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PRELIMINARY STUDY OF THE EFFECTS OF AGE AND TYPE-2 DIABETES ON THE RELEASE OF INTERLEUKIN (IL)-6, IL-10, TNF-ALPHA, AND CORTISOL IN RESPOSE TO ACUTE EXERCISE

LUDMILA COSIO-LIMA[1], PETRA SCHULER[1], KATY REYNOLDS[1], LEMUEL TAYLOR[1], GORDON KELLOG[1], JILL CERNEY[1], TABITHA HODGES[1], PAUL LEBLANC[2]

[1]Department of Health, Leisure, and Exercise Science/University of West Florida, Pensacola, FL [2]University of Alabama, Tuscaloosa, AL

ABSTRACT

Cosio-Lima LM, Schuler PB, Reynolds KL, Taylor L, Kellog G, Cerney J, Hodges T, LeBlanc PA. The effects of age and type-2 diabetes on the release of interleukin (IL)-6, IL-10, TNF-alpha, and cortisol in response to acute exercise. *JEPonline* 2008;11(3):33-41. The immune response to exercise results in the release of inflammatory markers. This study examined the effects of age and type-2 diabetes on cytokine response following an acute bout of exercise. Five older type-2 diabetics (OD) (mean age 82±3 years), 5 non-diabetic (79±6 years) older adults (OND), and 5 young healthy (YH) (26±3years) control subjects were studied before and following a sub-maximal incremental treadmill test. Venous blood samples were collected immediately pre- and 5 minutes post-exercise, and plasma was analyzed for interleukin (IL)-6, IL-10, tumor necrosis factor-a (TNF-a), C–reactive protein (CRP), and cortisol levels. Cortisol levels were significantly elevated post-exercise ($p < 0.05$) for all participants suggesting a sufficient exercise stimulus (>60%HRR). A significant main effect for group was found for IL-6 ($p < 0.05$) with post-hoc tests suggesting significantly higher levels in the older diabetic group (OD) compared to the young controls (YH). No significant group by time interaction was found for any of the variables studied. Present study findings suggest that age, rather than the presence of diabetes, affected resting plasma IL-6 levels. Furthermore, neither age nor diabetes influenced cytokine responses to an acute bout of exercise.

Key Words: Diabetics, Acute Exercise, Cytokine Response, Aging
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INTRODUCTION

It is well recognized that inflammatory mechanisms play a major role in the pathological processes of cardiovascular disease, type-2 diabetes, stroke, and chronic obstructive pulmonary disease (1). Patients with type-2 diabetes, metabolic syndrome, and obesity have elevated levels of cytokines such as interleukin-6 (IL-6) and C-reactive protein (CRP) (2). Additionally, atherosclerosis and coronary artery disease (CAD) are associated with greater levels of CRP, IL-6, and tumor necrosis factor alpha (TNF- a). TNF- a has been demonstrated to be a key player in systemic low-level inflammation, and it is associated with obesity and several chronic diseases, and this is because it promotes proatherosclerotic, procoagulant, and procachectic characteristics (3). An increase in pro-inflammatory cytokines also plays a role in the production of several acute-phase proteins involved in the inflammatory response (e.g. CRP) (4). Moreover, levels of inflammatory mediators are correlated with other risk factors in chronic disease processes, including levels of fibrinogen, albumin, cholesterol, arterial blood pressure, and body mass index (BMI) (3).

Aging is correlated with increased levels of inflammatory markers, which are predictors of mortality and disability (5). It has also been suggested that old age, even in the absence of disease, is associated with immunosenescence that results in a decline in phagocytic capacity, reduced dendritic cell traffic (DC), and an increase in pro-inflammatory cytokines such as IL-6 and TNF- a (6).

Physical disability in the elderly population is associated with a decline in muscle mass and muscle strength. This decline in physical stamina is sometimes caused by several diseases that are characterized by chronic inflammation and high levels of IL-6 (e.g. type-2 diabetes). It is unclear whether functional disability precedes or follows increases in IL-6 levels (7). Several epidemiological studies have shown that physical activity and physical performance are inversely associated with inflammatory markers. In physically active elderly (70-80 yrs old), decreased levels of IL-6, CRP, and TNF- a have been observed when compared to elderly with a sedentary lifestyle (3,8,9,10).

On the other hand, it has been suggested that anti-inflammatory cytokines, such as IL-10, might be involved in slowing the aging process and promoting longevity. IL-10 is elevated in the healthy elderly, but declined in the frail elderly along with DC antigen presenting function (11). In 2001, Moore et al reported that IL-10 is involved in regulating immune reactions and inflammatory responses and suppressing pro-inflammatory cytokines such as TNF- a, IL-6, IL-8, and IL-1ß (12).

The extent of interactions between an acute bout of aerobic exercise, aging, and the immune system is not fully understood, as the immune response to exercise depends on frequency, intensity, and duration of exercise (11). Healthy, physically active elderly people present with lower levels of inflammatory markers. However, controversies arise regarding the association of circulating levels of TNF- a and IL-6 during exercise in patients with type-2 diabetes (13). While some studies have observed elevated levels of plasma TNF- a in type 2 diabetics, other studies have not (14,15). Also, little is known regarding the relationship of IL-6 and type-2 diabetes during exercise. Febbraio et al. (13) demonstrated that exercise did not result in TNF-a release in both diabetics and controls, and there was a trend for augmented exercise-induced IL-6 release in diabetics compared to controls. To our knowledge, no previous studies have examined the effects of age and type-2 diabetes on cytokine release in response to acute exercise. Hence, further exercise studies examining the relationship between diabetes, inflammatory markers, and age are warranted. The primary purpose of this preliminary study was to determine whether age or type-2 diabetes influence cytokine responses to an acute bout of exercise. Specifically, we examined the response of IL-6 levels to exercise in healthy older subjects and older subjects with type-2 diabetes. In particular, we were interested in the

determination of IL-6 (pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) levels in serum of healthy old subjects and old subjects with type-2 diabetes (ages 73 to 85 years).

METHODS
Subjects
Five older male and female type- 2 diabetics (OD) (male = 3, females = 2), aged 82 ± 1 (mean ± SD), 5 older non-diabetic (OND) (79 ± 6 years, male = 2, females = 3), and 5 young, healthy (YH) (26 ± 3 years, male = 3, females = 2) control subjects participated in this study. Older subjects were matched for body mass index- (BMI), waist circumference, and VO$_2$max via extrapolation method from the last grade/speed achieved during a sub maximal treadmill-incremental test. None of the women was taking oral contraception or taking any form of estrogen therapy. Before participation in this study, all subjects signed an informed consent approved by the University of West Florida institutional review board and completed a comprehensive medical history which was reviewed by an onsite physician. Medical clearance by their primary care physician was also required prior to participation in the study. Subjects continued on their usual medication dosage during the study. All participants were in good health with no major complications related to diabetes, such as cardiovascular disease and neuropathies. None of the diabetic subjects were insulin dependant, one subject was taking actos (45mg daily) and the rest were taken hypoglycemic drugs (metformin). Only one participant was on beta-blockers medication (toprol). Participants with absolute or relative contraindications to exercise testing as described by the American College of Sports Medicine (2005) were excluded from this study (16).

Procedures
Blood sampling and questionnaires
Subjects reported to the University of West Florida Exercise Science Performance Laboratory between 7.00-8.00 am after a 12-hour overnight fast. Prior diet was not monitored on the subjects. Subjects were instructed to abstain from any type of exercise for 48 hours before testing. An onsite physician reviewed their medical history and physician consent forms prior to participation in the testing. Fasting plasma glucose levels, lipid concentrations (high-density lipoprotein [HDL], triglycerides [TRIG], and total cholesterol [TC] were measured via finger prick (CardioCheck, Christie Clinic, Illinois). Low-density lipoprotein [LDL was computed via the formula [LDL = TC – HDL – (TRIG/5)]. Following this sample, a meal replacement bar (170 calories; 4.5 g fat, 20 g protein, 17 g carbohydrates; Pure Protein, Bayport, NY) was given to the subjects. The meal bar was used to better reflect the response to a typical meal among all subjects and prevent blood glucose levels from elevating too high. If after the meal replacement bar blood glucose was still lower than 100 mg/dL, 4 ounces of orange juice were given to the subject. Height (cm), weight (kg), and waist circumference were measured in each participant. Body mass index (BMI) was calculated for each subject. Each participant then filled out the Physical Activity and Nutritional Health Questionnaires. Venous blood samples (10-mL) were collected immediately pre- and 5 minutes post-exercise, and plasma was analyzed for C–reactive protein (CRP), interleukin (IL)-6, IL-10, tumor necrosis factor-alpha (TNF-a), and cortisol levels using commercial ELISA kits (R & D Systems, Minneapolis, MN; and Diagnostic Systems Laboratories, Webster, TX, respectively).

Exercise Test
Approximately 15 minutes after breakfast, and after the venous blood sample was collected, a baseline 12-lead ECG was obtained on each subject. Both sitting and standing, resting heart rate (HR) and blood pressure (BP) measurements were recorded and 85% age-predicted maximal heart rate reserve (HRR) was determined for each subject [(220-age) – resting heart rate x 0.85]. The Modified Bruce Protocol was used to determine aerobic capacity using a motor driven treadmill. Serial 12-ECG recordings were obtained every three minutes throughout the exercise test to monitor for any

cardiac arrhythmias or other abnormalities. Blood pressure and rate of perceived exertion (RPE), on the Borg scale (6-20) measurements were also recorded every three minutes. Intensity was increased every three minutes, and once their 85% HRR was reached, the treadmill speed and grade were reduced and participants were encouraged to walk for a total of 20 minutes at 60% HRR. A sitting ECG, BP, and RPE were recorded every three minutes during a cool down period.

Blood Analysis

Blood was collected, centrifuged, and stored at -40^{0}C for later analysis. TNF-a, IL-6, and IL-10 concentrations were determined by a quantikine high-sensitivity enzyme-linked immunoabsorbent assay (ELISA) (R & D Systems, Minneapolis, MN) with assay sensitivity of 0.12, 0.04 ng/mL, and less than 0.5 pg/mL, respectively. The intra- and inter-assay coefficients of variation for TNF- a were 4.3% and 7.3%; for IL-6 7.8% and 7.2%; and for IL-10 8.5% and 10.2%. Plasma levels of cortisol, CRP, and insulin were determined by a commercial ELISA (Diagnostic System Laboratories, Webster, TX). The sensitivity for cortisol, CRP, and insulin were 0.1 µg/dL, .6 µg/dL, and 0.26 µg/dL, respectively. The intra- and inter-assay coefficient of variation for cortisol were 2.4% and 12%; for CRP were 3.5% and 14%, and for insulin 2.6% and 5.2%. All pre- and post-intervention samples were measured in the same assay. Insulin sensitivity, as percentages of a normal reference population (%S) was calculated through the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) equation by Tanaka et al (2000)(17).

Statistical Analyses

Repeated-measures ANOVA with Tukey post-hoc analyses were used to compare pre-and post-exercise values by group; one-way ANOVA was used to compare descriptive information between groups. Significance was set at $P < 0.05$. The statistical package SPSS (version 13, Chicago, IL) was used to compute these statistics.

RESULTS

Descriptive statistics for baseline characteristics of subjects are shown in Table 1. There were no significant differences in weight, height, BMI, insulin resistance, insulin sensitivity, waist circumference, or fasting triglycerides between, older diabetics (OD), old non-diabetic (OND), and young healthy (YH) subjects. Total cholesterol levels were higher ($P < 0.01$) in OND subjects compared to OD and YH. Fasting glucose was higher ($P < 0.02$) in OD subjects compared to OND and YH. VO$_2$max was higher ($P < 0.001$) in YH compared to OD and OND.

Table 1.Baseline Subject Characteristics

	Old Diabetic (OD)	Old Non-Diabetic (OND)	Young Healthy (YH)
Males	3*	2	3
Females	2	3	2
Age (Mean ± SD)	82.4 ± 3.0	79.2 ± 5.8	26.0 ± 2.5*
Waist circum. (inch)	36.8 ± 2.9	36.6 ± 6.2	32.8 ± 3.2
Height (m)	1.7 ± 0.6	1.7 ± 0.4	1.8 ± 0.8
Weight (kg)	74.0 ± 8.5	68.3 ± 12.0	82.0 ± 16.0
BMI (kg/m^2)	25.4 ± 2.0	24.4 ± 4.4	24.9 ± 3.8
TC (mg/dL) **	148.0 ± 24.0	192.0 ± 21.0*	151.0 ± 19.5
Glucose (mg/dL) **	103.0 ± 4.5*	80.0 ± 15.0	75.0 ± 14.0
TRIG (mg/dL)**	101.0 ± 90.5	73.0 ± 22.0	56.0 ± 15.2
VO$_{2max}$ (ml/kg/min)	32.4 ± 5.4	32.4 ± 5.4	53.7 ± 8.9*
Insulin resistance	2.2 ± 0.38	2.96 ± 1.50	1.9 ± 1.0
Insulin sensitivity	47.8 ± 7.8	60.5± 71.0	64.3 ± 26.1

*One participant on ß-blocker medication (actos, 45mg/q day)

** 12-hr fasting samples P < 0.05

Table 2. Mean cytokine levels between Pre-and Post-exercise in Old Diabetics (OD), Old Non-diabetics (OND) and Young Healthy (YH) Subjects

Cytokine	OD		OND		YH		
	Pre	Post	Pre	Post	Pre	Post	P
IL-6 (pg/mL)	2.1± 0.7	2.0± 1.2	1.3 ± 0.6	1.7 ± 0.8	0.6 ± 0.5	0.8 ± 0.5	0.034*
IL-10 (pg/mL)	2.3± 1.0	4.1 ± 3.4	3.3 ± 2.1	1.3 ± 1.3	6.8 ± 4.1	3.6 ± 1.3	0.094
TNF-a (pg/mL)	1.3± 0.5	0.8 ± 0.7	1.5 ± 1.4	1.1 ± 0.9	0.5 ± 0.1	1.0 ± 0.7	0.370
CRP (ng/L)	8.9± 15.7	8.2 ± 3.8	5.0 ± 5.2	5.4 ± 5.5	3.9 ± 5.8	4.1 ± 5.8	0.350

* Differences shown between groups
Mean ± SD   P < 0.05

Table 2 represents mean cytokine data in OD, OND, and YH pre and post-exercise. A significant main effect for group was found for IL-6 ($p<0.05$) with post-hoc tests suggesting significantly higher levels in the older diabetic group (OD) compared to the young controls (YH). No significant group by time interaction was found for Il-10, TNF- a, nor CRP. Table 3 represents the metabolic data (insulin and cortisol) in OD, OND, and YH pre and post-exercise. Cortisol levels were significantly elevated post-exercise ($p<0.05$) for all participants, whereas no significant differences were observed in insulin levels among participants pre- and post-exercise.

Table 3. Mean metabolic levels Pre-and Post-exercise in Old Diabetics (OD), Old Non-diabetics (OND) and Young Healthy (YH) Subjects

Metabolic Marker	OD		OND		YH		
	Pre	Post	Pre	Post	Pre	Post	P
Cortisol(µg/dL)	24.3 ± 6.9	25.7 ± 8.7	22.3 ± 6.7	28.9 ± 1.0	29.0 ± 0.2	38.9 ± 10.3	0.050*
Insulin(pg/mL)	16.2 ± 3.0	18.6 ± 17.0	24.6 ± 13.0	28.7 ± 3.9	5.4 ± 8.1	26.2 ± 21.7	0.589

*Differences shown within group
Mean ± SD   P < 0.05

DISCUSSION

This preliminary study demonstrates that IL-6 levels in older diabetics (OD) were considerably higher at rest when compared to young healthy (YH), and old no-diabetic (OND) study participants. Moreover, cortisol levels were significantly higher in all groups post-exercise. Furthermore, neither age nor diabetes influenced response of cytokines to an acute bout of exercise in the present study.

To our knowledge, this is the first preliminary study to demonstrate that age rather than the presence of type-2 diabetes affects resting plasma IL-6 levels. The higher IL-6 levels seen in the OD and OND in the current study are consistent with observations reported by Wannamethee et al. (18) where IL-6 was significantly associated with age and low physical activity. Interestingly, the older participants in the current study also demonstrated low physical activity scores in the Physical Activity and Nutritional Health Questionnaires. Wannamethee et al. (18) also showed no relationship between IL-6 levels and insulin resistance when adjusted for BMI. This supports the results in the present study demonstrating no significant differences in insulin resistance, insulin sensitivity, and BMI between the three groups.

Other researchers have proposed possible explanations for a pathophysiological relationship between IL-6 and aging (19). At rest, IL-6 is primarily produced by monocytes and macrophages,

fibroblasts, vascular endothelial cells, and adipose tissue (20,21). An age-related increase in IL-6 has been associated with the development of physical disability (7), disease (22), and mortality (6). Ferrucci et al. (7) suggest that cytokine, particularly IL-6, may be directly related to medical conditions and physical disabilities. They also suggest that IL-6 may be associated with sarcopenia and frailty. However, it is unclear whether elevated IL-6 levels or physical disability and disease develop first.

In addition to the differences resting in IL-6 in the older groups when compared with the YH, a trend for lower levels of IL-10 was observed in the OD and OND compared with the YH. This suggests that physical activity may play a role in these findings since IL-10 is influenced by physical fitness (3,11). The YH group in the present study had a much higher VO2max when compared to both the OD and OND groups. Also, physical activity levels reported by the OD and NOD groups were lower than the YH group. IL-10 is a potent anti-inflammatory cytokine that promotes cardiovascular and neuromuscular health (3). Jankard and Jemiolo (11) point out that exercise training increases IL-10 while decreasing IL-6 levels. These authors suggest that regular physical activity in older populations may lower the degenerative effects of aging by maintaining healthy levels of pro and anti-inflammatory cytokines.

No changes in insulin levels were observed after the exercise bout among the present study subjects. According to various researchers, the role of insulin response to an acute bout of exercise among patients with type-II diabetes has not been well documented (23). Due to subjects' time constraints we were not able to draw post-exercise glucose levels that would have enabled us to calculate post-exercise insulin resistance and insulin sensitivity. Therefore, it is difficult to confirm whether the post-exercise insulin response was due to insulin resistance.

Although statistically insignificant, and merely based on study-speculation trends, resting TNF-a levels in the OD and OND groups were higher when compared to the YH group. Bruunsgaard (3) states that TNF-a is inversely related to skeletal muscle protein synthesis, resulting in loss of muscle mass, strength, and functional capacity in the elderly. Moreover, he hypothesizes that low-level inflammation is caused indirectly by TNF-a production. The TNF-a-related pathological processes could explain our findings that both the OD and OND groups presented higher TNF-a levels than the YH group, which again suggests that age and low physical activity levels were important factors that influenced the trends observed in the present study. The small sample size in this study needs to be taken into consideration as a study limitation that could have influenced the power sample in this study.

Several interesting trends were noted in cytokine response post-exercise. The OND and YH groups responded similarly with increases in IL-6, IL-10 and a decrease in CRP. The OD group had the opposite responses in IL-6, IL-10, and CRP levels. It is well known that plasma IL-6 release is related to exercise intensity, duration, the mass of skeletal muscle recruited, and one's endurance capacity (24,25). This post-exercise IL-6 release has also been observed in elderly untrained subjects (26). The elevation in IL-6 seen in OND and YH groups post-exercise agrees with previous studies where the increase in IL-6 levels originates primarily from skeletal muscle (24). On the other hand, the OD presented with decreased IL-6 levels post-exercise. According to Lyngso et al. (27) the decrease in IL-6 levels observed in the OD is related to the IL-6 adipose tissue suppression and enhanced IL-6 clearance by the hepatosplanchnic viscera resulting in lower net levels of IL-6 post-exercise in patients with type-2 diabetes.

In conclusion, unlike the OND and the YH groups, the adipose tissue, as opposed to skeletal muscle, contributed to the cytokine response observed post-exercise in the OD group in the present study. Slight, but insignificant decreases in TNF-a levels post-exercise in the OD and OND groups were

observed while in the YH, TNF-a levels increased. This observation is in agreement with some studies (28,29) but not supported by other studies (14,15). These results agree with a study by Febbraio et al (13) where no statistical differences in arterial TNF-a were observed after exercise in neither control nor diabetic middle-aged groups. On the other hand, it has been observed that exercise can result in an increase in circulating TNF-a as a result in stress caused by muscle contraction as was the case in the present study with the YH group (13). Therefore, our study provides evidence that the patterns of IL-6 and IL-10 responses are different during exercise in older adults when compared to older diabetics. Controversy exists regarding the effect of TNF- and the pathogenesis of type-2 diabetes, and further studies are warranted to address this issue.

It is interesting that CRP levels paralleled the IL-6 responses while IL-10 responded inversely to IL-6 and CRP in both resting and post-exercise in all groups. This further supports previous studies showing that CRP and IL-6 are pro-inflammatory cytokines and IL-10 is an anti-inflammatory cytokine (3).

Limitations of the study
Our study had limitations that warrant discussion. The sample population in the present study was small, and therefore we were not able to match for gender or ethnicity. We are aware that a larger sample might have given us more statistical power. Ideally, we would like to have a larger sample size; however, the nature of the population and scheduling constraint by the subjects made the study difficult to execute. Also, because of the subjects' time constraints, we were not able to draw post-exercise glucose levels that would have allowed us to calculate post-exercise insulin resistance and insulin sensitivity.

CONCLUSIONS

In conclusion, the current study demonstrates that age may have a more profound effect on cytokine response at rest than diabetes in older individuals who have low physical activity levels. In contrast, the present study suggests that diabetes may mediate cytokine responses after exercise in older individuals.

Address for correspondence: Cosio-Lima L, PhD, CSCS, Department of Health, Leisure, and Exercise Science, University of West Florida, 11000 University Parkway, Building 72, Room 220, Pensacola, FL, USA, 32514. Phone (850) 473-7350; Email: lcosiolima@uwf.edu.

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