

Novel animal model for ventricular ejection fraction measured by MRI

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Highlights:

- First to demonstrate ventricular ejection fraction (VEF) per cine magnetic resonance imaging (MRI) in wild non-surgical or non-genetic mouse, the Tunac Arterial Plaque (TAP) model
- TAP model involved feeding the mouse with high fat diet and treatment with the ubiquitous environmental pollutant, PCB, which in combo created inflammation and produced arterial plaques.
- Disruption of arterial endothelial glycocalyx triggered plaque formation, which weakened the integrity of cardiac cells (myocytes) and resulted in a reduced % VEF.
- Plaque formation in aortal arch was confirmed by MRI, which involved multiple contrast weightings (e.g., T1, T2, and proton density).

INTRODUCTION

Ejection fraction and heart failure

An ejection fraction (EF) is the volume of blood ejected from the left ventricle with each heartbeat, which is used as a measure of the pumping efficiency of the heart. EF is calculated by dividing the volume of blood pumped by the volume collected at the end of diastolic filling. A reduction in EF is a sign of heart failure (HF), which can be subdivided into 3 categories:

1. normal or preserved ejection fraction (HFpEF): $\geq 50\%$ – referred to as diastolic heart failure where the heart muscle contracts normally but the ventricles do not relax during ventricular filling.
2. moderately reduced: 40–49%] (HFmrEF)
3. reduced ejection fraction (HFrEF): $< 40\%$ – referred to as systolic heart failure where the heart muscle does not contract effectively resulting in less oxygen-rich blood pumped out to the body

HF (aka, CHF, or chronic heart failure) is a global pandemic affecting at least 26 million people worldwide and is increasing in prevalence (2014. *ESC Heart Failure* 1:4–25) affecting approximately 1 - 2% of adult population. Of the estimated 5 million patients in the US diagnosed with HF, approximately 50% are preserved ejection fraction (HFpEF) and its prevalence is increasing by about 1% annually (2019. *Circ Res.* 124:1598-1617) relative to that of reduced ejection fraction (HFrEF) incidence (2016. *Circulation* 134:73-90). HF accounts for more than 80 000 deaths annually in the US and an estimated \$69.8 billion in annual health care spending by 2030 (2020. *Circulation* 141: e139-e596).

HF is a multifactorial, systemic disease triggered by damaged heart muscle (myocardium), followed by the activation of structural, neurohumoral, cellular, and molecular mechanisms to maintain physiological functioning (2011. *Eur Heart J.* 32:670–679). Currently, there are no

effective treatments for HfpEF, which is due to the lack of pre-clinical models to allow extensive imaging and therapeutic evaluation (2017. *Circ Res* 120: 1243–1245)

In this regard, there is a need for a murine model of HF to evaluate novel therapies, particularly the left ventricular ejection fraction (LVEF), which is a strong independent predictor of cardiovascular morbidity and death (1999. *Circulation*. 1035–1042). Currently, a typical animal model involves a genetically modified mouse lacking osteopontin, treated with aldosterone and 1% sodium chloride (NaCl) to induce blood pressure elevation, cardiac hypertrophy, and fibrosis (2004. *Am J Hypertens* 17:188–93). Another option is a mouse with coronary arteries surgically narrowed to mimic ischemic cardiomyopathy (ICM) as a means of triggering myocardial infarction (MI). For example, a C57BL/6 wildtype (WT) mouse surgical occluded at the left anterior descending artery (LAD) creating a MI then HF evaluated by MRI after 4 weeks (2006. *FASEB J*. 20:956–958).

The disruption of the endothelial glycocalyx is the upstream component of HF, which triggers coronary plaques and contributes to myocyte stiffness and reduced EF volume particularly in the left ventricle (2018. *Eur Heart J* 39: 3439–3450). Meanwhile, MI is the most visible symptom, which develops HF and a sequelae of downstream diseases including a cardio-renal syndrome, which is the endpoint of HF (Fig. 1).

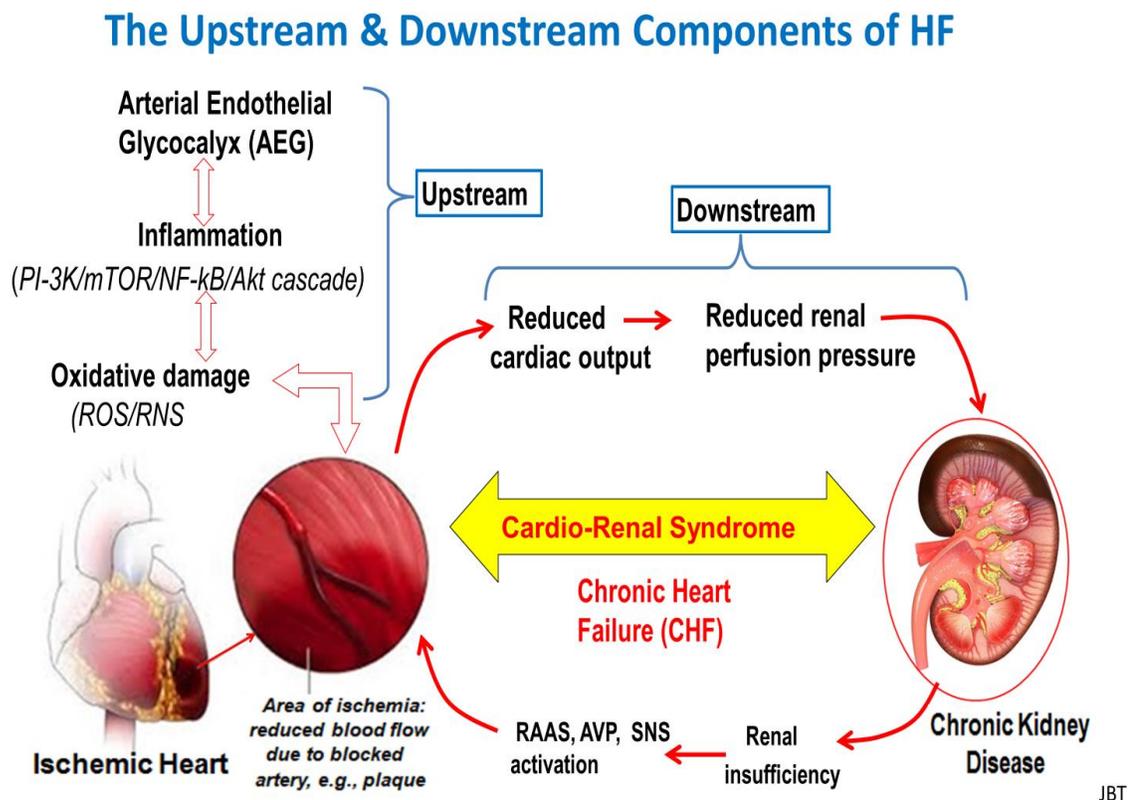


Figure 1. Disruption of the arterial endothelial glycocalyx triggers a cascade of diseases including the cardio-renal syndrome and chronic heart failure (CHF)

Cardiac magnetic resonance Imaging (MRI) for EF

EF can be determined using several invasive and non-invasive imaging modalities, either subjectively by visual estimation or objectively by quantitative methods [i.e., echocardiography, magnetic resonance imaging (MRI), computed tomography (CT), gated equilibrium radionuclide angiography (commonly referred to as multiple-gated acquisition [MUGA] scan) and gated myocardial perfusion imaging with either single-photon emission computed tomography (SPECT) or positron emission tomography (PET)]. In the clinical setting, cardiac MRI and blood pool SPECT are considered as gold standards for the assessment of LVEF (2011. *Nucl Med Commun.* 32:121–128), but have not found wide use for monitoring cardiac therapies in mice.

One of the early high-resolution MRI sequences for assessment of myocardial function and morphology in mice describes a cine performed with prospective ECG gating using a spoiled gradient echo technique and quantitative analysis using a semi-automated *approach* (2007. *J Nucl Med.* 48:288–294). Indeed, MRI has been used for calculation of EF function in both healthy and MI-induced mice (2006. *J Nuc Med.* 47:974–980), in mice with permanent ischemic cardiomyopathy (ICM) or transient LAD ligation (2009. *J Nuc Med.* 50:132–138), and in different mouse models of cardiovascular diseases (2012. *EJNMMI Res.* 2: 43-52)

OBJECTIVE

The objective of this study is to evaluate the Tunac Arterial Plaque (TAP) mouse (2017. *J Clin Exp Cardiol Suppl* 8:1) as a model for reduced EF. Traditional mouse models involve genetically modified or surgically altered animals, whereas the TAP model is a wild mouse (C57Bl/6 strain) fed with a high fat diet and treated with an environmental chemical pollutant (PCB) to mimic human lifestyle. Thus, the EF volume of treated and untreated mice will be measured per MRI, as well as, an assessment for the presence of arterial plaque.

MATERIALS AND METHODS

Mice

Ten ten-week-old male C57/Bl6 mice were obtained from Jackson Laboratories. Either normal or high fat (60% fat) diet (D12451, DIO series diet, Opensource Diets) were given per experiment group below upon receipt. Animals were acclimated for 1 week and then PCB was administered by gavage according to the following schedule.

- Group 1 (mice 1,2) – regular chow diet (Control group)
- Group 2 (mice 3-8) – high fat diet + PCB (2X per week for 2 weeks)
- Group 5 (mice 9,10) – high fat diet only

Treatments

3,3',4,4'-Tetrachlorobiphenyl (PCB-77) was obtained from Neosyn Laboratories. The dry chemical was suspended in 15.22 ml of corn oil to deliver 200 $\mu\text{mol/kg}$ in 0.2 ml by gavage per mouse according to the treatment schedule. All experiments were approved by the Henry Ford

Health System Institutional Animal Care and Use Committee. All reagents were acquired from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Magnetic resonance imaging (MRI)

All studies were performed using a 7-Tesla Varian magnet (Fig. 2) interfaced to a Bruker console running Paravision 6.0. Briefly, the heart was aligned to the proper orientation, then an intragate scan was carried out for a CINE presentation (motion sensitive MRI in which a series of static images are obtained at various stages of the cardiac cycle and then played back as a movie). We used a blackblood method that causes the blood to appear darker than the adjacent tissue). The CINE sequence required approximately 10 minutes and acquired hundreds of images that were then sorted and reordered by the software. Afterwards, another program (Medviso Segment), was used to auto detect end diastole/end systole and calculate percent ejection fraction (EF), heart rate (HR) and respiratory rate (RR).



Figure 2. A 7-Tesla Varian MRI system at the Henry Ford Health System laboratories

All animals received a baseline MRI scan including a 3-plane localizer scan that was used to position the animal within the magnet. This sequence was then followed by an intragate scan procedure (Bruker Corporation) which produced a series of 3 image sets that were used to align the heart to the proper orientation for a CINE scan. A cine MRI is a special type of MRI data set in which a series of static heart images are obtained at different times (i.e. different stages of the cardiac cycle) and then played back as a movie. Cine MRI is helpful in evaluating tissues that move such as the heart, flowing blood, or cerebral spinal fluid. It is also occasionally used in evaluating bowel, joint or muscle motion. The resulting images were then used to estimate the

ejection fraction. The CINE scan technique used is referred to as a blackblood method which causes the blood to appear darker than the adjacent tissue. The complete sequence required approximately 10 minutes and it acquires hundreds of images that are then sorted and reordered by the software. The resulting data set can subsequently be run as a movie, which was the basis for the estimation of the ejection fraction (EF), heart rate (HR) and respiratory rate (RR).

The MRI protocol used for the EF imaging was as follows.

- 1) The first step was to run a localizer sequence to position the heart at the center of magnet.
- 2) Step 2 was used to acquire a stack of axial images to localize the blood vessels at the top of heart (9 slices). These images were used to select different levels within either the heart or the aortic vessels for either ejection fraction estimates or aorta cross sectional images, respectively.
- 3) Step 3 generated a set of coronal images that were used to set up for ejection fraction imaging using the images from Step 2 to line up the next image that shows the 4 vessels at top of heart (produces an image along the long axis of the heart).
- 4) Step 4 was used to produce a 4-chamber heart image.
- 5) Lastly, Step 5 runs a CINE_blackblood_IG FLASH sequence. This sequence was used to set up the CINE procedure. It produce a set of images showing the heart at various stages of the cardiac cycle that can be run as movie. The sequence provided the image data set that was used to estimate the cardiac ejection fraction.

For the Aorta & carotid artery imaging, the following was used:

- 1) technique that involves multiple contrast weightings (e.g., T1, T2, and proton density). Localization of the correct plane angulation for the multislice experiments was planned from coronal and sagittal scout images.
- 2) The next step was to set up for imaging of the aorta, using some of the images from Step 1 to line up the slice with the aorta (produces an image showing the aortic arch, carotid and brachial arteries). The sequence was run on 3 slices, 0.5 mm thick.
- 3) Finally, we set up for cross sectional imaging of aorta (FOV 20 mm, 3-5 slices, 0.5 mm thick). The sequence was run 3 times with slices set to different angles.

Additional contrast enhanced scans were performed in some animals after the CINE and aorta imaging. Once the data were collected, they were transferred offline for processing. To estimate the ejection fraction, we used a program called Medviso Segment. After opening the data set and displaying the images in Segment, we used a command to auto detect end diastole and end systole. This provided the EF data as a %. All animal groups were studied at multiple time points after treatment except for the two that received the HFD only.

RESULTS:

a) General mouse health:

No mice died during the study, and no adverse effects were noted due to administration of PCB. No notable gross changes in weight, respiratory or heart rates attributable to the diet and PCB (Table1)

Table 1. Data on the experimental mice including weight, respiration, heart rate and % ejection fraction (EF%).

FileNam	PatienM	PatienC	Acquisi	ExperiM	Height[c	Weight[k	Sex	RespRa	HeartRa	R-R[ms]	LVM[ml]	LVM[g]	EDV[ml]	ESV[ml]	SV[ml]	EF[%]	CO[l/mir	PFR[ml]	PER[ml]
Bad data	JT01		2E-07		unable to estimate EF														
	JT01_01	JT01_01C	2E-07	#6		0.027	O	82	422	133.33	0.0146	0.0153	0.0091	0.0029	0.0062	68.451	0.0028	0.1977	0.1993
	JT01A_C	JT01A-C	2E-07			0.029	O	80	429	133.33	0.0166	0.0174	0.012	0.0027	0.0093	77.591	0.0042	0.1978	0.3157
	JT02_1I	JT02	2E-07	#7		0.025	M	91	493	133.33	0.0139	0.0146	0.0134	0.0047	0.0087	64.738	0.0039	0.3301	0.22
	JT02A_I	JT02A-C	2E-07	#7		0.036	O	67	486	133.33	0.0194	0.0204	0.0132	0.0036	0.0096	72.583	0.0043	0.3418	0.2533
noisy	JT03_1I	JT03_HI	2E-07	#10		0.036	M	97	522	133.33	0.0143	0.015	0.0131	0.0047	0.0084	64.155	0.0038	0.3536	0.2789
	JT03-HF	JT03-HF	2E-07	#6		0.051	O	80	464	133.33	0.0187	0.0196	0.0141	0.0056	0.0085	60.338	0.0038	0.1022	0.2005
noisy	JT03-HF	JT03-HF	2E-07	#7		0.053	O	72	431	133.33	0.018	0.0189	0.015	0.0053	0.0097	64.538	0.0044	0.1844	0.4118
	JT03_HI	JT03_HI	2E-07	#5		0.053	O	62	394	133.33	0.018	0.0189	0.0153	0.0054	0.0099	64.74	0.0045	0.3449	0.1757
	JT04_1I	JT04_HI	2E-07	#8		0.03	M	95	532	133.33	0.0153	0.0161	0.0129	0.0038	0.0091	70.577	0.0041	0.2365	0.2095
	JT04-HF	JT04-HF	2E-07	#6		0.046	O	69	385	133.33	0.016	0.0168	0.0141	0.0056	0.0085	60.405	0.0038	0.2814	0.3111
	JT04-HF	JT04-HF	2E-07	#7		0.047	O	83	474	133.33	0.016	0.0168	0.0135	0.0057	0.0079	58.144	0.0035	0.1577	0.2163
	JT04-HF	JT04-HF	2E-07	#6		0.048	O	82	417	133.33	0.0168	0.0177	0.0135	0.0054	0.0081	59.948	0.0037	0.3111	0.19
	JT04-HF	JT04-HF	2E-07			0.046	O	71	430	133.33	0.0179	0.0188	0.0129	0.0054	0.0075	58.345	0.0034	0.2154	0.2054
	JT05_HI	JT05	2E-07			0.037	M	97	436	133.33	0.0157	0.0165	0.013	0.0043	0.0087	67.045	0.0039	0.2519	0.3043
	JT05_01	JT05_HI	2E-07	#7		0.047	O	96	445	133.33	0.0162	0.017	0.0168	0.0055	0.0112	67.085	0.0051	0.4941	0.3904
	JT05_01	JT05_HI	2E-07	#7		0.046	O	81	433	133.33	0.0173	0.0181	0.0116	0.0049	0.0067	57.991	0.003	0.2198	0.2172
	JT05-HF	JT05-HF	2E-07	#6		0.042	O	88	486	133.33	0.0162	0.017	0.013	0.0053	0.0077	59.1	0.0035	0.1277	0.31
	JT05-HF	JT05-HF	2E-07	#6		0.04	O	67	459	133.33	0.0323	0.0339	0.0275	0.0122	0.0153	55.648	0.0069	0.3152	0.455
	JT05_HI	JT05_HI	2E-07	#6		0.041	O	83	515	133.33	0.0166	0.0175	0.0132	0.0044	0.0088	66.471	0.0039	0.2362	0.2876
	JT05_HI	JT05_HI	2E-07			0.041	O			133.33	0.0163	0.0171	0.0134	0.0051	0.0083	61.761	0.0037	0.2045	0.1675
noisy	JT06_HI	JT06	2E-07	#8		0.034	M	92	457	133.33	0.0154	0.0162	0.013	0.0047	0.0082	63.501	0.0037	0.3881	0.1635
noisy	JT06_01	JT06_HI	2E-07	#7		0.043	O	93	444	133.33	0.0147	0.0155	0.0141	0.006	0.0091	57.56	0.0037	0.3454	0.1799
	JT06_01	JT06_HI	2E-07	#6		0.04	O	85	499	133.33	0.0154	0.0162	0.0118	0.0052	0.0066	56.221	0.003	0.2207	0.1563
	JT06-HF	JT06-HF	2E-07	#8		0.038	O	69	539	133.33	0.0161	0.0169	0.0128	0.0055	0.0072	56.56	0.0032	0.28	0.1814
	JT06-HF	JT06-HF	2E-07	#7		0.039	O	64	453	133.33	0.0173	0.0182	0.0137	0.0065	0.0072	52.564	0.0032	0.2136	0.1613
	JT07-HF	JT07-HF	2E-07	#6		0.049	O	81	396	133.33	0.0175	0.0184	0.0158	0.0057	0.0101	64.021	0.0046	0.2547	0.2201
Bad data	JT07_HI	JT07_HI	2E-07	#10		0.055	O	52	436	133.33	0.041	0.0431	0.027	0.0078	0.0193	71.288	0.0087	0.473	0.6542
	JT08-HF	JT08-HF	2E-07	#7		0.043	O	80	431	133.33	0.0172	0.0181	0.0136	0.0038	0.0098	72.291	0.0044	0.3196	0.2165
little noisy	JT08_HI	JT08_HI	2E-07	#8		0.047	O	73	464	133.33	0.0171	0.018	0.0111	0.0043	0.0068	61.224	0.0031	0.3119	0.1655
	JT08_HI	JT08_HI	2E-07			0.047	O			133.33	0.0171	0.018	0.0111	0.0043	0.0068	61.224	0.0031	0.3119	0.1655
	JT09_01	JT09-HF	2E-07	#9		0.047	O	88	438	133.33	0.0163	0.0171	0.014	0.0053	0.0087	61.84	0.0039	0.1561	0.3281
noisy	JT10_01	JT10-HF	2E-07	#7		0.045	O	90	442	133.33	0.0167	0.0176	0.013	0.0034	0.0096	73.622	0.0043	0.336	0.284

b) Ejection fraction among treatments:

Mice fed with normal diet showed normal ejection fraction volume (75.1%). The high fat diet alone without PCB also effectively reduced EF% (67.7%), and the lowest reduction in EF were for mice fed with high fat and PCB (57.2%), Fig. 3.

% ejection fraction

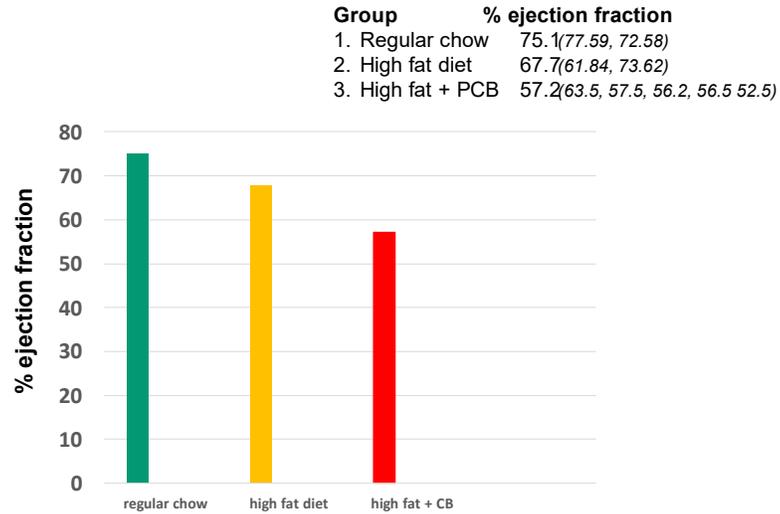


Figure 3. Ejection fraction profile in Tap mouse was most reduced when treated with high fat diet and PCB

c) Progression in reduction of ejection fraction (EF) volume;

Focusing on the high fat-PCB-treated group, there was a gradual reduction in % EF starting at 2 weeks with 65.2% EF and 52.5% at the 8-wk time point (Fig 4).

Time point

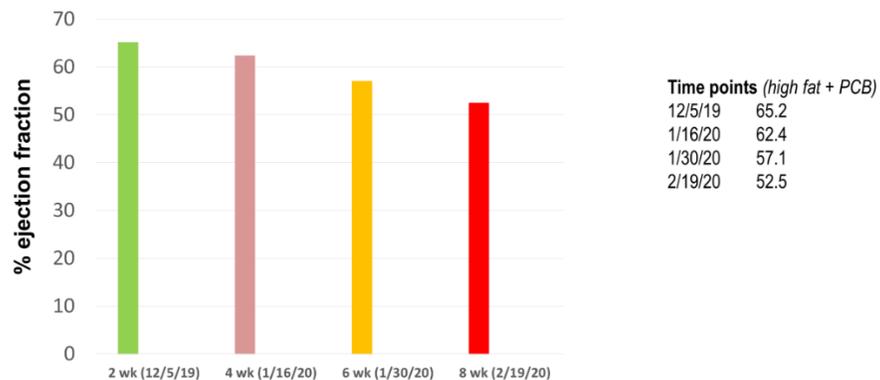


Figure 4. Progressive reduction in ejection volume from in the Tap mouse treated with high fat diet and PCB

d) MRI imaging of aortal arch.

Due to their small dimensions and nearly constant motion, MR imaging of coronary arteries for plaques is virtually impossible in mouse. The next option was an MRI scan of the aortal arch, which were found in mice fed with high fat diet and PCB treatment (Fig. 5).

Plaques in aortal arch identified by MRI

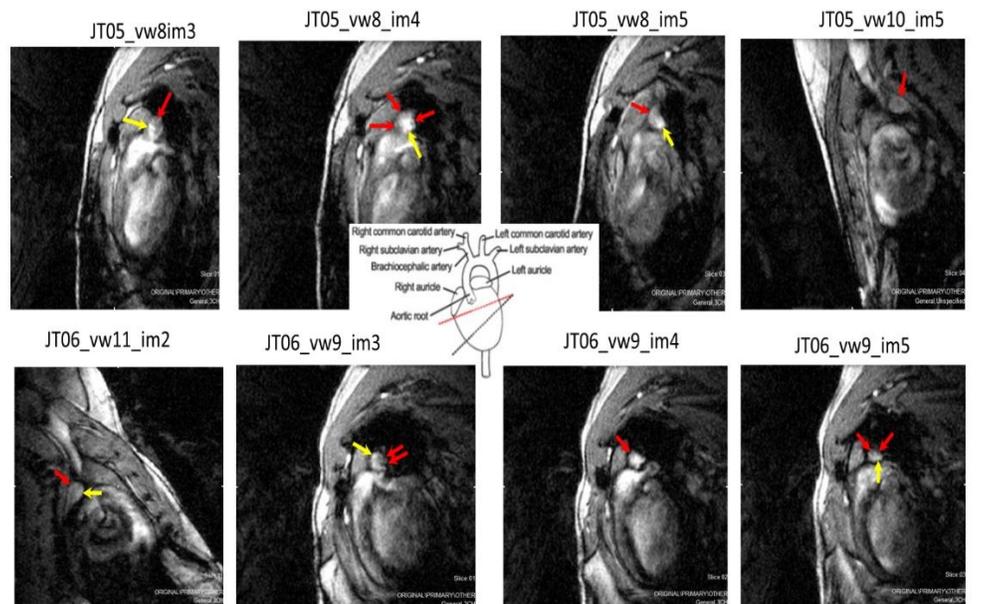


Figure 5. MRI scan of mice mouse (05) showing plaques in the aortal arch (red arrows)

DISCUSSION

Man-made chemical pollutants are part of the equation that triggers multifactorial diseases. The complex etiology of diseases is of multiple (plexic) origin, and disease manifestations are equally complex, herein called xenoplexic diseases including the family of CVD, inflammatory, neurological, immunological, hematological, endocrine disorders, cancer, macular dysfunction, etc. Pollutants are found in foods, beverages and packaging, clothes, cosmetics and cleaning products, furniture, paints, electronic equipment, plastics, and the list is long. Persistent pollutants including the lipophilic persistent organic pollutants (POPs) polychlorobiphenyls (PCBs) bioaccumulate in fatty tissues (2009. *Environ Health Perspect.*117:417–25). PCB was chosen for this study because they are the most ubiquitous environmental pollutant. PCBs are resistant to acids and bases as well as to heat, and have been used as an insulating material in electric equipment, such as transformers and capacitors, and also in heat transfer fluids and in lubricants, as plasticizers, surface coatings, inks, adhesives, flame-retardants, paints, and carbonless

duplicating paper. Since 1929 around 2 million ton of PCBs have been produced, about 10% of which remain in the environment today (2019. *Science Direct* 37: 399-407). Pollutants disrupt certain signaling and differentiation pathways and induce inflammation in the adipose tissue (2007. *Chemosphere* 67:1463-7). Pollutants and other blood debris trapped in a stagnant blood flow, due to high level of fat packaging lipoprotein (VLDL), triggers inflammation, disruption of endothelium, plaque formation and cardiovascular diseases (Fig 6)

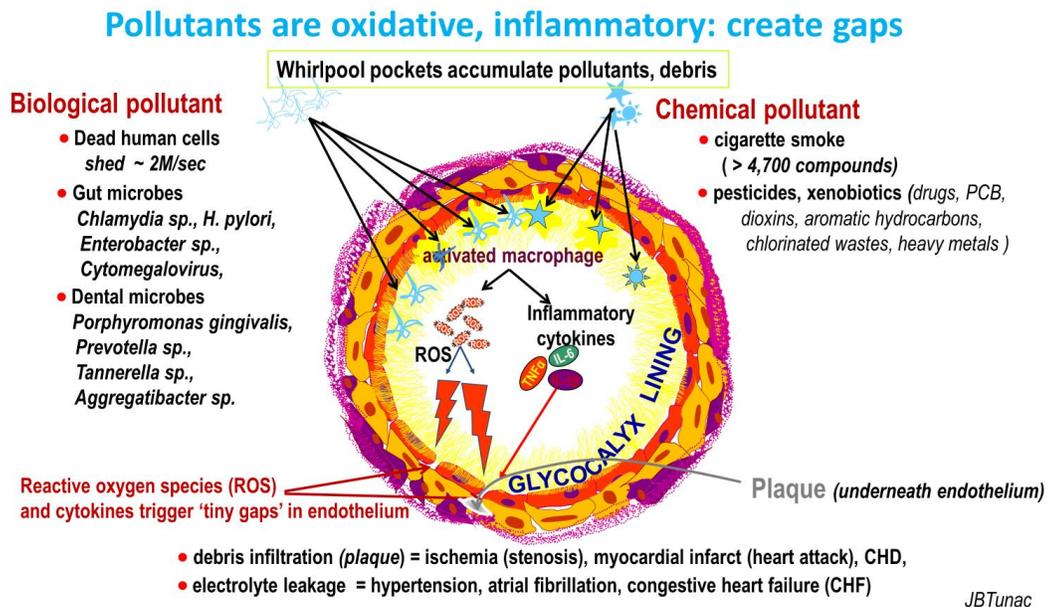
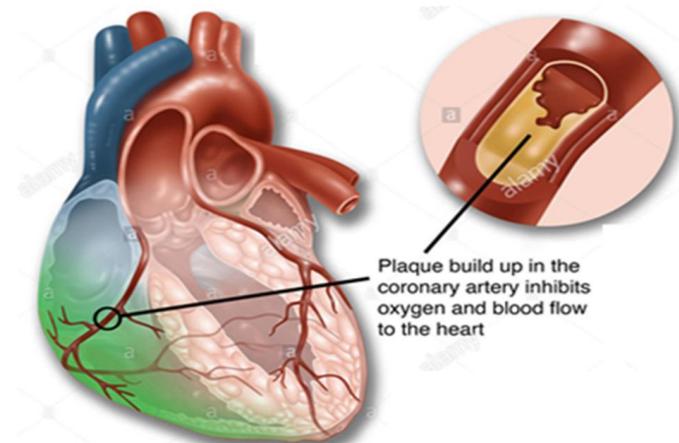


Figure 6. Biological and chemical pollutants in the arterial bends triggers inflammation, tiny endothelial gaps creating electrolyte leakage (hypertension) and debris infiltration (plaque)

Plaques stiffen myocytes or trigger apoptosis (2005. *Cardiovascular Res* 67: 21–29), disrupt pumping and reduced EFV (Fig 7)

Plaque and HF risk



- Green areas highlight death and damage to heart.
- Plaque build-up in the coronary artery inhibits oxygen and blood flow to the heart leading to stiff myocytes
- Over time weakens heart efficiency to pump blood leading to heart failure (HF)

JBTunac

Figure 7 Green areas highlight death and damage to heart due to plaque, which stiffens heart myocyte and reduce ejection fraction (EF) volume.

While MRI has become a routine imaging to assess the myocardium in the clinic, coronary artery imaging offers challenges because of both their small caliber and complex motion. In animal models, MRI has been used to detect plaques in aortal arch (2004. *Arterios Thrombosis Vasc Biol* 24:1714–1719). Because, of the impossibility of scanning plaques in the coronaries of mice, detection of plaques in the aorta infers plaque formation in the coronaries. Moreover, our previous study where mice with high fat diet and PCB (2017. *J Clin Exp Cardiol Suppl* 8:1) showed well-formed plaque formation in the brachiocephalic artery.

In summary, this study demonstrates plaque formation in the aortal arch, which is good indication of plaque formation in the coronary arteries.

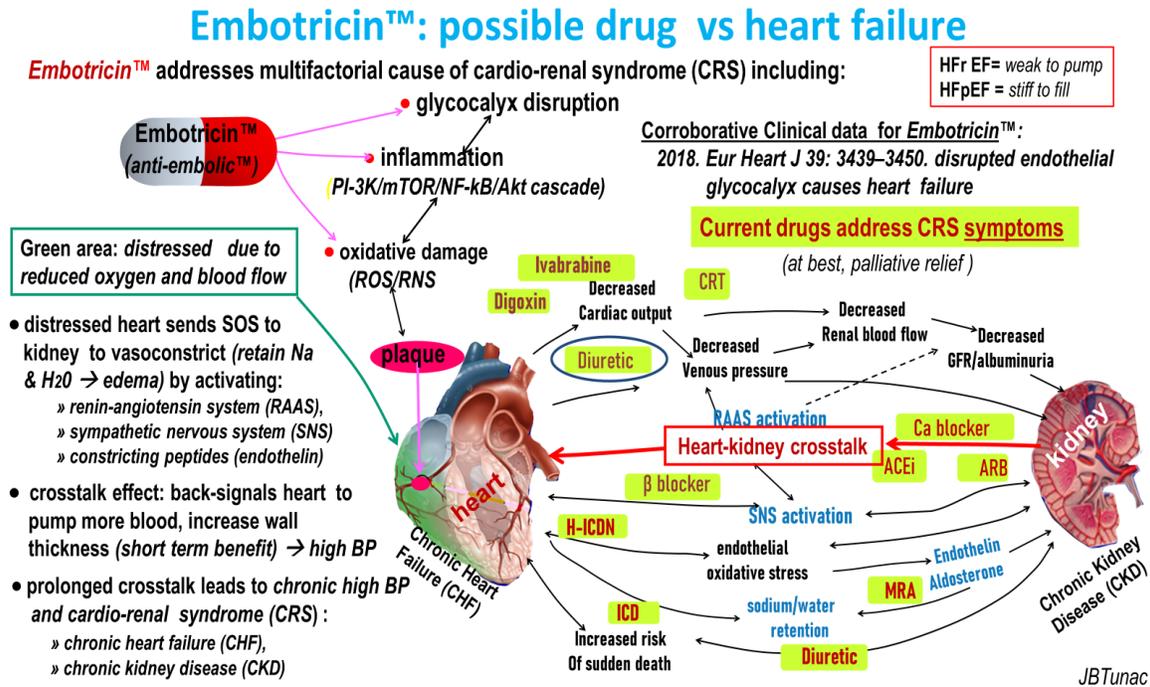


Figure 8. A compromised heart pumping result in reduced ejection fraction (EF) volume prompting heart kidney heart talk and a sequelae of diseases including heart failure (HF)

FUTURE EXPERIMENT

Several drugs have been developed vs HF (Fig 8) but are palliative at best because they are symptom targeted. A sequel to this project will be to use this model and evaluate Embotricin™ for its ability to restore the integrity of the arterial endothelial glycocalyx, reverse plaque formation and restore normal ejection fraction (EF) volume.