PROJECT PERIODIC REPORT

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[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Abbreviations and acronyms

AGB	Adjustable Gastric Banding
AgRP	Agouti-Related Peptide
AKT	Protein kinase B
ANOVA	Analysis of variance
ARC	Arcuate Nuclei
ARNT	Aryl hydrocarbon receptor nuclear translocator
BAT	Brown adipose tissue
BCT	Body Core Temperature
BDNF	Brain-derived neurotrophic factor
BMI	Body Mass Index
BOLD	Blood oxygen level dependent
BW	Body Weight
CCK	Cholecystokinine
CNS	Central Nervous System
CNV	Copy Number Variation
CSF	Cerebrospinal fluid
DAT	Dopamine Transporter
DES	Duodenal Endoluminal Sleeve
DIO	Dietary-induced obesity
ELISA	Enzyme-linked immunosorbent assay
eQTL	Expression quantitative trait locus
fMRI	Functional MRI
FTO	Fat mass and obesity-associated protein
GFP	Green Fluorescent Protein
GHSR	Ghrelin Receptor
GIANT	Genetic investigation of anthropometric traits
Gip	Gastric inhibitory polypeptide
GLP	Glucagon-Like peptide
GOAT	Ghrelin O-Acyl Transferase
GOOS	Genetics Of Obesity Study
GTT	Glucose Tolerance Test
GWAS	Genome-Wide Association Studies
HFD	High Fat Diet
ICV	Intracerebroventricular
IP	Intraperitoneal
IPA	Ingenuity pathway analysis
IV	Intravenous
JAK2	Janus kinase 2
K_{ATP}	ATP-Sensitive Potassium Channel
Kir6.2	Major subunit of K_{ATP}
KO	Knock-out
LC	Locus coeruleus
LCM	Laser-Capture Microdissection
LEPR	Human Gene encoding endospanin-1
MBH	Medio-basal hypothalamus
MC4R	Melanocortin 4 receptor
MCRs	Melanocortin receptors
ME	Median eminence
MEMRI	Manganese-Enhanced MRI
MRAP2	Melanocortin 2 receptor accessory protein 2
MRI	Magnetic Resonance Imaging
NAc	Nucleus Accumbens
ND	Normal diet/normal chow
NGS	Next generation sequencing

NPY	Neuropeptide Y
NSE	Neuron Specific Endolase
NTS	Nucleus Tractus Solitary
ob	Leptin gene
OB-R	Leptin receptor
OFC	Orbitofrontal Cortex
OLI	Oxyntomodulin-like immunoreactivity
OR	Odd ratio
OXM	oxyntomodulin
OXPHOS	Oxidative Phosphorylation
NEGR	Phosphofurin acidic cluster sorting protein 1
PACS1	Neuronal growth regulator
PCSK1	proprotein convertase subtilisin/kexin type 1
Pdk1	Phosphoinositide-dependent kinase-1
PI3K	Phosphoinositide 3-kinase
POMC	Pro-opiomelanocortin
PRKCH	Proteinase kinase C, eta
PVN	Paraventricular Nucleus
Q-PCR	Quantitative Polymerase Chain Reaction
RMST	Rhabdomyosarcoma 2 associated transcript
ROI	Region of interest
RVLM	Rostroventrolateral medulla
RYGB	Roux-en-Y gastric bypass
SCOOP	European ancestry with severe early-onset obesity
SH2B1	SH2B adapter protein 1
SIFT	Sorting intolerant from tolerant
SIM1	Single-minded homolog 1
SNA	Sympathetic Nerve Activity
SN	Substantia nigra
SNP	Single Nucleotide Polymorphism
STAT	Signal transducer and activator of transcription
SynI	Synapsin I
TH	Transmembrane protein
TMEM	Tyrosine Hydroxylase
TrkB	Receptor of BDNF
VAS	Visual Analogue Scale
VMN	Ventromedial nucleus
VSG	Vascular Sleeve Gastrectomy
VTA	Ventral Tegument Area
WAT	White adipose tissue
WES	Whole Exome Sequencing
WP	Work Package
WT	Wild Type

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1. Final publishable summary report

1.1 Executive summary

EurOCHIP aimed to improve our understanding of the physiology of food intake and body-weight control, as a necessary step in the development of effective therapeutic and preventative strategies for tackling obesity. Specifically, this project focused on the communication between the gastrointestinal tract and the brain.

1) Novel effector systems regulated by gut hormones in the hypothalamus and brainstem

We have identified novel signalling pathways and molecules in brain regions responsive to gut peptides and involved in the regulation of energy homeostasis. These have yielded important insights into the regulation of the dopaminergic feeding circuitry, revealed that HFD results in "resistance" to ghrelin in specific hypothalamic nuclei, and delineated in detail the role of GOAT in the production of acylated 'active' ghrelin. We demonstrated associations between OXPHOS gene expression, the central action of ghrelin, and nutritional status, and also showed that endospanin-1 is involved in the control of body weight, food intake and glucose homeostasis. We show that the benefits of bariatric surgery are ghrelin-independent, with initial evidence pointing to incretins as contributing factors. Also, deletion of leptin or insulin signalling in AgRP neurons is sufficient to alter body weight, but only blocking both signalling cascades can alter blood glucose levels. GLP-1 action on food intake does not depend on STAT3 signalling in POMC neurons.

2) Response to gut hormones of brain areas involved in higher cognitive and affective functions

Ghrelin effects on food intake/motivation exerted at the VTA level involve overlapping but divergent circuits, revealed through VTA delivery of NPY Y1 antagonists or mu-opioid receptor antagonists, or by delivery of D1/D2 dopamine receptor antagonists to the nucleus accumbens. Insulin signalling in catecholaminergic neurons is necessary for energy homeostasis and influences the ability of cocaine to involve the dopaminergic system. Work performed on human brain activation showed that (1) feeding and the infusion of gut hormones can reduce subsequent energy intake; (2) brain regions are activated upon presentation of food-salient visual stimuli; and (3) feeding and gut hormone administration can reduce brain activation by food images. A new role of K_{ATP}-channel-dependent neuronal excitability in maintaining thermogenic BAT sympathetic tone and energy homeostasis in catecholaminergic neurons was shown.

3) Impact of genetic variations on appetitive behaviour and predisposition to childhood obesity

A novel tool was developed to diagnose monogenic forms of obesity and diabetes. Candidate genes were sequenced from 'trios' comprising parents and severely obese children, allowing us to investigate the relation between gene variants/mutations and appetitive behaviour. Rare variants in SIM1 are associated with severe obesity and especially hyperphagic obesity of early onset. Loss-of-function in SH2B1 is associated with severe early-onset obesity, insulin resistance, and reduced final height. All mutations were associated with loss of function of GH/NGF-mediated signalling. Global or brain-specific inactivation of *Mrap2* causes obesity in mice, and rare heterozygous variants in *MRAP2* are associated with early-onset, severe obesity in humans. Combining SNP and CNV analysis and focusing on severe obesity allowed identifying four new obesity susceptibility loci. Severe obesity without developmental delay is associated with greater burden of rare, typically singleton CNVs, in parallel with findings in intellectual disability. Rare CNVs that delete genes involved in the neuronal regulation of energy homeostasis contribute to severe obesity.

4) Gut peptide milieu modulation through administration of exogenous gut peptides or dietary interventions

In humans, we demonstrated differences in gut hormone/peptide release in response to the macronutrient content of a meal, but do not translate into immediate differences in subsequent food intake. A study of the effects of co-administration of low doses of PYY_{3-36} and OXM in humans showed that the combined infusion has an additive anorectic effect in overweight and obese humans. These results and data from other recent studies suggest that Y2 receptor agonists and GLP-1 receptor agonists may be particularly suited to co-administration for the treatment of obesity.

These results from EurOCHIP, particularly the identification of novel genes, molecules and pathways involved in the control of food intake and body weight, will contribute not only to research breakthroughs in the treatments of diabetes and obesity, but also to the prevention and treatment of associated complications. The identification of new genetic determinants of obesity opens new avenues in the design of new therapeutic strategies against childhood obesity. EurOCHIP has also addressed the question of lifestyle modulation through the exploration of the effects of different dietary manipulations on gut hormone secretion and their effect on food intake. In terms of economic impacts, the project is expected to contribute to reducing the costs of obesity in Europe, which are currently above 40 billion Euros yearly and represent for each European country a burden of 0.1 to 0.6% of the gross domestic product.

1.2 Project context and objectives

Obesity is one of the greatest public health challenges of the 21st century. The prevalence of obesity has tripled in many countries in Europe since the 1980s (http://www.euro.who.int/obesity). It is currently responsible for 2-8% of health costs and 10-13% of deaths in different parts of Europe. Few would dispute that the obesity epidemic has been driven by lifestyle and environmental changes. However, although change in lifestyle is possible, the wholesale change of our environment to that of 50 years ago in an attempt to combat obesity is likely to be difficult to achieve. Yet, current obesity treatments are limited. Bariatric surgery is effective, but significant risks and costs limit its utility. Although previous licensed medical therapies that target central neurotransmitters were successful in reducing appetite and body weight, side effects frequently limit their application. Thus, treatments for obesity that are not associated with adverse effects are desperately needed.

How then do we proceed?
Individuals respond differently to these 'obesigenic' environmental changes, and these differential responses have a strong genetic element underlying them. It can therefore be argued that it is necessary to understand the physiology of the system to effectively tackle the pathology.

EurOCHIP believes that *improved understanding of the normal physiology of energy homeostasis and the endocrine control of food intake will have profound implications for the development of effective therapeutic and preventative strategies for human obesity.*

Energy homeostasis relies on an integrated system in which adipocyte-derived signals provide long-term information to the brain about the state of nutrient stores, whereas a variety of gastrointestinal peptides triggered by ingestive status and also signalling to the brain, have important roles in influencing meal initiation and termination. **Thus, the EurOCHIP consortium focused on the interaction between the gastrointestinal tract and the brain in the control of energy homeostasis.**

To this end, EurOCHIP had four main objectives split into four scientific work packages, each divided in a series of tasks addressing the specific objectives (and related deliverables) listed below.

WP2 **Identify novel effector systems in the hypothalamus and brainstem that are regulated by ghrelin, PYY_{3-36} and OXM and are involved in the regulation of energy homeostasis:**

- Determine which hypothalamic nuclei responded to gut hormones (D2.1)
- Determine the transcriptomic response of the hypothalamic nuclei to PYY_{3-36} and ghrelin by microarray analysis and identify candidate genes related to obesity (D2.5)
- Understand the role of GOAT in energy homeostasis by characterising the *Goat* KO and *Goat ob/ob* double KO phenotypes in mice (D2.2, D2.6)
- Set-up a strategy for silencing OB-RGRP (leptin receptor) and to determine the role of leptin on gut peptide (CCK, GLP-1 and OXM) effects in the brain (D2.7, D2.8)
- Determine how genetic models of hypothalamic insulin, leptin and melanocortin signalling respond to gut hormones, for instance by:
  - investigating the effect of deletion of *Stat3* in POMC and AgRP neurons on ghrelin, GLP-1 and OXM action (D2.3)
  - exploring how deletion of insulin receptor in POMC and AgRP neurons affects ICV GLP-1 and OXM action (D2.4)
- Establish bariatric surgery procedures in rodents and evaluate the impact of GOAT activity on the outcomes of bariatric surgery (D2.9).

WP3 **Examine the response of brain areas involved in higher cognitive and affective functions to ghrelin, PYY_{3-36} and OXM:**

- Study human brain activation in response to different macronutrients and dietary manipulations using fMRI (D3.1)
- Carry out investigations on human brain responses to ghrelin, GLP-1 and OXM using fMRI (D3.7)
- Identify the spatial distribution and temporal activation profile of murine high brain centres in response to injection of gut hormones PYY_{3-36} and OXM, using MEMRI (D3.3)
- Explore how pharmacological disruption of the mesolimbic reward system affects ghrelin action (D3.2)
- Study the importance of the reward system for action of ghrelin on food intake and body weight regulation (D3.5)
- Determine if ghrelin enhances the rewarding properties of food (D3.8)
- Explore the role of leptin signalling in dopaminergic neurons in mice using targeted gene disruption in mice, by studying the effect of deletion of *Stat3* and *Pdk1* in dopaminergic neurons on the action of gut hormones ghrelin, PYY_{3-36} and OXM action (D3.4)
- Determine how the targeted disruption of the insulin receptor in dopaminergic neurons affects the actions of gut hormones (D3.6)
- Investigate the role of glucose sensing in the dopaminergic system with respect to the action of gut hormones, by determining how the expression of a constitutive open mutant of Kir6.2 specifically in dopaminergic VTA-neurons affects the actions of ghrelin, PYY_{3-36} and OXM (D3.9).

WP4 **To determine if genetic variation in molecules involved in the brain response to gut peptides affects appetitive behaviour and predisposition to childhood obesity:**

- Develop Next Generation Sequencing (NGS) and Whole Exome Sequencing (WES) protocols for sequencing of genes from families with extreme forms of obesity
- Sequence candidate genes for obesity and identify common gene variants and their relation to appetite behaviour (D4.3)
- Perform monogenic screening and identify rare genetic variants (D4.2)
- Draw conclusions on genetic determinants of bariatric surgery (D4.1).

WP5 **To modulate the gut peptide milieu in humans by administration of exogenous gut peptides or through dietary intervention, to determine if they are potential therapeutic targets:**

- Determine the effects of different dietary manipulations on gut hormone secretion and food intake in humans (D5.1)
- Investigate the potential combined effects of PYY_{3-36} and OXM on food intake in humans (D5.2).

1.3 Main S&T results and foregrounds

WP2: PERIPHERAL MOLECULES AND THEIR INTERACTIONS WITH THE HYPOTHALAMUS AND BRAINSTEM

The general objective of WP2 consisted in identifying novel effector systems in the hypothalamus and brainstem which are regulated by ghrelin, PYY_{3-36} and OXM and involved in the regulation of energy homeostasis.

Task 2.1: Characterisation of the neurons activated by peripheral administration of PYY3-36 and OXM, and Task 2.2: Identification of the spatial distribution and temporal activation profile of hypothalamic and brainstem neurons activated in response to a single ip injection of PYY_{3-36} and OXM in mice using MEMRI (Tasks leader: ICL).

These tasks were implemented by ICL (Partner 2) during the first 18-month period of the project. **Detailed results were reported in Deliverable D2.1 "Characterisation of neurons activated by PYY_{3-36} and OXM" submitted by ICL on the 27th of May 2011.**

In order to control the rapid rise in obesity and its enormous health implications, it is necessary to understand the pathways involved in regulating appetite. The complex regulation of energy homeostasis occurs in the hypothalamus and brainstem. Post-prandially secreted gut hormones play a major role as satiety signals to the central nervous system (CNS), acting directly on neural circuits in the hypothalamus and brainstem, or via vagal afferents.

Numerous studies had shown that PYY_{3-36} and OXM are potent anorexigenic gut hormones. Both had been investigated as potential therapeutic agents for obesity, but their mechanism of action remained obscure. The aim of this task was to characterise the CNS neurons activated by peripheral administration of PYY_{3-36} and OXM in mice.

The specific objective of this work was to compare brains of mice injected with each peptide separately and both peptides together, using immunocytochemistry to identify areas of neuronal activation, coupled with *in situ* hybridisation (ISH) for specific neuropeptides with well characterised roles in the regulation of food intake, to see if they also mediate the anorexigenic effects of PYY_{3-36} and OXM.

This work showed that both PYY_{3-36} and OXM activate neurons in the arcuate nucleus of the hypothalamus.

Task 2.3: Identification of molecules and pathways that are regulated within the specific hypothalamic nuclei that respond to ghrelin, PYY_{3-36} and OXM (Task leader: Inserm)

This task was completed by Inserm (Partner 4) and UCAM (Partner 1) by July 2012 and is described in Deliverable D2.5 "Microarray analysis of OXM, PYY_{3-36} and ghrelin activated LCM hypothalamic nuclei; Data analyses of LCM microarray data, Q-PCR validation, selection of candidate genes for further analyses" submitted on the 23rd of July 2012.

The arcuate nucleus of hypothalamus is one of the CNS regions where ghrelin exerts its central control of food intake. Preliminary data obtained in the frame of Task 2.3 suggested certain associations between the OXPHOS status and the central ghrelin action. Furthermore, combining these findings with previous results, a link between the expression of OXPHOS genes and level of food intake or metabolic homeostasis in general began to emerge. However additional work was required to further elucidate this link and identify crucial molecular components.

During the first 18 months of the project, it was established that the arcuate nucleus of the hypothalamus (ARC) is one of the CNS regions in which the central control of food intake by ghrelin is taking place. It was shown that chronic ghrelin administration increases food intake. However, if the animals are fed a high fat diet (HFD) concurrent with the ghrelin treatment, the effect on food intake disappears. UCAM (Partner 1) and Inserm (Partner 4) have dissected the ARC by LCM, extracted, amplified and labelled RNA, and hybridized these onto Affymetrix Rat Gene ST arrays. They identified genes encoding components of the oxidative phosphorylation pathway (OXPHOS) that are down-regulated by ghrelin on a chow diet, but up-regulated by ghrelin on HFD.

UCAM had previously published data showing that leptin (which is an anorectic hormone and therefore mediates an opposite response in food intake to ghrelin) up-regulates OXPHOS genes. Therefore, it was concluded that OXPHOS genes are reciprocally regulated by leptin and ghrelin. More work was required to determine if this is a secondary response of the hypothalamus, or if this is a primary signalling mechanism. In particular, could differential regulation of the OXPHOS pathway underlie the ghrelin resistance seen in HFD? Additional work was also required to compare the responses of ARC and VMN.

From Month 19 to Month 34, the work focused on the response of different brain regions to ghrelin and the response of different brain nuclei to PYY_{3-36}. There appeared to be a nuclei-specific response to exposure to a HFD, such as ghrelin 'resistance', as assessed by changes in OXPHOS gene expression, and occurring in the ARC but not the VMN.

Genes that are differentially regulated by PYY_{3-36} in two hypothalamic nuclei and the VTA have been identified in the frame of EurOCHIP. The true value of the expression profiling experiments performed in this task will however need to be clarified through cross-referencing of the data obtained for genes responsive to different hormones or nutritional conditions.

One question to be actively pursued in the future will be to determine if the observed changes in OXPHOS gene expression result in a decrease of OXPHOS activity, and, if so, whether this change in OXPHOS is a primary change due to signalling by leptin and ghrelin, or secondary to the changing fuel status of specific neurons.

As mentioned above, a repertoire of genes differentially regulated by fasting, dependent on PYY_{3-36} but independent of leptin, has been identified in the ARC. Further data analyses should reveal new molecular mechanisms involved into the brain response to nutritional and hormonal cues.

Task 2.4: Testing the hypothesis that the novel gut enzyme ghrelin octanoyl acyl transferase (GOAT) is an essential regulator of the energy homeostasis and adiposity (Task leader: DIfE / HMGU)

This task was implemented by DIfE (Partner 6) and HMGU (Partner 9) and reported in Deliverable D2.2 "Report on *Goat* KO phenotype" submitted on the 24th of February 2012.

In order to test if ghrelin *O*-acyl transferase (GOAT) is an essential regulator of energy homeostasis, DIfE and HMGU tested if GOAT deficiency can rescue the obese phenotype of leptin deficient mice (*ob/ob* mice). For this purpose, a new mouse model was created that was simultaneously deficient in both leptin and GOAT (*Goat*$^{-/-}$ *ob/ob* mice). Energy homeostasis was extensively studied during different feeding regimes in *Goat*$^{-/-}$ *ob/ob* and *ob/ob* mice. On standard diet body weight, food intake and body composition were not different between 2 month old male or female *Goat*$^{-/-}$ *ob/ob* and *ob/ob* littermates.

Since the partners had shown that the phenotype of GOAT$^{-/-}$ mice is amplified on medium-chain-triglyceride (MCT) diet (Kirchner *et al*. 2009) a new generation of *Goat*$^{-/-}$ *ob/ob* and *ob/ob* mice was generated and fed with MCT diet early after weaning. At the time when MCT feeding was started *Goat*$^{-/-}$ *ob/ob* and *ob/ob* mice had similar body weights. However, over time *Goat*$^{-/-}$ *ob/ob* mice seemed to gain body weight more slowly than *ob/ob* littermates. Although the difference did not reach the level of statistical significance at the end of the observation period, *Goat*$^{-/-}$ *ob/ob* mice developed a strong trend towards decreased body weight. Further, fat mass tended to be decreased while fat free mass remained unchanged compared to *ob/ob* littermates.

To study whether the differences in body weight and fat mass between *Goat*$^{-/-}$ *ob/ob* and *ob/ob* mice were induced by changes in energy expenditure, activity or food intake, male mice were placed in an indirect calorimetry system six weeks after starting MCT feeding. This system measures energy expenditure, locomotor activity and food intake simultaneously. Food intake was not changed between the genotypes. Energy expenditure followed the expected circadian rhythm and was similar in both groups during the light and dark phases. The respiratory quotient strongly tended to be decreased in *Goat*$^{-/-}$ *ob/ob* mice during the light and dark phases. This indicated that *Goat*$^{-/-}$ *ob/ob* mice prefer fat oxidation to carbohydrate metabolism as fuel source, which might contribute to the lighter and leaner phenotype of *Goat*$^{-/-}$ *ob/ob* mice. Interestingly, locomotor activity was significantly increased in *Goat*$^{-/-}$ *ob/ob* compared to *ob/ob* mice.

Therefore, during the first 18-month period of the project, the comparison between leptin-deficient *ob/ob* and double KO *Goat*$^{-/-}$ *ob/ob* variant mice has established that *Goat*$^{-/-}$ *ob/ob* variants have the same food intake behaviour as *ob/ob* mice, whereas showing a higher trend to weight decrease. This phenomenon has been explained by a metabolism that is more oriented towards fat oxidation and increased locomotor activity.

During the second project period (Month 19 to Month 36), the partners in charge (DIfE and HMGU) have focused their efforts on the effects of GOAT deletion on the regulation of body core temperature (BCT). The data obtained indicated that mice lacking GOAT have an improved ability to maintain BCT at low ambient temperatures, as compared to wild-type controls. Additionally and potentially explaining the superior BCT maintenance in the cold, locomotor activity was increased in *Goat-KO* mice at a specific temperature range (25-22°C). Therefore, the absence of the enzyme GOAT enables mice to better sustain BCT at low external temperatures. Moreover, these data underline the previously reported importance to examine mouse metabolism phenotypes following genetic engineering or pharmacological intervention at a large range of environmental temperatures in order to thoroughly assess energy metabolism.

Task 2.5: Testing the hypothesis that GOAT is an essential regulator of glucose homeostasis and insulin sensitivity associated with obesity (Task leader: DIfE / HMGU)

This task was completed by September 2012 and is extensively described in periodic reports 1 and 2, and in Deliverable D2.6 "Phenotype of *Goat ob/ob* double KO" submitted by HMGU on the 27th of November 2012.

Acyl ghrelin increases food intake and adiposity and is the only known orexigenic gastrointestinal hormone. However, When EurOCHIP started, the reports illustrating the effects of ghrelin on glucose homeostasis were conflicting. Glucose tolerance tests in rodents and humans showed that systemic infusion of acyl ghrelin impaired glucose homeostasis. In contrast, earlier studies in rats suggested that ghrelin decreased blood glucose by increasing insulin release. Desacyl ghrelin was shown to either have no effect on glucose homeostasis in mice and humans, or to have beneficial effects on insulin sensitivity and secretion. Some reports further suggested that desacyl ghrelin might oppose acyl ghrelin-mediated glucose regulation, *e.g*. by improving insulin sensitivity or decreasing hepatic glucose output. A more recent report showed that animals lacking GOAT have an impaired ability to maintain glycaemic control during prolonged negative energy balance. Such impairment was prevented when mice were treated with acyl ghrelin, the only known product of GOAT activity. Here, DIfE (partner 6) and HMGU (Partner 9) aimed to systematically dissect the impact of ghrelin acylation on hypoglycaemic control. Chronic calorie restriction in mouse models with loss-of-function for ghrelin signalling was used to assess the protective role that ghrelin acylation has against the development of hypoglycaemia. Exposure to a calorie deprived diet in *Ghsr-KO* mice that lack acyl ghrelin signaling, *Goat null* mice that lack ghrelin acylation, and in *ghrelin-KO* mice that lack both acyl and desacyl ghrelin, were used to highlight the specific impact of the desacyl/acyl ghrelin ratio on glycaemic control under a negative energy balance.

During the first 18 months of the project (Period 1), studies have been conducted on *ghrelin-KO*, *Goat-KO* and *Ghsr-KO* mice variants under prolonged calorie restriction. The data obtained showed that, whereas no significant difference in glucose blood rate has been observed between wild-type and variants, the acyl/desacyl ghrelin ratio is a relevant determinant of glucose tolerance.
Such control is enhanced under conditions of caloric deprivation. Chronic energy depletion in mice deficient for GOAT, Ghrelin, Ghrelin receptor, or both, did not lead to more frequent or severe hypoglycaemia events when compared to wild type littermate controls. Older age (7-8 months) of mouse strains used in this study, as compared to previously published reports (Zhao et al., 2010) may represent one potential explanation for the inability to observe a substantial role for GOAT in the prevention of hypoglycaemia.

The work performed during the second period of the project (Month 19 to Month 36) focused on the putative protective role of GOAT ablation on glucose intolerance and body adiposity, using ob/ob variants and *Goat*$^{-/-}$ *ob/ob* double KO mice.

In summary, data obtained from the ablation of all three components of the GOAT-ghrelin-GHSR axis in mice on an ob/ob background convincingly demonstrated that neither desacyl- nor acyl-ghrelin (signalling) can reverse the massive obesity induced by leptin deficiency. The surprising finding of improved vs. impaired glucose homeostasis in *ghrelin-ob/ob* and *Ghsr-ob/ob* double mutants further points out a complex and only partially understood role of ghrelin in glucose control. The lack of effect in *Goat*$^{-/-}$ *ob/ob* double mutants suggests that it is not the increased ratio of desacyl/acyl-ghrelin that controls glucose homeostasis.

In conclusion, the paradoxical findings obtained by the EurOCHIP partners and others highlight the plurality and complexity of the GOAT-ghrelin-GHSR axis in controlling glucose and energy homeostasis. The discrepant findings between single and double mutants suggest a close interplay between leptin and ghrelin signalling pathways. They also indicate that impaired leptin signalling can potentially override any beneficial metabolic effects mediated via the GOAT-ghrelin system.

Further studies and novel models with abolished constitutive activity but an otherwise functional acyl-ghrelin signal transduction will be needed to delineate the detrimental effects of acyl-ghrelin signalling from the potential beneficial effects of GHSR constitutive activity in the absence of acyl-ghrelin. Such studies could also help to clarify why glucose tolerance was improved in both our *Goat-KO* and *Ghsr-KO* mice while leptin signalling was intact.

Task 2.6: Influence of altered GOAT activity on the immediate metabolic benefits of bariatric surgery (Task leader: DIfE / HMGU)

This task was completed by HMGU (Partner 9) and resulted in the production of Deliverable D2.9 "Report on whether the immediate metabolic benefits of bariatric surgery are mediated by GOAT" submitted on the 18th of November 2013.

Obesity and diabetes type 2 are major public health problems, and multiple efforts are currently underway to develop novel pharmacotherapies against this diabesity pandemic. Nevertheless, for multiple obese patients, bariatric surgery remains the only option for sustainable weight loss and immediate improvement of glycaemic control. The exact mechanistic underpinnings for the success of bariatric surgery, however, remain unknown. Bariatric surgery, at least initially, restricts calorie intake, which contributes to the observed weight loss. In addition, animal studies with careful pair-feeding revealed a non-restrictive component of weight loss, which may be attributed to altered gut hormone secretion. Profound changes in gut hormone secretion after weight loss surgery were demonstrated for Glp1, Gip, PYY as well as the stomach hormone ghrelin. While each of these gut hormones was previously shown to play a pivotal role in glycaemic control or body weight homeostasis, their individual roles in bariatric surgery remain elusive.

In task 2.6, HMGU (Partner 9) aimed to elucidate the role of acyl ghrelin in mediating benefits of bariatric surgery. Acyl ghrelin is the only circulating orexigenic hormone and exerts its action via binding the growth hormone secretagogue receptor. Acylation of ghrelin with a medium-chain fatty acid is catalysed by the recently identified enzyme ghrelin–O-Acyl transferase (GOAT). At present, ghrelin acylation is the only known function of GOAT, and gain- as well as loss-of-function models of GOAT suggest an involvement in energy and glucose homeostasis. However, at the start of the EurOCHIP project, no data were available on a potential involvement of GOAT and in general ghrelin physiology for bariatric surgery success.

The major aims of task 2.6 were the establishment of bariatric surgeries in rodents and evaluation of the impact of GOAT activity on the outcomes of bariatric surgery. Using different bariatric surgery models, the aim of task 2.6 was to deprive exocrine cells from nutrients that may induce the production (via the action of GOAT) and secretion of acyl-ghrelin or of incretin hormones such as GLP-1. Four types of bariatric surgery were evaluated: duodenal endoluminal sleeve (DES) and adjustable gastric banding as less invasive and merely malabsorptive procedures, and the highly invasive vertical sleeve gastrectomy and Roux-en-Y gastric bypass surgery (Figures 1 and 2).

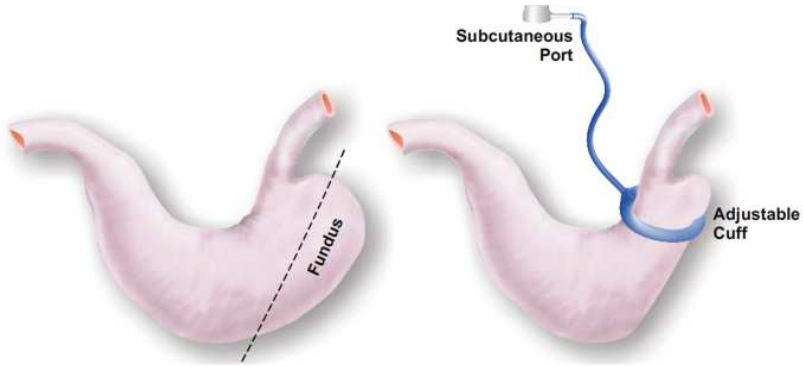

Figure 1: Schematic for fundectomy and adjustable gastric banding surgery in rats (Habegger et al., 2013).

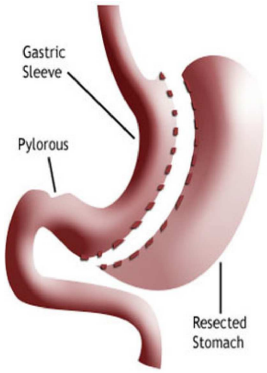

Figure 2: Schematic for vertical sleeve gastrectomy.

First results on the DES procedure further revealed that body weight loss was identical between DES rats and control rats pair-fed to the DES group. Such lack of difference suggests that metabolic effects of this procedure are purely based on malabsorption, and not on endocrine effects mediated by the duodenal GOAT-ghrelin or incretin system. Adjustable gastric banding surgery was successfully established in rats, yielding survival rates of 80% and more. Major obstacles in establishing the surgery were the choice of material for the gastric band, as well as the choice of material for the inflatable port. Further, placement and fixation of the band to the stomach proved to be critical. Nevertheless, after successfully defining the optimal surgery conditions in rats (see Habegger et al., 2013), AGB provided immediate metabolic benefits on food intake and body weight after inflation of the band. In contrast, fundectomy, *i.e.* the surgical removal of the whole fundus and thus of a substantial proportion of ghrelin-producing cells, had no effect on metabolism, suggesting that the GOAT-ghrelin system may not play a major role in adjustable gastric band surgery, at least in rats.

In mice, gastric banding surgery had to be substantially adapted in comparison to rat AGB surgery. Neither the anatomic configuration of mouse stomach nor the availability of suitable endocutter tools allowed for initial fundectomy as used in the rat model. Further, the lack of inflatable ports of small-enough size prevented adjusting banding pressure. Despite major efforts, all attempts to establish adjustable gastric banding surgery in mice failed, and high mortality rates and the lack of weight loss efficacy did not warrant mechanistic studies. In conclusion, due to the lack of suitable material and tools, AGB surgery in mice could not be established, and potential effects of AGB in mouse models of GOAT deficiency remain unknown. Attempts to establish Roux-en-Y gastric bypass (RYGB) surgery in mice yielded even higher mortality rates than gastric banding surgeries.

HMGU's collaborator Prof. Randy Seeley from the University of Cincinnati, however, could apply VSG surgery to ghrelin loss-of-function models that had been provided by HMGU. Ghrelin loss-of-function mice lack both acyl and desacyl ghrelin. Accordingly, GOAT KO mice with their lack of acyl ghrelin but abundance of desacyl ghrelin should behave similarly in response to VSG surgery since acyl ghrelin appears to be the only active constituent in ghrelin physiology. Desacyl ghrelin at physiological concentrations does not bind the ghrelin receptor, and genetic and pharmacological evidence do not suggest a major role for endogenous desacyl ghrelin in glucose or energy homeostasis (Heppner et al., 2012; 2013; Kirchner et al., 2013). Notably, VSG surgery in ghrelin KO mice, which represent a 100% physiological KO of GOAT activity and further exclude any biasing effects of the GOAT substrate desacyl-ghrelin, improved metabolic physiology in both WT and ghrelin KO mice. The collaborative data obtained thus suggests that the GOAT-ghrelin system is not essential for the success of VSG surgery (Chambers et al. 2013).

In summary, data obtained on bariatric surgery in ghrelin-loss-of-function suggests that the benefits of these surgical procedures are ghrelin-independent. However, first evidence points to incretins as contributing factors. Adjustable gastric banding was able to sensitize the rats for the weight lowering action of the Glp-1 mimetic Exendin 4 (Ex-4).

Further studies will need to reveal the exact mechanism for the synergism between bariatric surgery and incretin pharmacotherapy. Further studies should also assess potential synergism by combinations of pharmacotherapy and bariatric surgery. Most importantly, translational studies should aim to reveal potential synergism of incretin treatment in patients that underwent different forms of bariatric surgery. Ideally, such knowledge may one day help to develop a personalized therapeutic intervention strategy that provides a tailored combination of pharmacotherapy and bariatric surgery less invasive, thereby minimizing side effects and surgical complications and maximizing benefits such as sustainable weight loss and immediate improvements in glucose control.

Task 2.7 and Task 2.8: Influence of leptin sensitivity enhancement in the ARC or in the NTS (with OB-RGRP silencing) on CCK, GLP1 and OXM action (Task leader: Inserm)

Tasks 2.7 and 2.8 were completed by Inserm and resulted in the production of Deliverable D2.7 "Report on how silencing OB-RGRP in the ARC affects CCK, GLP-1 and OXM action" and Deliverable D2.8 "Report on whether enhanced leptin signalling in the NTS by silencing of OB-RGRP influences CCK, GLP-1 and OXM action", submitted by Inserm on the 28th and 15th of November 2013, respectively.

In order to explore how changes in leptin sensitivity in the brain modulate the effects of gut peptides, two strategies have been applied by Inserm (Partner 4) for deleting or silencing a negative regulator of the leptin receptor OB-RGRP (also known as Endospanin-1).

Strategy 1 aimed to generate OB-RGRP (Endospanin-1)-brain specific silenced mice by lentiviral transgenesis. In order to generate transgenic mice expressing a specific microRNA (miRNA) against Endospanin-1 in the brain by lentiviral transgenesis, two objectives had to be reached:

- The first objective was to identify and validate a strong brain-specific promoter allowing expression of the transgene in the lentiviral transgenesis approach. Synapsin I (SynI) and Neuron-Specific Enolase (NSE) promoters have been tested to allow strong gene expression restricted to neurons. The SynI promoter appeared to be strong and specific enough, and was thus chosen for continuing the experimentation.

- The second objective consisted in constructing lentiviral vectors allowing a good expression of the miRNA cassette, in two steps: construction and identification of Endospanin-1 miRNA, and assessment of efficiency of lentiviral vectors expressing the 2 Endospanin-1 miRNA.

In parallel, Endospanin-1 knock-out (KO) mice were also generated as an alternative strategy. Since the KO strategy was more successful, Partner 3 (Inserm) decided to focus on the 2nd strategy, which appears more promising.

Strategy 2 aimed to establish OB-RGRP (Endospanin-1) conditional KO mice, taking into acount that in humans, the *LEPR* gene leads to the expression of Endospanin-1 and LEPR transcripts, controlled by the same promoter, while in mice, the Endospanin-1 and LEPR transcripts originate from 2 different genes, allowing the establishment of specific *Endospanin-1 KO* mice. Basic

physical phenotyping of Endospanin-1$^{-/-}$ mice did not show any particularities. Endospanin-1 gene disruption is not lethal, and mice look healthy and can reproduce (even though *Endo 1 KO* mice seem to show lower reproduction rate compared to WT animals).

Inserm generated both female and male *Endospanin-1* $^{-/-}$ mice to respond to the question of whether Endospanin-1-induced leptin hypersensitivity affects sensitivity to gut peptides. To explore the effect of higher leptin sensitivity (through *Endospanin-1* silencing) on the anorectic response of gut hormones, several experiments were performed by Inserm, with peripheral administration of the GLP-1 agonist, exendin-4, or saline with subsequent measurement of food intake.

In a first series of experiments, intriguingly, Inserm observed that both male and female *Endospanin-1* $^{-/-}$ mice were less sensitive to the food intake suppressing effect of a suboptimal dose of exendin-4 relative to WT controls, suggesting that *Endospanin-1*$^{-/-}$ mice are less sensitive to exendin-4. In a second set of experiments with a greater number of animals, Inserm did not observe any difference in the capacity of exendin-4 to decrease food intake of KO mice compared to WT mice. Surprisingly, the profiling of plasma hormones after mixed-meal administration showed that *Endospanin-1 KO* mice have an altered metabolic hormonal secretion. Food-induced secretion of glucagon, insulin, amylin and GLP-1 is unexpectedly decreased in *Endospanin-1 KO* mice.

The observed decrease in meal-induced glucoregulatory pancreatic hormones and GLP-1, as well as the close link between glucose and energy homeostasis, prompted Inserm to investigate glucose homeostasis in *Endospanin-1 KO* mice. This was a crucial step to go further into the examination of gut peptide sensitivity in this model. The further characterisation of the model uncovered the complexity of *Endospanin-1 KO* mice. Indeed, Inserm observed that glucose homeostasis as well as body weight regulation was modified in the *Endospanin-1*-deficient, leptin hypersensitivity model.

Inserm observed that while Endospanin-1 silencing in the hypothalamic ARC improves body weight and decreases food intake, it impairs glucose homeostasis. Indeed, hypothalamic ARC Endospanin-1-silenced animals displayed fasting hyperglycaemia, glucose intolerance, and severely blunted glucose-stimulated insulin secretion, despite normal pancreatic insulin content.

Similarly to Endospanin-1 silenced mice, *Endospanin-1* KO mice developed glucose intolerance with age. At 3 months of age, *Endospanin-1* KO mice fed a chow diet displayed similar glucose metabolism to WT mice during intra-peritoneal glucose tolerance test (IPGTT) or oral GTT experiment. However, 6 month-old *Endospanin-1* KO mice exhibited glucose intolerance when chow-fed in addition to decreased glucose uptake in visceral fat during a radiolabeled 2-deoxyglucose uptake experiment.

The observed effects of Endospanin-1 silencing or deletion on glucose homeostasis were not expected since animals with higher leptin sensitivity should also be more sensitive to insulin and more glucose-tolerant. The differential effect on body weight and glucose homeostasis, which are typically associated with distinct intra-cellular signalling pathways, suggested a direct impact of Endospanin-1 on OBR signaling. Inserm therefore decided to further study the impact of Endospanin-1 on OBR signalling.

Yeast-Two–Hybrid data previously obtained by Inserm pointed out that Endospanin-1 interacts with the P85 protein, the regulatory subunit of PI3K. PI3K is a strategic kinase crucial in the regulation of several biological functions. More interestingly, central regulation of glucose homeostasis by leptin was suggested to depend on leptin-induced PI3K signalling. The PI3K/AKT pathway is also a crucial mediator of insulin receptor signalling, and crosstalk between insulin and leptin was revealed at this level. Inserm therefore confirmed that Endospanin-1 interacted with P85 and that Endospanin-1 and P85 were part of a same complex with OBR.

Interestingly, Endospanin-1 proved to be required for leptin-induced PI3K activity. While Endospanin-1 silencing increased leptin-induced STAT3 phosphorylation (regulation of body weight), it abolished the capacity of leptin to activate the PI3K/AKT pathway (regulation of glucose homeostasis) both in a cellular model and *in vivo*. Impaired leptin signalling through the PI3K/AKT pathway similarly abolished the capacity of insulin to activate this pathway. Inserm's data suggested that Endospanin-1 can be defined as a new potential master integrator of hypothalamic leptin and insulin signalling which has the unique property to regulate the balance between different OB-R signalling pathways, with important outcomes on leptin-induced decrease of energy intake and glucose homeostasis regulation.

In conclusion, GLP-1 does not display any differential effect on food intake in Endospanin-1 KO mice vs. WT controls. Most importantly, loss of Endospanin-1 increases leptin-induced STAT3 signalling, but leads to a decrease in leptin and insulin activation of the PI3K pathway. Altogether these findings are associated with decreased food intake and body weight, but alteration of glucose homeostasis in Endospanin-1-deficient mice. Therefore, Endospanin-1 KO mice do not appear to be an appropriate model of general leptin hypersensitivity because it is associated with selective effects on leptin signalling pathways. This differential effect of Endospanin-1 on leptin signalling led to lower food intake and body weight, but triggered glucose intolerance. Considering this unappreciated facet of Endospanin-1, Inserm concluded that Endospanin-1 KO or silenced model is complex and not adequate for the study of gut hormones in condition of general leptin hypersensitivity, since Endospanin-1 depletion leads to decreased leptin-induced PI3K activity. For those reasons, Inserm did not analyse CCK and OXM sensitivity in Endospanin-1 KO mice as originally planned. Results from such study would have been very difficult to interpret, knowing the dichotomy Endospanin-1 has on intracellular energy regulation pathways. Instead, the characterization of Endospanin-1 function was more meaningful and revealed an unprecedented regulation of leptin receptor function by a multifaceted protein.

As a consequence, additional relevant studies were carried out in the frame of EurOCHIP:

1) Impact of Endospanin-1 deletion in humans
Inserm had the opportunity to characterize a 7-year old patient with a homozygous 80 kb deletion in the chromosomal 1p31.3 region comprising the proximal promoter and exons 1 and 2 of the *LEPR* gene, encoding the leptin receptor and Endospanin-1. The obese phenotype was consistent with previously reported individuals with OB-R deficiency. The lack of any OB-R-independent phenotype of Endospanin-1 deficiency reinforces the specificity of Endospanin-1 to OB-R. As the patient was very young, it was not surprising not to see an alteration of glucose homeostasis.

2) Identification of small molecule compounds with potential to reverse leptin resistance
Inserm's work on Endospanin-1 indicates that cellular leptin sensitivity can be improved by increasing OB-R surface expression, suggesting a new therapeutic strategy to restore leptin sensitivity in obese patients. The prevention and reversal of leptin resistance is one of the major current goals of obesity research. Improvement of OB-R cell surface expression can thus be considered as an interesting anti-obesity therapeutic strategy. Inserm searched for small molecular weight molecules capable to increase OB-R cell surface expression. In order to identify such compounds, Inserm developed a cell-based, phenotypic assay to perform a high-content screen against a library of 50,000 chemical compounds. Inserm finally identified 4 compound clusters that increased OB-R cell surface exposure with no effect on total OB-R expression and cellular toxicity. Inserm showed that the compounds potentiated leptin-promoted signalling through the JAK2/STAT3 pathway, and could constitute an original therapeutic solution against obesity. If efficient, such compounds could be foreseen in a multi-therapy setting in combination with other food intake lowering and glucose homeostasis-normalizing drugs such as gut hormones.

3) Transport of peripheral homeostatic hormones into the medio-basal hypothalamus
Hormones such as leptin, insulin or gut peptides, which are secreted in the periphery, need to cross the blood brain barrier in order to target specific receptors in the brain and trigger any biological response on food intake. The mechanism allowing the active access of hormones into the cerebrospinal fluid (CSF) is under intense investigation. Leptin resistance was proposed to arise from defective leptin transport across the blood-brain barrier to the CSF. In a collaborative work, Inserm demonstrated that peripherally administered leptin sequentially activates OBR in tanycytes, specialized glial cells of the median eminence that form a blood-CSF barrier, followed by mediobasal hypothalamus (MBH) neurons. In mice deficient in the signal-transducing LepRb isoform or with diet-induced obesity, leptin taken up by tanycytes accumulates in the ME and fails to reach the MBH. Triggering specific signalling in tanycytes re-establishes leptin transport and its activation of MBH neurons in obese animals and accelerates the restoration of leptin sensitivity upon the return to a normal-fat diet. Leptin transport by tanycytes thus plays a critical role in the pathophysiology of leptin resistance, and holds therapeutic potential for treating obesity. This study opens new perspective and suggests that other hormones such as gut peptides could also follow this specialized route to reach the MBH to decrease food intake.

Task 2.9: Impact of deletion of insulin receptor in POMC and AgRP neurons on ICV GLP-1 and OXM action (Task leader: UKK)

The work performed by UKK (Partner 5) under tasks 2.9 resulted in the production of Deliverable D2.4 "Report on how deletion of insulin receptor in POMC and AgRP neurons affects ICV GLP-1 and OXM action", submitted on the 28th of November 2013.

Previous work performed by UKK (Partner 5) had demonstrated the critical role for insulin in modulating the electrical activity of AgRP and POMC neurons, mediated via Pi3K dependent control of KATP channels. Interestingly, while insulin hyperpolarizes and silences both POMC and AgRP neurons, leptin depolarizes POMC neurons to increase their firing rate, and hyperpolarizes AgRP neurons to inhibit their electrical activity.

In order to gain further insight into how the two peripheral hormones regulate the function of these neurons, UKK generated mice with insulin receptor and/or leptin receptor deficiency specifically in AgRP neurons. The aims were first to phenotypically characterize the different lines, and then study how insulin and leptin signalling affect GLP-1 and OXM action in these neuron subpopulations.

In order to directly investigate the role of the insulin receptor and leptin receptor in modulating the activity of the above mentioned neurons, breeding of mice with floxed alleles of the insulin receptor gene ($IR^{flox/flox}$), the leptin receptor gene ($LepRb^{flox/flox}$) or both ($IR^{flox/flox,}$ $LepRb^{flox/flox}$) were set up with mice expressing cre recombinase specifically in AgRP neurons. Mice were then analysed for a set of metabolic parameters (Body weight, blood glucose, insulin, and glucose sensitivity).

UKK generated a set of animals for the above mentioned mouse groups. Unfortunately, due to problems in its animal facility and the complexity derived from the combination of three different alleles, the team was able to generate only a much reduced number of animals to perform the planned investigations.

Preliminary analysis of the four groups showed differences in body weight gain for a period of 20 weeks for the groups $AgRP^{LepRbfl/fl}$ and $AgRP^{IRfl/fl,\ LepRb\ fl/fl}$ when compared to the control or the $AgRP^{IRfl/fl}$ group. Analysis of blood glucose levels showed a significant increase only for the double knockout animals. These differences in body weight versus glucose homeostasis suggest that

deficient leptin or insulin signalling in AgRP neurons alone is sufficient to alter body weight, but only the simultaneous lack of both signalling cascades can alter blood glucose levels.

With this approach, UKK intends to further dissect the individual contribution of leptin or insulin signalling in AgRP neurons for the control of energy homeostasis. The UKK team also aims to investigate how these two peripheral hormones interact with gut peptides such as GLP-1 for the regulation of food intake, glucose homeostasis and energy expenditure. The preliminary results obtained by UKK suggest that the two signalling pathways can have independent effects, but also converge to modulate AgRP neuron function. UKK needs to generate bigger groups of animals to confirm this observation and then be able to add GLP1 to the panel of peripheral signals acting in the arcuate nucleus.

Due to the difficulties met for generating mice of interest, part of the planned experiments from Deliverable D2.4 will be started beyond the end of the EurOCHIP project. Nevertheless, UKK used the time and resources available to extend its work program in D3.6 and D3.9, as described in detail hereafter, and which has yielded important novel insights into the regulation of the dopaminergic feeding circuitry.

Task 2.10: Impact of deletion of Stat3 in POMC and AGRP neurons on action of Ghrelin, GLP-1and OXM (Task leader: UKK)

The work performed by UKK (Partner 5) under tasks 2.10 resulted in the production of Deliverable D2.3 "Report on how deletion of *Stat3* in POMC and AgRP neurons affects ghrelin, GLP-1 and OXM action", submitted on the 28th of July 2012.

GLP1 is a proglucagon-derived peptide secreted by the L-cells of the small intestine known to act as an incretin (stimulating insulin secretion from the pancreas dependent on glucose). GLP1 can also induce anorexia through mechanisms that involve vagal and possibly central pathways, but those are not completely understood (Reviewed in Cummings DE 2007, Suzuki K 2010). Interestingly, GLP1 is expressed in different regions of the brain and it has been shown that intracerebroventricular injection of GLP1 or its agonist exendin 4 results in a reduction of food intake in rodents and humans (Turton MD 1996, Flint A 1998).

Leptin is an anorectic hormone secreted by adipocytes with a key role in the regulation of food intake by the Central Nervous System (CNS). Leptin exerts part of its function through the modulation of specific neuron sub-populations in the arcuate nucleus of the hypothalamus: the POMC (anorexigenic) and AgRP (orexigenic) neurons. Leptin signalling in these neurons requires binding to its receptor, which in turn activates the JAK/STAT pathway. STAT3 activation results in a subpopulation specific response, where POMC neurons get activated and AgRP neurons get inactivated to promote satiation.

There is some evidence for the interaction between leptin and GLP1, both in the periphery and the CNS. For example, the effect of peripherally injected GLP1 or exendin 4 is enhanced by a leptin pretreatment (Williams D. 2006) and leptin directly depolarizes preproglucagon hormones in the Nucleus Tractus Solitarius (Hisadome K 2010). Also, Leptin receptor expression in hindbrain GLP1-neurons regulates food intake and energy balance in mice (Scott MM 2011).

In order to study the interaction of Leptin and GLP1 in specific neuron subpopulations of the arcuate nucleus, UKK (Partner 5) decided to analyse the central effect of both hormones in a mouse model for deficient Leptin signalling. For that, UKK used mice with a modified STAT3 locus in which the exon containing a key tyrosine residue is flanked by loxP sequences and can therefore be

specifically eliminated by Cre-recombinase (Takeda 1998). Using mice that express Cre recombinase specifically in POMC or AgRP neurons, UKK has generated mice that lack an activatable form of STAT3 in these populations.

As expected, injection of GLP1 intracerebroventricularly into these mice resulted in a significant reduction in food intake in a dose-dependent manner. To avoid a too strong effect (that could mask interactions with leptin signalling) and malaise due to too elevated doses of GLP-1 UKK has decided to use 0.5 µg of GLP1 for its experiments.

As previously described, *Pomc* $^{Stat3-/-}$ male mice did not differ in body weight (BW) or food intake from control littermates, whereas *Pomc* $^{Stat3-/-}$ females showed increased body weight without significant differences in food intake during *ad libitum* feeding.

Icv injection of GLP1 into *Pomc* $^{Stat3-/-}$ males and females resulted in a significant reduction in food intake 2 and 4 hours after refeeding, similar to that observed in control littermates. Interestingly, *Pomc* $^{Stat3-/-}$ females showed a significant reduction in food intake at 2, 4 and 8h after saline injection compared to control littermates. This alteration in refeeding, independent of the treatment and only in females is consistent with previously published data (Xu AW, 2007). Leptin icv injection in control females reduced food intake relative to saline at all time-points, whereas *Pomc* $^{Stat3-/-}$ females showed this reduction only at 24h. In control mice, leptin and GLP1 did not show any synergistic effect when injected simultaneously. At time point 8h, injection of GLP1 and leptin together in control mice resulted in a slight increase in food intake compared to leptin alone. Interestingly, *Pomc* $^{Stat3-/-}$ mice at 8h showed a synergistic effect of leptin and GLP1, as injection of both components resulted in a reduction of food intake higher than that caused by any of them alone. Nevertheless, the results on GLP1 and Leptin interaction are very preliminary, since only a reduced number of animals received both treatments. Also, more experiments in male mice are needed before the effect of leptin and GLP1 on refeeding can be analysed.

At the tested doses, there is no synergism between leptin and GLP1 in control mice. Strikingly, in the presence of altered Stat-3 action in POMC-neurons, combined leptin and GLP1 action seems to be increased. These effects clearly deserve further studies including the ongoing analyses of leptin and GLP1 action in *AgRP* $^{Stat3-/-}$ mice.

WP3: PERIPHERAL MOLECULES AND THEIR ACTIONS ON HIGHER BRAIN CENTRES

The objective of WP3 is to examine the response of brain areas involved in higher cognitive and affective functions to ghrelin, PYY_{3-36} and OXM.

Task 3.1 and Task 3.2: Human brain activation using functional MRI in response to ghrelin, GLP1, OXM and to different macronutrients (Tasks leaders: ICL & UCAM)

Tasks 3.1 and 3.2 were completed and resulted in the production of Deliverable D3.1 "Report on different dietary manipulations on human brain response using fMRI" and Deliverable D3.7 "Report on effect of ghrelin, GLP-1 and OXM on human brain response using fMRI", submitted by UCAM on the 19th of October 2012 and on the 22nd of July 2013, respectively, based on the results obtained by ICL.

Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has recently been used as a tool to investigate the changes in brain activity associated with differences in nutritional status in humans. Activity of reward systems in the brain is increased in the fasted state compared to the fed state with presentation of food-relevant stimuli (LaBar *et al.*, 2001). However, there are only a few reports on the use of fMRI for characterization of brain activity following the systemic administration of hormones affecting appetite in humans (Baicy *et al.*, 2007; Batterham *et al*., 2007; Farooqi *et al*., 2007; Malik *et al*., 2008; Rosenbaum *et al*., 2008). Intravenous infusion of PYY_{3-36} to human subjects modulates activity in brain regions mediating appetitive behaviour and leads to reduced food intake (Batterham *et al*., 2007). However, there have been no human fMRI studies investigating effects of administration of GLP-1 or co-administration of PYY and GLP-1 on brain activity in humans.

In this work, ICL (Partner 2) used BOLD fMRI to investigate the changes in brain activity following PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ (either as single or combined administration) in fasted healthy human subjects, and compared the effects to those seen naturally following a meal. The results of these experiments are summarised below:

1) Both feeding and the infusion of PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ reduce subsequent energy intake, and the summed reduction in energy intake by each of the single hormone infusions was comparable with the reduction after the combined infusion;
2) Brain amygdala, caudate, insula, nucleus accumbens, orbitofrontal cortex and putamen are activatedupon presentation of food-salient visual stimuli, and there were similar reductions in mean percent change in BOLD signal for all of these six regions of interest (ROIs) following feeding or gut hormone administration, compared to control subjects;
3) Feeding, PYY, and GLP-1 administration reduce brain activation by food images. The reduction in mean percent BOLD signal change with the combined infusion of PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ was similar to the summed reduction in mean percent BOLD signal changes after individual administrations of the two hormones.

The results provide evidence in humans that the actions of PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ on brain responses to food-salient stimuli are additive, explaining the way in which gut hormones co-secreted physiologically after meals may work in concert to limit further food intake and cause satiety. Furthermore, the lack of any obvious differential activation pattern between PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ suggests that these hormones may be acting at the level of higher reward centres via a final common pathway.

These results are also interesting in relation to a previous study investigating the effects of ghrelin, the only known gut hormone which acutely increases food intake, on brain activity in normal-weight humans (Malik *et al*., 2008). Intravenous ghrelin infusion increased BOLD activation when subjects viewed images of food compared to when they viewed images of non-food in the amygdala, OFC, insula, visual areas, and striatum. Intravenous ghrelin infusion also resulted in increased food intake compared with saline infusion. By contrast, in the EuroCHIP study, the gut hormones PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$, which acutely inhibit food intake, resulted in a reduction in mean percent BOLD signal change in these ROIs. Collectively, these results and those of Malik *et al*. suggest that certain brain regions form CNS networks, which when activated by ghrelin mediate hunger and when inhibited by the anorectic gut hormones $PYY3_{-36}$ and $GLP\text{-}1_{7\text{-}36\ amide}$ mediate satiety.

In summary, research in this task has characterized the effects of single and combined administration of PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ on brain BOLD fMRI activations in humans. It has been shown that combined infusion of PYY_{3-36} and $GLP\text{-}1_{7\text{-}36\ amide}$ leads to an anorectic effect similar to that observed following a meal. In keeping with this, combined administration modulates brain activations implicated in appetite control to an extent similar to that observed physiologically after a meal. These findings provide direct evidence that the combined action of gut hormones including PYY_{3-36} and $GLP\text{-}1_{7\text{-}36}$ amide in the brain could explain postprandial satiety.

Task 3.3: Spatial distribution and temporal activation profile of higher centres activated in response to a single ip injection of PYY_{3-36} and OXM in mice using MEMRI (Task leader: ICL)

This task was implemented by ICL (Partner 2), and detailed results were reported in Deliverable D3.3 "Identification of distribution and activation of murine high brain centres by PYY_{3-36} and OXM using MEMRI" submitted on the 14th of October 2011.

In order to control the rapid rise in obesity and its enormous health implications, it is necessary to understand the pathways involved in regulating appetite. Post-prandially secreted gut hormones play a major role as satiety signals to the central nervous system (CNS), acting directly on neural circuits in the hypothalamus and brainstem, or via vagal afferents. Numerous studies show that PYY_{3-36} and OXM are potent anorexigenic gut hormones. Both are being investigated as potential therapeutic agents for obesity, but the specific centres in the brain which are activated by these potent anorexigenic gut hormones is not clear. The aim of this task was to identify the spatial distribution and temporal activation profile of brain centres activated in response to a single *ip* injection of PYY_{3-36} and OXM in mice using the imaging technique of manganese enhanced MRI (MEMRI).

In this work, ICL has used MEMRI to show distinct patterns of neuronal activation within the hypothalamus and brainstem of fasted mice in response to peripheral injection of the anorexigenic agents PYY_{3-36} and OXM.

The administration of these anorectic gut hormones results in a reduced rate of signal enhancement in specific areas of the brain, reflecting a reduction in neuronal activity. ICL concluded that MEMRI constitutes a powerful tool for the future investigation of the effects of drugs, hormones, and environmental influences on neuronal activity with a view to identifying novel pathways and new targets for the treatment of obesity.

T3.4: Impact of pharmacological disruption of the mesolimbic reward circuit on ghrelin and fasting induced food intake as well as ghrelin induced adiposity (Task leader: UGOT)

This task was completed by UGOT (Partner 7). The results are extensively described in Deliverable D3.2 "Report on how pharmacological disruption of the mesolimbic reward system affects ghrelin action", submitted on the 28th of November 2013.

In this task, partner 7 (UGOT) aimed to exploit nicotinic cholinergic blockade to interrupt the cholinergic-dopaminergic reward link and determine whether this circuit plays an important role in ghrelin-induced food intake and adiposity. For this purpose, GHS-R1A antagonist administration in fasted rats allowed to explore the impact of suppressing endogenous ghrelin secretion on food intake, as ghrelin levels are increased by fasting.

UGOT sought to determine whether ghrelin central effects on food intake can be interrupted by nicotine acetylcholine receptor (nAChR) blockade. Ghrelin regulates mesolimbic dopamine neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens, partly via cholinergic VTA afferents originating in the laterodorsal tegmental area (LDTg). Given that these cholinergic projections to the VTA have been implicated in natural as well as drug-induced reinforcement, UGOT sought to investigate the role of cholinergic signalling in ghrelin-induced food intake as well as fasting-induced food intake, for which endogenous ghrelin has been implicated. The following results were obtained during the first 18 months of the project:

1) Peripheral (ip) treatment with the non-selective centrally active nAChR antagonist mecamylamine decreased fasting-induced food intake in both mice and rats;
2) Central administration of mecamylamine decreased fasting-induced food intake in rats;
3) Icv ghrelin-induced food intake was suppressed by ip mecamylamine but not by ip hexamethonium, a peripheral nAChR antagonist;
4) Ip Mecamylamine blocked food intake following ghrelin injection into the VTA;
5) Expression of the ghrelin receptor, the growth hormone secretagogue receptor 1A, was found to co-localize with choline acetyltransferase, a marker of cholinergic neurons, in the LDTg, and mecamylamine ip treatment decreased the ability of palatable food to condition a place preference (a classic test showing a suppressed food reward).

These data suggested that ghrelin-induced food intake is partly mediated *via* nAChRs and that nicotinic blockade decreases the rewarding properties of food. This work is also mentioned in Deliverable D3.8 ("Report on whether ghrelin enhances the rewarding properties of food").

Subsequent activities sought to examine whether VTA-accumbal dopaminergic signalling is required for the effects of ghrelin on food reward and intake, and to determine whether endogenous ghrelin acting on the VTA-accumbal dopamine neurons enhances food reward.

Rats were trained in a progressive ratio sugar-induced operant behaviour schedule to measure food motivation and reward behaviour; in addition chow intake was measured subsequently to operant behaviour study. A D1R or D2R antagonist was injected into the NAc in combination with ghrelin microinjection into the VTA to investigate whether this blockade attenuates the ghrelin-induced food reward behaviour.

As a result, VTA injections of ghrelin, consistent with UGOT's previous data (Skibicka, K.P. et al, Neuroscience, 201;180:129-37), produced a significant increase in food reward behaviour and chow

intake. The reward effect of ghrelin was completely blocked by pre-treatment with either a D1R or a D2R antagonist in the NAc. However, chow intake was unaffected by both antagonist treatments.

Thus, pharmacological interruption of ghrelin effects on the VTA-NAc pathways using dopamine receptor antagonists appears to suppress food-motivated behaviour without affecting chow intake. These data identify the VTA to NAc dopaminergic projections, along with D1 and D2R receptors in the NAc as essential elements of the ghrelin responsive circuit controlling food reward behaviour. The results also suggest that food reward behaviour and simple intake of chow are controlled by divergent circuitry where NAc dopamine plays an important role in food reward but not food intake.

In addition, funded through external grants, UGOT decided to initiate additional studies in line with the work package objective, exploring other (non-cholinergic) pharmacological routes to interrupt ghrelin effects on food intake and reward. In particular, UGOT focused on other receptors and signalling systems linked to food intake and reward that include NPY receptor 1 (Y1), opioid (mu) receptors (published by UGOT in Skibicka et al., Endocrinology, 2012, 153:1194-205) and dopamine receptors D1/D2 (Skibicka et al., 2013; Neuropharmacology. 2013 Oct;73:274-83). A summary of these additional task results, published in articles that acknowledge EurOCHIP.

T3.5: Impact of lesion of the mesolimbic reward system on ghrelin-induced food intake and adiposity (Task leader: UGOT)

This task was implemented by UGOT (Partner 7). Detailed results were reported in Deliverable D3.5 "Report on how lesioning the mesolimbic reward system affects ghrelin action" submitted on the 19th of October 2012.

In this task, UGOT (Partner 7) aimed to study ghrelin-induced food intake and adiposity in rats with VTA lesion and/or selective lesion of the VTA dopaminergic system (6-OH dopamine lesion). UGOT had previously showed that the meso-accumbal dopamine (reward) system is a target for ghrelin. Thus, when administered centrally or into discrete reward nodes such as the VTA (the site or origin of the dopamine cells), this caused a rise in extracellular dopamine levels in the nucleus accumbens together with an increase in locomotor activity. Moreover, it emerged in subsequent studies performed by UGOT that ghrelin action at the level of the VTA is important for conferring reward from alcohol. Subsequently, UGOT started investigating whether ghrelin action at this site is important for reward from food. This work has now been published. Part of the work, that investigates the importance of this projection for ghrelin effect on food intake and body weight regulation, involved lesioning the VTA or the dopamine projections originating in VTA. The following results were obtained:

1) VTA lesion using ibotenic acid suppresses food-motivated behaviour.
2) Chemical lesion of the VTA in rats did not affect body weight gain or consumption of chow measured over the 7 days following surgery, indicating that the lesion did not induce hypophagia *per se*. Moreover, no difference in baseline locomotor activity was found between sham and VTA-lesioned rats.
3) Icv ghrelin injection increased 4-hour standard chow intake in both sham and VTA-lesioned rats compared with vehicle treatment. However, icv ghrelin-induced chow intake did not differ between sham and VTA-lesioned rats.
4) Icv ghrelin injection increased the consumption of peanut butter in both sham and VTA-lesioned rats compared with vehicle treatment. Ghrelin-induced peanut butter consumption was, however, attenuated in VTA-lesioned rats compared with sham rats.

5) In tests of motivation for a food reward, time spent exploring a peanut butter set-up was considerably decreased in ghrelin-treated VTA-lesioned rats compared with ghrelin-treated sham rats. The increased exploration time was not coupled to actual eating but rather to the effort of trying to eat and access remaining peanut butter left at the bottom of an Eppendorf tube. When individuals that did not explore/consume any of the peanut butter were excluded, no difference in consumption of peanut butter could be found between ghrelin-treated sham and VTA-lesioned rats. Importantly, the time spent exploring the peanut-filled Eppendorf (including eating) was still decreased by 52% in VTA-lesioned rats compared with sham rats. The proportion of rats not interested in peanut butter following ghrelin administration was greater (2/6) in the VTA-lesioned group than in the sham group (0/5).
6) The effects of long-term central ghrelin treatment on body weight and regular chow intake were not affected by bilateral 6-OH dopamine lesion of the VTA; these parameters were identical for the lesioned animals relative to sham controls.
7) Given this unexpected result in 6) above, UGOT sought to verify the extent of the success of the dopamine depletion by VTA 6-OH lesion. UGOT found that when the rats were exposed to a palatable food (peanut butter), there was an inverse relationship between intake of palatable food and the levels of dopamine in the NAc as well as the prefrontal cortex in ghrelin treated rats but not following vehicle treatment.

As a conclusion, The VTA is a primary site of action for ghrelin effects on food motivation. In particular, the dopamine cells in this region (that project to, *e.g.*, the accumbens and the prefrontal cortex) are required for these effects of ghrelin.

T3.6: Enhancement of the rewarding properties of food by ghrelin (Task leader: UGOT)

This task was implemented by UGOT (Partner 7). Detailed results were reported in Deliverable D3.8 "Report on whether ghrelin enhances the rewarding properties of food" submitted on the 27th of May 2011.

Using behavioural tests of food reward, UGOT (Partner 7) aimed to determine if the rewarding properties of palatable food are suppressed in animal models of altered ghrelin signalling (e.g. rats treated centrally with GHS-R1A agonists and antagonists or ghrelin receptor knockout mice).

Behavioural studies evaluating the time spent in food exploration (palatable food in a non-accessible container) were used to assess reward and motivation. In the two models mentioned above, partner 7 explored the impact of long-term exposure to highly palatable food (e.g. ensure chocolate drink), that appears to activate reward pathways and high fat feeding (separately/combined) on metabolic phenotypes, such as bodyweight, feeding, energy expenditure, respiratory quotient and body fat composition. These models have been used previously to study the impact of palatable foods in an obesigenic environment.
The following results were obtained:

1) Mice with a disrupted gene encoding the ghrelin receptor (GHS-R1A) and rats treated peripherally with a GHS-R1A antagonist both show suppressed intake of rewarding food in a free choice (chow/rewarding food) paradigm. Moreover, accumbal dopamine release induced by rewarding food was absent in GHS-R1A knockout mice.
2) Acute bilateral intra-VTA administration of ghrelin also increased 1-hour consumption of rewarding food but not standard chow.

3) The ability of rewarding food to condition a place preference was suppressed by the GHS-R1A antagonist in rats

Although UGOT originally planned to perform studies investigating motivated behaviour for food using the peanut-Eppendorf set-up (see task 3.5 - if the animal finds the food rewarding, it will increase exploration time) and lever pressing for food, it was finally decided to incorporate the models into studies investigating the impact of lesion of the VTA on ghrelin-induced motivated behaviour for food.

Another deviation concerned the fact that UGOT decided not to perform metabolic studies of energy expenditure, respiratory quotient and body fat composition in the free choice (palatable food/chow study). Indeed such parameters likely only reflect changes in fat that are secondary to the palatable diet and do not reflect effects of ghrelin. Data in line with the deliverable and objectives of this work package have however been collected using many additional models to those listed in the initial grant, including dopamine measurements, intra-VTA injections, food intake/preference, locomotor stimulation and the CPP test mentioned above.

As a conclusion, the data obtained support the hypothesis that central ghrelin signalling at the level of the VTA is important for the incentive value of palatable food. This is evidenced, first by food preference and intake studies comparing effects on palatable food versus normal chow. Thus, animals with suppressed ghrelin signalling (induced by a GHS-R1A antagonist or by GHS-R1A knockout) show normal chow intake, reduced intake of palatable/rewarding food and reduced preference for palatable/rewarding food. UGOT also showed that direct injection of ghrelin into a key reward node, the VTA, increases food intake and induces a locomotor response. The midbrain dopamine system, an established target for food reward (including motivated behaviour for food) was also shown to be a target for ghrelin-induced food intake.

T3.7: Effects of targeted genetic disruptions of Stat3 and Pdk1 in dopaminergic neurons on the actions of Ghrelin, PYY_{3-36} and OXM (Task leader: UKK)

The work completed by UKK (Partner 5) in task 3.7 resulted in the production of Deliverable D3.4 "Report on how deletion of *Stat3* and *Pdk1* in dopaminergic neurons affects ghrelin, PYY_{3-36} and OXM action" submitted on the 28th of November 2013.

In order to investigate the role of leptin signalling in dopaminergic neurons on the actions of gut hormones, UKK (Partner 5) generated mice with targeted disruption of the *Stat3* and mice lacking *Pdk1*, the principle mediator of PI3k-signalling, in dopaminergic neurons. STAT3 is a key mediator of leptin signalling, while PI3k is an important point of convergence between insulin and leptin signalling. Deviating from the intended plans, UKK also decided to generate mice lacking the alternative leptin receptor Stat5 instead of Stat3 specifically in dopaminergic neurons. Since the role of Stat5 is considerably less characterized in leptin signalling compared to that of Stat3 and PI3k, the resulting mice carry the potential of providing entirely new insights into the regulation of feeding.

The mice expressing a constitutively active Stat3 molecule in DAT cells, generated and characterized by UKK, did not yield a major metabolic phenotype. Similarly, mice with PTEN-deletion did not yield a metabolic phenotype either. Thus, pursuing the role of Stat5 rather than PI3k and Stat3 appeared a more promising approach than the initially proposed one.

Immunohistochemical analyses revealed a profound expression of DAT in the dopaminergic brain areas including the ventral tegmental area (VTA) and *substantia nigra* (SN).

Furthermore, Stat5 expression in the VTA/SN region was confirmed by Stat5 immunostaining in control mice (Stat5 fl/fl mice), whereas no Stat5 expression could be detected in *S5daKO* (Stat5 knock out) mice, confirming UKK's work on conditional gene ablation of Stat5 in dopaminergic neurons.

From 3 weeks of age, UKK monitored body weight of control and *S5daKO* mice under normal chow diet conditions. This analysis revealed no differences in body weight gain in *S5daKO* mice compared to controls. Although no change in body weight gain was detected, *S5daKO* mice showed a decreased fat mass and increased lean mass (at the age of 16 weeks) as compared to their control littermates. This profound metabolic phenotype was observed in female mice but not in male mice.

To determine the underlying mechanism of leanness in mice lacking Stat5 signalling in dopaminergic neurons, UK assessed food intake in *S5daKO* mice. This analysis showed that female *S5daKO* exhibit an increased food intake. Assessment of indirect calorimetry in control and *S5daKO* mice revealed that female *S5daKO* have an increased locomotor activity together with increased energy expenditure. Collectively, these analyses indicate that increased locomotion and increased energy expenditure account for the reduced adiposity in female *S5daKO* mice.

To address whether the obese phenotype of *S5daKO* mice also affects glucose metabolism,UKK performed insulin and glucose tolerance tests. Deletion of Stat5 in dopaminergic neurons improves insulin sensitivity. Furthermore, *S5daKO* mice show an improved resistance against thermal stress when exposed to cold.

T3.8: Effects of targeted disruption of the insulin receptor in dopaminergic neurons on the actions of Ghrelin, PYY_{3-36} and OXM (Task leader: UKK)

This task was implemented by UKK (Partner 5). **Detailed results were reported in Deliverable D3.6 "Report on how deletion of *insulin receptors* in dopaminergic neurons affects ghrelin, PYY_{3-36}, and OXM action" submitted on the 27th of November 2012.**

The hypothalamus integrates various hormonal and neuronal signals to regulate appetite and metabolism, and thus plays a crucial role in the homeostatic control of body weight. Additional neuronal circuits can influence and eventually override this system. One such system comprises mid-brain dopamine neurons, which play a pivotal role in reward and motivational aspects of feeding behaviour. Importantly, food palatability and reward are thought to be major factors involved in the regulation of food intake. Overriding of basic homeostatic control systems by the cognitive, rewarding, social, and emotional aspects of palatable food may contribute to the obesity epidemic.

Peripheral hormonal signals such as insulin and leptin directly target the hypothalamus to control energy homeostasis in a negative feedback regulation. Moreover, these signals also directly regulate the reward circuitry of the brain, and direct insulin action in dopaminergic midbrain neurons regulating reward-related behaviour associated with food have been recently demonstrated (Könner, Cell Metab, 2011; Hommel, Neuron, 2006), leading to the conclusion that the deletion of insulin signalling in these cells causes obesity in mice.

As a reminder, GLP1 is a proglucagon derived peptide which can induce anorexia through mechanisms that involve vagal and possibly central pathways. At present it is not known whether GLP1 could have an effect on the reward circuitry of the brain for the regulation of food intake.

To investigate whether insulin signalling in dopaminergic midbrain neurons plays a role in the central regulation of food intake by GLP1, UKK (Partner 5) specifically disrupted the insulin receptor in dopaminergic neurons. Male control and KO mice (lacking the insulin receptor in cells expressing tyrosine hydroxylase) were icv injected with GLP-1, and their food intake and body weight were then measured. Additionally, to define the role of insulin receptor signalling in dopaminergic midbrain neurons, UKK inactivated insulin receptor signalling in tyrosine hydroxylase (Th)-expressing cells of mice.

Metabolic phenotyping of mice with an insulin receptor inactivation in Th-positive mid-brain neurons revealed a significant body weight increase compared to control littermates. This increase in body weight was accompanied by an increase in fat mass and hyperphagia. Electrophysiological studies revealed that insulin acutely stimulated firing frequency in 50% of dopaminergic VTA/SN neurons. This response was abolished in mice lacking the insulin receptor in Th-expressing cells. Moreover, these mice exhibited an altered response to cocaine under food-restricted conditions as well as an increased preference for a sucrose solution. These findings, published in Könner *et al*. (Cell Metab, 2011, 6:720-8), provide direct evidence that insulin signalling in Th-positive mid-brain neurons is required for long-term control of energy homeostasis.

To analyse the effect of centrally applied GLP1 in mice lacking the insulin receptor in Th-expressing cells, UKK then injected male control and knockout mice icv with GLP-1 and measured food intake and body weight. Icv injection of GLP1 resulted in a significant reduction in food intake 2 and 4 hours after re-feeding, both in control and knockout mice, as compared to control animals.

The results on the characterization of the TH-Cre/IR mice have unveiled a new role for insulin signalling in control of energy metabolism through the reward system of the brain. The corresponding phenotype is described in details in the paper recently published by UKK (Könner, Cell Metab., 2011). Since GLP1 shares some properties with insulin, and since both are peripheral signals targeting as well the central nervous system, UKK investigated the possibility that GLP1 action on the brain for control of food intake could involve insulin signalling in catecholaminergic neurons. However, the results obtained on GLP1 icv treatment argue against this hypothesis.

T3.9: Effect of the expression of a constitutive open mutant of Kir6.2 specifically in dopaminergic VTA-neurons on the actions of Ghrelin, PYY_{3-36} and OXM (Task leader: UKK)

Task 3.9 was completed by UKK and resulted in the production of Deliverable D3.9 "Report on how expression of mutant *KIR6.2* in dopaminergic neurons affects ghrelin, PYY_{3-36} and OXM action" submitted on the 15th of November 2013.

Based on the unexpected results obtained on insulin signalling in task 3.8, UKK (Partner 5) decided to focus on another important peripheral signal involved in energy homeostasis, namely glucose. Glucose-mediated neuronal excitation is partly controlled by ATP-dependent closure of potassium (K_{ATP}) channels, which can play a fundamental role as metabolic sensors linking changes in cellular glucose metabolism to electrical activity. However, the specific role of K_{ATP} channels, widely expressed in defined neuronal populations involved in energy homeostasis, was only partly understood at the start of EurOCHIP. K_{ATP} channels exhibit a wide expression pattern in the central nervous system, particularly clustering in a broad range of catecholaminergic neurons. Hence, UKK investigated the function of K_{ATP} channels in these neurons in mediating the effect of glucose sensing on energy homeostasis. In that goal, UKK used a K_{ATP} channel variant called Kir6.2 that renders the channels resistant to closure, thereby disrupting glucose effect on neuronal activity.

This study led to a number of new findings, including the following main results:

1) The metabolic sensor Kir6.2 is expressed in most catecholaminergic TH-positive neurons.
2) *Kir6.2*^{THCre}-mice show a slight increase in body weight upon exposure to ND. The difference in body weight was more apparent in animals exposed to HFD.
3) Obesity in *Kir6.2*^{THCre}-mice was confirmed by the relative increase in fat mass under ND or HFD, and was further reflected by significant hyperleptinemia on HFD.
4) Morphological analysis of white adipose tissue revealed significant hyperplasia of adipocytes in *Kir6.2*^{THCre}-mice.
5) Energy expenditure was significantly reduced in *Kir6.2*^{THCre}-mice on HFD; decreased energy expenditure rather than increased food intake may account for the exacerbated obesity in *Kir6.2*^{THCre}-mice.
6) Blood glucose and plasma insulin analyses showed significant hyperglycaemia and hyperinsulinemia in HFD-fed *Kir6.2*^{THCre}-mice compared to controls.
7) *Kir6.2*^{THCre}-mice exhibited slightly impaired glucose tolerance and slightly impaired insulin tolerance, even under ND conditions.
8) HFD-mediated impairment in glucose tolerance was significantly enhanced in *Kir6.2*^{THCre}.
9) Expression of the variant Kir6.2 subunit in TH neurons impairs glucose tolerance and insulin sensitivity even under ND conditions, and this effect is enhanced on HFD.
10) The brown adipocytes in *Kir6.2*^{THCre}-mice show a macro-vacuolar, white-adipocyte-like phenotype.
11) BAT of *Kir6.2*^{THCre}-mice exhibited slightly reduced mRNA-expression of the brown adipocyte differentiation marker CIDEA as well as of PGC-1α and UCP-1 as key regulators of mitochondrial biogenesis and uncoupling. Moreover, protein expression of UCP-1 in BAT of *Kir6.2*^{THCre}-mice compared to controls was similarly reduced.
12) A significant reduction in BAT sympathetic nerve activity (SNA) is observed in *Kir6.2*^{THCre}-mice. The data obtained point towards a specific reduction of BAT SNA and not a generalized reduction of SNA in *Kir6.2*^{THCre}-mice. This notion was further supported by an unaltered regulation of heart rate and blood pressure in *Kir6.2*^{THCre}-mice.
13) Expression of the variant Kir6.2 subunit unlikely alters general stress responses.
14) Expression of an ATP-insensitive Kir6.2 subunit in catecholaminergic neurons of mice results in obesity and reduced sympathetic outflow to BAT as well as a lack of ability to increase BAT SNA in response to centrally applied glucose resulting in an appropriate thermogenic response under cold conditions.
15) TH-positive-neurons in the locus coeruleus (LC), which, *via* retrograde tracing experiments, have been implicated in the neurocircuitry that controls BAT innervation, respond to alterations in extracellular glucose concentrations with concomitant changes in firing properties in a K_{ATP}-channel-dependent manner.
16) Consistent with the increased weight gain upon selective expression of the variant Kir6.2-subunit in the LC, these mice exhibited significantly increased adiposity as indicated by the significant elevation in total body fat content, epigonadal fat pad mass as well as white adipose tissue (WAT) hyperplasia.

17) Glucose-elicited, K_{ATP}-channel-dependent control of neuronal activity of LC neurons contributes to the regulation of BAT SNA as well as adaptive responses to high fat feeding to maintain energy homeostasis.

As a conclusion, the results on the characterization of the *Kir6.2*^{THCre}-mice have unveiled a new role of K_{ATP}-channel-dependent neuronal excitability in catecholaminergic neurons in maintaining thermogenic BAT sympathetic tone and energy homeostasis.

WP4: HUMAN GENETICS

WP4 aimed to determine if genetic variation in molecules involved in the brain response to gut peptides affects appetitive behaviour and predisposes to childhood obesity. It examined the role of genetic influences, both monogenic and polygenic, on the response of the brain to gut hormones and the ability of an individual to respond to bariatric surgery, with a particular focus on severe childhood obesity.

T4.1: Identification of new genes contributing to variation in appetitive behaviour and risk for severe childhood obesity (Task leader: CNRS)

Task 4.1 was completed by UCAM (Partner 1) and CNRS (Partner 3) and resulted in the production of Deliverable D4.2 "Progress report on monogenic screening and rare genetic variants" submitted on the 15th of November 2013.

This task aimed to identify novel genetic aetiologies of severe early-onset obesity or more polygenic forms of obesity linked to appetite behaviour. In that goal, CNRS developed and validated a novel tool for rapid and cost efficient genetic diagnosis of monogenic obesity and diabetes using next-generation sequencing technologies. The sequencing target included 43 genes involved in monogenic forms of obesity or diabetes. A proof-of-concept study demonstrated the interest of the protocol allowing for the simultaneous analysis of dozens of genes for less than 300€.

CNRS then completed the screening and analysis of 230 candidate genes for obesity sequenced in DNA samples from 13 families, including a total of 43 severely obese individuals, 35 obese individuals, 46 overweight individuals and 79 subjects with normal body mass index. CNRS discovered the first heterozygous mutation in the obesity gene PCSK1 that co-segregates with obesity in a large pedigree. In addition, a putative new gene for obesity that seems to play a role in adipocyte metabolism was identified and submitted to further functional studies. Finally, whole-exome sequencing was completed in 6 trios including normal weight parents and severely obese children, to find putatively causal *de novo* mutations in the obese children.

In parallel, UCAM screened a number of candidate genes in its cohort of severely obese children and has potentially identified two new monogenic obesity syndromes involving *SH2B1* and *MRAP2*. UCAM has also begun to really characterize a multitude of missense mutations previously unreported in *SIM-1*. The data obtained by UCAM demonstrate that rare variants in *SIM1* are associated with severe obesity and should be considered in patients with hyperphagic obesity of early onset. The study of patients with *SIM1* variants that exhibit reduced activity *in vitro* provides strong evidence that this transcription factor plays a physiological role in central melanocortin signalling in a specific pathway that regulates food intake independently of energy expenditure, although the precise details of this circuitry remain to be established. Identifying the direct transcriptional targets of *SIM1* will be critical to understanding the molecular mechanisms underlying its role in the regulation of food intake and body weight.

UCAM has also demonstrated that loss-of-function mutations in *SH2B1* were associated with severe early-onset obesity, insulin resistance, and reduced final height. All the mutations were associated with loss of function in assays of GH/NGF-mediated signalling. Intriguingly, apart from the frame shift mutation, the other mutants did not impair leptin signalling. Although this discordance may reflect the differing sensitivities of the assays used, it is plausible that some of the effects of SH2B1 on energy homeostasis may be mediated by leptin-independent pathways.

Finally, UCAM has found that global or brain-specific inactivation of *Mrap2* causes obesity in mice and that rare heterozygous variants in *MRAP2* are associated with early-onset, severe obesity in humans. The mechanism(s) by which Mrap2 exerts its effects on body weight regulation remain to be firmly established but likely involve altered signalling through Mc4r and perhaps other MCRs.

T4.2: Common gene variants and their relation to appetitive behaviour (Task leader: CNRS)

Task 4.2 was completed by UCAM (Partner 1) and resulted in the production of Deliverable D4.3 "Progress report on common genetic variants" submitted on the 15th of November 2013.

Within a population that shares the same environment, a significant proportion of the variance in BMI is genetically determined. Meta-analyses of genome-wide association studies (GWAS) for obesity-related traits had led to the discovery of at least 52 loci at the project start. However, the fraction of BMI variance that can be attributed to common SNPs is, at the most, 2%. Candidate gene studies in severe obesity have led to the identification of several rare, highly penetrant mutations involving the hypothalamic leptin-melanocortin pathway, with variants in the *MC4R* gene being the most common, with a population prevalence of 1 in 1000. Although epistatic and gene-environment interactions may contribute to the unexplained heritability of obesity, it seems that a significant fraction is due to missing loci or established loci that have not yet been characterised.

In task 4.2, UCAM (Partner 1) performed a GWAS in 1509 UK children of European ancestry with severe early-onset obesity and 5380 Wellcome Trust Case Control Consortium 2 (WTCCC2) UK controls to identify new associations of common and low-frequency SNPs and CNVs with obesity.

By combining SNP and CNV analysis and focusing on severe obesity, UCAM identified four new obesity susceptibility loci, including an intermediate-frequency variant in *LEPR*. These findings support the idea that both common and rare variants around specific genes or loci (*LEPR*, *POMC*, *MC4R*, *BDNF*, *SH2B1*) are involved in the pathogenesis of obesity. UCAM showed that there is an incomplete overlap between the loci influencing risk of severe obesity and those that influence more common obesity, detected by studies in population-based cohorts, and that the relative contribution of each locus to severe vs. common obesity also differs. Furthermore, UCAM provided evidence that severe obesity without developmental delay is associated with a significantly greater burden of rare, typically singleton CNVs, in parallel with findings in intellectual disability. Using pathway analysis, UCAM found that rare CNVs that delete genes involved in the neuronal regulation of energy homeostasis contribute to severe obesity; looking for rare coding variants in these genes may be fruitful. As UCAM observed a significant enrichment for CNVs that deleted GPCRs, which are key targets for drug development, these findings may have potential therapeutic implications.

In this part of EurOCHIP, UCAM and CNRS played an important role in the international consortia that contributed to identify new genes/loci associated with BMI and risk for obesity in various populations. These studies included genotyping efforts and comprehensive statistical analyses that permitted to find dozens of new genes and to publish papers in top scientific journals.

During the course of EurOCHIP, UCAM and CNRS have fully succeeded in establishing cost efficient protocols using NGS for the genetic characterization of extreme forms of obesity. Exome sequencing is in routinely use in CNRS and UCAM labs and CNRS has recently published a paper demonstrating the interest of the targeted NGS of all known obesity gene for a cost efficient and rapid molecular diagnostics of monogenic obesity. In this respect the progress in NGS technology has allowed the use of this technology for both sensitive but expensive exome sequencing and more affordable targeted re-sequencing opening new insights towards personalized obesity medicine.

T4.3: Role of genetic determinants on the differences in metabolic response to bariatric surgery (Task leader: CNRS)

This task was completed by CNRS (Partner 3) and resulted in Deliverable D4.1 "Progress report on genetic determinants of bariatric surgery" submitted on the 27th of November 2012.

This part of the project aimed at identifying genetic determinants involved in the differences observed in metabolic responses to bariatric surgery.

CNRS (Partner 3) has genotyped the 24 obesity-associated genetic variants (same as above) in 1,785 Swiss individuals who have had a bariatric surgery (with a 6-year follow-up). CNRS assessed the effect of each SNP and the associated obesity genotype score (sum of the obesity risk alleles per individual) on the decrease (%) in body mass index.

WP5: HUMAN INTERVENTION AND THERAPEUTICS

WP5 aimed to modulate the gut peptide milieu in humans, either by administration of exogenous gut peptides or through dietary intervention, to determine if they are potential therapeutic targets.

T5.1: Investigation of the effects of co-administration of the gut hormones PYY_{3-36} and OXM on food intake in humans (Task leader: ICL)

This task was fully implemented by ICL (Partner 2). Detailed results were reported in Deliverable D5.1 "Report on different dietary manipulations on circulating gut hormones and food intake" submitted on the 15th of November 2013.

In task 5.1, the aim of ICL (Partner 2) was to intravenously administer the gut hormones PYY_{3-36} and OXM in combination to healthy overweight and obese volunteers (BMI 27-35kg/m^2) and measure their acute effects on food intake. The objective was to compare the effects of co-administration of PYY_{3-36} and OXM on appetite with the effects of each peptide alone.

Healthy male and female volunteers aged ≥18 years with a stable BMI of 25–40 kg/m^2 were recruited by advertisement. The study was approved by the Hammersmith and Queen Charlotte's and Chelsea Research Ethics Committee (reference number 06/Q0406/50). All participants gave written informed consent, and the study was planned and performed in accordance with the Declaration of Helsinki.

The study followed a randomized, double-blind, placebo-controlled crossover protocol comparing the effect on energy intake of six different pairs of infusions (Saline, Low-dose $PYY_{3–36}$, Low-dose OXM, High-dose $PYY_{3–36,}$ High-dose OXM, $PYY_{3–36}$ + OXM). Immediately before each blood sample was taken, subjects completed visual analogue scales (VAS) rating hunger, satiety, prospective food consumption, and nausea. ICL investigated the Effect of $PYY_{3–36}$ and oxyntomodulin infusions on energy intake and appetite, and measured plasma concentrations of $PYY_{3–36}$, OLI, insulin, and glucose.

In this study, ICL has shown that combined infusion of $PYY_{3–36}$ and oxyntomodulin appear to have an additive anorectic effect in overweight and obese humans. These results and data from other recent studies suggest that Y2 receptor agonists and GLP-1 receptor agonists may be particularly suited to co-administration for the treatment of obesity. However, further studies are required to establish whether chronic co-administration of gut hormones can increase the potential anorectic effect without inducing a parallel increase in nausea.

T5.2: Effects of different dietary manipulations on peripheral hormone secretion and ad libitum food intake (Task leader: UCAM)

This task was fully implemented by UCAM (Partner 1). Detailed results were reported in Deliverable D5.1 "Report on different dietary manipulations on circulating gut hormones and food intake" submitted on the 27th of May 2011.

In task 5.2, UCAM (Partner 1) examined the effects of different dietary manipulations on gut hormone secretion and *ad libitum* food intake.

Eight healthy volunteers participated in this randomized three-way crossover study (mean age of 32 years). Subjects were fasted from 22:00 the night before the study and were admitted to the clinical research facility at 07:00. Blood was drawn before and after a test meal of varying macronutrient composition (from 07:30 ending at 12:00, with a meal given at 08:00). Volunteers completed visual analogue scores to assess hunger and fullness immediately prior to each time point. Isocaloric, isovolaemic breakfasts were given at 08:00 and subjects were instructed to finish within 25 minutes. The calories given per breakfast were standardized for each participant to match 20% of the calculated energy requirements:

- Breakfast 1 – 60% CHO, 20% fat, 20% protein
- Breakfast 2 - 60% fat, 20% protein, 20% CHO
- Breakfast 3 – 60% protein, 20% fat, 20% CHO

The differences in energy density of the various ingredients of the breakfasts were matched by adding water making the three breakfasts isovolaemic. After the last sample was taken at 12:00, an ad-libitum lunch was served at 12:15. The macronutrient composition of the lunch was 50% carbohydrate, 30% fat and 20% protein.

The following results were obtained:

- Hunger scores decreased after food intake but there was no difference between breakfasts
- Ghrelin decreased after food intake equally with the three breakfasts
- PYY was highest after the protein breakfast, especially at 3-4 hours.
- GLP-1 was also highest after the protein breakfast
- Differences in gut hormone release were observed when macronutrients were altered
- The differences in gut hormones did not translate into differences in subsequent food intake

1.4 Potential impact, main dissemination activities and exploitation of results

A critical part of EurOCHIP has been the development of a strategy to appropriately exploit and disseminate the data generated throughout the course of the project. This was to ensure the maximum exposure of the project findings, thus leveraging the maximum impact. For a disease such as obesity, which affects so many in the EU, this is a critical step if we are to successfully tackle this problem.

The EurOCHIP exploitation plan has naturally considered how the produced data should be presented to the scientific community, and this is through the traditional means of scientific publications and presentations at conferences, congresses and symposia. However, this plan also takes seriously the public engagement aspect, to the broader public, as well as to students. These include public lectures, radio and TV interviews, as well as events targeted towards students interested in undertaking a science degree. And with regards to potential commercial exploitation, one of the partners (Inserm, Partner 4) has also applied, and been awarded two different patents.

The EurOCHIP scientific exploitation plan has been very successful. The consortium has at least 63 peer-reviewed publications that have resulted either directly or are related to results emerging from EurOCHIP, many in the highest impact journals, and most are openly accessible to the community at large. In terms of presentations at scientific meetings, EurOCHIP partners have been invited to speak at more than 75 international meetings, conferences, congresses and symposia. Thus the consortium has disseminated its data far and wide indeed to the scientific community.

With regards to public engagement, partners within EurOCHIP have also been very active. In addition to participating in annual science festivals that are the hallmark of most major University towns, the consortium members have been making a push to publicize their research to a far larger community. For example, Giles Yeo (UCAM, Partner 1) has this year participated in a TV programme ‘Fat Family Tree’ that screened during the ‘primetime slot’ on UK’s Channel 4, drawing in a million live viewers. Additional viewers tuned on later ‘on-demand’. Dr Yeo also participated in a UK literary festival, as part of the ‘Thinking aloud’ series, where the issues surrounding obesity were discussed with a literary audience. Steve Bloom (ICL, Partner 2) spoke to the ‘Lords Science and Technology Committee’ at the UK Parliament, ensuring that policy decisions surrounding obesity are raised at governmental level. Incidentally, two EurOCHIP participants, Profs Sir Stephen O’Rahilly (UCAM) and Sir Steve Bloom (ICL) were both ‘knighted’ by the Queen of England (thus the title ‘Sir’) during the course of the EurOCHIP project. The publicity the award of both these knighthoods and the contribution to science and obesity research made by both Sir Steves, ensured that obesity research, including findings emerging from EurOCHIP, was brought to the public attention. These are just some examples, and all of these are listed in detail in the project final report.

Finally, based in part from work emerging from EurOCHIP, Ralf Jockers (Inserm, Partner 4) has applied and been awarded two patents, one ‘Method for Detecting Leptin Receptor Ligands’ Issued Nov. 17 2009, US 7,618,818, and another ‘Oligonucleotides which Inhibit Expression of the OB-RGRP’ Issued Feb. 8 2011, US 7,884,084.

In conclusion, the coordinator and the consortium believe that EurOCHIP has been very scientifically successful. Importantly however, the EurOCHIP team have managed to ensure that the data was disseminated beyond the scientific community, and brought to the attention of the public and policy makers. In addition, one of the partners has also had two awarded patents, based in large on work emerging from EurOCHIP.

1.5 Project public website and relevant contact details

Address of the project website:

http://www.eurochip-obesity.com/

Contact details:

Dr Giles S.H. Yeo
University of Cambridge
e-mail: gshy2@cam.ac.uk
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2. Use and dissemination of foreground

Section A

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher and/or place of publication	Year of publication	Relevant pages	Permanent identifiers[3] (if available)	Is/Will open access[4] provided to this publication?
1	Diet-induced gene expression of isolated pancreatic islets from a polygenic mouse model for the metabolic syndrome.	T. Dreja (UCAM)	**Diabetologia**	2/53	Springer Verlag	2010	309-320	doi : 10.1007/s00125-009-1576-4	Yes
2	Morbid obesity exposes the association between *PNPLA3* I148M (rs738409) and indices of hepatic injury in individuals of European descent.	S Romeo (UCAM)	**International Journal of Obesity**	1/34	Nature Publishing Group	2010	190-194	doi : 10.1038/ijo.2009.216	Yes
3	Prevalence of loss of function *FTO* mutations in lean and obese individuals.	D. Meyre (UCAM)	**Diabetes**	1/59	American Diabetes Association Inc.	2010	311-318	doi : 10.2337/db09-0703	Yes
4	Leptin and the Control of Body Weight:A Review of Its Diverse Central Targets, Signaling Mechanisms, and Role in the Pathogenesis of Obesity.	Ashwini Oswal (UCAM)	**Obesity**	2/18	Nature Publishing Group	2010	221-229	doi : 10.1038/oby.2009.228	No
5	The Effects of Neurokinin B upon Gonadotrophin Release in Male Rodents	M. P. Corander (UCAM)	**Journal of Neuroendocrinology**	3/22	Blackwell Publishing	2010	181-187	doi : 10.1111/j.1365-2826.2009.01951.x	Yes
6	Hypothalamic-specific manipulation of Fto, the ortholog of the human obesity gene FTO, affects food intake in rats.	Yi-Chun Loraine Tung (UCAM)	**PLoS ONE**	1/5	Public Library of Science	2010	e8771	doi : 10.1371/journal.pone.0008771	Yes

[3] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).
[4] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

7	Identification of the global transcriptomic response of the hypothalamic arcuate nucleus to fasting and leptin.	Zorica Jovanovic (UCAM)	**Journal of Neuroendocrinology**	8/22	Blackwell Publishing	2010	915-925	doi : 10.1111/j.1365-2826.2010.02026.x	Yes
8	Central leptin signalling: Beyond the arcuate nucleus.	Zorica Jovanovic (UCAM)	**Autonomic Neuroscience: Basic and Clinical**	1-2/156	Elsevier	2010	8-14	doi : 10.1016/j.autneu.2010.05.008	Yes
9	Central melanocortin signalling regulates cholesterol.	Yi-Chun Loraine Tung (UCAM)	**Nature Neuroscience**	7/13	Nature Publishing Group	2010	779-780	doi : 10.1038/nn0710-779	Yes
10	Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs.	K. H. Zivraj (UCAM)	**Journal of Neuroscience**	46/30	Society for Neuroscience	2010	15464-15478.	doi : 10.1523/JNEUROSCI.1800-10.2010	Yes
11	Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localisation	Gumy L*, **Yeo GSH*** (UCAM)	**RNA**	1/17	Cold Spring Harbor Laboratory Press	2011	85-98.	doi : 10.1261/rna.2386111	Yes
12	Where next for GWAS?	G. S. H. Yeo (UCAM)	**Briefings in Functional Genomics**	2/10	Oxford University Press	2011	51	doi : 10.1093/bfgp/elr011	Yes
13	Hypothalamic transcriptome plasticity in two rodent species reveals divergent differential gene expression but conserved pathways.	L. Stewart (UCAM)	**Journal of Neuroendocrinology**	2/23	Blackwell Publishing	2011	177-185	doi : 10.1111/j.1365-2826.2010.02093.x	Yes
14	Where to go with FTO?	Rachel Larder (UCAM)	**Trends in Endocrinology and Metabolism**	2/22	Elsevier Inc.	2011	53-59	doi : 10.1016/j.tem.2010.11.001	Yes
15	Central leptin and ghrelin signalling: Comparing and contrasting their mechanisms of action in the brain.	Xiaoye Shan (UCAM)	**Rev Endocr Metab Disord**.	12(3)	Springer Netherlands	2011	197-209	doi : 10.1007/s11154-011-9171-7	Yes
16	From GWAS to Biology: Lessons from FTO.	Yi-Chun Loraine Tung (UCAM)	**Annals of the New York Academy of Sciences**	1/1220	Blackwell Publishing	2011	162-171	doi : 10.1111/j.1749-6632.2010.05903.x	Yes

17	FTO biology and obesity: why do a billion of us weigh 3 kg more?	Man-Ka Marcella Cheung (UCAM)	**Frontiers in Endocrinol**	/2	[Lausanne : Frontiers Research Foundation]	2011	-	doi : 10.3389/fendo.2011.00004	Yes
18	FTO and obesity: a problem for a billion people.	G. S. H. Yeo (UCAM)	**Journal of Neuroendocrinology**	2/24	Blackwell Publishing	2012	393-394	doi : 10.1111/j.1365-2826.2011.02254.x	Yes
19	BarraCUDA - a fast short read sequence aligner using graphics processing units.	Klus P (UCAM)	**BMC Research Notes**	January 2012 5:27	BIOMED CENTRAL LTD	2012	epub	doi : 0.1186/1756-0500-5-27	Yes
20	Human SH2B1 mutations are associated with maladaptive behaviors and obesity.	Michael E. Doche (UCAM)	**Journal of Clinical Investigation**	12/122	The American Society for Clinical Investigation	2012	4732-4736	doi : 10.1172/JCI62696	Yes
21	Kinetic analysis of FTO (Fat mass and obesity related) reveals that it is unlikely to function as a sensor for 2-oxoglutarate.	Ma M (UCAM)	**BIOCHEMICAL JOURNAL**	2/444	PORTLAND PRESS LTD	2012	epub	doi : 10.1042/BJ20120065	Yes
22	Overlap of Endocrine Hormone Expression in the Mouse Intestine Revealed by Transcriptional Profiling and Flow Cytometry.	Habib AM (UCAM)	**Endocrinology**	7/153	ENDOCRINE SOC	2012	3054-3065	doi : 10.1210/en.2011-2170	Yes
23	Uncovering the biology of FTO.	G. S. H. Yeo (UCAM)	**Molecular Metabolism**	1-2/1	Amsterdam, The Netherlands : Elsevier Science	2012	32-36	doi : 10.1016/j.molmet.2012.06.001	Yes
24	Unravelling the brain regulation of appetite: Lessons from genetics.	G. S. H. Yeo (UCAM)	**Nature Neuroscience**	October 2012 Vol15 No10	Nature publishing group	2012	1343-1349	doi : 10.1038/nn.3211	Yes
25	FTO expression is regulated by availability of essential amino acids.	Cheung MK (UCAM)	**INTERNATIONAL JOURNAL OF OBESITY**	May 2012	NATURE PUBLISHING GROUP	2013	744-747	doi : 10.1038/ijo.2012.77	Yes
26	Adult Onset Global Loss of the Fto Gene Alters Body Composition and Metabolism in the Mouse.	Fiona McMurray (UCAM)	**PLoS Genetics**	1/9	Public Library of Science	2013	e1003166	doi : 10.1371/journal.pgen.1003166	Yes
27	Role for the obesity-related FTO gene in the cellular sensing of amino acids.	P. Gulati (UCAM)	**Proceedings of the National Academy of Sciences of the United States**	7/110	National Academy of Sciences	2013	2557-2562	doi : 10.1073/pnas.1222796110	Yes

28	The biology of FTO: from nucleic acid demethylase to amino acid sensor.	Pawan Gulati (UCAM)	**Diabetologia**	10/56	Springer Verlag	2013	2113-2121	doi : 10.1007/s00125-013-2999-5	Yes
29	Genome-wide SNP and CNV analysis identifies common and low-frequency variants associated with severe early-onset obesity.	Eleanor Wheeler (UCAM)	**Nature Genetics**	5/45	Nature publishing group	2013	513-517	PMID: 23563609	Yes
30	High protein intake stimulates postprandial GLP1 and PYY release.	Agatha A. van der Klaauw (UCAM)	**Obesity**	8/21	Nature publishing group	2013	1602-1607	doi : 10.1002/oby.20154	Yes
31	Rare variants in single-minded 1 (SIM1) are associated with severe obesity.	Shwetha Ramachandrappa (UCAM)	**Journal of Clinical Investigation**	7/123	The American Society for Clinical Investigation	2013	3042-3050	doi : 10.1172/JCI68016	Yes
32	Loss of function of the melanocortin 2 receptor accessory protein 2 is associated with mammalian obesity.	M.Asai (UCAM)	**Science**	6143/341	American Association for the Advancement of Science	2013	275-278	doi : 10.1126/science.1233000	Yes
33	KSR2 Mutations Are Associated with Obesity, Insulin Resistance, and Impaired Cellular Fuel Oxidation.	Laura R Pearce (UCAM)	**Cell**	4/155	Cell press	2013	765-777	doi : 10.1016/j.cell.2013.09.058	Yes
34	The bigger picture of FTO – the first GWAS-identified obesity gene.	Ruth J. F. Loos (UCAM)	**Nature Reviews Endocrinology**	-	Nature publishing group	2013	-	doi : 10.1038/nrendo.2013.227	Yes
35	The hypothalamus and metabolism: integrating signals to control energy and glucose homeostasis.	Anthony P Coll (UCAM)	**Current Opinion in Pharmacology**	6/13	Elsevier BV	2013	970-976.	doi : 10.1016/j.coph.2013.09.010	Yes
36	PP2Ce: Fat and stressed out?	Goodall JC and Yeo GSH (UCAM)	**Molecular Metabolism**	In press	-	2013	-	-	Yes
37	Glucagon and GLP-1 inhibit food intake and increase c-fos expression in similar appetite regulating centres in the brainstem and amygdala.	JA Parker (ICL)	**International Journal of Obesity**	10/37	Nature Publishing Group	2013	1391-1398	doi: 10.1038/ijo.2012.227	yes
38	Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia.	T.M. Tan (ICL)	**Diabetes**	4/62	American Diabetes Association Inc.	2013	1131-1138	doi : 10.2337/db12-0797	yes

39	Peripheral administration of prokineticin 2 potently reduces food intake and body weight in mice via the brainstem.	KEL Beale (ICL)	**British Journal of Pharmacology**	2/168	Nature Publishing Group	2013	403-410	doi : 10.1111/j.1476-5381.2012.02191.x	yes
40	Selective ablation of peptide YY cells in adult mice reveals their role in beta cell survival.	Amir H. Sam (ICL)	**Gastroenterology**	2/143	W.B. Saunders Ltd	2012	459-468	doi : 10.1053/j.gastro.2012.04.047	yes
41	The Gut Hormones PYY3-36 and GLP-17-36 amide Reduce Food Intake and Modulate Brain Activity in Appetite Centers in Humans	Akila De Silva (ICL)	**Cell Metabolism**	5/14	Cell Press	2011	700-706	doi : 10.1016/j.cmet.2011.09.010	yes
42	A role for metalloendopeptidases in the breakdown of the gut hormone, PYY 3-36.	M.L. Addison (ICL)	**Endocrinology**	12/152	The Endocrine Society	2011	4630-4640	doi : 10.1210/en.2011-1195	yes
43	Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human	Atsuhiko Ichimura (CNRS)	**Nature**	7389/483	Nature Publishing Group	2012	350-354	doi : 10.1038/nature10798	no
44	A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance.	Alisa K Manning (CNRS)	**Nature Genetics**	6/44	Nature Publishing Group	2012	659-669	doi : 10.1038/ng.2274	no
45	A genome-wide association meta-analysis identifies new childhood obesity loci	Jonathan P Bradfield (CNRS)	**Nature Genetics**	5/44	Nature Publishing Group	2012	526-531	doi : 10.1038/ng.2247	no
46	Analysis of the contribution of FTO, NPC1, ENPP1, NEGR1, GNPDA2 and MC4R genes to obesity in Mexican children.	Aurora Mejía-Benítez (CNRS)	**BMC Medical Genetics**	1/14	BioMed Central	2013	21	doi : 10.1186/1471-2350-14-21	no
47	Loss-of-function mutations in SIM1 contribute to obesity and Prader-Willi-like features.	Amélie Bonnefond (CNRS)	**Journal of Clinical Investigation**	7/123	The American Society for Clinical Investigation	2013	3037-3041	doi : 10.1172/JCI68035	no
48	Contribution of 24 obesity-associated genetic variants to insulin resistance, pancreatic beta-cell function and type 2 diabetes risk in the French population	S. Robiou-du-Pont (CNRS)	**International Journal of Obesity**	7/37	Nature Publishing Group	2013	980-985	doi : 10.1038/ijo.2012.175	no

49	Common variants near BDNF and SH2B1 show nominal evidence of association with snacking behavior in European populations	S. Robiou-du-Pont (CNRS)	**Journal of Molecular Medicine**	9/91	Springer Verlag	2013	1109-1115	doi : 10.1007/s00109-013-1027-z	no
50	Highly sensitive diagnosis of 43 monogenic forms of diabetes or obesity, through one step PCR-based enrichment in combination with next-generation sequencing	Amélie Bonnefond and Julien Philippe (CNRS)	**Diabetes Care**	-	American Diabetes Association Inc.	2013	-	PMID : 24041679	no
51	Homozygous deletion of an 80 kb region comprising part of *DNAJC6* and *LEPR* genes on chromosome 1P31.3 is associated with early onset obesity, mental retardation and epilepsy.	Virginie Vauthier (INSERM)	**Molecular Genetics and Metabolism**	3/106	Academic Press Inc.	2012	345-350	doi : 10.1016/j.ymgme.2012.04.026	no
52	Anti-obesity phenotypic screening looking to increase OBR cell surface expression.	Vauthier V, Jockers R, Dam J (INSERM)	**Journal of Biomolecular Screening**	-	SAGE Publications Inc.	2013	In press	-	yes
53	Design and validation of a homogeneous time-resolved fluorescence-based leptin receptor binding assay.	Virginie Vauthier (INSERM)	**Analytical Biochemistry**	1/436	Academic Press Inc.	2013	1-9	doi : 10.1016/j.ab.2012.12.013	no
54	KATP-Channel-Dependent Regulation of Catecholaminergic Neurons Controls BAT Sympathetic Nerve Activity and Energy Homeostasis	Sulay Tovar (UKK)	**Cell Metabolism**	3/18	Cell Press	2013	445-455	doi : 10.1016/j.cmet.2013.08.006	no
55	Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis	Könner, A.C. (UKK)	**Cell Metabolism**	No 13, June 2011	Cell Press	2011	720-728	-	no
56	High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons	Klöckener T (UKK)	**Nature Neuroscience**	7/14	Nature Publishing Group	2011	911-918	doi : 10.1038/nn.2847	yes
57	Ablation of Ghrelin O-Acyltransferase Does Not Improve Glucose Intolerance or Body Adiposity in Mice on a Leptin-Deficient ob/ob Background	Henriette Kirchne (HMGU)	**PLoS ONE**	4/8	Public Library of Science	2013	e61822	doi : 10.1371/journal.pone.0061822	Yes
58	Tanycytes: an ERK-gated conduit for leptin into the hypothalamus	Dam J, Jockers R (INSERM)	**Cell Metabolism**	-	-	In Revision	-	-	-
59	Endospanin 1 silencing in the hypothalamic arcuate nucleus contributes to sustained weight loss of high fat diet obese mice	Vauthier V, Jockers R, Dam J (INSERM)	**Gene Therapy**	-	-	In Revision	-	-	-

60	Endospanin 1 dissociates body weight regulation and glucose homeostasis by differentially affecting hypothalamic leptin signalling	Vauthier V, Jockers R, Dam J (INSERM)	-	-	-	In preparation	-	-	-
61	Hypothalamic HDAC5 links epigenetic chromatin remodeling with the pathogenesis of obesity	Kabra, Dhiraj (INSERM)	-	In preparation	-	2014	-	-	-
62	Novel incretin glucagon triagonists exceed metabolic benefits of single- and dual-agonist therapies against 28diabetes and obesity	Tschöp, Matthias (HMGU)	-	In preparation	-	2014	-	-	-

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	**Type of activities[5]**	**Main leader**	**Title**	**Date/Period**	**Place**	**Type of audience[6]**	**Size of audience**	**Countries addressed**
1	Conference	G. Yeo	The Biology of FTO	11th-15th July 2010	International Congress for Neuroendocrinology, Rouen, France.	Scientific Community	200	International
2	Symposium	G. Yeo	Use of AAV to study the role of the FTO gene	28th September 2010	Annual symposium centre for integrative mammalian physiology and pharmacology, Imperial College, London	Scientific Community	200	UK
3	Conference	G. Yeo	Fat mass and obesity associated gene (FTO) and human obesity	14th-16th December 2010	British Pharmacological Society Winter Meeting, London. 'Emerging targets for obesity treatment' Symposium.	Scientific Community ademic	250	UK
4	Symposium	G. Yeo	Leptin and the Control of Body Weight: A Review of its Diverse Central Targets, Signaling Mechanisms, and Role in the Pathogenesis of Obesity	18th – 19th March, 2011	The Brain in Obesity. A conference hosted by the McGill World Platform for Health and Economic Convergence British Consulate-General Montreal, Canada	Scientific Community	300	International

[5] Choose among the following dissemination activities: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[6] Choose among the following types of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

5	Conference	G. Yeo	Dissecting the roles of specific hypothalamic nuclei in the control of energy homeostasis	9th – 13th April, 2011	Central Nervous System Section, FASEB Experimental Biology 2011 meeting, Washington DC, USA	Scientific Community	500	International
6	Conference	G. Yeo	The biology of FTO: Implications for the treatment of common obesity	22nd – 26th June, 2012	Symposium 'Pathogenesis of obesity', American Endocrine Society Annual Meeting, Houston, Texas, USA	Scientific Community	500	International
7	Conference	G. Yeo	Considering obesity as a chronic brain disease	2nd – 5th July, 2012	Symposium 'How to cure obesity', Physiological Society Annual Meeting, Edinburgh UK	Scientific Community	100	International
8	Conference	G. Yeo	FTO plays a role in the cellular response to amino acid deprivation	13th – 16th September, 2012	EMBL/EMBO Diabetes and Obesity Meeting, Heidelberg, Germany	Scientific Community	300	International
9	Conference	G. Yeo	Role of FTO in the CNS control of metabolism	1st – 5th October, 2012	Symposium 'Neuroendocrine control of glucose homeostasis', EASD2012 Berlin, Germany	Scientific Community	300	International
10	Conference	G. Yeo	Is the Obesity Related FTO a Viable Therapeutic Target?	17th – 22nd March, 2013	Keystone symposium 'Neuronal Control of Appetite, Metabolism and Weight', Banff, Alberta, Canada	Scientific Community	350	International

11	Conference	G. Yeo	A role for FTO in nutrient sensing	14th – 16th April, 2013	Programming Obesity: Central and Peripheral Contributors, Cambridge UK	Scientific Community	150	International
12	TV programme	G. Yeo	Fat family tree	23rd May 2013	Expert contributer Channel 4's 'Fat Family Tree'	Medias	National primetime audience >1 million live viewers	UK
13	Public engagement	Yeo	Thinking aloud 'Obesity'	13th April 2013	Wordfest 2013	Medias	150	UK
14	Conference	Invited Speaker	EASD Annual Meeting	02nd October 2009	Vienna	Scientific Community	850	All European countries
15	Conference	Plenary Speaker	Metabolism & Endocrinology Themed Meeting of The Physiological Society	24th March 2010	AstraZeneca Pharmaceuticals, Macclesfield	Scientific Community	250	All European countries
16	Conference	Invited Speaker	BRC summer school	18th June 2010	Berkhamstead	Scientific Community	150	UK
17	Conference	Invited Speaker	REGPEP 2010 - 18th International Symposium in Belfast	28th September 2009	Belfast	Scientific Community	250	All European countries
18	Conference	Invited Speaker	Centre for Obesity Research	14th January 2011	Birmingham	Scientific Community	150	All European countries
19	Conference	Invited Speaker	Society of Chemical Industry Conference	17th February 2011	Belgrave Square, London	Scientific Community	100	UK
20	Conference	Invited Speaker	Annual Advanced General Medical Conference	22nd to 24th February 2011	Muscat	Scientific Community	500	Worldwide
21	Conference	Invited Speaker	ICTU Therapeutic Group Seminar (International Centre for Circulatory Health)	7th April 2011	London	Scientific Community	50	UK
22	Conference	Invited Speaker	Bachem Spring Symposium	28th April 2011	Basel, Switzerland	Scientific Community	100	All European countries
23	Conference	Invited Speaker	Metabolic & Endocrine Specialty Group Meeting	18th May 2011	Victoria, London	Scientific Community	25	UK
24	Conference	Invited Speaker	Regional Physicians Meeting	25th May 2011	Oxford	Scientific Community	200	All European countries

25	Conference	Lecture	SfE Clinical Update	8th November 2011	The Hilton, Victoria Quays, Sheffield	Scientific Community	200	UK
26	Conference	Invited Speaker	Cure Parkinson's Trust – International Conference	01st December 2011	Royal Society of Medicine in London	Scientific Community	150	UK
27	Conference	Invited Speaker	World Diabetes Congress 2011	08th December 2011	Dubai, United Arab Emirates	Scientific Community	1250	Worldwide
28	Opening Ceremony	Invited Speaker	Imperial Centre for Translational and Experimental Medicine w/ Chancellor George Osbourne	28th May 2012	ICTEM Building, Hammersmith	Scientific Community	150	UK
29	Conference	Keynote Speaker	BPS Focused Meeting on Neuropeptides – in Association with the European Neuropeptide Club and the American Summer Neuropeptide Conference	08th June 2012	Kings College, London	Scientific Community	250	All European countries
30	Lords Science and Technology Committee	Comments	Lords Science and Technology Committee	12th June2012	Parliament Buildings, Westminster	Scientific Community	50	UK
31	Symposium	Plenary Speaker	19th International Symposium on Regulatory Peptides (RegPep 2012)	21st to 24th August 2012	Copenhagen	Scientific Community	500	All European countries
32	Conference	Invited Speaker	SPS Biomedicum - Tatemoto session	25th to 27th August 2012	Helsinki	Scientific Community	200	All European countries
33	Conference	Invited Speaker	Obesity 2012, The Management of Obesity and its Complications	25th October 2012	Hallam Conference Cnt, London	Scientific Community	250	All European countries

34	Conference	Invited Speaker	Celebration of the life of Sir Christopher Booth	06th December 2012	Regents Park, London	Scientific Community	50	UK
35	Imperial Hosting	Invited Speaker	John Fingleton Visit to Hammersmith Campus	20th December 2012	ICTEM Building, Hammersmith	Scientific Community	75	UK
36	Conference	Invited Speaker	Clinical update: Diabetes – management of chronic complications	30th January 2013	Wimpole Street, London	Scientific Community	150	UK
37	Conference	Invited Speaker	Society for Endocrinology BES 2013	22rd March 2013	Harrogate	Scientific Community	750	All European countries
38	Conference	Plenary Speaker	Programming Obesity: Central and Peripheral Contributors	16th April 2013	Cambridge	Scientific Community	250	All European countries
39	Annual Meeting	State of the Art Lecture	ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology & Nutrition)	09th May 2013	Excel Centre, London	Scientific Community	200	All European countries
40	Conference	Invited Speaker	UK Incretin Club Meeting	09th May 2013	Royal College of Physicians	Scientific Community	100	UK
41	Conference	Keynote Speaker	Type 2 diabetes	26th June 2013	BMA House, Tavistock Square	Scientific Community	75	UK
42	New Fellows Inaugural Ceremony	New Fellow Speech	New Fellows Inaugural Ceremony – The Royal Society	10th July 2013	The Royal Society Carlton House Terrace	Scientific Community	250	UK
43	Thesis	Vauthier	Endospanins, a new family of proteins regulating the leptin receptor	December 2011	Paris	Scientific Community	70	France
44	Poster	Vauthier Dam	International Conference on Obesity - *ICO* 2010	July 2010	*Stockholm*	Scientific Community, industry	1000	Sweden

45	Conference	Dam Jockers	Looking for a therapy against obesity	January 2011	Sanofi-Aventis, Frankfurt	Industry	20	Germany
46	Presentation	Dam Jockers	Central regulation of food intake	May 2011	Dourdan	Scientific Community	200	France
47	Poster	Pagnon Chen Dam Jockers	Central regulation of appetite by PYY 3-36	Sept 2010	Paris	Scientific Community	70	France
48	Presentation	Vauthier Dam Jockers	Endospanin1 a new regulator of leptin receptor function	Sept 2010	Paris	Scientific Community	70	France
49	Poster	Dam Jockers	Identification of new drugs to combat the obesity epidemic	Sept 2013	Paris	Scientific Community	50	France
50	Conference	Jockers	Impact of leptin receptor trafficking on obesity development	November 2009	Paris	Scientific Community	100	France
51	Radio Interview	Jockers	New perspectives for the treatment of obesity	2009	Paris	Civil society, Media	national	France
52	Interview	Jockers	Role of circadian rhythm perturbation in obesity and type 2 diabetes development	2012	Paris	Civil society, Media	national	France
53	Interview	Jockers	*Selection Reader's Digest* : Treatment for obesity	2009	Paris	Civil society, Media	national	France
54	Conference	Jockers	New therapeutic strategies for the leptin receptor in obesity treatment	2009	Suwon-city	Scientific Community	50	South Korea
55	Presentation	Jockers	Animal models for obesity research: from mice to large animals	2009	Paris	Scientific Community	60	France
56	Conference lecture	Jens C. Brüning	33rd Blankenese Conference “Nutrient Sensing: From Brain to Gut”	27.05. – 28.05.2013	Hamburg, Germany	Scientific Community	300	global
57	Conference lecture	Jens C. Brüning	Keystone Symposium “Neuronal control of Appetite, Metabolism and Weight”	17.03. – 22.03.2013	Banff, Alberta, Canada	Scientific Community	500- 1000	global

58	Conference lecture	Jens C. Brüning	New York Academy of Sciences conference: Inositol phospholipid signaling in physiology and disease	25. – 27.06.2012	New York, USA	Scientific Community	500	global
59	Conference lecture	Jens C. Brüning	Keystone Symposium "Genetic and Molecular Basis of Obesity and Body Weight"	29.01. – 03.02.2012	Santa Fe, NM, USA	Scientific Community	500 – 1000	global
60	Conference Lecture	Dr. Linda Verhagen	Congress of the Society for the Study of Ingestive Behaviour	10.07. – 13.07.2012	Zürich	Scientific Community	500	global
61	Conference	Tschöp	29. Jahrestagung der Deutschen Adipositas Gesellschaft	October 2013	Hannover, Germany	Scientific Community	700	International
62	Conference	Tschöp	4th Annual Symposium ITMO: Circulation, metabolism, nutrition: Central control of metabolic functions	October 2013	Paris, France	Scientific Community	80	Europe
63	Meeting	Tschöp	Annual Meeting of the Helmholtz Association	September 2013	Berlin, Germany	Scientific Community, Media, Civil Society	1000	Europe
64	Conference	Tschöp	IDEA Summit: Innovation in Diabetes – European Action Summit	September 2013	Lund, Sweden	Scientific Community, Industry	600	International
65	Conference	Tschöp	The 36th Naito Conference on Molecular Aspects of Energy Balance and Feeding Behavior	September 2013	Sapporo, Hokkaido, Japan	Scientific Community	200	International
66	Conference	Tschöp	FENS-IBRO Summer School: Training School in Neuroendocrinology	July 2013	Prato, Italy	Scientific Community	400	International
67	Conference	Tschöp	Cell Symposium: Immunometabolism: From Mechanisms to Therapy	June 2013	Toronto, Canada	Scientific Community	900	International

68	Conference	Tschöp	The Endocrine Society Annual Meeting	June 2012	San Francisco, USA	Scientific Community	900	International
69	Conference	Tschöp	International Congress of Endocrinology	May 2012	Florence, Italy	Scientific Community	900	International
70	Conference	Tschöp	3rd Schloss Elmau Meeting "Resitance and Disease promoting Principles of Innate Immunity"	May 2012	Ellmau, Austria	Scientific Community	80	Europe
71	Film	Tschöp	Matthias Tschöp – Alexander von Humboldt Professur	May 2012	n/a	Scientific Community, Civil Society	You tube video: 1500 viewings	International
72	Conference	Tschöp	European Society for Endocrinology (ESE)	November 2011	Zyprus, Greece	Scientific Community	200	Europe
73	Workshop	Tschöp	The Obese Species Workshop	October 2011	Erice, Sicily, Italy	Scientific Community	100	Europe
74	Symposium	Tschöp	The Ramanbhai Foundation, 4th Int. Symposium	February 2011	Ahmedabad, India	Scientific Community	400	International
75	Conference	Tschöp	The Obesity Society 28th Annual Scientific Meeting	October 2010	San Diego, CA, USA	Scientific Community	400	International
76	Conference	Tschöp	11th International Congress of Obesity (ICO)	July 2010	Stockholm, Sweden	Scientific Community	300	International
77	Conference	Tschöp	American Diabetes Association 70th Scientific Sessions	June 2010	Orlando, Florida	Scientific Community	1000	International
78	Conference	Tschöp	2nd USC Childhood Obesity Research Conference	October 2009	Los Angeles, CA, USA	Scientific Community	400	International
79	Conference	Tschöp	21st Annual American Peptide Conference	June 2009	Bloomington, Indiana, USA	Scientific Community	500	International
80	Conference	P. Pfluger	Deutsche Diabetes Gessellschaft Kongress 2013	Leipzig, Germany	Leipzig, Germany	Scientific Community	-	Germany
81	Conference	P. Pfluger	Joint FEPS & Spanish Physiological Society Scientific Congress 2012	Santiago de Compostela, Spain	September 2012	Scientific Community	-	International

82	Conference	P. Pfluger	Joint 15th International Congress of Endocrinology & 14th European Congress of Endocrinology (ICE/ECE)	May 2012	Florence, Italy	Scientific Community	-	International
83	Conference	P. Pfluger	'Frontiers in Gastrointestinal Translational Research Scientific symposium	May 2011	Leuwen, Belgium	Scientific Community	-	International
84	Conference	P. Pfluger	International Life Sciences Institute (ILSI) North America, Technical Committee on Dietary Lipids – Food, Nutrition & Safety Program, Winter Meeting 2010	Washington DC, USA	December 2010	Scientific Community	-	International
85	Conference	P. Pfluger	Obesity 2010 - 28th Annual Scientific Meeting	San Diego, USA	October 2010	Scientific Community	-	International
86	Workshop	P. Pfluger	Ascona Workshop „ The intestinal wall - the regulatory interface in energy homeostasis"	Ascona, Switzerland	June 2010	Scientific Community	-	International
87	Teaching	P. Pfluger	Nutrition Obesity Research Center Seminar	Birmingham, Alabama, USA	March 2010	Scientific Community	-	International

Press releases (ICL):

http://www.dailymail.co.uk/health/article-2053804/How-diet-pill-using-gut-hormones-fool-brains-thinking-full.html?ito=feeds-newsxml
http://www.121doc.co.uk/news/gut-instinct-to-push-through-obesity-treatment-6618.html
http://www.medicalnewstoday.com/articles/236596.php
http://www.sciencecodex.com/read/simple_gut_hormone_combo_makes_our_brains_think_were_full-80343
http://www.science20.com/science_20/pyy_and_glp1_gut_hormones_trick_your_brain_feeling_full-83947
http://www.guardian.co.uk/science/2011/sep/11/obesity-food-appetite-suppressant
http://www.dailymail.co.uk/news/article-194833/Jab-set-cure-obesity.html
http://www.telegraph.co.uk/science/science-news/10177794/Obesity-drug-could-end-need-for-gastric-bypass-surgery.html

Section B

PART B1

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[7]	**Confidential** (YES/NO)	**Foreseen embargo date** (dd/mm/yyyy)	**Application reference(s)** (e.g. EP123456)	**Subject or title of application**	**Applicant (s) (as on the application)**
Patent	NO	Issued Nov 17 2009	US 7,618,818	Method for Detecing Leptin Receptor Ligands	Jockers, Couturier
Patent	NO	Issued Feb 8 2011	US 7,884,084	Oligonucleotides which Inhibit Expression of the OB-RGRP	Jockers, Couturier, Uhlmann

PART B2

Type of Exploitable Foreground[8]	**Description of exploitable foreground**	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application**[9]	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
NOT APPLICABLE								

[7] Choose among the following types of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

3. Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	241592
Title of Project:	EurOCHIP
Name and Title of Coordinator:	Dr Giles YEO, University of Cambridge

B Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***Yes*** ***Yes***
2. Please indicate whether your project involved any of the following issues :	
RESEARCH ON HUMANS	
• Did the project involve children?	***Yes***
• Did the project involve patients?	***Yes***
• Did the project involve persons not able to give consent?	***Yes***
• Did the project involve adult healthy volunteers?	***Yes***
• Did the project involve Human genetic material?	***Yes***
• Did the project involve Human biological samples?	***Yes***
• Did the project involve Human data collection?	***Yes***
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	***Yes***
• Did the project involve tracking the location or observation of people?	***Yes***
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	***Yes***
• Were those animals transgenic small laboratory animals?	***Yes***
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	***Yes***
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	***Yes***
DUAL USE	
• Research having direct military use	
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	3
Work package leaders	1	6
Experienced researchers (i.e. PhD holders)	16	16
PhD Students	7	1
Other	7	-

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	5
Of which, indicate the number of men:	2

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project? **Yes**

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
☒	Design and implement an equal opportunity policy	○	○	○	○	X
☒	Set targets to achieve a gender balance in the workforce	○	○	○	X	○
☒	Organise conferences and workshops on gender	X	○	○	○	○
☒	Actions to improve work-life balance	○	○	X	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X **No**

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

X Yes- please specify

UCAM:

G. Yeo: University of Cambridge expanding participation programmes
2011-present, Mature Students' Summer School

G. Yeo: Year 11 BAME (formerly GEEMA) Summer School, 14 – 16 August, Churchill College

G. Yeo: Wordfest 2013, 'Thinking aloud – Obesity' ADC Theatre, 13th April 2013
http://www.cambridgewordfest.co.uk/festivals/spring/event/view/thinkingaloudobesity

G. Yeo: Contributor, Channel 4's 'Fat Family Tree', 23rd May, 2013 (and on C4 OD)

G. Yeo: Cambridge Alumni lecture series 2011 - 2012

G. Yeo: Schools masterclass presentation to Year 11 students, Cambridge Science Festival, 2009-2011

ICL:

W. Dhillo spoke to the public at the Cheltenham Science Festival 'Hormone Fight Club', Cheltenham, UK, June 2013

W. Dhillo Chaired British Society of Neuroscience Public Event on Obesity, Barbican London, UK, April 2013

Gut reactions: http://www.wellcomecollection.org/whats-on/events/gut-reactions.aspx
Wellcome Trust, London UK, Sept 2011

Inserm:

School pupils

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

- ○ Yes- please specify
- X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

- X Main discipline[10]: Public health (3.3)
- X Associated discipline[10]: Clinical medicine (3.2)
- X Associated discipline[10]: Biochemistry (1.5)

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	○ X	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

- ○ No
- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- ○ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

[10] Insert number from list below (Frascati Manual).

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?		84
To how many of these is open access[11] provided?		2
How many of these are published in open access journals?		1
How many of these are published in open repositories?		1
To how many of these is open access not provided?		82
Please check all applicable reasons for not providing open access:		
☒ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ☒ no funds available to publish in an open access journal ☒ lack of time and resources ❑ lack of information on open access ❑ other[12]: ……		
15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		2
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	0
	Registered design	0
	Other	0
17. How many spin-off companies were created / are planned as a direct result of the project?		0
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

☒	Increase in employment	☒	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
☒	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs: ☒ Difficult to estimate / not possible to quantify	*Indicate figure:*

[11] Open Access is defined as free of charge access for anyone via Internet.
[12] For instance: classification for security project.

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

X Yes ○ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

X Yes ○ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

☒	Press Release	☒	Coverage in specialist press
❑	Media briefing	☒	Coverage in general (non-specialist) press
☒	TV coverage / report	☒	Coverage in national press
☒	Radio coverage / report	☒	Coverage in international press
☒	Brochures /posters / flyers	☒	Website for the general public / internet
☒	DVD /Film /Multimedia	☒	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

☒	Language of the coordinator	☒	English
☒	Other language(s): French		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

4. Final report on the distribution of the European Union financial contribution

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	***Final amount of EU contribution per beneficiary in Euros***
1.UCAM	
2. ICL	
3. CNRS	
4. Inserm	
5. UKK	
6. DIfE	
7. UGOT	
8. EQY	
9. HMGU	
Total	