RGC Ref. No.:
UGC/FDS11/M02/15
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Investigation of the differential roles of centrally located GLP-1 receptors in emesis and feeding in *Suncus murinus*

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. CHAN Sze-wa / Associate Professor	School of Health Sciences, Caritas Institute of Higher Education
Co-Investigator(s)	Prof. RUDD John A / Professor	School of Biomedical Sciences, The Chinese University of Hong Kong
Others		

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	01/01/2016		
Project Completion Date	31/12/2018	30/06/2019	Institution Approval granted on 23/11/2018
Duration *(in month)*	36	42	Institution Approval granted on 23/11/2018
Deadline for Submission of Completion Report	31/12/2019	30/06/2020	

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To determine if a "non-classical" GLP-1 receptor involved in the anorectic and emetic effects of GLP-1 receptor agonists involves the hypothalamus and/or amygdala.
2. To determine if the effects of GLP-1 (7-36) amide and exendin-4 to induce emesis and inhibit feeding are mediated via an exendin (9-39)-insensitive mechanism. These studies may indicate if exendin-4 acts differentially from endogenous GLP-1.
3. To determine if "non-classical" GLP-1 receptor mediates vasopressin release and changes gastric myoelectric activity, independent of effects on gastric emptying.

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

1.

2.

3. ....

5.3 Realisation of the objectives

(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

GLP-1 receptor agonists are used in the treatment of type 2 diabetes but they can be associated with reduced appetite, nausea and emesis. Three objectives were set up to investigate if GLP-1 receptors have differential roles in emesis and feeding control and the objectives have been achieved as follow: Objective 2. We first determined the *in vivo* potency of the endogenous GLP-1 [GLP-1 (7-36) amide]. Using an intraperitoneal glucose tolerance test, we found that central administration of GLP-1 (7-36) amide reduced blood glucose levels via an exendin (9-39)-sensitive pathway. Next, we investigated the effect of central administration of GLP-1 (7-36) amide on emesis, spontaneous activities and food and water intake over a period of 1 hour. We showed that GLP-1 (7-36) amide reduced the total distance travelled by the animals but the effect was not antagonized by exendin (9-39). In addition, GLP-1 (7-36) amide induced emesis and inhibited food and water intake and the effect was antagonized by exendin (9-39). To investigate the mechanism of GLP-1 (7-36) amide to induce emesis and modulate feeding, we determined the expression of c-Fos immunoreactivity in the brain using immunohistochemistry. We showed that GLP-1 (7-36) amide induced c-Fos expression in the brainstem and several hypothalamic nuclei. The increased c-Fos expression in the brainstem and hypothalamus was antagonized by exendin (9-39), excepting for the bed nucleus of the stria terminalis. Objective 1. Following our original plan, we determined the stereotaxic coordinates of the paraventricular hypothalamic nucleus (PVH) and central nucleus of the amygdala (CeA) based on the brain atlas of *S. murinus* established by our laboratory. We believed that the PVH is the key site in regulating emesis and feeding behaviour, thus, studies were progressed to investigate if intracerebral PVH administration of GLP-1 (7-36) amide and exendin-4 induce emesis and inhibit feeding. Our results showed that both exendin-4 and GLP-1 (7-36) amide inhibited food and water intake but only exendin-4 induced emesis. Objective 3. We investigated cardiovascular and gastrointestinal functions using radiotelemetry devices during the first 4 h, 4 – 5 h and 5 – 24 h post-drug administration. Changes in gastric myoelectric activity are known to be altered during nausea and emesis. We showed that both exendin-4 and GLP-1 (7-36) amide increased body temperature and systolic and diastolic blood pressure; exendin-4, but not GLP-1 (7-36) amide, also modulated heart rate and gastric myoelectrical activity. We then proceeded to investigate if the physiological changes were induced via an exendin (9-39)-sensitive pathway. We showed that exendin (9-39) alone had no effect on the parameters measured and it failed to antagonize the physiological changes induced by exendin-4. As central administration of exendin-4 can reliably induce emesis, we proceeded to investigate if a "non-classical" GLP-1 receptor modulates gastric emptying. Although the In-vivo Xtreme imaging system with a novel near-infrared (NIR) fluorescent imaging agent may permit a real time monitoring and quantification of gastric emptying rates, the signal detected using the 2-D imaging system did not reliably reflect the 3-D nature of the gastric contents. Thus, measuring a fluorescent signal using the current available equipment did not meet our requirements for determining gastric emptying. Therefore, we used the conventional charcoal meal test to evaluate the effects of centrally administered exendin-4 on gastric transit. In this study, we focused on the measurement of small intestinal transit time (the position of the charcoal front as a percentage of the total length of the gastrointestinal tract). Our results showed that central administration of exendin-4 inhibited gastric transit.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To determine if a "non-classical" GLP-1 receptor involved in the anorectic and emetic effects of GLP-1 receptor agonists involves the hypothalamus and/or amygdala.	✓	100%
2. To determine if the effects of GLP-1 (7-36) amide and exendin-4 to induce emesis and inhibit feeding are mediated via an exendin (9-39)-insensitive mechanism. These studies may indicate if exendin-4 acts differentially from endogenous GLP-1.	✓	100%
3. To determine if "non-classical" GLP-1 receptor mediates vasopressin release and changes gastric myoelectric activity, independent of effects on gastric emptying.	✓	100%

6. Research Outcome

6.1 Major findings and research outcome

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

The results from the intraperitoneal glucose tolerance test showed that GLP-1 (7-36) amide reduces blood glucose level in a dose-dependent manner and the glucose-lowering effect of GLP-1 (7-36) amide is mediated via central GLP-1 receptors. The failure of exendin (9-39) to modify blood glucose level suggests that the central GLP-1 receptor may not mediate a tonic inhibitory influence on systemic glucose homoeostasis in anaesthetized *S. murinus*. In conscious, fasted freely moving animals, GLP-1 (7-36) amide inhibited food and water intake dose-dependently. In contrast to exendin-4, the effect of GLP-1 (7-36) amide to inhibit food and water intake was mediated via an exendin (9-39)-sensitive pathway. GLP-1 (7-36) amide induced emesis in 15 – 30% animals and the effect was predictively antagonized by exendin (9-39). Moreover, GLP-1 (7-36) amide reduced the total distance travelled by the animals. Pretreatment with exendin (9-39) antagonized the increase in the time the animals spent lying flat but it did not antagonize significantly the reduced locomotor activity induced by GLP-1 (7-36) amide. In addition, GLP-1 (7-36) amide induced a characteristic pattern of c-Fos expression in the brain similar to that induced by exendin-4. Exendin (9-39) inhibited the increased c-Fos expression in the hypothalamus and brainstem, except for that in the bed nucleus of the stria terminalis (BNST). These data suggest that the action of GLP-1 (7-36) amide to induce emesis and inhibit food and water intake may involve GLP-1 receptors in the hypothalamus and brainstem. In a separate set of studies, intracerebral PVH administration of exendin-4 induced emesis and inhibited food and water intake significantly. Conversely, GLP-1 (7-36) amide had a trend to inhibit food and water intake but did not induce emesis. These data suggest that the GLP-1 receptors in the PVH are differentially involved in feeding and emesis control. In the telemetry studies, we demonstrated that exendin-4 increased body temperature, systolic and diastolic blood pressure, heart rate and increased dominant frequency (with a decrease in the bradygastria range, and an increase in the tachygastria range, whereas the normogastria range was unaffected) during the first 4 h post-injection. Exendin-4 also significantly inhibited food and water intake during 4 – 5 and 5 – 24 h post-injection periods. GLP-1 (7-36) amide increased body temperature, systolic and diastolic blood pressure but failed to modulate heart rate and gastric myoelectrical activity and did not affect food and water intake. In another set of studies, we showed that exendin-4 inhibited food and water intake up to 24 h; exendin-4 also induced emesis and increased heart rate and decreased heart rate variability during the first 4 h post-drug administration; exendin (9-39) alone had no effect on the physiological changes and failed to antagonize the action induced by exendin-4. In a separate set of studies, GLP-1 (7-36) amide did not induce significant changes in any of these physiological parameters. Furthermore, we demonstrated that exendin-4 significantly prolonged the gastric transit time, in addition, the weight of the stomach was higher in the exendin-4-treated animals compared to that in saline-treated animals.

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

We demonstrated that the non-classical GLP-1 receptor in the brain stem and hypothalamus may have differential roles in glucose homeostasis, emesis and feeding control in *Suncus murinus*. For further development, we will need to investigate the downstream modulators of the non-classical GLP-1 receptor activation in feeding and emesis control. Studies may also extend to dissect the effect of exendin-4 from modulating blood glucose level and other physiological parameters. These studies will allow us to assess if the non-classical GLP-1 receptor represents a potential target for drugs to ameliorate chemotherapy-induced emesis and also for anti-obesity development.

7. Layman's Summary

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

GLP-1 receptor agonists used in the treatment of type 2 diabetes can be associated with nausea, emesis and reduced appetite in man. In this study, we investigated the differential roles of centrally located GLP-1 receptor in emesis and feeding control using *Suncus murinus*, a species capable of emesis. We showed a non-classical GLP-1 receptor in the central nervous system was involved in the regulation of multiple physiological responses, including glucose homeostasis, locomotor activity, emesis, feeding, cardiovascular and gastrointestinal functions. Importantly, the synthetic GLP-1 analogue exendin-4, acts differently from endogenous GLP-1 in feeding control. The understanding of the differential roles of central GLP-1 receptor allows us to access if GLP-1 receptor ligands are potential targets for anti-emetic and anti-obesity development.

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)*	**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)*						
		Yes		Zengbing Lu, Sze Wa Chan* Longlong Tu, Man Piu Ngan and John A. Rudd	GLP-1 receptors are involved in the GLP-1 (7-36) amide-induced modulation of glucose homoeostasis, emesis and feeding in Suncus murinus (house musk shrew)/ European Journal of Pharmacology, Manuscript Number EJP-54241	No	No	Yes	No

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)*	**Accessible from the Institutional Repository** *(Yes or No)*
Nov/2016 / Australia	Action of centrally administered glucagon-like peptide-1 (7-36) amide to modulate blood glucose, feeding and induce emesis in *Suncus murinus*	Joint Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) and Molecular Pharmacology of G Protein-Coupled Receptors (MPGPCR) Scientific Meeting	Yes	Yes	Yes	No
Sep/2017/ Korea	Centrally administered GLP-1 (7-36) amide reduces plasma glucose levels in *Suncus murinus* via exendin-(9-39)-sensitive mechanisms	2017 International Congress of Diabetes and Metabolism	No	Yes	Yes	No
Dec/2017 /Australia	GLP-1-induced anorectic and emetic responses are mediated via exendin (9-39)-sensitive mechanisms in *Suncus murinus*	APSA-ASCEPT 2017 Joint Scientific Meeting	No	Yes	Yes	No
Jun/2018/ U.S.	Central GLP-1 receptors are differentially involved in emesis and feeding control in *Suncus murinus* (House Musk Shrew)	Digestive Disease Week® (DDW) 2018	No	Yes	Yes	No
Jul/2018/Japan	The Central Actions of the Glucagon-like Peptide-1 Receptor Agonist, Exendin-4, in *Suncus murinus*: A telemetric study	18th World Congress of Basic and Clinical Pharmacology	No	Yes	Yes	No

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

New knowledge of the pharmacology of the glucagon-like peptide-1 receptor system, mechanism of chemotherapy-induced nausea and emesis and neuroendocrine control of feeding have contributed to the teaching and learning of several courses including Human Anatomy and Physiology, Human Pathophysiology and Pharmacology and Therapeutics.

11. 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
Nil	Nil	Nil	Nil

12. Other Impact
(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

This project was conducted in collaboration with the School of Biomedical Sciences, the Chinese University of Hong Kong.

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
No. of outputs arising directly from this research project	1	5	0	0	Type	No.

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
Nil	Nil