Positive correlation between tumor necrosis factor (TNF)- α and cardiorespiratory fitness after six-months of regular aerobic exercise in Peruvian Amerindian women

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ABSTRACT

Background: Adipose tissue is a source of inflammatory cytokines which may influence insulin action. **Aim:** To evaluate exercise effects on plasma (p)-levels of tumor necrosis factor (TNF)- α , and interleukin (IL)-6 in Peruvian Amerindian women. **Material and Methods:** After five years of observation during which p-glucose deteriorated and weight increased, 44 Peruvian Amerindian women aged 20-59 years took part in a 6-month exercise program. Plasma TNF- α and IL-6 had been measured 5 years earlier, and were reassessed before and after exercise, using ELISA kits. Cardiorespiratory fitness was derived from the results of the twelve minutes' walk and expressed as maximum oxygen uptake (VO_{2max}). **Results:** After the training program, estimated VO_{2max} increased from 18.55 ± 3.79 to 20.91 ± 4.61 mL $kg^{-1} min^{-1}$ ($p < 0.01$) and p-TNF- α increased from 3.2 ± 3.4 to 4.3 ± 6.6 pg/mL ($p = 0.03$). There were reductions in p-glucose from 5.19 ± 1.59 to 4.32 ± 1.62 mmol/L ($p < 0.01$) and in waist circumference ($p=0.01$). Weight, p-insulin, and IL-6 did not change. Levels of p-glucose and p-TNF- α observed after the training period were identical to values 5 years earlier. P-TNF- α was positively associated with estimated VO_{2max} prior to ($r = .414$, $p < 0.01$) as well as after ($r = .362$, $p < 0.05$) exercise, independently of adiposity. P-IL-6 was associated with waist circumference but not with estimated VO_{2max} . **Conclusions:** P-TNF- α correlated with estimated VO_{2max} independently of adiposity. Beneficial effects of exercise on p-glucose were observed in spite of an increase in p-TNF- α .

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Key words: Adiposity; Exercise; Interleukin-6; Physical fitness; Tumor necrosis factor-alpha.

Asociación entre factor de necrosis tumoral α sérico y capacidad aeróbica en mujeres amerindias peruanas, después de seis meses de entrenamiento

Antecedentes: El tejido adiposo es una fuente de citoquinas inflamatorias, que pueden influenciar la acción de insulina. **Objetivo:** Evaluar el efecto del ejercicio sobre los niveles de factor de necrosis tumoral α (TNF α) e interleukina 6 (IL-6) en mujeres amerindias. **Material y Métodos:** Después de cinco años de observación,

en los cuales la glicemia y el peso aumentaron, 44 mujeres amerindias de 20 a 59 años participaron en un programa de ejercicio que duró seis meses. TNF α e IL-6 se habían medido cinco años antes y se re evaluaron antes y después del ejercicio, usando kits de ELISA. La capacidad cardiorespiratoria se estimó a partir de la prueba de marcha de 12 minutos y se expresó como capacidad aeróbica máxima (VO_{2max}). **Resultados:** Después del programa de ejercicio, la VO_{2max} estimada aumentó de $18,55 \pm 3,79$ a $20,91 \pm 4,61$ mL kg⁻¹ min⁻¹ ($p < 0,01$) y el TNF α aumentó de $3,2 \pm 3,4$ a $4,3 \pm 6,6$ pg/mL ($p = 0,03$). Se observó una reducción de la glicemia de $5,19 \pm 1,59$ a $4,32 \pm 1,62$ mmol/L ($p < 0,01$) y de la circunferencia de cintura ($p = 0,01$). El peso, insulinemia e IL-6 no cambiaron. Los niveles de glicemia y TNF α observados después del período de entrenamiento, eran iguales a los determinados cinco años antes. Los niveles de TNF α se correlacionaron con el VO_{2max} estimado antes y después del período de entrenamiento, y no dependieron de la adiposidad. Los niveles de IL-6 se correlacionaron con la circunferencia de cintura pero no con el VO_{2max} estimado. **Conclusiones:** Los niveles de TNF α se correlacionaron con el VO_{2max} estimado independientemente del nivel de adiposidad. Los efectos beneficiosos del ejercicio sobre la glicemia se observaron a pesar de un aumento de los niveles de TNF α .

Several prospective studies have shown lower cardiorespiratory fitness to be associated with higher risk of glucose intolerance and type 2 diabetes in normoglycemic individuals. This association is independent of adiposity, and a potential underlying mechanism may be that lower physical fitness increases systemic inflammatory activity¹. Improved physical fitness increases insulin sensitivity but the mechanisms for these effects are still somewhat unclear. One possible pathway may be through anti-inflammatory effects attributed to regular physical activity.

Whether the cytokine interleukin (IL)-6 has positive or negative effects on metabolic processes is a subject of continuing controversy². Adipose tissue is an important source of IL-6, and increased adiposity is known to be associated with reduced physical fitness³. Similarly, lower physical fitness is associated with higher plasma (p) levels of IL-6¹, and several prospective studies of exercise training have demonstrated reductions of p-IL-6.

Tumor necrosis factor (TNF)- α is considered to contribute to insulin resistance by inhibiting the expression of genes essential for insulin signalling and adipocyte differentiation⁴. P-levels of TNF- α do not reflect regular physical activity in healthy non-diabetic women and are not associated with fitness after adjusting for visceral adiposity⁵.

Studies on the effect of aerobic exercise on fitness and adipocytokines in subjects with deteriorating p-glucose derived from other ethnic groups than Caucasians are lacking. We have previously

shown that Amerindian women from the capital of Peru, Lima, are characterized by substantial hyperinsulinemia, increased body fat mass and waist circumference but lower p-glucose and leptin than in a matched white population⁶. Five years later the participants in this prospective study had developed significantly ($p < 0.0001$) higher fasting p-glucose with unaltered p-insulin and leptin⁷. However, a six-months supervised aerobic training period resulted in an increase of physical fitness (VO_{2 max}), and decreased p-glucose⁸.

As regular aerobic exercise may improve insulin sensitivity by modulating plasma content and/or function of adipocytokines, the present study focussed on potential effects of aerobic training on circulating p-levels of IL-6 and TNF- α .

We investigated whether cardiorespiratory endurance (VO_{2 max}) prior to and after aerobic training was associated with changes in p-IL-6 and TNF- α independently of visceral adipose tissue accumulation, and whether p-IL-6, TNF- α , glucose and fitness prior to the exercise programme were determinants of p-glucose after the exercise period.

Material and Methods

Study subjects

"Alternativa", a nongovernmental organization, was founded 20 years ago as a "Center for Social Research and Popular Education" and is working

on topics related to urban poverty in the district of San Martín de Porres in Lima. Alternativa is working with community kitchens organized by women, facing nourishment problems by collective cooking of the main meal.

About 15 to 20 families constitute each kitchen in the district of San Martín de Porres. Women were invited from 14 community kitchens to the Department of Nutrition and Health Education of Alternativa.

The study population consisted of 182 Peruvian Amerindian women who took part in the baseline study in 1999, and of those two were excluded due to a baseline p-glucose level exceeding 7.0 mmol/L⁶. A migration antecedent from indigenous Andean communities in Peru was identified in all participants. The age range was 20-59 years, with a mean age of 41 years. Fatty acid composition in serum was measured in 141 subjects⁷. This subgroup was invited to take part in the follow-up examination in 2004. A total of 83 women (59%) participated in the second examination. Of these, 76 consented to take part in the aerobic training study, and 59 completed it. Fitness was measured and data on p-TNF- α and IL-6 were available from 44 of these participants⁸. These 44 women did not differ significantly from the remaining participants regarding baseline measurements of body weight, body mass index (BMI), waist circumference, p-glucose, p-insulin and p-leptin. None of the participants had ongoing cardiovascular disease but two subjects had developed diabetic fasting p-glucose concentrations at the reexamination in 2004⁷. Approval for the original study had been given in 1998 and 1999 by the Ethics Committees of Lund University and San Martín University Hospital in Lima. Furthermore, the follow-up and training studies were separately approved by the Ethics Committee of San Martín University Hospital. The study was undertaken in accord with the Helsinki Declaration of 1975, as revised in 1983.

Methods

The examination procedures were the same at all three occasions. The examinations took place in the morning after an overnight fast at Alternativa. All subjects refrained from any severe physical activity 48 h before measurements. Body weight and height were measured with the subject wearing light clothes. Abdominal obesity was

determined as waist circumference measured in a standing position. BMI was calculated in kg/m². Cardiorespiratory fitness was assessed as $\text{VO}_{2\text{max}}$ (mL kg⁻¹ min⁻¹) indirectly after subjects ran around two cones spaced 20 m apart for 12 minutes, as previously described⁹.

Exercise training

Exercise sessions were undertaken once or three times per week⁸, and always supervised by a physiotherapist and took place outdoors on a square with a concrete surface. Of the 44 women presented in this paper, 23 exercised once a week, and 24 subjects attended three training sessions/week. There was a close association between the number of training sessions and improvement in $\text{VO}_{2\text{max}}$ ($p = 0.007$). A warm-up with stretching, light jogging and flexibility movements was followed by aerobic dancing for a total of 60 minutes. The average metabolic equivalent (MET) score calculated for total physical activity performed per training session was 275 or 4.6 MET-minutes / session¹⁰.

Dietary intake

Twenty-four-hour recall was used to evaluate the composition of the diet at the baseline examination, and analyzed with the use of the Peruvian tables of food composition from the Ministry of Health (7th edition), Lima, Peru, 1996.

Measurements

Serum or plasma was separated from venous blood and stored within 1 hour at minus 20 °C and then brought to Sweden for analysis. Insulin was measured with double-antibody radioimmunoassay techniques with the use of guinea pig anti-human insulin antibodies and human insulin as the standard (Linco Research, St Charles, MO). Leptin was measured with a radioimmunoassay specific to human leptin (Linco Research).

P-glucose was measured by using the glucose oxidase procedure. P-TNF- α and p-IL-6 were measured by ELISA using commercially available test kits (Pharmingen, San Diego, CA, USA). Detection limits were 0.12 pg/ml and 0.70 pg/ml¹¹, intraassay coefficients of variation (CV) were 8.8% and 4.4%, and interassay CV were 16.7% and 6.4%, respectively.

Statistical analyses

Data are expressed as mean $\pm$ SD. Comparisons between values at baseline and examinations before and after the training period were determined by use of Wilcoxon signed rank tests. The relation between waist circumference, VO_{2max} and inflammatory markers were assessed by adjusted and unadjusted Spearman rank correlations. Results were declared significant at $p < 0.05$. P-TNF- α , IL-6 and glucose as well as VO_{2max} values were logarithmically transformed to reduce skewness. Multiple regression analysis was undertaken to examine which variables predicted p-glucose concentration after the intervention period. The selected variables were forced into the model based on analysis (Table 2). All statistical analyses were conducted by using the statistical package Stat-

View (version 5.0.1, for Macintosh; SAS Institute Inc, Cary, NC).

Results

Anthropometric and biochemical characteristics

Table 1 presents anthropometric data, physical fitness, and related biochemical characteristics at baseline and five years later, both prior to and after the aerobic training program. An increase in aerobic fitness by 12.7 % (from 18.55 to 20.91 mL $\cdot$ min $^{-1}$ kg $^{-1}$; $p < 0.0001$) was achieved during the aerobic training programme. Body weight and BMI were unchanged in contrast to waist circumference which decreased by 1.31 % ($p = 0.0128$). Exercise training did not influence fasting p-insulin and p-leptin, although mean p-glucose was significantly

Table 1. Data from baseline and 5 years later prior to and after exercise intervention in 44 Peruvian Amerindian women

	Baseline		Before training		After training
Body weight (kg)	59.6 $\pm$ 12.0	$p = 0.0002$	62.3 $\pm$ 12.6		62.7 $\pm$ 12.3
Waist circumference (cm)	84.6 $\pm$ 11.0	$p < 0.0001$	91.7 $\pm$ 10.6	$p = 0.0128$	90.5 $\pm$ 10.4
Body mass index (kg/m 2)	26.2 $\pm$ 5.2	$p < 0.0001$	27.8 $\pm$ 5.2		27.5 $\pm$ 5.2
VO_{2max} (mL $\times$ kg $^{-1}$ $\times$ min $^{-1}$)	ND		18.55 $\pm$ 3.79	$p < 0.0001$	20.91 $\pm$ 4.61
P-glucose (mmol/L)	4.16 $\pm$ 0.48	$p < 0.0001$	5.19 $\pm$ 1.59	$p < 0.0001$	4.32 $\pm$ 1.62
P-insulin (pmol/L)	85 $\pm$ 42		86 $\pm$ 52		87 $\pm$ 57
P-leptin (ng/mL)	14.3 $\pm$ 7.5		13.9 $\pm$ 7.1		14.3 $\pm$ 9.3
P-TNF- α (pg/mL)	4.3 $\pm$ 4.6	$p = 0.0221$	3.2 $\pm$ 3.4	$p = 0.0266$	4.3 $\pm$ 6.6
P-interleukin-6 (pg/mL)	2.0 $\pm$ 2.5	$p = 0.0461$	1.8 $\pm$ 1.2	$p = 0.098$	1.4 $\pm$ 1.1
Total energy intake (kcal/d)	1587 $\pm$ 618		ND		ND
Carbohydrate (%)	69 $\pm$ 9				
Protein (%)	12 $\pm$ 3				
Fat (%)	19 $\pm$ 5				

Mean $\pm$ SD. No intervention took place between baseline and the training programme. ND=not done, P=plasma, TNF=tumor necrosis factor.

Table 2. Unadjusted and adjusted Spearman correlations between waist circumference, cardiorespiratory fitness (CRF), and inflammatory markers tumor necrosis factor (TNF)- α and interleukin (IL)-6 in 44 Peruvian Amerindian women before and after exercise intervention

	Before training		After training	
	TNF- α	IL-6	TNF- α	IL-6
Waist				
Unadjusted	-.16	.428**	-.08	.513**
Adjusted for CRF	.133	.427**	.046	.477**
CRF				
Unadjusted	.414**	-.095	.362*	-.102
Adjusted for waist	.266*	-.022	.356*	.181

*= $p < 0.05$, **= $p < 0.01$.

Table 3. Multiple regression with plasma (p)-tumor necrosis factor (TNF)- α , interleukin-6, glucose and VO_{2max} prior to exercise intervention as independent variables and p-glucose measured after six-months exercise as dependent variable in 44 Peruvian Amerindian women

	Coefficient	SE	Standardized coefficient	P-value
Intercept	0.57	0.26	0.57	0.036
P-TNF- α	-1.00	0.8	-0.16	0.225
P-interleukin-6	-0.01	0.06	-.02	0.881
P-glucose	0.83	0.18	0.62	< 0.0001
VO_{2max}	-0.39	0.18	-0.29	0.035

($p < 0.0001$) lower after the intervention period. P-TNF- α was higher ($p = 0.027$) after aerobic training, and was almost the same as baseline values five years earlier. P-IL-6 before and after training were not significantly different from each other ($p = 0.152$).

Associations between fitness, p-TNF- α and IL-6

Regression analysis showed that log TNF- α was positively correlated to log VO_{2max} , both prior to ($p = 0.0024$) and after ($p = 0.0197$) the exercise programme, whereas there were no significant associations between p-IL-6 and log VO_{2max} .

Associations of p-TNF- α and IL-6 with abdominal adiposity and fitness

Spearman correlation coefficients between waist circumference, cardiorespiratory physical fitness (CRF) and p-TNF- α and IL-6 prior and after the training period are presented in Table 2. Waist circumference correlated positively with p-IL-6 before and after adjustment for CRF, whe-

reas no correlation was found between p-TNF- α and waist circumference.

CRF was positively correlated with p-TNF- α before and after adjustment for waist circumference, whereas no relation was observed between CRF and p-IL-6. The correlation between CRF and p-TNF- α was significant both prior to and after the exercise training period.

Predictors of p-glucose at the end of the exercise training period

In a multiple regression analysis including p-glucose, p-TNF- α , p-IL-6, and VO_{2max} prior to the exercise training as independent variables, p-glucose and VO_{2max} were determinants of p-glucose after training (Table 3).

Discussion and conclusions

The main objective of the present study was to investigate the effect of aerobic training on

p- TNF- α and IL-6 in a cohort of sedentary Amerindian women characterized by deteriorating p-glucose and low cardiorespiratory fitness⁸. Furthermore, we wanted to assess the respective contribution of abdominal adiposity and cardiorespiratory fitness to variation of p-TNF- α and p-IL-6 prior to as well as after the aerobic training.

The six-month exercise training programme resulted in an increase of subject's VO_{2max} by an average of 12.7 %, and promoted a significant ($p = 0.0266$) increase of p-TNF- α simultaneously with a reduction ($p < 0.0001$) in p-glucose (Table 1). P-TNF- α was positively associated with VO_{2max} , prior to as well after the aerobic training period, a relation that was independent of the influence of abdominal adiposity (Table 2). P-IL-6 was associated with waist circumference but not with fitness.

The strong association between p-IL-6 and waist circumference, as well as the fact that this relation is independent of the influence of fitness are both in agreement with other studies⁵, whereas the positive relation between p-TNF- α and VO_{2max} is a novel observation.

Weight reduction in obese subjects following a hypocaloric diet for 12 months resulted in significant decreases of p-TNF- α and p-IL-6¹², whereas in contrast p-TNF- α concentrations were not influenced by exercise training or induced weight losses in non-obese^{13,14} and obese pre-¹⁵ and postmenopausal¹⁶ women. In the present study exercise training did not change body weight, p-leptin or p-insulin, whereas mean elevated p-glucose was significantly lower after the intervention period. Thus, it is likely that the improved insulin sensitivity was not a consequence of changed body fat mass, but instead associated with increased fitness and p-TNF- α .

TNF- α is secreted from a variety of cells including macrophages, muscle, and adipose tissue. Most of the TNF- α secreted by adipose tissue is derived from macrophage infiltration or other nonfat cell sources. Subcutaneous adipose tissue has been claimed to produce more TNF- α than visceral¹⁷, but in contrast to for IL-6 no net release of TNF- α to plasma has been detected from this source¹⁸. As p-levels of TNF- α were not associated with gene expression of TNF- α in subcutaneous adipose tissue, neither at the beginning nor at the end of three months aerobic training¹⁵, the most likely explanation is that the elevated p-TNF- α levels after long term aerobic training program-

mes are derived from other sources than adipose cells per se.

In the present study mean p-TNF- α increased 28% after aerobic training, and reached the same values that had been observed five years earlier when all women had normal fasting glucose concentrations. A likely explanation for this discrepancy from other investigations resulting in reductions or moderate increases of mean p-TNF- α concentrations¹³⁻¹⁶, is that the food consumption pattern in our study population is characterized by a low percentage of calories from fat, 19 %, Table 1, in comparison with reports from industrialized countries⁷. Thus, the observation by Meksawan et al. that the magnitude of the increase in p-TNF- α concentration at post-exercise on a 19% fat diet is greater than that on 30% or 50% fat diets¹⁹ may in part explain our findings.

To reconcile the apparently conflicting data on whether changed p-TNF- α concentrations at muscular work are deleterious or beneficial to insulin sensitivity, recent evidence suggesting that inflammation may have two different roles in the regulation of metabolism are of importance²⁰. The first may be inhibition of insulin sensitivity (a negative effect), as suggested by many studies of low grade inflammation^{4,21,22}, and the second may be induction of energy expenditure (a positive effect)²⁰.

Our study population is characterized by obesity, a sedentary lifestyle and low cardiorespiratory fitness⁷. Only 36% of the women had a normal BMI ($< 25 \text{ kg/m}^2$) when the training period started. That is comparable to figures reported by Jacoby et al²³ in a health survey performed in six Peruvian cities. It is most likely that the increases of body weight and waist circumference during the 5 years non-intervention period prior to the aerobic training were due to reduced energy expenditure. In transgenic mice with deficiencies in TNF- α , IL-1, and IL-6 levels or their receptors, body weight gain is enhanced²⁴. In contrast, when cytokine activity is stimulated, energy expenditure is increased and body weight gain is attenuated²⁵.

VO_{2max} , evaluated prior to the start of the intervention programme was an independent predictor of post-exercise p-glucose, in contrast to p-TNF- α and p-IL-6 (Table 3).

In a large prospective study p-TNF- α was not an independent determinant of future type 2 diabetes development²⁶. This is also in agreement

with the observation of no effect of circulating TNF- α on whole-body insulin sensitivity in predominantly nondiabetic subjects²⁷. The effect of p-TNF- α on insulin resistance is therefore questionable, and the association with improved p-glucose in the present study may be indirect through the beneficial impact of physical fitness on glucose tolerance. Our study did not include a control group, and this issue needs to be studied under controlled conditions with greater power in larger materials. Furthermore, it is apparent that the present study provides no evidence that IL-6 is directly involved in exercise related improvements of glucose tolerance. At the baseline examination all Amerindian women had normal fasting p-glucose but increased p-insulin in comparison to Caucasian subjects⁶, and already before any exercise intervention we found disassociations between p-TNF- α and p-IL-6 in relation to changes of body weight and waist circumference in our material²⁸. The extent to which our findings may be generalized to subjects of other ethnic backgrounds is not known, however. Aboriginal Canadian women with normal fasting p-glucose showed no increased p-TNF- α values compared to white women, despite an increased insulin resistance²⁹.

In conclusion, the present study indicates that p-concentrations of TNF- α , but not of IL-6, are positively associated with cardiorespiratory fitness in Amerindian women prior to as well as after six-months of endurance physical exercise, a relation that is independent of the influence of abdominal adiposity. Improved aerobic fitness and elevated p-TNF- α concentrations are associated with lower fasting p-glucose values when these women participate in a long-term aerobic exercise programme.

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