RGC Ref. No.:
UGC/FDS11/M02/16
(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

Potential involvement of NUCB2/nesfatin-1 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	CHAN Sze-wa/ Associate Professor	School of Health Sciences / Caritas Institute of Higher Education
Co-Investigator(s)	RUDD John A/ Professor	School of Biomedical Sciences / The Chinese University of Hong Kong
Co-Investigator(s)	LIN Ge/ Professor	School of Biomedical Sciences / The Chinese University of Hong Kong
Co-Investigator(s)	SAKAI Takafumi/ Professor and Dean	Graduate School of Science and Engineering / Saitama University, Japan
Co-Investigator(s)	SAKATA Ichiro/ Associate Professor	Graduate School of Science and Engineering / Saitama University, Japan

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	1 Jan 2017	N/A	N/A
Project Completion Date	31 Dec 2019	30 June 2020	Institution Approval granted on 9/12/2019
Duration *(in month)*	36	42	Institution Approval granted on 9/12/2019
Deadline for Submission of Completion Report	31 Dec 2020	30 June 2021	N/A

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To characterize the expression and distribution of NUCB2/nesfatin-1 and potency of nesfatin-1 that will be used in experiments in conscious animals.
2. To determine if nesfatin-1 pathway activation inhibit food and water intake, induce emesis and potential activation of vasopressin circuitry in the brainstem and forebrain, induce changes in GMA and modulate hypothalamic monoamines.
3. To determine if the action of nesfatin-1 to reduce food intake and to induce emesis is prevented by conventional anti-emetic drugs. These studies may indicate if nesfatin-1 acts differentially to modulate feeding and emesis.

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)

Nesfatin-1 is an anorectic peptide derived from nucleobindin2 (NUCB2). NUCB2/nesfatin-1 have been identified in peripheral tissues and brain areas involved in the regulation of feeding, emotion and emesis. However, no study has investigated the role of NUCB2/nesfatin-1 in a species capable of emesis. In this project, we investigated the role of NUCB2/nesfatin-1 in mechanisms of feeding, biomarkers of nausea, and mechanisms of emesis in *Suncus murinus*. Objective 1: The amino acid sequence of *S. murinus* nesfatin-1 was deduced using *in silico* cloning. The amino acid sequence of nesfatin-1 is highly conserved among humans, rats, mice, and *S. murinus*. The expression of NUCB2 mRNA and nesfatin-1 protein in the brain and gastrointestinal tissues were identified using conventional RT-PCR and western blot, respectively. NUCB2 mRNA was detected in the entire brain as well as in the gastrointestinal tissues. Western blot confirmed the expression of nesfatin-1 protein in these tissues. Utilising quantitative real-time PCR methods, the expression of NUCB2 mRNA between *ad libitum*-fed animals and animals deprived of food for 24 h were evaluated. The expression of nesfatin-1 mRNA in the hypothalamus, hippocampus and brainstem were down-regulated, but up-regulated in the striatum following food deprivation. Next, the distribution of nesfatin-1 immunoreactivity was identified using immunohistochemistry. Nesfatin-1 immunoreactivity was localized in the brainstem and hypothalamus. Positive nesfestin-1 immunoreactive cells were also detected in the gastric antrum, duodenum, ileum and colon. The effect of nesfatin-1 on isolated gastric antrum and intestine was determined isometrically and it was found that nesfatin-1 failed to either contract or relax isolated gastrointestinal tissues.

Objective 2: To investigate the effect of a central administration of nesfatin-1 on feeding, emesis and locomotor activity, nesfatin-1 was administered intracerebroventricularly into conscious, freely moving animals after an overnight fast. Nesfatin-1 induced emesis and inhibited food and water intake for up to 24 h, without any effect on locomotor activity. To investigate the central pathway activated by nesfatin-1, brains were sectioned and processed for c-Fos immunohistochemistry. We found that nesfatin-1 elevated c-Fos expression in the brainstem and hypothalamus, however, c-Fos was not elevated in vasopressin neurons. To examine if nesfatin-1 modulates hypothalamic monoamine levels, brain tissues were collected after drug administration and the levels of hypothalamic 5-hydroxytryptophan (5-HTP), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline and dopamine, were assayed using LC-MS. There is some evidence that glutamate and γ-aminobutyric acid (GABA) may be involved in emesis, thus, glutamate and GABA were also measured. There was no significant change in any of the neurotransmitters measured. Changes in gastric myoelectric activity (GMA) are known to be altered during nausea and emesis, thus, we investigated if nesfatin-1 modulates cardiovascular and gastrointestinal functions using radiotelemetry devices during the first 4 h, 4 – 5 h and 5 – 24 h post-drug administration. Nesfatin-1 has no significant effect on body temperature, GMA and cardiovascular functions. To investigate if the action of nesfatin-1 involves the central corticotropin-releasing factor 2 (CRF2) signalling system, the CRF2 receptor antagonist, astressin 2B or saline was administered prior to nesfatin-1 or saline. Astressin 2B did not attenuate the anorectic effect of nesfatin-1.

Objective 3: Based on our findings, we believed that the mechanism of action of nesfatin-1 may involve receptor systems that regulate emesis and also play a pivotal role in feeding control. Thus, studies were progressed to examine if the action of nesfatin-1 can be antagonised by the glucagon-like peptide-1 (GLP-1) receptor antagonist exendin-9. In these experiments, exendin-9 or saline was administered prior to nesfatin-1 or saline. Exendin-9 did not antagonise the emetic and anorectic actions of nesfatin-1. Next, we examined if the action of nesfatin-1 can be antagonised by ghrelin which is known to increase food intake. Ghrelin or saline was administered prior to nesfatin-1 or saline. Ghrelin failed to antagonise the anorectic action of nesfatin-1.

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 characterize the expression and distribution of NUCB2/nesfatin-1 and potency of nesfatin-1 that will be used in experiments in conscious animals.	✓	100%
2. To determine if nesfatin-1 pathway activation inhibit food and water intake, induce emesis and potential activation of vasopressin circuitry in the brainstem and forebrain, induce changes in GMA and modulate hypothalamic monoamines.	✓	100%
3. To determine if the action of nesfatin-1 to reduce food intake and to induce emesis is prevented by conventional anti-emetic drugs. These studies may indicate if nesfatin-1 acts differentially to modulate feeding and emesis.	✓	100%

6. Research Outcome

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

We confirmed that the amino acid sequence of *Suncus murinus* nesfatin-1 shared over 85% homology with humans, rats, and mice. RT-PCR analysis showed that NUCB2 mRNA was detected in the entire brain and also in the stomach and gut. Western blot analysis confirmed the expression of nesfatin-1 protein. NUCB2 mRNA in the hypothalamus, hippocampus and brainstem were down-regulated following 24 h food deprivation, whereas, the mRNA level in the striatum was up-regulated. Nesfatin-1 immunoreactivity was detected in the hypothalamus, brainstem, gastric antrum and gut. Taken together, our results suggest that NUCB2/nesfatin-1 may have a wider physiological role, in addition to metabolic homeostasis and appetite in *S. murinus*. Furthermore, NUCB2/nesfatin-1 may be regulated by nutritional status and associated with emotional change during fasting. Organ bath assays found that nesfatin-1 failed to either contract or relax isolated gastric antrum and intestinal preparations even though nesfatin-1 immunoreactive cells were detected in these tissues. These findings suggest that the inhibitory factor to inhibit gastric motility may probably act via central pathway that could not be replicated using isolated gastrointestinal tissues. In conscious, freely moving animals, intracerebroventricular administration of nesfatin-1 induced emesis and suppressed significantly 6-h cumulative food intake, without affecting the latency to feeding and locomotor activity. Nesfatin-1 did not produce a significant effect on water intake in the first six hours, however, it inhibited significantly both food and water intake at 24 h post-administration. In a separate set of experiments, nesfatin-1 elevated c-Fos expression in the nucleus tractus solitaries and several hypothalamic nuclei, including the arcuate nucleus, paraventricular nucleus, central nucleus of the amygdala and supraoptic nucleus. These data suggest that the action of nesfatin-1 may be mediated via neuronal circuitry in the hypothalamus and brainstem. LC-MS analysis of neurotransmitters showed that nesfatin-1 did not modify the levels of hypothalamic monoamines, glutamate and GABA. Radiotelemetry studies showed that nesfatin-1 did not modify significantly body temperature, blood pressure, heart rate, heart rate variability and GMA. In a separate set of studies, nesfatin-1 reduced food intake but it failed to induce emesis. Pretreatment with astressin 2B did not affect the action of nesfatin-1. In another set of studies, nesfatin-1 induced emesis and inhibited food intake. Pretreatment with exendin-9 failed to antagonise the action of nesfatin-1. In other studies, nesfatin-1 inhibited food intake but did not induce emesis. Pretreatment with ghrelin failed to antagonise the action of nesfatin-1. These data suggest that nesfatin-1 has produced effects via an unknown mechanism that is unique and requires further investigation.

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

We demonstrated for the first time that nesfatin-1 pathway activation inhibits food and water intake, induces emesis and elevates c-Fos expression in the brainstem and hypothalamus in *Suncus murinus*. We found that nesfatin-1 did not activate the key brain area connecting the brainstem emetic reflex centre to the forebrain. Furthermore, we demonstrated that the action of nesfatin-1 acts differentially to modulate feeding and emesis and via an unique unknown mechanism. Further experiments may reveal new targets to develop drugs for the treatment of nausea, emesis, and perhaps obesity-related diseases. Identification of the NUCB2/nesfatin-1 receptor will allow subsequent development of selective probes to dissect the mechanism of action of nesfatin-1.

7. Layman's Summary

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

Nesfatin-1 is an anorectic peptide derived from nucleobindin2 (NUCB2). This project investigated the mechanisms of the action of nesfatin-1 in emesis and feeding control in *S. murinus*. We demonstrated that nesfatin-1 is highly conserved in *S. murinus*, humans, and rodents. NUCB2/nesfatin-1 was expressed in the gastrointestinal tract and also brain areas involved in the regulation of feeding, emotion and emesis in *S. murinus*. The expression of NUCB2 mRNA in the brain may be associated with nutritional status. In conscious, freely moving animals, we demonstrated that central administration of nesfatin-1 induced emesis and inhibited food and water intake, but not locomotor activity. Furthermore, the action of nesfatin-1 may involve neuronal circuitry in the hypothalamus and brainstem and via an unique unknown mechanism.

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, Dexuan Cui, Julia Yuen Hang Liu, Bin Jiang, Ichiro Sakata, Shota Takemi, Takafumi Sakai, Man Piu Ngan, Ge Lin, Sze Wa Chan* and John A Rudd	The actions of centrally administered nesfatin-1 on gastrointestinal contractility, emesis, feeding, and locomotor activity in Suncus murinus (House Musk Shrew)/ *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* (manuscript number R-00162-2021)	No	Yes (Attachment 1)	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)*
12/ 2017/ Australia	Nesfatin-1 suppresses feeding and induces emesis in *Suncus murinus* (House Musk Shrew)	Australasian Pharmaceutical Science Association (APSA) and Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) 2017 Joint Scientific Meeting	2018	Yes (Attachment 2)	Yes	No
7/ 2018/ Japan	The role of nesfatin-1 in the regulation of feeding and emesis in *Suncus murinus* (House Musk Shrew)	18th World Congress of Basic and Clinical Pharmacology	No	Yes (Attachment 3)	Yes	No
9/ 2019 / Portugal	Effects of astressin 2B and nesfatin-1 on food and water intake in *Suncus murinus*	4th Biennial Meeting of the European Society of Neurogastroenterology and Motility	No	Yes (Attachment 4)	Yes	No

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

Research experience and new knowledge of the role of NUCB2/nesfatin-1 and mechanism of chemotherapy-induced nausea and emesis has contributed to 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			

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 and Graduate School of Science and Engineering, Saitama University, Japan.

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	3	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