CRF 8G (Revised Dec 08)

RGC Reference HKU4/CRF/10
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

A Multi-disciplinary Approach to Investigate Vascular Dysfunction in Obesity and Diabetes: From Molecular Mechanism to Therapeutic Intervention
對肥胖、糖尿病相關血管病變的跨學科研究: 從分子機制到幹預治療

2. Investigator(s) and Academic Department/Units Involved

Research Team	Name/Post	Unit/Department/Institution
Project Coordinator	Aimin Xu/Professor	Medicine, and Pharmacology & Pharmacy, HKU
Co-investigator(s)	Yu Huang/Professor Karen Lam/Professor Paul Vanhoutte/Professor Yu Wang/Associate Professor	School of Biomedical Sciences, CUHK Medicine, HKU Pharmacology & Pharmacy, HKU Pharmacology & Pharmacy, HKU
Others	Peter Libby/Professor Eugene Y Chen/Professor Jianglin Fan/Professor	Medicine/Harvard Medical School Cardiovascular Center/University of Michigan University of Yamanashi

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	25/06/2011		
Project Completion Date	24/06/2014		
Duration (*in month*)	36		
Deadline for Submission of Completion Report	24/03/2015		

5. Project Objectives

5.1 Objectives as per original application

1. To study the detailed mechanisms whereby adiponectin prevents endothelial injury induced by obesity and diabetes;

2. To determine the molecular pathways whereby A-FABP mediates endothelial dysfunction and vascular inflammation;

3. To elucidate the roles of adiponectin and A-FABP in the pathogenesis of vascular disease in genetically engineered rabbits;

4. To further evaluate the clinical association of the three adipokines with vascular disease and to explore their prognostic value as biomarkers for risk prediction and early diagnosis in large study cohorts.

5.2 Revised objectives

N.A.

6. Research Outcome

6.1 Major findings and research outcome

(maximum 1 page; please make reference to Part C where necessary)

A. **Adiponectin exerts its vascular protective effects via multiple mechanisms**: First, adiponectin acts in an endocrine and paracrine manner on endothelial layer of blood vessels to stimulate AMP-activated protein kinase, which in turn protects diabetes-induced endothelial damage by reducing oxidative stress. Such an effect of adiponectin is indispensable for the vascular protective benefits of the anti-diabetic drug rosiglitazone (*see item-1, part-C*). Second, activation of AMPK by adiponectin can also lead to induction of heme oxygenase (HO)-1 and SDF-1, which in turn promotes proliferation and mobilization of endothelial progenitor cells (EPCs), thereby leading to augmented revascularization and EPCs-mediated endothelial repair (*see item-2, in panel C*). This AMPK-mediated protection of EPC number and functionality represents a key mechanism by which adiponectin prevents diabetes-induced impairment in vascular regeneration. Third, it may suppress the protein kinase CDK5, and subsequently enhances SirT1 activity via dephosphorylation at its NH2-terminus. Increased sirT1 activity in turns prevent age-related endothelial dysfunction and atherosclerosis by blocking premature cellular senescence (see

item-3, part-C). Fourth, adiponectin may also suppress obesity-induced oxidative stress by upregulating uncoupling protein-2 (UCP-2) in endothelial cells (see item-6). Last, the adaptor protein APPL1 serves as an obligatory downstream signaling molecule to mediate the insulin-sensitizing actions of adiponectin, by activation of the protein kinase Akt, which in turn activate eNOS to promote endothelial NO production. In obesity, reduced expression of APPL1 in blood vessels shifts the actions of insulin from Akt to ERK activation, thereby leading to vasoconstriction and endothelial dysfunction (*see item-4, part-C*). In addition, APPL1 may alleviate diabetes-induced endothelial dysfunction indirectly by coupling insulin actions to insulin secretion in pancreatic β-cells, which in turn maintains glycemic homeostasis (see item-5 & 30). We have made a comprehensive summary on adiponectin signaling pathways in several invited review papers (*see items 20, 21, 22, 25, 37*)

B. **A-FABP and lipocalin-2 acts as a pro-inflammatory adipokine to link obesity with vascular dysfunction via its actions in both macrophages and endothelial cells**. In endothelial cells, A-FABP potentiates toxic lipids-induced suppression of Akt activation, thereby leading to impaired eNOS activity and endothelial NO production (*see item-9*). In macrophages, A-FABP suppresses autophagy and subsequently causes ER stress, which in turn triggers JNK activation and production of pro-inflammatory cytokines (*see item-7*). In addition, A-FABP may potentiate the oxidative stress by upregulating NADPH oxidase isoforms 1 and 4 via TLR4 (*see item#27*). On the other hand, lipocalin-2 induces endothelial dysfunction and causes cardiovascular disease via inhibition of cytochrome P450 2C, disruption of mitochondrial functions and alteration of phospholipid remodeling, and also induction of apoptosis (see items #13, #14, #15 and #35). A-FABP and lipocalin-2, both of which are lipid-binding binding adipokines, are the key mediator that links lipid toxicity with vascular inflammation in obesity (see item #21&23). In both rodents and large animals, we have provided evidence demonstrating that pharmacological inhibition of A-FABP and lipocalin-2 may represent an effective strategy for treatment and prevention of vascular diseases associated with obesity and diabetes.

C. Increased circulating levels of A-FABP are an independent predictor for heart failure in a 5-year prospective study in patients with diabetes, and also can be used for prediction of future cardiovascular event in a 12-yearcommunity-based study in Hong Kong. On the other hand, elevated lipocalin-2 in urine samples can be used for early detection of those individuals who are at the high risk of cardiovascular disease [US patent 8030097 (B2), Japanese patent 2011519037 (A)].

In summary, this study unequivocally established the roles of dysregulated adiponectin, A-FABP and lipocalin-2 as an important mediator of vascular dysfunction in obesity and diabetes, and suggests that these adipokines can be used as both biomarkers for early diagnosis and drug target fir therapeutic intervention of cardiovascular diseases. We have published 40 papers and have been awarded with three patents related to this project.

6.2 Potential for further development of the research and the proposed course of action *(maximum half a page)*

A. Basic and clinical research: During this study, we have made several novel discoveries that deserve further investigation. First, we have identified several novel microRNAs (miR-34a and miR-883b) as regulators of adipokine production and macrophage-mediated inflammation in obese adipose tissue. Furthermore, we have obtained novel evidence showing that the two lipid-binding adipokines (A-FABP and lipocalin-2) are actively involved in promoting M2→M1 polarization of macrophages in obese adipose tissues. Notably, miR-883b is under the control of adiponectin whereas miR-34a controls the production of adipokines. Based on these observations, we believe that the aforementioned microRNAs and adipokines form a complex network to fine-tine the number and polarity of adipose-resident macrophages, and their dysregulation plays important roles in initiating

and/or perpetuating obesity-associated macrophages-mediated metabolic and vascular inflammation We have secured another CRF grant in the 2014/2015 round (grant no C7055-14G, 8,780,000 HKD) to continue our study in both animals and humans.

B. Translational and applied research: Based on the findings from this completed project, we have been awarded with several US and international patents [US 8030097, US 2010310578 (A1)], claiming the use of the adipokines as diagnostic biomarkers and therapeutic targets for cardiovascular diseases. In addition, we have already developed a series of immunoassays for these adipokines via the antibody and immunoassay service (AIS) established by the PI (see www.antibody.hku.hk). We have successfully commercialized these assays for basic and clinical research, clinical diagnostics and high throughput drug screening. Furthermore, we have secured an applied research fund from ITC via University and Industrial matching scheme (UIM/270, 3998000 HKD) to develop chemiluminecence-based assays for these biomarkers to be used for early diagnosis of diabetic vascular complications (in collaboration with Pian Zian Huang Pharmaceutical Company, which was listed in stock market in Mainland China). Our industrial partner will work together with us for obtaining approval for CFDA and for promotion of clinical applications in Mainland China. In addition, Dr. Yu Wang and Prof. Paul Vanhoutte (co-PIs) have secured over 5 million HKD via strategic alliance with Servier (an European drug company) to develop drug candidates by targeting adipokines, taking advantage of the patents, animal models and platforms established in this project.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

All our major research outcomes were based on the close collaborations among PI and co-PIs, as evidenced by significant contribution of each investigator to our joint research platform, joint supervision of postgraduate students, joint lab meeting, sharing of research resources, joint publications and joint commercialization activities.

1. We have organized 5 meetings among the PI and co-PIs to discuss the funding allocation and to update the research progress for this project. During the meeting, the PI and co-PIs discussed the detailed plan for collaboration for each study objective.

2. **Joint lab meeting:** The research staff and postgraduate students from A Xu, K Lam, P Vanhoutte and Y Wang's groups held joint lab meeting every two weeks. The joint research meeting and journal club among the research staff from A Xu, Y Wang and PM Vanhoutte's lab were held on a biweekly basis. In addition, lab members from A Xu's group and Y Huang's group at CUHK held joint lab meeting every two months, and the staff/students involved in this project presented their data each time during the joint meeting. We also organized joint conference on last December.

3. **Joint supervision of postgraduate students and postdoctoral fellows.** We trained 10 RPG (8 Ph.D. and 2 M.Phil.) students during the funding period. There are currently 14 (13 Ph.D. and 1 M.Phil.) students under the joint supervision of the PI and co-PIs, including two students under the joint supervision of A Xu and PM Vanhoutte (Baretella Oliver Marc and Detremmerie Charlotte), four students under the joint supervision of K Lam and A Xu (Lingling Shu, Zhong Ling, Pan Yong and Huang Zhe), four students under the joint supervision of Y Wang and A Xu (Wang Baile, Li Jing, Yan Liang and Liang Yan), and three under the joint supervision of Y Wang, PM Vanhoutte and A Xu (Cheong Lai Yee, Feng Tianshi and Cai Yu). A Xu is also an academic advisor for Y Huang's PhD student (Liu Jian) at CUHK. These students and supervisors met any least once a month to discuss the progress of the research projects related to this study. With the support from this CRF project, PI and four co-PIs at HKU have successfully bided for four postdoctoral fellow positions from the University, and these four postdoctoral fellows (Dr. Di Zhu, Dr. Gu Ping, Dr. Larry Liang and Dr. Ye Dewei) are

currently under the joint supervision of the PI and co-PIs, to facilitate the collaboration between each research group.

4. **Collaborative research activities on applied research related to this project**. With the support from PM Vanhoutte and K Lam, PI has established a HKU antibody and immunoassay service center to commercialize the adipokine-related immunoassay products (www.antibody.hku.hk). Through the close collaboration between PM Vanhoutte, A Xu and Yu Wang, we have established a strategic alliance with Servier (a multi-national drug company based on France) to identify lead compounds that can be potentially used for the treatment of obesity-related cardiometabolic complications by targeting adipokines and adipose-vascular axis. So far, we have been awarded with three contract projects with a total amount of ~15 million HKD.

5. **Joint publications.** All the original publications listed in part C were based on the collaborative work between PI and co-PIs. In all these joint publications, both PI and each co-PI has made genuine contributions. The contribution of each investigator to this project is also evidenced by the fact that both PI and each co-PI have contributed to these joint publications as a senior/corresponding author.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Cardiovascular disease (CVD), including stroke, heart attack and periphery artery disease, is the major cause of death and hospitalization in our rapidly ageing population. Much of the high incidence of CVD is attributed to the rapid rise in the prevalence of obesity and diabetes. Unfortunately, none of the current therapies can reverse the progression of CVD, partly due to a lack of the understanding of the pathogenesis of these chronic diseases. In this study, we have identified several factors secreted from fat tissues that are involved in the early pathogenesis of vascular disease in obesity and diabetes. These adipocyte-secreted factors serve as the important regulators for both systemic energy metabolism and vascular homeostasis, and their dysregulation in obese fat can cause insulin resistance and vascular damage. Furthermore, we have demonstrated that pharmacological or genetic interventions targeting these molecules can prevent vascular diseases associated with obesity and diabetes. These new findings provide scientific base for the future development of non-invasive diagnostic methods for risk prediction and early diagnosis of CVD, and more effective therapeutic interventions that can reverse or cure these chronic diseases by targeting endothelial dysfunction associated with obesity and diabetes.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*
Year of publication	Year of Acceptance (For paper accepted but not yet published)	Under Review	Under Preparation (optional)					

2011 (Item-1)				Wong WT, Tian XY, **Xu A***, Yu J, Lau CW, Hoo R, **Wang Y**, Lee VW, **Lam KSL, Vanhoutte PM, and Huang Y***.	Adiponectin Is Required for PPAR γ -Mediated Improvement of Endothelial Function in Diabetic Mice. ***Cell Metabolism,*** 16:101-15	Yes (2012)	No	Yes
2012 (Item-2)				Li Y, Lam KSL, Tse HF, Chen C, **Wang Y, Vanhoutte PM, and Xu A***.	Endothelium-selective Activation of AMP-activated Protein Kinase Prevents Diabetes-induced Impairment of Vascular Function and Re-endothelialization via Induction of Heme Oxygenase-1 in Mice. ***Circulation***, 126: 1267-77	Yes (2012)	No	Yes
2012 (Item-3)				Bai B, Liang Y, Xu C, Lee MY, **Xu A**, Wu D, **Vanhoutte PM*, Wang Y***.	CDK5-Mediated Hyperphosphorylation of SIRT1 Contributes to the Development of No Endothelial Senescence and Atherosclerosis. ***Circulation.*** 7;126(6):729-40	Yes (2012)	No	Yes
2011 (Item-4)				Wang Y, Cheng KK, **Lam KSL,** Wu D, Wang Y, **Huang Y, Vanhoutte PM**, Sweeney G, Li Y, **Xu A*.**	APPL1 counteracts obesity-induced vascular insulin resistance and endothelial dysfunction by modulating the endothelial production of nitric oxide and endothelin-1 in mice. ***Diabetes,*** 60: 3044-54	Yes (2012)	No	Yes

2012 (Item-5)				Cheng KK, **Lam KSL**, Wu D, **Wang Y**, Sweeney G, Hoo R, Zhang J and **Xu A*** (*Highlighted by a commentary*)	APPL1 potentiates insulin secretion in pancreatic beta-cells by increasing Akt-dependent expression of SNARE proteins in mice. ***PNAS,*** 109:8919-27	Yes (2012)	No	Yes
2012 (Item-6)				Tian XY, Wong WT, **Xu A**, Lu Y, Zhang Y, Wang L, Cheang WS, **Wang Y**, Yao X, **Huang Y***	Uncoupling Protein-2 Protects Endothelial Function in Diet-induced Obese Mice. ***Circ Res.*** 110: 1211-6	Yes (2012)	No	Yes
2013 (Item-7)				Hoo RL, Lee IP, Zhou M, Wong JY, Hui X, **Xu A***, **Lam KSL*.**	Pharmacological Inhibition of Adipocyte Fatty Acid Binding Protein Alleviates Both Acute Liver Injury and Non-alcoholic Steatohepatitis in Mice. ***J Hepatol,*** 58(2): 358-364	Yes (2012)	No	Yes
2012 (Item-8)				Ye D, Li Y, **Lam KS**, Li H, Jia W, **Wang Y**, Man K, Li X and **Xu A*** (*Highlighted by a commentary*)	TLR4 mediates obesity-induced nonalcoholic steatohepatitis through activation of X-box binding protein in mice. ***Gut,*** 61:1058-67	Yes (2012)	No	Yes

2013 (Item-9)				Chan C, Laio S, Zhang Y, Lee M, **Xu A**, Tse H, **Vanhoutte P** *	A-FABP and oxidative stress underlie the impairment of endothelium dependent relaxation to serotonin and the neointimal thickening in the porcine coronary artery with regenerated endothelium. ***ASC Chem Neuroscience*** 4(1): 122-129	Yes (2012)	No	Yes
2013 (Item-10)				Liu M, Zhou M, Bao Y, Xu Z, Li H, Zhang H, Zhu W, Zhang J, **Xu A**, Wei M*, Jia W*.	Circulating adipocyte fatty acid-binding protein levels are independently associated with heart failure. ***Clin. Sci.*** 124(2):115-22.	Yes (2012)	No	Yes
2013 (Item-11)				Chow WS, Tso AWK, **Xu A**, Yuen MMA, Fong CHY, Lam TH, Lo SV, Tse HF, Woo YC, Yeung CY, Cheung BMY*, **Lam KSL***	Elevated Circulating Adipocyte-Fatty Acid Binding Protein Levels Predict Incident Cardiovascular Events in a Community-based Cohort: A 12-Year Prospective Study ***JAHA*** 15;2(1):e004176.	Yes (2012)	No	Yes
2012 (Item-12)				Yuen CY, Wong SL, Lau CW, Tsang SY, **Xu A**, Zhu Z, Ng CF, Yao X, Kong SK, Lee HK, **Huang Y***.	From Skeleton to Cytoskeleton: Osteocalcin Transforms Vascular Fibroblasts to Myofibroblasts Via Angiotensin II and Toll-Like Receptor 4. ***Circ Res.*** 11(3):e55-66	Yes (2012)	No	Yes

2012 (Item-13)				Xu G, Ahn J, Chang S, Eguchi M, Ogier A, Han S, Park Y, Shim C, Jang Y, Yang B, **Xu A, Wang Y**, Sweeney G*.	Lipocalin-2 induces cardiomyocyte apoptosis by increasing intracellular iron accumulation. ***J Biol Chem.*** 287(7):4808-17.	No	No	Yes
2012 (Item-14)				Liu JT, Song E, **Xu A**, Berger T, Mak TW, Tse HF, Law IK, Huang B, Liang Y, **Vanhoutte PM*, Wang Y***.	Lipocalin-2 deficiency prevents endothelial dysfunction associated with dietary obesity: role of cytochrome P450 2C inhibition. ***Br J Pharmacol.*** 165(2):520-31	Yes (2012)	No	Yes
2012 (Item-15)				Yang B, Fan P, **Xu A, Lam KSL**, Berger T, Mak TW, Tse HF, Yue JWS , Song E, **Vanhoutte PM**, Sweeney G, and **Wang Y**∗	Improved functional recovery to I/R injury in hearts from lipocalin-2 deficiency mice: restoration of mitochondrial function and phospholipids remodeling ***Am J Transl Res.*** 4(1): 60–71.	Yes (2012)	No	Yes
2012 (Item-16)				Ji Y, Sun S, **Xu A**, Bhargava P, Yang L, **Lam KSL**, Gao B, Lee CH, Kersten S, Qi L*	Activation of natural killer T cells promotes M2 macrophage polarization in adipose tissue and improves systemic glucose tolerance via the IL-4/STAT6 signaling axis in obesity. ***J Biol Chem.*** 287(17):13561-71.	Yes (2012)	No	Yes

2012 (Item-17)				Liu L, Liu J, Wong WT, Tian XY, Lau CW, Wang YX, Xu G, Pu Y, Zhu Z, **Xu A, Lam KSL**, Chen ZY, Ng CF, Yao X, **Huang Y***.	Dipeptidyl peptidase 4 inhibitor sitagliptin protects endothelial function in hypertension through a glucagon-like peptide 1-dependent mechanism ***Hypertension.*** 60(3):833-41	Yes (2012)	No	Yes
2011 (Item-18)				Tian XY, Wong WT, **Xu A**, Chen ZY, Lu Y, Liu LM, Lee VW, Lau CW, Yao X, and **Huang Y***	Rosuvastatin improves endothelial function in *db/db* mice: role of angiotensin II type 1 receptors and oxidative stress ***Br J Pharmacol.*** 164(2b): 598–606.	Yes (2012)	No	Yes
2011 (Item-19)				Wong SL*, Lau CW, Wong WT, **Xu A**, Au CL, Ng CF, Ng SS, Gollasch M, Yao X, **Huang Y***.	Pivotal role of protein kinase Cξ in angiotensin II-induced endothelial cyclooxygenase II expression: A link to vascular inflammation. ***Arterioscler Thromb Vasc Biol.*** 31(5):1169-76	Yes (2012)	No	Yes
2012 (Item-20)				Li FY, **Lam KSL** and **Xu A***.	Therapeutic perspectives for adiponectin: an update. ***Curr Med Chem.*** 19(32):5513-23.	Yes (2012)	No	Yes

2012 (Item-21)				**Xu A** and **Vanhoutte PM***.	Adiponectin and adipocyte fatty acid binding proteins in the pathogenesis of cardiovascular disease. ***Am J Physiol Heart Circ Physiol.*** 302(6):H1231-40.	Yes (2012)	No	Yes
2012 (Item-22)				Hui X**, Lam KSL**, V**anhoutte PM** and **Xu A***.	Adiponectin and cardiovascular health, an update. ***British Journal of Pharmacology*** 165(3):574-90.	Yes (2012)	No	Yes
2012 (Item-23)				**Wang Y***.	Small lipid-binding proteins in regulating endothelial and vascular functions: Focusing on adipocyte fatty acid binding protein and lipocalin-2 ***British Journal of Pharmacology*** 165(3):603-21.	Yes (2012)	No	Yes
2013 (Item-24)				Lin Z, Tian H, **Lam KS**, Lin S, Hoo RC, Konishi M, Itoh N, **Wang Y**, Bornstein SR, **Xu A***, Li X*.	Adiponectin Mediates the Metabolic Effects of FGF21 on Glucose Homeostasis and Insulin Sensitivity in Mice. ***Cell Metab***. 2013, 17:779-89.	Yes (2013)	No	Yes
2013 (Item-25)				Gu P, **Xu A*.**	Interplay between adipose tissue and blood vessels in obesity and vascular dysfunction. ***Rev Endocr Metab Disord***. 2013Mar; 14(1):49-58	Yes (2013)	No	Yes

2013 (Item-26)				Liu Y, Turdi S, Park T, Morris NJ, Deshaies Y, **Xu A**, Sweeney G.	Adiponectin corrects high-fat diet-induced disturbances in muscle metabolomics profile and whole-body glucose homeostasis. ***Diabetes***. 2013 Mar; 62(3):743-52	Yes (2013)	No	Yes
2013 (Item-27)				Liang CF, Liu JT, **Wang Y, Xu A, Vanhoutte PM*.**	Toll-like receptor 4 mutation protects obese mice against endothelial dysfunction by decreasing NADPH oxidase isoforms 1 and 4. ***Arterioscler Thromb Vasc Biol***. 2013Apr; 33(4):777-84.	Yes (2013)	No	Yes
2013 (Item-28)				Tonks KT, Ng Y, Miller S, Coster AC, Samocha-Bonet D, Iseli TJ, **Xu A***, Patrick E, Yang JY, Junutula JR, Modrusan Z, Kolumam G, Stöckli J, Chisholm DJ, James DE, Greenfield JR.	Impaired Akt phosphorylation in insulin-resistant human muscle is accompanied by selective and heterogeneous downstream defects. ***Diabetologia***. 2013 Apr; 56(4):875-85.	Yes (2013)	No	Yes
2013 (Item-29)				Vu V, Bui P, Eguchi M, **Xu A**, Sweeney G.	Globular adiponectin induces LKB1/AMPK-dependent glucose uptake via actin cytoskeleton remodeling. ***J Mol Endocrinol***. 2013 51: 155-165	No	Yes	Yes

2013 (Item-30)				Park M, Wu D, Park T, Choi CS, Li RK, Cheng KK, **Xu A**, Sweeney G.	APPL1 transgenic mice are protected from high-fat diet-induced cardiac dysfunction. ***Am J Physiol Endocrinol Metab***. 2013 Oct 1; 305 (7): E795-804.	No	Yes	Yes
2013 (Item-31)				Heilbronn LK, Campbell LV, **Xu A,** Samocha-Bonet D.	Metabolically protective cytokines adiponectin and fibroblast growth factor-21 are increased by acute overfeeding in healthy humans. PLoS One. 2013 Oct 18;8(10):e78864	No	Yes	Yes
2014 (Item-32)				Qiu B, Shi X, Wong ET, Lim J, Bezzi M, Low D, Zhou Q, Akıncılar SC, Lakshmanan N, Swa HLF, Tham JML, Gunaratne J, Cheng KKY, Hong W, **Lam KSL**, Ikawa M, Guccione E, **Xu A**, Han W, Tergaonkar V.	NUCKS Is a Positive Transcriptional Regulator of Insulin Signaling ***Cell Reports*** 2014 Jun; 7(6): 1876-1886	No	Yes	Yes
2014 (Item-33)				Li H, Wei S, Cheng K, Gounko NV, Ericksen RE, **Xu A**, Hong W* and Han W*.	BIG3 inhibits insulin granule biogenesis and insulin secretion ***EMBO Reports*** 2014 Jun; 15(6): 714-722	No	Yes	Yes

2014 (Item-34)				Cheng KKY, **Lam KSL**, Wang B, **Xu A*.**	Signaling mechanisms underlying the insulin-sensitizing effects of adiponectin ***Best Practice & Research: Clinical Endocrinology & Metabolism*** 2014 Jan; 28(1): 3-13	No	Yes	Yes
2014 (Item-35)				Song E, Fan P, Huang B, Deng B, Cheung BMY, F é é tou M, Vilaine J, Villeneuve N, **Xu A, Vanhoutte PM, Wang Y**.	Deamidated lipocalin-2 induces endothelial dysfunction and hypertension in dietary obese mice ***JAHA*** 2014; 3: e000837	No	Yes	Yes
2014 (Item-36)				Lin Z, Wu F, Lin S, Pan X, Jin L, Lu T, Shi L, **Wang Y**, **Xu A***, Li X*.	Adiponectin protects against acetaminophen-in duced mitochondrial dysfunction and acute liver injury by promoting autophagy in mice ***Journal of Hepatology*** 61(4): 825-831	No	Yes	Yes
2014 (Item-37)				**Wang Y***	Molecular Links between Caloric Restriction and Sir2/SIRT1 Activation. ***Diabetes & Metabolism Journal*** 38(5); 321-329	No	Yes	Yes
2014 (Item-38)				Bai B**, Vanhoutte PM, Wang Y.**	Loss-of-SIRT1 function during vascular ageing: hyperphosphorylat ion mediated by cyclin-dependent kinase 5. ***Trends Cardiovasc Med.*** 24 (2): 81-4.	No	Yes	Yes

2014 (Item-39)				Chan CK, Liao SY, Zhang YL**,** **Xu A,** Tse HF, **Vanhoutte PM.**	Protective effects of histamine on Gq-mediated relaxation in regenerated endothelium. ***Am J Physiol Heart Circ Physiol.*** 15;306(2):H286-90.	No	Yes	Yes
2014 (Item-40)				Wang Y, Xiao Y, Zhong L, Ye D, Zhang J, Tu Y, Bornstein SR, Zhou Z, **Lam KS, Xu A.**	Increased neutrophil elastase and proteinase 3 and augmented NETosis are closely associated with β-cell autoimmunity in patients with type 1 diabetes. ***Diabetes.*** 63(12):4239-48.	No	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each conference abstract)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of RGC *(Yes or No)*
March/2014/ Kuala Lumpur, Malaysia	Interplay Between Adipokines And Hepatokines In Obesity-related Metabolic Complications	12th International congress on obesity, 2014	*No*	*Yes*	*Yes*
November/2014/Suntec City, Singapore	Adiponectin, FGF21 and metabolic homeostasis	10th International Diabetes Federation-Western Pacific Region Congress	*No*	*Yes*	*Yes*

10. ***Student(s) trained*** (please attach a copy of the title page of the thesis)

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
1. Zhao Yingzi	Doctor of Philosophy	September 01, 2010	August 31, 2014
2. Manio Micheal Magtoto	Doctor of Philosophy	September 01, 2010	August 31, 2014
3. Xu Cheng	Doctor of Philosophy	September 01. 2009	August 31, 2013
4. Lee Pei Chi	Master of Philosophy	September 01, 2011	August 31, 2013
5. Bai Bo	Doctor of Philosophy	January 01, 2010	December 31, 2013
6. Liang Yan	Doctor of Philosophy	September 01. 2009	August 31, 2013
7. Li Jie	Master of Philosophy	September 01. 2009	August 31, 2013
8. Fan Pencheng	Doctor of Philosophy	September 01, 2010	August 31, 2014
9. Song Erfei	Doctor of Philosophy	September 01, 2010	August 31, 2014
10. Wang Yu-Dong	Doctor of Philosophy	September 01, 2010	August 31, 2014
11. Baretella Oliver Marc	Doctor of Philosophy	September 01, 2011	August 31, 2015
12. Cai Yin	Doctor of Philosophy	January 01, 2011	December 31, 2014
13. Zhong Ling	Doctor of Philosophy	September 01, 2011	August 31, 2015
14. Detremmerie Charlotte	Doctor of Philosophy	October 02, 2012	October 01, 2016
15. Li Jin	Doctor of Philosophy	September 01, 2012	August 31, 2016
16. Shu Lingling	Doctor of Philosophy	September 01, 2012	August 31, 2016
17. Wang Baile	Doctor of Philosophy	September 01, 2012	August 31, 2016
18. Huang Zhe	Doctor of Philosophy	September 01, 2012	August 31, 2016
19. Liu Jian	Doctor of Philosophy	January 01, 2011	December 31, 2014
20. Cai Yu	Doctor of Philosophy	September 01, 2010	August 31, 2014
21. Feng Tianshi	Doctor of Philosophy	September 01, 2014	August 31, 2018
22. Cheong Lai Yee	Doctor of Philosophy	September 01. 2014	August 31, 20188
23. Pan Yong	Doctor of Philosophy	September 01, 2013	August 31, 2017
24. Chen Zhanrui	Master of Philosophy	September 01, 2013	August 31, 2015

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

A. Technology transfer and applied research: (1) So far, we have obtained two patents for lipocalin-2 (8,030,097 B2 and 7,645,616). As lipocalin-2 is one of the key adipokines studied in this project, acquisition of this patent will provide us a unique advantage for continuation of both basic and applied research in this field. (2) Through the HKU antibody and immunoassay services (AIS) established by PI and co-PIs (www.antibody.hku.hk), we have developed a number of monoclonal antibody-based immunoassays for quantifying serum levels of various biomarkers related to diabetes and cardiovascular disease, which have been licensed to several European and US companies (Biovendor, Abcam, Novo Nordisk, Merck) for basic and clinical research, clinical trials, and clinical diagnosis. (3) We are also working closely with a Shenzhen-based company to apply for SFDA approval in China for our rapid lipocalin-2 diagnostic kit for risk detection of cardiovascular diseases. (4) Through our strategic alliance with Servier, we have now secured three contract research grants with a total amount of ~15 million HKD to use our high throughput assays and animal models to develop pharmacological inhibitors of A-FABP, lipocalin-2 and TLR4 for treating obesity-related metabolic and cardiovascular diseases. (5) With the support of University-industry matching fund (UIM/270, 3,998,000 HKD) from Hong Kong ITC obtained this year, we collaborate with Zhang Long Co. Ltd. for development of innovative chemiluminescent immunoassays for diabetes and cardiovascular diseases.

B. International collaborations: With this grant support, we have also established an extensive collaboration network on both basic and clinical studies of adipokines and adipose tissue inflammation. (1) In collaboration with Prof. Li Xiaokun at Wenzhou Medical University, we have

investigated the protective effect of adiponectin against acetaminophen-induced mitochondrial dysfunction and acute liver injury by promoting autophagy in mice (see publication item-24 and 36); (2) In collaboration with Prof. Samocha-Bonet at University of Adelaide (Australia), we demonstrated that the metabolically protective adipokines adiponectin and fibroblast growth factor-21 are increased by acute overfeeding in healthy humans (see publication item-31). (3) In collaboration with Dr. Qi Lin at Cornell University, we have investigated the roles of NTK cells in the onset and progression of adipose tissue inflammation in obese mice and humans (see item-16). Our HKU team conducted studies in human adipose tissue in this manuscript and provided animal models. (4) In collaboration with Dr. Gary Sweeney at York University, Canada, we have investigated the global metabolic effects of adiponectin using an integrated metabolomics approach (Liu Y, Xu A and Sweeney G, Diabetes, 2012, item 13, 15, 26, 29 and 30). (5) In collaboration with Dr. Jerry Greenfield at Australia Garvan Institute of Medical Health, we have compared the levels of adiponectin, A-FABP and lipocalin-2 among lean individuals, obese metabolically healthy subjects, obese insulin resistant subjects and obese diabetic subjects, and discovered that progressively increased A-FABP, but decreased adiponectin from lean to obese with diabetic patients (Tong C, Xu A and Greenfield G, Diabetologia, 2013, item-28). (6) We have also extended our long-term collaboration with Prof. Donghai Wu at Guangzhou Institute of Biomedicine and Science (GIBH) to work on the role of adaptor protein APPL1 in mediating adiponectin and insulin actions (see item items 3-5 and 30). For all these collaborations, our HKU team provided adipokine assays, animal models, performed part of the experiments and contributed to the conceptual design of the studies.