Impact of cigarette smoking cessation on plasma α-klotho levels

- VN-SEESAW-Klotho Study -

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Short title: Cigarette smoking cessation and α-klotho

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Abstract

Smoking cessation reduces the risk of cardiovascular disease and improves clinical outcomes. We studied the effect of smoking cessation on plasma levels of α-klotho, which is an anti-aging protein. We treated 28 smokers (male:female=23:5, 46±12y) with varenicline (n=14) or a transdermal nicotine patch (n=14) as part of a 12-week smoking cessation program (the VN-SEESAW Study). Pulse rate, blood pressure, plasma levels of α-klotho, fibroblast growth factor (FGF)-19 and FGF-21, hemoglobin (Hb) and expiratory carbon monoxide (CO) concentration were measured before and after the anti-smoking intervention. Smoking cessation significantly decreased pulse rate, α-klotho, Hb and CO concentration, but not FGF-19 or FGF-21 in all subjects. On the other hand, body mass index significantly increased after the intervention. Changes in α-klotho levels (values at week 12 minus the values at week 0) were negatively associated with α-klotho levels at week 0 and positively associated with changes in Hb levels. In addition, the successful smoking cessation group (n=21) showed significant reductions in pulse rate, systolic blood pressure, α-klotho, Hb and CO concentration. In conclusions, smoking cessation significantly decreased serum levels of the anti-aging molecule α-klotho. Our results are consistent with a previous report that an increase in α-klotho might be a compensatory response to smoking stress.

Abbreviations: AMI = acute myocardial infarction, VN-SEESAW study = a trial of varenicline vs nicotine patch in adult smokers: efficacy, safety and withdrawal symptoms, CO = end-expiratory carbon monoxide, BW = body weight, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic BP, PR = pulse rate, IRB = Independent Review Board, FGF = fibroblast growth factor, Hb = hemoglobin

Key words: smoking cessation; α-klotho; hemoglobin; expiratory CO.

1. Introduction

Cigarette smoking is a risk factor for various diseases and is one of the most critical coronary risk factors [1-3]. Smoking cessation reduces the risk of cardiovascular disease and improves clinical outcomes [4, 5]. The risk of acute myocardial infarction (AMI) has been reported to be substantially reduced one year after smoking cessation [6]. We previously reported that the spread of a non-smoking policy significantly decreased the in-hospital onset of AMI in our hospital [7]. Thus, smoking cessation is being actively promoted worldwide.

Klotho is a well-known anti-aging molecule [8-12]. Overexpression of klotho in transgenic mice significantly extended their life span compared to that of wild-type mice [9]. Higher klotho levels have been associated with a lower prevalence of cardiovascular disease [10]. Low serum klotho levels have been associated with increased carotid artery intima-media thickness and epicardial fat thickness and with decreased flow-mediated dilation of the brachial artery, indicating that a low serum klotho level is a predictor of atherosclerosis [11]. On the other hand, in another study, serum levels of α-klotho in never-smokers were low, and smokers showed highly increased serum levels of α-klotho [12].

The life expectancy of smokers is at least one decade less than that of subjects who have never smoked. Smoking cessation before the age of 40 years reduces the risk of death associated with continued smoking by about 90 % [13]. Smokers may exhibit low-grade inflammation and inflammation in blood vessels may be improved by smoking cessation [14, 15]. The serum levels of the inflammation-related cytokine interleukin-6 were significantly higher in smokers than in never-smokers [12]. In addition, serum levels of α-klotho were correlated with interleukin-6 in middle-aged

never-smokers, but not in smokers [12]. However, little is known about the association between smoking cessation and this anti-aging molecule. Thus, we believe that it is important to study this association in Japanese smokers. In this study, we hypothesized that smoking cessation could lead to a compensatory response in α-klotho as an anti-aging molecule, and therefore we evaluated α-klotho in participants before and after smoking cessation.

2. Methods

2.1. Study design

Tsukahara *et al*. previously reported a randomized controlled open comparative trial of varenicline vs nicotine patch in adult smokers: efficacy, safety and withdrawal symptoms (the VN-SEESAW study) [16]. Briefly, 32 Japanese adult smokers were enrolled for treatment at Fukuoka University Hospital. Participants either received varenicline for 12 weeks or wore a transdermal nicotine patch on the chest for 8 weeks. Successful smoking cessation was identified by both a self-assessment and the end-expiratory carbon monoxide (CO) concentration (<8 ppm). Body weight (BW), body mass index (BMI), systolic blood pressure (SBP), diastolic BP (DBP), and pulse rate (PR) were measured. The study protocol was approved by the Independent Review Board (IRB) of Fukuoka University Hospital [#7-05(08-27)], and all participants gave their written informed consent. Takata *et al*. performed an additional examination [VN-SEESAW-HDL Study; IRB (#14-5-13)] of plasma lipid profiles [low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride levels], high-density lipoprotein subfractions, and cholesterol efflux capacity [17]. In the current investigation (VN-SEESAW-Klotho Study), we measured α-klotho, fibroblast growth

factor (FGF)-19, FGF-21 and hemoglobin (Hb) in blood samples obtained at baseline (0 weeks) and at 12 weeks in both the varenicline and nicotine patch groups. The current study after modification was also approved by the IRB of Fukuoka University Hospital.

2.2. Measurement of plasma levels of α-klotho, FGF-19 and FGF-21

Plasma levels of α-klotho, FGF-19 and FGF-21 were measured using commercial sandwich enzyme immunoassay kits according to the respective manufacturer's instructions (Quantikine® ELISA Human FGF-19 and FGF-21 Immunoassay kits were purchased from R&D Systems, Minneapolis, MN, and a Human soluble α-Klotho Assay Kit was purchased from Immuno-Biological Laboratories Co, Ltd., Gumma, Japan)

2.3. Statistical analysis

Data are presented as the mean ± standard deviation. Changes were calculated as the values after 12 weeks minus the values at week 0. Differences between values at baseline and after 12 weeks were evaluated with the paired t-test. The associations between two parameters were assessed by Spearman's rank correlation coefficient. Differences between the successful and unsuccessful smoking cessation groups or the varenicline and nicotine patch groups were evaluated with the unpaired t-test. A P value of less than 0.05 was considered statistically significant. Analyses were performed using the Stat View statistical software package (Stat View 5; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1.Patient characteristics and various parameters at weeks 0 and 12 in all participants

Twenty-eight participants (14 for varenicline, 14 for a nicotine patch) were analyzed (Table 1, Figure 1). In all participants, PR, plasma levels of α-klotho (Figure 1A), Hb and CO concentration were significantly decreased after 12 weeks, whereas there were no changes in FGF-19 or FGF-21 (Figure 1BC). On the other hand, there were significant increases in BW and BMI after 12 weeks.

3.2. Patient characteristics and various parameters at weeks 0 and 12 in the successful and unsuccessful smoking cessation groups

Seven of the 28 participants did not stop smoking (Table 2). The proportions of participants in the unsuccessful smoking cessation group were not significantly different between those who received varenicline (4 participants) and a nicotine patch (3 participants). There were no statistically significant differences in BW, BMI, α-klotho, FGF-19, FGF-21, CO concentration or Hb between the successful and unsuccessful groups at week 0. After 12 weeks, both groups showed significant reductions in α-klotho (Figure 2A), Hb and CO concentration. PR and SBP in the successful group, but not the unsuccessful group, significantly decreased between baseline and 12 weeks, whereas there were significant increases in BW and BMI after 12 weeks in the successful group.

3.3. Patient characteristics and various parameters at weeks 0 and 12 in the varenicline and nicotine patch groups

We also analyzed the differences in characteristics of the participants and various

parameters between the varenicline and nicotine patch groups (Table 3). There were no statistically significant differences in BW, BMI, α-klotho, FGF-19, FGF-21, CO concentration or Hb between the at week 0. After 12 weeks, both groups showed significant reductions in α-klotho (Figure 2B), CO concentration and Hb. BW and BMI in both groups significantly increased after 12 weeks.

3.4. Associations between α-klotho and Hb or CO concentration

Figure 3 shows the associations between α-klotho and Hb or CO concentration. The change in the plasma level of α-klotho from week 0 to week 12 (Δ= the value at week 12 minus the value at week 0) was negatively associated with α-klotho at week 0 and positively associated with ΔHb (Figure 3AB). On the other hand, there were no associations between Δα-klotho and ΔCO or ΔHb (Figure 3CD).

4. Discussion

In this study, smoking cessation significantly decreased serum levels of the anti-aging molecule α-klotho, which suggests that an increase in α-klotho may be a compensatory response to smoking stress.

We hypothesized that smoking cessation could lead to a compensatory response in α-klotho as an anti-aging molecule. We found that smoking cessation significantly decreased plasma levels of α-klotho. Higher klotho levels have been associated with a reduced risk of cardiovascular disease, and lower levels were associated with the progression of atherosclerosis [10, 11]. Since all of the participants in this study were smokers, we did not analyze α-klotho levels before they began smoking. Nakanishi *et al*. reported that α-klotho levels in never-smokers were low, while those in smokers

were very high [12]. In addition, serum α-klotho was positively correlated with the pro-inflammatory molecule interleukin-6 [12], and has been reported to act as an anti-inflammatory molecule [18]. The positive correlation suggested that the increase in serum levels of α-klotho might be a compensatory response to smoking stress. In this study, Δα-klotho was negatively associated with α-klotho at week 0. Thus, the α-klotho levels in our participants were upregulated by smoking, and smoking cessation may produce a compensatory decrease in these levels. Our results were in agreement with those in a previous report [12].

The levels of FGF-19 and FGF-21 were not significantly decreased after 12 weeks in this study. β-klotho, which is a homolog of α-klotho, interacts with FGF-19, which is a known regulator of bile acid homeostasis [19], and with FGF-21, which regulates the metabolism [20]. Although smoking cessation should reduce FGF-21 levels, since smoking increased serum levels of FGF-21 [12], these levels did not change and BMI significantly increased after smoking cessation in this study. Serum levels of FGF-21 have been shown to increase in subjects with metabolic syndrome and atherosclerosis [21], and this could explain why FGF-21 levels did not change after smoking cessation. Since no prior studies have examined the influence of smoking cessation on changes in FGF-19 and FGF-21, further studies are needed to clarify these changes.

Another important result in this study was that PR, Hb and CO concentration significantly decreased after 12 weeks in all patients. The successful smoking cessation group showed significant reductions in PR, SBP, Hb and CO concentration, and all of these reductions in this group were reasonable. A meta-analysis supported an association between heavy smoking and a high resting heart rate [22]. Moreover, heavy

smoking is associated with a persistent rise in BP and with an increase in BP variability [23]. Since smokers had significantly higher levels of Hb [24], the level of Hb may significantly decrease after smoking cessation, with a reduction of the CO concentration. Δα-klotho was positively associated with ΔHb. Decreased α-klotho levels are associated with decreased Hb in patients with chronic kidney disease [25]. Under hypoxic conditions, erythropoietin stimulates the differentiation of erythroid progenitor cells and normoblasts to increase the amount of red blood cells [26]. Erythropoietin promotes the expression of α-klotho and an increase in α-klotho suppresses the production of erythropoietin [26, 27]. After smoking cessation, hypoxic conditions improve and erythropoietin may not be needed to stimulate differentiation to increase red blood cells, and consequently erythropoietin may not promote the expression of α-klotho.

This study has several limitations. For example, the sample size was small, which limits our ability to generalize the results. Furthermore, this study assessed α-klotho levels only over the short term. The long-term effect of smoking cessation on α-klotho will need to be elucidated in the future.

In conclusion, our results agree with those of a previous report that an increase in α-klotho might be a compensatory response to smoking stress.

Conflict of interest

KS and SM are Directors of NPO Clinical and Applied Science, Fukuoka, Japan. KS and SM received a grant from the Public Interest Incorporated Foundation of "Clinical Research Promotion Foundation" in Fukuoka, Japan, and part of this work was transferred to NPO Clinical and Applied Science, Fukuoka, Japan. KS has an Endowed

Department of Molecular Cardiovascular Therapeutics (SM), Fukuoka University, supported by MSD Co., Ltd.

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Figure legends

Figure 1.

Plasma levels of α-klotho (A), fibroblast growth factor (FGF)-19 (B) and FGF-21 (C) at weeks 0 and 12 in all patients.

Figure 2.

Plasma levels of α-klotho in the successful and unsuccessful smoking cessation groups (A) and in the varenicline and nicotine patch groups (B).

Figure 3.

Associations between Δα-klotho and the α-klotho level at week 0 (A), Δα-klotho and ΔHb (B), Δα-klotho and ΔCO concentration (C), and ΔHb and ΔCO concentration (D). Δ indicates the value at week 12 minus the value at week 0.

Table 1. Patient characteristics and various parameters at weeks 0 and 12 in all participants.

	Week 0	Week 12	p value
BW, kg	66.7±11.2	68.7±11.5	<0.001
BMI, kg/m^2	23.7±3.4	24.4±3.5	<0.001
PR, bpm	78±9	72±10	0.007
SBP, mmHg	129±18	125±15	0.164
DBP, mmHg	78±10	77±11	0.625
α-klotho, pg/ml	520±92	465±85	<0.001
FGF-19, pg/ml	248±134	266±260	0.735
FGF-21, pg/ml	240±260	198±150	0.245
CO, ppm	26.6±11.2	7.2±8.2	<0.001
Cr, mg/dl	0.8±0.1	0.8±0.2	0.259
Hb, g/dl	15.0±1.2	14.5±1.1	<0.001

Data are presented as the mean ± standard deviation. BW, body weight; BMI, body mass index; PR, pulse rate; SBP, systolic blood pressure; DBP, diastolic BP; FGF, fibroblast growth factors; CO, carbon monoxide; Cr, creatinine; Hb, hemoglobin.

Table 2. Patient characteristics and various parameters at weeks 0 and 12 in the successful and unsuccessful smoking cessation groups.

	Successful group (n=21)			Unsuccessful group (n=7)		
	Week 0	Week 12	p value	Week 0	Week 12	p value
BW, kg	67.1±12.7	69.1±12.9	<0.0001	65.8±4.9	67.5±5.9	0.087
BMI, kg/m^2	23.7±3.8	24.5±3.8	<0.0001	23.4±2.0	24.1±2.7	0.079
PR, bpm	77±9	70±11	0.016	79±10	76±7	0.230
SBP, mmHg	130±19	124±15	0.039	125±14	130±16	0.327
DBP, mmHg	78±11	70±11	0.473	79±5	80±11	0.782
α-klotho, pg/ml	538±97	487±84	<0.0001	467±43	399±50	0.010
FGF-19, pg/ml	237±128	254±280	0.807	277±155	297±225	0.791
FGF-21, pg/ml	230±277	181±131	0.359	260±232	232±195	0.398
CO, ppm	22.3±9.6	4.1±1.8	<0.0001	37.9±7.9	16.6±12.4	0.002
Cr, mg/dl	0.8±0.1	0.8±0.2	0.452	0.8±0.2	0.9±0.2	0.356
Hb, g/dl	15.0±1.2	14.5±1.1	0.001	15.3±1.0	14.6±1.4	0.018

Data are presented as the mean ± standard deviation. BW, body weight; BMI, body mass index; PR, pulse rate; SBP, systolic blood pressure; DBP, diastolic BP; FGF, fibroblast growth factors; CO, carbon monoxide; Cr, creatinine; Hb, hemoglobin.

Table 3. Patient characteristics and various parameters at weeks 0 and 12 in the varenicline and nicotine patch groups.

	Varenicline group (n=14)			Nicotine patch group (n=14)		
	Week 0	Week 12	p value	Week 0	Week 12	p value
BW, kg	67.3±9.0	69.2±9.6	0.005	66.2±13.3	68.3±13.4	0.002
BMI, kg/m^2	23.7±3.3	24.4±3.4	0.004	23.6±3.6	24.4±3.7	0.002
PR, bpm	79±11	73±9	0.610	77±8	71±12	0.070
SBP, mmHg	127±16	126±16	0.863	131±20	124±16	0.105
DBP, mmHg	77±11	76±11	0.440	78±10	78±11	0.966
α-klotho, pg/ml	488±87	440±83	0.004	553±87	489±84	<0.0001
FGF-19, pg/ml	285±145	239±155	0.419	215±120	291±335	0.403
FGF-21, pg/ml	131±73	172±110	0.044	349±328	224±185	0.057
CO, ppm	27.9±9.8	5.4±4.1	<0.0001	25.3±12.7	9.1±10.7	<0.0001
Cr, mg/dl	0.8±0.1	0.8±0.2	0.583	0.8±0.1	0.8±0.1	0.272
Hb, g/dl	14.9±1.1	14.3±1.1	0.001	15.2±1.2	14.7±1.2	0.018

Data are presented as the mean ± standard deviation. BW, body weight; BMI, body mass index; PR, pulse rate; SBP, systolic blood pressure; DBP, diastolic BP; FGF, fibroblast growth factors; CO, carbon monoxide; Cr, creatinine; Hb, hemoglobin.

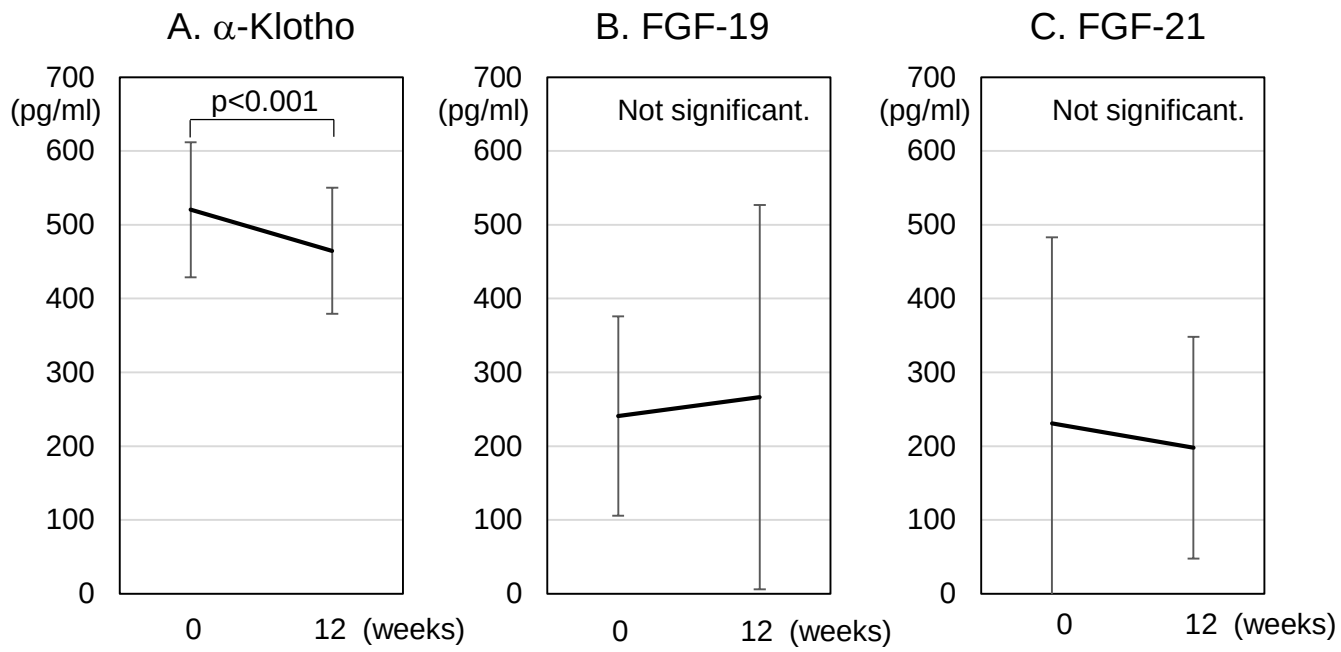

Figure 1.
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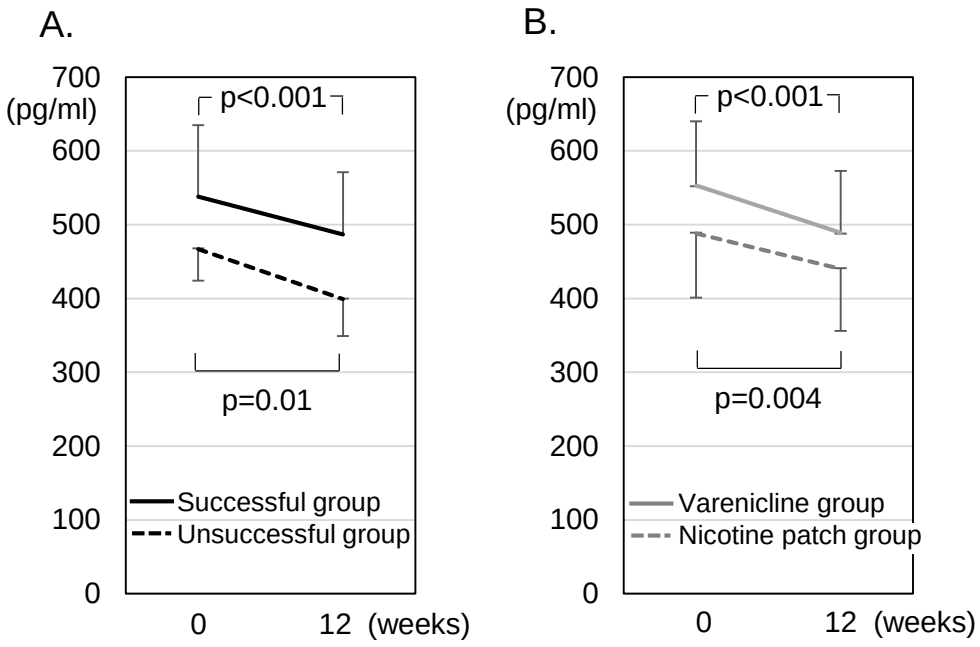

Figure 2.
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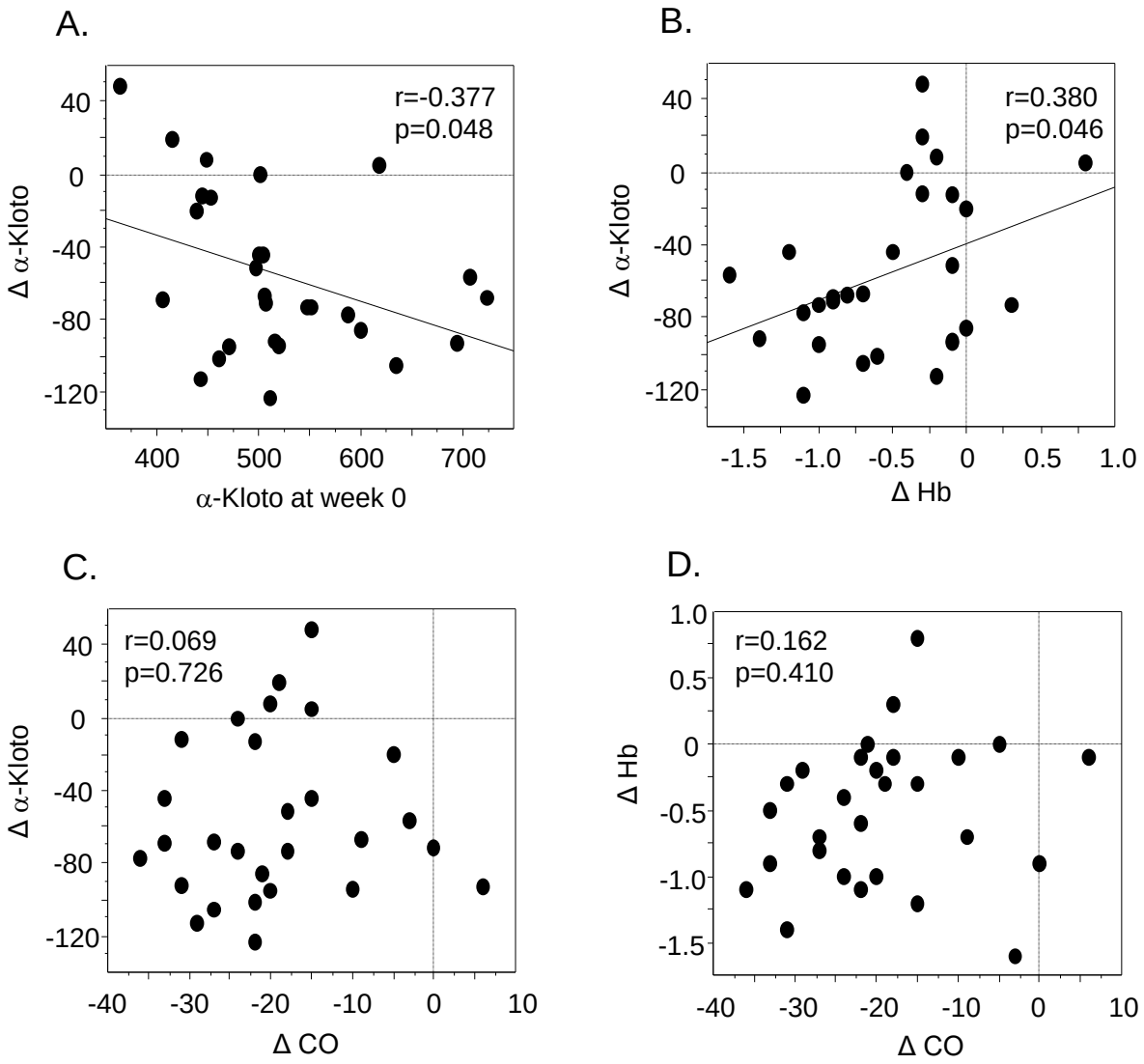

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