Suppression of Age-Dependent Increase in Insulinemia in Early Middle-Aged Females with Exercise Habit

Tung-Hsiung Hsu[1], Yuh-Feng Liu[1], Shin-Da Lee[2], Shu-Man Chen[3], Jo-Ping Lee[2], Chin-Lung Fang[4], Te-Chih Liu[1], and Chia-Hua Kuo[1]

[1]*Laboratory of Exercise Biochemistry*
Taipei Physical Education College, Taipei
[2]*Department of Physical Therapy*
China Medical University, Taichung
[3]*Committee of General Studies*
Shih Hsin University, Taipei
and
[4]*Department of Physical Education*
National Taiwan Normal University, Taipei
Taiwan, Republic of China

Abstract

Circulating insulin concentration has been suggested as a biomarker for human longevity. The goal of the study was to determine the insulin levels under a glucose-challenged condition for the sedentary and physical active females in early middle age. We measured serum insulin levels for following groups: young sedentary (Y-SED, age 19.7 ± 0.2 years), middle-aged sedentary (M-SED, age 42.3 ± 3.1 years), young physically active (Y-EX, age 20.7 ± 0.5 years), and middle-aged physically active (M-EX, age 40.3 ± 2.8 years). Oral glucose tolerance test (OGTT) and insulin measurement were performed under overnight fasted condition. Triglyceride, cholesterol, body mass index (BMI), and waist-to-hip ratio (WHR) were also determined in all subjects. While fasted glucose and insulin levels were not different among 4 groups, glucose and insulin levels under OGTT were greater in the M-SED group than those in the Y-SED group. The M-EX subjects exhibited lower insulin levels on OGTT, as compared to the M-SED group, and were similar to the level of Y-SED. BMI and WHR of the M-SED group were comparable to those of the M-EX group. Triglyceride and cholesterol levels were highly associated with age and WHR but not the level of physical activity. The current study found a substantially greater insulin response on OGTT in the healthy sedentary females aged ~40, as compared to those in the young sedentary and the middle-aged physically active females, independent of weight status. The result of the study also suggests that accumulating 150 min of weekly exercise is sufficient to counteract the adverse effect of age on insulin sensitivity.

Key Words: exercise, aging, cholesterol, triglyceride, insulin, glucose tolerance

Introduction

Insulin, produced by the islets of Langerhans in pancreas, is a major anabolic hormone in human body. The amount of pancreatic insulin output to circulation is a function of circulating glucose and peripheral insulin sensitivity. The blood level of this hormone has recently been suggested as a predictor for human longevity according to the combined results from Baltimore Longitudinal Study of Aging and NIA Primate Aging Study (21), in which the group with greater circulating insulin level exhibits faster rate of age-dependent decline

Corresponding author: Dr. Chia-Hua Kuo, Laboratory of Exercise Biochemistry, Taipei Physical Education College, 101, Jhongcheng Rd., Sec. 2, Shihlin, Taipei 111, Taiwan, ROC. Tel: +886-2-28718288 ext. 5101, Fax: +886-2-28753383, E-mail: kch@tpec.edu.tw
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in survival. Increased insulin level can be regarded as a sign of insulin resistance, which is known to be associated with the development of various metabolic diseases (8, 20). Insulin resistance is present in the great majority of elderly with states of glucose intolerance, but frank decompensation of glucose homeostasis does not occur if individuals can maintain a state of compensatory hyperinsulinemia (10, 22).

Regular exercise is generally considered a good intervention preventing development of insulin resistance or hyperinsulinemia for healthy individuals (14). It is well established that the development of insulin resistance is concurrently associated with both aging and reduced physical activity (23). Early prevention for the development of insulin resistance is important before the onset of overt clinical syndromes with advancing age. Information was inadequate on how early in human lifetime that insulin level starts to increase substantially and how effective of keeping physically active lifestyle on insulin sensitivity at early middle age. In this study, insulin resistance measures, including glucose tolerance, insulin, triglyceride, and cholesterol, were determined among young and early middle-aged female subjects with or without regular physical activity within the same community. The female subjects that we investigated in this study were ~40 years old. Females at this age level have generally no immediate health problem and is particularly easy to ignore the importance of keeping physically active lifestyle due to distraction of heavy family responsibility. The current study provides the answers on whether aging effect on insulin can occur in the females at early middle age and how regular physical activity affects insulinemia at this age level.

Materials and Methods

Human Subjects

Thirty-four healthy premenapausal women, including sedentary young (Y-SED, age 19.7 ± 0.2 years, N = 9), physically active young (Y-EX, age 20.7 ± 0.5 years, N = 9), sedentary middle-aged (M-SED, age 42.3 ± 3.1 years, N = 8), and physically active middle-aged (M-EX, age 40.3 ± 2.8 years, N = 8) groups were voluntarily enrolled this community-based study. The definition of "physically active" is that an individual sustains exercise habit for > 3 times a week, > 30 min each time. To recruit subjects, advertisement was delivered *via* poster on the campus bulletin board. All experimental procedures were explained to all subjects before they signed the human experiment consent. None of the subjects had a history of chronic metabolic diseases, and no acute illness was reported from subjects within the most recent month of measurement. The subjects under menopause or medication were precluded. Ethical approval for the study was obtained from the Human Subject Committee of Taipei Physical Education College. Their age and exercise time (min per week) were self-reported according to questionnaire, which defines exercise in criteria of heart rate above 130 beat per min or perceived exertion ratings above 12 on the Borg Scale for any form of physical activity (rating 6-20% effort; 7-30% effort - very, very light (Rest); 8-40% effort; 9-50% effort - very light - gentle walking; 10-55% effort; 11-60% effort - fairly light; 12-65% effort; 13-70% effort - somewhat hard - steady pace; 14-75% effort; 15-80% effort - hard; 16-85% effort; 17-90% effort - very hard; 18-95% effort; 19-100% effort - very, very hard; 20- Exhaustion). Body mass index (BMI) and waist-to-hip ratio (WHR) were measured at the day of performing oral glucose tolerance test (OGTT). Circumference of the waist (umbilical level) and hip (maximum of buttocks) (WHR) were measured to the centimeter, and the waist-to-hip ratio was calculated to estimate the degree of central fat distribution. BMI (body mass index) was calculated as kilogram per square meter. Oral glucose tolerance and metabolic measures were performed under morning fasted condition. To avoid the confounding effect of acute exercise on measurements, subjects were remained sedentary 24 h before OGTT.

OGTT and Insulin Response

Measurements for OGTT and insulin response during OGTT were performed to all subjects from each group. On the day of the OGTT, subject reported to the experimenters between 8-9 a.m. after an overnight fast. The test procedure was performed according to the method previously described by Wang *et al.* (24). A 75-gram of glucose (Roquette Italia S.P.A., Cassano Spinola, Alessandria, Italy) was orally delivered with 500 ml of water. Blood samples were collected from the fingertips at 0 (fasting value), 30, 60, and 90 min. Area under curve of glucose (GAUC) and insulin (IAUC) were calculated using the increment of glucose and insulin levels from baseline. A Lifescan glucose analyzer (Los Angeles, CA, USA) was utilized for glucose concentration determination in the method of glucose oxidase. Serum samples from approximately 200 μl of fingertip blood were used for insulin determination by the method of ELISA on TECAN Genios ELISA analyzer (Salzburg, Austria) using a commercial ELISA kit (Diagnostic Systems Laboratories, Inc. Webster, TX, USA).

Blood Triglycerides and Cholesterol

Total serum cholesterol and triglyceride were measured on a Beckman spectrophotometer analyzer

Table 1. Subject characteristics. For sedentary groups, body mass index (BMI) of the middle-aged subjects was significantly greater than those of the young subjects. For both sedentary and physically active groups, waist-to-hip ratio (WHR) of the middle-aged subjects was significantly greater than those of the young subjects. *Significance against the young group on the same physical activity status, *P* < 0.05.

	Sedentary		Physically Active	
	Young	Middle-aged	Young	Middle-aged
Age (yr)	19.7 ± 0.2	42.3 ± 3.1	20.7 ± 0.5	40.3 ± 2.8
Weight (kg)	57.1 ± 3.2	61.9 ± 1.1	59.8 ± 2.0	60.8 ± 2.7
Height (cm)	164.3 ± 1.3	160.1 ± 2.2	163.0 ± 2.0	161.6 ± 1.9
Exercise time (min/wk)	43.3 ± 10.1	37.5 ± 13.6	273.3 ± 39.3	150.0 ± 12.7
BMI	21.1 ± 1.0	24.2 ± 0.8*	22.5 ± 0.5	23.4 ± 1.2
WHR	0.76 ± 0.02	0.82 ± 0.02*	0.76 ± 0.01	0.80 ± 0.02*

Table 2. Triglyceride and cholesterol levels. Both serum lipid levels of the middle-aged groups were significantly greater than those of the young groups regardless of the physical activity status. *Significance against the young group on the same physical activity status, *P* < 0.05.

	Sedentary		Physically Active	
	Young	Middle-aged	Young	Middle-aged
Triglyceride (mg/dl)	81.5 ± 12.1	128.4 ± 25.1*	92.4 ± 9.3	141.4 ± 14.8*
Cholesterol (mg/dl)	142.8 ± 8.1	178.6 ± 6.3*	129.7 ± 9.5	164.5 ± 9.2*

with Sigma Trinder's reaction (Sigma, St. Louis, MO, USA), according to the manufacturer's procedure.

Statistical Analysis

Two-way ANOVA was used to determine the age-activity interaction of all measurements, and one-way ANOVA was used to compare the mean difference of all measured variables among 4 groups. Fisher PLSD post hoc test used to determine the significant difference between each of the two groups. Pearson correlation and partial correlation were also used to determine the significant correlation among variables. On the basis of preliminary estimates of the corresponding standard deviations, we determined that at least 7 subjects would be required in each group for the study to have a statistical power of 95% to detect meaningful differences between groups. A level of $P < 0.05$ was set for significance to all tests, and all values are expressed as means ± SE.

Results

Subject characteristics are shown in Table 1. WHR was significantly greater in the middle-aged subjects than those in the young subjects. For both age levels, WHR of the physically active subjects was similar to those of the sedentary subjects. Total cholesterol and triglyceride levels of the middle-aged groups were significantly greater than those of the young groups, for both sedentary and physically active subjects (Table 2).

Figure 1 shows the result of OGTT and insulin response from the 4 groups. Both fasted glucose concentrations and OGTT of the middle-aged sedentary subjects were significantly greater than those of the young sedentary subjects. Similarly, the middle-aged sedentary subjects exhibited a substantially greater insulin response on OGTT compared to the sedentary young subjects. The glucose tolerance of the middle-aged physically active group was better than those of the middle-aged sedentary group, but poorer than those of the young physically active group. The insulin levels on OGTT for the middle-aged subjects with regular physical activity were also lower than those of the sedentary middle-aged counterparts, and similar to the rest of young groups. No significant difference was found in fasted insulin and insulin levels on OGTT between the sedentary and physically active young subjects. Weekly exercise time was significantly correlated with that of the area under curve of insulin (IAUC) when WHR was controlled ($r = -0.56$, $P < 0.001$), and the correlation with the remaining variables becomes non-significant.

When all subjects were pooled together, the variables that were significantly correlated with age

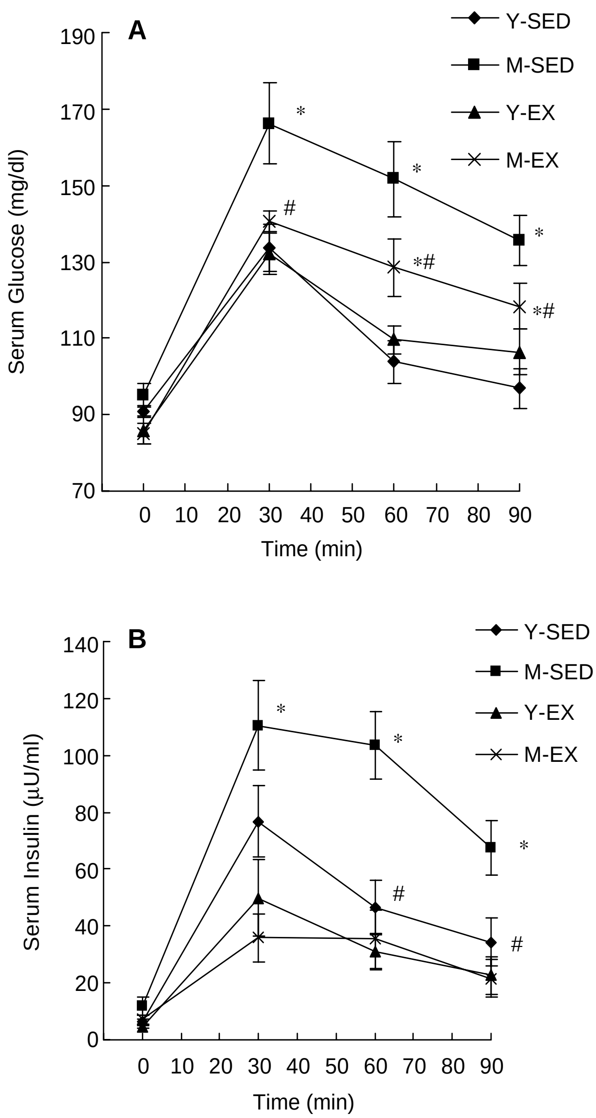

Fig. 1. OGTT (A) and insulin responses (B). Concentrations of serum glucose and insulin after an oral glucose challenge in the sedentary middle-aged subjects were significantly greater than those in the physically active middle-aged group and both young groups. Insulin sensitivity in the physically active middle-aged subjects was only partially maintained against aging effect as their glucose response was slightly higher than in the young subjects during the OGTT. *Significance against the young group on the same physical activity status, $P < 0.05$; #Significance against the sedentary group on the same age level, $P < 0.05$. Y-SED: sedentary young group; M-SED: sedentary middle-aged; Y-EX: physically active young; M-EX: physically active middle-aged.

are listed in Table 3, which includes BMI, WHR, fasted insulin, GAUC and IAUC, triglyceride, and cholestrerol.

Discussion

At the group level, the present finding indicates a significant aging effect on postprandial insulin level with small age difference. This metabolic change is apparent by age ~40 if the subject do not exercise regularly. A noteworthy result is that the fasted glucose and insulin in the early middle-aged female groups were not much different from the young female groups, indicating that using fasted levels of both glucose and insulin concentrations would not be sensitive enough to disclose the aging effect. We must know that the glucose and insulin levels of the sedentary middle-aged subjects in this study is still far from clinical stage, and could be considered normal or healthy by clinician.

Table 3. Correlation coefficients for the physiological variables and age

Variables	R to age	*P*
BMI	0.462	0.006
WHR	0.586	< 0.001
Fasted glucose (mg/dl)	0.262	0.134
Fasted insulin (µU/ml)	0.410	0.016
GAUC	0.646	< 0.001
IAUC	0.349	0.043
Triglyceride (mg/dl)	0.553	0.001
Cholesterol (mg/dl)	0.532	0.001

Abbreviation: BMI: body mass index; WHR: waist-to-hip ratio; GAUC: area under curve of glucose concentrations; IAUC: area under curve of insulin concentrations

Age is generally known as a major risk factor for development of type 2 diabetes (11), based on the evidence that the significantly higher prevalence of impaired glucose tolerance and type 2 diabetes was observed in adults older than 65 years (12). In the younger age group, decreased physical activity and increased adiposity are known to contribute to the reduced insulin sensitivity (1, 15). This is initially compensated with an increase in insulin secretion (2, 5). However, the appearance of impaired glucose tolerance in type 2 diabetes suggests that adequate insulin secretion cannot be maintained when the hyperinsulinemia persists a for longer period (5). The result of the present study implicates the importance of physically active lifestyle on the biomarker of human longevity and also highlights the importance of using insulin measurement on OGTT for early prevention in age-related metabolic disorders such as type 2 diabetes.

The result of the study found that regular physical activity did not make significant differences in the glucose and insulin levels under glucose-challenged condition for the young subjects at ~20 years old. However, this difference in exercise effect became substantial when the subject age was close to 40 years old, despite that the weekly exercise time was lower in the middle-aged females, as compared to the young physically active counterparts. Furthermore, regular physical activity at ~150 min per week was sufficient to generate a suppressive effect on the age-associated increase in insulinemia as the insulin levels was completely normalized to ~20 years of age level. This suggests that the adverse effect of physical inactivity on postprandial insulin levels is unmasked with age. In fact, insulin sensitivity is not completely maintained in active middle-aged subjects as the glucose response was slightly higher than that in the young subjects during the OGTT.

Abdominal obesity is known as a major contributor to insulin resistance and hyperinsulinemia by age (4, 19). Most of the previous epidemiological studies were performed with a wide range of age. For example, Colman *et al.* (6) reported that total adiposity and central body fat distribution are significant determinants of the increase in fasting insulin levels, whereas age, dietary intake, and levels of aerobic fitness and physical activity are less important contributors to the variation in fasting insulin concentrations in women aged from 18 to 90. In this study, we found that both the sedentary and physically active middle-aged subjects were more obese, as compared to their younger age control groups. Yet, only insulin levels under OGTT, but not fasting insulin level, was significantly correlated with WHR. This indicates that, evaluating cohort with younger age range, fasting insulin is not as sensitive as the insulin under OGTT for determining the age-dependent metabolic deterioration. The aging effect on insulinemia appears to be mediated by increased fatness. This is suggested by the evidence of the present study that the significant correlations between age and both GAUC and IAUC becomes non-significant when data was controlled for WHR.

The current study demonstrated that regular physical activity is an independent determinant for the insulinemia under OGTT in the middle-aged females. However, this effect is not mediated by changing weight status or abdominal obesity, since the correlation coefficient between regular physical activity and insulinemia remained significant when the factor of WHR was controlled. It is likely that the regular physical activity exerts its insulin-lowering effect by increasing skeletal muscle insulin sensitivity. Under insulin stimulated condition, skeletal muscle is the main site for postprandial glucose disposal (7). Therefore, skeletal muscle insulin sensitivity directly affects the whole-body insulin sensitivity (14). Exercise training has been found to increase muscle insulin-responsive glucose transport (16).

Although it has been frequently reported that reduced physical activity is closely associated with the development of obesity (17), in this study the weight status for the sedentary and physically active middle-aged female subjects were not much different. This is probably due to the gender-specific factor that the early middle-aged female subjects in the study had previous pregnancy experience. Parity has been reported to be related to adiposity and the prevalence of obesity (13). Another possibility is that the middle-aged female subjects in the study initiated their regular physical activity when they found their body weight was unacceptable.

Although BMI was not significantly different between sedentary and physically active middle-aged females, WHR was slightly lower for those physically active female subjects, which suggests that the factor of regular physical activity is more related to body composition than weight status. One limitation of the study that may confound our observation of the exercise effect on weight is that the data for weekly exercise time was relying on a self-reported questionnaire, which can not accurately indicate their intensity of physical activity. Exercise intensity has previously been considered as an important factor on body fat status (3).

The current study found that total cholesterol value was associated with WHR, but not the level of physical activity. This result is different from previous study performed by Gandapur *et al.* (9) that a 6-month of aerobic exercise training program significantly lowers total cholesterol level. One major difference from the current study is that the BMI level of the aerobic trained subjects was significantly lowered. Thus the cholesterol changes appear to be related to weight status change. Inter-individual variation in endocrine condition, such as baseline DHEA-S level, has also been reported to implicate for the different findings of exercise adaptation on cholesterol and other metabolic outcomes (13, 18).

In conclusion, the current study found that insulin concentration under a standardized glucose-challenged condition, was substantially greater in the healthy female aged ~40, as compared to those in the young control group. This age-dependent metabolic change was suppressed to the young level in the same age group regularly participating physical activity. The exercise time ~150 min per week appears to be sufficient to normalize the insulin concentration to the young level. Additionally, the age-dependent increases in triglyceride and cholesterol were closely associated with the degree of central fatness independent of weekly exercise time.

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