

Supplementary Information

Klotho-related Molecules Upregulated by Smoking Habit

in Apparently Healthy Men:

A Cross-sectional Study

Kaori Nakanishi, Makoto Nishida, Masaya Harada, Tohru Ohama, Noritaka Kawada,

Masaaki Murakami, Toshiki Moriyama, Keiko Yamauchi-Takihara.

Supplementary Table S1. Correlation between smoking severity and serum levels of fibroblast growth factor (FGF)-21, soluble alpha Klotho (α Kl), and interleukin (IL)-6

Smoker (n = 40)	FGF-21		α Kl		IL-6	
	r	P-value	r	P-value	r	P-value
Cigarettes smoked per day	-0.05	0.71	0.01	0.95	*0.326	0.046
Brinkman index	0.08	0.49	-0.01	0.91	*0.301	0.038

* P<0.05.

FGF-21, fibroblast growth factor-21; α Kl, soluble alpha-Klotho; IL-6, interleukin-6

We examined the correlation between smoking severity (using the number of cigarette smoked per day and Brinkman index) and serum levels of fibroblast growth factor (FGF)-21, soluble alpha Klotho (α Kl), and interleukin (IL)-6. Serum levels of IL-6 significantly correlated with smoking severity, however FGF-21 and α Kl did not show any correlations.

Supplementary Table S2. Correlation between age and serum levels of soluble**alpha Klotho (α Kl) in middle-aged group**

	All (n = 80)		Smoker (n =40)		Never-smoker (n =40)	
	r	P-value	r	P-value	r	P-value
age	-0.05	0.55	0.02	0.90	-0.09	0.45

To confirm the age influence on serum levels of soluble alpha Klotho (α Kl) in the middle-aged subjects (46.1 ± 5.1 years), we examined the correlation between age and serum levels of α Kl. There were no correlation between age and α Kl in the study subjects.

Supplementary Table S3. Characteristics of the subjects in aged group

	All (n = 60)	Smoker (n = 36)	Never-smoker (n = 24)	P-value
Age (years)	60.3 ± 1.7	60.1 ± 1.7	60.4 ± 1.8	0.56
BMI (kg/m ²)	23.5 ± 2.2	23.7 ± 2.3	23.2 ± 2.1	0.34
WC (cm)	83.3 ± 6.8	84.3 ± 6.9	81.7 ± 6.4	0.14
SBP (mmHg)	126 ± 14	126 ± 16	126 ± 10	0.98
DBP (mmHg)	81 ± 10	82 ± 11	80 ± 9	0.42
Cr (mg/dl)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.14
UA (mg/dl)	5.8 ± 1.1	5.9 ± 1.1	5.6 ± 1.2	0.34
TC (mg/dl)	208 ± 30	209 ± 30	208 ± 29	0.92
TG (mg/dl)	125 ± 57	*138 ± 64	105 ± 36	0.02
HDL-C (mg/dl)	56 ± 14	55 ± 16	56 ± 11	0.82
FPG (mg/dl)	97 ± 18	96 ± 20	97 ± 14	0.88
HbA1c (%)	5.3 ± 0.5	5.2 ± 0.6	5.4 ± 0.3	0.07
sαKl (pg/ml)	497 ± 175	**590 ± 143	350 ± 107	<0.001
IL-6 (pg/ml)	1.6 ± 0.7	1.7 ± 0.9	1.4 ± 0.5	0.117

Data are expressed as mean ± SD. * P<0.05, ** P<0.001 versus never-smokers.

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; UA, uric acid; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; FPG, fasting plasma glucose; sαKl, soluble alpha-Klotho; IL-6, interleukin-6

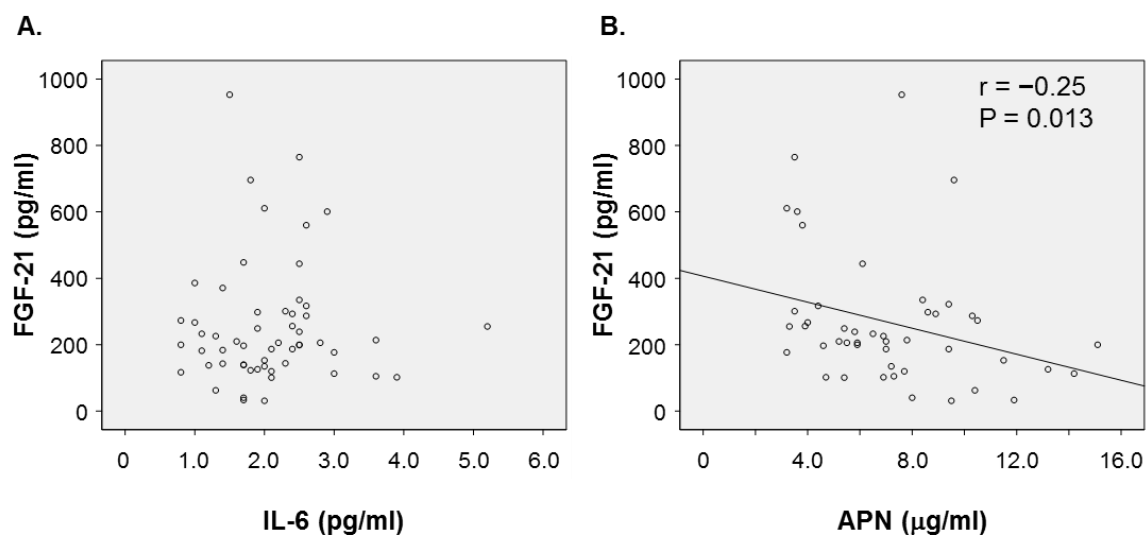
Serum levels of triglycerides (TG) and soluble alpha Klotho (sαKl) were significantly higher in smokers than in never-smokers (P = 0.02 and P < 0.001).

Supplementary Table S4. Details of smoking habit in aged smokers

Smoker (n = 36)	n (%)
Cigarettes smoked per day	
10 - 20 cigarettes	30 (83.3)
21 - 30 cigarettes	3 (8.3)
31 - 40 cigarettes	3 (8.3)
Duration of smoking	
11 - 15 years	1 (2.8)
16 - 20 years	4 (11.1)
> 20 years	31 (86.1)

The details of smoking habit in aged smokers are shown in Supplementary Table S4.

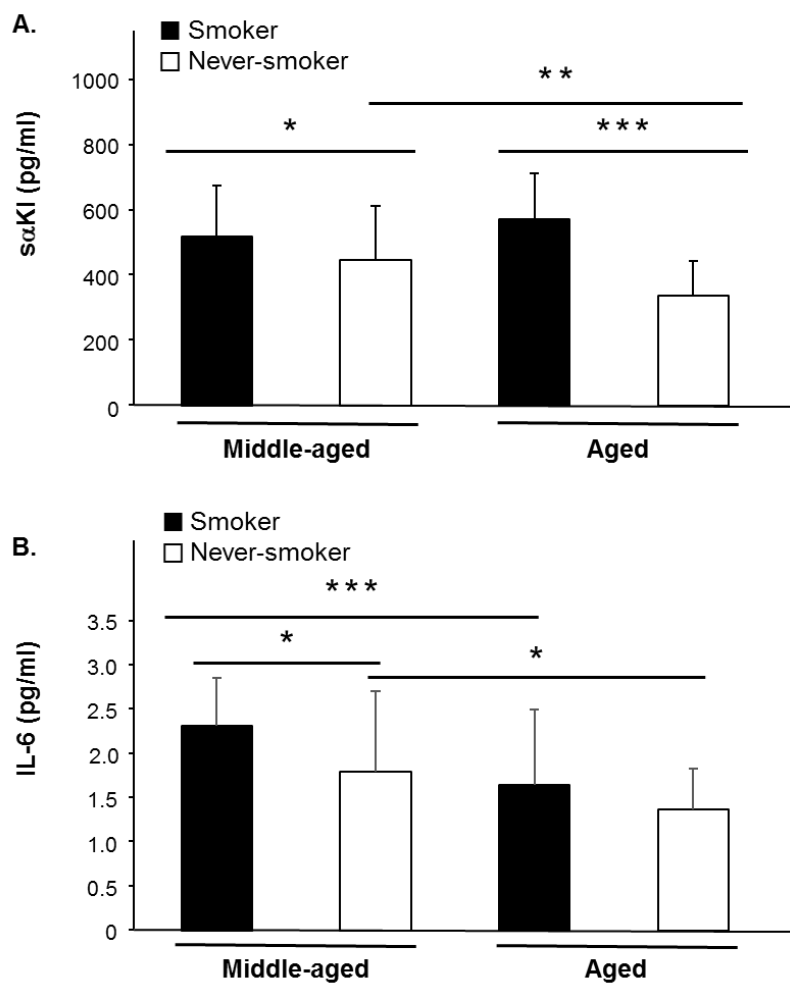
Supplementary Figure S1. Correlation between serum levels of fibroblast growth factor (FGF)-21 and interleukin (IL)-6 and adiponectin (APN)



Correlations between serum levels of fibroblast growth factor (FGF)-21 and (A) interleukin (IL)-6 and (B) adiponectin (APN) (n = 80).

Serum levels of FGF-21 were correlated only with APN ($r = -0.25$, $P = 0.013$) and no significant correlation was found between FGF-21 and IL-6.

Supplementary Figure S2. Comparing the cytokines among middle-aged and aged subjects



Comparing the serum levels of (A) soluble alpha Klotho (sαKI) and (B) interleukin (IL)-6 among middle-aged (46.1 ± 5.1 years, 40 smokers and 40 never-smokers) and aged (60.3 ± 1.7 years, 36 smokers and 24 never-smokers) subjects.

*P<0.05, **P<0.005, ***P<0.001.

In aged never-smokers, serum levels of α KI and IL-6 were significantly lower to those of middle-aged never-smokers ($P = 0.002$, $P = 0.035$). Moreover, serum levels of IL-6 were significantly lower in aged smokers than in middle-aged smokers ($P < 0.001$).

However, aged smokers showed strongly and significantly increased serum levels of α KI than aged never-smokers ($P < 0.001$).