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SYMPOSIUM REVIEW

Adipocyte function and the development of cardiometabolic disease

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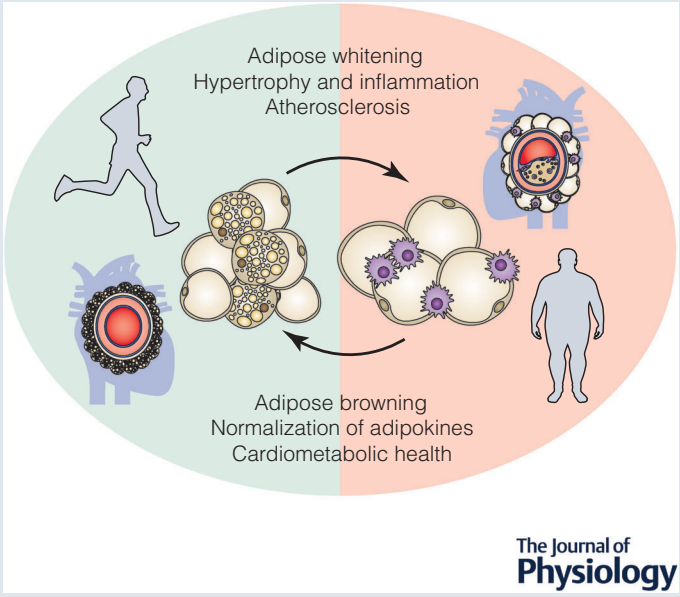

Abstract Obesity is a medical disorder caused by multiple mechanisms of dysregulated energy balance. A major consequence of obesity is an increased risk to develop diabetes, diabetic

Maude Giroud has been working on adipose tissue metabolism and obesity since 2012, when she started her PhD on adipose tissue plasticity in the team of Zoubir Amri at the university of Nice Sophia Antipolis in France. She pursued her scientific career as a postdoc at the Helmholtz Centre Munich in the team of Stefan Herzig where she studied novel transcription factors in adipogenesis as well as the role of long non-coding RNAs in thermogenic adipocytes. In 2020–2021, Maude was a senior postdoc in the lab of Alexander Bartelt and gained expertise in the field of obesity-related cardiovascular diseases. After her degree in veterinary medicine, **Henrika Jodeleit** received her doctoral degree from LMU Munich for her work on autoimmune responses in chronic inflammatory diseases. She continued this work during her postdoc and was also involved in the development of preclinical models for IBD and other inflammatory diseases. She joined Alexander Bartelt's lab in 2019 as a senior postdoc working on cardiovascular diseases. Her work mainly focuses on adaptive mechanisms in models of myocardial infarction.

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complications and cardiovascular disease. While a better understanding of the molecular mechanisms linking obesity, insulin resistance and cardiovascular disease is needed, translational research of the human pathology is hampered by the available cellular and rodent model systems. Major barriers are the species-specific differences in energy balance, vascular biology and adipose tissue physiology, especially related to white and brown adipocytes, and adipose tissue browning. In rodents, non-shivering thermogenesis is responsible for a large part of energy expenditure, but humans possess much less thermogenic fat, which means temperature is an important variable in translational research. Mouse models with predisposition to dyslipidaemia housed at thermoneutrality and fed a high-fat diet more closely reflect human physiology. Also, adipocytes play a key role in the endocrine regulation of cardiovascular function. Adipocytes secrete a variety of hormones, lipid mediators and other metabolites that directly influence the local microenvironment as well as distant tissues. This is specifically apparent in perivascular depots, where adipocytes modulate vascular function and inflammation. Altogether, these mechanisms highlight the critical role of adipocytes in the development of cardiometabolic disease.

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Abstract figure legend While cardiometabolic health is associated with adipose tissue browning and beneficial adipokine profiles, obesity leads to adipose tissue whitening, inflammation and atherosclerosis.

Introduction

Advancements in public awareness, preventative healthcare and medical procedures have led to a pronounced global decline in cardiac mortality in recent decades, reaching an all-time low in the late 1990s (Ezzati *et al.* 2015). However, while the interventions following a cardiac event have improved immensely, factors contributing to cardiovascular disease (CVD) including heart failure, coronary artery disease (CAD) and underlying atherosclerosis are increasing in prevalence, and are predicted to drive an increase in overall cardiac mortality in the coming decades. One main contributor to the increase in CVD prevalence is the global epidemic of obesity, defined as a BMI $\geq$ 30 kg/m^2 (Hruby & Hu, 2015). Affecting both industrialized and rural populations, the demographics of obesity are also changing, with one in five children in the United States being classified as obese according to the Centers for Disease Control and Prevention. One of the underlying factors connecting obesity and CVD is the strong association of obesity with insulin resistance and type 2 diabetes (T2D), resulting in diabetic cardiomyopathy and CAD (Saltiel & Olefsky, 2017; Jia *et al.* 2018). Thus, the increasing prevalence of obesity, particularly at younger ages, is prolonging the lifetime exposure to insulin resistance, T2D and associated cardiovascular complications. Adipocytes are found throughout the body, displaying a broad range of phenotypes depending on developmental origin, functional plasticity and environmental conditions. This review focuses on the biology of adipocytes, as these cells are critical pillars of a healthy metabolism. We discuss the biology of classical white and thermogenic adipocytes, also considering the highly diverse nature of perivascular adipose tissue (PVAT). In addition, we discuss the mechanisms by which adipocyte dysfunction in obesity contributes to the development of cardiovascular disease in humans, and how these processes can be studied in the mouse as a model organism.

CVD, obesity and adipose tissue

Obesity and associated metabolic disorders

Excess adipose tissue accumulation and adipocyte dysfunction have been extensively studied as being causally related to the development of obesity-associated metabolic disease. Under conditions of overnutrition, excess calories are stored by adipocytes in the form of triglycerides, which are subsequently liberated by lipolysis under conditions of nutrient deprivation. Over thousands of years, human metabolism has evolved to efficiently preserve energy stores, allowing us to survive periods of food scarcity. Nowadays, however, conditions of starvation or nutrient deprivation are uncommon, and instead humans are exposed to continuous nutrient excess, resulting in high rates of obesity (McLaughlin *et al.* 2016). Despite adipocytes having a remarkable lipid storage capacity, their size is metabolically and mechanically limited. Proper adipose tissue expansion is healthy but limited vascular oxygen supply and spatial constraints represent

major challenges for adipose tissue expanding properly. Once pushed beyond their expansion limit, adipocytes lose the ability to respond appropriately to metabolic cues, which results in impaired mitochondrial function and endoplasmic reticulum (ER) stress. This stress response is closely linked to insulin resistance, uncontrolled lipolysis, aberrant adipokine secretion, and the secretion of proinflammatory cytokines and chemokines (Prentice *et al*. 2019). The recruitment of immune cells into adipose tissue is initially advantageous for tissue repair, but in the condition of obesity it becomes maladaptive, and chronic adipose tissue inflammation further promotes adipose tissue dysfunction.

Obesity and dyslipidaemia

In obesity, adipose tissue dysfunction is causally related to the collapse of systemic metabolism, as metabolic tissues including the liver, muscle and pancreatic β-cells are no longer able to maintain metabolic homeostasis. It is important to realize, however, that while adipose dysfunction is the origin of many metabolic problems in obesity, it is not solely responsible for the pathological outcomes. If the other tissues maintain homeostasis, adipose dysfunction alone has little impact. The dysfunction of the peripheral tissues is partially driven by ectopic lipid accumulation and exposure to lipotoxic conditions associated with the failure of adipocytes to adequately store and maintain lipid homeostasis. These perturbations further induce resistance to several key hormones such as insulin and leptin, while inflammatory mediators pathologically impair proper interorgan communication (Longo *et al*. 2019). The manifestation of metabolic disease is typically associated with a distinct pathological plasma profile, including elevated glucose, insulin and lipids, as well as lower levels of the adipokine adiponectin, and increased inflammatory cytokines and chemokines. While this scenario is greatly improved by weight loss therapies and various lipid-lowering and insulin sensitizing medications, chronic lipotoxic exposure ultimately results in failure of the β-cells to compensate for insulin resistance by producing and secreting more insulin, marking a point of no return to developing T2D (Kusminski *et al*. 2016; Longo *et al*. 2019). Diabetic dyslipidaemia, which is the insulin resistance-linked increase in atherogenic plasma lipoproteins, is a strong risk factor for developing CAD and CVD, providing a direct link between obesity-associated adipocyte dysfunction and manifestation of CVD. The role of adipocyte dysfunction in CVD is further supported by the inverse condition to obesity, lipodystrophy, wherein the virtual absence of adipose tissue contributes to increased CVD risk. Much like obesity, lipodystrophic patients are also incapable of appropriately storing lipids; however, in this case it is due to the virtual absence of functional adipocytes. Lipodystrophic patients, for example, subjects carrying loss-of-function mutations in the gene coding for the critical adipocyte transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), present the whole spectrum of metabolic deterioration usually seen in patients with genetic or acquired obesity, including insulin resistance, non-alcoholic fatty liver disease, dyslipidaemia, hypertension and atherosclerosis (Mirza *et al*. 2019; Sollier *et al*. 2020). Thus, healthy adipose tissue, and most critically appropriate adipocyte functionality, is central to the prevention of diabetes and associated CVD.

Mouse models for studying adipocyte function in obesity and CVD

As with most diseases, basic investigations of the molecular mechanisms linking adipose dysfunction and CVD heavily rely on the use of model organisms. While the association of adipocyte function, obesity and CVD in humans is evident, it is important to realize that using the mouse as a model organism has limitations. In most cases, genetic and/or dietary interventions are required to induce obesity or CVD; however, most obesity models do not develop CVD, and vice versa. The most commonly used models for the study of obesity are genetically lacking critical components of satiety signalling, for example, loss-of-function mutations in the gene coding for the adipokine leptin (the *obese ob*/*ob* mouse) or its receptor (the *diabetes db*/*db* mouse), which are associated with excessive food intake, severe obesity and systemic metabolic dysfunction (Wang *et al*. 2014*a*). In these cases, the mouse model relatively closely recapitulates the human condition, as humans with these corresponding mutations also develop severe early-onset obesity associated with excessive food intake and metabolic disease. Of course, this condition is exceptionally rare in the human population, thus models of diet-induced obesity (DIO), wherein mice are fed with obesogenic high-fat diets, are an important alternative and complementary model allowing for the study of the effects of obesity on tissue function, plasma parameters and overall manifestation of diabetes. Studying the connection between obesity and atherosclerosis, however, is complicated by the fact that plasma lipid metabolism and the propensity for developing CVD differs greatly between humans and most mouse models. Generally, atherosclerosis is a chronic immunometabolic disease, initiated by the retention of lipoprotein particles in the vessel wall, and driven by a complex immune response and abnormal growth of vascular smooth muscle. Years of chronic exposure to atherogenic conditions leads

to the formation of advanced atherosclerotic plaques, which narrow the vessel wall, limiting perfusion and oxygen supply. Particularly dangerous are vulnerable plaques that have a high propensity to rupture, and the subsequent thrombus formation leads to local and distant occlusion of blood vessels, ultimately resulting in life-threatening, potentially fatal myocardial infarction, stroke, or embolism (Bentzon *et al.* 2014). In humans, a major atherogenic factor is low-density lipoprotein (LDL). Following decades of research, it has been established that there is an unequivocal causal role for elevated plasma LDL cholesterol in atherosclerosis (Steinberg & Witztum, 2010). However, in regular mice, plasma cholesterol is almost exclusively found in high-density lipoproteins (HDL), while atherogenic LDL and lipoprotein remnant levels are too low to initiate significant atherosclerotic lesion formation (Camus *et al.* 1983; Gordon *et al.* 2015). In addition, in both genetic and dietary mouse models of obesity, only a minor increase in atherogenic lipoproteins is observed, even upon feeding with a cholesterol-rich diet. Hence, most atherosclerosis studies rely on genetic mouse models, in which critical parts of lipoprotein metabolism are absent or dysfunctional.

The most frequently used animal models to study atherosclerosis are apolipoprotein E (ApoE) and LDL receptor (LDLR) KO mice (Getz & Reardon, 2012). ApoE is a small glycoprotein found on triglyceride-rich lipoproteins and HDL, and serves several important functions in lipoprotein metabolism, including the removal of cholesterol-rich remnant lipoproteins as well as proper HDL turnover. ApoE is a ligand for the LDLR, which also binds apolipoprotein B-containing lipoproteins. Hence, ApoE and LDLR KO mice are primarily characterized by high levels of atherogenic cholesterol-rich lipoproteins and the development of atherosclerotic plaques, which is enhanced when the mice are fed a cholesterol-rich Western diet (Nakashima *et al.* 1994; Johnson *et al.* 2014). However, an atherogenic diet only causes mild insulin resistance compared to DIO, and both ApoE and LDLR KO mice are relatively resistant to DIO (Bartelt *et al.* 2010). To overcome these limitations, ApoE KO mice can be crossed with genetically obese leptin receptor-deficient *db*/*db* mice, which by itself leads to hyperinsulinaemia and insulin resistance (DePaoli, 2014). ApoE KO *db*/*db* mice are more susceptible to atherosclerosis than ApoE KO mice (Getz & Reardon, 2016). Intriguingly, when ApoE mice are crossed with the corresponding leptin deficient *ob*/*ob* model, the ApoE KO *ob*/*ob* mice develop fewer plaques than ApoE KO mice, indicating that leptin accelerates the development of atherosclerosis. In both *ob*/*ob* and *db*/*db* mice lacking LDLR a decrease in atherosclerotic plaques can be observed when compared to LDLR KO mice (Chiba *et al.* 2008). Altogether, while these studies indicate that leptin plays an important role in atherosclerosis development in ApoE and LDLR KO models, the natural state of obesity is more difficult to study in these mice.

Another general difference between mouse models and human atherosclerosis is that both ApoE and LDLR KO mice mainly develop atherosclerotic lesions in the large arteries such as the aorta (Isobe *et al.* 2006). Even ApoE and LDLR double KO mice require prolonged cholesterol exposure to cause lesion formation in coronary arteries, which is still insufficient to cause myocardial infarction. This general limitation of mouse models can be partly overcome by surgical transverse aortic constriction (TAC) or ligation of the left anterior descending artery (LAD), which both generate phenotypes more closely resembling human CAD. The TAC procedure causes hypertension in ApoE KO mice, leading to atherosclerosis in coronary arteries (Marino *et al.* 2019), and, when subjected to physical exercise, these mice reveal signs of myocardial infarction (Golforoush *et al.* 2020). Likewise, the ligation of the LAD limits blood supply, immediately causing an ischaemic event, which mimics myocardial infarction. When LAD ligation was performed on ApoE KO, an additional effect on atherogenesis was observed (Wright *et al.* 2010). Considering these limitations, alternative mouse models have been studied over the last two decades that offer translational and technical advantages. The discovery of apolipoprotein E3-Leiden, a variant of human apolipoprotein E associated with familial type III hyperlipoproteinaemia (Havekes *et al.* 1986) led to the development of a mouse model carrying this variant as a transgene (van den Maagdenberg *et al.* 1993). In recent years, the human cholesteryl ester transfer protein (CETP), which is naturally absent in mice, was added as a transgene to give rise a model with a humanized lipoprotein profile (Berbée *et al.* 2015). The discovery of mutations in the gene *PCSK9* (*proprotein convertase subtilisin/kexin type 9*) that cause autosomal dominant hypercholesterolaemia (Abifadel *et al.* 2003) have led to a simplified approach to induce atherosclerosis in mice, irrespectively of the genetic background. Viral delivery of PCSK9 to the liver of mice induces overexpression of protein in circulation. As PCSK9 degrades the LDLR, these mice develop a lipoprotein profile and atherosclerotic lesions similar to common LDLR KO mice (Maxwell & Breslow, 2004). Viral delivery of PCSK9 now enables inducing atherosclerosis without lengthy breeding of genetic models of interest into the above-mentioned atherogenic backgrounds of ApoE and LDLR KO. These findings highlight the roadblocks for studying obesity-associated atherosclerosis in these mouse models and demonstrate potential experimental approaches for enhancing the translation of mouse research into human CVD mechanisms (Fig. 1).

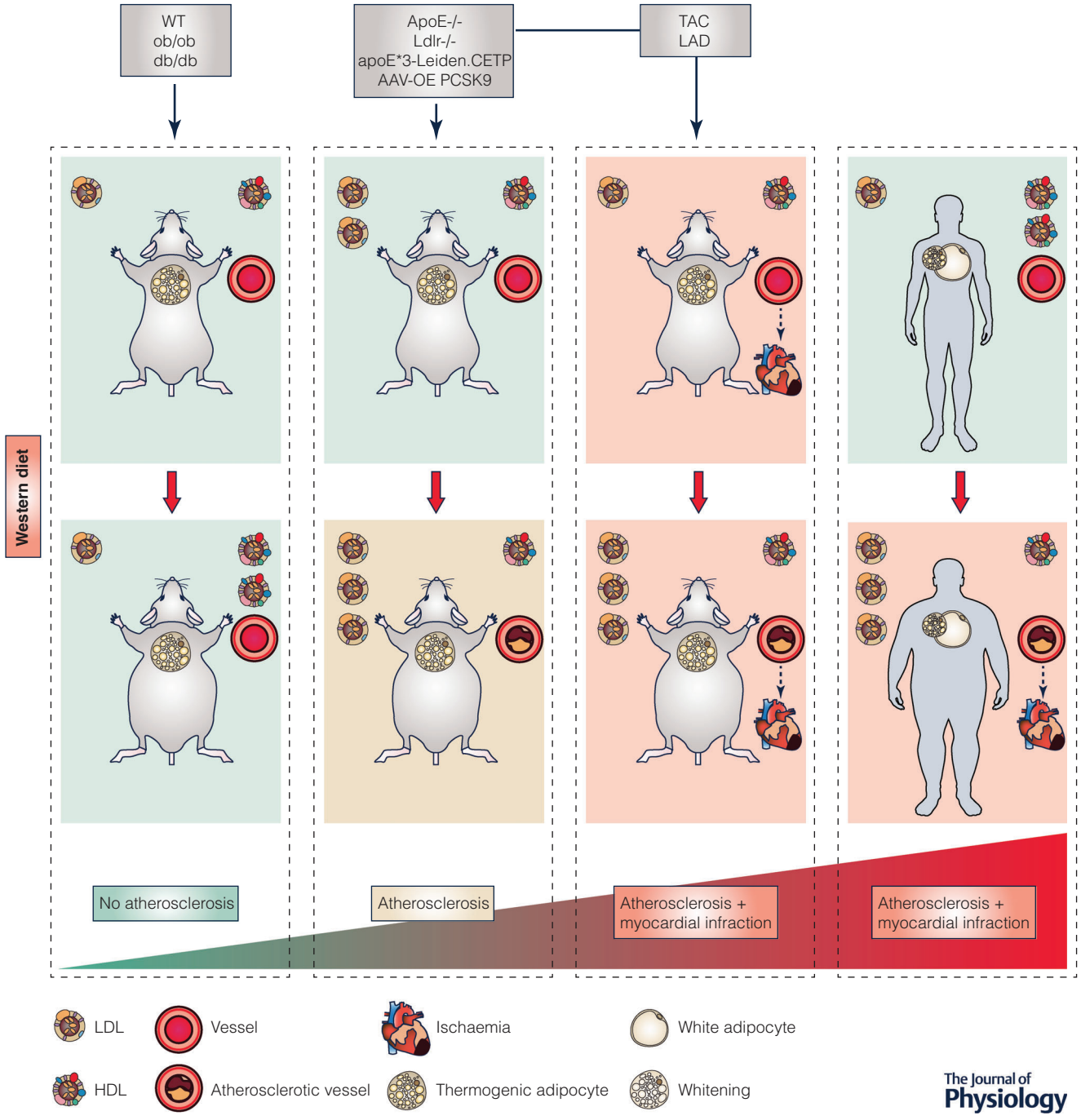

Figure 1. Mouse models for studying cardiovascular disease and obesity
The mouse is a suitable model organism for studying obesity or atherosclerosis but investigating the relationship between obesity and atherosclerosis simultaneously is complicated by differences in plasma lipid metabolism between human and mice. Wild-type (WT) mice or mice with genetic obesity (*ob/ob* or *db/db)* do not develop atherosclerosis, even on cholesterol-rich diets. For studying atherosclerosis Apolipoprotein E (ApoE) and LDL receptor (LDLR) KO mice are the most used models. APOE3∗Leiden.CETP transgenic mice carrying a knock-in for the human hyperlipidaemic *APOE* variant and cholesteryl transfer protein (CETP) display a humanized lipoprotein profile. Injection of adeno-associated virus (AAV) for overexpression (OE) of PCSK9 in the liver is a versatile tool to induce hyperlipidaemia and atherosclerosis independently of the above-mentioned genetic models. Myocardial infarction is surgically induced by transverse aortic constriction (TAC) or ligation of the left anterior descending artery (LAD).

White adipocyte function and dysfunction

The adipose organ is organized in discrete depots that expand with increasing adiposity, both by adipocyte diameter (hypertrophy) as well as by *de novo* adipocyte formation from precursor cells (hyperplasia). Excessive growth of the individual depots can ultimately lead to depots connecting to form a continuous organ. In principle, adipose depots can be generally classified as visceral or subcutaneous, but more specifically there are also cutaneous, perivascular, epicardial, pericardial, intramuscular and thoracic depots, each with novel emerging roles in cardiometabolic health. In healthy adults the majority of white adipose tissue (WAT) is found subcutaneously, serving as an insulator, but also as the body's main energy storage depot (Bjørndal *et al.* 2011). In obesity, accumulation of adipose tissue in the waist area, usually referred to as 'belly fat', confers a significantly higher cardiometabolic risk than adipose around the hips and legs. Thus, in addition to the relatively crude classification of obesity by BMI, an important aspect of assessing cardiometabolic risk is determining the hip-to-waist ratio, which indicates the relative distribution of adipose tissue depots in the body. The human condition of lipodystrophy provides further insight into the relative contribution of specific adipose depots to CVD risk. In patients where subcutaneous depots are specifically absent, there is accumulation of visceral fat, dyslipidaemia, ectopic lipid storage, insulin resistance and accelerated atherosclerosis (Mann & Savage, 2019). The fundamental differences between adipose depots and associated CVD risk mainly arise from the differences in physical expandability, metabolic activity and endocrine targeting of subcutaneous *vs.* visceral fat depots. Understanding the mechanisms regulating adipogenesis is of importance to modulate lipid metabolism and prevent CVD. Several transcription factors have been associated with adipocyte differentiation, primarily PPAR and CCAAT/enhancer-binding protein (C/EBP) protein families. PPARγ is the master regulator of adipogenesis, and is both necessary and sufficient for adipocyte differentiation, acting to regulate expression of numerous adipogenesis genes including C/EBPα. PPARγ and C/EBPα function synergistically to potentiate an adipocyte-specific gene expression profile, and thus regulate adipocyte development and function. Adipocyte-specific PPARγ KO mice exhibit severe lipodystrophy, abnormal lipid accumulation in the liver, insulin resistance, and increased serum triglyceride and free fatty acid levels (Wang *et al.* 2013). Over the years, many more transcriptional regulators of adipocyte differentiation have been discovered, including some that are expressed by both adipocytes and cells of the cardiovascular system, such as heart- and neural crest derivatives-expressed protein 2 (HAND2) (Giroud *et al.* Diabetologia 2021). Overall, the ability of adipocytes to properly differentiate and function is critical to prevent dyslipidaemia and CVD.

During catabolic phases such as fasting or exercise, adipocytes break down triglycerides into non-esterified fatty acids (NEFAs) that are released into the circulation, repackaged by the liver into very low-density lipoproteins (VLDL), and released to supply the body with energy (Lammers *et al.* 2011). Conversely, during the postprandial phase, lipids enter the blood stream as chylomicrons. Both VLDL and chylomicrons are rapidly hydrolysed by lipoprotein lipase located on the surface of vascular endothelial cells, releasing NEFAs that are taken up by peripheral tissues (Fielding, 2011). NEFAs are internalized by the fatty acid translocase cluster of differentiation-36 (CD36) and fatty acid transport proteins (FATPs). Once in the cytoplasm, NEFAs are shuttled by lipid chaperones of the fatty acid binding protein (FABP) family and are converted into acyl-CoA by fatty acyl-coA synthetase. The resulting acyl-CoA can then either be converted into acylcarnitine, allowing it to be transported into the mitochondria where it enters β-oxidation, or to be converted to lipid metabolites for storage, depending on the energy status of the organism. Under anabolic conditions, the acyl-CoA is converted into triacylglycerol (TAG) lipid droplets by glycerol-3-phosphate acyltransferases (GPATs and AGPATs) and the diacylglycerol transferase (DGAT) enzymes in the ER membrane. Accumulation of TAG in the ER builds nascent lipid droplets that bud from the ER to form intracellular lipid droplets (Guo *et al.* 2009). This process is stimulated by anabolic hormones, most notably insulin.

If energy intake exceeds energy expenditure, the accumulation and growth of lipid droplets causes adipocytes grow in diameter, a process named hypertrophy (Liu *et al.* 2020). In addition, preadipocytes in the adipose depots are also recruited to differentiate into adipocytes (hyperplasia). In most cases, the dominant process is hypertrophy over hyperplasia. However, adipocytes are limited in their capacity to grow in diameter, and beyond a certain threshold of size, hypertrophic cells lose their functionality and are characterized by decreased secretion of adipokines such as adiponectin, even though they exhibit increased lipolysis and release of NEFAs caused by insulin resistance (Steppan *et al.* 2001). With increasing diameter but unchanged vascular supply, hypertrophy causes hypoxia, which further compromises adipocyte health and activates expression of hypoxia-inducible factor-1α (HIF1α). Adipocyte-specific HIF1α KO mice are protected from insulin resistance whereas mice overexpressing HIF1α develop insulin resistance and adipose tissue inflammation (Lee *et al.* 2014). Immunostaining of adipose tissue shows a correlation between areas of hypoxia and sites of

macrophage infiltration, consistent with the impaired survival and function of hypertrophic, hypoxic adipocytes (Saltiel & Olefsky, 2017). The reduction in adiponectin secretion further exacerbates this process, as adiponectin directly stimulates the production of nitric oxide (NO) in endothelial cells, which suppresses inflammation and immune cell infiltration (Chen *et al.* 2003). Other adipokines including omentin, apelin, visfatin, FABP4 and chemerin also play a role in CVD through the regulation of inflammation, hypoxia, apoptosis and lipid metabolism (Oikonomou & Antoniades, 2019). Hence, altered lipid metabolism as a hallmark of adipocyte dysfunction is a key factor for the development of CVD in obesity (Fig. 2).

On the tissue level, obesity is characterized by maladaptive adipose tissue remodelling, hypoxia, inflammation, and insulin resistance (Bartelt *et al.* 2017). Intracellularly, aberrant accumulation of lipids results in organelle dysfunction and an inability to maintain metabolic balance. The concerted activity of ER, mitochondria and lipid droplets are paramount to regular adipocyte function and obesity is characterized by ER stress. Constant nutrient exposure imposes diverse challenges on the ER, including lipotoxicity and insufficient protein folding. In mouse models of obesity, a chronic upregulation of the unfolded protein response (UPR) has been demonstrated, which results in the activation of inflammatory pathways and direct inhibition of insulin action (Hotamisligil *et al.* 1994). Organelle stress does not occur in isolation, and the ER is physically and functionally connected to all organelles through ER contact sites (Lemmer *et al.* 2021). Lipid droplets arise from the ER, are surrounded by many structural and functional proteins named perilipins. Perilipin 1 (PLIN1) and perilipin 2 (PLIN2) are highly abundant in adipocytes and play a role of protection of the lipid droplets against hydrolases

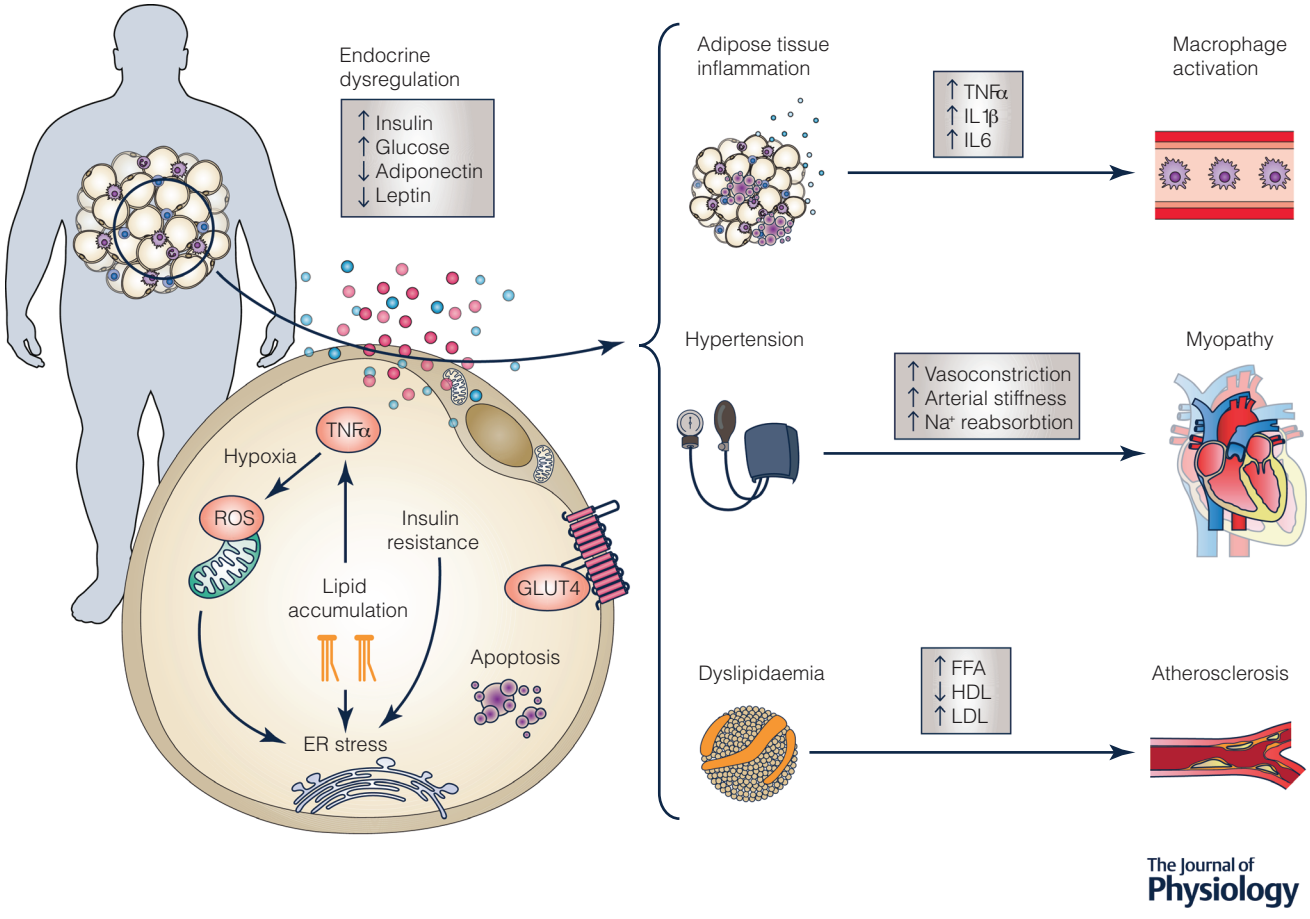

Figure 2. Cardiovascular complications caused by obesity-related endocrine dysregulation
Obesity is a challenging condition for the adipocyte, as lipid accumulation is a linked to hypoxia, mitochondrial and ER stress, ultimately leading to apoptosis and inflammation. Also, the endocrine profile of the adipocyte is changed in obesity, resulting in insulin resistance, diminished adiponectin levels and hyperleptinaemia. Altogether, this local and endocrine dysfunction affects systemic metabolism. Blood pressure is also affected by an increase in vasoconstriction, arterial stiffness, and Na^+ reabsorption, leading to severe myopathy. Finally, in obesity, dyslipidaemia with increased levels of LDL cholesterol and decreased levels of HDL cholesterol is a major cardiovascular risk factor.

such as adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoacylglycerol lipase (MGL). Upon β-adrenergic receptor activation, PLIN1 is phosphorylated by protein kinase-A, allowing lipolysis and lipid release (Tansey *et al.* 2003; Zimmermann *et al.* 2004). Usually, insulin action suppresses lipolysis, but in obesity, insulin resistance is associated with uncontrolled lipolysis. The increase in lipolysis not only increases circulating lipid levels, but also causes intracellular NEFA accumulation. A part of the NEFA is converted to acyl-CoA and subsequently to acylcarnitine by the mitochondrial protein carnitine palmitoyltransferase 1 (CPT1). Acylcarnitine can then transverse the mitochondrial membrane into the matrix, where it is reversed to acyl-CoA by CPT2 and utilized in β-oxidation for the generation of chemical energy and acetyl-CoA (Bose *et al.* 2019). Under normal conditions, adipocytes primarily generate acetyl-CoA through glycolysis, with a secondary contribution from β-oxidation. In obesity, the increase in β-oxidation is associated with induction of oxidative stress and inflammation, which further exacerbates adipose dysfunction. Therefore, the ability of adipocytes to regulate intracellular and systemic metabolism is dependent on the concerted activity and health of the ER, lipid droplets and mitochondria

Thermogenic adipocyte function and dysfunction

Types of thermogenic adipocytes and where to find them

In addition to WAT, mammals also possess brown adipose tissue (BAT), which mainly consists of thermogenic brown adipocytes. In contrast to white adipocytes, brown adipocytes are characterized by high mitochondrial content and multiple small lipid droplets (Scheja & Heeren, 2016). While sharing several basic adipogenic and lipolytic features with white adipocytes, thermogenic adipocytes are distinct in their ability to transform chemical energy into heat to support the maintenance of body temperature in homeotherm animals. In humans, BAT depots are located around the claviculae, the sternum and in the paravertebral, epicardial and perirenal regions. For decades, its relevance in adult humans was considered relatively minor compared to rodents. However, interest in the development and function of BAT was renewed when active BAT depots were detected as hotspots of high glucose tracer uptake in positron emission computed tomography (PET-CT) in humans (Nedergaard *et al.* 2007). There is an inverse correlation between presence of BAT in PET-CTs and BMI, indicating that BAT, or lack thereof, may play a role in obesity (Wang *et al.* 2015). More recent evidence has corroborated the notion that BAT activity is inversely correlated with parameters of cardiometabolic disease (Becher *et al.* 2021). Activating brown adipocytes in obese patients could be a natural way to burn extra calories, reduce lipid accumulation and prevent CVD. However, compared to rodents, BAT in humans is relatively scarce. Using BAT as a therapy for humans is further complicated by the fact that BAT activity decreases in obesity and with age (Cypess *et al.* 2009).

The primary mediator of non-shivering thermogenesis (NST) is uncoupling protein-1 (Ucp1), which is a fatty acid-activated proton carrier that uncouples the electron transport chain from ATP production. NST is heat production due to metabolic energy transformation that does not involve contraction of skeletal muscles. Other, probably more ancient mechanisms of futile cycling of creatine and calcium have been described in the absence of Ucp1 (Roesler & Kazak, 2020). Some studies have proposed that, based on transcription profiles, human BAT resembles inducible brown-like cells in mouse WAT depots, so-called 'beige' adipocytes (Wu *et al.* 2012). However, human BAT is diverse and both beige and classical brown adipocyte phenotypes are found (Wu *et al.* 2012; Cypess *et al.* 2013). Beige adipocytes are phenotypically flexible and resemble white adipocytes at warmer temperatures but adopt a thermogenic phenotype by cold stimulation, the so-called 'browning' of WAT (Abdullahi & Jeschke, 2016). In principle, beige adipocytes are as thermogenic as brown adipocytes (Petrovic *et al.* 2010) but BAT as a depot has features that make it a superior heater such as higher vascularization. Beige adipocytes develop either postnatally in WAT through cold and adrenergic stimulation, or through trans-differentiation of white adipocytes and are further characterized by an inducible Ucp1 expression (Wu *et al.* 2012). Interestingly, the epicardial adipose depot (EAT) in the peri-coronary area and perivascular adipose tissue (PVAT) surrounding blood vessels show characteristics of both white and brown adipocytes depending on their locations in the thoracic or abdominal regions (Fitzgibbons *et al.* 2011). Due to its proximity to the vessel, these adipose depots directly regulate vascular biology by providing mechanical support and by regulating vascular tension (Akoumianakis *et al.* 2017). In summary, these metabolic and anatomic cues suggest thermogenic adipocytes may play a role in obesity and related CVD.

Concept of thermoneutrality and thermogenesis: role of β-adrenergic signalling

Unlike ectotherms, endotherm animals such as mammals maintain their temperature at a metabolically favourable level. The basal metabolic rate is the minimum energy required to sustain vital functions while maintaining body temperature. It is measured at thermoneutrality

in a fasted state at complete rest. Consequently, at thermoneutrality, which for many mouse strains and models is approximately 28°C–30°C, mice require and display minimal thermogenesis. As in the thermoneutral zone their thermogenic function is obsolete, brown and beige adipocytes adopt a more lipid-laden, white phenotype with low mitochondrial and low Ucp1 content. Conversely, at standard laboratory housing temperatures (∼22°C) the animals are below thermoneutrality, BAT is activated, and there is considerable adipose browning with thermogenic adipocytes expressing high levels of UCP1, high nutrient uptake and smaller lipid droplets. Under these conditions, the appearance of thermogenic adipocytes in WAT depots originates either by *de novo* differentiation from mesenchymal stem cells or trans-differentiation from dormant beige adipocytes (Rosenwald *et al.* 2013). The best characterized and natural mechanism for brown adipocyte activation and adipose browning is cold exposure. When exposed to temperatures below the thermoneutral zone, sympathetic neurons secrete noradrenaline that binds to β-adrenergic receptors of brown adipocytes, the most powerful stimulus for non-shivering thermogenesis. Prolonged activation of β-adrenergic signalling increases BAT capacity and adipose browning in many ways, which is reviewed elsewhere (Ramseyer & Granneman, 2016).

Activation of BAT: impact on obesity and CVD

Cold acclimatization is a complex process that not only involves the recruitment of thermogenic adipocytes but also numerous other physiological changes, such as higher food intake and higher blood pressure. In general, short-term cold exposure of a few hours increases glucose and lipid uptake by thermogenic adipocytes (Bartelt *et al.* 2011), whereas prolonged exposure beyond 24 h leads to cellular remodelling and recruitment of precursor cells (Wang & Seale, 2016). The systemic activation of brown and beige adipocytes by cold (1) quickly induces utilization of intracellular glycogen and lipid stores, (2) increases uptake of glucose and lipoprotein derived NEFAs, and (3) drains remote nutrient stores in liver and WAT. In mice, this leads to enhanced insulin sensitivity as well as improved oral glucose and fat tolerance in as little as 24 h. In hyperlipidaemic mice, cold exposure corrects plasma lipid levels, and in diet-induced obese mice it improves insulin resistance and induces weight loss (Bartelt *et al.* 2011). In humans, BAT activity is linked to lower BMI and improved insulin sensitivity (Becher *et al.* 2021). While these obesity data are in line with studies in mice, the outcomes of cold exposure on CVD are complicated by the mouse models used. A potential caveat is the pronounced increase in food intake, which approximately doubles from 22°C to 4°C. Considering that atherosclerosis is usually studied in models with genetic disruption of lipid metabolism such as ApoE or LDLR knockouts, models that are extremely sensitive to dietary cholesterol, cold acclimatization increases atherogenesis in these mice (Dong *et al.* 2013) caused by the cold-induced increase in food intake (Berbée *et al.* 2015). Alternatively, BAT can also be pharmacologically activated with β3-adrenergic agonists like CL316,243. When ApoE and LDLR KO mice are pair-fed and treated with CL316,243, atherogenesis is similar compared to control mice (Worthmann *et al.* 2019). However, in APOE3∗Leiden.CETP mice with a humanized lipoprotein profile, both cold and CL316,243 treatment lower plasma cholesterol and limit atherosclerosis (Berbée *et al.* 2015). In addition, HDL and reverse cholesterol transport is stimulated by cold and CL316,243 treatment, and inversely correlated to atherogenesis (Bartelt *et al.* 2017). Human pre-clinical studies using mirabegron, an β3-adrenergic agonist, showed that one injection was sufficient to increase BAT activity, energy expenditure, lipolysis and liver fatty acid oxidation, while chronic treatment with mirabegron increased HDL cholesterol and ApoA1 levels (O'Mara *et al.* 2020). Altogether, these studies in mouse models and humans illustrate that the beneficial effects of thermogenic activity on parameters of CVD and imply that the inverse correlation of BAT and metabolic disease observed in humans might underlie a causal relationship. However, regardless of these beneficial effects of BAT, cold-induced activation of adrenergic receptors may have detrimental side effects on the cardiovascular system. Indeed, mirabegron was reported to have cardiovascular side effects at higher doses, most notably increasing blood pressure. Other mechanisms independent of adrenergic receptors also contribute to the systemic adaptation to cold, including cardiac natriuretic peptides, fibroblast growth factor-21 (FGF21), adenosine, prostaglandins and other compounds. Findings from 'Betaless' mice that genetically lack all three β-adrenergic receptors showed that BAT properties remain largely intact; nevertheless, browning requires the β3-adrenergic receptor and depends on the mouse strain. In FVB/N but not in C57BL/6J or 129Sv the browning of WAT is dependent on β3-adrenergic signalling (de Jong *et al.* 2017). Overall, targeting β3-adrenergic signalling is a potential approach to reduce obesity induced CVD via thermogenic adipocyte activation, yet major cardiovascular side effects complicate the utilization of the β3-adrenergic receptor agonist as a safe medication (O'Mara *et al.* 2020) (Fig. 3).

UCP1-dependent and -independent thermogenesis

As UCP1 is a critical mediator of NST, UCP1 KO mice have been extensively studied and this model is often used as a tool for testing specific mechanisms in the absence of NST. Interestingly, in the first reports on

UCP1 KO mice, histological differences in lipid droplet size, being larger in the KO animals, were noted, but no major changes in thermogenic markers were observed. Additionally, no mitochondrial deficiencies were detected in the BAT of UCP1 KO mice originally, but more recent studies indicated the contrary (Stier *et al.* 2014; Kazak *et al.* 2017). Even though it was anticipated that in the absence of Ucp1-mediated NST the energy balance should be positive, KO animals were not prone to weight gain, but were sensitive to acute cold exposure. UCP1 KO mice need to be slowly acclimatized to cold, and there are conflicting reports on whether there is increased muscle

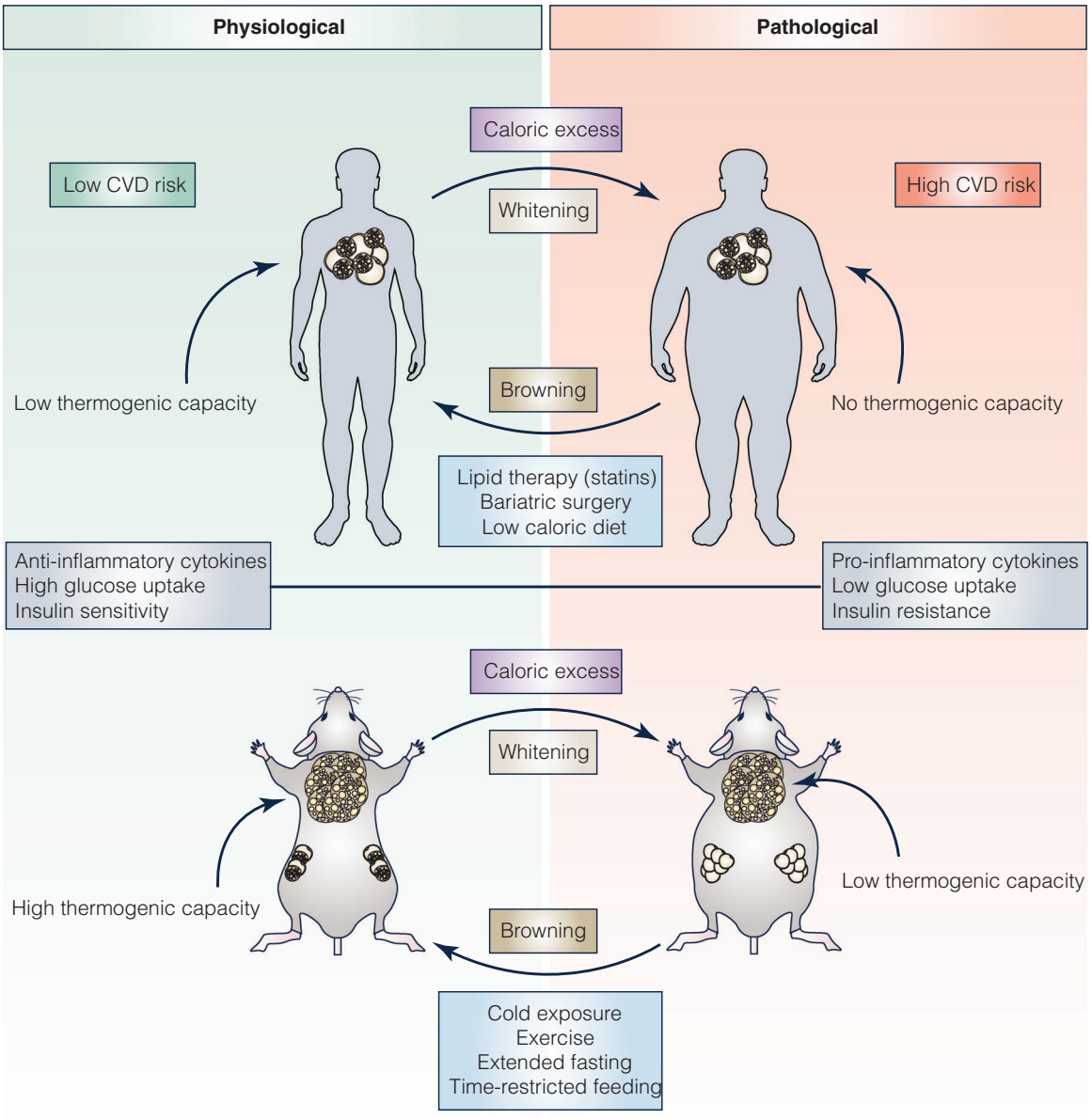

Figure 3. Adipose tissue plasticity and metabolic health
In humans and mice caloric excess induces a pathological metabolic status followed by significant changes in adipocyte physiology including whitening of brown adipose tissue. Consequently, the risk of developing cardiovascular disease increases, caused by an increase in inflammatory cytokine secretion, the development of insulin resistance, and a decrease of glucose uptake. To recover a healthy metabolic status several options are available, e.g. low calorie diet, lipid therapy or bariatric surgery. In mice, cold exposure, exercise and different fasting induce browning of white adipose tissue, restoring the metabolic functions impaired by caloric excess. The low thermogenic capacity of humans is best mimicked in mice housed at thermoneutrality (30°C).

shivering in this model (Enerbäck *et al.* 1997). In fact, at room temperature or under conditions of long-term cold adaptation, several studies have shown that UCP1 is dispensable for maintaining body temperature. Indeed, in UCP1 KO mice, the compromised BAT activity is compensated by UCP1-independent phenotypic WAT browning and activation (Keipert *et al.* 2017). However, at thermoneutrality, UCP1 KO mice develop obesity when fed high-fat diets, indicating a 'thermogenic' effect of fatty foods, the basis of diet-induced thermogenesis (von Essen *et al.* 2017). Interestingly, in UCP1 KO mice, isoproterenol infusion showed exaggerated myocardial injury and hypertrophy, indicating that the absence of proper NST affects CVD beyond just heat generation (Thoonen *et al.* 2015). In contrast, transgenic expression of UCP1 directly in cardiomyocytes shows improvement in recovery of perfusion after ischaemia (Hoerter *et al.* 2004). Overall, these studies highlight that UCP1-mediated NST is linked to cardiovascular function. As an alternative to UCP1, the traffic of creatine kinase B in mitochondria has been shown to induce thermogenesis, triggered by the futile creatine cycle. In this model, a mitochondrial creatine kinase induces the phosphorylation of creatine, hydrolysing mitochondrial ATP into ADP, which is a necessary substrate for the mitochondrial electron transport chain. Then, a phospho-creatine phosphatase hydrolyses phospho-creatine into creatine initiating the futile creatine cycle (Kazak *et al.* 2015; Wallimann *et al.* 2020; Connell *et al.* 2021). Other models have proposed calcium cycling in beige adipocytes as a thermogenic mechanism (Mottillo *et al.* 2018).

Adaptive mechanisms of sustained cold acclimatization

BAT undergoes significant remodelling during cold adaptation and, conversely, when the cold stress is alleviated, during involution of BAT. These processes require enhanced quality control and recycling mechanisms, as the abundance of lipids, proteins and entire organelles is dynamically matched to the metabolic need of NST. This represents a pronounced challenge for proteostasis, the proper production and timely elimination of proteins (Bartelt & Widenmaier, 2020). Both brown and beige adipocytes rely on proteasomal protein degradation to match increased abundance of ubiquitinated proteins. This process is mediated by the cold-inducible transcription factor Nfe2l1, which drives the transcription of proteasome subunits and is required for cold adaptation (Bartelt *et al.* 2018). In the absence of Nfe2l1, brown and beige adipocytes progressively lose their thermogenic capacity, as cellular stress originating from, but not limited to, the ER drives adipocyte dysfunction. This leads to whitening of BAT and diminished adipose tissue browning with marked inflammation of the fat pads (Bartelt *et al.* 2018). This does not necessarily lead to a reduction in energy expenditure, rather the organism adapts to the absence of NST. While high-fat diet-induced weight gain is similar, mice deficient in thermogenic adipocyte Nfe2l1 are more glucose intolerant and insulin resistant (Bartelt *et al.* 2018). How Nfe2l1 impacts on CVD is an active area of investigation. One additional mechanism by which proteins, and particularly mitochondria, are being recycled is autophagy (Akoumianakis *et al.* 2017). In mice, inhibition of autophagy in adipocytes results in decreased mitochondrial clearance and increased insulin sensitivity, which are caused by a preservation of the thermogenic phenotype at thermoneutrality (Altshuler-Keylin *et al.* 2016). The age-dependent decline of BAT in adults (Nedergaard *et al.* 2007) suggest that reduced autophagic activity observed in older animals might be distinct from the acute remodelling mechanisms. Conversely, endogenous lipid synthesis is an important buffer system in the transition from cold to thermoneutrality, and the carbohydrate-responsive element binding protein (ChREBP) transcription factor is required for involution of BAT (Schlein *et al.* 2021).

When exposed to cold, the first line of thermogenesis is muscle shivering, so it is perhaps not surprising that there are endocrine connections between myocytes and thermogenic adipocytes. Interestingly, exercise is linked to BAT function, but with exercise being thermogenic itself, increase of BAT thermogenic activity is probably secondary and associated with exercise-induced sympathetic activation of β-adrenoreceptors (Vosselman *et al.* 2012). Somewhat contrary to expectations, extended fasting, which like sustained cold is a catabolic condition, decreases sympathetic activity and desensitizes BAT to noradrenaline (norepinephrine) in mice housed at room temperature (22°C) (Shin *et al.* 2017; Nakamura & Nakamura, 2018). In parallel, prolonged fasting is also followed by the secretion of several hormones including glucocorticoids, glucagon, FABP4 and FGF21, as well as the reduction of insulin and thyroid hormone secretion (Patel *et al.* 2015; Martinez *et al.* 2017; Prentice *et al.* 2019). Both in BAT and WAT, sympathetic tone is increased upon fasting, and there is a switch from glucose-based energy production to fatty acid oxidation (Li *et al.* 2017). Overall, somewhat paradoxically, these mechanisms highlight that conditions of negative energy balance are linked to increased NST and adipose browning (Reinisch *et al.* 2020).

Hormonal activity of BAT

Even though the thermogenic function of BAT is most notable, it also has secretory function. Several so-called 'BATokines' have been described to enhance BAT activity

and positively influence systemic metabolism in an endocrine, paracrine, or autocrine manner (Villarroya *et al*. 2017). In an autocrine feed-forward loop, the lipid hormones 12,13-diHOME and 12-HEPE stimulate fatty acid and glucose disposal into BAT (Lynes *et al*. 2017; Leiria *et al*. 2019). In a paracrine fashion, BAT initiates crosstalk with the local environment. Endothelial cells are controlled via the secretion of vascular endothelial growth factor A (VEGFA), which increases vascularization and similarly the release of nitric oxide, altogether increasing blood flow to and from BAT and facilitating the transport of heat to the rest of the body. The related homologue, VEGFB, stimulates fatty acid transport by endothelial cells (Hagberg *et al*. 2010). BAT also releases nerve growth factor (NGF) to communicate with the central nervous system and promote sympathetic innervation. Furthermore, brown adipocytes release endocrine factors including triiodothyronine (T3), neuregulin 4 (NRG4), interleukin-6 (IL6) and FGF21. Indeed, BAT contributes, at a systemic level, to the increase in T3 after cold activation, which has been shown to induce UCP1 (Silva & Larsen, 1983). NRG4 secreted by BAT attenuates hepatic lipogenesis (Wang *et al*. 2014*b*), while another well-studied endocrine factor reportedly released by BAT, FGF21, regulates nutrient disposal into adipose tissue (Schlein *et al*. 2016). While FGF21 has also been reported to influence browning (only caused by genetic deficiency and developmental issues), these findings have been refuted, as has the hypothesis of a compensation of UCP1 function by FGF21. Keipert *et al*. (2017) showed that long-term cold adaptation requires neither FGF21 nor UCP1. Brown adipocytes not only produce beneficial hormones, but also secrete deleterious molecules, most notably inflammatory mediators. In obesity, brown adipocytes undergo a phenotypic change that is best described as 'whitening', associated with the accumulation of lipids and mitochondrial loss (Shimizu & Walsh, 2015). Therefore, dysfunctional BAT could in fact prove to be an additional risk factor for CVD pathogenesis by contributing to hyperlipidaemia and atherosclerosis (van Dam *et al*. 2017). This is illustrated under conditions of enhanced BAT whitening, for example, at thermoneutrality or in the absence of Nfe2l1, which leads to the formation of crown-like structures in BAT and increased levels of inflammatory cytokines and chemokines systemically (Bartelt *et al*. 2018). Knockout of the insulin receptor in BAT of ApoE KO mice creates lipoatrophic BAT resulting in high plasma triglyceride levels. Moreover, these mice exhibit increased infiltration of pro-inflammatory cells when compared to controls (Wang & Seale, 2016). Thus, pro-inflammatory cytokine expression is enhanced in BAT in obesity, potentially contributing to diminished clearance of plasma lipids and atherogenesis (McGregor *et al*. 2013; Villarroya *et al*. 2017).

Epicardial and perivascular adipose tissue in CVD

BAT contributes to a healthy metabolism and is protective against CVD, notably via its thermogenic and secretory functions. However, the ability to secrete hormones is not restricted to BAT, as visceral and subcutaneous WAT depots have well-established roles here as well (Scheja & Heeren, 2019). Much less is known about epicardial (EAT), pericardial (PAT) and perivascular adipose tissue (PVAT) depots, even though there is accumulating evidence that they secrete adipokines and affect CVD in a paracrine and vasocrine fashion. Foremost, fatty acids released by adipocytes are important metabolites necessary to produce cardiac ATP, mainly generated by mitochondrial β-oxidation and oxidative phosphorylation (Doehner *et al*. 2014). Interestingly, EAT contains more thermogenic cells than most other types of WAT, especially in the peri-coronary area. Yet, it is important to distinguish EAT from PAT, which is anatomically, embryologically and functionally distinct (Lima-Martínez *et al*. 2013). EAT is closer to the myocardium, between the outer wall of the myocardium and the visceral layer of pericardium, and shares the same microcirculation, explaining the vasocrine crosstalk between these two tissues. In contrast, PAT is anterior to the EAT, further to the myocardium and located between the parietal and visceral pericardium (Iacobellis *et al*. 2005). EAT is more of interest for the study of the bidirectional crosstalk between adipose tissue and heart, which can be beneficial or deleterious depending on the metabolic context. For example, it has been shown that the secretion of adiponectin has antioxidant effects in cardiomyocytes during arrhythmogenesis (Nanayakkara *et al*. 2012). EAT can also modulate myocardial insulin resistance by modulation of glucose transporter-4 expression (Salgado-Somoza *et al*. 2012). Other evidence shows that EAT from diabetic patients impairs cardiomyocyte contractility and mitochondrial β-oxidation via the renin-angiotensin-aldosterone-system and natriuretic peptides (Blumensatt *et al*. 2017). EAT is also implicated in cardiac fibrosis, inflammation and heart hypertrophy and failure (Antonopoulos *et al*. 2014). Furthermore, IL-6, which is usually released in myocardial diseases, induces lipolysis in the EAT and is potentially at the origin of the negative correlation between left ventricular ejection fraction and obesity (Blomstrand *et al*. 2018). Whereas the pathological accumulation of EAT impairs myocardial metabolism, the dramatic loss of lean and fat mass observed in severe cases of cachexia or cardiac insufficiency seems to also influence cardiovascular parameters (von Haehling *et al*. 2017). Recent publications report a dysregulation of EAT lipid metabolism in cachexia due to natriuretic peptides and cardiolipin. However, how secretion from EAT affects

heart metabolism in this specific pathology is still unclear (Janovska *et al*. 2020). Interestingly, there is a positive correlation between the abundance of peri-coronary EAT and CAD (Prati *et al*. 2003). Indeed, the paracrine effect of WAT is also found along the vascular network throughout the body and, even if anatomically different, EAT and PVAT influence atherosclerotic plaque formation in the same way.

PVAT is morphologically and functionally heterogenous. In humans, it surrounds most blood vessels, with the exceptions of the cerebral and pulmonary vasculature (Gao, 2007), whereas in mice PVAT cannot be found around coronary arteries. PVAT has gained wider recognition in the past 30 years with the discovery that thoracic PVAT decreases vessel reactivity after treatment with noradrenaline (Soltis & Cassis, 1991). The phenotype of PVAT strongly correlates with its anatomic location, and it displays characteristics of both white and brown adipocytes. In mice, PVAT surrounding the thoracic aorta primarily features characteristics of BAT whereas PVAT of the abdominal aorta bears a closer resemblance to WAT (Qi *et al*. 2018). Gene expression analysis has revealed only 228 significantly different genes when thoracic PVAT and classical BAT were compared. Additionally, genes typically associated with BAT, namely UCP1 and PPARγ, are also found in PVAT, consistent with PVAT displaying thermogenic ability (Fitzgibbons *et al*. 2011). On the other hand, abdominal PVAT displays a mostly mixed brown and white-like phenotype, with mesenteric, carotid and femoral perivascular tissue resembling WAT in obesity (Kim *et al*. 2019). The PVAT phenotype not only differs depending on its anatomic location, but also on the type of blood vessel: Larger conduit vessels are mostly surrounded by brown-like PVAT, whereas the smaller resistance vessels are supported by white-like PVAT (Huang Cao *et al*. 2017).

Just as complex and heterogeneous as the phenotypes of PVAT are its physiological functions. First, due to its localization adjacent to the vessel wall, PVAT provides structural support. Second, PVAT secretes pro- as well as anti-inflammatory cytokines and vasoactive substances controlling vascular homeostasis in an endocrine and paracrine manner (Akoumianakis *et al*. 2017). Secreted cytokines include adipokines such as leptin, adiponectin, visfatin and resistin as well as TNFα, IL-6, IL-8, or MCP-1 that contribute to vasorelaxation, SMC proliferation, inflammation and oxidative stress. In lean individuals a balanced secretion of pro- and anti-inflammatory mediators prevails (van Dam *et al*. 2017). In metabolic disorders like obesity PVAT becomes dysfunctional. Adiponectin, for example, causes vasodilatation of small arteries, but this function is lost in obesity (Greenstein *et al*. 2009; Fitzgibbons & Czech, 2014). The cytokine profile switches towards a pro-inflammatory profile, which is linked to decreased thermogenic capacity, local vascular inflammation, remodelling of the vascular wall and vasoconstriction (Fitzgibbons & Czech, 2014). This is also relevant for the development of CVDs such as hypertension and atherosclerosis. Its proximity to coronary arteries in humans further indicates its potential relevance for CVD, especially atherosclerosis (Bartelt *et al*. 2019).

In general, a pro-inflammatory phenotype of thoracic PVAT is associated with an increased risk to develop CVD (Okamoto *et al*. 2001), and smoking also reportedly increases PVAT inflammation (Rossi *et al*. 2014). Still, how exactly PVAT exerts its effects on CVD is not proven. Considering the thermogenic capacity, cold activation enhances lipid clearance by PVAT and therefore helps to prevent atherosclerotic lesions. Due to its proximity to the vessel, biologically active molecules produced by PVAT also directly influence vascular homeostasis. Adiponectin, nitric oxide, hydrogen sulfide, prostacyclin, angiotensin, or reactive oxygen species influence vascular tone (Xia & Li, 2017). However, research on PVAT is limited due to its heterogeneity, species-specific differences, and the lack of specific transgenic models. Available mouse models include atrophic mice, like the A-ZIP/F mouse that has no WAT and little BAT and PVAT (Moitra *et al*. 1998), or the inducible FAT-ATTAC mouse (Pajvani *et al*. 2005). A more promising model carries the deletion of PPARγ specifically in vascular smooth muscle cells, which results in normal WAT and BAT, but no PVAT depots (Brown *et al*. 2014). However, as all vascular smooth muscle cells are affected, it is not possible to study PVAT-specific effects as smooth muscle cells are also affected. In conclusion, perivascular adipocytes are important for maintaining proper vessel function, and a correlation between dysfunctional PVAT and CVD has been shown. Whether PVAT dysfunction favours vascular diseases or vice versa remains to be further investigated (Fig. 4).

Outlook

The correlation between adipose tissue dysfunction and CVD is well established. In most cases CVD is a consequence of general metabolic dysregulation, associated with increased blood pressure, impaired glucose regulation, dyslipidaemia and chronic inflammation. As of today, treating obesity remains challenging, as the evolutionarily acquired adaptative mechanisms of the body favour energy storage over energy expenditure. Current treatments mainly target lipid absorption and satiety, or use bariatric surgery. However, a new generation of hormonal therapies, including targeting GLP-1/GLP-1R or SGLT2, appear to have profound antidiabetic and cardiovascular benefit, and are associated with significant weight loss (Nagahisa & Saisho, 2019). Using BAT as a target for the treatment

of obesity by increasing thermogenesis in a controlled fashion is considered an interesting, yet premature approach. β3-Agonists are indeed already prescribed at low doses for the treatment of overactive bladder (O'Mara *et al.* 2020). At the higher doses that would be required to enable the recruitment and activation of thermogenic adipocytes in humans, potential cardiovascular side effects of this class of drugs makes them unsuitable for obesity treatment. Another approach would be the use of thermogenic uncouplers mimicking UCP1 action, but as of today, most uncouplers act systemically, and advancements to enable targeted uncoupling only in adipocytes would be required for therapeutic use. Targeting UCP1-independent futile cycles could allow for an increase in thermogenesis by bypassing β-adrenergic signalling, and potentially minimizing side effects. However, it is important to consider the net difference in thermogenic capacity between humans and rodent models. Indeed, while mice possess abundant brown fat, human thermogenic cells are scarce and are mostly found dispersed throughout the body. The origin of these cells remains controversial, but it is now well accepted that human BAT is thermogenic and has beneficial effects on metabolic health, (Becher *et al.* 2021). However, the thermogenic capacity of human BAT remains insufficient for promoting meaningful weight loss and metabolic improvement in obese patients, yet a thermogenic lifestyle might very well contribute to a negative energy balance. Beyond these systemic effects of human BAT on energy balance and obesity, the reported local crosstalk between adipocytes and the cells of the cardiovascular system may open new therapeutic horizons.

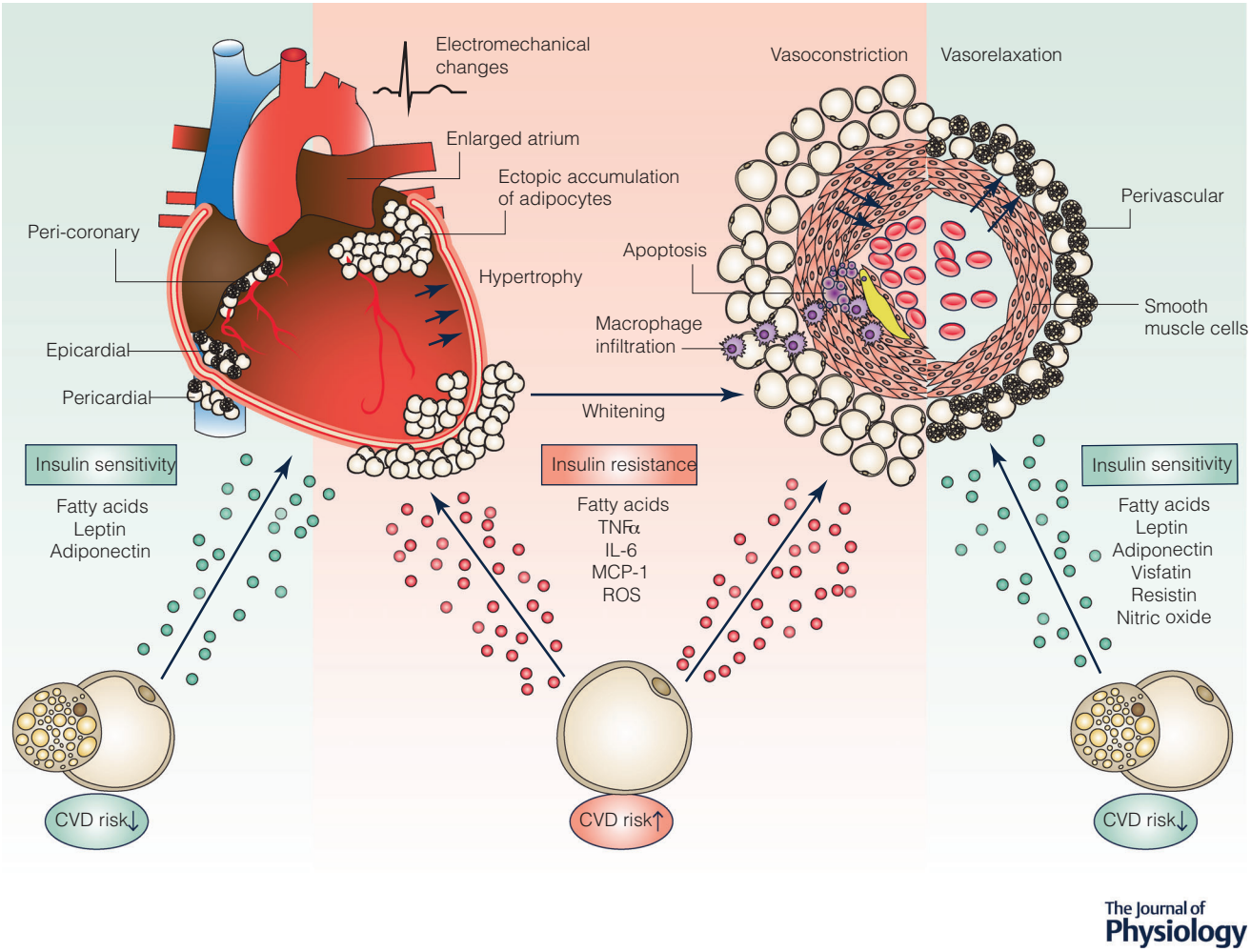

Figure 4. Ectopic adipose tissue depots of the heart and cardiovascular disease
Under physiological circumstances epicardial and perivascular adipose tissue maintain a healthy vasculature and heart function. Secreted mediators increase insulin sensitivity and thereby decrease cardiovascular risk. Metabolic disorders result in whitening and ectopic accumulation of adipose tissue, which promotes vasoconstriction and ventricle hypertrophy.

Concluding remarks

Obesity and cardiovascular disease are linked through multiple endocrine and paracrine mechanisms, with adipocytes playing a major role in both preservation and imbalance of metabolic homeostasis. On the road to systemic collapse of the hormonal networks regulating metabolism, adipocyte dysfunction is a critical hallmark that enables the development of insulin resistance, dyslipidaemia and atherogenesis. Conversely, thermogenic adipocytes exert beneficial effects on metabolism, both by efficiently dissipating energy-dense nutrients as well as by producing favourable cytokines. During evolution, these physiological mechanisms have been finetuned to function in a gene-environment relationship that is no longer present in our modern societies, as calories are ample, nutrients are refined to be absorbed quickly, physical activity is obsolete, and we are hardly ever cold. This changed environment poses new challenges to our metabolism, and the rise in obesity and associated CVD is a result of a maladaptive response by our bodies to these challenges. The classification of obesity as a medical pathology is a first step in the public health strategy to tackle this disease as a society, but there is also exciting new molecular understanding of adipocyte biology and its impact on metabolic adaptation. Adipocytes are both friend and foe and future research will show how we can get the best out of our adipocytes.

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Additional information

Competing interests

None.

Author contributions

All authors conceptualized the outline of the review. The manuscript and figures were prepared by M.G. and H.J. with help from K.J.P. and A.B. All authors edited and commented on text and figures. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Supporting information

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