



HAL
open science

Lipid and glucose metabolism in white adipocytes: pathways, dysfunction and therapeutics

Pauline Morigny, Jeremie Boucher, Peter Arner, Dominique Langin

► **To cite this version:**

Pauline Morigny, Jeremie Boucher, Peter Arner, Dominique Langin. Lipid and glucose metabolism in white adipocytes: pathways, dysfunction and therapeutics. *Nature Reviews Endocrinology*, 2021, 17 (5), pp.276-295. 10.1038/s41574-021-00471-8 . hal-03320059

HAL Id: hal-03320059

<https://ut3-toulouseinp.hal.science/hal-03320059v1>

Submitted on 13 Aug 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Lipid and glucose metabolism in white adipocytes: pathways, dysfunction and**
2 **therapeutics**

3

4 Pauline Morigny^{1,2,3,4,5}, Jeremie Boucher^{6,7,8}, Peter Arner⁹ and Dominique
5 Langin^{1,2,10,11†}

6 ¹Institute of Metabolic and Cardiovascular Diseases (I2MC), Institut National de la
7 Santé et de la Recherche Médicale (Inserm), UMR1297, Toulouse, France

8 ²University of Toulouse, Paul Sabatier University, I2MC, UMR1297, Toulouse, France

9 ³Institute for Diabetes and Cancer, Helmholtz Center Munich, Neuherberg, Germany,

10 ⁴Joint Heidelberg-IDC Translational Diabetes Program, Inner Medicine 1, Heidelberg
11 University Hospital, Heidelberg, Germany

12 ⁵German Center for Diabetes Research (DZD), Neuherberg, Germany

13 ⁶Bioscience Metabolism, Research and Early Development, Cardiovascular, Renal and
14 Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden

15 ⁷The Lundberg Laboratory for Diabetes Research, University of Gothenburg, Sweden

16 ⁸Wallenberg Centre for Molecular and Translational Medicine, University of
17 Gothenburg, Sweden

18 ⁹Department of Medicine (H7), Karolinska Institutet, Stockholm, Sweden

19 ¹⁰Franco-Czech Laboratory for Clinical Research on Obesity, Third Faculty of Medicine,
20 Prague and Paul Sabatier University, Toulouse, France

21 ¹¹Toulouse University Hospitals, Laboratory of Clinical Biochemistry, Toulouse, France

22 †email: dominique.langin@inserm.fr

23

24

25

26

1 **Abstract**

2 In mammals, the white adipocyte is a cell type that is specialized for storage of energy,
3 in the form of triacylglycerols, and for energy mobilization, as fatty acids. White
4 adipocyte metabolism confers an essential role to adipose tissue in whole-body
5 homeostasis. Dysfunction of white adipocyte metabolism is a cardinal event in the
6 development of insulin resistance and associated disorders. This Review focuses on
7 our current understanding of lipid and glucose metabolic pathways in the white
8 adipocyte. We survey recent advances in humans on the importance of adipocyte
9 hypertrophy and on in vivo turnover of adipocytes and stored lipids. At the molecular
10 level, identification of novel regulators and interplay between metabolic pathways
11 explains the fine-tuning between anabolic and catabolic fates of fatty acids and glucose
12 in different physiological states. We also examine the metabolic alterations involved in
13 the genesis of obesity-associated metabolic disorders, lipodystrophic states, cancers
14 and cancer-associated cachexia. New challenges include defining the heterogeneity of
15 white adipocytes in different anatomical locations throughout the lifespan and
16 investigating the importance of rhythmic processes. Targeting white fat metabolism
17 offers opportunities for improved patient stratification and a wide, yet not exploited,
18 range of therapeutic opportunities.

1 **Introduction**

2 White adipose tissue (WAT) was long considered an inactive tissue that primarily
3 served a thermal insulation purpose¹. In the mid-20th century, it became increasingly
4 apparent that WAT is important for energy homeostasis, as it is able to utilize glucose
5 as well as to store and release energy-rich fatty acids (FIG. 1). Lipids are stored mostly
6 as triacylglycerol (TAG), in a single lipid droplet in mature white adipocytes or in
7 multiple lipid droplets in most other cell types, including brown adipocytes². In 1964, a
8 major breakthrough was the development of collagenase digestion, which enabled the
9 isolation of fairly pure preparations of adipocytes³. This breakthrough paved the way
10 for studies of TAG metabolism, including uptake, synthesis and hydrolysis, a process
11 known as lipolysis. Defects of glucose metabolism in hypertrophic adipocytes were
12 identified in 1976⁴. In 1980, insulin was shown to stimulate the translocation of the
13 glucose transport machinery to the plasma membrane in adipocytes^{5,6}. The discovery,
14 in the early 1990s, that WAT is an endocrine and inflammatory organ, notably with the
15 identification of the hormones leptin and adiponectin, added new dimensions to
16 research in this field⁷⁻¹¹. In parallel, knowledge was progressing on WAT being a major
17 metabolic regulator. Historical aspects of adipocyte biology have been
18 comprehensively reviewed elsewhere¹².

19 Among the many cell types in WAT (BOX 1), the adipocyte is the one that is
20 specialized in energy metabolism. Metabolic alterations drive changes from healthy to
21 dysfunctional adipocytes, with systemic consequences. This Review focuses on the
22 current understanding of glucose and lipid metabolic pathways in white adipocytes and
23 dysfunctions reported in situations of excess WAT (for example, obesity and the related
24 condition type 2 diabetes mellitus (T2DM)), or of WAT paucity (such as lipodystrophy
25 and cachexia). We also address therapeutic perspectives in targeting white fat

1 metabolism and some of the outstanding questions in this field of research. Other
2 aspects of adipose biology, such as the role of brown and beige fat and the endocrine
3 function of WAT, have been reviewed elsewhere¹³⁻¹⁷.

4

5 **Adipocyte size and turnover**

6 Cell size and turnover are major determinants of white adipocyte metabolism and
7 fat mass, the alterations of which are associated with pathological conditions (FIG. 2a,
8 b). Since the development of methods to determine the size of adipocytes in the late
9 1960s and 1970s, it became clear that adipocyte size varies considerably within fat
10 depots of the same person and between individuals, and that the average adipocyte
11 size increases during body weight gain and decreases after weight loss¹⁸. From a
12 clinical point of view, the most important aspect of adipocyte size is its relation to
13 cardiometabolic status (FIG. 2b). Numerous studies have shown a strong association
14 between large adipocytes and cardiometabolic disorders¹⁹⁻²¹, including risk of
15 developing T2DM, as well as associations with insulin resistance, dyslipidaemia and
16 hypertension (BOX 2). Adipose mass can develop in two ways, by adipose tissue
17 hypertrophy or adipose tissue hyperplasia, which respectively define the WAT
18 morphologies termed hypertrophic WAT and hyperplastic WAT (FIG. 2b). Hypertrophic
19 WAT is characterized by large adipocytes, which can be formed through two processes
20 during build-up of fat mass: formation of few large-sized adipocytes, or storage of more
21 lipids in pre-existing fat cells. Hyperplastic WAT shows a greater number of adipocytes
22 of a smaller diameter than in normal or hypertrophic WAT. When fat mass develops,
23 there is an increase in the number of smaller adipocytes through differentiation of
24 progenitor cells. In humans, the two morphologies are found irrespective of body
25 weight status, although obesity is usually characterized by a combination of WAT

1 hypertrophy and hyperplasia²². The hypertrophic morphology is associated with
2 adverse cardiometabolic profile^{23,24}. Advances in understanding of the origin of the new
3 adipocytes formed during adipogenesis have been reviewed elsewhere^{16,17,25}.

4 De novo adipocyte formation and differentiation are complex processes that are
5 subject to regulation by multiple signalling pathways, including, amongst others,
6 signalling by the nuclear receptor peroxisome proliferator-activated receptor- γ
7 (PPAR γ) and its co-activator PGC1 α , as well as by WNT and NOTCH signalling
8 pathways²⁶⁻²⁸. Studies in mice have shed new light on the control of adipogenesis.
9 The process is inhibited by low steady-state oxidative stress, which alters
10 mitochondrial function in adipocyte precursors²⁹. Increased adipogenesis in obese
11 mice is mediated, at least in part, by the mechanosensitive cationic channel Piezo1
12 through the FGF1 signalling pathway³⁰. The adipocyte insulin receptor also seems to
13 be important in the turnover of adipocytes, as demonstrated with conditional knock-out
14 experiments³¹. Finally, mammary adipocyte turnover may be governed by site-specific
15 regulation. These adipocytes dedifferentiate during pregnancy and remain
16 dedifferentiated during lactation. Upon weaning, dedifferentiated cells proliferate and
17 redifferentiate into adipocytes³². Multiple origins of precursor cells have now been
18 identified in mice³³ and in humans³⁴. One source of adipocyte precursors in humans is
19 stem cells from the bone marrow^{35,36}. On average, bone marrow precursors contribute
20 10% to the total adipocyte pool, a figure that is doubled in obesity (FIG. 2a).

21 The anatomical location of the fat depot also influences adipose tissue morphology.
22 Subcutaneous WAT comprises more than 80% of total body fat, whereas visceral fat
23 comprises up to 10 or 20% of total body fat in women or men, respectively³⁷. The small
24 visceral depot is often considered to be more pernicious than the larger subcutaneous
25 depot. Visceral WAT hypertrophy has been associated with insulin resistance and

1 cardiometabolic disorders²³. Nevertheless, the pathophysiological consequences of
2 regional adipose tissue morphology are complex. Two independent studies
3 demonstrated that visceral adipocyte hypertrophy is predominantly associated with
4 dyslipidaemia whereas subcutaneous adipocyte hypertrophy is mainly associated with
5 insulin resistance in humans^{38,39}. Moreover, a reduction in adipocyte size in
6 subcutaneous WAT improves insulin sensitivity in individuals with obesity¹⁸. In visceral
7 but not in subcutaneous WAT, the association between adipocyte hypertrophy and M1-
8 like macrophage-mediated and/or B-cell-mediated inflammation may be involved in
9 insulin resistance⁴⁰. It should be emphasized that, in these human studies, the
10 associations between adipocyte size and clinical phenotypes are not evidence of a
11 causal link. An exception might be a possible role of adipocyte size in T2DM, as
12 prospective studies reveal that enlarged subcutaneous adipocytes confer increased
13 risk of developing T2DM^{41,42}.

14 At the whole-body level, WAT mass is determined by the dynamics of adipocyte and
15 lipid turnover⁴³. A breakthrough in investigating cell turnover in vivo came with methods
16 to study incorporation of atmospheric ¹⁴C into DNA of free-living individuals⁴⁴. The
17 method has been used to determine the age and turnover of human subcutaneous
18 adipocytes⁴⁵. On average, 10% of these cells are renewed each year. In obesity,
19 adipocyte turnover at the whole-body level increases about two-fold, owing to an
20 acceleration of adipogenesis. Importantly, hypertrophic WAT is associated with low
21 generation rate of new adipocytes, irrespective of body fat mass⁴⁶.

22

23 **Lipid turnover in white adipocytes**

24 The ¹⁴C method has been further used to determine the age and turnover
25 parameters for lipids within human adipocytes (FIG. 2c). The lipid content of a human

1 adipocyte is renewed six times on average during its ~10-year life span⁴⁷, a turnover
2 rate that has been confirmed using multi-isotope imaging mass spectrometry⁴⁸. Lipid
3 age data can be modelled to determine parameters reflecting the adipocyte capacity
4 for storage (that is, lipid input; K_{in}) and removal (that is, lipid output; K_{out})^{36,47-49}. Cross-
5 sectional studies show that weight gain is associated with increased lipid age (reflecting
6 decreased turnover) in the subcutaneous region, which already appears in the
7 overweight state, owing to a combination of increased K_{in} and decreased K_{out} ^{36,47}. In
8 the visceral region, lipid turnover is decreased only among very obese individuals⁴⁹.
9 This regional difference may explain why lipid mobilization is usually more rapid in the
10 visceral compared to subcutaneous WAT among overweight and obese individuals^{50,51}.
11 It could also partly explain why visceral fat is more pernicious than subcutaneous fat
12 because a high output of fatty acids from visceral fat to the liver via the portal vein has
13 direct effects on liver metabolism. Longitudinal studies of subcutaneous WAT show
14 that lipid turnover decreases (that is, lipid age increases) over time irrespective of
15 variations in body weight⁵². If the decrease in K_{out} is not counterbalanced by a decrease
16 in K_{in} , then body fat will accumulate over time (FIG. 2c). In those individuals with obesity
17 whose body weight decreases markedly following bariatric surgery, adipocyte lipid
18 turnover increases and the initial weight reduction is maintained. Conversely, those
19 individuals who do not show an increase in lipid turnover experience long-term weight
20 regain. Moreover, adipocyte lipid turnover is decreased in insulin-resistant individuals
21 and in patients with familial or common dyslipidaemic conditions^{47,52}. These data
22 highlight the physiological and pathophysiological importance of adipocyte and lipid
23 turnover^{36,45-49,52}, although the short-term regulation of these two processes in
24 physiological and pathological states remains largely unknown⁴³.

25

1 **Fat storage**

2 Most of the fatty acid stored in adipocytes originates from circulating TAG that is carried
3 by lipoproteins, such as very-low-density lipoproteins and chylomicrons (FIG. 3).
4 Following hydrolysis by lipoprotein lipase, fatty acids rapidly enter the adipocyte, both
5 passively by diffusion and actively by protein-mediated processes involving fatty acid
6 transporters^{53,54}. The fatty acid translocase CD36, which is localized in plasma
7 membrane lipid microdomains, acts in concert with fatty acid-binding proteins in the
8 plasma membrane to promote uptake of long-chain fatty acids. Fatty acid transport
9 proteins and long-chain acyl-CoA synthetases are responsible for uptake and
10 conversion of fatty acids into acyl-CoA derivatives that can be esterified on the glycerol-
11 3-phosphate backbone to be stored as TAG in the lipid droplet. The various steps
12 leading to storage of fat as TAG are stimulated by insulin (FIG. 3). In the fed state,
13 glycerol-3-phosphate is produced from glucose during glycolysis. The esterification
14 process is performed by the sequential action of glycerol-3-phosphate
15 acyltransferases, 1-acyl-glycerol-3-phosphate-acyltransferases (such as AGPAT2),
16 phosphatidic acid phosphatases (also known as lipins) and diacylglycerol
17 acyltransferases (DGATs)⁵⁵. There is emerging evidence that large protein assemblies
18 ensure compartmentalization of enzyme pathways and high local concentrations of
19 substrates and intermediates⁵⁶. The protein complexes allowing proper channelling of
20 fatty acids and acyl-CoAs into specific pathways of the white adipocyte have not yet
21 been characterized. DGATs catalyse the formation of TAG from diacylglycerol (DAG)
22 and are important regulators of this pathway. Mice with adipocyte-specific ablation of
23 either *Dgat1* or *Dgat2* fed a chow diet display normal adipose tissue development,
24 indicating that either isoform is sufficient for TAG production and storage⁵⁷. However,
25 in mice fed a high-fat diet, adipocyte-specific ablation of *Dgat1*, but not *Dgat2*, leads to

1 a slight decrease in WAT mass, ectopic lipid storage in liver and skeletal muscle, and
2 insulin resistance⁵⁷. DGAT1 may be protective against insulin resistance by preventing
3 fatty acid-induced endoplasmic reticulum stress and inflammation in adipocytes, which
4 is achieved by promoting fatty acid re-esterification during increased lipolysis^{57,58}.

5 In addition to exogenous lipid sources, fatty acids stored in adipocytes can also
6 originate from endogenous synthesis from glucose, a pathway termed de novo
7 lipogenesis (DNL)^{59,60} (FIG. 3). After entering the adipocyte through insulin-sensitive
8 (GLUT4) and non-insulin-sensitive (GLUT1) glucose transporters, glucose is
9 metabolized through glycolysis and the tricarboxylic acid (TCA) cycle to produce citrate
10 molecules that are required for DNL. ATP citrate lyase (ACLY) and acetyl-CoA
11 carboxylase (ACC1) respectively produce acetyl-CoA and malonyl-CoA, which are
12 used by fatty acid synthase (FASN) to generate palmitic acid. Another source of acetyl-
13 CoA in white adipocytes comes from the conversion of acetate by the acyl-CoA
14 synthetase ACSS2⁶¹. The relative contribution of ACLY and ACSS2 to the acetyl-CoA
15 pool in various pathophysiological conditions is unclear at present. Fatty acid
16 elongases and desaturases subsequently modify the length and the degree of
17 unsaturation of newly synthesized palmitic acid⁶². In vivo, the assessment of adipose
18 tissue DNL is complicated by the contribution of hepatic DNL, as fatty acids newly
19 synthesized in the liver are exported to adipose tissue and stored as TAG. During
20 chronic glucose infusion, de novo production of fatty acids in human adipose tissue
21 has been demonstrated^{63,64}. In habitual dietary conditions, the contribution of adipose
22 DNL to fatty acids stored in human adipose tissue seems limited⁶⁵.

23
24
25

1 **Fat mobilization**

2 The mobilization of WAT lipids after hydrolysis of TAG is maximal during periods of
3 energy demands, such as fasting or physical exercise. Neuroendocrine control of
4 lipolysis and the associated signalling pathways have been extensively reviewed
5 elsewhere^{66,67}. In human adipocytes, catecholamines, natriuretic peptides and insulin
6 are the main hormonal regulators of lipolysis (FIG. 4). In addition, many autocrine and
7 paracrine factors act through activation of anti-lipolytic G-protein-coupled receptors.
8 Furthermore, locally produced inflammatory mediators, in particular tumour necrosis
9 factor, act through specific signal transduction pathways to regulate basal lipolysis⁶⁸.

10 In adipocytes, three neutral lipases are involved in TAG breakdown. Adipose
11 triglyceride lipase (ATGL; encoded by *PNPLA2*) is the main enzyme responsible for
12 TAG hydrolysis to DAG. Whole-body⁶⁹ as well as adipocyte-specific *Pnpla2*-knockout
13 mice show drastically reduced basal and stimulated lipolysis^{70,71}. *PNPLA2* knockdown
14 in human adipocytes also reduces basal and stimulated lipolysis⁷². Accordingly, human
15 mutations leading to inactive ATGL are associated with decreased rate of glycerol and
16 fatty acids formation in response to lipolytic agents as well as with neutral lipid storage
17 disease with myopathy^{73,74}. An unexpected aspect of the phenotype in this storage
18 disease is the lack of marked alteration of fat mass in carriers of these mutations. The
19 second enzyme, hormone-sensitive lipase (HSL; encoded by *LIPE*), hydrolyses DAG,
20 although it also displays TAG hydrolysis activity. Whole-body HSL-deficiency leads to
21 DAG accumulation within adipose tissue and decreased stimulated lipolysis in both
22 mice⁷⁵ and humans^{72,76}. The third enzyme, monoglyceride lipase, catalyses the
23 hydrolysis of monoacylglycerol to glycerol and a fatty acid. Studies of monoglyceride
24 lipase-deficient mice show that HSL also participates in WAT monoacylglycerol
25 hydrolysis⁷⁷.

1 The activity of lipases depends on their intracellular localization and interactions
2 with cofactors (FIG. 4). ATGL is located on lipid droplets and different lipid droplet-
3 associated proteins regulate its activity. For example, CGI-58 (encoded by *ABHD5*) is
4 essential for full ATGL activation in stimulated lipolytic states. Mutations in *ABHD5* lead
5 to TAG accumulation in adipose tissue in mice and humans^{78,79}. Conversely, ATGL
6 activity is inhibited by G0S2 and CIDEA (also known as FSP27) by their direct
7 interaction with ATGL⁸⁰⁻⁸³. Perilipin 1 (PLIN1) is a major protein of the adipocyte lipid
8 droplet and inhibits ATGL function by sequestering CGI-58 in basal conditions⁸⁴.
9 When lipolysis is stimulated, phosphorylation of PLIN1 promotes the release of CGI-
10 58 from PLIN1, allowing it to interact with ATGL. PLIN1 also increases HSL activity by
11 binding directly to HSL⁸⁵. The fatty acid-binding protein FABP4 also binds to the
12 phosphorylated HSL and translocates to the lipid droplet to regulate HSL lipolytic
13 activity^{86,87}. PTRF (encoded by *CAVIN1*) interacts with caveolin 1 (encoded by *CAV1*)
14 to stabilize caveolae, which are small invaginations of the plasma membrane that
15 control lipid trafficking; PTRF promotes lipolysis by recruiting HSL to caveolae^{88,89}.
16 Human adipocytes express another member of the CIDE family of proteins, CIDEA, at
17 higher levels than mouse white adipocytes^{90,91}. CIDEA is localized to lipid droplets,
18 where it controls basal lipolysis, but also to the nucleus, where it acts as a transcription
19 cofactor⁹²⁻⁹⁴. In white adipocytes, shuttling of proteins between lipid droplets and the
20 nucleus is a novel and potentially important level of regulation connecting
21 transcriptional control and metabolic pathways. Proteomic studies have identified other
22 lipid droplet-associated proteins that are involved in lipid droplet maturation and fatty
23 acid storage as well as proteins with unknown roles, which require further study to
24 determine their function⁹⁵.

1 In addition to the classic lipolysis pathway involving neutral lipases, other
2 lipolytic pathways have been described. In vitro experiments suggest that lipophagy
3 contributes to β -adrenergic-receptor-stimulated lipolysis⁹⁶. Mice with adipocyte-
4 specific knockout of *Atg7*, a crucial macroautophagy gene, had decreased WAT mass
5 with appearance of brown fat-like adipocytes^{97,98}, a phenotype that might result from
6 an impairment of adipocyte differentiation rather than an inhibition of lipolysis in mature
7 adipocytes. In addition, a lipase-independent pathway of lipid mobilization through
8 release of lipid droplet-derived exosome-like vesicles has been described⁹⁹. In mice,
9 WAT releases ~1–2% of its lipid content each day via exosomes. Overall, several new
10 lipid degradation pathways that are independent of neutral lipases have been
11 described in the last few years. However, their importance in lipid droplet TAG
12 hydrolysis and regulation in pathophysiological conditions remains unclear.

13

14 **Glucose metabolism**

15 It has now been repeatedly shown that adipose tissue expression of lipogenic
16 enzymes and cognate transcription factors, notably carbohydrate-responsive element-
17 binding protein (ChREBP), is strongly and positively correlated with insulin sensitivity,
18 suggesting that DNL plays a part in adipocyte and whole-body metabolism that extends
19 beyond the simple production of fatty acids for storage¹⁰⁰⁻¹⁰³. There is evidence that
20 DNL serves as a regulator of adipocyte membrane fluidity and insulin signalling^{103,104}.
21 As described below, DNL may also interact with other metabolic pathways and
22 modulate the production of lipid species that control systemic insulin sensitivity.
23 Furthermore, adipocyte-specific *Fasn*-deficiency in mice suggests that products of
24 DNL are involved in sympathetic neuronal signalling, conversion of white adipocytes

1 into beige adipocytes and in the promotion of thermogenic activity in brown adipose
2 tissue (BAT) ^{105,106}.

3 Somewhat surprisingly, the important early steps of glucose metabolism have
4 not been thoroughly studied in adipocytes; notably, the role of adipocyte glycolysis is
5 poorly documented. During fasting and starvation, induction of aerobic glycolysis is
6 mediated by the forkhead transcription factors FOXK1 and FOXK2¹⁰⁷. This
7 reprogramming of cellular metabolism results in enhanced production of lactate (FIG.
8 3). Three decades ago, white adipocytes were shown to be important producers of
9 lactate ¹⁰⁸, which was later confirmed in humans and has been recently reassessed
10 using tracer labelling^{109,110}. In vivo, the impairment of lactate production by the
11 *Drosophila* fat body, which has features of mammalian WAT and liver, results in
12 enhanced whole-body glucose utilization¹¹⁰. Lactate production by WAT therefore
13 seems to contribute to whole-body lactate turnover. Lactate is a metabolic intermediate
14 that can feed into the TCA cycle in most tissues, including the liver, for
15 gluconeogenesis ¹¹¹. Lactate production enables the uncoupling of glycolysis and the
16 TCA cycle, notably in the fasted state¹¹². This uncoupling may reduce whole-body
17 glucose utilization and be part of a complex regulation of glucose carbon fate in the
18 adipocyte that includes lactate production, glycerol-3-phosphate synthesis, DNL and
19 glucose oxidation. The investigation of functional heterogeneity among white
20 adipocytes identified a population with enhanced glycolytic metabolism whereas
21 another population showed increased DNL ^{113,114}. These populations co-exist within a
22 single fat depot but their relative proportions differ from one depot to another. WAT
23 heterogeneity has been reviewed elsewhere ^{115,116}, however, the physiological and
24 pathophysiological importance of this heterogeneity remains to be established.

25

1 **Energy dissipation**

2 Mammalian cells rely on several mechanisms to dissipate energy. Mitochondrial
3 oxidative respiration can be a major contributor to heat production. Substrate cycles,
4 sometimes improperly referred to as futile cycles, may also contribute to energy
5 dissipation and are viewed as essential for metabolic control¹¹⁷. Brown and beige
6 adipocytes have the unique capacity to oxidize fatty acids at very high rates. Compared
7 with white adipocytes, thermogenic adipocytes are uniquely equipped to dissipate
8 energy as heat instead of storing energy in chemical forms. Strong evidence exists to
9 support *de novo* differentiation of thermogenic adipocytes from progenitor cells¹¹⁸.
10 Early studies suggesting direct conversion of white adipocytes into beige adipocytes
11 during cold exposure or treatments with β_3 -adrenergic and PPAR agonists have been
12 confirmed¹¹⁹⁻¹²². In this Review, we focus on the interconversion of unilocular white
13 adipocytes into beige adipocytes.

14 The prototypical adaptive thermogenesis pathway in beige adipocytes involves
15 uncoupling protein 1 (UCP1)¹⁴. UCP1 is located in the mitochondrial inner membrane,
16 where it dissipates the proton electrochemical gradient across the lipid bilayer, which
17 is then no longer available to be used for ATP synthesis (FIG. 5a). UCP1 is one among
18 many components that constitute a cellular machinery that allows energy dissipation
19 as heat. During white-to-beige conversion of human adipocytes, mitochondrial
20 fragmentation from sustained fission results in enhanced UCP1-dependent uncoupling
21 of respiration¹²³. Moreover, the interconversion provokes a major metabolic
22 reprogramming with induction of fatty acid anabolic and catabolic pathways in the
23 cytosol and the mitochondria¹²⁴. Inhibition of the pyruvate dehydrogenase complex
24 through its phosphorylation by pyruvate dehydrogenase kinase 4 redirects glucose
25 from oxidation towards TAG synthesis and favours the use of fatty acids as an energy

1 source by uncoupled mitochondria. Independently of the control of UCP1 expression
2 and activity by the adrenergic signalling pathway, succinate, an intermediate in the TCA
3 cycle, participates in activation of UCP1-mediated thermogenesis by stimulating the
4 production of reactive oxygen species in brown and beige fat¹²⁵. Moreover,
5 extracellular succinate is readily taken up by brown adipocytes to be oxidized. The
6 response of human beige adipocytes to extracellular succinate has not been
7 established. Whether succinate activates thermogenesis during conditions that are
8 known to induce its release, such as physical exercise and ischaemia, is not
9 known^{125,126}.

10 Conversely, brown-to-white adipocyte conversion is observed during adaptation
11 to thermoneutral environments in mice and during ageing in both mice and humans.
12 Whitened brown adipocytes show a unilocular lipid droplet that is typical of white
13 adipocytes but their mitochondria, with low UCP1 content, retain brown-adipocyte-like
14 features. As in hypertrophic and dysfunctional white adipocytes, whitened brown
15 adipocytes show inflammasome activation that may favour pyroptotic cell death ¹²⁷.
16 During warming, beige but not brown adipocytes display chromatin remodelling
17 towards that of the white state¹²⁸. However, beige adipocytes retain an epigenomic
18 memory that allows reactivation of a thermogenic programme following re-exposure to
19 cold. These data support a full bidirectional interconversion between white and beige
20 adipocytes, whereas unilocular white-like adipocytes in BAT may constitute hidden
21 brown adipocytes. Specific chromatin-remodelling enzymes modulate this
22 interconversion. Histone methylation is chemically stable and thus may act as a long-
23 term cell memory mechanism. Several enzymes that regulate methylation of histone
24 lysine residues, notably members of the lysine (K)-specific demethylase (KDM) family,
25 are involved in the control of beige adipocyte metabolism¹²⁹. The most studied family

1 member is KDM1A, which activates a beiging programme while repressing WAT-
2 specific genes by interacting with the thermogenic transcription factors ZFP516 and
3 PRDM16¹³⁰⁻¹³². Collectively, data on the various enzymes point to an essential role of
4 demethylation of histone H3 lysine residues in the maintenance of a beige phenotype.

5 In recent years, several UCP1-independent thermogenic processes in beige
6 and white adipocyte metabolism have been characterized ¹⁴ (FIG. 5b). The TAG–fatty
7 acid cycle is a long-recognized substrate cycle in adipocytes. During lipolysis,
8 breakdown of TAG by lipases releases fatty acids and glycerol, which are either
9 exported from the adipocyte or oxidized. The phosphorylation of glycerol by glycerol
10 kinase and activation of fatty acids to form acyl-CoAs allow re-esterification to TAG.
11 The expression and activity of glycerol kinase is much lower in human white adipocytes
12 than in brown or beige adipocytes¹²⁴. However, glycerol kinase can be induced in white
13 adipocytes following adrenergic activation and PPAR agonist treatments, allowing the
14 fine-tuning of fatty acid fate between release, oxidation and esterification^{133,134}. As
15 such, the energy cost of the TAG–fatty acid cycle is low. However, when other
16 pathways such as DNL and fatty acid oxidation are integrated, prototypical white fat
17 may significantly contribute to energy dissipation and a lean phenotype in mice¹³⁵. In
18 malignant hyperthermia, an uncontrolled release of intracellular Ca²⁺ from skeletal
19 muscle sarcoplasmic reticulum results in hypermetabolism and heat production.
20 Similarly, cold-induced thermogenesis may occur in beige fat when ATP-dependent
21 Ca²⁺ cycling by sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase and the ryanodine
22 receptor is enhanced¹³⁶. The functional importance of the ATP-dependent Ca²⁺ cycling
23 was shown in beige adipocytes in pigs (*Sus scrofa domestica*) lacking functional
24 UCP1¹³⁶. In addition, evidence exists for a mitochondrial substrate cycle that is
25 regulated by creatine to drive thermogenic respiration when ADP is limiting in beige

1 fat^{137,138}. Irrespective of the occurrence or not of UCP1-dependent thermogenic proton
2 leak, beige adipocytes exhibit this futile creatine cycling, which contributes to the basal
3 metabolic rate of cells rather than being a response to acute adrenergic stimulation¹³⁹.
4 The selective reduction of creatine transport in adipocytes results in impaired
5 adrenergic thermogenesis¹⁴⁰. The expression of the creatine transporter in human
6 subcutaneous adipocytes is negatively correlated with obesity and insulin
7 resistance¹⁴⁰. These data therefore suggest a role for extracellular creatine in the
8 control of beige-fat-mediated energy expenditure. An additional example of UCP1-
9 independent thermogenesis in subcutaneous WAT is provided by mice with genetic
10 activation of AMP kinase that show increased energy expenditure¹⁴¹, although the
11 molecular pathway involved has yet to be identified.

12 From a metabolic standpoint, the colour of an adipocyte can therefore be
13 considered to be defined by the metabolic machinery that enables the use of different
14 substrates.

15

16 **Crosstalk between metabolic pathways**

17 Lipolysis and lipogenesis in WAT are typically viewed as independent pathways
18 with opposite outcomes. However, chronic adrenergic activation promotes not only
19 TAG hydrolysis but also DNL and lipid turnover in mouse WAT¹⁴². Interestingly,
20 ablation of *Pnpla2* diminishes lipolysis, as expected, but also leads to a decrease in
21 lipid turnover owing to downregulation of DNL enzymes (FIG. 6). The exact molecular
22 mechanism underlying the ATGL-mediated and/or lipolysis-mediated induction of DNL
23 following adrenergic activation and its relevance in human WAT are not known.
24 Another example of crosstalk is provided by studies of another neutral lipase highly
25 expressed in adipocytes, HSL. Partial deficiency in HSL improves whole-body insulin

1 sensitivity in obese mice without changes in plasma fatty acid levels, suggesting that
2 mechanisms other than lipolysis are involved¹⁴³. The genetic inhibition of HSL in
3 human adipocytes and mouse WAT also results in enhanced insulin sensitivity and
4 induction of DNL. The fatty acid elongase of very-long chain fatty acid 6 (ELOVL6)
5 shows the highest upregulation among DNL enzymes¹⁰³. ELOVL6, together with
6 stearoyl desaturase, promotes an increase in phospholipid oleic acid content, which
7 increases plasma membrane fluidity and enhances insulin signalling¹⁰³. In adipocytes,
8 ELOVL6 is the main transcriptional target of the glucose-responsive transcription factor
9 ChREBP (encoded by *MLXIPL*). Mechanistically, HSL physically interacts with
10 ChREBP α , thereby impairing ChREBP α translocation to the nucleus and blocking
11 ChREBP α -mediated induction of the transcriptionally highly active isoform ChREBP β
12 (which is produced from an alternative transcription start site in *MLXIPL*)¹⁰³. The
13 expression of ChREBP β in WAT is strongly associated with whole-body insulin
14 sensitivity¹⁰¹⁻¹⁰³. Glucose metabolism is also linked to the metabolism of the branched-
15 chain amino acids (BCAA) leucine, isoleucine and valine. In contrast to the beneficial
16 effects of BCAAs on protein synthesis in conditions such as ageing or cachexia,
17 elevated blood levels of BCAAs are associated with obesity, insulin resistance, T2DM
18 and cardiovascular diseases in humans^{144,145}. Mendelian randomization analysis in a
19 large number of individuals is consistent with a causal role of BCAA metabolism in the
20 aetiology of T2DM¹⁴⁶. The increased circulating BCAA levels are related in part to
21 decreased oxidation in WAT owing to suppressed expression of catabolic
22 enzymes^{147,148}. Protein catabolism may provide BCAAs to support mitochondrial
23 metabolism and DNL. *In vitro*, catabolized BCAAs can account for up to one third of
24 the lipogenic acetyl-CoA pool in mouse and human white adipocytes¹⁴⁹. Moreover, an
25 unexpected link between mitochondrial BCAA catabolism and DNL has been identified.

1 In adipose tissue, enzyme promiscuity of fatty acid synthase and carnitine
2 acetyltransferase supports the synthesis of monomethyl branched-chain fatty acids
3 from BCAAs, which are incorporated into TAG¹⁵⁰. The physiological conditions in which
4 this pathway is important have not yet been established. Branched-chain are mobilized
5 during fasting and their turnover is decreased with a high-fat diet, although their role
6 and importance in these conditions is unknown. Conversely, mice with enhanced
7 glucose transport in adipocytes show decreased expression of BCAA-metabolizing
8 enzymes in WAT¹⁵¹. How glucose metabolism regulates BCAA enzyme expression
9 has not yet been described. These studies describe a few examples of the interplay
10 between glucose, fatty acid and amino acid metabolism in the adipocyte. Advanced
11 systems-biology approaches aimed at building genome-scale metabolic models of the
12 adipocyte may help us to better understand the crosstalk between metabolic
13 pathways^{152,153}.

14

15 **Adipocyte metabolic dysfunction**

16 ***Adipocyte lipid droplet disorders***

17 An excess or a lack of WAT may cause similar pathological conditions. In both
18 situations, inadequate storage in subcutaneous WAT favours lipid spill-over to other
19 depots and organs, such as visceral fat, liver, skeletal muscle and pancreatic β -
20 cells^{154,155}. The resulting lipid toxicity leads to altered metabolic function in these organs
21 and subsequently causes an adverse cardiometabolic phenotype. In this respect,
22 inherited lipodystrophies are valuable models of impaired adipocyte metabolism with
23 clinical relevance. Disease-associated variants in *AGPAT2* and *CAVIN1* cause forms
24 of congenital generalized lipodystrophies, which are rare autosomal recessive
25 disorders characterized by a near complete lack of adipose tissue^{156,157} (FIG. 3 and 4).

1 Mutations in the genes encoding the three lipid-droplet-associated proteins PLIN1, HSL
2 and CIDEA cause forms of familial partial lipodystrophies, which are autosomal
3 recessive or autosomal dominant disorders characterized by varying degrees of body-
4 fat loss in different fat depots^{76,158,159}. Broadly speaking, the extent of fat loss governs
5 the severity of complications, such as insulin resistance, dyslipidaemia, hepatic
6 steatosis and polycystic ovary syndrome. In the general population, there is genetic
7 evidence that a limited capacity of peripheral WAT to store surplus energy is implicated
8 in human insulin resistance¹⁶⁰ (BOX 2), suggesting that common genetic variation
9 influences cardiometabolic disease risk through lipodystrophy-like mechanisms. Of
10 note, the inability to form or expand fat depots may occur despite an increase in
11 adipocyte size. Collectively, studies in humans and transgenic mouse models reveal
12 that the metabolic dysfunction in lipodystrophies and obesity are similar^{24,155}.

13

14 ***Hepatic glucose production***

15 Compelling evidence exists that dysfunction of fatty acid metabolism in
16 adipocytes has a systemic impact. The basal rate of lipolysis is positively associated
17 with insulin resistance, independently of body mass index and age¹⁴³. In prospective
18 cohorts, high basal and low stimulated lipolysis at baseline predict later development
19 of insulin resistance¹⁶¹. Evidence for the contribution of adipocyte lipolysis to insulin
20 resistance also comes from lipase-deficient mouse models and patients with *PLIN1*-
21 deficiency^{70,71,143,158}. An impaired insulin-mediated suppression of hepatic glucose
22 production is a prominent feature of insulin resistance (FIG. 7). Acute suppression of
23 hepatic glucose production by insulin involves insulin-induced inhibition of WAT
24 lipolysis. A reduction in fatty acid flux to the liver lowers hepatic acetyl-CoA
25 concentrations and glucose production through decreased pyruvate carboxylase

1 activity¹⁶². The antilipolytic action of insulin is impaired in rodents with insulin resistance
2 induced by a high-fat diet, thereby promoting hepatic glucose production. However,
3 several studies in rodents and dogs suggest that the direct effects of insulin on
4 hepatocytes are dominant over the contributions of extrahepatic tissues (such as
5 adipose tissue) in the control of hepatic glucose production^{163,164}. Whether the direct or
6 the indirect (that is, anti-lipolytic) effects of insulin are more important in the regulation
7 of hepatic glucose production seems to depend on the experimental context. Despite
8 a wealth of studies, several questions remain unanswered. Lipolysis-derived fatty acids
9 in hepatocytes have several fates when entering the liver. The relative contributions of
10 fatty acids to different metabolic pathways, notably TAG synthesis and fatty acid
11 oxidation, which vary according to physiological and pathological states, will modulate
12 the impact of insulin in control of hepatic glucose metabolism. The importance of
13 chronic delivery of lipolysis-derived fatty acids in obesity-associated insulin resistance
14 is not firmly established in humans. A systematic review of the literature revealed that
15 circulating levels of non-esterified fatty acids (NEFAs) in fasting conditions are poorly
16 correlated with body fat and insulin sensitivity in humans^{165,166}. The kinetics of when
17 insulin resistance occurs in the liver and WAT are also not well-characterized in
18 humans. The onset of insulin resistance in the two tissues may differ among obese
19 individuals.

20

21 ***Tumour aggressiveness and cachexia***

22 In addition to their role in diabetes and cardiovascular risk, adipocyte lipolysis
23 and white fat metabolism play a role in the development of some cancers and in cancer-
24 associated cachexia¹⁶⁷. In breast cancer, the secretory activity of tumour cells
25 promotes depletion of lipids in surrounding adipocytes, which results in a massive

1 release of fatty acids¹⁶⁸. The uptake of these fatty acids by breast cancer cells induces
2 a profound metabolic remodelling leading to enhanced tumour aggressiveness¹⁶⁸.
3 Cancer-associated cachexia is a life-threatening condition in which loss of fat mass
4 may precede the loss of lean mass^{169,170}. Increased stimulated lipolysis and increased
5 circulating NEFA and glycerol levels are observed in some patients and animal models
6 with cancer-associated cachexia^{169,171,172}. A reduced loss of body weight and skeletal
7 muscle mass has been reported in tumour-bearing ATGL-deficient mice¹⁷¹. The
8 inhibition of lipolysis and DNL in white adipocytes by blocking the interaction between
9 CIDEA and the cellular energy homeostasis regulator AMP kinase partially protects
10 against loss of fat mass and prolongs the resistance of tumour-bearing mice to
11 cachexia¹⁷². One can hypothesize that increased production of fatty acids favours the
12 accumulation of lipids and lipotoxic species in skeletal muscle, which participate in the
13 development of muscular atrophy^{173,174}.

14

15 ***Effects of fatty acids on macrophages***

16 White adipocyte-derived fatty acids have emerged as important modulators of
17 macrophage metabolism. In conditions of chronic activation of lipolysis, fatty acids
18 released from adipocytes are taken up by macrophages, leading to lipid accumulation
19 in these immune cells^{175,176}. This fatty acid-scavenging role is reminiscent of foam cell
20 accumulation of cholesteryl esters in atherosclerotic plaques. In mouse models, obesity
21 is associated with an accumulation of lipid droplets in macrophages and activation of
22 lysosome biogenesis, resulting in TAG catabolism¹⁷⁷. Alternatively, as mentioned
23 above, these lipids in adipose tissue macrophages may originate from lipid-droplet-
24 derived exosome-like vesicles released by adipocytes⁹⁹. Therefore, the net release of
25 fatty acids from WAT could be controlled concomitantly by adipocyte lipid mobilization

1 and adipose tissue macrophage lysosomal activity. The importance of adipocytes and
2 macrophages in WAT fatty acid release in humans is currently unknown. Whether
3 these buffering mechanisms substantially affect the circulating levels of fatty acids that
4 are available for storage as ectopic lipids in other organs, and thus mitigate the
5 detrimental effect of exacerbated WAT lipolysis, remains to be determined.

6 Earlier studies suggested that fatty acids released from adipocytes may have an
7 impact on other adipose cell types and thereby have an indirect systemic effect. The
8 crosstalk between adipocytes and other cell types in WAT contributes to modulation of
9 the immune response and fibrosis¹⁷⁸ (BOX1, FIG. 7). This tissue remodelling alters the
10 secretory profile of stromavascular cells, which produce molecules that may have
11 endocrine actions¹⁵. Hypertrophic adipocytes under severe metabolic stress are prone
12 to pyroptosis, a pro-inflammatory form of programmed cell death¹⁷⁹. The increased
13 number of dying adipocytes in obese WAT provokes recruitment of macrophages,
14 forming crown-like structures^{180,181}. The recruited macrophages show a pro-
15 inflammatory M1-like phenotype and produce an array of cytokines and chemokines.
16 The pathogenetic role of so-called low-grade inflammation in adipose tissue in the
17 development of obesity and insulin resistance is well-documented¹⁸². However, the
18 relative importance of the pro-inflammatory M1-like phenotype and lipid trafficking in
19 WAT macrophages during obesity and fasting in humans and mice is unclear and may
20 vary according to the anatomical location of fat depots. Notwithstanding these features
21 of adipose tissue macrophages, the studies summarized here indicate that adipocyte
22 metabolism may be the main driver of the immune response in WAT. Accordingly,
23 transcriptomic analysis of WAT from women with different degrees of obesity and
24 metabolic impairment showed a tight inverse correlation between the expression of
25 adipocyte genes involved in lipid and glucose metabolism and of macrophage genes

1 involved in the immune response, both in subcutaneous and in visceral fat depots¹⁴⁷.
2 Of note, the induction of insulin resistance in mouse white adipocytes induces a
3 macrophage pro-inflammatory response¹⁸³. Adipose tissue inflammation may therefore
4 be considered a local adaptation to primary dysfunction in adipocyte metabolism.
5 Different metabolic impairments in the adipocyte may be envisaged to have different
6 local consequences. This viewpoint may help to reconcile apparently contradictory
7 findings, such as the possible occurrence of insulin resistance in the absence of WAT
8 inflammation, as observed in *Cidec*-null mice fed a high-fat diet¹⁸⁴.

9

10 ***Bioactive lipid and lipocalin secretion***

11 In addition to the production of fatty acids, adipocytes can modulate systemic
12 insulin sensitivity through the secretion of other bioactive lipid products (FIG. 7). The
13 monounsaturated fatty acid (MUFA) palmitoleate (C16:1n-7) is the second most
14 abundant MUFA in human blood and adipose tissue¹⁸⁵. Palmitoleate was identified as
15 an adipocyte-specific, DNL-derived fatty acid with insulin-sensitizing properties in
16 mice¹⁸⁶. However, the positive association between circulating levels of palmitoleate
17 and insulin sensitivity in humans is debated¹⁸⁷. Differences in synthesis between the
18 main sites of production of circulating palmitoleate, WAT and liver, as well as
19 differences in DNL between rodents and humans may explain some of the
20 discrepancies⁵⁹. Nevertheless, a recent longitudinal study of a large cohort of non-
21 diabetic individuals showed, after adjustment for potential confounders, notably NEFA,
22 that circulating palmitoleate is an independent determinant of insulin sensitivity¹⁸⁸.
23 Large-scale intervention studies are now warranted to establish the causal role of
24 palmitoleate in preserving insulin sensitivity. A new class of fatty acids, fatty acid-
25 hydroxyl-fatty acids (FAHFAs), has been identified in WAT and serum of mice

1 overexpressing GLUT4 in adipocytes¹⁸⁹. FAHFAs can be stored in adipocyte TAGs
2 and mobilized through lipolysis¹⁹⁰. In humans, levels of FAHFAs composed of palmitic
3 and stearic acids have been reported to be lower in serum and adipocytes of insulin-
4 resistant individuals^{189,191}. FAHFAs exert a beneficial effect on insulin sensitivity,
5 through the promotion of insulin secretion, an increase in adipocyte glucose uptake
6 and the inhibition of WAT inflammation and hepatic glucose production^{189,192,193}. The
7 pharmacological administration of FAHFAs in mice has yielded conflicting data on
8 insulin sensitivity^{189,192,194}. There are methodological issues in studying this class of
9 lipids that require cross-validation between laboratories^{194,195}. In WAT, FAHFA levels
10 are under the control of ChREBP¹⁹⁶. The threonine hydrolases AIG1 and ATRP have
11 been shown to participate in the degradation of FAHFAs but the enzymes responsible
12 for their synthesis are still unknown¹⁹⁷. The combined deficiency of these two threonine
13 hydrolases resulted in increased FAHFA levels in WAT but not in plasma and does not
14 restore insulin sensitivity in mice fed a high fat diet. An inhibitor of the threonine
15 hydrolases that can be administered in vivo has been synthesized¹⁹⁷. Whether chronic
16 treatment with this inhibitor would improve insulin sensitivity in rodent models of insulin
17 resistance needs to be assessed. In brown and beige fat, an oxidized metabolite of
18 linoleic acid, 12,13-diHOME, is produced during cold exposure and promotes
19 thermogenesis by increasing fatty acid uptake in adipocytes¹⁹⁸. Plasma levels of 12,13-
20 diHOME are negatively associated with body mass index and insulin resistance in
21 different cohorts of individuals with various degrees of fat mass and glucose
22 tolerance^{198,199}. In addition to a paracrine effect on BAT, increased secretion of 12,13-
23 diHOME in response to exercise promotes fatty acid uptake in skeletal muscle^{198,200}.
24 Ceramides are potential lipid mediators of insulin resistance²⁰¹. In adipocytes, inhibiting
25 the synthesis or activating the degradation of ceramide leads to systemic improvement

1 in metabolic parameters, notably by reducing adipose tissue inflammation and liver
2 steatosis²⁰¹⁻²⁰³. Of note, the modulation of ceramide metabolism has similar effects in
3 the liver and there is efficient exchange of these lipid species between WAT and liver
4 to maintain metabolic homeostasis.

5 Adipocytes also secrete a wide range of lipocalins, which transport fatty acids
6 and other lipid species. A prototypical example is the fatty acid-binding protein
7 FABP4¹⁵, which plays a part in intracellular lipolysis but is also secreted through a non-
8 classical pathway when lipolysis is stimulated²⁰⁴. Circulating FABP4 activates
9 gluconeogenesis and stimulates hepatic glucose production, favouring the
10 development of diabetes in obese mice²⁰⁵. The retinol-binding protein RBP4 is another
11 example of a lipocalin that deleteriously affects insulin sensitivity^{15,206}. The expression
12 of RBP4 is elevated in mice with defective adipose tissue glucose transport²⁰⁷. RBP4
13 contributes to the development of insulin resistance through both metabolic and
14 inflammatory effects^{207,208}.

15

16 **Therapeutic targeting of WAT metabolism**

17 Drugs that act on WAT metabolism can be effective at treating T2DM, even in
18 the absence of body weight lowering (FIG. 7). Thiazolidinediones (TZDs) provide a
19 proof of principle: this class of drug, which comprises rosiglitazone and pioglitazone,
20 acts on PPAR γ , a nuclear factor that is essential for adipogenesis. Whereas high-
21 affinity synthetic agonists such as TZDs are potent adipogenesis activators, the identity
22 of endogenous PPAR γ ligands is an old yet unresolved question in the field. Based on
23 the nature of the ligands in this class of nuclear receptors, PPAR γ ligands are predicted
24 to be lipids or their derivatives, with eicosanoids and fatty acid metabolites proposed
25 as natural ligands²⁰⁹. TZDs promote lipid storage in WAT, improve the secretory profile

1 of adipocytes and decrease WAT inflammation, resulting overall in a robust insulin
2 sensitization²¹⁰. Despite a safety profile that precludes the widespread use of TZDs,
3 there is substantial evidence for the beneficial effects of TZDs beyond plasma glucose
4 lowering and insulin sensitization, notably on atherosclerosis, cardiovascular events
5 and nonalcoholic steatohepatitis^{211,212}. Together, these studies show that drugs acting
6 on WAT have the potential to treat diabetes and to decrease the risk of cardiometabolic
7 diseases.

8 Controlling fatty acid release from WAT is an attractive avenue to achieve insulin
9 sensitization. In mice, the chronic inhibition of lipolysis using selective inhibitors of HSL
10 or ATGL results in improvement in insulin sensitivity^{143,213}. Agonists of anti-lipolytic G_i-
11 coupled receptors are postulated to have similar effects. One such drug is nicotinic
12 acid, which acts through HCAR2 (also known as GPR109A), resulting in acute
13 reduction in NEFA levels⁶⁶ (FIG. 4). However, HCAR2-independent mechanisms also
14 contribute to the chronic lipid-lowering effects observed with nicotinic acid
15 treatment^{214,215}. The worsening, rather than expected improvement, of glycaemic
16 control observed during chronic nicotinic acid treatment may be due to the
17 development of tolerance that occurs with prolonged nicotinic acid treatment and/or to
18 a major rebound in NEFA levels observed during rapid nicotinic acid washout²¹⁶. An
19 intermittent dosing strategy is successful in retaining the ability of nicotinic acid to lower
20 NEFA levels and improves insulin sensitivity^{217,218}. A well-defined nicotinic acid
21 exposure, timed to feeding periods, profoundly improves metabolic profile in obese
22 Zucker rats. Inhibiting lipolysis via other receptors may be able to circumvent the
23 problems of tolerance and NEFA rebound observed with HCAR2 agonists.

24 HCAR1 (also known as GPR81) is, like HCAR2, an anti-lipolytic G_i-coupled
25 receptor⁶⁶. Chronic dosing with HCAR1 agonists in obese and insulin-resistant mice

1 leads to robust insulin-sensitizing and antidiabetic effects in the absence of body weight
2 changes²¹⁹. However, an unexpected hypertensive effect is observed owing to
3 activation of HCAR1 in the microvasculature of the kidney, which precluded further
4 testing in humans²¹⁹. Nevertheless, these results show that the inhibition of lipolysis
5 holds promise for improving insulin sensitivity. The inhibition of adipocyte lipolysis could
6 also counteract the development of cancer-associated cachexia, as convincingly
7 shown in mice¹⁶⁹.

8 Conversely, activating lipolysis coupled to fatty acid utilization is another
9 strategy to modulate blood glucose levels and insulin sensitivity. The mobilization of
10 fatty acids from WAT is crucial in providing substrates to promote energy expenditure.
11 Such a link is probably altered during ageing, which is associated with a decline in
12 various components of energy expenditure²²⁰. A recent longitudinal study in women
13 revealed an age-related decrease in catecholamine-induced lipolysis in subcutaneous
14 WAT²²¹. The stimulation of adipocyte lipolysis may be considered as a therapeutic
15 approach only if fatty acid utilization is not rate-limiting as, otherwise, fatty acids are
16 likely to be deposited in non-adipose tissues and contribute to a worsening of insulin
17 resistance. In this regard, caution should be exercised when comparing the effects of
18 lipolysis activation in humans and rodents, as mice and rats have larger amounts of
19 active BAT and a higher capacity to oxidize lipids²²². Combined with the interspecific
20 differences in tissue distribution of the β_3 adrenoceptor among fat depots, these
21 differences explain the much greater beneficial effects of β_3 adrenergic agonists in
22 rodents than in humans.

23 Targeting adipose tissue also has the potential to achieve a safe increase in
24 energy expenditure by increasing thermogenesis through either browning of WAT or
25 acting on substrate cycles and UCP1-independent thermogenic processes in WAT²²³

1 (FIG. 5). However, a negative energy balance seems to be a prerequisite for weight
2 reduction; increasing fatty acid oxidation alone has little impact on overall adiposity and
3 body weight²²⁴. Moreover, the relative contribution of different targetable thermogenic
4 pathways in various fat depots to increased energy expenditure is still not firmly
5 established in adult humans. Other recently identified pathways in white adipocytes
6 may be of interest. For example, activating white adipocyte DNL may be beneficial
7 given the strong positive association between this pathway and insulin sensitivity in
8 humans^{100,102}. However, activating DNL in the liver is generally considered to be
9 detrimental, as hepatic DNL is increased during the development of fatty liver
10 disease⁵⁹. As HSL is expressed at very low levels in the liver, disrupting the interaction
11 between HSL and ChREBP may constitute an adipocyte-specific mechanism to
12 enhance DNL and insulin signalling¹⁰³.

13 As illustrated with the use of TZDs and HCAR2 and HCAR1 agonists, safety
14 concerns can derail clinical development of molecules targeting WAT metabolism. The
15 development of more targeted drugs or restricting the action of a drug to an intended
16 tissue or cell type may avoid off-target effects in the future. Genes with tissue-specific
17 expression are enriched among targets of marketed non-oncology drugs²²⁵ but such
18 an enrichment is not found among drugs in early-phase clinical trials. For novel
19 therapeutic targets under consideration, priority should therefore be given to those
20 drugs that target genes that are highly or exclusively expressed in adipocytes.

21

22 **Conclusions and future perspectives**

23 An outstanding issue in adipose research is WAT heterogeneity, which may
24 comprise a minimum of four levels. First, WAT is distributed among many different fat
25 depots that differ in their anatomical location and function^{226,227}. Subcutaneous and

1 visceral adipose depots are generally considered to have opposite roles in the
2 development of insulin resistance and diabetes. However, a full parallel metabolic
3 characterization of adipocytes in these two depots is still lacking in humans. The
4 contribution of adipocytes in smaller depots, such as bone marrow, perivascular,
5 mammary, epicardial, joint, dermal, retro-orbital and plantar WAT, to overall
6 metabolism and organ function is not yet resolved. Second, sex differences in WAT
7 exist, and dynamic changes occur in WAT over a lifetime²²⁸⁻²³⁰. Ageing-related and
8 sex-specific physiological states, such as pregnancy, lactation and menopause, are
9 accompanied by changes in adipocyte metabolism, which are not well characterized.
10 Regarding these two layers of heterogeneity, differences between mice and humans
11 require that extrapolating insights from mouse studies to humans must be done with
12 extreme caution.

13 Each fat depot contains many different cell types in the stroma-vascular fraction,
14 conferring a third layer of heterogeneity (BOX 1). The extent of immune cell infiltration,
15 vascularization and innervation differs greatly between the depots^{226,227}. Adipocytes
16 themselves come in different colours, that is, white, brown and beige, which are
17 associated with different intrinsic properties; the recently recognized diversity among
18 each category of adipocytes within a fat depot represents a fourth layer of
19 heterogeneity. Several populations of white adipocytes with unique metabolic
20 properties and differential responses to exogenous stimuli have been
21 characterized^{113,114}. A subset of human adipocytes lacking the lipolytic β_2 -adrenoceptor
22 has recently been shown to be enriched in subcutaneous WAT of metabolically
23 impaired individuals with obesity²³¹. Similarly, not all beige adipocytes share similar
24 metabolic features. A population with high glucose uptake and oxidation capacity has
25 been identified in mice lacking β -adrenergic signalling²³². Whether this specific

1 population exists in substantial amounts in human fat depots is unknown. Single-
2 nucleus RNA sequencing brought new information on adipocyte heterogeneity in
3 mouse and human WAT. A subpopulation of acetate-producing adipocytes decreases
4 the thermogenic capacity of neighbouring adipocytes²³³. As this subpopulation is more
5 represented in human than in mouse adipose tissue, it may contribute to the lower
6 energy dissipation capacity of human adipose tissue.

7 Recognition of the importance of rhythmic processes and metabolic flexibility is
8 increasing. Besides oscillations in hormones, temperature and feeding behaviour,
9 endogenous circadian clocks found in metabolic tissues ensure proper rhythmicity of
10 metabolism²³⁴. WAT itself is subject to large variations in gene expression that follow
11 circadian patterns, both in mice and humans. Disruptions in these rhythms, which
12 cause physiological processes to be out of alignment with internal clocks, contribute to
13 insulin resistance²³⁵. Furthermore, obesity and insulin resistance are associated with a
14 state of metabolic inflexibility, that is, the inability to switch between carbohydrate and
15 lipid utilization during the fed and fasted states, respectively²³⁶. However, chronically
16 forcing the utilization of a particular energy substrate is contrary to normal physiological
17 processes. Drug and food administration to restricted and specific time periods may
18 avoid some of the deleterious consequences observed with the constant chronic
19 therapeutic manipulation of metabolic pathways^{217,237}.

20 Pharmacotherapy is rarely equally effective in all treated patients. This is the
21 case for TZDs, where a substantial fraction of patients with T2DM do not show
22 improvement in insulin sensitivity with treatment²³⁸. T2DM is a highly heterogeneous
23 disease: cluster analysis based on six simple variables identified five subgroups of
24 patients with T2DM that differed in disease progression and risk of diabetic
25 complications^{239,240}. Recent studies indicate the importance of WAT function for the

1 development of whole-body insulin resistance and diabetes in obese individuals.
2 Characterizing the extent of alterations in adipocyte metabolism may allow refined
3 patient stratification and help in identifying individuals who could benefit the most from
4 existing and future drugs in both metabolic diseases and cancer^{161,241}.

5 White adipocytes are definitely much more than inanimate fat-laden entities. We
6 believe that targeting WAT holds promise for the treatment of cardiometabolic diseases
7 and other conditions with dysregulation of adipocyte metabolism. Future novel
8 adipocyte-based strategies for the treatment of metabolic diseases may include the
9 conversion of energy-storing white adipocytes into energy-consuming brown-like
10 adipocytes, exploiting both UCP1-dependent and UCP1-independent mechanisms to
11 increase energy expenditure, promoting adipocyte lipid storage and oxidation, and
12 time-dependent activation of glucose and lipid utilization to restore metabolic flexibility.
13 Combinatorial approaches with other pharmacological agents that reduce food intake
14 or increase energy expenditure may be required to promote a catabolic state and fully
15 harness the potential of adipocyte-based therapies.

1 **References**

- 2 1 Pond, C. M. An evolutionary and functional view of mammalian adipose tissue.
3 *Proc Nutr Soc* **51**, 367-377 (1992).
- 4 2 Thiam, A. R. & Beller, M. The why, when and how of lipid droplet diversity. *J*
5 *Cell Sci* **130**, 315-324 (2017).
- 6 3 Rodbell, M. Metabolism of Isolated Fat Cells. I. Effects of Hormones on Glucose
7 Metabolism and Lipolysis. *J Biol Chem* **239**, 375-380 (1964).
- 8 4 Czech, M. P. Cellular basis of insulin insensitivity in large rat adipocytes. *J Clin*
9 *Invest* **57**, 1523-1532 (1976).
- 10 5 Cushman, S. W. & Wardzala, L. J. Potential mechanism of insulin action on
11 glucose transport in the isolated rat adipose cell. Apparent translocation of
12 intracellular transport systems to the plasma membrane. *J Biol Chem* **255**,
13 4758-4762 (1980).
- 14 6 Suzuki, K. & Kono, T. Evidence that insulin causes translocation of glucose
15 transport activity to the plasma membrane from an intracellular storage site.
16 *Proc Natl Acad Sci U S A* **77**, 2542-2545 (1980).
- 17 7 Hotamisligil, G. S., Shargill, N. S. & Spiegelman, B. M. Adipose expression of
18 tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance.
19 *Science* **259**, 87-91 (1993).
- 20 8 Hu, E., Liang, P. & Spiegelman, B. M. AdipoQ is a novel adipose-specific gene
21 dysregulated in obesity. *J Biol Chem* **271**, 10697-10703 (1996).
- 22 9 Maeda, K. *et al.* cDNA cloning and expression of a novel adipose specific
23 collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem*
24 *Biophys Res Commun* **221**, 286-289 (1996).

- 1 10 Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. A novel
2 serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem*
3 **270**, 26746-26749 (1995).
- 4 11 Zhang, Y. *et al.* Positional cloning of the mouse obese gene and its human
5 homologue. *Nature* **372**, 425-432 (1994).
- 6 12 Lafontan, M. Historical perspectives in fat cell biology: the fat cell as a model for
7 the investigation of hormonal and metabolic pathways. *Am J Physiol Cell*
8 *Physiol* **302**, C327-359 (2012).
- 9 13 Guilherme, A., Henriques, F., Bedard, A. H. & Czech, M. P. Molecular pathways
10 linking adipose innervation to insulin action in obesity and diabetes mellitus. *Nat*
11 *Rev Endocrinol* **15**, 207-225 (2019).
- 12 14 Chouchani, E. T. & Kajimura, S. Metabolic adaptation and maladaptation in
13 adipose tissue. *Nat. Metab.* **1**, 189–200 (2019).
- 14 15 Scheja, L. & Heeren, J. The endocrine function of adipose tissues in health and
15 cardiometabolic disease. *Nat Rev Endocrinol* **15**, 507-524 (2019).
- 16 16 Vishvanath, L. & Gupta, R. K. Contribution of adipogenesis to healthy adipose
17 tissue expansion in obesity. *J Clin Invest* **129**, 4022-4031 (2019).
- 18 17 Ghaben, A. L. & Scherer, P. E. Adipogenesis and metabolic health. *Nat Rev Mol*
19 *Cell Biol* **20**, 242-258 (2019).
- 20 18 Stenkula, K. G. & Erlanson-Albertsson, C. Adipose cell size: importance in
21 health and disease. *Am J Physiol Regul Integr Comp Physiol* **315**, R284-R295
22 (2018).
- 23 19 Engfeldt, P. & Arner, P. Lipolysis in human adipocytes, effects of cell size, age
24 and of regional differences. *Horm Metab Res Suppl* **19**, 26-29 (1988).

- 1 20 Laforest, S., Labrecque, J., Michaud, A., Cianflone, K. & Tchernof, A. Adipocyte
2 size as a determinant of metabolic disease and adipose tissue dysfunction. *Crit*
3 *Rev Clin Lab Sci* **52**, 301-313 (2015).
- 4 21 Pausova, Z. From big fat cells to high blood pressure: a pathway to obesity-
5 associated hypertension. *Curr Opin Nephrol Hypertens* **15**, 173-178 (2006).
- 6 22 Arner, P. & Spalding, K. L. Fat cell turnover in humans. *Biochem Biophys Res*
7 *Commun* **396**, 101-104 (2010).
- 8 23 Tandon, P., Wafer, R. & Minchin, J. E. N. Adipose morphology and metabolic
9 disease. *J Exp Biol* **221** (2018).
- 10 24 Rutkowski, J. M., Stern, J. H. & Scherer, P. E. The cell biology of fat expansion.
11 *J Cell Biol* **208**, 501-512 (2015).
- 12 25 Berry, R., Jeffery, E. & Rodeheffer, M. S. Weighing in on adipocyte precursors.
13 *Cell Metab* **19**, 8-20 (2014).
- 14 26 Christodoulides, C., Lagathu, C., Sethi, J. K. & Vidal-Puig, A. Adipogenesis and
15 WNT signalling. *Trends Endocrinol Metab* **20**, 16-24 (2009).
- 16 27 Ma, X., Wang, D., Zhao, W. & Xu, L. Deciphering the Roles of PPARgamma in
17 Adipocytes via Dynamic Change of Transcription Complex. *Front Endocrinol*
18 *(Lausanne)* **9**, 473 (2018).
- 19 28 Shan, T., Liu, J., Wu, W., Xu, Z. & Wang, Y. Roles of Notch Signaling in
20 Adipocyte Progenitor Cells and Mature Adipocytes. *J Cell Physiol* **232**, 1258-
21 1261 (2017).
- 22 29 Fernando, R. *et al.* Low steady-state oxidative stress inhibits adipogenesis by
23 altering mitochondrial dynamics and decreasing cellular respiration. *Redox Biol*
24 **32**, 101507 (2020).

- 1 30 Wang, S. *et al.* Adipocyte Piezo1 mediates obesogenic adipogenesis through
2 the FGF1/FGFR1 signaling pathway in mice. *Nat Commun* **11**, 2303 (2020).
- 3 31 Sakaguchi, M. *et al.* Adipocyte Dynamics and Reversible Metabolic Syndrome
4 in Mice with an Inducible Adipocyte-Specific Deletion of the Insulin Receptor.
5 *Cell Metab* **25**, 448-462 (2017).
- 6 32 Wang, Q. A. *et al.* Reversible De-differentiation of Mature White Adipocytes into
7 Preadipocyte-like Precursors during Lactation. *Cell Metab* **28**, 282-288 e283
8 (2018).
- 9 33 Sebo, Z. L. & Rodeheffer, M. S. Assembling the adipose organ: adipocyte
10 lineage segregation and adipogenesis in vivo. *Development* **146** (2019).
- 11 34 Raajendiran, A. *et al.* Identification of Metabolically Distinct Adipocyte
12 Progenitor Cells in Human Adipose Tissues. *Cell Rep* **27**, 1528-1540 e1527
13 (2019).
- 14 35 Gavin, K. M. *et al.* De novo generation of adipocytes from circulating progenitor
15 cells in mouse and human adipose tissue. *FASEB J* **30**, 1096-1108 (2016).
- 16 36 Ryden, M., Andersson, D. P., Bernard, S., Spalding, K. & Arner, P. Adipocyte
17 triglyceride turnover and lipolysis in lean and overweight subjects. *J Lipid Res*
18 **54**, 2909-2913 (2013).
- 19 37 Walker, G. E., Marzullo, P., Ricotti, R., Bona, G. & Prodam, F. The
20 pathophysiology of abdominal adipose tissue depots in health and disease.
21 *Horm Mol Biol Clin Investig* **19**, 57-74 (2014).
- 22 38 Hoffstedt, J. *et al.* Regional impact of adipose tissue morphology on the
23 metabolic profile in morbid obesity. *Diabetologia* **53**, 2496-2503 (2010).

1 39 Veilleux, A., Caron-Jobin, M., Noel, S., Laberge, P. Y. & Tchernof, A. Visceral
2 adipocyte hypertrophy is associated with dyslipidemia independent of body
3 composition and fat distribution in women. *Diabetes* **60**, 1504-1511 (2011).

4 40 Verboven, K. *et al.* Abdominal subcutaneous and visceral adipocyte size,
5 lipolysis and inflammation relate to insulin resistance in male obese humans.
6 *Sci Rep* **8**, 4677 (2018).

7 41 Lonn, M., Mehlig, K., Bengtsson, C. & Lissner, L. Adipocyte size predicts
8 incidence of type 2 diabetes in women. *FASEB J* **24**, 326-331 (2010).

9 42 Weyer, C., Foley, J. E., Bogardus, C., Tataranni, P. A. & Pratley, R. E. Enlarged
10 subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II
11 diabetes independent of insulin resistance. *Diabetologia* **43**, 1498-1506 (2000).

12 43 White, U. & Ravussin, E. Dynamics of adipose tissue turnover in human
13 metabolic health and disease. *Diabetologia* **62**, 17-23 (2019).

14 44 Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H. & Frisen, J.
15 Retrospective birth dating of cells in humans. *Cell* **122**, 133-143 (2005).

16 45 Spalding, K. L. *et al.* Dynamics of fat cell turnover in humans. *Nature* **453**, 783-
17 787 (2008).

18 46 Arner, E. *et al.* Adipocyte turnover: relevance to human adipose tissue
19 morphology. *Diabetes* **59**, 105-109 (2010).

20 47 Arner, P. *et al.* Dynamics of human adipose lipid turnover in health and
21 metabolic disease. *Nature* **478**, 110-113 (2011).

22 48 Guillermier, C. *et al.* Imaging mass spectrometry demonstrates age-related
23 decline in human adipose plasticity. *JCI Insight* **2**, e90349 (2017).

24 49 Spalding, K. L. *et al.* Impact of fat mass and distribution on lipid turnover in
25 human adipose tissue. *Nat Commun* **8**, 15253 (2017).

1 50 Ibrahim, M. M. Subcutaneous and visceral adipose tissue: structural and
2 functional differences. *Obes Rev* **11**, 11-18 (2010).

3 51 Lee, M. J., Wu, Y. & Fried, S. K. Adipose tissue heterogeneity: implication of
4 depot differences in adipose tissue for obesity complications. *Mol Aspects Med*
5 **34**, 1-11 (2013).

6 52 Arner, P. *et al.* Adipose lipid turnover and long-term changes in body weight.
7 *Nat Med* **25**, 1385-1389 (2019).

8 53 Kersten, S. Physiological regulation of lipoprotein lipase. *Biochim Biophys Acta*
9 **1841**, 919-933 (2014).

10 54 Thompson, B. R., Lobo, S. & Bernlohr, D. A. Fatty acid flux in adipocytes: the
11 in's and out's of fat cell lipid trafficking. *Mol Cell Endocrinol* **318**, 24-33 (2010).

12 55 Coleman, R. A. & Mashek, D. G. Mammalian triacylglycerol metabolism:
13 synthesis, lipolysis, and signaling. *Chem Rev* **111**, 6359-6386 (2011).

14 56 Coleman, R. A. It takes a village: channeling fatty acid metabolism and
15 triacylglycerol formation via protein interactomes. *J Lipid Res* **60**, 490-497
16 (2019).

17 57 Chitraju, C., Walther, T. C. & Farese, R. V., Jr. The triglyceride synthesis
18 enzymes DGAT1 and DGAT2 have distinct and overlapping functions in
19 adipocytes. *J Lipid Res* **60**, 1112-1120 (2019).

20 58 Chitraju, C. *et al.* Triglyceride Synthesis by DGAT1 Protects Adipocytes from
21 Lipid-Induced ER Stress during Lipolysis. *Cell Metab* **26**, 407-418 e403 (2017).

22 59 Solinas, G., Boren, J. & Dulloo, A. G. De novo lipogenesis in metabolic
23 homeostasis: More friend than foe? *Mol Metab* **4**, 367-377 (2015).

24 60 Wallace, M. & Metallo, C. M. Tracing insights into de novo lipogenesis in liver
25 and adipose tissues. *Semin Cell Dev Biol* (2020).

1 61 Zhao, S. *et al.* ATP-Citrate Lyase Controls a Glucose-to-Acetate Metabolic
2 Switch. *Cell Rep* **17**, 1037-1052 (2016).

3 62 Guillou, H., Zadavec, D., Martin, P. G. & Jacobsson, A. The key roles of
4 elongases and desaturases in mammalian fatty acid metabolism: Insights from
5 transgenic mice. *Prog Lipid Res* **49**, 186-199 (2010).

6 63 Aarsland, A., Chinkes, D. & Wolfe, R. R. Hepatic and whole-body fat synthesis
7 in humans during carbohydrate overfeeding. *Am J Clin Nutr* **65**, 1774-1782
8 (1997).

9 64 Diraison, F. *et al.* Differences in the regulation of adipose tissue and liver
10 lipogenesis by carbohydrates in humans. *J Lipid Res* **44**, 846-853 (2003).

11 65 Smith, G. I. *et al.* Insulin resistance drives hepatic de novo lipogenesis in
12 nonalcoholic fatty liver disease. *J Clin Invest* (2019).

13 66 Lafontan, M. & Langin, D. Lipolysis and lipid mobilization in human adipose
14 tissue. *Prog Lipid Res* **48**, 275-297 (2009).

15 67 Morigny, P., Houssier, M., Mouisel, E. & Langin, D. Adipocyte lipolysis and
16 insulin resistance. *Biochimie* **125**, 259-266 (2016).

17 68 Langin, D. & Arner, P. Importance of TNFalpha and neutral lipases in human
18 adipose tissue lipolysis. *Trends Endocrinol Metab* **17**, 314-320 (2006).

19 69 Haemmerle, G. *et al.* Defective lipolysis and altered energy metabolism in mice
20 lacking adipose triglyceride lipase. *Science* **312**, 734-737 (2006).

21 70 Ahmadian, M. *et al.* Desnutrin/ATGL is regulated by AMPK and is required for
22 a brown adipose phenotype. *Cell Metab* **13**, 739-748 (2011).

23 71 Schoiswohl, G. *et al.* Impact of Reduced ATGL-Mediated Adipocyte Lipolysis on
24 Obesity-Associated Insulin Resistance and Inflammation in Male Mice.
25 *Endocrinology* **156**, 3610-3624 (2015).

- 1 72 Bezaire, V. *et al.* Contribution of adipose triglyceride lipase and hormone-
2 sensitive lipase to lipolysis in hMADS adipocytes. *J Biol Chem* **284**, 18282-
3 18291 (2009).
- 4 73 Fischer, J. *et al.* The gene encoding adipose triglyceride lipase (PNPLA2) is
5 mutated in neutral lipid storage disease with myopathy. *Nat Genet* **39**, 28-30
6 (2007).
- 7 74 Natali, A. *et al.* Metabolic consequences of adipose triglyceride lipase deficiency
8 in humans: an in vivo study in patients with neutral lipid storage disease with
9 myopathy. *J Clin Endocrinol Metab* **98**, E1540-1548 (2013).
- 10 75 Haemmerle, G. *et al.* Hormone-sensitive lipase deficiency in mice causes
11 diglyceride accumulation in adipose tissue, muscle, and testis. *J Biol Chem* **277**,
12 4806-4815 (2002).
- 13 76 Albert, J. S. *et al.* Null mutation in hormone-sensitive lipase gene and risk of
14 type 2 diabetes. *N Engl J Med* **370**, 2307-2315 (2014).
- 15 77 Taschler, U. *et al.* Monoglyceride lipase deficiency in mice impairs lipolysis and
16 attenuates diet-induced insulin resistance. *J Biol Chem* **286**, 17467-17477
17 (2011).
- 18 78 Lass, A. *et al.* Adipose triglyceride lipase-mediated lipolysis of cellular fat stores
19 is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. *Cell*
20 *Metab* **3**, 309-319 (2006).
- 21 79 Radner, F. P. *et al.* Growth retardation, impaired triacylglycerol catabolism,
22 hepatic steatosis, and lethal skin barrier defect in mice lacking comparative
23 gene identification-58 (CGI-58). *J Biol Chem* **285**, 7300-7311 (2010).
- 24 80 El-Assaad, W. *et al.* Deletion of the gene encoding G0/G 1 switch protein 2
25 (G0s2) alleviates high-fat-diet-induced weight gain and insulin resistance, and

1 promotes browning of white adipose tissue in mice. *Diabetologia* **58**, 149-157
2 (2015).

3 81 Yang, X. *et al.* The G(0)/G(1) switch gene 2 regulates adipose lipolysis through
4 association with adipose triglyceride lipase. *Cell Metab* **11**, 194-205 (2010).

5 82 Grahn, T. H. *et al.* Fat-specific protein 27 (FSP27) interacts with adipose
6 triglyceride lipase (ATGL) to regulate lipolysis and insulin sensitivity in human
7 adipocytes. *J Biol Chem* **289**, 12029-12039 (2014).

8 83 Nishino, N. *et al.* FSP27 contributes to efficient energy storage in murine white
9 adipocytes by promoting the formation of unilocular lipid droplets. *J Clin Invest*
10 **118**, 2808-2821 (2008).

11 84 Granneman, J. G., Moore, H. P., Krishnamoorthy, R. & Rathod, M. Perilipin
12 controls lipolysis by regulating the interactions of AB-hydrolase containing 5
13 (Abhd5) and adipose triglyceride lipase (Atgl). *J Biol Chem* **284**, 34538-34544
14 (2009).

15 85 Wang, H. *et al.* Activation of hormone-sensitive lipase requires two steps,
16 protein phosphorylation and binding to the PAT-1 domain of lipid droplet coat
17 proteins. *J Biol Chem* **284**, 32116-32125 (2009).

18 86 Shen, W. J. *et al.* Characterization of the functional interaction of adipocyte lipid-
19 binding protein with hormone-sensitive lipase. *J Biol Chem* **276**, 49443-49448
20 (2001).

21 87 Smith, A. J. *et al.* Physical association between the adipocyte fatty acid-binding
22 protein and hormone-sensitive lipase: a fluorescence resonance energy transfer
23 analysis. *J Biol Chem* **279**, 52399-52405 (2004).

- 1 88 Aboulaich, N., Ortegren, U., Vener, A. V. & Stralfors, P. Association and insulin
2 regulated translocation of hormone-sensitive lipase with PTRF. *Biochem*
3 *Biophys Res Commun* **350**, 657-661 (2006).
- 4 89 Zhou, S. R. *et al.* Acetylation of Cavin-1 Promotes Lipolysis in White Adipose
5 Tissue. *Mol Cell Biol* **37** (2017).
- 6 90 Nordstrom, E. A. *et al.* A human-specific role of cell death-inducing DFFA (DNA
7 fragmentation factor-alpha)-like effector A (CIDEA) in adipocyte lipolysis and
8 obesity. *Diabetes* **54**, 1726-1734 (2005).
- 9 91 Puri, V. *et al.* Cidea is associated with lipid droplets and insulin sensitivity in
10 humans. *Proc Natl Acad Sci U S A* **105**, 7833-7838 (2008).
- 11 92 Jash, S., Banerjee, S., Lee, M. J., Farmer, S. R. & Puri, V. CIDEA
12 Transcriptionally Regulates UCP1 for Britening and Thermogenesis in Human
13 Fat Cells. *iScience* **20**, 73-89 (2019).
- 14 93 Kulyte, A. *et al.* CIDEA interacts with liver X receptors in white fat cells. *FEBS*
15 *Lett* **585**, 744-748 (2011).
- 16 94 Wang, W. *et al.* Cidea is an essential transcriptional coactivator regulating
17 mammary gland secretion of milk lipids. *Nat Med* **18**, 235-243 (2012).
- 18 95 Zhang, C. & Liu, P. The New Face of the Lipid Droplet: Lipid Droplet Proteins.
19 *Proteomics* **19**, e1700223 (2019).
- 20 96 Lizaso, A., Tan, K. T. & Lee, Y. H. beta-adrenergic receptor-stimulated lipolysis
21 requires the RAB7-mediated autolysosomal lipid degradation. *Autophagy* **9**,
22 1228-1243 (2013).
- 23 97 Singh, R. *et al.* Autophagy regulates adipose mass and differentiation in mice.
24 *J Clin Invest* **119**, 3329-3339 (2009).

- 1 98 Zhang, Y. *et al.* Adipose-specific deletion of autophagy-related gene 7 (atg7) in
2 mice reveals a role in adipogenesis. *Proc Natl Acad Sci U S A* **106**, 19860-
3 19865 (2009).
- 4 99 Flaherty, S. E., 3rd *et al.* A lipase-independent pathway of lipid release and
5 immune modulation by adipocytes. *Science* **363**, 989-993 (2019).
- 6 100 Eissing, L. *et al.* De novo lipogenesis in human fat and liver is linked to ChREBP-
7 beta and metabolic health. *Nat Commun* **4**, 1528 (2013).
- 8 101 Herman, M. A. *et al.* A novel ChREBP isoform in adipose tissue regulates
9 systemic glucose metabolism. *Nature* **484**, 333-338 (2012).
- 10 102 Kursawe, R. *et al.* Decreased transcription of ChREBP-alpha/beta isoforms in
11 abdominal subcutaneous adipose tissue of obese adolescents with prediabetes
12 or early type 2 diabetes: associations with insulin resistance and hyperglycemia.
13 *Diabetes* **62**, 837-844 (2013).
- 14 103 Morigny, P. *et al.* Interaction between Hormone-Sensitive Lipase and ChREBP
15 in Fat Cells Controls Insulin Sensitivity. *Nature Metabolism* **1**, 133-146 (2019).
- 16 104 Collins, J. M., Neville, M. J., Hoppa, M. B. & Frayn, K. N. De novo lipogenesis
17 and stearoyl-CoA desaturase are coordinately regulated in the human adipocyte
18 and protect against palmitate-induced cell injury. *J Biol Chem* **285**, 6044-6052
19 (2010).
- 20 105 Guilherme, A. *et al.* Adipocyte lipid synthesis coupled to neuronal control of
21 thermogenic programming. *Mol Metab* **6**, 781-796 (2017).
- 22 106 Guilherme, A. *et al.* Neuronal modulation of brown adipose activity through
23 perturbation of white adipocyte lipogenesis. *Mol Metab* **16**, 116-125 (2018).
- 24 107 Sukonina, V. *et al.* FOXK1 and FOXK2 regulate aerobic glycolysis. *Nature* **566**,
25 279-283 (2019).

- 1 108 DiGirolamo, M., Newby, F. D. & Lovejoy, J. Lactate production in adipose tissue:
2 a regulated function with extra-adipose implications. *FASEB J* **6**, 2405-2412
3 (1992).
- 4 109 Jansson, P. A., Larsson, A., Smith, U. & Lonroth, P. Lactate release from the
5 subcutaneous tissue in lean and obese men. *J Clin Invest* **93**, 240-246 (1994).
- 6 110 Krycer, J. R. *et al.* Lactate production is a prioritized feature of adipocyte
7 metabolism. *J Biol Chem* **295**, 83-98 (2020).
- 8 111 Hui, S. *et al.* Glucose feeds the TCA cycle via circulating lactate. *Nature* **551**,
9 115-118 (2017).
- 10 112 Rabinowitz, J. D. & Enerbäck, S. Lactate: the ugly duckling of energy
11 metabolism. *Nature Metab* **in press** (2020).
- 12 113 Lee, K. Y. *et al.* Developmental and functional heterogeneity of white adipocytes
13 within a single fat depot. *EMBO J* **38** (2019).
- 14 114 Lee, K. Y. *et al.* Tbx15 Defines a Glycolytic Subpopulation and White Adipocyte
15 Heterogeneity. *Diabetes* **66**, 2822-2829 (2017).
- 16 115 Luong, Q., Huang, J. & Lee, K. Y. Deciphering White Adipose Tissue
17 Heterogeneity. *Biology (Basel)* **8** (2019).
- 18 116 Lynes, M. D. & Tseng, Y. H. Deciphering adipose tissue heterogeneity. *Ann N*
19 *Y Acad Sci* **1411**, 5-20 (2018).
- 20 117 Newsholme, E. A. & Crabtree, B. Substrate cycles in metabolic regulation and
21 in heat generation. *Biochem Soc Symp*, 61-109 (1976).
- 22 118 Sanchez-Gurmaches, J., Hung, C. M. & Guertin, D. A. Emerging Complexities
23 in Adipocyte Origins and Identity. *Trends Cell Biol* **26**, 313-326 (2016).

- 1 119 Harms, M. J. *et al.* Mature Human White Adipocytes Cultured under Membranes
2 Maintain Identity, Function, and Can Transdifferentiate into Brown-like
3 Adipocytes. *Cell Rep* **27**, 213-225 e215 (2019).
- 4 120 Kroon, T. *et al.* PPARgamma and PPARalpha synergize to induce robust
5 browning of white fat in vivo. *Mol. Metab.* **36**, 100964 (2020).
- 6 121 Tiraby, C. *et al.* Acquisition of brown fat cell features by human white
7 adipocytes. *J Biol Chem* **278**, 33370-33376 (2003).
- 8 122 Wang, W. & Seale, P. Control of brown and beige fat development. *Nat Rev Mol*
9 *Cell Biol* **17**, 691-702 (2016).
- 10 123 Pisani, D. F. *et al.* Mitochondrial fission is associated with UCP1 activity in
11 human brite/beige adipocytes. *Mol Metab* **7**, 35-44 (2018).
- 12 124 Barquissau, V. *et al.* White-to-brite conversion in human adipocytes promotes
13 metabolic reprogramming towards fatty acid anabolic and catabolic pathways.
14 *Mol Metab* **5**, 352-365 (2016).
- 15 125 Mills, E. L. *et al.* Accumulation of succinate controls activation of adipose tissue
16 thermogenesis. *Nature* **560**, 102-106 (2018).
- 17 126 Murphy, M. P. & O'Neill, L. A. J. Krebs Cycle Reimagined: The Emerging Roles
18 of Succinate and Itaconate as Signal Transducers. *Cell* **174**, 780-784 (2018).
- 19 127 Kotzbeck, P. *et al.* Brown adipose tissue whitening leads to brown adipocyte
20 death and adipose tissue inflammation. *J Lipid Res* **59**, 784-794 (2018).
- 21 128 Roh, H. C. *et al.* Warming Induces Significant Reprogramming of Beige, but Not
22 Brown, Adipocyte Cellular Identity. *Cell Metab* **27**, 1121-1137 e1125 (2018).
- 23 129 Inagaki, T. Histone demethylases regulate adipocyte thermogenesis. *Diabetol*
24 *Int* **9**, 215-223 (2018).

- 1 130 Duteil, D. *et al.* LSD1 promotes oxidative metabolism of white adipose tissue.
2 *Nat Commun* **5**, 4093 (2014).
- 3 131 Sambeat, A. *et al.* LSD1 Interacts with Zfp516 to Promote UCP1 Transcription
4 and Brown Fat Program. *Cell Rep* **15**, 2536-2549 (2016).
- 5 132 Zeng, X. *et al.* Lysine-specific demethylase 1 promotes brown adipose tissue
6 thermogenesis via repressing glucocorticoid activation. *Genes Dev* **30**, 1822-
7 1836 (2016).
- 8 133 Guan, H. P. *et al.* A futile metabolic cycle activated in adipocytes by antidiabetic
9 agents. *Nat Med* **8**, 1122-1128 (2002).
- 10 134 Mazzucotelli, A. *et al.* The transcriptional coactivator peroxisome proliferator
11 activated receptor (PPAR)gamma coactivator-1 alpha and the nuclear receptor
12 PPAR alpha control the expression of glycerol kinase and metabolism genes
13 independently of PPAR gamma activation in human white adipocytes. *Diabetes*
14 **56**, 2467-2475 (2007).
- 15 135 Flachs, P. *et al.* Induction of lipogenesis in white fat during cold exposure in
16 mice: link to lean phenotype. *Int J Obes (Lond)* **41**, 372-380 (2017).
- 17 136 Ikeda, K. *et al.* UCP1-independent signaling involving SERCA2b-mediated
18 calcium cycling regulates beige fat thermogenesis and systemic glucose
19 homeostasis. *Nat Med* **23**, 1454-1465 (2017).
- 20 137 Chouchani, E. T., Kazak, L. & Spiegelman, B. M. New Advances in Adaptive
21 Thermogenesis: UCP1 and Beyond. *Cell Metab* **29**, 27-37 (2019).
- 22 138 Kazak, L. *et al.* A creatine-driven substrate cycle enhances energy expenditure
23 and thermogenesis in beige fat. *Cell* **163**, 643-655 (2015).

- 1 139 Bertholet, A. M. *et al.* Mitochondrial Patch Clamp of Beige Adipocytes Reveals
2 UCP1-Positive and UCP1-Negative Cells Both Exhibiting Futile Creatine
3 Cycling. *Cell Metab* **25**, 811-822 e814 (2017).
- 4 140 Kazak, L. *et al.* Ablation of adipocyte creatine transport impairs thermogenesis
5 and causes diet-induced obesity. *Nat Metab* **1**, 360-370 (2019).
- 6 141 Pollard, A. E. *et al.* AMPK activation protects against diet induced obesity
7 through Ucp1-independent thermogenesis in subcutaneous white adipose
8 tissue. *Nat Metab* **1**, 340-349 (2019).
- 9 142 Mottillo, E. P. *et al.* Coupling of lipolysis and de novo lipogenesis in brown,
10 beige, and white adipose tissues during chronic beta3-adrenergic receptor
11 activation. *J Lipid Res* **55**, 2276-2286 (2014).
- 12 143 Grousse, A. *et al.* Partial inhibition of adipose tissue lipolysis improves glucose
13 metabolism and insulin sensitivity without alteration of fat mass. *PLoS Biol* **11**,
14 e1001485 (2013).
- 15 144 Newgard, C. B. *et al.* A branched-chain amino acid-related metabolic signature
16 that differentiates obese and lean humans and contributes to insulin resistance.
17 *Cell Metab* **9**, 311-326 (2009).
- 18 145 White, P. J. & Newgard, C. B. Branched-chain amino acids in disease. *Science*
19 **363**, 582-583 (2019).
- 20 146 Lotta, L. A. *et al.* Genetic Predisposition to an Impaired Metabolism of the
21 Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian
22 Randomisation Analysis. *PLoS Med* **13**, e1002179 (2016).
- 23 147 Klimcakova, E. *et al.* Worsening of obesity and metabolic status yields similar
24 molecular adaptations in human subcutaneous and visceral adipose tissue:

1 decreased metabolism and increased immune response. *J Clin Endocrinol*
2 *Metab* **96**, E73-82 (2011).

3 148 Pietilainen, K. H. *et al.* Global transcript profiles of fat in monozygotic twins
4 discordant for BMI: pathways behind acquired obesity. *PLoS Med* **5**, e51 (2008).

5 149 Green, C. R. *et al.* Branched-chain amino acid catabolism fuels adipocyte
6 differentiation and lipogenesis. *Nat Chem Biol* **12**, 15-21 (2016).

7 150 Wallace, M. *et al.* Enzyme promiscuity drives branched-chain fatty acid
8 synthesis in adipose tissues. *Nat Chem Biol* **14**, 1021-1031 (2018).

9 151 Herman, M. A., She, P., Peroni, O. D., Lynch, C. J. & Kahn, B. B. Adipose tissue
10 branched chain amino acid (BCAA) metabolism modulates circulating BCAA
11 levels. *J Biol Chem* **285**, 11348-11356 (2010).

12 152 Mardinoglu, A. *et al.* Integration of clinical data with a genome-scale metabolic
13 model of the human adipocyte. *Mol Syst Biol* **9**, 649 (2013).

14 153 Ramirez, A. K. *et al.* Integrating Extracellular Flux Measurements and Genome-
15 Scale Modeling Reveals Differences between Brown and White Adipocytes.
16 *Cell Rep* **21**, 3040-3048 (2017).

17 154 Patni, N. & Garg, A. Congenital generalized lipodystrophies--new insights into
18 metabolic dysfunction. *Nat Rev Endocrinol* **11**, 522-534 (2015).

19 155 Mann, J. P. & Savage, D. B. What lipodystrophies teach us about the metabolic
20 syndrome. *J Clin Invest* **130**, 4009-4021 (2019).

21 156 Agarwal, A. K. *et al.* AGPAT2 is mutated in congenital generalized lipodystrophy
22 linked to chromosome 9q34. *Nat Genet* **31**, 21-23 (2002).

23 157 Hayashi, Y. K. *et al.* Human PTRF mutations cause secondary deficiency of
24 caveolins resulting in muscular dystrophy with generalized lipodystrophy. *J Clin*
25 *Invest* **119**, 2623-2633 (2009).

- 1 158 Gandotra, S. *et al.* Perilipin deficiency and autosomal dominant partial
2 lipodystrophy. *N Engl J Med* **364**, 740-748 (2011).
- 3 159 Rubio-Cabezas, O. *et al.* Partial lipodystrophy and insulin resistant diabetes in
4 a patient with a homozygous nonsense mutation in CIDEC. *EMBO Mol Med* **1**,
5 280-287 (2009).
- 6 160 Lotta, L. A. *et al.* Integrative genomic analysis implicates limited peripheral
7 adipose storage capacity in the pathogenesis of human insulin resistance. *Nat*
8 *Genet* **49**, 17-26 (2017).
- 9 161 Arner, P., Andersson, D. P., Backdahl, J., Dahlman, I. & Ryden, M. Weight Gain
10 and Impaired Glucose Metabolism in Women Are Predicted by Inefficient
11 Subcutaneous Fat Cell Lipolysis. *Cell Metab* **28**, 45-54 e43 (2018).
- 12 162 Perry, R. J. *et al.* Hepatic acetyl CoA links adipose tissue inflammation to
13 hepatic insulin resistance and type 2 diabetes. *Cell* **160**, 745-758 (2015).
- 14 163 Titchenell, P. M., Lazar, M. A. & Birnbaum, M. J. Unraveling the Regulation of
15 Hepatic Metabolism by Insulin. *Trends Endocrinol Metab* **28**, 497-505 (2017).
- 16 164 Edgerton, D. S. *et al.* Targeting insulin to the liver corrects defects in glucose
17 metabolism caused by peripheral insulin delivery. *JCI Insight* **5** (2019).
- 18 165 Hodson, L. & Karpe, F. Hyperinsulinaemia: does it tip the balance toward
19 intrahepatic fat accumulation? *Endocr Connect* **8**, R157-R168 (2019).
- 20 166 Karpe, F., Dickmann, J. R. & Frayn, K. N. Fatty acids, obesity, and insulin
21 resistance: time for a reevaluation. *Diabetes* **60**, 2441-2449 (2011).
- 22 167 Rohm, M., Zeigerer, A., Machado, J. & Herzig, S. Energy metabolism in
23 cachexia. *EMBO Rep* **20** (2019).
- 24 168 Duong, M. N. *et al.* The fat and the bad: Mature adipocytes, key actors in tumor
25 progression and resistance. *Oncotarget* **8**, 57622-57641 (2017).

- 1 169 Agustsson, T. *et al.* Mechanism of increased lipolysis in cancer cachexia.
2 *Cancer Res* **67**, 5531-5537 (2007).
- 3 170 Fouladiun, M. *et al.* Body composition and time course changes in regional
4 distribution of fat and lean tissue in unselected cancer patients on palliative
5 care--correlations with food intake, metabolism, exercise capacity, and
6 hormones. *Cancer* **103**, 2189-2198 (2005).
- 7 171 Das, S. K. *et al.* Adipose triglyceride lipase contributes to cancer-associated
8 cachexia. *Science* **333**, 233-238 (2011).
- 9 172 Rohm, M. *et al.* An AMP-activated protein kinase-stabilizing peptide ameliorates
10 adipose tissue wasting in cancer cachexia in mice. *Nat Med* **22**, 1120-1130
11 (2016).
- 12 173 Lipina, C. & Hundal, H. S. Lipid modulation of skeletal muscle mass and
13 function. *J Cachexia Sarcopenia Muscle* **8**, 190-201 (2017).
- 14 174 Stephens, N. A. *et al.* Intramyocellular lipid droplets increase with progression
15 of cachexia in cancer patients. *J Cachexia Sarcopenia Muscle* **2**, 111-117
16 (2011).
- 17 175 Caspar-Bauguil, S. *et al.* Fatty acids from fat cell lipolysis do not activate an
18 inflammatory response but are stored as triacylglycerols in adipose tissue
19 macrophages. *Diabetologia* **58**, 2627-2636 (2015).
- 20 176 Kosteli, A. *et al.* Weight loss and lipolysis promote a dynamic immune response
21 in murine adipose tissue. *J Clin Invest* **120**, 3466-3479 (2010).
- 22 177 Xu, X. *et al.* Obesity activates a program of lysosomal-dependent lipid
23 metabolism in adipose tissue macrophages independently of classic activation.
24 *Cell Metab* **18**, 816-830 (2013).

- 1 178 Sun, K., Kusminski, C. M. & Scherer, P. E. Adipose tissue remodeling and
2 obesity. *J Clin Invest* **121**, 2094-2101 (2011).
- 3 179 Giordano, A. *et al.* Obese adipocytes show ultrastructural features of stressed
4 cells and die of pyroptosis. *J Lipid Res* **54**, 2423-2436 (2013).
- 5 180 Canello, R. *et al.* Reduction of macrophage infiltration and chemoattractant
6 gene expression changes in white adipose tissue of morbidly obese subjects
7 after surgery-induced weight loss. *Diabetes* **54**, 2277-2286 (2005).
- 8 181 Cinti, S. *et al.* Adipocyte death defines macrophage localization and function in
9 adipose tissue of obese mice and humans. *J Lipid Res* **46**, 2347-2355 (2005).
- 10 182 Zatterale, F. *et al.* Chronic Adipose Tissue Inflammation Linking Obesity to
11 Insulin Resistance and Type 2 Diabetes. *Front Physiol* **10**, 1607 (2019).
- 12 183 Shimobayashi, M. *et al.* Insulin resistance causes inflammation in adipose
13 tissue. *J Clin Invest* **128**, 1538-1550 (2018).
- 14 184 Zhou, L. *et al.* Insulin resistance and white adipose tissue inflammation are
15 uncoupled in energetically challenged Fsp27-deficient mice. *Nat Commun* **6**,
16 5949 (2015).
- 17 185 Hodson, L., Skeaff, C. M. & Fielding, B. A. Fatty acid composition of adipose
18 tissue and blood in humans and its use as a biomarker of dietary intake. *Prog*
19 *Lipid Res* **47**, 348-380 (2008).
- 20 186 Cao, H. *et al.* Identification of a lipokine, a lipid hormone linking adipose tissue
21 to systemic metabolism. *Cell* **134**, 933-944 (2008).
- 22 187 Frigolet, M. E. & Gutierrez-Aguilar, R. The Role of the Novel Lipokine Palmitoleic
23 Acid in Health and Disease. *Adv Nutr* **8**, 173S-181S (2017).

- 1 188 Trico, D. *et al.* Circulating palmitoleic acid is an independent determinant of
2 insulin sensitivity, beta cell function and glucose tolerance in non-diabetic
3 individuals: a longitudinal analysis. *Diabetologia* **63**, 206-218 (2020).
- 4 189 Yore, M. M. *et al.* Discovery of a class of endogenous mammalian lipids with
5 anti-diabetic and anti-inflammatory effects. *Cell* **159**, 318-332 (2014).
- 6 190 Tan, D. *et al.* Discovery of FAHFA-Containing Triacylglycerols and Their
7 Metabolic Regulation. *J Am Chem Soc* **141**, 8798-8806 (2019).
- 8 191 Hammarstedt, A. *et al.* Adipose tissue dysfunction is associated with low levels
9 of the novel Palmitic Acid Hydroxystearic Acids. *Sci Rep* **8**, 15757 (2018).
- 10 192 Syed, I. *et al.* Palmitic Acid Hydroxystearic Acids Activate GPR40, Which Is
11 Involved in Their Beneficial Effects on Glucose Homeostasis. *Cell Metab* **27**,
12 419-427 e414 (2018).
- 13 193 Zhou, P. *et al.* PAHSAs enhance hepatic and systemic insulin sensitivity through
14 direct and indirect mechanisms. *J Clin Invest* **129**, 4138-4150 (2019).
- 15 194 Pflimlin, E. *et al.* Acute and Repeated Treatment with 5-PAHSA or 9-PAHSA
16 Isomers Does Not Improve Glucose Control in Mice. *Cell Metab* **28**, 217-227
17 e213 (2018).
- 18 195 Syed, I. *et al.* Methodological Issues in Studying PAHSA Biology: Masking
19 PAHSA Effects. *Cell Metab* **28**, 543-546 (2018).
- 20 196 Vijayakumar, A. *et al.* Absence of Carbohydrate Response Element Binding
21 Protein in Adipocytes Causes Systemic Insulin Resistance and Impairs Glucose
22 Transport. *Cell Rep* **21**, 1021-1035 (2017).
- 23 197 Erikci Ertunc, M. *et al.* AIG1 and ADTRP are endogenous hydrolases of fatty
24 acid esters of hydroxy fatty acids (FAHFAs) in mice. *J Biol Chem* **295**, 5891-
25 5905 (2020).

- 1 198 Lynes, M. D. *et al.* The cold-induced lipokine 12,13-diHOME promotes fatty acid
2 transport into brown adipose tissue. *Nat Med* **23**, 631-637 (2017).
- 3 199 Vasan, S. K. *et al.* The proposed systemic thermogenic metabolites succinate
4 and 12,13-diHOME are inversely associated with adiposity and related
5 metabolic traits: evidence from a large human cross-sectional study.
6 *Diabetologia* (2019).
- 7 200 Stanford, K. I. *et al.* 12,13-diHOME: An Exercise-Induced Lipokine that
8 Increases Skeletal Muscle Fatty Acid Uptake. *Cell Metab* **27**, 1111-1120 e1113
9 (2018).
- 10 201 Funcke, J. B. & Scherer, P. E. Beyond adiponectin and leptin: adipose tissue-
11 derived mediators of inter-organ communication. *J Lipid Res* (2019).
- 12 202 Xia, J. Y. *et al.* Targeted Induction of Ceramide Degradation Leads to Improved
13 Systemic Metabolism and Reduced Hepatic Steatosis. *Cell Metab* **22**, 266-278
14 (2015).
- 15 203 Chaurasia, B. *et al.* Targeting a ceramide double bond improves insulin
16 resistance and hepatic steatosis. *Science* **365**, 386-392 (2019).
- 17 204 Ertunc, M. E. *et al.* Secretion of fatty acid binding protein aP2 from adipocytes
18 through a nonclassical pathway in response to adipocyte lipase activity. *J Lipid*
19 *Res* **56**, 423-434 (2015).
- 20 205 Cao, H. *et al.* Adipocyte lipid chaperone AP2 is a secreted adipokine regulating
21 hepatic glucose production. *Cell Metab* **17**, 768-778 (2013).
- 22 206 Oikonomou, E. K. & Antoniadou, C. The role of adipose tissue in cardiovascular
23 health and disease. *Nat Rev Cardiol* **16**, 83-99 (2019).
- 24 207 Yang, Q. *et al.* Serum retinol binding protein 4 contributes to insulin resistance
25 in obesity and type 2 diabetes. *Nature* **436**, 356-362 (2005).

- 1 208 Moraes-Vieira, P. M. *et al.* RBP4 activates antigen-presenting cells, leading to
2 adipose tissue inflammation and systemic insulin resistance. *Cell Metab* **19**,
3 512-526 (2014).
- 4 209 Hallenborg, P. *et al.* The elusive endogenous adipogenic PPARgamma
5 agonists: Lining up the suspects. *Prog Lipid Res* **61**, 149-162 (2016).
- 6 210 Soccio, R. E., Chen, E. R. & Lazar, M. A. Thiazolidinediones and the promise
7 of insulin sensitization in type 2 diabetes. *Cell Metab* **20**, 573-591 (2014).
- 8 211 Cusi, K. *et al.* Long-Term Pioglitazone Treatment for Patients With Nonalcoholic
9 Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized
10 Trial. *Ann Intern Med* **165**, 305-315 (2016).
- 11 212 DeFronzo, R. A., Inzucchi, S., Abdul-Ghani, M. & Nissen, S. E. Pioglitazone:
12 The forgotten, cost-effective cardioprotective drug for type 2 diabetes. *Diab*
13 *Vasc Dis Res* **16**, 133-143 (2019).
- 14 213 Schweiger, M. *et al.* Pharmacological inhibition of adipose triglyceride lipase
15 corrects high-fat diet-induced insulin resistance and hepatosteatosis in mice.
16 *Nat Commun* **8**, 14859 (2017).
- 17 214 Lauring, B. *et al.* Niacin lipid efficacy is independent of both the niacin receptor
18 GPR109A and free fatty acid suppression. *Sci Transl Med* **4**, 148ra115 (2012).
- 19 215 Romani, M., Hofer, D. C., Katsyuba, E. & Auwerx, J. Niacin: an old lipid drug in
20 a new NAD(+) dress. *J Lipid Res* **60**, 741-746 (2019).
- 21 216 Goldie, C. *et al.* Niacin therapy and the risk of new-onset diabetes: a meta-
22 analysis of randomised controlled trials. *Heart* **102**, 198-203 (2016).
- 23 217 Kroon, T., Baccega, T., Olsen, A., Gabrielsson, J. & Oakes, N. D. Nicotinic acid
24 timed to feeding reverses tissue lipid accumulation and improves glucose
25 control in obese Zucker rats[S]. *J Lipid Res* **58**, 31-41 (2017).

- 1 218 Kroon, T., Kjellstedt, A., Thalen, P., Gabrielsson, J. & Oakes, N. D. Dosing
2 profile profoundly influences nicotinic acid's ability to improve metabolic control
3 in rats. *J Lipid Res* **56**, 1679-1690 (2015).
- 4 219 Wallenius, K. *et al.* Involvement of the metabolic sensor GPR81 in
5 cardiovascular control. *JCI Insight* **2**, e92564 (2017).
- 6 220 Manini, T. M. Energy expenditure and aging. *Ageing Res Rev* **9**, 1-11 (2010).
- 7 221 Ryden, M., Gao, H. & Arner, P. Influence of ageing and menstrual status on
8 subcutaneous fat cell lipolysis. *J. Clin. Endocrinol. Metab.* **105**, dgz245 (2019).
- 9 222 Reitman, M. L. Of mice and men - environmental temperature, body
10 temperature, and treatment of obesity. *FEBS Lett* **592**, 2098-2107 (2018).
- 11 223 Maurer, S., Harms, M. & Boucher, J. The colorful versatility of adipocytes: white-
12 to-brown transdifferentiation and its therapeutic potential in man. *FEBS J*
13 (2020).
- 14 224 Hoehn, K. L. *et al.* Acute or chronic upregulation of mitochondrial fatty acid
15 oxidation has no net effect on whole-body energy expenditure or adiposity. *Cell*
16 *Metab* **11**, 70-76 (2010).
- 17 225 Ryaboshapkina, M. & Hammar, M. Tissue-specific genes as an underutilized
18 resource in drug discovery. *Sci Rep* **9**, 7233 (2019).
- 19 226 Schoettl, T., Fischer, I. P. & Ussar, S. Heterogeneity of adipose tissue in
20 development and metabolic function. *J Exp Biol* **221** (2018).
- 21 227 Zwick, R. K., Guerrero-Juarez, C. F., Horsley, V. & Plikus, M. V. Anatomical,
22 Physiological, and Functional Diversity of Adipose Tissue. *Cell Metab* **27**, 68-83
23 (2018).

- 1 228 Macotela, Y., Boucher, J., Tran, T. T. & Kahn, C. R. Sex and depot differences
2 in adipocyte insulin sensitivity and glucose metabolism. *Diabetes* **58**, 803-812
3 (2009).
- 4 229 Palmer, B. F. & Clegg, D. J. The sexual dimorphism of obesity. *Mol Cell*
5 *Endocrinol* **402**, 113-119 (2015).
- 6 230 Stout, M. B., Justice, J. N., Nicklas, B. J. & Kirkland, J. L. Physiological Aging:
7 Links Among Adipose Tissue Dysfunction, Diabetes, and Frailty. *Physiology*
8 (*Bethesda*) **32**, 9-19 (2017).
- 9 231 Hagberg, C. E. *et al.* Flow Cytometry of Mouse and Human Adipocytes for the
10 Analysis of Browning and Cellular Heterogeneity. *Cell Rep* **24**, 2746-2756
11 e2745 (2018).
- 12 232 Chen, Y. *et al.* Thermal stress induces glycolytic beige fat formation via a
13 myogenic state. *Nature* **565**, 180-185 (2019).
- 14 233 Sun, W. *et al.* snRNA-seq reveals a subpopulation of adipocytes that regulates
15 thermogenesis. *Nature* **587**, 98-102 (2020).
- 16 234 Panda, S. Circadian physiology of metabolism. *Science* **354**, 1008-1015 (2016).
- 17 235 Stenvers, D. J., Scheer, F., Schrauwen, P., la Fleur, S. E. & Kalsbeek, A.
18 Circadian clocks and insulin resistance. *Nat Rev Endocrinol* **15**, 75-89 (2019).
- 19 236 Goodpaster, B. H. & Sparks, L. M. Metabolic Flexibility in Health and Disease.
20 *Cell Metab* **25**, 1027-1036 (2017).
- 21 237 Chaix, A., Manoogian, E. N. C., Melkani, G. C. & Panda, S. Time-Restricted
22 Eating to Prevent and Manage Chronic Metabolic Diseases. *Annu Rev Nutr*
23 (2019).

- 1 238 Sears, D. D. *et al.* Mechanisms of human insulin resistance and
2 thiazolidinedione-mediated insulin sensitization. *Proc Natl Acad Sci U S A* **106**,
3 18745-18750 (2009).
- 4 239 Ahlqvist, E. *et al.* Novel subgroups of adult-onset diabetes and their association
5 with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes*
6 *Endocrinol* **6**, 361-369 (2018).
- 7 240 Zaharia, O. P. *et al.* Risk of diabetes-associated diseases in subgroups of
8 patients with recent-onset diabetes: a 5-year follow-up study. *Lancet Diabetes*
9 *Endocrinol* **7**, 684-694 (2019).
- 10 241 Cao, Y. Adipocyte and lipid metabolism in cancer drug resistance. *J Clin Invest*
11 **129**, 3006-3017 (2019).
- 12 242 Shapiro, B. & Wertheimer, E. The synthesis of fatty acids in adipose tissue in
13 vitro. *J Biol Chem* **173**, 725-728 (1948).
- 14 243 Wertheimer, E. & Shapiro, B. The physiology of adipose tissue. *Physiol Rev* **28**,
15 451-464 (1948).
- 16 244 Hausberger, F. X., Milstein, S. W. & Rutman, R. J. The influence of insulin on
17 glucose utilization in adipose and hepatic tissues in vitro. *J Biol Chem* **208**, 431-
18 438 (1954).
- 19 245 Korn, E. D. & Quigley, T. W., Jr. Studies on lipoprotein lipase of rat heart and
20 adipose tissue. *Biochim Biophys Acta* **18**, 143-145 (1955).
- 21 246 Wadstrom, L. B. Lipolytic effect of the injection of adrenaline on fat depots.
22 *Nature* **179**, 259-260 (1957).
- 23 247 Vaughan, M., Berger, J. E. & Steinberg, D. Hormone-Sensitive Lipase and
24 Monoglyceride Lipase Activities in Adipose Tissue. *J Biol Chem* **239**, 401-409
25 (1964).

- 1 248 Fain, J. N., Kovacev, V. P. & Scow, R. O. Antilipolytic effect of insulin in isolated
2 fat cells of the rat. *Endocrinology* **78**, 773-778 (1966).
- 3 249 Hirsch, J. & Gallian, E. Methods for the determination of adipose cell size in man
4 and animals. *J Lipid Res* **9**, 110-119 (1968).
- 5 250 Fujita, T. *et al.* Reduction of insulin resistance in obese and/or diabetic animals
6 by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878,
7 U-63,287, ciglitazone), a new antidiabetic agent. *Diabetes* **32**, 804-810 (1983).
- 8 251 Loncar, D. Convertible adipose tissue in mice. *Cell Tissue Res* **266**, 149-161
9 (1991).
- 10 252 Tontonoz, P., Hu, E. & Spiegelman, B. M. Stimulation of adipogenesis in
11 fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79**,
12 1147-1156 (1994).
- 13 253 Lehmann, J. M. *et al.* An antidiabetic thiazolidinedione is a high affinity ligand
14 for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol*
15 *Chem* **270**, 12953-12956 (1995).
- 16 254 Montague, C. T. *et al.* Congenital leptin deficiency is associated with severe
17 early-onset obesity in humans. *Nature* **387**, 903-908 (1997).
- 18 255 Sengenès, C., Berlan, M., De Glisezinski, I., Lafontan, M. & Galitzky, J.
19 Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J* **14**,
20 1345-1351 (2000).
- 21 256 Abel, E. D. *et al.* Adipose-selective targeting of the GLUT4 gene impairs insulin
22 action in muscle and liver. *Nature* **409**, 729-733 (2001).
- 23 257 Weisberg, S. P. *et al.* Obesity is associated with macrophage accumulation in
24 adipose tissue. *J Clin Invest* **112**, 1796-1808 (2003).

- 1 258 Jenkins, C. M. *et al.* Identification, cloning, expression, and purification of three
2 novel human calcium-independent phospholipase A2 family members
3 possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol*
4 *Chem* **279**, 48968-48975 (2004).
- 5 259 Villena, J. A., Roy, S., Sarkadi-Nagy, E., Kim, K. H. & Sul, H. S. Desnutrin, an
6 adipocyte gene encoding a novel patatin domain-containing protein, is induced
7 by fasting and glucocorticoids: ectopic expression of desnutrin increases
8 triglyceride hydrolysis. *J Biol Chem* **279**, 47066-47075 (2004).
- 9 260 Zimmermann, R. *et al.* Fat mobilization in adipose tissue is promoted by adipose
10 triglyceride lipase. *Science* **306**, 1383-1386 (2004).
- 11 261 Wu, J. *et al.* Beige adipocytes are a distinct type of thermogenic fat cell in mouse
12 and human. *Cell* **150**, 366-376 (2012).
- 13 262 Rosenwald, M., Perdikari, A., Rulicke, T. & Wolfrum, C. Bi-directional
14 interconversion of brite and white adipocytes. *Nat Cell Biol* **15**, 659-667 (2013).
- 15 263 Thomou, T. *et al.* Adipose-derived circulating miRNAs regulate gene expression
16 in other tissues. *Nature* **542**, 450-455 (2017).
- 17 264 Ying, W. *et al.* Adipose Tissue Macrophage-Derived Exosomal miRNAs Can
18 Modulate In Vivo and In Vitro Insulin Sensitivity. *Cell* **171**, 372-384 e312 (2017).
- 19 265 Crewe, C. *et al.* An Endothelial-to-Adipocyte Extracellular Vesicle Axis
20 Governed by Metabolic State. *Cell* **175**, 695-708 e613 (2018).
- 21 266 Muller, S., Kulenkampff, E. & Wolfrum, C. Adipose Tissue Stem Cells. *Handb*
22 *Exp Pharmacol* **233**, 251-263 (2016).
- 23 267 Caslin, H. L., Bhanot, M., Bolus, W. R. & Hasty, A. H. Adipose tissue
24 macrophages: Unique polarization and bioenergetics in obesity. *Immunol Rev*
25 **295**, 101-113 (2020).

- 1 268 Sun, K., Tordjman, J., Clement, K. & Scherer, P. E. Fibrosis and adipose tissue
2 dysfunction. *Cell Metab* **18**, 470-477 (2013).
- 3 269 Roden, M. & Shulman, G. I. The integrative biology of type 2 diabetes. *Nature*
4 **576**, 51-60 (2019).
- 5 270 Canfora, E. E., Meex, R. C. R., Venema, K. & Blaak, E. E. Gut microbial
6 metabolites in obesity, NAFLD and T2DM. *Nat Rev Endocrinol* **15**, 261-273
7 (2019).
- 8 271 Schlaich, M., Straznicky, N., Lambert, E. & Lambert, G. Metabolic syndrome: a
9 sympathetic disease? *Lancet Diabetes Endocrinol* **3**, 148-157 (2015).
- 10 272 Ulrich-Lai, Y. M. & Ryan, K. K. Neuroendocrine circuits governing energy
11 balance and stress regulation: functional overlap and therapeutic implications.
12 *Cell Metab* **19**, 910-925 (2014).

13

1 **Acknowledgments**

2 The rapid growth of research in adipocyte metabolism made it impossible to cite a large
3 number of excellent studies relevant to the topic of this review. D.L. is supported by
4 Inserm, Paul Sabatier University, European Research Council (ERC) under the
5 European Union's Horizon 2020 research and innovation programme (SPHERES,
6 ERC Synergy Grant agreement No. 856404), Fondation pour la Recherche Médicale
7 (DEQ20170336720), Agence Nationale de la Recherche (ANR-17-CE14-
8 0015Hepadialogue), Région Occitanie (DIALOGUE projects), FORCE/F-CRIN and
9 AstraZeneca France. D.L. is a member of Institut Universitaire de France.

10

11 **Author contributions**

12 D.L. conceived the initial version of the article. P.M., J.B., P.A. and D.L. wrote the
13 article. P.M. and P.A. prepared the figures. D.L. integrated contributions and produced
14 the submitted version with input from P.M., J.B. and P.A. All authors approved the final
15 version of the article.

16

17 **Competing interests**

18 J.B. is an employee of AstraZeneca. The other authors declare no competing interests.

19

20 **Peer review information**

21 *Nature Reviews Endocrinology* thanks J.-H. Chen, who co-reviewed with S. O'Rahilly;
22 M. Czech; and the other, anonymous, reviewer(s) for their contribution to the peer
23 review of this work.

24

1 **Key points**

- 2 • White adipocyte size and turnover are determinants of systemic insulin
3 sensitivity and cardiometabolic phenotype in humans.
- 4 • White adipocytes are specialized in fat storage and mobilization; the underlying
5 lipid metabolic pathways are tightly connected with those governing the
6 intracellular fate of glucose.
- 7 • In some fat depots, there is a bidirectional switch between white and beige
8 adipocytes, which display an oxidative phenotype with energy dissipation
9 through uncoupling protein 1 (UCP1)-dependent and UCP1-independent
10 pathways.
- 11 • White adipocyte metabolic pathways control the secretion of proteins and lipids,
12 with local and systemic effects on inflammation and insulin sensitivity.
- 13 • Adipocyte metabolism offers promising targets for the treatment of
14 cardiometabolic diseases and cancer-associated disorders.
- 15 • Future research will include the in-depth characterization of adipocyte diversity
16 associated with anatomical location, age, sex and physiological rhythms.

17

18

1 **Figure legends**

2

3 **Fig. 1. Timeline of important advances and promising discoveries in white**
4 **adipose tissue research**

5 Main references for each year are listed here: 1948^{242,243}; 1954²⁴⁴; 1955²⁴⁵; 1957²⁴⁶;
6 1964^{3,247}; 1966²⁴⁸; 1968²⁴⁹; 1976⁴; 1980^{5,6}; 1983²⁵⁰; 1991²⁵¹; 1993⁷; 1994^{11,252}; 1996⁸⁻
7 ¹⁰; 1995–1997^{253,254}; 2000²⁵⁵; 2001²⁵⁶; 2003^{121,257}; 2004²⁵⁸⁻²⁶⁰; 2008–2011^{45,52}; 2012²⁶¹;
8 2013²⁶²; 2015¹⁶²; 2017^{263,264}; 2015–2019^{103,136,138}; 2018–2019^{99,265}. The graph depicts
9 the number of publications published each year using the following search query in an
10 August 2020 search of Pubmed: “adipocyte” or “fat cell” or “adipose tissue”
11 [Title/Abstract]. ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase;
12 MGL, monoglyceride lipase; PPAR, peroxisome proliferator-activated receptor; TNF,
13 tumor necrosis factor; TZDs, thiazolidinediones.

14

15 **Fig. 2. Turnover of human white adipose tissue.** Schematic overview of the links
16 between adipocyte formation and pathological conditions. **a** | Adipocyte generation and
17 cell death occur constantly. Progenitor cells proliferate in different niches within fat
18 depots. A fraction of these cells originates from bone marrow. In obesity, the generation
19 rate of new large and/or small adipocytes is increased. When the generation rate is
20 decreased, fewer, larger adipocytes form, a process referred to as hypertrophy. **b** | Fat
21 mass can develop in two ways. The formation of a few large adipocytes or the
22 accumulation of lipids in pre-existing cells result in hypertrophy. Alternatively, precursor
23 cells proliferate and differentiate into a large number of small adipocytes, a process
24 termed hyperplasia. Adipocyte hypertrophy is associated with an adverse
25 cardiometabolic phenotype whereas adipocyte hyperplasia at the same fat mass has

1 benign effects. **c** | Adipocyte lipid turnover decreases with ageing, as reflected by
2 increased lipid age. This reduced turnover decreases the rate of lipid removal (K_{out})
3 from adipocytes. If a reduced K_{out} is counterbalanced by a decreased rate of lipid
4 storage (K_{in}), fat mass remains unchanged, whereas fat mass expands over time if K_{in}
5 does not decrease (or increases).

6
7 **Fig. 3. Fat storage and glucose metabolism in white adipocytes.** GLUT4 and
8 GLUT1 are, respectively, the insulin-sensitive and non-insulin-sensitive glucose
9 transporters in adipocytes. During glycolysis, some glucose is converted into glycerol-
10 3-phosphate (glycerol-3P), which constitutes the backbone of triacylglycerol (TAG).
11 Adipocytes also make a substantial contribution to whole-body lactate turnover. After
12 glycolysis, glucose can be oxidized in the tricarboxylic acid cycle (TCA) to produce
13 energy or be converted into fatty acids by de novo lipogenesis (DNL). This pathway,
14 which is under the control of insulin, involves the sequential action of ATP citrate lyase
15 (ACLY), acetyl-CoA carboxylase (ACC1) and fatty acid synthase (FASN). Palmitic acid
16 produced by these enzymes can be further elongated and desaturated by elongase of
17 very-long-chain fatty acid 6 (ELOVL6) and stearoyl-CoA desaturase SCD, respectively.
18 DNL-derived fatty acids can be used as components of phospholipids in cellular
19 membranes, serve as extracellular signalling molecules or, to a lesser extent than
20 extracellular fatty acids, be stored as TAG in the lipid droplet. Insulin promotes
21 hydrolysis of fatty-acid-loaded lipoproteins by lipoprotein lipase (LPL) and entry of the
22 released fatty acids through specific fatty acid transporters. Insulin also stimulates fatty
23 acid esterification, which occurs by the sequential action of acetyl-CoA synthetase
24 (ACS), glycerol-3-phosphate-acyltransferase (GPAT), 1-acyl-glycerol-3-phosphate-
25 acyltransferase (AGPAT), phosphatidic acid phosphatase (PAP; also known as lipin)

1 and diacylglycerol acyltransferase (DGAT) enzymes. ER, endoplasmic reticulum;
2 MCT, monocarboxylate transporters.

3

4 **Fig. 4. Fat mobilization in white adipocytes.** In basal conditions, insulin inhibits
5 lipolysis through activation of the cAMP-degrading enzyme phosphodiesterase 3B
6 (PDE3B) (part **a**). The inhibition of cAMP synthesis is mediated by activation of anti-
7 lipolytic G-protein coupled receptors (GPCRs) coupled to $G_{\alpha i}$. Through clearance of
8 atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) promoted by
9 natriuretic peptide receptor C (NPRC), these natriuretic peptides (NPs) do not exert a
10 lipolytic effect. Adipose triglyceride lipase (ATGL)-mediated triacylglycerol (TAG)
11 hydrolysis is minimal owing to interaction of the ATGL activator CGI58 with the lipid-
12 droplet coating protein perilipin 1 (PLIN1). FSP27 and G0S2 are negative regulators of
13 ATGL activity. Like FSP27, CIDEA is also a member of the CIDE family and controls
14 basal lipolysis. The net action of NPs and catecholamines results from the balance
15 between binding of these molecules to inhibitory receptor complexes (part **a**) and
16 activatory receptor complexes (part **b**). In stimulated conditions, ANP and BNP
17 (through NPRA) and catecholamines (through β -adrenergic receptors coupled to $G_{\alpha s}$)
18 induce an increase in cGMP and cAMP levels, respectively (part **b**). The protein
19 kinases PKG and PKA phosphorylate hormone-sensitive lipase (HSL) and thereby
20 promote its translocation from cytosol to the lipid droplet, where it interacts with PLIN1.
21 HSL is bound to the fatty-acid-binding protein FABP4. Dissociation of CGI58 from
22 phosphorylated PLIN1 allows CGI58 to interact with ATGL. ATGL, HSL and
23 monoglyceride lipase (not shown) catalyse the sequential hydrolysis of TAG into fatty
24 acids and glycerol, which are released from the adipocyte through dedicated
25 transporters or oxidized in the tricarboxylic acid cycle (TCA). PTRF (also known as

1 cavin 1) is involved in HSL recruitment to the lipid droplet and is a constituent of
2 caveolae (together with caveolin 1 (CAV1)), where it participates in fatty acid trafficking.

3

4 **Fig. 5. Energy dissipation in adipocytes. a |** Uncoupling protein 1 (UCP1)-dependent
5 thermogenesis involves β -oxidation of fatty acids and production of reactive oxygen
6 species (ROS) induced by succinate oxidation. In response to thermogenic stimuli,
7 fatty acids, glucose and succinate are imported from the circulation. Fatty acids can be
8 mobilized from intracellular stores of triacylglycerol (TAG). Fatty acid oxidation is
9 favoured over glucose oxidation because of inhibition of the pyruvate dehydrogenase
10 (PDH) complex by phosphorylation of PDH components by pyruvate dehydrogenase
11 kinase 4 (PDK4). **b |** Several UCP1-independent pathways promote energy
12 dissipation. The substrate cycle of TAG synthesis and hydrolysis includes lipolysis and
13 phosphorylation of glycerol into glycerol 3-phosphate (glycerol-3P) to allow re-
14 esterification of fatty acids. Another branch feeding the cycle is the synthesis of fatty
15 acids from glucose (de novo lipogenesis). The contribution of the TAG–fatty acid
16 substrate cycle to heat production is debated. The SERCA2–ryanodine receptor (RyR)
17 pathway in the endoplasmic reticulum (ER) induces Ca^{2+} cycling. In the mitochondria,
18 creatine substrate cycling also results in energy dissipation. The two latter pathways
19 are coupled to ATP synthesis and consumption and generate heat. ETC, electron
20 transport chain; IMS, intermembrane space; RCC, respiratory chain complex; S, F1/F0
21 ATP synthase; TCA, tricarboxylic acid cycle.

22

23 **Fig. 6. Crosstalk between metabolic pathways in the white adipocyte.** The
24 interaction between hormone sensitive lipase (HSL) and the carbohydrate-responsive
25 element-binding protein α (ChREBP α) inhibits ChREBP α translocation into the

1 nucleus. Disruption of the HSL–ChREBP α interaction promotes ChREBP α
2 transcription activity, involving induction of expression of the highly transcriptionally
3 active isoform ChREBP β and of lipogenic enzymes, such as ATP-citrate lyase (ACLY),
4 acetyl-CoA carboxylase (ACC1), fatty-acid synthase (FASN), elongase of very-long-
5 chain fatty acid 6 (ELOVL6), and stearoyl-CoA desaturase (SCD). Specific induction
6 of ELOVL6, a preferential target of ChREBP, promotes oleic acid synthesis and
7 incorporation into plasma membrane phospholipids, thereby increasing plasma
8 membrane fluidity and insulin signalling. Conversely, β_3 -adrenergic receptor signalling
9 promotes an adipose triglyceride lipase (ATGL)-dependent induction of lipogenic
10 enzyme expression and *de novo* lipogenesis (DNL). Branched-chain amino acids
11 (BCAAs) are another important source of substrate for DNL. BCAAs can be oxidized
12 in the tricarboxylic acid cycle (TCA) and contribute to the lipogenic acetyl-CoA pool.
13 Branched-chain CoA intermediates of BCAA catabolism (BC-CoAs) are produced in
14 mitochondria but can also be exported to the cytosol through carnitine acyl transferase
15 (CrAT). CrAT and FASN promiscuity favours BC-CoA elongation and monomethyl
16 branched-chain fatty acid (mmBCFA) production. mmBCFA can be stored by
17 incorporation in triacylglycerol (TAG) and mobilized during fasting. Glucose metabolism
18 inhibits BCAA metabolism through unknown mechanisms.

19

20 **Fig. 7. Systemic impact of adipocyte metabolism and therapeutic perspectives.**

21 In non-pathological conditions, adipocytes exhibit a beneficial metabolism that
22 supports systemic insulin sensitivity (green pathways). This metabolic phenotype
23 promotes healthy lipid storage in the form of triacylglycerol (TAG) in lipid droplets, low
24 basal lipolysis, *de novo* lipogenesis (DNL) and secretion of beneficial bioactive lipids
25 (such as fatty acid esters of hydroxy fatty acids (FAHFAs) and 12,13-diHOME) and

1 adipokines (such as leptin and adiponectin). During obesity or lipodystrophy,
2 adipocytes show opposite features, secreting factors (such as fatty acids, ceramides,
3 cytokines and the fatty acid binding protein FABP4 and retinol-binding protein RBP4)
4 that promote chronic inflammation and systemic insulin resistance (red pathways).
5 Multiple aspects of adipocyte metabolism are valuable targets for drug development
6 (blue stars). The inhibition of the interaction between hormone-sensitive lipase and
7 carbohydrate-responsive element-binding protein (ChREBP) results in activation of
8 DNL and enhanced insulin signalling through increased content of monounsaturated
9 fatty acids (MUFA) in plasma membrane phospholipids. Enhanced DNL is associated
10 with the synthesis of beneficial lipid species such as FAHFA, which have systemic
11 insulin-sensitizing effects. The inhibition of ceramide synthesis and/or activation of
12 ceramide degradation protect against systemic insulin resistance. The inhibition of
13 lipolysis using $G_{\alpha i}$ -coupled receptor (GPCR) agonists is another promising strategy.
14 Peroxisome proliferator-activated receptor- γ (PPAR γ) agonists promote healthy lipid
15 storage and DNL, decrease inflammation and induce a beneficial adipokine profile,
16 which together improve systemic insulin sensitivity. Finally, conversion of white
17 adipocytes into beige adipocytes and stimulation of energy dissipation in beige
18 adipocytes is an attractive strategy to increase energy expenditure. ER, endoplasmic
19 reticulum; GLP-1, glucagon-like peptide 1; glycerol-3P, glycerol-3-phosphate; UCP1,
20 uncoupling protein 1.

21

22

1 **Box 1. Diversity of cell types in white adipose tissue**

2 White adipose tissue (WAT) contains various cell types that support a diversity of roles
3 and functions. The white adipocyte is the prototypical cell of white fat that imparts its
4 colour to the tissue. This cell type is specialized in metabolism, notably the storage of
5 chemical energy as TAG and its release as fatty acids. Glucose metabolic pathways
6 are associated with lipid metabolism, notably through de novo synthesis of fatty acids
7 and glycerol. Depending on the anatomical location and physiological conditions (for
8 example, cold exposure and season), white fat depots contain various amounts of
9 beige adipocytes, which can derive from differentiation of precursor cells or reversible
10 interconversion of white adipocytes to beige adipocytes ^{118,124}. Beige adipocytes are
11 enriched in mitochondria and equipped with several pathways that allow energy
12 dissipation as heat. The stromovascular fraction of WAT contains immune and non-
13 immune cells. Non-immune cells in this fraction include progenitor cells and endothelial
14 cells ^{118,266}. Progenitor cells have the potential to differentiate into white or beige
15 adipocytes and, together with fibroblasts, play an important part in extracellular matrix
16 production and fibrosis. Endothelial cells form the endothelium, which plays a key part
17 as a barrier and exchange area between blood and adipose tissue. A growing number
18 of immune cells have been identified in WAT, with macrophages being the most
19 abundant. The initial binary classification of adipose tissue macrophages as pro-
20 inflammatory or anti-inflammatory cells may be considered obsolete²⁶⁷. Some
21 macrophages are specialized in lipid scavenging. Other myeloid cells (such as
22 dendritic cells and neutrophils) and various lymphocyte populations participate in the
23 immune response and tissue remodelling ²⁶⁸. Adipocytes and some cell types in the
24 stromovascular fraction (notably macrophages), secrete peptides termed adipokines
25 (such as leptin, adiponectin, RBP4, FABP4 and tumor necrosis factor) and lipid

1 molecules termed lipokines (such as FAHFA and 12,13-diHOME)¹⁵. These factors can
2 act locally on neighbouring cells (paracrine action) or remotely on cells in other organs
3 (endocrine action). Moreover, immune cells and endothelial cells communicate with
4 local nerve fibres¹³. This interplay leads to neurohumoral signalling that regulates
5 whole-body metabolism.

6

7 **Box 2. Insulin resistance**

8 Insulin resistance is a pathophysiological state characterized by an impairment of
9 insulin-mediated control of glucose and fat metabolism. In skeletal muscle and white
10 adipose tissue (WAT), insulin is less efficient in stimulating glucose uptake while, in
11 the liver, the hormone loses its ability to inhibit endogenous glucose production. Insulin
12 resistance also results in uncontrolled release of fatty acids by WAT as the result of
13 diminished anti-lipolytic action of insulin. Insulin resistance can be organ-specific (for
14 example, affecting the liver but not the skeletal muscle and vice versa) or pathway-
15 specific (for example, impairment of insulin action on hepatic gluconeogenesis but not
16 on de novo lipogenesis). This selectivity is explained by molecular defects that affect
17 various levels of insulin signalling pathways. A current view, which proposes adipocyte
18 dysfunction as an early cardinal event in insulin resistance, is that WAT drives
19 metabolic fluxes that impact the liver and skeletal muscle²⁶⁹. However, the kinetics of
20 dysfunction in different metabolic organs is not well-characterized in humans. The gut,
21 through microbiota and digestion-related functions, and the brain, through the
22 neuroendocrine control of metabolism, may also have an important role in insulin
23 resistance²⁷⁰⁻²⁷². Insulin resistance results in an increased burden on pancreatic islet
24 β -cells to secrete insulin as a compensatory mechanism for the loss of sensitivity to

1 the hormone. When β -cell function declines, fasting and postprandial blood glucose
2 levels increase, signalling the onset of type 2 diabetes mellitus. Insulin resistance is
3 also found in various pathological conditions, such as polycystic ovary syndrome,
4 lipodystrophies, non-alcoholic fatty liver disease, cardiovascular disease and some
5 cancers.

6

7 **Glossary terms**

8 Adipose tissue hypertrophy

9 Adipose tissue expansion through an increase in adipocyte size.

10 Adipose tissue hyperplasia

11 Adipose tissue expansion through the generation of new adipocytes.

12 M1-like macrophages

13 Subtype of macrophages characterized by the secretion of pro-inflammatory

14 cytokines and chemokines such as IL-6 and TNF.

15 Lipophagy

16 Triacylglycerol hydrolysis by lysosomal acid lipases after engulfment of a lipid droplet

17 by an autophagosome, which fuses with lysosomes.

18 Beige adipocyte

19 Also known as brown-in-white (brite) adipocytes. A subtype of thermogenic

20 adipocytes located in white fat depots and uniquely equipped to dissipate energy as

21 heat.

- 1 Pyroptotic cell death
- 2 Cell death triggered by pro-inflammatory signals and subsequent activation of the
- 3 NLRP3 inflammasome.
- 4 Lipocalins
- 5 Small extracellular proteins that are responsible for the transport of hydrophobic
- 6 molecules, such as lipids, steroids and retinoids, in the circulation.

Figure 1

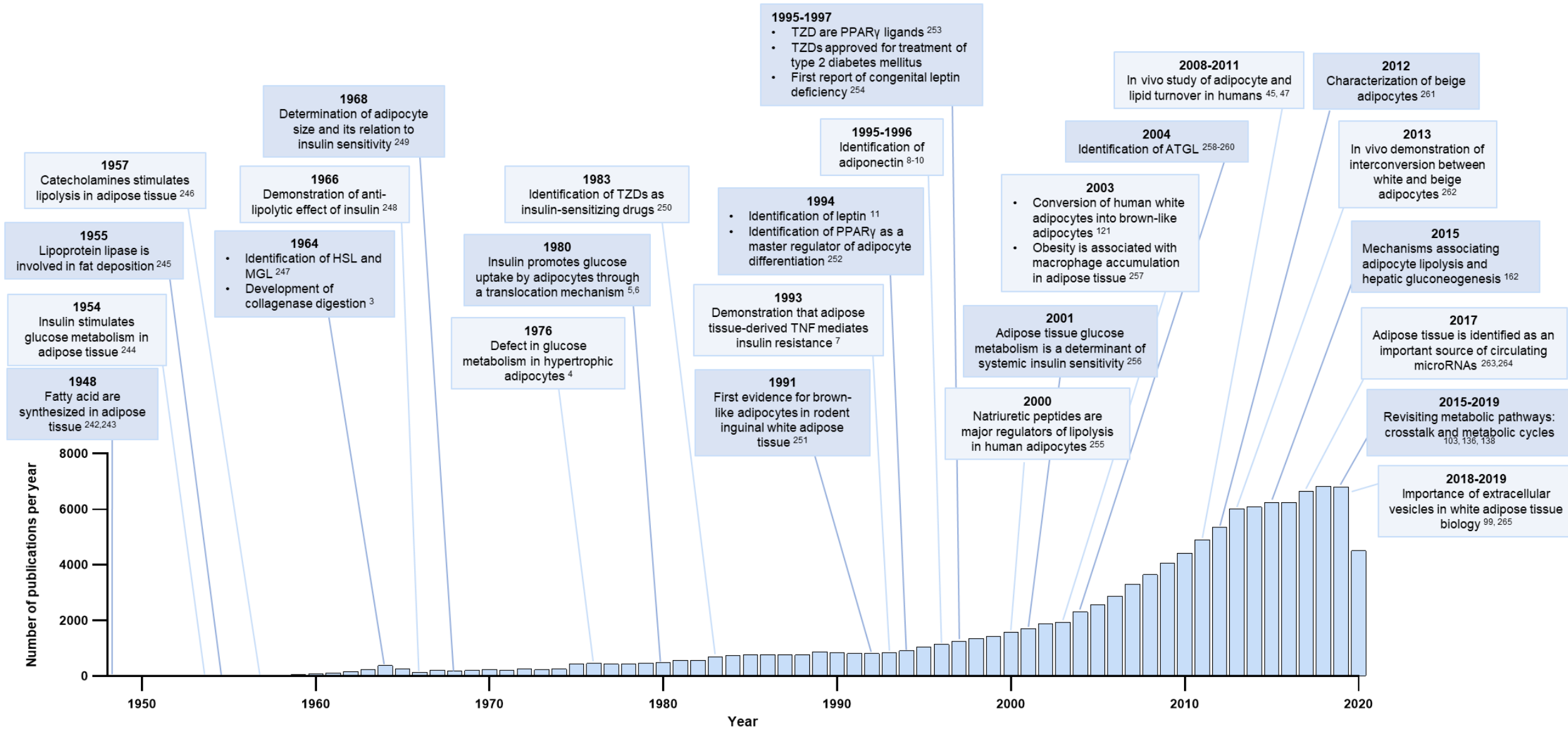
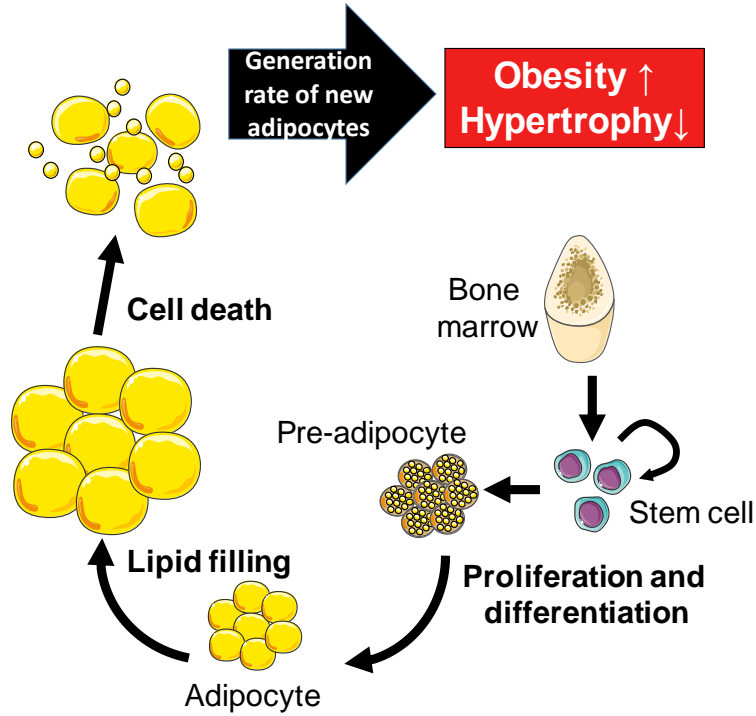
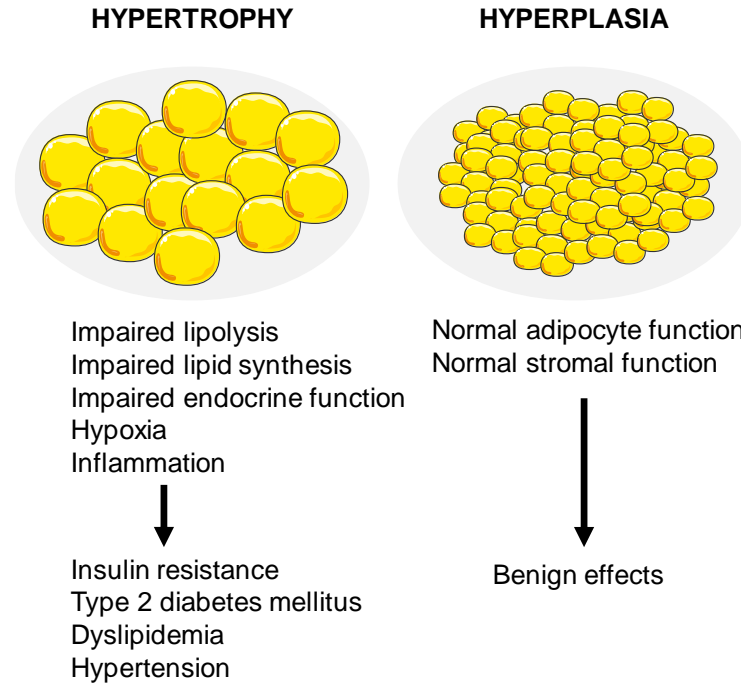


Figure 2

a



b



c

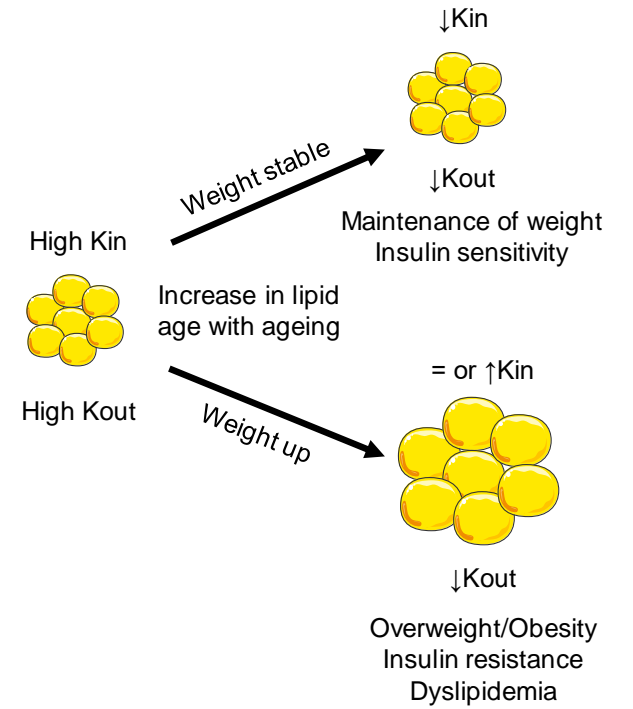


Figure 3

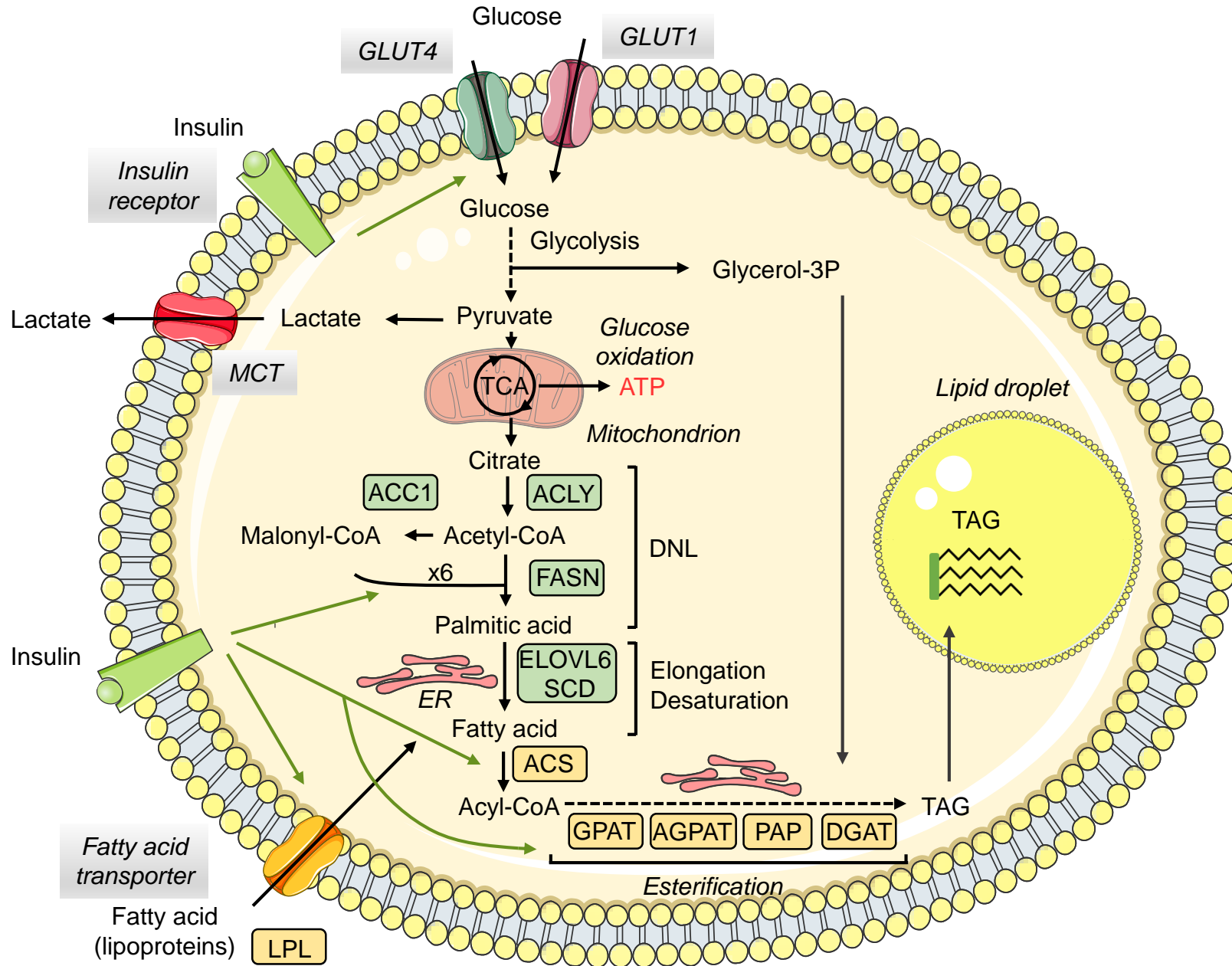
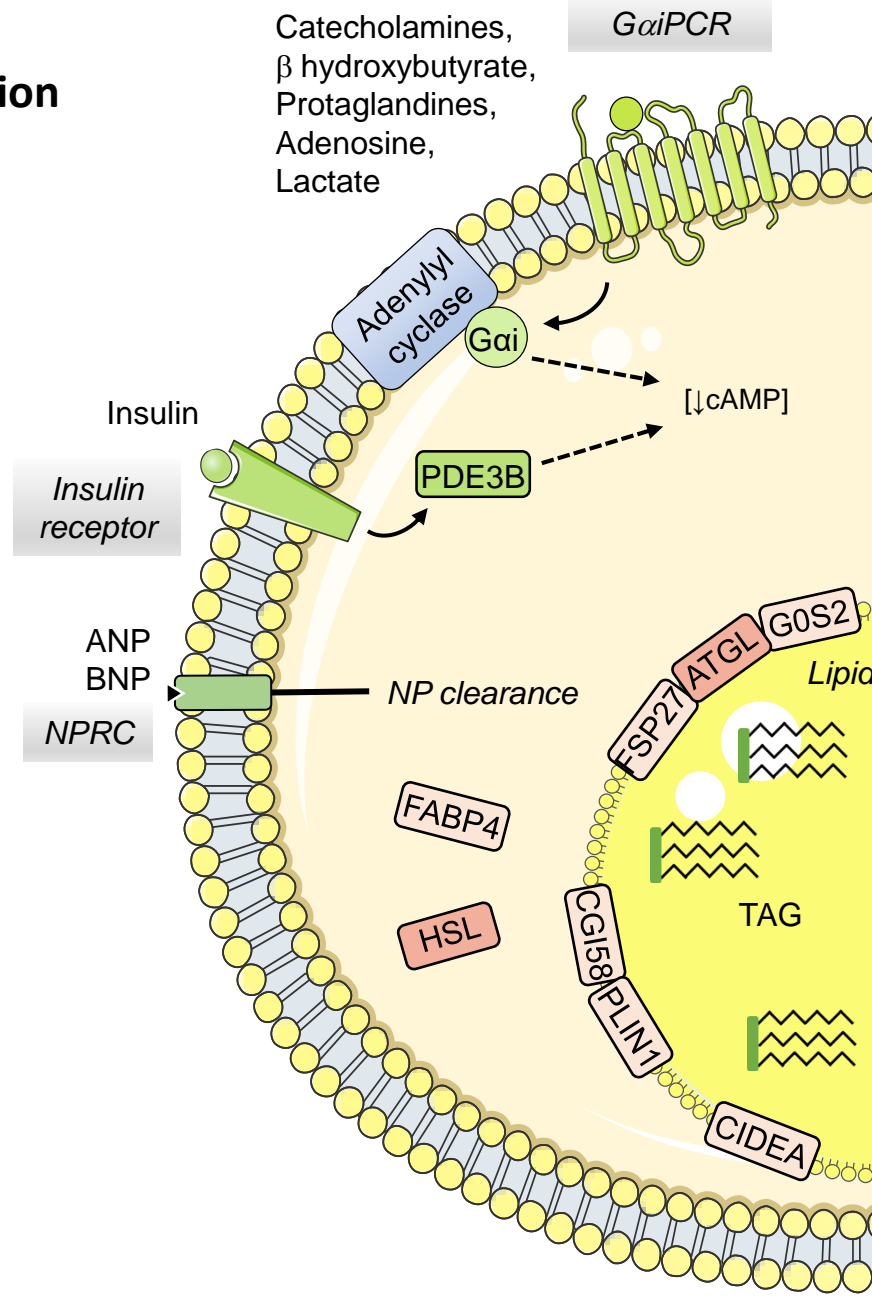


Figure 4

a. Basal condition



b. Stimulated condition

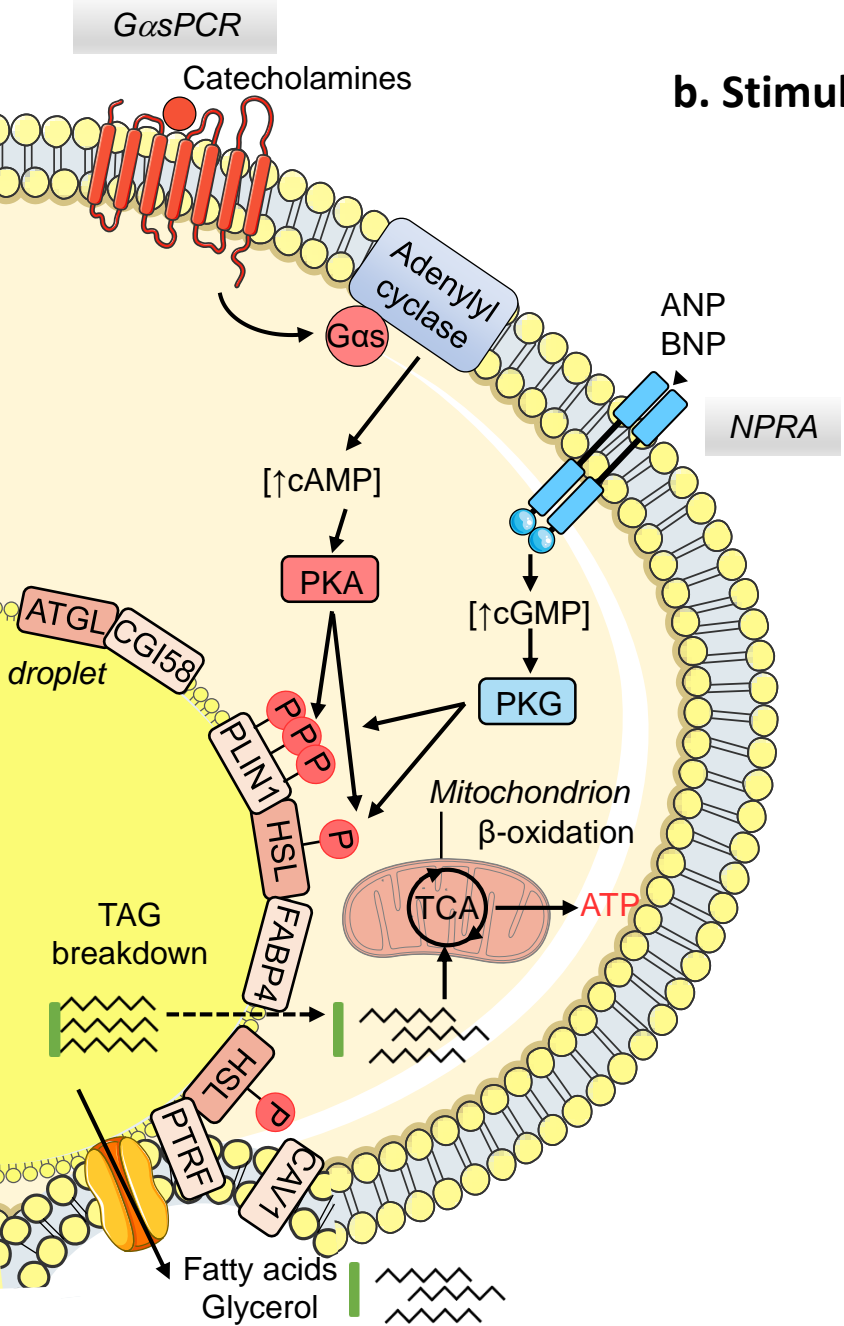
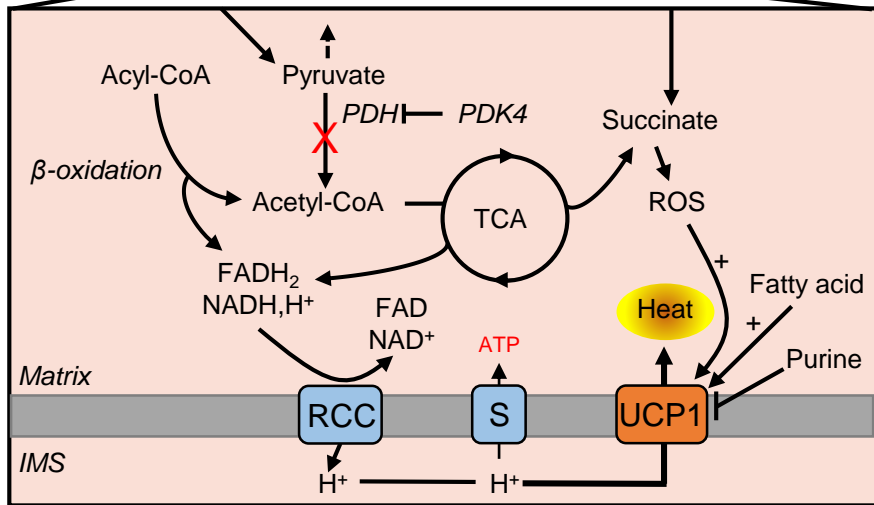
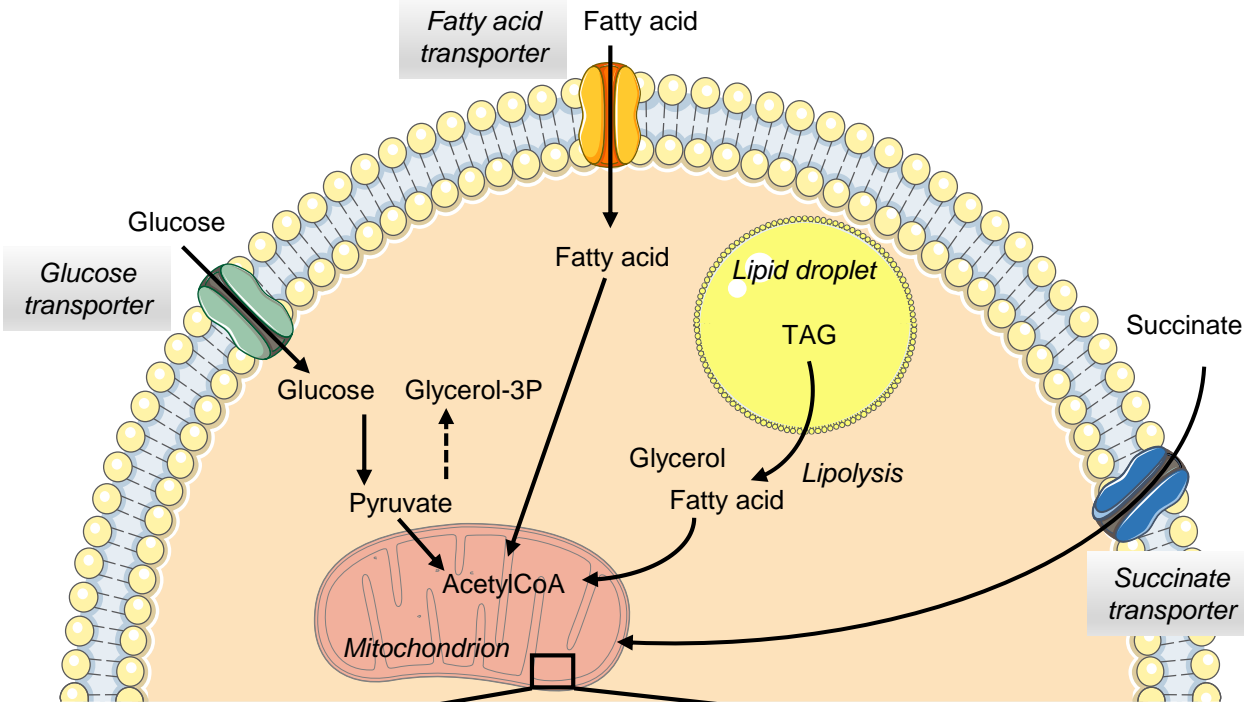


Figure 5

a. UCP1-dependent energy dissipation



b. UCP1-independent energy dissipation

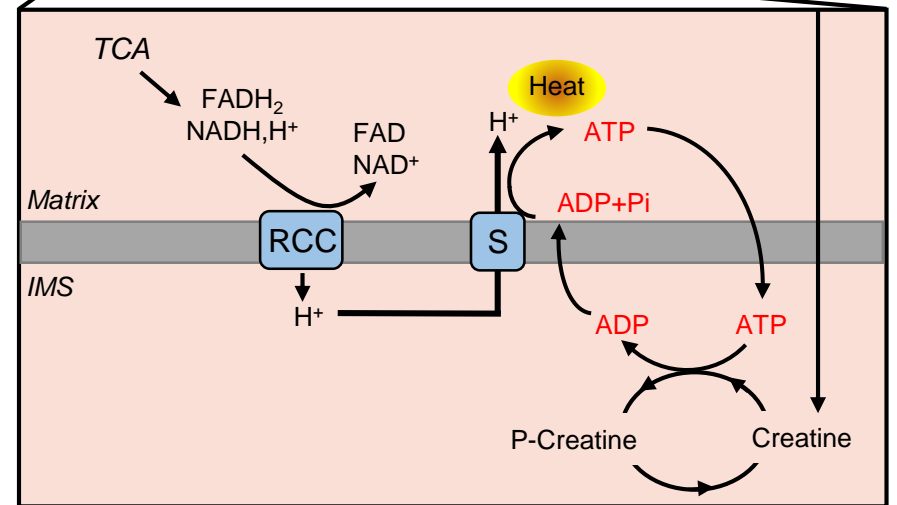
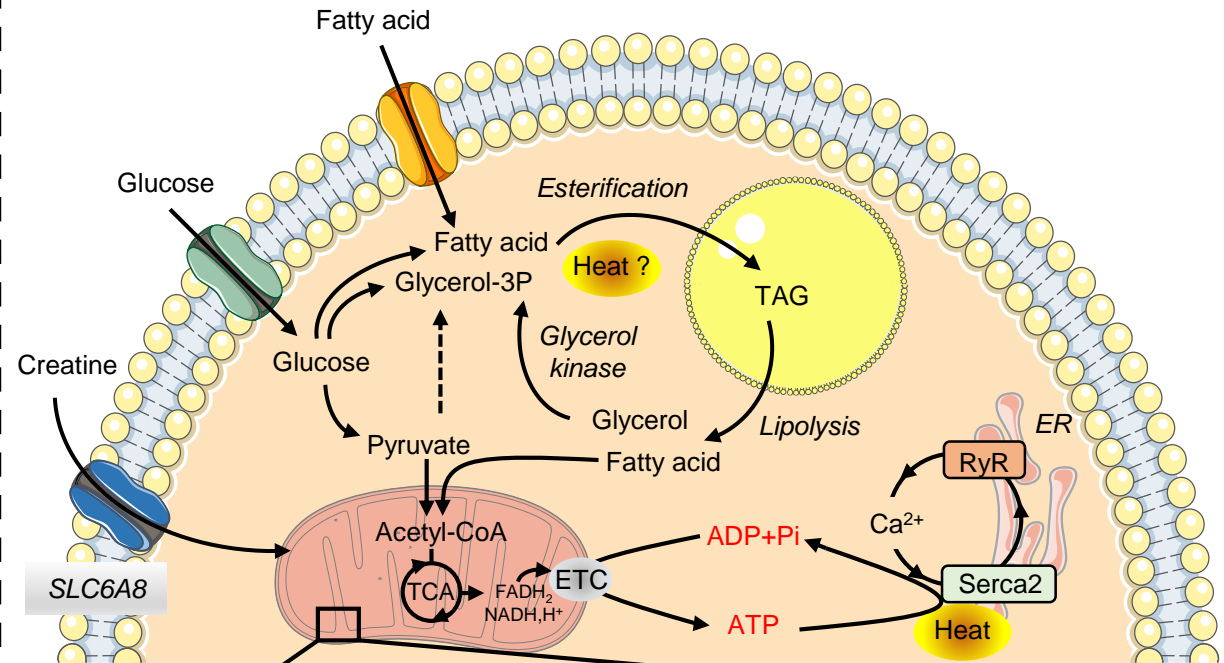


Figure 6

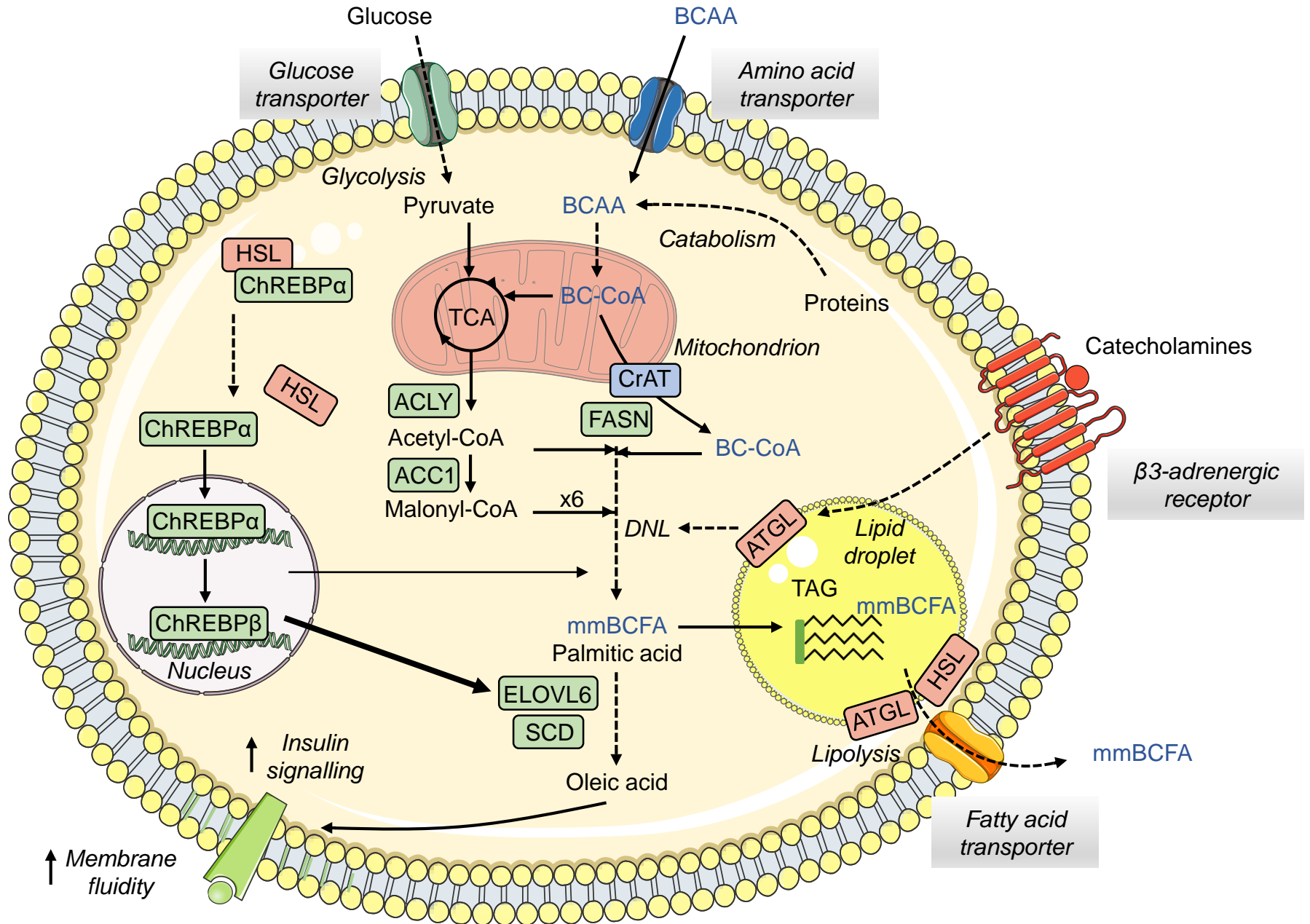


Figure 7

