Executive Summary:

Obesity represents the major risk factor for the cardiometabolic syndrome, which is an epidemic disease that generates a severe global socio-economic burden for the public health systems. Enhanced production of proinflammatory adipocytokines by expanded adipose tissue is now considered as a key event in the pathogenesis of this syndrome. This process involves:

i) the systemic release of adipokines, preferentially by visceral abdominal fat; and
ii) the paracrine, adipokine-mediated crosstalk between periorganic fat and different organs including skeletal and cardiac muscle.

Members of the ADAPT consortium have pioneered this novel view of adipose tissue as an active endocrine organ. However, there is very limited knowledge if adipokines and their downstream signalling pathways may represent "drugable" targets potentially opening new avenues to combat the devastating complications linked to obesity and the cardiometabolic syndrome. Therefore, the major goal of ADAPT was to identify novel or existing adipocytokines as drug targets that could be used to reverse obesity-associated inflammation and adverse reactions related to excess fat.

Specific objectives of ADAPT were:

I) to characterise the cellular origin of adipokines and to identify novel members of this family,
II) to investigate the adipokine-mediated intraorgan cross-talk between different adipose tissue cell types and to determine how adipokines regulate adipocyte lipolysis and/or fatty acid oxidation resulting in changes of fatty acid release in obesity-induced inflammation to define potential drug targets,
III) to study the inter-organ crosstalk between adipose tissue and skeletal muscle and to define the functional implications of ectopic brown fat in the protection against adipokine-induced inflammation,
IV) to better understand the pathophysiological relevance of periorgan fat with emphasis on epicardial fat and its implications for altered cardiac metabolism and cardiac function,
V) to determine whether new adipokines are "drugable" targets, to perform pharmacological evaluation and virtual screening in order to identify new chemical tools, and
VI) to harmonise the existing infrastructure for clinical studies on new targets and to validate in vitro data and animal data.

The jointly executed research programme provided a number of novel findings with substantial impact both for scientific progress in the field and the future development of novel anti-diabetic drugs. These findings include:

i) the identification and characterisation of new adipokines like follistatin, FGF, TRAP, chemerin, CCL5, CTSK, INHBA and CCL20,
ii) the prediction and testing of new LXR ligands by virtual screen,
iii) an advanced analysis on the role of HSL in insulin sensitivity, resulting in the demonstration that a reduction in lipolytic capacity can reshape lipid fluxes and improve insulin sensitivity without modification of fat mass,

iv) the identification of serum amyloid A and CCL5 in the crosstalk between macrophages and fat cells,
v) the development of antibodies and ELISA assays for TRAP, a feedback signal between fatty acids and adipokines, and
vi) the clinical analysis of potential novel biomarkers like chemerin and Cystatin C,
vii) the identification of PEDF as one of the most abundant proteins released by adipocytes,
viii) the description and jointly conducted analysis of DPP4 as a new adipokine potentially linking obesity to the metabolic syndrome,
ix) a detailed analysis of the role of hypoxia in adipokine production in both animal models and humans,
x) a new view on the crosstalk between preadipocytes and macrophages,
xi) a seminal contribution regarding the dynamics of human adipose lipid turnover in health and disease,
xii) novel clinical data on the analysis of cardiac mass and function in relation to abdominal and thoracic fat tissue depots, and
xiii) extensive investigations on the correlation of adipokine expression and clinical phenotypes.

Spreading of excellence was successfully carried out both in terms of public information and the generation of new knowledge presented at scientific meetings and published in high-ranking scientific journals. The ADAPT website has registered an increasing number of visits with up to 1000 visits per month. ADAPT has published more than 130 papers including 20 joint papers.

Project context and objectives:

ADAPT is an EC-funded collaborative project of top European laboratories focusing on adipose tissue as a major endocrine organ. The major goal of this project is to identify novel or existing adipocytokines as drug targets that could be used to reverse obesity-associated inflammation and adverse reactions related to excess fat. The multidisciplinary ADAPT consortium involves 11 partners and integrates basic and clinical science, bioinformatics, and in silico drug design. The research within ADAPT is organized in 4 tightly interacting research lines, as outlined below.

In light of substantial scientific evidence that proinflammatory adipokines are central to the initiation of pathophysiological processes related to excess fat, it must be considered as a great challenge to identify novel or existing adipokines as targets for drug design. For this purpose a multidisciplinary approach would be required that integrates expertise from diverse fields including basic and clinical science, bioinformatics, in silico drug design and the specific capacities of a large pharmaceutical company, as fulfilled by the ADAPT consortium. The overall concept involves four research lines that build up a stepwise strategy including:

i) the identification of novel adipokines and the cellular sources and regulation of adipokine producton,
ii) the analysis of intraorgan crosstalk within adipose tissue which plays a pivotal role in adipose tissue inflammation,
iii) the assessment of interorgan crosstalk with a focus on skeletal and cardiac muscle and the role of brown fat, and
iv) the pharmacological and clinical evaluation of adipokines as drug targets and potential biomarkers.

The four research lines of ADAPT jointly addressed the following key topics:

RL 1 Adipokine expression and secretion
- Assessment of cell type specific adipokine production including subtypes of macrophages
- Regulation of adipokine production in the respective cell types, in particular by other adipokines and by fatty acids through lipolysis
- Discovery of novel adipokines.

RL 2 Autocrine/paracrine role of adipokines and their link to inflammation
- Understanding the communication of the different cell types within adipose tissue using adipokines
- Defining the use of adipokines by endothelial cells to remodel and re-vascularise adipose tissue in response to inflammation
- Effect of new adipokines and protective factors on the lipolytic process in adipose tissue.

RL 3 The role of periorganic fat
- Identification of specific adipokines produced by periorganic fat depots (pericardial, brown fat)

- Assessment of the cross talk of the periorganic fat cells (muscle, heart) with the surrounding tissue cells (myocytes, cardiomyocytes)
- Characterisation of the clinical role of epicardial fat.

RL 4 Evaluation of drug targets
- Definition of adipokines or steps in their signal transduction pathways suitable as drug targets
- Assessment of adipokine-directed antibodies or commercially available drugs models for treatment of adverse adipokine reactions
- Potential use of adipose gene expression of a particular adipokine as a predictor of adverse adipokine reactions in man.

Based on the research lines described above, the key objectives of ADAPT were:

- to characterise the cellular origin of adipokines and to identify novel members of this family
- to assess the regulation of adipokine production after dietary intervention in humans and under different controlled in vitro conditions
- to investigate the adipokine-mediated intraorgan cross-talk between different adipose tissue cell types and to determine how adipokines regulate adipocyte lipolysis and/or fatty acid oxidation resulting in changes of fatty acid release in obesity-induced inflammation to define potential drug targets
- to study the inter-organ crosstalk between adipose tissue and skeletal muscle and to define the functional implications of ectopic brown fat in the protection against adipokine-induced inflammation
- to better understand the pathophysiological relevance of periorgan fat with emphasis on epicardial fat and its implications for altered cardiac metabolism and cardiac function
- to determine whether new adipokines are “drugable” targets, to perform pharmacological evaluation and virtual screening in order to identify new chemical tools
- to harmonise the existing infrastructure for clinical studies on new targets and to validate in vitro data and animal data

Project results:

Work package (WP) 1.1 “Cellular origin of adipokines in adipose tissue”

Objective:
The main objectives of WP1.1 were to identify the cellular origin of adipokines and to broaden our knowledge on the diversity of products secreted by adipose tissue by using novel methods to detect and identify these factors.

Deliverables:
The work in WP1.1 started in month 1 and the first deliverables (D1 and D3) were in month 18 and two additional deliverables (D2 and D4) were in month 36. These deliverables were detailed in the previous periodic reports. The activities in WP1.1 progressed as expected including the interactions between partners. No deviations from Annex 1 are reported. The main achievements are highlighted below including summaries of Deliverables.

Tasks and detailed achievements in WP1.1

Task 1: Cell source of adipokines within human adipose tissue (INST, GSK)
Human abdominal subcutaneous adipose tissue (AT) was obtained from overweight women undergoing plastic surgery. INST collected the native various cell populations from the stroma-vascular fraction and the mature adipocytes. A survey of transcript expression of adipokines was performed for each cell type using pangenomic DNA microarrays and reverse transcription-real-time PCR. In parallel, isolated cells were maintained ex vivo in basal culture medium for 24 hours and their conditioned media were collected and analyzed to identify previously defined and novel adipokines using a shotgun proteomic approach (NanoLC/ESI LTQ-Orbitrap MS/MS).

Task 2: Novel methods for identification and detection of adipokines secreted from human adipose tissue (GDC, INST, CHAR, MHH)
INST used NanoLC/ESI LTQ-Orbitrap MS/MS for proteome coverage with maximum sensitivity and dynamic range of 24h culture media from different human adipose tissue cell types. GDC established an integrated proteomic platform using two complementary technics, i.e. 1DE-LC-ESI-MS/MS and 2DE-MALDI-MS, to allow comprehensive characterization of the human adipocyte secretome (adipokinome). MHH developed new protocols to recover and detect complex large-size and lipophilic molecules by microdialysis in human adipose tissue. The relevance of adipokine secretion in/from adipose tissue with respect to insulin resistance and other metabolic abnormalities in obesity and diabetes has been investigated in prospective human clinical studies. CHAR and INST completed the studies.

Deliverables:

D1. Adipokine gene expression profiles in the different cell types composing human adipose tissue
Microarray analysis yielded a list of genes for adipocytes, progenitors, endothelial capillary cells, macrophages, endothelial lymphatic cells and negative fraction. Gene functions and biological processes were investigated using the PANTHER

Classification System using binomial test and Bonferroni correction. Signal transduction was the most represented biological process in macrophages and blood endothelial cells. Genes for immunity were mostly expressed in macrophages and negative fraction. Most genes expressed in adipocytes represented metabolism while those in progenitors encoded factors involved in cell differentiation. Endothelial lymphatic cells expressed genes involved in transcriptional regulation.

D2. Relative contribution of the different cell types composing human adipose tissue in the production of adipokines
Proteomic analysis of different cell types composing human adipose tissue was performed by INST and led to the identification of 1383 non redundant proteins secreted by the different cellular fractions. D3. Validation of 2-D-DIGE, CE-MS and microdialysis for the study of adipokine production.

Novel proteomics methods to detect human adipose tissue secreted products have been developed and validated (INST, GDC) including the analysis of microdialysates sampled by relatively new catheters with a cut off at 100 kDa (MHH). Mapping of the human adipocyte secretome has been successfully initiated by GDC and will be further developed for identification of novel targets. Microdialysis study of adipokines was validated by CHAR and used in a clinical protocol.

D4. Adipokine detection and identification using 2-D-DIGE, CE-MS and microdialysis
A large number of novel adipokines were identified in conditioned media from different cell types of human adipose tissue using different proteomic techniques (INST, GDC). All of these candidates have been confirmed to be expressed and released from human adipocytes and all were more highly expressed in adipocytes as compared to cells from stroma-vascular fraction isolated from human adipose tissue. The characterization was completed by in vitro experiment as well as in vivo studies using in situ microdialysis showing regulation of adipokine production during hypersinsulinemia, dietary restriction and physical excercise (CHAR).

In further exploring the use of adipose tissue microdialysis to study adipokines, MHH aimed to measure ADMA, a well described endogenous inhibitor of nitric oxide synthases. These enzyme influence adipose tissue blood flow, glucose uptake, and lipolysis by production of nitric oxide under normal conditions. We determined ADMA by using GC-MS/MS and described microdialysate concentrations of 0.2 µmol/l, corresponding to about 50% of plasma concentrations. In vitro experiments revealed nearly 100% recovery. Neither obesity or diabetes, nor an oral glucose load, or weight loss after bariatric surgery had any acute or chronic influence on interstitial ADMA concentrations (n=20 in total). Thus, in our view, interstitial adipose ADMA is not influenced by obesity, and may thus be of minor importance for obesity-associated adipose tissue dysfunction (manuscript in preparation).

WP 1.2 “Regulation of adipokine production”

Objective:
The main objectives of WP1.2 are to determine the factors influencing in vivo the secretion of adipokines, to investigate the relationship between adipokine expression/

secretion and insulin sensitivity and to elucidate the mechanisms underlying the modulation of adipokine production in vivo in response to hormones, lipolytic products and hypoxia.

Deliverables:
The work in WP1.2 started in month 6 and the first deliverables were in month 18 (D6) and 30 (D5). These deliverables were detailed in the previous periodic reports. Additional deliverables were in month 42 (D7) and 48 (D8 and D9). The activities in WP1.2 detailed in the previous progress report (month 36) have progressed according to the described project plan including the interaction between partners. No deviations from Annex 1 are reported. The main achievements are highlighted below including summaries of Deliverables.

Tasks and detailed achievements in WP1.2

Task 1: Influence of obesity and fat distribution on adipokine secretion (INST, CHAR)
INST and CHAR compared SAT and VAT gene expression according to obesity, visceral fat accumulation, insulin resistance, and presence of the metabolic syndrome. CHAR recruited 56 women who were divided into 4 groups (lean, overweight, obese and metabolic syndrome). Anthropometric measurements, euglycemic hyperinsulinemic clamp, blood analysis and computed tomography scans were performed. Paired samples of subcutaneous and visceral fat were obtained and whole AT was used for gene expression analysis of macrophage-specific markers and adipokines by RT-qPCR (CHAR, INST). INST performed DNA microarray analysis on a subset of 8 patients per group.

Task 2: Determination of the regulation of adipokine expression and production at different time points in a multiple phase dietary intervention programme (CHAR, INST)
A multiple phase weight loss program composed of an energy restriction phase with a 4-week very-low-calorie diet and a weight stabilization period composed of a 2-month low-calorie diet followed by 3–4 months of a weight maintenance diet has been performed at CHAR on 48 obese subjects. A thorough phenotypical characterization was performed at each time point, including euglycemic hyperinsulinemic clamp and subcutaneous adipose tissue biopsy. INST and CHAR measured adipose tissue adipokine and inflammatory marker gene expression using DNA microarrays, evaluated adipokine secretion from biopsy-derived explants of the whole adipose tissue using LUMINEX technology, and quantified and quantitation of adipose tissue macrophage numbers. mRNA levels of the respective adipokines were also measured using microfluidic cards (CHAR, INST). Plasma levels of adipokines were measured in the entire group.

Task 3: Effects of adipose tissue lipolysis on adipocyte and stroma-vascular cell secretory activities (INST, KI, SU, GSK, PHYS)
INST conducted a series of in vitro experiments on human adipocytes and macrophage cell lines. Lipolytic challenges were performed on human hMADS adipocytes with an appropriate lipolytic agonist and the resulting conditioned-media were used to treat human THP1 macrophages. The activity of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) were knocked down using

siRNA or inhibited using specific molecules in human hMADS adipocytes. KI generated conditioned media from human mature adipocytes stimulated with different lipolytic agonists and conditioned medium from human adipose tissue explants incubated under basal (unstimulated) conditions. KI also obtained from UPMC conditioned medium from human subcutaneous adipose tissue macrophages to be tested for effects on the function of differentiated human fat cells. Finally, INST addressed the adipose tissue inflammatory profile and secretory capacity in HSL heterozygous mice (HSL+/-) and mice treated with a HSL inhibitor.

Task 4: Effects of hypoxia on adipocyte and stromal-vascular cell secretory activities (INST, UPMC)
INST tested hypoxic culture conditions (1% oxygen) on human subcutaneous adipose tissue mature adipocytes, maintained in fibrin gels or in sealed microchambers, and immunoselected macrophages (CD34-/CD14+ cells). Impact of the hypoxic conditions was studied on adipocyte metabolism (free fatty acid and glycerol release, glucose uptake and lactacte release). Expression level of selected genes was measured by RT-qPCR in macrophage and adipocytes. UPMC assessed the effect of hypoxia on WAT gene expression in vivo using a model in which rats were progressively submitted to reduced barometric pressure in hypobaric chambers, until reaching the equivalent of 5,500 m altitude. Quantitative RT-PCR-based gene expression analysis was performed in epididymal WAT, after 1 and 3 weeks of hypobaric exposure. UPMC also explored the effect of chronic hypoxia on adipose tissue macrophage accumulation as well as potential association with liver injuries (i.e NASH and fibrosis) in a clinical study with obese subjects.

Deliverables

D5. Influence of anatomical distribution of fat and obesity state on adipokine production
DNA microarray-based gene expression profiling showed that 1125 genes were more and 1025 genes were less expressed in lean compared with metabolic syndrome subjects. Functional annotation clustering revealed, from lean to metabolic syndrome subjects, progressive down-regulation of metabolic pathways and up-regulation of immune response genes involved in toll-like receptor, tumour necrosis factors, nuclear factor-kappaB, and apoptosis pathways. These associations were similar in SAT and VAT. Expression of adipose tissue macrophage-specific genes increases with the degree of adiposity and correlates with markers of insulin resistance and the metabolic syndrome to a similar degree in SAT and in VAT (INST, CHAR). Altogether, these results suggest that an increase in adiposity and a worsening of metabolic status are associated with a coordinated down-regulation of metabolism-related and up-regulation of immune response-related and adipose tissue macrophage-specific gene expression. The pattern of secretion of adiponectin multimeric forms in VAT and SAT has been investigated in a group of 20 lean and obese subjects. There is a decrease of high molecular weight multimeric form in both SAT and VAT in obese subjects, the decrease being more patent in VAT.

D6. Adipokine and inflammatory marker gene expression during a multiple phase dietary intervention program
Transcriptome profiling performed by INST and CHAR revealed two main patterns of variations. The first involved 464 mostly adipocyte genes involved in metabolism that

were down-regulated during energy restriction, up-regulated during weight stabilization, and unchanged during the dietary restriction. The second comprised 511 mainly macrophage genes involved in inflammatory pathways that were up-regulated during energy restriction and down-regulated during weight stabilization and dietary intervention. In conclusion, adipose tissue macrophages and adipocytes show distinct patterns of gene regulation and association with insulin sensitivity during the various phases of a dietary weight loss program.

D7. In vitro production and plasma levels of adipokines by adipose tissue during caloric restriction programs

Diet-induced changes of adipokine production in subcutaneous abdominal adipose tissue (SAT) were investigated during a 6-months multi-phase weight-reducing dietary intervention. 48 obese women who followed a dietary intervention consisting of 4 weeks´ very low-calorie diet (VLCD) followed by weight stabilization (WS) period composed of 2 months´ low-calorie diet and 3 months of weight maintenance diet. Before and at the end of VLCD and WS, samples of plasma and SAT were obtained. Plasma levels, SAT mRNA expression and secretion rates of adipocyte-derived adipokines (leptin, serum amyloid, and haptoglobin) decreased during VLCD and increased during WS. Adipokines derived mainly from stroma-vascular cells (IL-6, IL-8, IL-10, IL-1Ra, TNFalpha, PAI-1 and MCP-1) increased or did not change during VLCD and decreased to levels equal or lower than the pre-diet ones during WS. The diet-induced changes of HOMA-IR correlated with changes of leptin plasma levels and secretion from SAT. Regulation of adipokine production in SAT differs according to their cellular origin (adipocytes vs. stroma-vascular cells) and the diet phase (VLCD vs. WS).

In another cohort of obese women CHAR investigated the effect of 28 days´ very-low-calorie-diet on the distribution of adiponectin isoforms (i.e. high-, middle-, and low-molecular weight isoforms (HMW, MMW and LMW, respectively)) in plasma and on their secretion in the explants derived from needle-biopsy-obtained samples of SAT (Kovacova et al. 2009). The distribution of adiponectin polymers in plasma was different from that secreted in SAT explants. VLCD neither induced change in total adiponectin level nor in the ratio of HMW to total adiponectin in plasma and in culture media of SAT explants. Consequently, no association between the diet-induced improvement of insulin sensitivity and those of adiponectin isoforms was found.

D8. Effects of hormones and lipolysis products on adipokine production by adipose tissue cells

Few effects of hMADs-derived lipolytic products were observed by INST on THP1 macrophage activation or differentiation. Among all the genes tested, only IL6, a pro-inflammatory marker, showed a significant increase in gene expression in THP1 macrophages in response to stimulated lipolysis from hMADs adipocytes, an effect that was completely blunted when activity of HSL was inhibited. There was no significant change in secretion of pro- or anti-inflammatory proteins by THP1 cells in response to the different hMADS conditioned media. Nevertheless, these experiments showed that THP1 macrophages were able to readily accumulate triglycerides when faced with increased FA level in the media, mimicking foam cell appearance. KI studied the relationship between lipolytic conditions (cachexia and semi-fasting) and the production of Zinc-alpha2 glycoprotein. KI has together with INST investigated

the role of twist1, a transcription factor regulated by weigh changes. Further studies demonstrated that attenuated twist1 expression resulted in an increased release of inflammatory cytokines. KI, INST and UMPC studied the effects of LXR on human adipocytes in culture. This study showed that LXR induces lipolysis which could be inhibited by HSL inhibitors. Results from the experiments performed by INST on HSL+/- mice showed that the HSL haploinsufficiency was associated with reduction in global free fatty acid turn-over (investigated by PHYS) and improvement of insulin sensitivity measured in vivo without modification of body weight and fat mass. This enhancement was not attributable to a modulation of AT inflammation. Treatment with a specific HSL inhibitor attenuated insulin resistance in high fat diet-fed mice. This work demonstrates that a reduction in lipolytic capacity can reshape lipid fluxes and improve insulin sensitivity without modification of fat mass.

D9. Effect of hypoxia on adipokine production
INST maintained subcutaneous adipocytes and macrophages under low oxygen tension in culture (1% oxygen). The results from this experiment demonstrated that oxygen tension was a potent regulator of the secretory function of both mature adipocytes and macrophages and appeared to be distinct between adipose tissue locations in human. UPMC tested the hypothesis that hypobaric hypoxia would lead to macrophage infiltration in rat adipose tissue. These data showed that hypoxia readily stimulates macrophage infiltration in the rat epididymal adipose tissue after 3 weeks of chronic hypoxia. However, the absence of crown like structures suggested that distinct or additional stimuli occur in obesity, potentially emerging from hypertrophied moribund adipocytes. In the clinical study, the results suggested that in morbidly obese patients, chronic hypoxia was strongly associated with liver injuries but did not worsen obesity induced-macrophage accumulation already present in adipose tissue.

WP 2.1 “Intraorgan crosstalk: Role of adipokines in adipose tissue inflammation

Objective:
The main objective of WP2.1 is to investigate the adipokine mediated intraorgan cross-talk between adipose tissue cells. The subtypes of residing and infiltrating macrophages are explored. Their ability to produce common or subtype specific adipokines and interaction with residing fat cells is investigated. For endothelial cells cross-talk with the mature fat cells as well as their ability to induce angiogenesis of adipose tissue are studied. The cross-talks between preadipocytes, adipocytes and other adipose tissue cells (including macrophages) have been extended.

Deliverables:
The following deliverables have been completed:

D10 Definition of macrophage subtypes present in human adipose tissue (task 1, month 18)
D11.Definition of adipocyte cross-talk with macrophage subtypes (task 1, month 36) as well as deliverables due to month 42
D12 Effect of adipokines on endothelial cell activation and angiogenesis (INST)
D13 Definition of macrophage subtype crosstalk with preadipocytes (UPMC)

Tasks and detailed achievements in WP2.1

Task 1: Characterization of the different macrophages in adipose tissue and their adipokine cross-talk with fat cells (INST, UPMC, KI, MAB, CHAR)
First partners (INST, UPMC, KI) pursued the generation of conditioned media from adipose tissue cells used for macrophages, adipocytes and preadipocytes cultures. This has been extended to other adipose cell types as endothelial cells and mast cells. A protocol to isolate these very fragile mast cells from human adipose tissue has been developed by UPMC.

CHAR also explored the methodology for quantification of adipose tissue macrophages and their subtypes in needle biopsy-derived samples of subcutaneous adipose tissue by flow cytometry (see D10). Moreover, INST prepared conditioned media from human native subcutaneous adipose tissue macrophages that were applied to human mature adipocytes and their effects on adipocyte lipolysis and insulin-mediated up-regulation of lipoprotein lipase and fatty acid synthase as well as CCL20 expression were studied. Adipose tissue macrophage-conditioned media did not modulate the basal or the stimulated lipolytic activities nor the insulin-mediated up-regulation of LPL and FAS. It increased the adipocyte expression of CCL20 (Duffaut et al., 2010).

UPMC pursued the analysis of macrophages in adipose tissue depots by immunohistochemistry focusing on WAT fibrotic area. UMPC showed that both M1 (stained with CD40 surface markers) and M2 (stained with CD163 and CD206) types are located in fibrotic bundles found in human adipose tissue both located in omental and subcutaneous depots. Lymphocytes and mast cells were co localized in these bundles. Adipocytes located in these fibrosis areas were of smaller size than adipocytes located in the parenchyma. These results suggest that these inflammatory cells might contribute directly or indirectly to fibrosis development and to adipocyte biology. The work on the characterization of fibrotic depots in human WAT and association with inflammatory cells and obesity phenotypes was published (Divoux et al., Diabetes 2010). In addition, the work showing that weight loss induced by gastric surgery rapidly modifies the activation state of adipose tissue macrophages in obese subjects towards a less pro-inflammatory profile (i.e. decrease of CD40+ and increase of CD206+ cells counts after weight loss) was also published (Aron et al., JCEM 2009).

The adipocyte macrophage media crosstalk was addressed in the model of gastric surgery. Adipose tissue explants were prepared from 20 obese subjects before and 3, 6, 12 months after the surgical procedure and from lean subjects. This task was finalized for the following activities:

- The completion of analysis regarding the implication of adipokines in fat cells' crosstalks and biology.
Exploring preadipocyte genes induced by human macrophage media (WP2.1 task 3) revealed a strong upregulation of several mediators; as CLL5/Rantes, Inhibin betaA, and CXCL2, which was deeper explored (see task 2) for its interaction with endothelial cells. UPMC showed that CCL5 significantly triggers monocyte adhesion and transmigration to/through WAT endothelial cells perpetuating WAT inflammation. CCL5 protected macrophages from free cholesterol apoptosis allowing

their lipid scavenging function to clear necrotic adipocytes, a process dependant on ERK and AKT pathway activation. This work shows that CCL5 is important in mediating adipocyte-macrophage cross talks (Keophipath, ATVB, 2010).
- The search of new targets involved in inflammation. UPMC and KI combined microarray analysis from lean and obese subjects (160 subjects) and from isolated adipocytes vs. SVF as well as from differentiated adipocytes with or without macrophage media.
Bioinformatic analysis showed that a series of genes strongly expressed in isolated adipocytes are downregulated with obesity and in presence of inflammation.
- The impact of adipose tissue medium on inflammatory cell phenotype evaluated by UPMC and INST. INST studied the impact of conditioned media of subcutaneous mature adipocytes on subcutaneous adipose tissue macrophage phenotype.
Real time PCT experiments showed that adipocyte-derived secretions increased the expression of IL-6, MCP-1, MMP-9, TGFbeta and IL10. However this exeperiment did not alter the expression of VEGF-A and LYVE-1 (Bourlier et al., Plos One, 2012).
UPMC showed that conditioned media of adipose tissue obtained in obese subjects also induced a pro-inflammatory activation of blood monocyte-derived macrophages, characterized by a striking increase in IL1 and NLRP3 inflammasome gene expression.

Mabtech has developed new technologies (ELISpot and FluoroSpot) that enable sensitive measurement of secretion of a large number of pro-inflammatory (e.g. TNF-a, IL-1b, IL-6, IL-12, IL-23, GM-CSF) as well as anti-inflammatory (IL-10, TGF-b) cytokines at the single cell level. With these techniques and using monocytes as a model for tissue macrophages it has been possible to define several functionally distinct subsets of cells as characterized by their cytokine secreting profiles (Smedman et al., Scand. J. Immunol. 2011). The same techniques have been demonstrated to work for the ex vivo analysis of macrophages derived from adipose tissue and will be used to provide a quantitative and qualitative characterization of macrophages isolated from obese and lean subjects. In collaboration with KI, Mabtech has also developed new monoclonal antibodies against the TRAP protein which will enable a similar investigation of this protein. The same methods will also be applied for studying the cross-talk between adipose and immune cells where e.g. the effect of conditioned medium from adipocyte cultures on monocytes and macrophages and their secretion of pro- and anti-inflammatory cytokines is investigated.

Task 2: Adipokine-mediated cross talk between vasculature and adipose tissue cells (INST, UPMC, CHAR, KI)
INST isolated the capillary endothelial cells (CD34+/CD31+) from the human subcutaneous adipose tissues from patients with distinct body mass index by the immunoselection/depletion method and their expression of various genes (angiogenic factors and receptors, adhesion molecules and chemokines, growth factors) are studied by real time PCR. CHAR explored the methodology for quantification of adipose tissue cells (incl. macrophages) in needle biopsy-derived samples of subcutaneous adipose tissue by flow cytometry (see Task 1 and D10).

Similar studies were performed on endothelial cells isolated from paired biopsies from subcutaneous and visceral adipose tissues from obese patients. Visceral adipose tissue endothelial cells compared to subcutaneous ones were characterized by

enhanced expression of inflammatory markers and angiogenic receptors (CCL20, CXCL8, ICAM-1) as well as angiogenic receptor (VEGFR2, Leptin Receptor and neuropilin 1 and 2) and senescent markers (IGFBP3). Immunohistochemical approaches showed also increased activation (ICAM1) and senescent state (gammaH2AX) of endothelial cells from visceral adipose tissues compared to subcutaneous ones (Villaret et al., Diabetes, 2010). KI has generated the recombinant TRAP proteins and variants thereof which are used in studies of adipocytes and endothelial cells. Methods for TRAP crosstalk between macrophages and fat cells are developed in collaboration with INST and it was shown that TRAP is expressed by human adipose tissue macrophages (Lang, Int J Obes, 2011). KI has identified SEMA3c as a fat cell-derived adipokine that stimulates extracellular matrix production from resident preadipocytes. In contrast to studies in mice, INST has shown that recombinant human SEMA3C has no effects on human endothelial cell proliferation. A joint manuscript from KI and INST detailing these as well as other findings is currently under preparation

Task 3: Adipokine-mediated crosstalk between preadipocytes and macrophage subtypes (INST, UPMC, CHAR, KI)
Native progenitor cells population (CD34+/CD31- cells) is studied in vitro by INST. Media are tested to identify a minimal culture condition allowing the differentiation of the native progenitor cells into adipocytes. Several adipocyte differentiation markers, well described in mouse preadipocyte cell lines, are studied to identify true human adipocyte differentiation markers andpreadipocyte markers. In addition, CHAR explored the methodology for quantification of adipose tissue progenitor cells characterized by combination of CD markers CD 34+/CD31- and lymphocytes CD3+/CD4+ and CD3+/CD8+ in needle biopsy-derived samples of subcutaneous adipose tissue by flow cytometry (see also Task 1 and D10). Response of progenitor cells to dietary intervention was explored in a preliminary clinical study.

UPMC pursued the analysis of the experimental model using primary human preadipocytes cultured with secreted factors from monocyte-derived and adipose-tissue macrophages. These conditions lead to inflammatory preadipocytes with diminished adipogenesis and a profibrotic phenotype. Human inflammatory preadipocytes produce many inflammatory molecules and profibrotic mediators. In the previous 18 month report, UPMC showed that one of these players, inhibin beta A, has a mediating role in the production of profibrotic proteins by preadipocytes in contact with an inflammatory microenvironment ((Keophiphath, Mol. Endoc 2009). Importantly in a collaborative work between INST, UPMC and a C Danis' group in Nice, they confirmed that Inhibin beta is also induced by obese adipose tissue and is expressed in several adipose cell types as macrophages (MolEndocrinol. 2009). This work published in Diabetes conferred additional role to the dimer of Inhibin beta Ai.e. Activin A and showed that this adipokine represents also a novel crucial player controlling self-renewal of human adipose progenitors. Activin A appears to be a critical mediator of inflammation secreted by macrophages and inflammatory preadipocytes accumulated in adipose tissue of obese subjects. To support this hypothesis, INST showed that ATM-derived factors and most specifically TGF1 induced a myofibroblast-like phenotype of AT progenitor cells. Such an effect appeared to be mediated, at least in part, through the induction of INHBA/activinA expression by the progenitor cells. Moreover, native human AT progenitor cells exhibited an increased expression of myofibroblast markers including Snail and Slug,

with obesity (Bourlier et al., Plos One, 2012) UPMC also pursued the bioinformatic analysis of the transcriptomic profile of the human preadipocytes treated by macrophages isolated from obese adipose tissue. Among the most over-expressed genes, were found genes corresponding to the functions “chemokine signalling pathways” with CXCL2 (see above) and “cytokine-cytokine receptors” with G-CSF as a new candidate which role in adipose tissue biology remains to be deciphered.

WP 2.2 “Role of adipokines in the release of fatty acids from the inflamed adipose tissue”

Objective:
The main objectives of WP2.2 were to define how adipokines regulate fatty acid turnover (lipolysis and oxidation) including the molecular mechanisms involved and whether fatty acids influence adipokine secretion/function. In addition, a main task was to evaluate novel drug targets in human and animal models.

Deliverables:
The work in WP2.2 started in month 6 and the first deliverable (D15) was detailed in the previous periodic report. Additional deliverables were in month 42 (D14 and D16) and 48 (D17).

Tasks and detailed achievements in WP2.2

Task 1: Interactions between TNF-alpha, CIDEA and LXRs (KI, SU, INST, MED)
During the last year, KI has together with INST been able to establish that LXR interacts directly with CIDEA1. MED has developed a fragment-based in silico analysis to design potentially isoform-selective LXR agonists and antagonists and has identified several novel molecules which are potential non-selective LXR blockers. SU has generated a transgenic mouse model over-expressing human CIDEA selectively in adipose tissues. These animals have now been subjected to a detailed phenotypic analysis following either regular chow or high fat diet. These assessments have demonstrated that CIDEA Tg-animals have somewhat higher insulin-sensitivity, which is in agreement with observational data in humans. SU has identified different human CIDEA isoforms, the role of which is currently under investigation. As reported previously, KI has together with INST studied the role of twist1, a transcription factor that regulates the expression and secretion of inflammatory adipokines (including TNF-alpha) in human adipocytes2, 3. These studies have now proceeded with a micro-array analysis of human adipocytes over expressing twist1. This has enabled the identification of novel twist1-regulated genes, which has been confirmed by ChIP and ChIP-seq analysis performed in collaboration with the SciLife laboratory at KI.

Task 3: Feed-back signals between fatty acids and adipokines (KI, SU, MAB)
As reported previously, using non-selective antibodies, KI has developed an ELISA assay for uncleaved TRAP to study samples from human adipose tissue and serum. KI has performed a clinical study on TRAP as a human adipokine and the results were recently published4. This study demonstrated that TRAP is secreted in vivo from human adipose tissue and its secretion is fully accounted for by macrophages in the tissue. In addition, secretion rates are strongly influenced by the size and number of

fat cells (increased secretion rate among those having hypertrophy-few but large fat cells). However, TRAP secretion is not related to the rate of adipocyte lipolysis. The transgenic TRAP mice that were generated for in vivo studies did not breed sufficiently enough, possibly because of attenuated fertility. We have therefore not been able to finalize the phenotypic assessments in these animals and have consequently chosen another in vivo approach. In preliminary experiments we have injected recombinant TRAP directly into subcutaneous fat tissue depots of wt mice. We found that there was a marked increase in adipogenesis at the place of injection in comparison with the contra lateral side where only vehicle was administered. These studies demonstrate that TRAP stimulates adipogenesis in vivo.

To investigate the influence of fatty acids released by lipolysis on AT inflammation at a cellular level, INST has conducted a series of in vitro experiments on human adipocytes and macrophage cell lines. In brief, lipolytic challenges were performed on human hMADS adipocytes with an appropriate lipolytic agonist and the resulting conditioned-media were used to treat human THP1 macrophages. GSK has provided pharmacological tools for these studies as well as SOPs for cultivating human THP1 monocytes/macrophages. The activity of HSL and adipose triglyceride lipase (ATGL), another lipase essential in the lipolytic process, were knocked down using siRNA or inhibited using specific molecules in human hMADS adipocytes. In THP1 cells, the involvement of the TLR4 signaling pathway was assessed using the specific inhibitor TAK-242. Messenger RNA levels of interleukins and pro- and anti-inflammatory cytokines were determined in THP1 cells by RT-qPCR. Secretion of inflammatory and anti-inflammatory cytokines was measured in the collected THP1 cell media using ELISA. The levels and composition of FAs released by differentiated hMADS adipocytes under different conditions of lipolytic activation were determined using enzymatic methods and gas chromatography. Cellular triglyceride content was assessed using enzymatic methods and specific fluorescent staining. INST also investigated the influence of lipolysis on AT inflammation and the implication of TLR4 in vivo using WT or TLR4-mutated mice fed a high fat diet and treated or not with an HSL inhibitor. Immune cell numbers were quantified by flow cytometry analyses. Insulin sensitivity was studied by in vivo tolerance tests.

Key deliverables:

D14. Identification of how TNF-alpha, CIDEA and LXRs interact on fatty acid metabolism in vitro and in vivo
This WP has demonstrated that LXR activation increases fatty acid oxidation5 and lipolysis6 in human white adipocytes. The former is mediated via an increase in pyruvate dehydrogenase kinase 4 expression while the latter is mediated by a downregulation in perilipin levels. Results from this WP have also shown that CIDEA increases fatty acid oxidation via increase pyruvate dehydrogenase kinase 1 and 4 expression, a mechanism that is very similar to that induced by LXR 7. In contrast, previous results from KI have demonstrated that CIDEA downregulates lipolysis. Taken together, these results show that CIDEA and LXR display similar effects on fatty acid oxidation but opposite effects on human fat cell lipolysis. These differences can possibly be explained by very recent data obtained in this WP suggesting a direct interaction between CIDEA and LXR1. These results indicate that CIDEA can modulate LXR function both positively and negatively.

D16. Characterisation of the actions of fatty acids as receptor agonists for TLR4
Few effects of hMADs-derived lipolytic products were observed by INST on THP1 activation or differentiation. Among all the genes tested, only IL6, a pro-inflammatory marker, showed a significant increase in gene expression in THP1 in response to conditioned media from lipolysis-stimulated hMADs cells, an effect that was completely blunted when the activity of HSL was inhibited. No significant effect of TLR4 inhibition was observed on IL6 gene expression. There was no significant change in the secretion of pro- or anti-inflammatory proteins by THP1 cells in response to the different hMADS conditioned media. Nevertheless, these experiments showed that THP1 macrophages were able to readily accumulate triglycerides when faced with increased FA levels in the media, mimicking a foam cell phenotype. This observation suggests a role for adipose tissue resident macrophages in scavenging excess amounts of locally released lipids. Experiments performed in vivo corroborated these results. Thus, mice with defective TLR4 displayed reduced inflammation and improved insulin sensitivity compared with their WT littermates. In WT mice, HFD induced adipose tissue macrophage accumulation and fibrosis in perigonadal fat pads. In contrast, HFD in TLR4 -/- mice resulted in a significantly attenuated macrophage content and adipose tissue fibrosis. In both genotypes, treatment with HSL inhibitor increased their insulin sensitivity but failed to improve their adipose tissue inflammatory profile. Taken together, these in vitro and in vivo data did not provide evidence for TLR4 influencing the effects of lipolysis-derived fatty acids on adipose tissue inflammation. However, results obtained in the animal models suggested an association between adipose tissue fibrosis and insulin resistance, which was TLR4 dependent. The causal link between adipose tissue fibrosis and insulin resistance needs to be further explored.

D17. Characterisation of TRAP as a feed-back regulator of interactions between fatty acids and adipokines
KI demonstrated in murine models that TRAP is an adipokine which regulates the size and number of fat cells and which could indirectly affect lipolysis and fatty acid release8. In human adipose tissue, TRAP is predominantly produced by macrophages and the levels correlate positively with rates of local inflammation as well as the number and size of adipocytes4 The work on this project involved the development of an in-house TRAP-ELISA. Recombinant TRAP protein was produced by KI. Given the difficulties in generating transgenic TRAP mice (most probably due to reduced fertility), studies have been completed by administration of recombinant TRAP into murine subcutaneous adipose tissue in vivo. Preliminary results suggest that this stimulates local adipogenesis since TRAP injection resulted in the appearance of small fat cells with multilocular lipid droplets (the typical morphology of newly developed fat cells). In contrast, no effects were observed in the contra lateral site where only vehicle or inactivated TRAP was injected.

WP 3.1 “The crosstalk between adipose tissue and skeletal muscle cells”

Objective:
The main objectives of WP3.1 are to elucidate the critical targets in skeletal muscle cells that interact with adipokines released from adipose tissue and lead to insulin resistance in muscle. Adiponectin is a major adipokine in this respect. The gene targets of fatty acids in muscle cells are also of particular significance. The

assessment of a role for brown fat in adipokine-induced inflammation is also to be determined.

Deliverables:
The work in WP3.1 started in month 12 and the first deliverable was due in month 18 (D18) with additional deliverables in months 30 (D19), 36 (D20) and 48 (D21-23). The activities in WP3.1 have progressed according to the described project plan including the interaction between the different beneficiaries. In Task 2, GDC has focused on the novel adipokine DPP4 (see WP1.1) instead of a detailed analysis of adiponectin (see below). In the four tasks detailed below, WP3.1 has achieved a number of results, the most important of which are high-lighted below (see also reference list at the end of this report).

Tasks and detailed achievements in WP3.1

Task 1: Provision of conditioned media and studies of effects on insulin action in human muscle cells (GDC, INST, UPMC)
GDC, INST and UPMC generated conditioned media (CM) from a variety of different cell sources and studied the crosstalk and signalling pathways of these media (see D18 for detailed description). Specifically, conditioned media have now been obtained and characterized from mature adipocytes, and from macrophages, endothelial cells, progenitor cells and lymphocytes from adipose tissue of patients with distinct BMI (UPMC). Further, conditioned media have also been prepared froma variety of tissue explants including epi- and pericardial fat, perivascular fat, and subcutaneous and visceral abdominal fat (GDC). Together with KI, GDC has studied in detail the functional implications of chemerin and its receptor ChemR23 (CMKLR1) in human skeletal muscle cells (see D19 for details). Overall, we show for the first time pro-inflammatory signalling of chemerin in human skeletal muscle and a critical role of ERK1/2 for the induction of insulin resistance. Proteomic analysis of conditioned media revealed PEDF (pigment epithelium-derived factor) as one of the most abundant proteins released by human adipocytes. In a recent study, GDC showed that PEDF secretion is inversely regulated by insulin and hypoxia. PEDF induces insulin resistance in adipocytes and hSkMC and leads to inflammatory signalling in hSMC. Because of these diverse actions, PEDF is a key adipokine, which could have an important role in diabetes and obesity-related disorders.

Task 2: Role of adiponectin in adipose tissue crosstalk function (GDC, CHAR)
At this stage, the work towards D22 (due month 48) includes the proteomic analysis of the adipocyte secretome in the absence and presence of adiponectin, based on the methodology described in WP1.1 (see also D3). Besides several other proteins, GDC identified PEDF as being regulated by adiponectin and released by human adipocytes at high concentrations (see also Task 1). GDC has started to characterize the functional impact of the novel adipokine DPP4 for skeletal muscle insulin resistance. This will probably result in a novel deliverable to be provided in month 48.

In a set of other studies the antilipolytic effect of adiponectine was investigated: in isolated adipocytes obtained from surgical biopsies adiponectin was shown to inhibit, in physiological concentrations, spontaneous as well as catecholamine-induced lipolysis (published in 2010, see below). This effect tended to be lower in obese individuals. The role of AMPK is being studied. Furthermore, in an ongoing study,

the antilipolytic action of adiponectin is being investigated in isolated adipocytes derived from paired samples of visceral and subcutaneous fat in lean and obese subjects and the action in the two fat depot compared.

Task 3: Modulation of gene regulatory targets in skeletal muscle cells by adipokines and fatty acids (GDC, KI)
GDC observed a strong upregulation of the fatty acid transporter CD36 in skeletal muscle cells treated with CM that was paralleled by increased fatty acid uptake and lipid accumulation. Protein levels of the transcription factor myogenin were increased by oleic acid alone, while incubation with palmitic acid (PA) + CM nearly abrogated myogenin protein abundance. However, myogenic transcription factor MyoD and the glucose transporter GLUT4 remained unaltered. Mitochondrial integrity was impaired by CM- and FA-treatment, with the most profound defects induced by co-application of PA+CM. Consequently, FA oxidation was nearly abolished in the presence of PA+CM. Our data indicate an increased synergistic lipotoxic risk emerging from adipokines and FA specifically PA, which may be mediated via CD36 (for details, see D20).

Task 4: Significance of ectopic brown fat within skeletal muscle for leanness and thus low inflammation levels (SU, GDC)
By the signal sequence trap method, about 120 secreted proteins have been identified in cultured brown adipocytes. Of these 27 have been studied in more detail and could be considered potential paracrine signals. One example is chemerin, which is well expressed and its expression is increased after high fat diet but decreased by cold exposure. Chemerin expression in brown adipose tissue correlates positively with body weight after high fat diet treatment. Expression of the chemerin receptor CMKLR1 is decreased by both treatments. Further studies are ongoing concerning other selected adipokines from brown adipocytes, such as lipocalin 2, adrenomedullin, lumican, Niemann Pick type c2. Several of these proteins have been proposed to be associated with obesity, insulin resistance and inflammation.

Conditioned media from brown adipocytes have been generated by a SOP in a special medium (hunger medium without FCS and insulin) in order to allow its testing on skeletal muscle cells. In addition, white adipocytes from the same source were cultured and conditioned medium generated under the same conditions as a positive control. Effects of these media are currently being investigated on skeletal muscle cells. Preliminary data indicate that both CM from brown and white adipocytes affect insulin signaling in skeletal muscle cells to the same extent.

Deliverables

D21. Identification of downstream targets of myogenin and impact for metabolic performance of skeletal muscle
GDC has further elaborated the in vitro model of skeletal muscle cells treated with adipokines and oleic/palmitic acid. This model is characterized by adipokine-induced upregulation of the fatty acid transporter CD36 in skeletal muscle cells triggering increased fatty acid uptake and lipid accumulation in the presence of oleic and palmitic acid. The metabolic performance of skeletal muscle cells treated with palmitic acid and adipokines was significantly perturbated on the level of lipid oxidation in parallel to impaired mitochondrial integrity in this situation. Protein

levels of the transcription factor myogenin were dramatically reduced after treatment with adipokines and palmitic acid. SU has found myogenin expression in classical brown adipocytes but not in brown adipocytes emerging in white adipose depots (brite adipocytes). This confirms the distinct origin of brite adipocytes. It is currently unclear what is the role of brown adipose myogenin. As we observed a significant anti-inflammatory action of electric pulse stimulation of skeletal muscle cells (EPS), this method was used to stimulate skeletal muscle cells pre-treated with adipokines and oleic/palmitic acid. EPS significantly decreases TNFalpha-induced NF-kB signaling in skeletal muscle cells. EPS does not change lipid oxidation in unstimulated skeletal muscle cells. However, EPS-treatment completely prevents oleic acid- and adipokine-induced reduction of lipid oxidation. The strong inhibition of lipid oxidation by palmitic acid in combination with adipokines remained unaltered.

D22. Detailed understanding of the autocrine/paracrine function of adiponectin
In a proteomic approach mapping the secretome of human adipocytes, GDC has identified DPP4 as a novel adipokine. Due to the special interest in DPP4 as a drug target for the treatment of type 2 diabetes and a complete lack of data on the role of DPP4 in adipose tissue and as an adipokine, this target was chosen to replace adiponectin. In vitro, DPP4 expression and release increase over differentiation of adipocytes. In differentiated adipocytes, DPP4 release is increased after stimulation with TNFalpha and insulin. In cooperation with KI, DPP4 release was analyzed from isolated adipocytes originating from biopsies of subcutaneous adipose tissue. Release of DPP4 is increased from adipocytes of obese patients compared to lean controls. Weight loss of obese patients leads to normalized DPP4 release. In all patients, DPP4 release correlates with adipocyte size, leptin secretion, BMI and circulating triglycerides. DPP4 serum concentrations are also significantly higher in obese patients as compared to lean controls. DPP4 exerts autocrine and paracrine/endocrine functions which were tested in several primary human cell models. DPP4 impairs insulin signaling in adipocytes, skeletal and smooth muscle cells, an effect that can be prevented by addition of a DPP4 inhibitor. In smooth muscle cells, DPP4 additionally induces proliferation that can be prevented by a specific inhibitor.

D23. Characterization of brown fat adipokines and their influence on skeletal muscle cells
A large number of brown adipokines have been identified, including chemerin, lipocalin 2, adrenomedullin, lumican, Niemann Pick type 2c, and very recently, in collaboration with colleagues at Cambridge university, BMP8b and lipocalin-like prostaglandin D2 synthase (L-PGDS). The secretion of these brown adipokines is strongly influenced by the differentiation state of the adipocytes. BMP8b increases adrenergic sensitivity in brown adipose tissue. L-PGDS expression correlates positively with brown adipose tissue activity.

WP 3.2 „Epicardial fat and its implications for altered cardiac metabolism and cardiac function”

Objective:
It is the overall goal of WP3.2 to investigate the secretory function of epicardial fat and to elucidate the pathophysiological role of this fat depot for cardiomyopathy associated with obesity and type 2 diabetes. In this context, experimental studies aim

to assess the crosstalk between epicardial fat and cardiomyocytes, whereas clinical studies aim to show if this fat depot is critically related to cardiac structure and function and can be mobilized by dietary intervention.

Deliverables:
The following deliverables have been completed:

D24: Characterisation of the adipokine secretory profile of epicardial fat
D25. Effects of ageing and high-fat diet on expression of cytokines and secretory activity of epicardial fat
D26. Characterisation of human epicardial fat
D27. Implication of epicardial fat for cardiomyocyte function and metabolic performance
D28. Gene expression of candidate adipokines in subcutaneous and epicardial fat in paired subjects.

The final deliverable D29 was due in month 42 and has been completed.

Tasks and detailed achievements in WP3.2

Task 1: Analysis of adipokine expression and secretion from epicardial fat and crosstalk with cardiomyoctes using the guinea pig model (GDC, KI)
GDC in collaboration with INST has used the guinea pig model and applied a high-fat diet (HFD) to induce insulin resistance and cardiac dysfunction. Conditioned media (CM) generated from epicardial and subcutaneous adipose tissue explants were subjected to cytokine profiling using antibody arrays. Eleven factors were differentially secreted by epicardial adipose tissue when compared to subcutaneous adipose tissue. Furthermore, secretion of 30 factors by epicardial adipose tissue was affected by HFD-feeding (for details, see D25). In cardiomyocytes, CM from epicardial adipose tissue of HFD-fed animals increased SMAD2-phosphorylation, decreased SERCA2a expression, and reduced insulin-mediated phosphorylation of Akt-Ser473 versus CM from subcutaneous adipose tissue and standard diet-fed animals. Finally, CM from epicardial adipose tissue of HFD-fed animals as compared to CM from the other groups markedly reduced sarcomere shortening and cytosolic Ca(2+) -fluxes in cardiomyocytes (for details, see D27). GDC has extended this work to human epicardial fat from control and type 2 diabetic subjects. It was found that activin A is substantially upregulated in type 2 diabetics and may play an important role in the dysregulation of cardiac function under these conditions (Greulich et al, Circulation, in revision).

Task 2: Clinical studies on epicardial fat (MHH, UPMC)
MHH has continued the analysis of cardiac mass and function in relationship to abdominal and thoracic fat tissue depots in much more detail. In a cross-sectional analysis, we now published our findings on the indirect relationship between myocardiocyte lipid accumulation (MTG – myocardiocyte triglycerides) and physical fitness. As MTG are increased in subjects with insulin resistance, positively correlated to increased left ventricular remodeling, and negatively correlated to diastolic function, they may contribute to obesity-associated heart failure in the long run (Utz et al, Heart 2011). Weight loss as achieved by hypocaloric diets low in carbohydrate or fat content reduced MTG in our study population but this reduction was not

associated with changes in cardiac function (paper submitted). This finding does not speak against our hypothesis, because cardiac size and performance in this population with no overt signs of diabetes and heart disease were clearly altered, but only to a modest degree. We now published the weight loss data in this cohort in detail (Haufe et al, Hepatology 2011). Interestingly, whereas pericardial adipose tissue is reduced by weight loss, epicardial fat mass did not chage. Thus, our previous notion of a stronger correlation between pericardial adipose tissue and visceral adipose tissue is supported by this responsiveness towards weight loss. For epicardial adipose tissue, the relationship to visceral adipose tissue is much smaller. Weight loss significantly reduced left ventricular mass, which is the hallmark of early obesity-associated cardiac changes (Haufe et al, Hypertension 2012). Whereas the reduction of left ventricular mass was independent of the macronutrient composition of the diet, and only partly associated with hemodynamic changes, we found that the increased intake of n-3 polyunsaturated fatty acids is a predictor of left ventricular mass reduction. Furthermore, we have reevaluated FABP4 as candidate adipokine of obesity-associated heart failure and found that subjects with the highest tertile of circulating FABP4 are prone to increased cardiac mass and reduced longitudinal contractility as determined by cardiac MRI. These data in principal confirm the in vitro data for FABP4 (paper submitted). Other candidate adipokines are now analyzed in our study population together with GDC. Further details on the heart can be found in Deliverable 29.

WP 4.1 “Pharmacological Evaluation and Identification of Drug Targets”

Objective:
The main objectives of WP4.1 are:

- to determine whether the new adipokines identified in the course of this project are potentially druggable and represent a starting point for pharmacological intervention
- to identify adipokine receptors and signalling pathways to find other points for classical pharmacological interventions taking advantage of the bioinformatics analysis
- to perform virtual screening to identify potential new chemical tools

Deliverables:
Activities related to WP4.1 started month 12 and the final deliverable (D33) has been completed as detailed below. The main achievements of WP4.1 are shortly summarized and Task 3 with D33 related to this period is described in detail.

Tasks and detailed achievements in WP4.1

Task 1: Bioinformatic analysis of new adipokines and their potential receptors identified
This task was completed month 18 and delivered a confidential pathway analysis of adipokines analysed within ADAPT (see D30).

Task 2: Provision of new animal models to study in vivo the influence of inflamed adipose tissue on whole body metabolism (PHYS, SU, INST, UPMC)

Different animal models have been investigated to study the influence of inflamed adipose tissue on whole body metabolism. All these studies used overexpression or inactivation of specific genes that could play a role in metabolism. In depth analysis of several metabolic parameters were monitored in these animal models including glucose and lipid homeostasis, inflammation of adipose tissue and body weight. Major outcomes have been reported in D31.

Task 3: Influence of adipokine overexpression and antibody blockade in rodent models (GSK, PHYS, SU, INST, MAB)
The identification of novel proteins of interest coming from proteomic studies has brought a number of candidate targets. Further analysis and filtering has led to the selection of 13 targets for which Adenovirus and/or blocking Antibodies to be used for target overexpression or neutralization will be prepared in anticipation of in vitro/in vivo experiments. Very recently, a panel of adenovirus tools has been prepared by GSK for the partners within ADAPT.

SU, in collaboration with colleagues in Cambridge, has evaluated the metabolic effects of novel adipokines by measurement of whole body metabolism in animals with an ablation of the adipokine gene. Two studies have been performed, one with mice lacking the gene for the TGFbeta superfamily member BMP8b. The KO animals demonstrate an obese phenotype and show a reduced ability to recruit brown fat thermogenesis in a cold environment, indicating a role for BMP8b in energy balance. For mice lacking the gene for lipocalin-like prostaglandin D synthase, energy expenditure in a cold environment remains markedly dependent upon carbohydrate oxidation, rather than switching to fat oxidation, indicating a role for the gene in substrate selection.

GSK and PHYS worked together to investigate the impact of a pharmacological inhibition of Stearoyl-CoA Desaturase 1 (SCD1), an enzyme that catalyzes the biosynthesis of monounsaturated fatty acids from saturated fatty acids, on metabolic parameters in the rat. GSK993 is a potent and orally available SCD1 inhibitor. In Zuckerfa/fa rats, GSK993 exerted a marked reduction in hepatic lipids as well as a significant improvement of glucose tolerance. Furthermore, in a diet-induced insulin resistant rat model, GSK993 induced a very strong reduction in Triton-induced hepatic Very Low Density Lipoprotein-Triglyceride production. In addition, following a hyperinsulinemic–euglycemic clamp in GSK993-treated animals, we observed an improvement in the whole body insulin sensitivity as reflected by an increase in the glucose infusion rate. Taken together, these findings demonstrate that the pharmacological inhibition of SCD1 translates into improved lipid and glucose metabolic profiles and raises the interest of SCD1 inhibitors as potential new drugs for the treatment of insulin resistance. However, particular, attention should be focused on the impact of SCD1 inhibition in the skin. Indeed, we observed that SCD1 inhibition caused some atrophy of sebaceous and meibomian glands due to depletion of wax esters in the skin and the eyelid. Therefore, future development of SCD1 inhibitor for chronic treatment will require careful determination of the therapeutic window between the dose that confers the metabolic effects and the dose that confers these side effects.

GSK and PHYS explored the effect of Liver X receptor (LXR) activation on reverse cholesterol transport (RCT) in a hamster model (a species expressing CETP) using an

LXR agonist identified by GSK, GW3965. Overall, GW3965 failed to improve both dyslipidemia and liver steatosis. However, after (3)H-cholesterol labeled macrophage injection, GW3965 treatment significantly increased the (3)H-tracer appearance by 30% in plasma over 72 h, while fecal (3)H-cholesterol excretion increased by 156% ($P < 0.001$). This study allowed to show that despite a lack of beneficial effect on circulating lipids, LXR activation promotes macrophage-to-feces RCT in dyslipidemic hamsters. These results emphasize the use of species with a more human-like lipoprotein metabolism for drug profiling.

Both Task 4 and Task 5 have been successfully completed and the corresponding deliverables (D34 and D35) have been reported.

WP 4.2 „Clinical evaluation of adipokines as a drug target"

Objective:
In WP4.2, the consortiums aims to evaluate adipokines as identified and described in other work packages as possible drug targets. This includes the detailed examination of the regulation of these adipokines under different clinical situations. Several groups of the consortium are active in clinical research, and one main goal of WP4.2 is to harmonize the existing infrastructure for clinical studies on new targets or compounds.

Deliverables:
In WP4.2, Milestone 22 ("Clinical evaluation of adipokines as drug targets for the adverse effects of obesity-associated inflammation and insulin resistance") and Deliverable 38 ("Determination of adipokine mRNA and circulating levels in existing populations and their correlation with phenotypic data") are due at month 48. Both are closely linked and related to task 2 (see below).

Tasks and detailed achievements in WP4.2

Task 1: Further improving the existing infrastructure for clinical studies (CHAR, INST, MHH, KI, UPMC)
Most of the work related to this task has been already reported in the 2nd periodic report (including Deliverables 36 and 37, and Milestone 8). All partners have cooperated during the second half of the funding period on the basis of common SOPs and clinical databases. Further progress was made in sharing extensive details regarding formats of microarray data (UPMC, INST, CHAR, MHH).

Task 2: Correlation of adipokine expression and clinical phenotypes (MHH, CHAR, INST, UPMC, KI)
UPMC explored monocyte subtypes in obesity in more detail. Indeed systemic low-grade inflammation in obesity is not only related to circulating adipokines, but also to CD16+ subpopulations of monocytes (namely, CD14+CD16+, and CD14dimCD16+). Both monocyte subsets were increased in obese subjects, with a significant enrichment of the CD14dimCD16+ subpopulation in obese diabetics. The percentage of CD14dimCD16+ monocytes and glycemia was positively correlated, independent of fat mass. Weight loss led to a sharp decrease of this subpopulation, and fat mass changes strongly determined the change. A diminution of the CD14+CD16+ subpopulation was also observed during weight loss and was associated with a

decrease in intima-media thickness (Poitou C et al., ATVB 2011). In another cohort of normal-weight women and obese women who were followed over 2 y after Roux-en-Y gastric bypass, multiplex proteomics was used to assay 27 cytokines and growth factors in serum. Most of these factors were found expressed and dysregulated in obese adipose tissue by ADAPT partners. Concentrations of IL-9, IL-1-RA, IL-10, IF-inducible protein 10, macrophage inflammatory protein 1, MCP-1, IL-8, RANTES, and VEGF were elevated in obese subjects. IL-10 was further elevated in diabetic obese patients, whereas eotaxin was increased only in diabetic patients. After surgery, many factors showed a biphasic pattern of variation, decreasing sharply at month 3 before rising back to pre-surgical values at month 6; these changes closely tracked similar changes in calorie and carbohydrate intake. These coordinated changes suggest an early influence of energy and carbohydrate intake, whereas a long-term reduction in body weight might prevail in regulating circulating cytokine concentrations (Dalmas at al., Am J Clin Nutr 2011).

KI has assessed the secretion of inflammatory cytokines in subcutaneous adipose tissue from healthy lean women. These studies demonstrated that TNF?, but none of the other factors, correlated significantly with adipose tissue morphology, i.e. secretion was increased in subjects with adipocyte hypertrophy characterized by the presence of few large adipocytes (Arner et al., NEJM 2010). These results demonstrate that TNF? and local inflammation may play a role in adipogenesis and adipose plasticity also in normal-weight conditions. KI together with GDC identified DPP4 as a novel adipokine and the expression was assessed in different clinical cohorts (Lamers D et al., Diabetes 2011). DPP4 expression was increased in obesity and the metabolic syndrome and correlated positively with fat cell size. In addition, in vitro experiments demonstrated that DPP4 attenuated insulin sensitivity in human adipocytes and myocytes. Taken together, our results suggest that increased DPP4 expression in obesity may be a contributing mechanism promoting insulin resistance. Finally, KI has also studied the expression of Zinc-alpha2-glycoprotein (ZAG) and could demonstrate that it is a catabolic marker in conditions of both voluntary and involuntary weight loss (Rydén et al., J Intern Med 2011). However, the functional role of ZAG in human adipocytes is controversial and remains to be established.

CHAR pursued the multiple period dietary intervention design (energy restriction phase with a 4-week very-low-calorie diet and a weight stabilization period composed of a 2-month low-calorie diet followed by 3 months of a weight maintenance diet). In a close collaboration with INST, associations between various characteristics of SAT and clinical parameters such as adiposity, insulin resistance and other indices of metabolic syndrome were analyzed. Plasma leptin appeared as the only variable that could be associated with the diet-induced improvement of insulin sensitivity (paper submitted). Adrenergic regulation of lipolysis in SAT was found to vary with respect to the dietary phase. However, no association between the time-course of lipolysis regulation and that of insulin sensitivity was found (Koppo K et al, AJP 2011). Gene expression response (for a large number of genes including adipokines, macrophage-specific markers, lymphocyte markers) to this dietary protocol was compared between abdominal and gluteal SAT. We did not find differences between the two depots before the diet. The general pattern of the diet-induced response was similar for a majority of genes (except for leptin and adiponectin). Thus, these results do not support the often cited “protective” role of gluteal adipose tissue (manuscript in preparation). CHAR and INST studied in a cross-sectional design paired samples of

abdominal subcutaneous (SAAT) and visceral (VAT) adipose tissue in 56 subjects. A similar distinct regulation of adipocyte-derived and macrophage-derived genes with respect to obesity and insulin resistance was found in SAAT and in VAT (Klimcakova et al., JCEM, 2011). Expression of macrophage-specific genes increased with increasing adiposity and insulin resistance in both, SAAT and VAT (Klimcakova et al, Diabetologia 2011). In both fat depots, macrophage gene expression was higher in obese subjects with metabolic syndrome when compared with obese subjects without metabolic syndrome. These results support the opinion that SAAT is as important as VAT in the pathogenesis of obesity-related metabolic disorders.

MHH together with INST analyzed gene expression differences in SAT of lean and obese, normotensive and hypertensive subjects. The original hypothesis that SAT gene expression differs widely between obese normotensive and hypertensive subjects was not confirmed, only two differentially expressed genes were found. MHH also analyzed FABP4. FABP4 blood concentrations correlated positively with body fat mass and insulin resistance, and were reduced by dietary weight reduction. In contrast, FABP4 gene expression was not related to obesity, and not changed by weight loss. Thus, other mechanisms than transcriptional regulation influence the amount of secreted FABP4 in obesity (manuscript submitted). MHH has established a dietary intervention which modulates fat and carbohydrate intake in a cross-sectional manner over two week periods without changing body weight and body composition. In a first analysis, we determined plasma ADMA in lean and obese subjects during the dietary intervention. Although suggested differently by other authors, neither obesity, nor a fat rich diet increased circulating ADMA. Similarly, neither diet had any influence on DDAH1 and 2 gene expression in SAT. DDAH is the principe enzyme for ADMA degradation (Engeli et al., NMCD 2011). We are currently analyzing other adipokines and genes within the framework of this experimental approach. Finally, MHH analyzed the influence of different kinds of body fat distribution on the value of bioelectrical impedance analysis. This technique is widely used, and we clearly showed that the precise determination of body fat mass by BIA is influenced by body shape. These findings are important for future studies of adipokines when precise phenotyping is of relevance (Haas et al., EJCN 2011).

Potential impact:

The ADAPT project aims to improve the health of European citizens, to increase the competitiveness and innovative capacity of European health-related industries and business, while addressing obesity and its associated complications, a global health issue and an emerging epidemic. ADAPT is focused on translational research with the ultimate goal to develop and validate new therapeutic approaches. The impact of the ADAPT Consortium rests on its ability to provide advances to combat the Cardiometabolic Syndrome, especially by clarifying the relationships between adipose tissue inflammation and insulin resistance, the identification of novel adipokines, the analysis of interorgan crosstalk and the evaluation of adipokines as drug targets to prevent the devastating consequences of excess fat accumulation.

The final goal of the ADAPT consortium was the identification of novel targets in the crosstalk between adipose tissue and other organs. This will contribute to development of new strategies for characterization and diagnosis, and in a longer perspective development of new drugs for treatment and/or prevention of the Metabolic Syndrome. The result will have a great potential impact on combating this syndrome, which is a crucial intermediate step between obesity and the associated risk of type 2 diabetes, hypertension, and cardiovascular diseases. In view of the rapidly-developing obesity epidemic, combating the Metabolic Syndrome will lead to improvement of the health of the European populations.

Health Impacts in Europe

The results from ADAPT will contribute to the development of a fundamentally new understanding of these pathophysiological mechanisms in order to enable us to design drugs that reduce or reverse adipokine-mediated adverse interactions and the obesity-associated inflammatory drift. The design of new drugs and their ultimate approval for use in Europe will take a further six to ten years but they will then have the potential to make an important new contribution towards dealing with one of the most serious health problems facing Europe.

The prevalence of obesity has risen up to three-fold in the last two decades. Half of all adults and one in five children in the WHO European Region are overweight. Of these, one third are already obese, and numbers are increasing fast. Overweight and obesity contribute to a large proportion of non-communicable diseases, shortening life expectancy and adversely affecting the quality of life. In 2005 it was estimated that there were more than one million deaths in the European Region due to diseases related to excess body weight annually. This trend is particularly alarming in children and adolescents, thus passing the epidemic into adulthood and creating a growing health burden for the next generation. The annual rate of increase in the prevalence of childhood obesity has been rising steadily and is currently up to ten times higher than it was in 1970.

Obesity also strongly affects economic and social development. Adult obesity and overweight are responsible for up to 6% of health care expenditure in the European Region; in addition, they impose indirect costs (due to the loss of lives, productivity and related income) that are at least two times higher. Overweight and obesity most

affect people in lower socioeconomic groups, and this in turn contributes to a widening of health and other inequalities. There is a need for new approaches to both prevention and treatment, which include the type of approaches and medium to long-term therapeutic outcomes that have been made possible by the advances made by ADAPT.

Contributions of ADAPT to solve the societal problems associated with obesity

The ADAPT objectives are complementary in their approach to the search for a better understanding of the development of the Metabolic Syndrome in relation to obesity and adipose tissue inflammation. The project has generated novel fundamental knowledge by studies on animal models, but also directly applicable knowledge in humans by studies of human samples, of human integrative physiology and human populations. These two types of knowledge together are necessary to envisage development of new drugs for treatment or prevention of the Metabolic Syndrome.

Adipokines have been recognised as key players in the initiation of insulin resistance and the Metabolic Syndrome and they are of great potential significance both as biomarkers and drug targets. By understanding the complex intra- and inter-organ crosstalk and the specific role of adipokines ADAPT has generated an essential platform for evaluating adipokines as “drugable” targets. Further, the identification of novel adipokines represents an essential step beyond the current state-of-the-art and may open new avenues for drug design targeted to combat the Metabolic Syndrome and its complications.

Reinforcement of European competitiveness

Another strong potential impact is the reinforcement of European competitiveness at both the scientific and economic levels within the field of adipose tissue biology and related drug design. ADAPT has created this partnership of 7 academic research groups and 3 European biotech companies and one large pharmaceutical company as active dedicated members, including some of the world leaders in the fields of Metabolic Syndrome, insulin signalling, adipose tissue biology, inflammation and cytokine function and biotechnology for the first time. Having these partners collaborating within the frame of a single European collaborative R&D project has led to the following:

- Increase in the scientific competitiveness:

Well-experienced scientific leaders are linked in the ADAPT Consortium, and some of whom have already cooperated in previous projects. The Consortium of research groups, laboratories, clinical departments, and companies will further develop the quality of the research pursued, and leverage these European teams further on towards worldwide leadership on essential components of adipose tissue inflammation and the specific role of adipokines and their exploitation for development of diagnostics and drug targets.

- Progression of economic competitiveness:

The ADAPT Consortium contributes to achieve the Lisbon objectives defined in March 2000 at the Lisbon European Council where the Heads of State and Government set the Union the goal of becoming by 2010 the most competitive and dynamic knowledge-based economy in the world, capable of sustainable economic growth with more and better jobs and greater social cohesion. The ADAPT Consortium aims at driving more innovation towards European SMEs, and has an important position in this move; thus, the synergistic interaction between three SMSs specialized in different fields of drug design and a large pharmaceutical company with an established programme targeted towards treatment of the Metabolic Syndrome provides a platform for successful joint economic programmes. New innovations and target development programs emerging from the ADAPT research will therefore increase the cooperation between European biotech companies themselves, between academic and industrial partners, as well as between biotech companies and worldwide pharmaceutical industries.

Innovation and industrial impacts

The potential industrial impacts will follow on from the pharmacological and clinical evaluation of adipokines as drug targets and potential biomarkers. The ADAPT Consortium is strongly focused on identifying and developing innovations, at different levels:

- Developing new technologies & tools:

The complementarities of the partners and the breadth of the research into adipose tissue biology and adipokine function, from the molecular biology addressing the mechanistic level to the clinical level, will give the ADAPT Consortium opportunities to further develop the technologies and tools in new settings and contexts. The concerted approaches are expected to lead to the identification and development of novel technologies for such areas as expression profiling, screening assays, bio-informatics, statistical methods, characterization of new animal models, etc. Physiogenex is aiming to develop a new patent on its animal model regarding its usefulness in studying new drug targeting the cross-talk between adipose and peripheral organs. The animal model will then be made commercially available for these purposes.

- Identifying new diagnostics tools:

One of the important goals of the project is to identify specific adipokines and/or associated signalling pathways that could be used as drug targets to prevent the development of the Metabolic Syndrome. Identification of this type of molecule will be helpful for developing strategies for early diagnosis and intervention in this syndrome and its associated-diseases. Mabtech is a world leader within the field of ELISpot and has several patents and patent applications relating to this technique. The results from ADAPT will enable it to further advance the development of its ELISpot equipment and to maintain its scientific and commercial competitive advantage.

- Optimizing novel drug target candidates:

The ADAPT project is based on the strong unmet clinical needs and major unexploited opportunities for new drug development related to obesity and the Metabolic Syndrome. The task is to further elucidate the biological mechanisms that

may identify adipokines as new drug targets, and the ADAPT Consortium is a strong platform for contributing to those worldwide efforts towards combating the Metabolic Syndrome.

Education and Training Impacts

The results from ADAPT have been incorporated in the undergraduate and postgraduate training programmes of the seven teaching institutions participating in ADAPT. The development of new chemical tools has been used in their laboratories and other techniques and published results were extremely useful for progress in the different groups. ADAPT involved more than 50 young researchers as post docs or PhD students for at least part of their training.

The researchers recruited had the opportunities to gain experience in the conduct and management of interdisciplinary research that brings together concepts and techniques from complementary areas such as physiology, molecular biology, cell biology, bioinformatics and genetics. They received training in state of the art techniques that were valuable to them in their research careers in either universities or in industry including the following:

- The proteomics group at GDC has established and successfully used multiplex 2D-gel electrophoresis coupled to a MALDI-TOF/TOF platform and in-house data bank analysis.
- The use of mass spectrometry to identify novel adipokines.
- Capillary electrophoresis-mass spectrometry (CE-MS).
- The identification of new markers using nLC/MS-MS type FT Orbitrap.
- Microdialysis.

They had access to excellent experimental facilities for microarray, RNAi, large-scale genotyping, human specific antibodies, metabolomics and proteomics. The examples above are illustrative rather than comprehensive, as the partners have very extensive experimental capacities.

The interactions between the researchers in the academic partners and those in the four companies were valuable both in terms of contributing to the production of the immediate research outcomes and the training impacts.

Dissemination and exploitation:

ADAPT has used multiple dissemination channels including publication in peer reviewed Journals, presentations at scientific meetings in Europe and overseas, and organization of workshops and summer schools, and the ADAPT website.

- Publication in peer reviewed journals

The ADAPT consortium has been following a very active and successful publication strategy and has produced more than 130 publications directly related to ADAPT including 20 joint papers. The partners have strong publication records and have typically published in top Journals in the field of endocrinology, metabolism, obesity and diabetes such as Diabetes, Diabetologia, Endocrinology, American Journal of Physiology etc. Importantly, landmark results of ADAPT were published in Nature, Nature Medicine, New England Journal of Medicine, Cell Metabolism and Circulation. This outstanding publication record shows that the ADAPT consortium has generated novel research results of the highest calibre.

- Presentations at scientific meetings

Presentations have been made at both national and European meetings and international conferences such as the European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) Annual Meetings. Members of the ADAPT consortium have presented results as invited speakers at a huge number of meetings including Keystone Symposia, ECO-, EASD-, ADA-meeting, EASO Björntorp symposium, NIH workshops, and many others.

- Workshops for researchers including those from institutes outside the consortium and from SMEs concerned with drug development

Organisation of workshops and symposia for scientists from SMEs and academia outside the ADAPT consortium was another key tool for efficient dissemination of the research results of this project. A summer school on proteomics was organised in Duesseldorf and a joint symposium took place in Toulouse in 2010 organised by Prof. D. Langin.

- The ADAPT website

The ADAPT website has developed as a very important tool for the visibility and awareness of the ADAPT research activity. The information published here included regular newsletters and brief reports on the development in obesity and adipokine research, written in a way understandable to lay persons. The website has found great interest with more than 1000 visits per month at this time.

The ADAPT consortium has taken steps to protect all the new knowledge generated which is identified as having potential economic value. This was done through patenting, taking account of the interests of all partners who have contributed directly to the relevant discoveries. Details are presented in section B.
Where target genes were identified, this was treated as confidential information pending discussion with companies about contract research or licensing.

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