From Medicine to Discovery:
A Clinician Scientist's Journey

By Professor Melvin Leow Khee Shing

About the Author

MBBS, MMed (Int Med), FAMS, FACE, FACP, FRCP (Edin), FRCPath, PhD

Professional Appointments

- Senior Consultant Endocrinologist, Dept of Endocrinology, TTSH
- Senior Principal Investigator, A*STAR
- Clinician Scientist, College of Clinician Scientists, Academy of Medicine and NHG
- Adjunct Professor, Department of Medicine, Yong Loo Lin School of Medicine, NUS
- Adjunct Professor, Duke-NUS Medical School
- Professor, Lee Kong Chian School of Medicine, NTU

Early Research and Clinical Foundations

It takes time, often years, for any research results to be validated and accepted by the medical community.

Although none of my current research has changed medical practice thus far, I believe some of my research may eventually find clinical applications based on the citations of my publications by scientists round the world. Undeniably, it takes time, often years, for any research results to be validated and accepted by the medical community.

In terms of research experience, I started out in the 'Endocrine Unit' of the Department of General Medicine at Tan Tock Seng Hospital as a co-investigator for various Phase 3 clinical trials sponsored by pharmaceutical companies from 1999 till Singapore got struck by SARS in 2003.

It was then that I decided to get my feet wet in PI-initiated studies. My first one was a BMRC-funded study that revealed hypocortisolism as one of several post-SARS sequelae which won me the NHG Doctor Award for translational research. Then I got awarded a NMRC IRG to investigate latent tuberculosis infection in people with diabetes. We all learned in medical school that diabetic patients are at risk for tuberculosis, but we don't know how rampant this is in Singapore. Our study revealed that up to 25% with diabetes in our clinics have latent tuberculosis infection, a staggering figure. Interestingly, those on metformin were less likely to have latent tuberculosis than those on non-metformin based anti-diabetic therapy. The protective effect of metformin against tuberculosis was then proven in mice by A*STAR scientists collaborating with my clinical team that included Prof. Sonny Wang and A/Prof Cynthia Chee. This was published in Science Translational Medicine and supports the use of metformin not only as an antiglycemic agent but also for its anti-TB properties in diabetes.

A Prophetic Tale

My research life and clinical journey in endocrinology drew some uncanny parallels with a certain novel.

At this point, it's timely to share a little tale. My research life and clinical journey in endocrinology drew some uncanny parallels with a certain novel. During my army days back in 1984 prior to entering med school, a lady military staff (Sgt. Vasugi) loan me a science fiction book entitled, "Informed Consent" authored by an endocrinologist named Dr. Neil Ravin. She said I should enjoy reading it because she knew I was intending to become a medical doctor. Although she said I could take a fortnight before returning to her, I got so riveted by the story I literally finished the 368 pages book within a couple of days.

It was a medical thriller based on a patient with an exceptionally rare adrenal tumour called a pheochromocytoma. Although meant to be fictional, plenty of stuff written in that book were factually correct and technically decades ahead of its time. It discussed the life of an endocrinologist called Dr. Brophy who took a leap of faith to switch career to become a clinician scientist. He was a dedicated and caring doctor but who also harboured a deep sense of curiosity and passion for science. His endocrine training helped him correctly diagnose a lawyer with pheochromocytoma when he presented with hypercalcemia and paroxysmal hypertension. This was a time when I was still doing my national service and had no inkling what specialty I would enter even if I should be selected to study medicine.

My clinical path after I graduated as a young doctor was anything but smooth. Endocrinology was not my first choice as a specialty, but I grabbed the traineeship offer in 1999 due to my need for a better salary to support my wife and young daughter. Soon after, I would serendipitously encounter three pheochromocytoma patients all admitted sequentially within a few months in a fashion eerily reminiscent of the ghosts of Christmas in the classic Charles Dickens story. In fact, the very first ‘pheo’ patient I was called to see at the ICU came as a surprise when the medical registrar on call contacted me while fireworks pyrotechnics lit the skies on our National Day in 1999. This was phenomenal because the annual incidence of this disease is less than 1 per 100,000 person-years.

Importantly, all three ‘pheo cases’ had hypertensive crises after they were administered metoclopramide as an anti-emetic agent. At that time, none of my medical team had known of this connection. But as an endocrine trainee, I had an instant epiphany as the “metoclopramide stimulation test for pheo” was something I had just read some weeks prior. In each instance, I observed how the ‘pheo crisis’ was temporally linked to metoclopramide and realized the mortal danger this ‘normally innocuous’ drug became when injected into patients harbouring ‘pheos’.

When we publish good science, that knowledge can impact positively on human lives beyond our shores and our imagination.

One might say that the ‘pheo’ is analogous to a time bomb ready to be set off by suitable triggers such as metoclopramide. Since most emergency physicians and general medicine ward doctors were oblivious of such risks, to prevent future catastrophes, I quickly published this as a case series to raise awareness. To my surprise, the European Medicines Agency (the equivalent of the FDA in the US) responded to my paper soon after and contacted me to request for more detailed anonymized data regarding the time-relationship in each of the cases. They subsequently issued a cautionary warning on prescribing metoclopramide across Europe that anyone with a history of paroxysmal or refractory hypertension should be viewed with circumspect prior to prescribing metoclopramide and contraindicated its use in those with proven pheochromocytoma. It was the first time I realized that when we publish good science, that knowledge can impact positively on human lives beyond our shores and our imagination.

This most astounding 'fiction' turned out to be scientifically correct.

Incredibly, this fictional book published by Neil Ravin in 1984 also elaborated towards the final chapter that so called "metastases" found during surgery to extirpate the lawyer’s adrenal tumour turned out to be brown fat induced by catecholamines secreted by the pheochromocytoma. On hindsight, this most astounding 'fiction' turned out to be scientifically correct, as a PubMed search revealed that brown fat was characteristically found among adult patients with pheochromocytoma in 10 papers published between 1962-1982. As fate would have it, brown fat would subsequently become pivotal in my research journey some three decades later. Given the scarcity of such knowledge and that PubMed only became available worldwide from 1996, I was amazed how the author knew about this association between

brown fat and pheochromocytoma, unless he witnessed such brown fat deposits within a real adult pheo patient he personally managed. Ironically, it was accepted as fact that brown adipose tissue (BAT) ceases to persist beyond infancy, which suggested this novelist was bold enough to challenge the mainstream mindset of that era. The ultimate proof for the presence of BAT in healthy adults would only emerge nearly a decade after the start of the new millennium. I should confess that I did not even know what brown fat was prior to entering university, though I later learned from medical school lecturers in histology and embryology in 1985 that brown fat was supposedly present only in neonates and infants but non-existent in adults, this fallacy being based on a single paper published by Dr Shinkishi Hatai in an anatomical journal in 1902 which sounded the 'death knell' for brown fat for over a century. That old paper turned out wrong when three independent research groups coincidentally published in the same issue and volume of the New England Journal of Medicine in 2009 that functional brown fat unequivocally exists in healthy adult humans.

By chance, the first author of one of the three key landmark papers (Dr. Aaron Cypess) was a house officer with whom I conducted daily ward rounds at Beth Israel Deaconess Medical Center and the Brigham and Women's Hospital during my clinical fellowship in Harvard over two decades ago. As an endocrinologist, I was naturally excited by this discovery. Based on the physiology that only a mere 50 grams of healthy brown adipose tissue (BAT) is sufficient to oxidize fat and glucose to keep us free from metabolic decompensation, it certainly holds therapeutic promise and potentials for obesity and diabetes, making it an area worthwhile investigating.

All said, it is timely to introduce the thyroid at this juncture, since unlike pheochromocytoma, thyroid conditions are much more common by far, being second to diabetes in prevalence. Thyroid hormones are intimately associated with BAT mass and activity in the body since it orchestrates the differentiation of preadipocytes into brown fat cells and regulate the conversion of unhealthy white fat into brown fat, a process known as 'browning'. Thyrotoxicosis is characterized by increased metabolic rate, thermogenesis and weight loss partly due to overactive brown fat. By contrast, people with hypothyroidism tend to be easily obese because they are deficient in brown fat. For reasons only God would know, thyroid homeostasis, metabolic physiology and adipose tissue biology ultimately became that 'triangle' shaping my research career and focus.

Sometimes it pays to be persistent in the face of insurmountable challenges.

It stands up to logic of leaving no stones unturned when humanity is confronted with prevalent diseases that presently have no cures. Whether BAT can or cannot improve the population's metabolic health remains hypothetical until someone proves or disproves that. While I am passionate to run experiments to test such hypotheses, I cannot execute anything without grant funding. Grant agencies are not so easily convinced by ideas that sound too esoteric or at high risks for failure. As well, failure is often not easy 'pill' to swallow. Yet scientists are constantly reminded that successes are preceded by many failures. I have thankfully received national competitive funding by the NMRC for the fourth round to do brown fat research since 2015. I know of drug companies which have given up on BAT research on grounds that it is a difficult 'moonshot' without disruptive technology. Sometimes it pays to be persistent in the face of insurmountable challenges. I can resonate with those words of US President John F. Kennedy who famously said, "We choose to go to the moon not because it is easy, but because it is hard". Hopefully, my team and I can discover new ways to wrest the power of brown fat and exploit it effectively as an additional weapon in the therapeutic arsenal against obesity and diabetes. If this works, it can be impactful by reducing healthcare costs and facilitating healthy ageing.

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US President John F. Kennedy

The Journey to Becoming a Clinician Scientist

Be true to yourself.

Everyone has a unique story. Mine is no exception. Prior to embarking on my research career, I have heard that "clinician scientists are a self-selected group". There's some truth in that. Someone also remarked that "it's hard for the uninitiated to be initiated". Still others warned that "the time and effort well exceed that of a full-time clinician", not to mention the "substantial risk of failures at grant attempts". These daunting statements got me thinking about my own situation as I weighed the pros and cons of embarking on such a seemingly 'unstable' career track. It was a time when I was busy training to become an endocrinologist with lots of competing priorities and limited bandwidth. But a good friend in my department (Dr. Winston Kon) also told me this, 'Be true to yourself'.

This brought back to mind the day I was being interviewed by a panel of professors when I was shortlisted for medical school. When asked why I wanted to join the medical profession, I waxed lyrical about how I hope to be a doctor to better understand medicine so that I can do research to advance medical science and benefit patients round the world. Despite being forewarned that it could be dangerous to bring up research during the interview, I was surprised the panellists remarked that they were all very impressed.

Research was not as well charted back in the eighties compared to now in Singapore. There was no formal research course in our local medical curriculum and mentors were sorely lacking. I relied purely on instinct and a strong, persistent passion to hone myself in various skillsets and knowledge territories that I believe could equip me to become a clinician scientist. I spent more time in the university libraries reading research journal papers than I did on my medical textbooks. A classmate thought I was crazy and wasting my precious time.

Nothing you learned will ever be wasted.

At one point, I was teaching myself subjects that appear a total disconnect from medicine. For instance, I taught myself engineering mathematics not because it was part of the medical curriculum but because I believed that various natural phenomena can be mathematically

described. I delved into special and general relativity, cosmology, astronomy, particle physics and quantum mechanics. Spending my Saturday afternoons during my preclinical years learning vector fields, Killing vector, tensor calculus, Lagrangian optimization, Jacobians and differential geometry probably differed from the typical medical student's routine. Getting stuck with Laplacian operator in polar coordinates and learning the various special functions (eg. Legendre polynomials, Bessel functions, Laguerre function) to crack the Schroedinger's equation were self-imposed challenges. These proved useful years later when I was pursuing my PhD and had to solve the crystal structures of protein molecules based on X-ray diffraction data in my structural biology course. I appreciated how my understanding of wave mechanics and Fourier transforms becomes handy.

This showed me that the knowledge gained years ago can turn out helpful one day in research. In this respect, the words from a fellow endocrine peer (Dr. Winston Kon again) once wisely remarked that "Nothing you learned will ever be wasted" proved so true. Till today, especially when mulling over tricky clinical questions, my natural tendency is to frame it in a way that a mathematical model can be intuitively constructed to analyse, simulate or solve the problem. I find this works for various aspects of endocrine physiology and appreciate the insights such math models yield when certain medical questions aren't readily tractable to laboratory or clinical experimentation.

The Language of Science and Medicine

Clinician scientists develop a certain mental dexterity to be comfortable in both worlds.

Basic science, bench skills and the language of science are quite foreign to most clinicians. There was indeed a steep learning curve to master laboratory skills and operation of various high tech scientific laboratory instruments. I recalled a time soon after the SARS outbreak when I took up many costly self-sponsored molecular biology courses. This contrasted greatly from the familiar routine of seeing patients in the hospital wards and the outpatient clinics.
The efforts paid off well as it prepared me when I transitioned from a full-time clinician to engage in basic science and molecular biology in an epigenetics laboratory at the Brenner Centre for Molecular Medicine in 2008. The typical boffin has a vocabulary of common wet lab words like 'Invitrogen', 'Qiagen', 'Eppendorf', 'sonicator', 'Falcon tube', 'parafilm', 'ddH2O', 'Mr. Frosty', 'NanoDrop', 'Thermal cyclers', 'Agilent MassHunter', 'PBS', 'PCR', 'Westerns', 'Amplicon' and '1st BASE'.

Medicine is the tutor of biology.

Clinician scientists develop a certain mental dexterity to be comfortable with 'Son-of-Sevenless (SOS)', 'sonic hedgehog', 'PI3K-AKT-mTORC1', 'BLAST', 'UniProt', 'SWI/SNF', 'KEGG', 'PyMOL' and 'AlphaFold2' on the one hand while discussing with lab scientists, and switching vocabulary to 'MODY', 'Novo Nordisk', 'Eli Lilly', 'Merck', 'chylomicronemia', 'Refetoff syndrome', '5'-deiodinase', 'germline', 'TRAb', 'propylthiouracil', 'Burch-Wartofsky Score' and 'Graves' disease' when speaking with clinicians. Over time, I began to acquire and familiarize myself with the lingo of scientists and blending with medical jargon that clinicians are used to. It helped me to understand, troubleshoot and critically review scientific data, to figure out why experiments failed and appreciate scientific papers from the perspective of a basic scientist. Familiarizing with the technical language of science and medicine allowed me to interact more effectively and meaningfully with basic scientists and in turn translate the information meaningfully to clinicians.

Often, our research hypotheses began from clinical observations that range from subtle to intriguing, from puzzling to blatant pain points. Also, debilitating diseases with no cures tend to motivate clinician scientists to start thinking about solutions. It could also be something physicians have been treating for decades but not much progress has been made, or that the available treatment modalities are associated with much side effects. Many diseases are likened to 'experiments of nature gone awry'. During my endocrine fellowship in Boston, Massachusetts, a Harvard professor (Prof. Gordon Strewler), who autographed an endocrinology textbook I used during my advanced traineeship, gave an interesting lecture entitled, 'Medicine is the tutor of biology'. He explained how our knowledge of normal biological processes mostly came from better understanding of disease mechanisms. In that regard, those diseases with still unsolved mechanisms within any medical discipline can be the start point for scientific hypotheses and formal research.

Making a Difference Through Discovery

Much credit goes to the splendid teamwork of fellow scientists and clinical collaborators without which I could not have possibly achieved anything much

What difference have I made to the world? On my own, probably very little. Much credit goes to the splendid teamwork of fellow scientists and clinical collaborators without which I could not have possibly achieved anything much. I shall highlight a few discoveries my team and I made which could prove useful at the bedside one day.

The first of such discoveries began with an unexpected phone call from a Dutch mathematical engineer named Simon Goede in 2012. He apparently read a mathematical model of the hypothalamus-pituitary-thyroid axis I published back in 2007 as the solo author. He believed that this model could be modified so that it could be applied to treat thyroid patients in a personalized manner by tweaking some of its parameters. He invited me to his home in the Netherlands and we ended up brainstorming mathematics daily. Our mathematical calculations ultimately succeeded when we jointly showed using three independent techniques – maximum curvature theory, loop gain theory and Newton-Raphson roots for non-linear simultaneous equations that converged nicely to derive the unique homeostatic euthyroid set point of thyroid patients so that we can treat each patient on an individual basis. The key twin formulae to pin down any individual's unique euthyroid set point were named the 'Leow-Goede equations of euthyroid equilibrium'. We co-published an 18-chapter textbook on mathematical thyroidology entitled, "Thyroid Systems Engineering – A Primer in Mathematical Modeling of the Hypothalamus-Pituitary-Thyroid Axis' that is available in the libraries of NUS and NTU and the shelves of Amazon and Kinokuniya bookstores. From these equations, we formulated a software with the help of a German thyroidologist (Johannes Dietrich) that became developed into an app called 'Thyroid-SPOT' (Set Point Optimization and Targeting), a strategy well aligned to the tenets of personalized and precision medicine which I hope will bring much benefit and relief to those suffering from thyroid hormone imbalance globally. As a motivational update, an independent pair of brilliant mathematicians from Austria very recently used highly advanced mathematics to independently prove that the Leow-Goede equations are

mathematically correct. This is notable as they showed that these equations we derived unlocked the 'hidden secret' of the euthyroid set point. Analogous to how Newton's laws of motion underpin mechanics, the Leow-Goede equations represent the first principle governing the homeostasis of the hypothalamus-pituitary-thyroid axis and are fundamental to the elucidation of the unique and individualized euthyroid set point of any given individual, thereby opening the door to personalized thyroidology. It is likely that the drifting of this set point alters the metabolic rate as humans age and could account for the surge in obesity and diabetes with aging. Knowing the personalized set point of a person holds the promise of improving metabolic health by instituting various pharmacological, nutraceutical or non-pharmacological measures to nudge the hypothalamus-pituitary-thyroid axis and restore the set point to its original optimal position. My hope is that the Thyroid-SPOT software can be made available free of charge and accessible by any physician in the world one day.

The second notable discovery was my quest to develop a method of inter-converting the laboratory results of thyroid function test from one assay system to another. For decades till the present, the harmonization of thyroid function test assays has not been very successful. Hence, a patient being assessed on serum free thyroxine (FT4) and thyroid stimulating hormone (TSH) using one laboratory's assay system can lead to results that differ considerably when the same blood sample was analyzed using a different assay system in a different clinic or hospital. This presents difficulty when for the patient who consults a doctor with a stack of FT4/TSH results from different labs as it will prove difficult ascertaining to what extent the numbers are different due to assay differences versus actual biological changes in response to treatment. I first derived a mathematical equation to solve this issue and decided to share it with a NHG mathematician (Dr. Meng Fanwen) who confirmed I was right. We then discussed further and jointly developed a linear transformation algorithm to allow the interconversion of thyroid function test results between assay systems which will aid doctors in interpreting changes in the context of therapy with thyroid medications. Together with Fanwen, we also improved the speed of computations by using matrix multiplications. I subsequently extended the mapping using mathematics inspired by Albert Einstein who famously applied it in his general relativity model of gravitation – namely differential geometry and tensor calculus which provided more powerful and accurate conversions when huge datasets are available. The complicated tensor equation reduces to exactly the same affine equation outlined above when data is limited. Our equations were validated using a database from an American thyroidologist (Prof. Jacqueline

Jonklaas) with comparisons across platforms including immunoassays, mass spectrometry and equilibrium dialysis, a gold standard. We then developed a mobile phone software app called 'ThyroConvert' with the help of an ITE lecturer (Mr. Chionh Puay Kiat) and made it available for free on Android phones. Unfortunately, we ran out of funds to develop an iOS compatible 'ThyroConvert' for iPhones. More recently, with the help of an app developer who happened to be the brother of one of my thyroid patients, we produced a web-based ThyroConvert accessible by any mobile phone platform and by laptops/desktops. We also integrated ThyroComvert into Thyroid-SPOT for more accurate and precise computation of the euthyroid set point. I hope that we can somehow eventually get this available free of charge to all doctors round the world as this conversion program allows the rapid alignment of plasma free thyroxine and TSH readouts from different labs featuring disparate normal population ranges.

The third discovery arose from my NMRC-funded project on brown adipose tissue (BAT). It was interesting to find that when BAT is activated, the level of thyroid hormones increased momentarily associated with slight TSH suppression. This implied that a yet unknown and uncharacterised molecule secreted by active BAT probably served as a stimulator of thyroid hormone synthesis by thyroid follicular cells. This means that we have inadvertently discovered the existence of a novel "thyroid-BAT hormonal axis" which might translate into a therapeutic target for hyperthyroidism and hypothyroidism in the years to come.

The fourth discovery was our detection of a mitochondrial protein – MTHFD1L which is overexpressed and released into the circulation as molecular cargoes within extracellular vesicles called exosomes when BAT is activated. I was very committed to deciphering the BAT secretome and discovering which molecule could be used as a convenient biomarker for BAT activity. This finding is currently being patented, and we are now doing further experiments to aid in the development of point-of-care test (POCT) to facilitate the assessment of brown fat activation by cold stimulation, functional foods or nutraceuticals instead of relying on the present gold standard but costly PET-CT or PET-MRI to determine BAT. This can catalyse and accelerate BAT research by food, nutraceutical and pharmaceutical companies because the current bottleneck is due to the high costs and ionizing radiation posed by PET scanning.

The fifth notable contribution to the BAT field was our development of infrared thermography (IRT) as a non-invasive and cost-effective tool to study BAT activation. This appeared to be quite precise and reliable when compared against PET-MRI, and our group had subsequently published several papers applying IRT to human BAT studies. Interestingly, a basic scientist and bioengineer near my laboratory requested me to utilize IRT to image BAT of mice for which my team and I experienced considerable difficulty. This posed a conundrum which occupied my mind for weeks. After mulling over the problem, I finally did some algebraic calculations which culminated into an elegant mathematical proof of the limitations of IRT when used for small animals like mice though it is pretty accurate for humans. I published this mathematical treatise in a respectable endocrine journal a couple of years ago. My hope is that IRT can become integrated into mobile phones so that people can assess how much their lifestyle (exercise, foods) can or cannot activate their BAT via infrared selfies. Such portable IRT can motivate people to live healthily and reduce morbidity and healthcare costs.

The sixth significant scientific discovery came about through a serendipitous collaboration with a molecular biologist group at the Institute of Cell and Molecular Biology (IMCB) in A*STAR. This concerned our discovery of an exceptionally rare and novel obesity syndrome yet to be named due to a mutation in a gene responsible for the formation of lipid droplets in adipocytes such that people carrying such a mutation will have under-functioning brown fat cells. Our research group in collaboration with a large team of brilliant international scientists and doctors are the world's first to stumble upon and then fully characterize this yet unnamed disease at the molecular level and deeply phenotype this condition. While the hard work in our labs took nearly four years to complete, every single day felt surreal and was filled with excitement. Although on the surface, this rare disorder may seem unlikely to be encountered by doctors in general, the clinical importance is that understanding the unique disease mechanism can potentially unlock innovative methods of enhancing brown fat function among those with little brown fat. That can potentially lead to an entirely new class of treatment targeting lipid droplet formation in adipocytes and benefit obese patients. Perhaps polymorphisms of this gene might also contribute to some of the more common forms of obesity. It was subsequently revealed to me that this esteemed group had unanimously decided to name this new disease after me and two other physicians – a compound eponym of three names to be adopted for this newly discovered brown fat malady in honour of our co-discovery and contributions to better understanding of the pathology of this novel disease so that generations of medical students

and doctors down the road will be able to read and learn about it in textbooks and published scientific literature in the future. In this respect, I am truly humbled to be able to leave an indelible footprint and become a part of the illustrious history of medicine and science.

Finally, I should also include my fair share of 'failures' just so to be realistic. Just to cite a couple here, I am still trying to figure out why two of my funded projects have been hitting 'roadblocks'. One is my quest to solve the crystal structure of the catalytic ligand binding site of the thyroperoxidase (TPO) enzyme. We have not been successful in crystallizing human TPO despite using a variety of molecular strategies including deglycosylation and using an insect protein expression system. The recent advent of AlphaFold AI by two Nobel laureates to elucidate the 3D protein structure becomes a natural tool of choice for our more recent attempts. The hope is that we can find new ligands that can lead to either safer and more effective drugs or even naturally occurring phytochemicals that can alleviate hyperthyroidism. The other is another funded study that examines the structure of thyroid autoantibodies with the hope of elucidating the usual binding partners which could represent the environmental antigens that trigger autoimmune thyroid diseases. If successful, this could lead to better preventive actions that can reduce the prevalence of autoimmune thyroid disease in the general population. Failures teach us lessons to better prepare us to scale subsequent challenges with courage. However, I remain optimistic that solutions to some of these challenges may eventually be found with enough perseverance.

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