1. FINAL PUBLISHABLE SUMMARY REPORT

The project “hypothalamic T3” was initiated to investigate the potential of hypothalamic thyroid hormone in the neuroendocrine regulation of appetite, energy balance and glucose homeostasis. A role for thyroid hormone in the regulation of energy expenditure has long been known with overt clinical consequences of energy imbalance in patients with thyroid dysfunction. Until recently the effects of thyroid hormone on metabolism were thought to be mediated by the action of T3 directly in peripheral tissues, but new evidence clearly points to another facet of thyroid hormone action – one of action in the hypothalamus to regulate appetite, energy balance and glucose metabolism. However, which homeostatic mechnisms thyroid hormone triggers in the brain is largely unknown.
The aim of this project was to identify components of T3 action on appetite and energy balance regulatory mechanisms the hypothalamus.

The project contained four specific research objectives:

Objective 1 – **Investigate chronic T3 release in the hypothalamus on central and peripheral hormones and glucose homeostasis.**
Chronic T3 release into the hypothalamus prevents seasonal body weight loss and reduces food intake in the photoperiodic Siberian hamster (Barrett et. al. 2007, Endocrinol 148:3608-17). We have used a similar approach in the Sprague Dawley rat to provide a non-photoperiodic model with constant elevated hypothalamic T3.
Silastic implants with embedded crystalline T3 were bilaterally placed into the hypothalamus of Sprague Dawley rats. After surgery half of the animals was put on a high fat (HF), the other half on a chow diet and body weight, body composition, food intake and body temperature were monitored over a period of 8 weeks. HF diet increased the body weight of rats from day 18 despite lower food intake and body fat mass was elevated in those animals by the end of the experiment as determined by MRI. An intraperitoneal (ip) glucose tolerance test during the last week revealed decreased glucose clearance in the HF rats. However no effect of chronic T3 release into the hypothalamus could be determined on body weight, food intake or body composition on either diet. Post mortem serum fatty acids were increased in the HF rats but not affected by T3 implants and serum thyroid hormones remained constant in all groups. *In situ* hybridisations on implanted rat brains were carried out for genes involved in of the regulation of hypothalamic thyroid hormone synthesis and the central control of energy balance. No effect of diet could be seen on any of these genes, and chronic T3 release had only limited effects on 2 of the investigated components. However, *TRH* expression in the paraventricular nucleus was significantly decreased in the T3 implanted animals of both diet groups indicating correct T3 release from the implants. Overall, chronic T3 release did not show the expected effects on whole body energy balance or central control centres in neither animals on chow nor animals challenged with a HF diet. This might indicate that in the non-photoperiodic Sprague Dawley rat some compensatory mechanism prevents long term physiological changes in response to chronic T3, unlike in photoperiodic species.

Objective 2 - **Identification of hypothalamic genes regulated by T3**
Sprague Dawley rats were chemically thyroidectomised by giving 0.025% methimazole in the drinking water for 22 days. On day 21 half of the rats received a saline injection, whereas the other half was injected with T3. A third group of 20 rats remained untreated and served as a naive control group. Thryroidectomy decreased food intake and body weight in these rats . Body composition revealed differences in lean mass but not in fat mass between thyroidectomised groups and control from day 14 of the experiment. Post mortem serum thyroid hormones revealed the expected pronounced differences in T3, T4 and TSH between all 3 groups, indicating successful thyroidectomy and T3 injection. Serum fatty acids as well as Insulin were affected by thyroidectomy. Glucose clearance was reduced and insulin levels decreased in the thyroidectomised animals during a glucose tolerance test on day 20. In the brain, *in situ* hybridisation revealed marked changes in gene expression of

genes involved in thyroid hormone regulation and central control of energy balance. Hence, we have generated and well characterized a model for the investigation of T3 action in the brain. RNA was extracted from hypothalamic blocks spanning the arcuate nucleus and analyzed on microarrays to screen for differential expressed genes regulated by T3.

Objective 3 - **Bioinformatic analysis of differentially expressed genes.**
Analysis of array data was made with Genespring software for most significant gene changes. Moreover a TopGO Fisher classic analysis for “biological processes” was carried out. Genes involved in a variety of biological processes such as eg. glial cell proliferation, regulation of hormone secretion, retinoic acid signalling, lipid metabolic processes or temperature homeostasis were noted as being regulated in the presence of T3.

Objective 4 - **Analysis of expression of differentially expressed genes in the hypothalamus of animal models of body weight regulation.**
Several candidate genes, eg involved in insulin signalling, neurogenesis and synaptic plasticity, have so far been chosen from the arrays for further investigation. They have been tested and differential expression been verified in various animal models of long and short-term body weight regulation, including rats models of chronic and acute T3 administration from the above described experiments, Siberian hamsters, diet induced obese (DIO) mice and fasted rats. So far the results have demonstrated differential expression of 4 candidate genes in different animal models, indicating a widespread significance of T3 and T3 regulated genes in the regulation of energy balance. Further studies will investigate the most promising pathways (eg in insulin signalling and neurogenesis) in more detail.

Conclusions and any socio-economic impacts of the project
The project was initiated with the hypothesis that thyroid hormone regulates the expression of hypothalamic genes involved in homeostatic mechanisms including appetite and energy balance.

The main findings were:

- Chronic T3 release in the hypothalamus of Sprague Dawley rats did not affect energy balance
- Developed and charactarized of a model to investigate T3 action in the brain
- Thyroid hormone availability affects gene expression in the adult brain of Sprague Dawley rats involved in a number of biological processes
- Thyroid hormone regulates genes involved in many centrally regulated processes including insulin signallng, neurogenesis, synaptic plasticity, retinoic acid signalling and regulation of hormone secretion. Some of these genes are differentially expressed in the hypothalamus of other animal models of long and short term energy balance

This project has set the essential basis for the identification of new mechanisms of T3 action in the brain and provides significant new information on genes regulated by thyroid hormone. Hypothyroidism during pregnancy or subsequent growth of new born children if untreated can have wide ranging impact on health and neurodevelopment. The identification of T3 regulated genes involved in neurogenesis and synaptic plasticity has potentially large significance in understanding the consequences of hypothyroidism during this critical stage of development. In addition we now that understand neurogenesis and synaptic plasticity are important processes in adults. Further development of data from our findings could lead to treatments to try and ameliorate the consequence of hypothyroidism or hyperthyroidism at different stages of development.