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Running title: Soccer training and insulin action in type 2 diabetes patients

Abstract

Background: We investigated the biomarkers of insulin action as well as changes in free fatty acids and lactate concentration after an acute soccer session pre and post training with caloric-restricted diet versus diet alone in type 2 diabetes (T2D) patients. Methods: Fifty-one middle-aged (61.1±6.4 years) T2D patients were randomly allocated to the soccer+diet group (SDG) or the diet group (DG). The control group comprised T2D patients observing a caloric-restricted diet who did not receive soccer training. Over 12 weeks, SDG performed 3x40 min per week of soccer training. Results: The first soccer session for SDG induced acute increases in blood lactate (1.4±0.1 to 6.0±0.7 mmol/L, $P<0.05$) and glucagon levels (112.1±6.2 to 142.9±8.0 pg/mL, $P<0.05$), whereas glucose and insulin levels remained unchanged. Moreover, this session showed suppressed insulin levels as well as higher free fatty acids, lactate levels and glucagon/insulin ratio compared to DG ($p<0.05$). After 12 weeks, a baseline decrease was observed in glucagon, leptin and lactate levels in SDG and DG ($p<0.05$), whereas HOMA-IR, Adipo-IR and glucose levels were lower only in SDG ($p<0.05$). At the last soccer training session, the blood lactate response was significantly lower than for the first session (4.0±0.4 vs 6.0 ± 0.7 mmol/L). At 48 h pre intervention, a decrease was observed in leptin levels ($p<0.05$), which remained lower post intervention. The positive correlation between leptin and insulin, and the lower levels after training, could be attributed to the improved insulin sensitivity along with the weight loss observed in both groups (~3.4 kg for DG and 3.7 kg for SDG).

Conclusion: Acute soccer sessions markedly improved insulin action markers in T2D patients, while the cumulative effects enhanced insulin sensitivity and decreased risk

factors associated with cardiovascular disease after 12 weeks of intervention better than caloric-restricted diet.

Key words: Insulin sensitivity; lactate; exercise; high-intensity training; free fatty acids; glucagon

1. Introduction

Diabetes mellitus is one of the major worldwide health problems and the leading cause of blindness, kidney failure, lower extremity amputation and cardiovascular disease [1,2].

Diabetes education, nutritional counselling, physical activity, self-monitoring and training in self-management principles constitute the foundation of all diabetes management plans. There is now abundant evidence that optimal glycaemic control prevents the development of diabetes complications [1]. Moreover, lifestyle modification, exemplified by optimal dietary practices and increased physical activity, has been demonstrated to prevent type 2 diabetes (T2D) and induce remission of early T2D [3].

Structured programmes of regular physical activity (150 min per week) and dietary strategies, including a reduction in calories, dietary sugar, fat and sodium, and an increase in dietary fibre and whole grains, are recommended for optimal metabolic health and weight control [4].

Exercise training or caloric restriction can reduce total and regional fat mass, especially visceral fat, in overweight or obese individuals, enhancing insulin sensitivity [5].

Several studies have demonstrated that very low to moderate caloric restriction can enhance insulin sensitivity, irrespective of weight loss [6,7]. The magnitude of insulin sensitivity increase was greater for the lower-calorie diet [7]. However, an increase in skeletal muscle glucose transporter (GLUT-4) during caloric restriction is not evident [8]. Conversely, exercise training has repeatedly been shown to improve muscle function, including enhanced insulin responsiveness [9] and GLUT-4 expression [10] as well as oxidative capacity [11] and mitochondrial content in skeletal muscle [12]. The greatest effect is on oxidative fibres [13], where several cytokines and myokines, including leptin, promote fatty acid oxidation, leading to decreased skeletal muscle lipid deposition [14].

The impact of exercise training on metabolic parameters also depends on the type of exercise. Alkahtani et al. [15] have shown a greater decrease in blood lactate in obese men after 12 weeks of high-intensity interval training than after 12 weeks of moderate-intensity interval training, while fat oxidation was increased regardless of the exercise intensity. However, Gordon et al. [16] have shown that insulin sensitivity is not modified after acute resistance exercise in T2D patients. Conversely, high-intensity interval training (~20-min bouts) performed by T2D patients over 2 weeks improved metabolic health and glucose control [17].

Similarly, soccer is a vigorous exercise activity interspersed with periods of rest [18,19] and may be an attractive option for implementing higher-intensity training in patients. The training load can be controlled using tools as heart rate monitors and global positioning system (GPS) allowed to meet the standard recommendations concerning physical activity for patients with diabetes [20]. Moreover, it was recognized that soccer training using small-sided games is more enjoyable and the adherence was higher than other kind of training such as running or cycling [18]. Consequently, by using tools to control the training load, soccer training can be recommended for patients with diabetes [18, 19, 21]. Given its particular popularity with Brazilians, it might therefore be a good intervention strategy for preventing or treating diabetes.

As caloric restriction and physical training can each improve insulin sensitivity, we hypothesised that their combined effects should exceed their individual impacts. To test this hypothesis, we compared the impact of caloric-restricted diet with recreational soccer training versus caloric-restricted diet in T2D patients.

As widely investigated in several studies conducted by Krustrup et al [22] recreational soccer training can be defined as warm-up, pair-based drills and small-sided football

training that is done in a friendly and motivating atmosphere, without competitive games against local rivals.

The soccer training program proposed in the current study was in accordance with the recommendations of the American Diabetes Association - ADA [20, 23]. In fact it was demonstrated that most clinical trials evaluating exercise interventions in people with type 2 diabetes have used a three times per week frequency [20, 23, 24]. The study conducted by Boulé et al. [25] provide support for encouraging type 2 diabetic individuals who are already exercising at moderate intensity to consider increasing the intensity of their exercise in order to obtain additional benefits in both aerobic fitness and glycemic control [25]. The amount and intensity recommended for exercise vary according to goals. To reduce cardiovascular disease risk, to increase glycemic control and to control weight maintenance it was recommended at least 150 min/week of moderate-intensity aerobic physical activity (40–60% of VO_{2max} or 50–70% of maximum heart rate) and/or at least 90 min/week of vigorous aerobic exercise (60% of VO_{2max} or 70% of maximum heart rate) [23]. According to ADA position statement, the medications adjustments should be done for those who show a tendency to hypotension or hypoglycaemia during or after training sessions [20].

In our previous preliminary observation [26], 12 weeks of soccer training and caloric-restricted diet decreased total body fat, enhanced VO_2max, reduced total cholesterol and triglyceride levels, and improved insulin sensitivity. In this study, we specifically assessed the combination of recreational soccer training and caloric-restricted diet on acute glucoregulatory and liporegulatory physiology at fasting and post-exercise, comparing caloric-restricted diet.

2. Materials and methods

2.1 Participants and study design

Fifty-one T2D subjects (29 females and 22 males) aged 48–68 years with 32.8±0.7 kg/m^2 body mass index (BMI) and 8.2±1.0 years of diabetes participated in the study. The recruitment process is illustrated in Figure 1.

None of the participants had been involved in any regular exercise training for the preceding 24 months and all the women were postmenopausal. The participants were on antihyperglycaemic medications and all were non-smokers and free from complications such as diabetic nephropathy, diabetic retinopathy, and cerebrovascular and cardiovascular disease. The protocol was approved by the Local Committee on the Ethics of Human Research and written informed consent was obtained from all subjects.

This study represented an independent element of a comprehensive interventional protocol investigating molecular, hormonal and metabolic adaptations in T2D patients enrolled in a diet and physical training programme. The participants were randomly allocated to either a soccer+diet group (SDG, n=22) or a diet group (DG, n=29).

The subjects in SDG performed 40 min of supervised recreational soccer training three times a week for 12 weeks. Before the intervention, the two groups were similar in respect of age, duration of diabetes, HbA1c, maximal oxygen uptake (VO_2max) and BMI. Ten subjects dropped out of the study (SDG, n=3; DG, n=7) due to: non-adherence to programme (n=4); cycling accident (n=1); cardiovascular disease and hyperparathyroidism (n=1); diabetic foot (n=1); introduction of insulin therapy (n=1); could not continue with training schedule (n=2). These figures are illustrated in Figure 1. Also, the number of participants in both groups remained similar at baseline after dropped out (Table 1, Figure 1). A total of 41 participants completed the study, of whom 19 were in SDG (9 females and 10 males) and 22 in DG (12 females and 10 males). The groups did not differ by gender (p=0.9). The baseline characteristics of the participants are given in Table 1.

Thirty of the subjects were taking antihypertensive agents, 19 were undergoing lipid-lowering therapy (statins) and all were taking oral hypoglycaemic medications (metformin, 17; sulphonylureas, 1; combination of metformin and sulphonylureas, 23; Table 1).

2.2 Preliminary procedures and general design

On admission to the study, all participants underwent screening by medical examination and echocardiography, followed by assessment of anthropometric parameters by DEXA, aerobic capacity by evaluation of maximal oxygen consumption (VO_2max) and biochemical profile.

Furthermore, a complete food history was collected prior to the start of the study for the prescription of individual diets. Following these evaluations, all participants were enrolled in the Nutritional Intervention Programme for weight reduction with (SDG) or without (DG) soccer training. Besides the chronic effects of the intervention, the acute impact was also evaluated to establish the hormonal and metabolic response to recreational training. Thus, blood samples were collected from the subjects in fasting state and after 1 h postprandial breakfast in both SDG and DG (Figure 2). SDG had another blood sample collected in fasting state post 48 h recovery, giving a total of six time points as follows:

- fasting pre-treatment (0 weeks);
- post breakfast and soccer session (1 h postprandial);
- after soccer session (post 48 h recovery);
- fasting post-treatment (12 weeks);
- post breakfast and soccer session (1 h postprandial);
- after soccer session (post 48 h recovery).

Following fasting blood sampling, the patients consumed a standardised breakfast (energy 253 kcal, carbohydrate 29.5 g, protein 15.3 g, fat 1.8 g and fibre 2.3 g), and additional postprandial samples were collected after 1 h for both groups (Figure 2). After breakfast, the patients in SDG performed a 40-min soccer session, lasting 1 h in total, and had their blood drawn immediately after the exercise session and the postprandial period. After 12 weeks, all participants were re-evaluated. For DG, there were four different time points; fasting and 1 h post-prandially before and after the treatment (Figure 2). For ethical reasons, medications were adjusted during the intervention programme to attain target levels and account for reduced needs.

2.3 Nutritional intervention programme

During follow-up, all participants received weekly nutritional counselling from experts. The dietary plan was individually calculated to provide a reduction in energy intake of 500–1,000 kcal per day for each of the participants. The prescribed diet was balanced and rich in fibre (minimum 20 g/day), as per current Brazilian guidelines. The dietary plan consisted of 45–60% carbohydrate, 15–20% protein and 20–30% fat. Individual nutritional counselling was provided every 2 weeks to reinforce and support the dietary restrictions and monitor caloric intake (Figure 2). If necessary, adjustments were made to the diet to improve compliance. Additionally, every 2 weeks all participants were enrolled in the Nutritional Education Programme, which ran in groups of no more than seven participants per 1-h session (Figure 2). This education programme included lectures on nutritional care and physical activity, glucose control and glucose management for T2D patients.

Pre and post intervention, the subjects were given a form to record the types and quantities of all food consumed by 3-day record. The nutrient calculations were made using the

Avanutri nutritional efficacy software (2010) and the TACO Brazilian Table of Food Composition (2006).

2.4 Training intervention

Forty minutes of supervised soccer training was performed three times per week for 12 weeks. The training sessions consisted of ordinary small-sided (3v3 to 7v7) friendly games [18, 27] performed outdoors on a 30–40-m wide and 45–60-m long natural grass pitch or indoors on a wooden court on rainy days. None of the players were the goal keepers. Each training session lasted 40 min. The sessions involved a 10-min low-intensity warm-up, 3 min of passive rest, followed by 2x12-min periods of play interspersed with 3 min of passive rest (Figure 2).

2.5 Measurements during training

In SDG, heart rate was recorded at 5-s intervals using chest belts during all training sessions (Polar Team System, Polar Oy, Kempele, Finland). Perceived exertion was recorded during and immediately after training sessions using an adapted Borg's 6-20 scale [28].

Before each training session, blood pressure was measured using an automatic wrist blood pressure monitor (HEM-629, OMRON, Illinois, USA). Before and after each training session, capillary blood glucose was collected from the fingertip and measured using a blood glucose monitor (ACCU-CHEK® Aviva Nano, Roche Diagnostics, Basel, Switzerland) (Figure 2).

2.6 Maximal oxygen uptake, maximal heart rate and ventilatory thresholds

Before and after the intervention period, VO_2max (mL/min/kg) and maximal heart rate (HRmax; bpm) were measured during an incremental treadmill test (Centurion 200, Micromed, Brasilia, Brazil) with speed and slope increments determined individually depending on the physiological responses (e.g. HR & BP). In fact, after 3 minutes resting period on the treadmill the test started with a speed increasing each minute during 3 minutes with 0% inclination. Then, at a fixed speed, the slope was increased by 2% each minute until voluntary exhaustion. The heart rate was obtained using a Polar Team 2 Heart Rate monitors (Polar Electro Oy, Kempele, Finland). Ventilatory variables were obtained using a metabolic analyser (CPX/D, MedGraphics® Saint Paul, MN, USA), which allowed the acquisition, processing and storage of breath-to-breath ventilation variables. Ventilatory variables were expressed as means of the last 30 s of recording during the exercise period. The ventilatory thresholds were determined using the ratios of ventilation / volume of oxygen consumed (V_E / V_{O_2}) and ventilation / volume of carbon dioxide production (V_E / V_{CO_2}) *vs.* power output [29].

2.7 Anthropometric measurements

Waist circumference

Using a tape measure, waist circumference was determined to the nearest 0.1 cm at the midpoint between the highest point of the iliac crest and the lower margin of the last palpable rib.

Body composition

The body composition of the participants was determined 3 to 5 days pre intervention and again post intervention. Body composition, including body fat percentage, fat mass, and regional and whole-body fat free mass, was determined by dual-energy X-ray

absorptiometry (DXA) using Hologic 4500 QDR densitometry equipment (Bedford, MA, USA).

2.8 Blood sample collection and analysis

Blood samples were collected from the subjects in fasting state and after 1 h postprandial breakfast in both SDG and DG (Figure 2). SDG had another blood sample collected in fasting state post 48 h recovery, giving a total of six time points as follows: fasting, 1 h postprandially (1 h post breakfast) and after soccer session (post 48 h recovery), pre and post-treatment. For DG, samples were collected at four different time points: fasting and 1 h postprandially before (0 weeks) and after the treatment (12 weeks) (Figure 2). All participants were re-evaluated 3–5 days after the 12-week intervention.

All samples were collected at 7 a.m. after 12 h of fasting. Participants were advised to keep the medications prior to the blood tests.

Venous blood samples were collected using BD Vacutainer® tubes (K2 EDTA and SST™ II Advance tubes) from Becton Dickinson (Franklin Lakes, NJ, USA). For the glucose, lactate and glucagon determinations, the EDTA tubes of blood were kept on ice and the plasma separated within the first hour. Aprotinin (SIGMA A6279, St Louis, MS, USA) was added for glucagon preservation. The blood samples were centrifuged at 2000 g in a refrigerated centrifuge (4ºC) for 15 min. The plasma glucose concentration was determined using the enzymatic-calorimetric method (glucose oxidase-peroxidase) with a commercial kit (Glucose Liquiform from Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). The plasma lactate was evaluated using a lactate oxidase-peroxidase colorimetric commercial kit (Enzymatic Lactate from Labtest Diagnóstica S.A.). HbA1c was assayed by high-performance liquid chromatography (HPLC) using a commercial kit (Biorad, CA, USA). Free fatty acid (FFA) levels were measured using NEFA-HR kits (ACS.ACOD

method) from Wako Diagnostics (Wako Chemicals Inc., USA). Leptin levels were obtained by the ELISA method using an R&D commercial kit (R&D Systems, Inc. Minneapolis, USA). Millipore Corporation's radioimmunoassay kits were used to measure insulin and glucagon (Millipore Corporation, Billerica, MA, USA). CRP levels were determined by the ELISA method using an R&D commercial kit (R&D Systems, Inc. Minneapolis, USA). Intra-assay and inter-assay precision (%CV) was 4.5% and 6.5% for CRP and, for hormonal analyses, 6.8% and 9.6% for insulin, 4.4% and 6.5% for glucagon, and 4% and 5% for leptin. Insulin sensitivity was estimated using the homeostasis model assessment (HOMA-IR) method [30]. Adipo-IR was calculated from fasting insulin and fasting FFA concentrations.

2.9 Statistical analysis

Data are reported as means±SEM, unless otherwise stated. All variables were examined for normality of distribution using the Shapiro-Wilk test. An unpaired t-test was applied between groups pre and post intervention. To compare the difference between time points, a Friedman test was used, followed by pairwise Wilcoxon tests. Nonparametric statistical methods were used to analyse variables that were not normally distributed. A multiple regression analysis was performed to identify associations between hormonal variables, adjusted by fatty body mass. A chi-square test was used to compare the difference in frequencies between interventions groups with regard to gender and medication adjustments. P-values below 0.05 were considered statistically significant.

3. Results

3.1 Clinical Characteristics

The clinical characteristics of the study population are shown in the Table 1. 41 type 2 diabetic patients aged 61.1±6.4 with a similar sex distribution (20 male and 21 female), BMI of 32.9±4.3 Kg/m², HbA1c of 7.3±1.3% and mean duration of diabetes of 8.2 ± 6.1 years had concluded the study. Of these patients 54.5% reported antihypertensive agents, and 36.4 were in use of statins. All patients were in use of hypoglycemic medications as follow metformin (38.6%), sulphonylurea (1%), and combination of metformin and sulphonylurea (52.3%, Table 1).

3.2 Training Effect

The mean HR zone during the training sessions was 19±9.3% (HR zone 50-59%), 19.8±8.1% (HR zone 60-69%), 23±8.5% (HR zone 70-79%), 18±8.3% (HR zone 80-89%), and 20±11.2% (HR zone 90-100%) of HRmax of the total training time. The zones above 70% HRmax prevailed for more than 60% of the total training time, corresponding to moderate and high intensity in a vast majority of the training time. The zone above 90% HRmax, corresponding to maximal training intensity, was 20±3% of the total training time while the zones below 70% HRmax, corresponding from moderate to lower intensities training were less than 40% of the total training time. The heart rate zones were similar for the first and last soccer training sessions ($p>0.05$).

After 12 weeks, VO_2max had increased by 11.4% in SDG ($p<0.05$). In contrast, VO_2max had decreased by 5.7% in DG ($p<0.05$, Table 2). The HRmax values obtained during the treadmill tests performed prior to the intervention were similar for SDG and DG (161.4±3.1 and 159.2±4.0 bpm, respectively) and did not alter over the 12-week period (163.4±2.7 and 158.6±3.8 bpm, respectively, Table 2).

3.3 Body fat percentage reduction after the 12-week intervention

Total body fat percentage decreased significantly in both SDG and DG, by 2.5±0.3% and 1.9±0.5%, respectively (Table 2). No significant changes were observed in fat percentage reduction between groups (Table 2).

Total body weight decreased by ~3.7 kg in SDG and by ~3.4 kg in DG after 12 weeks. Fat mass was lowered ($P<0.05$) in both groups (SDG by ~3.4 kg and DG by ~2.7 kg) after 12 weeks, with no differences between groups in the post-intervention period. Fat-free mass remained similar in SDG and decreased by ~0.8 kg in DG post intervention, although it did not reach a statistical difference. Waist circumference was lower post intervention in both SDG and DG, by 5.4±0.6 and 4.9±1.4cm, respectively (Table 2).

3.4 Effect of the 12-week intervention on medication adjustments

The adjustments in medications were constantly done through the intervention period by the physicians. These precautions helped us to avoid serious hypoglycaemic and hypotension events- which were rare, light and needed no personal support.

After the 12-week intervention, the number of patients in SDG and DG, respectively, who had their medication reduced or suspended was as follows: antihypertensive agents (3 and 6); lipid-lowering therapy (statins) (3 and 1); oral hypoglycemic medication (metformin, 4 and 1; combination of metformin and sulfonylureas, 4 and 11). No post-intervention differences were observed in the frequency of medication adjustments for SDG and DG ($p>0.05$).

3.5 Acute responses to soccer intervention at the beginning and end of the 12-week training programme

Glucose and lactate responses

In the SDG, blood glucose increased (162.1±14.8 mg/dL) following breakfast and post exercise session (postprandial) when compared to fasting (139.5±8.0 mg/dL), however its increase did not reach statistical significance ($p>0.05$). In the DG, glucose response increased significantly following breakfast (postprandial) 184.2±8.8 mg/dL than fasting (135.6±7.6 mg/dL, $p<0.05$; Fig. 3A).

At 3 months, postprandial glucose concentrations were significantly higher in both groups compared to fasting levels. Glucose concentration did not change 48 h after the first and last soccer training sessions (Fig. 3A).

Lactate concentrations were significantly higher after the first (1.4±0.1 to 6.0±0.7 mmol/L) and last (1.1±0.1 to 4.0±0.4 mmol/L) soccer training sessions and returned to fasting levels after 48 h of recovery. At the last training session, the blood lactate response was significantly lower than at the first session (4.0±0.4 vs 6.0 ± 0.7 mmol/L, Fig. 3C).

After 12 weeks of training, fasting glucose levels were significantly reduced in SDG (119.0±4.7 vs 139.5±8.0 mg/dL, $P<0.001$) (Fig. 3A), whereas no significant changes were observed in DG. Fasting lactate levels were significantly lower after the intervention programme in both SDG and DG (Fig. 3C).

Insulin, glucagon, HOMA-IR and Adipo-IR

Soccer practice reduced postprandial glucose levels and consequently the insulin requirement were significantly lower than in DG ($P<0.001$), both at the beginning of the study and after 12 weeks of intervention (Fig. 4A). Glucagon concentrations increased in the postprandial period in both groups ($P<0.01$). This response was similar on the first and last days of the intervention programme (Fig. 4B).

The glucagon/insulin ratio increased sharply after the first (8.7±0.8 vs 3.0±0.3) and last training sessions (7.4±1.2 vs 2.7±0.3) in SDG compared to DG ($P<0.001$, Fig. 4C).

Conversely, the glucagon/insulin ratio was significantly lower in DG during the postprandial period before and after the intervention (Fig. 4C).

The cumulative effect of the 12-week intervention significantly lowered fasting glucagon concentrations in both groups (Fig. 4B). However, fasting glucagon concentrations were lower in SDG than in DG (93.4±4.4 vs 106.9±3.3 pg/mL, respectively, P<0.01) at the end of the intervention. Fasting insulin response and glucagon/insulin ratio were similar after the 12-week intervention in both groups (Figs 4A, 4C).

The surrogate index of insulin resistance (as estimated by HOMA-IR) was significantly lower in SDG after 12 weeks (6.3±0.7 vs 4.7±0.5, P<0.05), suggesting an increase in insulin sensitivity, but was not significantly altered from baseline in DG (Table 2). Similarly, adipose tissue insulin sensitivity (as estimated by Adipo-IR) was observed only in SDG post 12 weeks (p<0.001; Table 2, Fig. 5).

Leptin

In SDG, leptin concentration decreased 48 h after the first soccer session (14.8±1.8 ng/mL vs. 16.0±2.1 at fasting levels; p<0.01) and remained below basal levels throughout the training programme (Fig. 6). After 12 weeks of the lifestyle programme, leptin levels were significantly reduced both in SDG (12.0±1.6 vs 16.0±2.1 ng/ml) and in DG (19.0±2.8 vs 22.5±3.8 ng/ml) (Fig. 6).

As expected, leptin levels and body fatty mass were highly correlated in both SDG and DG (P<0.0001).

Free fatty acid concentrations

Peak plasma FFA concentration measured following breakfast and immediately after the first soccer training session was higher in SDG than in DG (0.7±0.1 vs 0.4±0.0 mEq/L, P<0.05) (Fig. 3B). Postprandial FFA levels decreased significantly in DG as a result of lower lipolytic response compared to SDG (Fig. 3B). Plasma FFA levels were not

significantly different in the groups at the end of the intervention period (Fig. 3B). However, a tendency towards a decrease in FFA was observed in SDG over the intervention period (0.7±0.0 vs 0.5±0.0 mEq/L, P=0.076).

4. Discussion

As far as we know, this is the first detailed assessment of the impact of an acute session of recreational soccer pre and post 12 weeks of training on metabolic and hormonal endpoints in middle-aged adults with T2D. The high heart rates and alterations in circulating lactate, FFA, glucose and glucagon levels indicate that the patients in this study attained a significant level of exertion during the recreational soccer sessions. The present findings reinforce our previous preliminary observation [26] that 12 weeks of soccer training and restricted diet decreased total body fat, enhanced VO_2max, reduced cholesterol and triglyceride levels, and improved insulin sensitivity.

Lactate concentrations reached 6 mmol/L on average after the first acute soccer session and 4 mmol/L after the last session, similar to values reached for high-level players as well as young recreational soccer players [18].

A tendency towards a decrease in FFA levels was observed in the soccer group over the intervention period (p=0.076). Besides that, the higher FFA levels observed after acute training are a result of increased lipolytic activity and reduced insulin levels. Exercise-induced neuroendocrine changes, especially increased catecholamines, favour the mobilisation of fat as an energy substrate [31]. Conversely, the decrease in postprandial FFA levels observed in DG indicates suppression of lipolysis [32]. This is further corroborated by the findings of higher insulin levels and lower glucagon/insulin ratio, which favour anabolism rather than catabolism.

In the present study baseline lactate levels decreased in both groups (diet and diet + soccer) after 12 weeks. The weight loss evidenced in both groups is accompanied by increased oxidative capacity of adipose tissue and skeletal muscle favoring lower levels of lactate concentration post-intervention. Also, the increase in insulin sensitivity with the intervention program resulted in lower glucose concentrations, and consequently in less substrate for lactate production.

The improvements in baseline blood lactate, glucose and FFA levels reflect peripheral metabolic adaptations. These peripheral adaptations include reduced lactate accumulation, glycogenolysis and lipolysis, which were detected after 12 weeks of soccer training in our study population of previously sedentary T2D patients.

We found no significant differences between SDG and DG in gross measures of body mass, fat mass, waist circumference and body fat percentage due to successful dietary regimen. Therefore, in this study dietary advise was more effective than exercise on anthropometric profile.

Unfortunately, we could not obtain direct measurements of visceral fat in the present study. Nonetheless, a reduction in visceral fat induced by soccer training could have contributed to the improved biomarkers of insulin action observed in the present study. Visceral fat is known to be particularly sensitive to the effects of a moderate-to-high-intensity aerobic training programme [33, 34]. The aerobic intensity during the soccer sessions were moderate-to-high for the male and female patients. As reported by Krustrup[10] and Randers[35], even with the modification of the rules, pitch size and number of players during small-sided soccer are enough to achieve high-intensity exercise for all participants.

Regarding C-reactive protein, although the reduction on its levels did not reach statistical significance after 12 weeks in both SDG and DG groups, the observed decrease of 1

mg/dL may have lowered inflammation, endothelial dysfunction and cardiovascular events risks.

The lower plasma glucose levels observed after cessation of acute training are consistent with increased utilisation of this substrate during soccer practice. This was observed despite the exercise-induced increase in glucagon and FFA, and the decrease in insulin levels, mechanisms that favour gluconeogenesis and provision of fuel to the working muscles [36]. Exercise training increases muscle capillarisation [31], AMPK activity and GLUT-4 translocation to cell membrane, thereby facilitating glucose uptake despite low insulin levels [10].

The improvements in blood glucose levels observed in this study are concordant with the reports by Adams [37] and Little et al. [17]. In the study of Little et al. [17], 2 weeks of brief high-intensity exercise reduced the average of 24-h blood monitoring glucose levels by 13% at ~48 h to 72 h post exercise and increased GLUT-4 expression by 369%. Furthermore, long-term training has been shown to induce adaptations in pancreatic B-cells, leading to a reduction in glucose-induced insulin secretion [38]. Insulin sensitivity was improved after soccer training, as shown by HOMA-IR and Adipo-IR. Adipo-IR has been recently validated by Søndergaard et al. [39, 40] as a good predictor of adipose tissue insulin sensitivity. In this regard, lower leptin levels have also been reported as indicative of improved insulin sensitivity [41]. Thus, the lower leptin levels in our study subjects could be attributed to their improved insulin sensitivity together with the considerable weight loss observed in both groups, whereas several studies have shown that leptin correlates with body fat mass (41, 42, 43). This weight loss is accompanied by increased oxidative capacity of adipose tissue and skeletal muscle [38].

Other possible mechanisms for the acute exercise effect on leptin include elevated plasma FFA levels [42, 43], the inhibitory effect of stress hormones, especially epinephrine and

norepinephrine [44], and a counter-regulatory response to the high energy expenditure during soccer that suppresses leptin in order to permit energy intake [43]. Moreover, there are conflicting reports regarding the effect of acute or short-term exercise on leptin levels. Our findings showing decreased leptin are in accordance with the observation by Jürimäe and Jürimäe [45], that leptin may be sensitive to short-term intensive exercise involving all major muscles.

5. Conclusion

Acute metabolic effects of a single soccer session on FFA and lactate changes are significantly attenuated after 3 months of training in middle-aged T2D patients. Also, soccer training improved markers of glucoregulation and insulin sensitivity in T2D patients, decreasing risk factors associated with cardiovascular disease after 12 weeks better than caloric-restricted diet. Besides being enthusiastically received, recreational soccer training is safe for T2D, non-insulin dependent, with none complications. The aerobic intensity during the soccer sessions were moderate-to-high for the male and female patients. Also, the training load and the medical treatment met the American Diabetes Association recommendations for T2D patients. These precautions helped us to avoid serious hypoglycaemic and hypotension events- which were rare, light and needed no personal support.

Thus, recreational soccer can be an attractive option for lifestyle intervention in Brazilian T2D patients. Our study suggests that popular national sports can be adapted as the physical activity component in the management of chronic diseases that require lifestyle modification.

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References

[1] S. Dagogo-Jack, Glycemic control and chronic diabetes complications. In: Umpierrez GE, editor. Therapy for Diabetes Mellitus and Related Disorders. 6th ed. Alexandria, VA: American Diabetes Association (2014) 668-695.

[2] Y. Hayashino, JL. Jackson, T. Hirata, N. Fukumori, F. Nakamura, S. Fukuhara, S. Tsujii, H. Ishii, Effects of exercise on C-reactive protein, inflammatory cytokine and adipokine in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. Metabolism 63 (2014) 431-40.

[3] EW. Gregg, H. Chen, LE. Wagenknecht, JM. Clark, LM. Delahanty, J. Bantle, HJ. Pownall, KC. Johnson, MM. Safford, AE. Kitabchi, FX. Pi-Sunyer, RR. Wing, AG. Bertoni, Association of an Intensive Lifestyle Intervention With Remission of Type 2 Diabetes. JAMA 308 (2012) 2489-2496.

[4] American Diabetes Association. Summary of revisions to the 2014 Clinical Practice Recommendations. Diabetes Care 37 Suppl 1 (2014) S4.

[5] Y. Mavros, S. Kay, KA. Anderberg, MK. Baker, Y. Wang, R. Zhao, J. Meiklejohn, M. Climstein, A. O'Sullivan, N. de Vos, BT. Baune, SN. Blair, D. Simar, K. Rooney, N. Singh, MA. Fiatarone Singh, Changes in Insulin Resistance and HbA_{1c} Are Related to Exercise-Mediated Changes in Body Composition in Older Adults With Type 2 Diabetes. Diabetes Care 36 (2013) 2372-2379.

[6] DE. Kelley , R. Wing, C. Buonocore , J. Sturis, K. Polonsky, M. Fitzsimmons, Relative effects of calorie restriction and weight loss in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 77 (1993) 1287-93.

[7] RR. Wing, EH. Blair, P. Bononi, MD. Marcus, R. Watanabe, RN. Bergman, Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. Diabetes Care 17 (1994) 30-36.

[8] DA. Sequea, N. Sharma, EB. Arias, GD. Cartee, Calorie restriction enhances insulin-stimulated glucose uptake and Akt phosphorylation in both fast-twitch and slow-twitch skeletal muscle of 24-month-old rats. J Gerontol A Biol Sci Med Sci 67 (2012) 1279-1285.

[9] JA. Hawley, SJ. Lessard, Exercise training-induced improvements in insulin action. Acta Physiol 192 (2012) 127-135.

[10] EA. Richter, M. Hargreaves, Exercise, GLUT4, and skeletal muscle glucose uptake. Physiol Rev 93 (2013) 993-1017.

[11] MS. Hood, JP. Little, MA. Tarnopolsky, F. Myslik, MJ. Gibala, Low-volume interval training improves muscle oxidative capacity in sedentary adults. Med Sci Sports Exerc 43 (2011) 1849-1856.

[12] AM. Joseph, DO. Hood, Relationships between Exercise, Mitochondrial Biogenesis and Type 2 Diabetes. Med Sport Sci 60 (2014) 48-61.

[13] A. Bouassida, K. Chamari, Y. Feki, A. Zbidi, Z. Tabka, Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. Br J Sports Med 44 (2010) 620-630.

[14] S. Weins, J. Ukropec, D. Gasperíková, I. Klimes, E. Seböková, Concerted action of leptin, in regulation of fatty acid oxidation in skeletal muscle and liver. Exp Clin Endocrinol Diabetes 115 (2007) 244-251.

[15] SA. Alkahtani, NA. King, AP. Hills, NM. Byrne, Effect of interval training intensity on fat oxidation, blood lactate and the rate of perceived exertion in obese men. Springer plus 2 (2013) 532.

[16] BA. Gordon, SF. Fraser, SR. Bird, AC. Benson, Insulin sensitivity not modulated 24 to 78 h after acute resistance exercise in type 2 diabetes patients. Diabetes Obes Metab 15 (2013) 478-480.

[17] JP. Little, JB. Gillen, ME. Percival, A. Safdar, MA. Tarnopolsky, Z. Punthakee, ME. Jung, MJ. Gibala, Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. J Appl Physiol 111 (2011) 1554-1560.

[18] P. Krustrup, JF. Christensen, MB. Randers, H. Pedersen, E. Sundstrup, MD. Jakobsen, BR. Krustrup, JJ. Nielsen, C. Suetta, L. Nybo, J. Bangsbo, Muscle adaptations and performance enhancements of soccer training for untrained men. Eur J Appl Physiol 108 (2010) 1247-1258.

[19] P. Krustrup, EW. Helge, PR. Hansen, P. Aagaard, M. Hagman, MB. Randers, M. de Sousa,

M. Mohr. Effects of recreational football on women's fitness and health: adaptations and mechanisms. Eur J Appl Physiol. 118 (2018) 11-32.

[20] SR. Colberg, RJ. Sigal, JE. Yardley, MC. Riddell, DW. Dunstan, PC. Dempsey, ES. Horton, K. Castorino, DF. Tate. Physical Activity/Exercise and Diabetes: A Position Statement of the American Diabetes Association. Diabetes Care. 39 (2016) 2065-2079.

[21] Krustrup P, Krustrup BR. Football is medicine: it is time for patients to play! Br J Sports Med. 52 (2018) 1412-1414.

[22] P. Krustrup, CA. Williams, M. Mohr et al. The "Football is Medicine" platform-scientific evidence, large-scale implementation of evidence-based concepts and future perspectives. Scand J Med Sci Sports. 28 (2018) 3-7.

[23] RJ. Sigal , GP. Kenny , DH. Wasserman , C. Castaneda-Sceppa , RD. White . Physical activity/exercise and type 2 diabetes: a consensus statement from the American Diabetes Association. Diabetes Care. 29 (2006) 1433-8.

[24] NG. Boule´, E. Haddad, GP. Kenny, GA. Wells, RJ. Sigal. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of con- trolled clinical trials. JAMA 286 (2001) 1218 – 1227.

[25] NG. Boule´, GP. Kenny, E. Haddad, GA. Wells, RJ. Sigal. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in type 2 diabetes mellitus. Diabetologia 46 (2003) 1071–1081.

[26] MV. de Sousa, R. Fukui, P. Krustrup, RM. Pereira, PR. Silva, AC. Rodrigues, JL. de Andrade, AJ. Hernandez, ME. da Silva, Positive effects of soccer on fitness, lipid profile, and insulin resistance in Brazilian patients with type 2 diabetes. Scand J Med Sci Sports 24 (2014) 57-65.

[27] MB. Randers, L. Nybo, J. Petersen , JJ. Nielsen, L. Christiansen, M. Bendiksen, J. Brito, J. Bangsbo, P. Krustrup. Activity profile and physiological response to football training for untrained males and females, elderly and youngsters: influence of the number of players. Scand J Med Sci Sports. 20 (2010) 14-23.

[28] CA. Foster, Monitoring training in athletes with reference to overtraining syndrome. Med Sci Sports Exer. 30 (1998) 1164-8.

[29] K. Wasserman, J. Hansen, D. Sue, W. Stringer, B. Whipp, eds. Principles of Exercise Testing and Interpretation. Lippincott Williams & Wilkins, Philadelphia, USA. 4th edn. ISBN 7-7817-4876-3 (2004); 612 pages.

[30] DR. Matthews, JP. Hosker, AS. Rudenski, BA. Naylor, DF. Treacher, RC. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 (1985) 412-419.

[31] ML. Goodwin, J. Blood, Glucose Regulation during Prolonged, Submaximal, Continuous Exercise: A Guide for Clinicians. Diabetes Sci Technol 4 (2010) 694-705.

[32] M. Sousa, HG. Simões, CC. Castro, MC. Otaduy, CE. Negrão, RM. Pereira, K. Madsen, ME. Silva, Carbohydrate supplementation increases intramyocellular lipid stores in elite runners. Metabolism 61 (2012) 1189-1196.

[33] BA. Irving, CK. Davis, DW. Brock, JY. Weltman, D. Swift, EJ. Barrett, GA. Gaesser, A. Weltman, Effect of exercise training intensity on abdominal visceral fat and body composition. Med Sci Sports Exerc 40 (2008) 1863-1872.

[34] Y. Minokoshi, C. Toda, S. Okamoto, Regulatory role of leptin in glucose and lipid metabolism in skeletal muscle. Indian J Endocrinol Metab 16 Suppl 3 (2012) S562-568.

[35] Randers MB, Ørntoft C, Hagman M, Nielsen JJ, Krustrup P (2018). Movement pattern and physiological response in recreational small-sided football - effect of number of players with a fixed pitch size. J Sports Sci. 36(13):1549-1556.

[36] CJ. Ramnanan, DS. Edgerton, G. Kraft, AD. Cherrington, Physiologic action of glucagon on liver glucose metabolism. Diabetes Obes Metab 13 (2011) 118-125.

[37] P. Adams, The impact of brief high-intensity exercise on blood glucose levels. Diabetes Metab Syndr Obes 6 (2013) 113-122.

[38] L. Norton, K. Norton, N. Lewis, Exercise training improves fasting glucose control. J Sports Med 16 (2012) 209-214.

[39] E. Søndergaard, MD. Jensen, Quantification of adipose tissue insulin sensitivity. J Investig Med 64 (2016) 989-991.

[40] E. Søndergaard, AE. Espinosa De Ycaza, M. Morgan-Bathke, MD. Jensen, How to Measure Adipose Tissue Insulin Sensitivity. J Clin Endocrinol Metab 102 (2017) 1193-1199.

[41] H. Askari, G. Tykodi, J. Liu, S. Dagogo-Jack, Fasting Plasma Leptin Level Is a Surrogate Measure of Insulin Sensitivity. J Clin Endocrinol Metab 95 (2010) 3836-3843.

[42] M. Duclos, JB. Corcuff, A. Ruffie, P. Roger, G. Manier, Rapid leptin decrease in immediate post-exercise recovery. Clin Endocrinol 50 (1999) 337-342.

[43] RR. Kraemer, H. Chu, VD. Castracane, Leptin and exercise. Exp Biol Med 227 (2002) 701-708.

[44] JS. Fisher, RE. Van Pelt, O. Zinder, M. Landt, WM. Kohrt, Acute exercise effect on postabsorptive serum leptin. J Appl Physiol 91 (2001) 680-686.

[45] J. Jürimae, T. Jürimae, Leptin responses to short term exercise in college level male rowers. Br J Sports Med 39 (2005) 6-9.

Tables

Figures

Figure 6. Acute and chronic effects of recreational soccer training and a nutritional intervention programme for type 2 diabetes patients on leptin levels. Diet group (n=21). Soccer+Diet group (n=19). Means (SEM) are presented.

Table 1. Baseline characteristics of subjects (n=41)

Variable (mean±SD)	Subjects (n=41)
Male, n (%)	20 (48.8)
Female, n (%)	21 (51.2)
Age (years)	61.1±6.4
Duration of T2D (years)	8.2±6.1
BMI (kg/m^2)	32.9±4.3
HbA1c (%)	7.3±1.3
Energy intake (kcal/ day)	1789.0±510.5
Drug therapy	
Antihypertensive agents, n (%)	24 (54.5)
Statins, n (%)	16 (36.4)
Metformin, n (%)	17 (38.6)
Metformin + sulphonylurea, n (%)	23 (52.3)
Sulphonylurea, n (%)	1 (2.3)

Table 2. Change in parameters at 12 weeks in type 2 diabetes patients by intervention group

	Diet (n=22)		Soccer+Diet (n=19)	
	0 weeks	12 weeks	0 weeks	12 weeks
Weight (kg)	84.1±2.9	80.7±2.6*	88.9±3.7	85.2±3.2*
Waist circumference (cm)	104.3±1.1	99.4±1.1*	106.9±2.7	101.5±2.6*
BMI (kg/m^2)	32.7±1.0	30.8±0.8*	33.0±0.9	31.3±0.7*
Body fat (%)	34.1±1.7	32.2±7.5*	34.6±1.6	32.1±1.6*

Fat mass (kg)	28.8±1.9	26.1±1.6*	30.7±2.0	27.3±1.6*
Fat-free mass (kg)	55.4±2.2	54.6±2.1	58.1±2.7	57.9±2.4
Glucose (mg/dL)	135.6±7.7	123.3±5.5	139.5±8.0	119.0±4.7*
Lactate (mmol/L)	1.6±0.1	1.3±0.1*	1.4±0.1	1.1±0.1*
Insulin (µU/mL)	20.8±1.9	18.6±1.5	18.0±1.6	16.0±1.4
Glucagon (pg/mL)	120.1±5.4	107.0±3.3*	112.2±6.2	93.4±4.4*#
FFA (mEq/L)	0.7±0.0	0.6±0.0	0.7±0.0	0.5±0.0
Adipo-IR (mmol x pmol/L)	2.2±0.3	1.9±0.3	1.7±0.1	1.3±0.1*
Leptin (ng/mL)	22.5±3.8	19.0±2.8*	16.0±2.1	12.0±1.6*
HbA1c (%)	7.3±0.3	6.3±0.9*	7.3±1.2	6.5±0.6*
HOMA-IR	7.4±0.7	5.8±0.5	6.3±0.7	4.7±0.5*
CRP (mg/L)	5.1±1.0	3.9±0.7	4.0±0.6	3.1±0.5
VO_2max (mL/min/kg)	22.7±3.5	21.4±3.5*	22.9±4.9	25.5±5.5*#
VT1-VO_{2max} (mL/min/kg)	15.9±3.0	14.8±2.5	14.8±3.0	16.6±4.0*
VT2-VO_{2max} (mL/min/kg)	20.1±3.4	18.4±2.5*	19.8±4.7	22.5±4.9*#
VT1- HRmax (bpm)	125.4±14.6	124.7±16.1	119.1±19.5	115.6±17.1
VT2- HRmax (bpm)	144.8±15.3	145.2±14.3	141.4±15.9	145.4±13.5
HRmax (bpm)	159.2± 4.0	158.6±3.8	161.4±3.1	163.4±2.7
HRrest (bpm)	75.1±3.5	76.9±3.9	74.5±3.1	69.6±2.6

Values are means±SE. * $p<0.05$ compared to fasting 0 weeks, # $p<0.05$ compared to diet group

Highlights

- Acute soccer sessions markedly improved insulin action markers in T2D patients,
- Acute metabolic effects of a single soccer session on FFA and lactate changes are significantly attenuated after 3 months of training in middle-aged T2D patients.
- Soccer training enhanced insulin sensitivity and decreased risk factors associated with cardiovascular disease in middle-aged T2D patients.