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Title of research project	The effects of TFAs on cardiovascular disease in Japan NOW.
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【Abstract】

Objectives; It is well-established that Trans-fatty acids (TFAs) are the risk factor of cardiovascular disease. Therefore, many countries strictly limit the intake of TFAs, and force food companies to describe the collect amount of TFAs included. In Japan, Indication of TFAs levels are not required because of less opportunity of lipid intake than Western countries. However Western-style food has become widespread around Japan, many people, especially young generation; eat hamburger, fried potato, beef, and so on. There are big issues that increasing number of younger people who suffer from acute coronary syndrome. Taken together, we have to evaluate the serum TFAs levels in Japanese now and its effects on the onset of cardiovascular disease. In addition, the mechanism how TFAs accelerate cardiovascular disease is not fully understood yet; therefore we approached to figure out these mechanisms with basic researches.

Method; Patients with and without coronary artery disease (CAD) in Kobe University Hospital were eligible (n=907) with written informed consent. Biochemical analyses were done on fasting serum samples. TFAs (i.e. elaidic acid or linoelaidic acid) were measured using gas chromatography/mass spectrometry. In mice model, atherosclerosis-prone LDL-receptor knockout (LDLR-KO) mice and wild-type C57BL/6 mice were used. The progression of atherosclerosis was evaluated in cholesterol and TFAs fed LDLR-KO mice and the acceleration of thrombus formation was evaluated in TFAs fed wild-type mice.

Results; Serum TFAs concentrations were positively correlated with body mass index and waist circumference, and inversely correlated with age. In lipid profiles, TFAs showed a positive correlation with LDL-C, triglycerides, apolipoprotein B and remnant like particles cholesterol, and showed a negative correlation with HDL-C and apolipoprotein AI. TFAs in CAD patients (n=439) was significantly lower than that in non-CAD patients (n=463) ($p<0.05$), because many CAD patients received intense statin therapy with and showed low LDL-C levels. Thus, we evaluated the TFAs levels standardized by phospholipids (PL) levels which represent total lipoprotein levels. TFAs/PL in CAD patients (n=439) was significantly higher than that in non-CAD patients (n=463) ($p<0.05$) and this tendency was particularly stronger in young (age≤58 years old) patients ($p<0.005$). After dividing participants into 4 groups by age, in youngest group (n=173) TFAs themselves in CAD patients was significantly higher than that in non-CAD patients ($p<0.05$). In addition, TFA in young patients displaying metabolic syndrome was higher than that in patients without metabolic syndrome ($p<0.05$). Complementary animal studies were performed using LDL receptor knockout mice fed with 5% elaidic acids or 5% oleate (control). Feeding the mice with elaidic acids resulted in a marked increase in atherosclerotic lesion sizes compared with the oleate treatment. In addition, plasma or tissue levels of inflammatory cytokines

were increased in response to dietary intake of elaidic acids. Next, Male C57BL6 mouse were treated with TFA (2% elaidic acid), cis-fatty acid (2% oleic acid) or normal diet for 4 weeks and thrombus formations in the carotid artery induced by He-Ne laser irradiation was evaluated. The gene expressions of thrombomodulin (TM), tissue factor pathway inhibitor (TFPI) and plasminogen activator inhibitor (PAI-1) were evaluated by real-time PCR.The treatment of mice with high TFA diet significantly promoted thrombus formations compared to normal diet ($p<0.001$) and cis-fatty acid treated mice ($p<0.05$). The plasma levels of total cholesterol, prothrombin time and activated partial thromboplastin time were not different among 3groups The level of D-dimer after thrombus formation was significantly higher in TFA-treated mice than normal diet mice ($p<0.01$). The aortic expressions of anti-thrombogenic genes such as TM and TFPI were decreased, and that of thrombogenic genes including PAI-1 were increased in TFA-treated mice compared to other two groups ($p<0.05$).

Conclusion; The serum TFA concentration is a risk factor of CAD in Japan, and would cause a serious health problem particularly in the young generation. We also discovered that TFAs accelerate the progression of atherosclerosis and the acceleration of thrombus formation which causes acute coronary syndrome. TFAs have unrecognized adverse health effects in atherosclerosis. In Japan, we consider it desirable to set guideline for the intake TFAs with recognizing TFAs as the risk factor of cardiovascular disease.