to be a more effective intervention for overweight and obese women than performing HIIT alone [32].

In our study, three sessions per week of HIIT and CT during 16-week period provided a physiological stimulus for improving body composition. We identify two potential modes of exercise training prescription that can result in FM loss while also preserving SMM or even building SMM, in four month. Fisher, et al. [24] proposed that HIIT could provide a potent anabolic response for overweight and obese women SMM, and in our study, this statement was observed briefly. This is a preliminary result that needs to be more investigated.

Previous studies with similar intervention periods have demonstrated the benefits of HIIT and CT in women body composition, but, some aspects are not clear in overweight and obese women yet. Respect to HIIT, Trapp, et al. [10] carried out a 15 weeks of HIIT intervention (20 min per session, 60 repetitions, 8 s maximal effort intervals with 12 s rest, 3 times per week performed on a cycle-ergometer) in obese young women (20.2 ± 2.0 years old) and their results show significant reductions in FM (Δ% = -11.11%) and BF% (Δ% = -7.69%) without significant changes in SMM (p > 0.05). Comparing our results with Trapp, et al. [10], a protocol based on cycle-ergometer is better to decrease FM and BF%, but not to improve SMM. In this context, our protocol intervention shows be potentially more effective than cycle-ergometer HIIT for building SMM, and less effective, but similarly beneficial to decrease FM and BF% tested by DXA. Of course, this relationship should be analyzed in a forward experimental study.

Shorter periods of HIIT should appear being less effective to improve body composition in overweight women. Trilk, et al. [22] applied a HIIT protocol in a period of 4 weeks (4-7 intervals of 30 s with 4 min rest, 3 times per week performed on a cycle-ergometer) and they did not meet significant changes in BW, FM, BF% (p > 0.05) of overweight women (30.1 ± 6.8 years old) tested by DXA. In contrast, Kordi, et al. [11] in a HIIT period of 6 weeks met significant decreases in BF% (-11.78%) tested by skinfolds in obese young women (18.64 ± 0.6 years old), while Airin, et al. [12], at the same period exercise, obtained significant changes in BW (-1.9%), BF% (-2.2%) and SMM (+1.15%) by bio-impedance in overweight women (20.5 ± 1.9 years old).

In the Trapp, et al. [10] study and our study, the results were not as high as those obtained by Kordi, et al. [11] in BF% and both contrast with the results met by Trilk, et al. [22] tested by DXA. According to this context, the training duration of HIIT and the instrumental used to measure body composition variables could be fundamental to explain the role of HIIT in overweight and obese women. The changes in body composition reported by shorter studies applying skinfolds and bio-impedance how its measure’s instrument have to be carefully analyzed.

Respect to CT, Ali-Mohamadi, et al. [23] carried out a CT intervention for 8 weeks in overweight women (23.2 ± 2.4-years-old). They performed a ST program (6 exercises, 3 sets of 8 repetitions at 80% 1RM, 2-3 min rest between sets and 3-5 min between each exercise) and ET program (10 min + 30 s per session at 75-80% HRmax) 3 times per week. The results of this study show significant reductions (p < 0.05) in FM (Δ = -26.95%) and BF% (Δ = -23.23%), but not in SMM (p > 0.05). Compared to our study, the ST program (8-9 exercises, 2-4 sets of 15-8 repetitions at 65-75% 1RM, 1-2 between sets and 2-3 min between each exercise) and the ET program (20-30 min per session at 70-80% HRmax) were more physically demanding for a longer duration and overweight women participants presented values in body composition (BW = 73.7 ± 9 kg, FM = 17.7 ± 2.9 kg, BF% = 24 ± 2.3) similar to our study (BW = 76.6 ± 17.9, FM = 34.4 ± 11.9 kg and, BF% = 45.9 ± 5.4), but, our results appear to be less effective than this research.

Due to these similarities in exercise protocol, the body composition characteristics of the sample and the shorter duration of the intervention, the lack of effectiveness observed in our results could be related to the instrument application for these authors in body composition. Skinfolds measurements method seems to include a systematic error in the fat analysis because of its high vulnerability to external factors such as researcher experience or instrument calibration [33] that demand to analyze conclusions using this technique with caution.

The results reported in this study were similar to those reported by Trapp, et al. [10] where intermittent exercises were appropriate and was observed to provide significant improvements in BM and BF. In contrast, when the exercise modality was different, based on cycle-ergometer, the same author Trapp, et al. [10] reported contradictory results in the SMM of obese women compared with our study. Others authors [22] not reported significant results when the exercise modality was different in time of intervention and some were confused when the difference was the instrument employed to measure body composition [11,12,23].

Physiologically, the improvement of both training groups in FM and BF% could be explain by the role of post-exercise lipolysis and energy expenditure for demanding and specific exercise. HIIT and CT require glucose catabolism and in consequence the glucose metabolism is turned away to restore mus-
The most important findings of the present study were the improvements of body composition in overweight and obese women. In this context, although the results were not highly marked, CT seems to be a better approach for the prevention and management of the women overweight and obesity than HIIT. However, the decision on the type of exercise prescription should be individualized to optimize health status variables’ improvement and to enjoy the exercise.

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In regards to the SMM, the continue training sessions suggest important metabolic demands, such as endogenous reserve exhaustion (hepatic glycogen and muscular), body fluid and electocute, hormonal imbalance, and/or imbalance in muscular proteins [19,35] that could originate a chronic muscular hypertrophy due to the increment in the muscular fiber, more actin and myosin, sarcoplasmic, connective tissue or any combination of them if the stimulus keeps constant [36].

Some limitations should be mentioned in this research study design. In order to reduce a potential drop out of the study, and to take account some recommendations in previous experiences, we did not control feeding habits neither tested the follicular stage of the menstrual cycle in overweight and obese women because we did not want to put the living habits of the participants under much stress. This strategy was insufficient to prevent drop-out, therefore, we investigated a small sample of overweight and obese women (n = 67).

Another important aspect of this study was the adherence to exercise despite of drop-out. It is noted that 9 subjects were eliminated from CT for failure to complete the required training sessions, while only 5 were eliminated from HIIT. The time session was important according to some explanations of the participants. Protocols such as HIIT has to be considered in designing training protocols for overweight and obese women, because of it probably contributes to lower drop-out rates compared to CT.

Nevertheless, given the extreme nature of the exercise protocol, it may be doubtful that the general population could safely or practically adopt the model of HIIT and some displeasure could occur for the overweight/obese adults and some other clinical populations [8].

Conclusion

In regards to the SMM, the continue training sessions suggest important metabolic demands, such as endogenous reserve exhaustion (hepatic glycogen and muscular), body fluid and electocute, hormonal imbalance, and/or imbalance in muscular proteins [19,35] that could originate a chronic muscular hypertrophy due to the increment in the muscular fiber, more actin and myosin, sarcoplasmic, connective tissue or any combination of them if the stimulus keeps constant [36].

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Nevertheless, given the extreme nature of the exercise protocol, it may be doubtful that the general population could safely or practically adopt the model of HIIT and some displeasure could occur for the overweight/obese adults and some other clinical populations [8].

Institutional Revision

This study was designed based on the participants’ rights of the National Health Board (Consejo Nacional de Salud, CONIS, for its acronym in Spanish) of the Costa Rican Ministry of Health and it was ensured by the Ethical Scientific Committee of the Vicerrectoría de Investigación of the University of Costa Rica (Session No. 293, VI-2964-2015).

All procedures were fully explained to the participants who signed a written informed consent form meeting the principles of the Ethical Scientific Committee of the Vicerrectoría de Investigación of the University of Costa Rica (Session No. 293, VI-2964-2015).

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