RGC Ref.: A-HKU705/13
(please insert ref. above)

The Research Grants Council of Hong Kong
ANR/RGC Joint Research Scheme
Completion Report

(Please attach a copy of the completion report submitted to the ANR by the French researcher)

Part A: The Project and Investigator(s)

1. 1. Project Title (ANR Acronym)

The gut microbiota-adipose tissue axis in the pathogenesis of obesity and its related metabolic disorders: molecular mechanism and clinical implications
腸道菌群-脂肪組織軸在肥胖相關代謝疾病發病中作用的分子機理研究及臨床意義探討

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

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Prof. Aimin Xu	Prof. Amar Jacques
Post	Professor	Professor
Unit / Department / Institution	Dept. of Medicine & Dept. of Pharmacology and Pharmacy	Inserm's institute of metabolic and cardiovascular disease and Hospital Rangueil, France
Co-investigator(s) *(with title)*	Prof. Karen S L Lam Prof. Jun Wang	Prof. Remy Burcelin

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	April 01, 2014	April 01, 2014	
Project Completion date	March 31, 2017	September 30, 2017	May 02, 2017
Duration *(in month)*	36	42	
Deadline for Submission of Completion Report	March 31, 2018	September 30, 2018	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. ***To interrogate the dynamic relationship of gut microbiota with adipose tissue inflammation and adipokine production in the pathogenesis of obesity and its related metabolic disorders in mice***

2. ***To investigate whether adipose tissue inflammation and adipokines are the key mediators of gut microbiota-induced systemic insulin resistance and metabolic dysregulation in mice***

3. ***To comprehensively identify and compare bacterial species and DNA sequences in both intestine and adipose tissues of lean and diet-induced obese mice with different metabolic status ((By French team)***

4) ***To elucidate how diabetes-associated microorganisms induce adipose tissue inflammation, aberrant production of adipokine/cytokines and metabolic dysfunction (By French team)***

5): ***To characterize the composition of bacterial species in plasma and adipose tissues of lean and obese subjects, and to correlate tissue bacteria with inflammation, adipokine secretion and metabolic profiles in humans***

5.2 Revised Objectives: N/A
Date of approval from the RGC: _____

Reasons for the change: _____

6. Research Outcome

Major findings and research outcome *(maximum 1 page; please make reference to Part C where necessary)*

In this study, we have discovered novel crosstalks between gut microbiota and adipose tissues in maintaining metabolic and vascular homeostasis, and impaired gut microbiota-adipose axis is an important contributor to obesity-related metabolic complications and atherosclerosis.

Firstly, we have found that dysbiosis of gut microbiota participates in the onset and progression of adipose inflammation and aberrant production of adipokines using germ-free mice and fecal transplantation studies. In germ-free mice, transplantation of gut microbiota from obese donors is sufficient to trigger macrophage infiltration into adipose tissues, increase proinflammatory cytokine production, but decrease adiponectin. Such a detrimental effect of obese gut microbiota is caused by increased gut permeability, which in turn leads to the translocation of endotoxin from gut into bloodstream and then to adipose tissues. Furthermore, we identified decreased abundance of *A muciniphila* in the gut as a major culprit whereby gut microbiota dysbiosis causes endotoxinemia and metabolic inflammation by increasing gut permeability. Replenishment of live A muciniphila, but not heat-inactivated one, is sufficient to reverse high fat high cholesterol diet (HFHC)-induced endotoxinemia, and reduce inflammation in both adipose tissues and vascular walls, which are accompanied by a marked alleviation of atherosclerosis and suppression of pro-inflammatory adipokines and chemokines. Mechanistically, we discovered that the secreted products from A muciniphila can induce the expression of intestinal expression of the tight junction proteins (zona occuldens protein-1 and occludin), thereby maintaining the gut permeability. Therefore, these findings not only uncovered a novel mechanism whereby gut microbiota dysbiosis causes chronic endotoxinemia, adipose tissue inflammation and metabolic disturbance in obesity, but also provide solid scientific evidence supporting the use of *A muciniphila* as probiotics for treatment and prevention of obesity-related metabolic complications (*see publication-1, part c*).

Secondly, we demonstrated that adipose tissues can also modulate the composition and function of gut microbiota, through secretion of adipokines and metabolites. Through a series of fecal transplantation experiment, we showed that the insulin-sensitizing, anti-diabetic and anti-inflammatory effects of adiponectin, a major adipokine secreted from adipocytes, are mediated at least in part by modulating the composition and function of gut microbiota. Gut microbiota in ADNKO mice fed on HFD was sufficient to worsen host insulin resistance and glucose intolerance compared to their WT littermates fed on HFD. Meanwhile, gut microbiota from WT mice fed on HFD could partially alleviate HFD-induced insulin resistance in ADNKO mice. The improvement of insulin sensitivity caused by gut microbiota was accompanied by decreased adipose tissue inflammation. Through an integrated shot-gun metagenomics analysis, we have identified the decreased abundances of Lactobacillus reuteri, Alistipes unclassified and Anaerotruncus sp. G3 in ADNKO mice fed on HFD compared to their WT littermates fed on HFD. In addition to the alterations of gut microbiota composition, the microbial functional pathways were also different between these two groups. Amino acids biosynthesis pathways (including L-lysine, L-arginine and S-adenosyl-L-methionine) were decreased in ADNKO mice fed on HFD compared to their WT littermates fed on HFD. hese findings indicate adiponectin can mediate the cross-talk between adipose tissue and gut microbiota to exert its insulin-sensitizing and anti-inflammatory effects. Furthermore, we made an unique lipodystrophic (fatless) mouse model by adipocyte-specific ablation of the murine double minute 2 (MDM2) gene using the cre-mice driven by the adiponectin promoter, and found that this adipocyte- MDM2-null mice progressively loss almost all the adipose tissues during ageing, accompanied by severe insulin resistance, diabetes, hyperlipidemia, fatty liver and heart dysfunction. Furthermore, plasma adiponectin is almost undetectable in these mice. All these abnormalities can be partially reversed by transplantation of adipose tissues. Importantly, we discovered that the progressive loss of adipose tissue leads to a drastic change in the composition of gut microbiota and its metabolites, whereas transplantation of gut microbiota from wild-type mice into the lipodystrophic mice can partially reverse insulin resistance, diabetes, and fatty liver disease. These findings provide novel evidences showing that

adipose tissues can control systemic metabolism and insulin sensitivity by modulating gut microbiota. Furthermore, this unique fatless mouse model (which is the first model with progressive loss of fat during ageing) established will allow us to dissect the detailed molecular pathways whereby different adipose depots and their secreted adipokines modulate the function and composition of gut microbiota by transplantation of adipose tissues and/or injection of adipokines (*see publication-2, part-c, and another manuscript under preparation, see attached*). Our French collaborator also made a major finding on how altered gut microbiota, through regulation of ileum IL17 cells, causes metabolic inflammation and diabetes (*Garidu L, Cell Metabolism, 2015, 22:100-112*).

Thirdly, through analysis of adipose tissues and plasma from Chinese individuals ranging from lean, overweight to obesity, we found that the endotoxin levels in both circulation and adipose tissues are closely associated with insulin resistance, adipose inflammatory and expression of pro-inflammatory adipokines, whereas the presence of adipose resident microbiota is not an important contributor. These clinical evidences support our animal-based studies that gut microbiota dysbiosis causes increased gut permeability and translocation of endotoxin from gut into bloodstream and adipose tissues, thereby triggering adipose inflammation, aberrant adipokine production and ensuing insulin resistance.

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

The findings, platforms and animal models established from this study provide us a unique opportunity to further study the gut microbiota-adipose tissue crosstalk and the roles of such crosstalk in maintaining metabolic and vascular homeostasis, and to interrogate how dysfunctional gut microbiota-adipose tissue axis causes obesity-related metabolic complications. Firstly, we plan to expand our study into morbidly obese Chinese receiving bariatric surgery. To this end, we are establishing one of the largest adipose tissue-feces biobank from around 500 obese Chinese with a spectrum of metabolic phenotypes (metabolically healthy and unhealthy), with subcutaneous, visceral white adipose tissues (WAT), feces, blood and urine samples collected. We will conduct metagenomics, metatranscriptomics and metabolomics studies for gut microbiota, and RNA-seq analysis for different adipose depots of these patients, to further dissect the molecular and functional links between gut microbiota and adipose tissues in Chinese, and to develop effective strategies for therapeutic intervention. Furthermore, we will take advantage of the unique mouse models of lipodystrophy established in this study to further dissect how adipose tissues and their derived adipokines, through modulation of gut immunity, regulate the composition, diversity and metabolism of gut microbiota, and will further identify individual bacterial species affected by adipose dysfunction or adipokines to better understand the pathogenesis of cardiometabolic diseases caused by the dysfunctional gut microbiota-adipose tissue axis.

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)

Gut microbiota, a complex ecosystem that is composed of approximately 400-500 bacterial species with over 300 million genes, is an integral part of human body that plays crucial roles in the maintenance of metabolic homeostasis. Altered composition of gut microbiota (dysbiosis) is an important mediator of obesity and diabetes, the prevalence of which has reached epidemic

proportions globally. The present study uncovered a key mechanism whereby altered gut microbiota, through affecting the functions of adipose tissues (fat), triggers insulin resistance and diabetes. We have identified changes of specific gut microbial species can cause leaky gut and penetration of endotoxin from the gastrointestinal tracts into the bloodstream and adipose tissues, where it instigates metabolic inflammation, systemic insulin resistance and metabolic disturbance. On the other hand, dysfunctional adipose tissues can further worsen the gut microbial dysbiosis by altering secretion of adipokines. Our interventional studies in rodents demonstrated that administration of a single bacterial species, Akkermansia muciniphila, is sufficient to reverse obesity-related metabolic inflammation and atherosclerosis by preserving gut integrity. Our findings suggest that therapeutic interventions targeting the gut microbiota-adipose tissue axis may represent a promising strategy for prevention and treatment of obesity, diabetes and its vascular complications.

Part C: Research Output

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

(Please attach a copy of each 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) ***(bold** the authors belonging to the project teams and 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 this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2016				**Jin Li**, Shaoqiang Lin, Paul M. Vanhoutte, Connie W. Woo, **Aimin Xu***	Akkermansia Muciniphila Protects Against Atherosclerosis by Preventing Metabolic Endotoxemia-Induced Inflammation in Apoe-/- Mice. **Circulation** 2016 Jun 14;133(24):2434-46	No	Yes	Yes	Yes
2018				**Zhuohao Liu**, Leigang Jin, Jin-Kui Yang, Baile Wang, Kelvin K.L. Wu, Philip Hallenborg, **Aimin Xu*,** Kenneth K.Y. Cheng	The Dysfunctional MDM2-p53 Axis in Adipocytes Contributes to Ageing Related Metabolic Complications by Induction of Lipodystrophy. **Diabetes** 2018 Aug 21. pii: db180684. doi: 10.2337/db18-0684. [Epub ahead of print]	No	Yes	Yes	Yes

			2018	Jin LI, Jun LI, Hau Tak Chau, Connie W. Woo, **Aimin Xu***	Adiponectin alleviates dietary obesity-induced metabolic disorders via modulation of gut microbiota, to be submitted to PNAS	No	Yes	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 delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

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 this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
22th June-26th June/2018/Orlando, Florida, United States	Loss of Adipocyte MDM2 Causes Lipodystrophy by Inducing P53-Dependent Apoptosis and Senescence	American Diabetes Association 78th Scientific Sessions	No	Yes	Yes	Yes
4th March-8th March/2018/Banff, Alberta, Canada	Adiponectin antagonizes dietary-induced metabolic diseases via modulation of gut microbiota	Keystone Symposium: Manipulation of the Gut Microbiota for Metabolic Health	No	Yes	Yes	Yes
7th May-10th May/2015/San Francisco, California, United States	Role of Gut Microbiota in the Pathogenesis and Progression of Atherosclerosis in mice	Arteriosclerosis, Thrombosis, and Vascular Biology | Peripheral Vascular Disease 2015 Scientific Sessions	No	Yes	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
JIN LI	Doctor of Philosophy	01/09/2012	31/08/2016
ZHUOHAO LIU	Doctor of Philosophy	01/09/2014	31/08/2018
WENGJIN LIU	Master of Medical Sciences	01/09/2016	31/08/2017

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

1. Adiponectin antagonizes dietary-induced metabolic diseases via modulation of gut microbiota

Jin Li, Aimin Xu; 2018 Best Abstract in Basic Science&Translational, 23rd Medical Research Conference, The University of Hong Kong

2. Akkermansia Muciniphila Protects Against Atherosclerosis by Preventing Metabolic Endotoxemia-Induced Inflammation in Apoe−/− Mice, Jin Li, Connie Woo, Aimin Xu 2017 Faculty Outstanding Research Output Award, The University of Hong Kong

3. The Protective Role of Gut microbiota Akkermansia municiphila in the Pathogenesis and Progression of Atherosclerosis in mice, Jin Li, Connie Woo, Aimin Xu, 2015 Top 10 percent abstract, Arteriosclerosis, Thrombosis, and Vascular Biology | Peripheral Vascular Disease 2015 Scientific Sessions