Appendix 3

ADAPTIVE THERMOGENESIS

BROWN FAT – WHITE FAT

As discussed in chapter 3, internal heat production, or thermogenesis, by the chemical dinitrophenol (DNP) is dangerous and potentially lethal. But scientists have known for some time that thermogenesis can also occur in a planned biological manner, as a mechanism for smaller mammals to avoid freezing to death from hypothermia.

Much of the research into thermogenesis has been performed on mice. Scientists have for many years known that mice can keep themselves warm in cold environments by converting their food energy directly into heat using their own mini-heaters. Mice have developed two different types of fat tissue, called brown and white adipose (fat) tissue - named because of their distinctive colours. The white fat tissue does what we would expect fat to do, it stores energy which can be accessed any time. The brown fat tissue however has an opposite role to play. It consumes energy and converts it directly into heat, the brown fat is the mice's internal mini-heater.

This process of heat production is called 'futile cycling', or 'uncoupling' (think of riding a bike when the chain is uncoupled – you won't go anywhere but may get hot) and is driven by a protein on the mitochondrial surface called thermogenin (or uncoupling protein-1, UCP-1). Thermogenin elicits the same reaction that DNP produces, but this time it is safe, because biological processes are protecting against any energy loss that is too excessive. The mouse is warmed but not overheated. Thermogenin is only found in brown fat tissue.

Any small mammal that exists in cold environments will rely on their brown fat to keep them warm. The smaller the animal the more heat that is lost to the atmosphere through convection and the more the risk of hypothermia. This rule applies to our species as well.

Human babies, because of their large surface area to volume ratio are at risk of hypothermia and not surprisingly when you analyse their fat deposits you discover that they also harbour brown, warming, fat tissues. What happens when we grow up? We do not dissipate as much heat into the environment and so our brown fat tissues remain quite small. The brown fat

deposits are present, particularly around the base of our necks but we sort of outgrow them, they remain the same size as a babies' brown fat deposits.

If human tissue could convert chemical (or food) energy into heat this would be a great strategy to target for obesity treatment. Many labs have, for years, presented their research in this area at the obesity conferences that I attend, in the hope that they could somehow develop a drug which could activate brown fat, to burn energy. Recently, an exciting discovery was that some white fat could be encouraged to produce heat. This fat turned into a cross between brown and white - producing beige fat which used a similar uncoupling protein, this one called UCP-2[ii]. Unfortunately, the only way that scientists have so far found to stimulate brown and beige human fat to let off heat, and therefore expend energy, is uncomfortable. Long term exposure to extreme cold temperatures! Most people would prefer to stick to the dieting rather than freezing, however unsuccessful.

If brown, and the newer beige fat tissue do not expend much energy as heat in humans, unless we take ice baths, which other organ may be using thermogenesis in metabolic adaptation? I had always thought for many years that the most likely candidate was our muscle tissue. This is where DPT (the dangerous weight loss drug of the 1930s) worked so there must be a natural way for muscles to burn energy to heat. It would also fit in with our natural biology - muscles are just below our skin so warming them would lead to easy convection of the heat into the atmosphere.

As animals get larger they rely less on brown fat to keep warm. In conditions of sudden cold they will use the shivering response. The muscles twitch involuntarily and this releases heat. Birds, marsupials and pigs have a complete absence of brown fat tissue, and in addition they don't shiver in response to the cold – how do they keep warm? Many scientists think that they are able to keep warm in cold environments by non-shivering thermogenesis. The muscles do not need to twitch or move to produce heat, they produce it just like the processes occurring in brown fat tissue. Let's explore this more because this has important implications for our own understanding of weight regulation.

PORCINE STRESS SYNDROME

Recent studies have shown that brown fat originates from muscle tissue and therefore has more in common with muscle than white fat. Pigs, who have no brown fat, must rely on muscular thermogenesis to keep warm. Pigs rarely shiver in the cold (preferring to huddle together), maybe they utilise non-shivering thermogenesis in their muscles, just like a dose of DPT, to keep warm?

A terrible affliction of pigs is a condition called malignant hyperthermia (or porcine stress syndrome). It is not unusual for pigs to die of this whilst travelling in crowded freight trains to the abattoir. It is thought that the stress response (SNS) caused by their predicament sends signals to their muscles causing thermogenesis. The animals are not moving or shivering but the muscles channel energy into heat in an uncontrolled way and the animals' temperature shoots up. Once the energy of the muscles is spent calcium rushes into the cell and the animal dies in paralysis, just like a DPT overdose. The pigs flesh turns yellow, becomes rancid and acidic and is rejected by the abattoir meat inspectors.

Birds are another animal with a complete absence of brown fat tissue. They have a very different composition to mammals, 70% of their body weight is muscle compared to 40% in terrestrial living mammals who need a heavier skeleton. Birds inhabit some of the coldest climates on earth from the artic to the Antarctic. Do they keep warm by having to move and fly to warm their muscles or do we witness them shivering? It is more likely that they can produce heat in their muscles when they are at rest in order to maintain their core temperature in the extreme cold? Clearly this will take extra energy and if this is not available as food in their environment they will fly south to where the metabolic energy equation is more favourable – more food, less cold.

WARM BLOODED, WARM MUSCLES

If you compare similar sized cold blooded animals (ectotherms) to warm blooded animals (endotherms) you see big differences in their muscular capacity. A mammal will have 30% greater muscle mass than a reptile and when you look deep into the muscle cells you find that mammals mitochondria (their cellular engines) have 220% greater mitochondrial surface area compared to reptiles. More muscle and more metabolically active muscle in animals needing to keep themselves warm to survive, another big clue into muscular thermogenesis.

For many years the thermogenin protein, the one that controls thermogenesis in brown fat, could not be located in muscle tissue so scientists did not think that muscular thermogenesis was an active part of human biology. It was thought to be a something we held in reserve only in specialised fat tissue for times of extreme cold. The energy that we seemed to burn off in excess when we over ate was thought by many to come from 'fidgeting'. Not gym exercise or shivering but just general fidgeting (like shaking your leg when nervous or bored). It was never explained how someone could fidget so much over a day that they could burn off the 500 extra kcals that most of us over-consume per day. I never really bought into this theory. It seemed highly unlikely and, in addition, the animal evidence seemed to point to muscular thermogenesis.

SARCOLIPIN – A NATURAL MUSCLE WARMER

The concept of adaptive thermogenesis (muscles turning food into heat to stop excessive weight gain) is currently being proven to be correct in animal studies. At 'Obesity Week 2017' in Washington DC, Dr Muthu Periasamy from Sanford Prebys Hospitals' Medical Discovery Institute presented ground breaking new evidence that muscular thermogenesis, without movement of shivering, may occur[iii]. A new protein called Sarcolipin that is found in the mitochondrial engines within muscles cells has been shown to exhibit uncoupling. In a similar way to UTP-1's DNP's action on brown fat tissue muscle, sarcolipin is able to signal to the muscle cell to transfer glucose into heat. Unlike DNP, sarcolipin is biologically natural and therefore safe. The Sanford team performed studies on mice whose warming brown fat had been removed. These mice were therefore going to be susceptible to hypothermia in cold conditions unless another mechanism could also work to keep them warm. Half of the mice had been bred not to produce sarcolipin, the muscle warming controller, and the other half had normal amounts of sarcolipin in their muscles. When the scientists tested their reaction to cold they found that despite not having any brown fat to keep them warm the mice with SLN were able to somehow compensate and keep warm. The mice with no brown fat and no SLN in their muscles became hypothermic quickly. The conclusion of this ground-breaking study was that there was indeed another process for mammals to produce heat

apart from brown fat. The sarcolipin in muscles seemed to be the crucial factor, the mice were compensating for no brown fat by using non-shivering muscular thermogenesis. Heat was being produced direct from glucose in their muscles.

MUSCULAR THERMOGENESIS PRODUCES WEIGHT LOSS

Our original hypothesis was that muscular thermogenesis (converting food energy to heat in muscle tissue) may be important in burning excess calories in times for food excess. This theory was tested, by the Stanford researchers, on mice. Mice can be fed either 'chow', which is a substitute for a diet they would find in the wild or 'canteen food', a surrogate of the processed high sugar and fat Western diet. It has long been known that mice fed canteen food will put on a lot more weight than those eating chow (just like cows and humans).

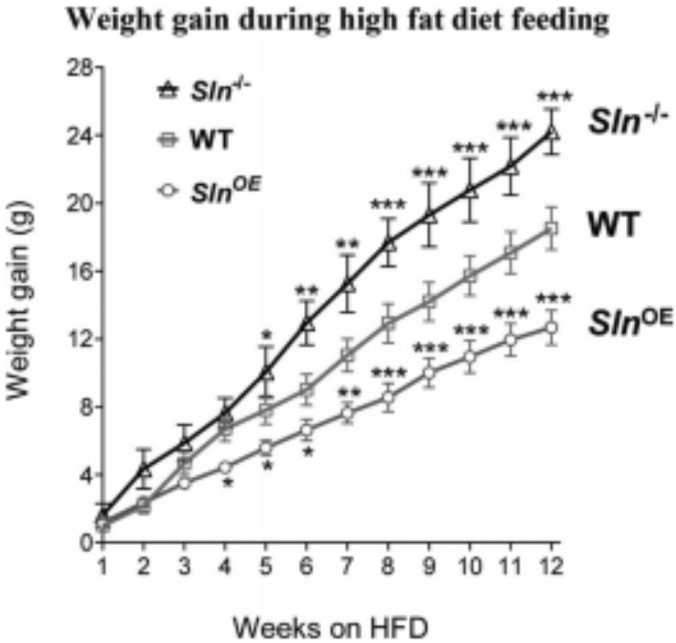
What would happen to the weight of the mice eating canteen food who had been bred with absent sarcolipin and therefore could not produce heat from their muscles (SLN-negative mice) and mice that had been bred with excess sarcolipin and could therefore burn off even more calories than normal mice when overfed?.

Three types of mice were compared to see how they would respond to a high fat diet. These were;

- Wild Type Mice (normal)
- Sarcolipin-negative Mice (those bred without any sarcolipin in their muscle – so they would not be able to burn excess energy off)
- Sarcolipin-positive Mice (those bred with excess sarcolipin in their muscle – so they would be ultra-efficient at burning off extra food energy)

The results were stunning…

The Sarcolipin-negative mice gained 22% in weight (compared to wild type) and the Sarcolipin-positive mice lost 23% of their weight - despite eating more calories than the wild type and Sarcolipin-negative mice.



The conclusion of the study was that sarcolipin was acting in the muscles to burn off some of the excess energy consumed, but that when sarcolipin was removed mice gained weight much more rapidly as they were unable to burn off the excess calories[iv].

The fascinating results of the two studies looking at the effect of sarcolipin on temperature and weight regulation confirm that muscular thermogenesis in mammals works in two ways. The first is, as expected, to produce heat and keep the bodies temperature within normal levels in times of cold exposure. The second mechanism is to burn off excess calories when they are overconsumed – adaptive thermogenesis working within our muscles.

The activation of adaptive thermogenesis in both brown fat and muscle cells is via the sympathetic nervous system (the messenger hormones are called beta-adrenergic because they originate in the adrenal glands). This neatly ties in with our theory of the SNS being activated in response to overeating. Energy is dissipated via both the mechanical work of a faster beating heart but also by heat generation in muscle cells. The SNS and muscular adaptive thermogenesis working neatly together to maintain an ideal level of energy reserve*.

*The studies on muscular thermogenesis are new, and I am sure will stimulate further researchers to explore this area as a potential treatment of obesity. Some scientists may point to previous studies that have looked at human core temperature during overeating and had found no increase. They may say this

evidence goes against my theory of adaptive muscular thermogenesis. I think these studies are flawed. Muscular thermogenesis occurs in our skeletal muscles, i.e. the muscles used to move us around. These are peripheral muscles and any heat generated from these would be easily lost to the atmosphere as convection. In addition, humans developed hyper-efficient cooling (sweating) mechanisms after we lost our body hair (a development only possible with the discovery of fire and the use of clothing to prevent us freezing) - the reason humans are able to wear down animals on a long hunt. Any heat entering blood vessels would stimulate the normal thermogenic response of sweating, core temperature would not rise.

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[i] (Klingenberg, 1999)
[ii] (Marlatt, 2017)
[iii] (Periasamy, 2017)
[iv] (Periasamy, 2015) (Periasamy, 2017) (Bal, 2012)