INFLAMED FAT: immune modulation of adipose tissue and lipid metabolism

Dam, A.D. van; Dam A.D. van

Chapter 7

General discussion and future perspectives

Adapted from:

Targeting white, brown and perivascular adipose tissue in atherosclerosis development.
Eur J Pharmacol 2017; in press

Immune modulation of brown(ing) adipose tissue in obesity.
Endocr Rev 2017; 38: 46-68

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The current worldwide obesity epidemic requires novel preventive and curative strategies. These strategies should not only aim at reducing obesity, but above all combat its related morbidities including type 2 diabetes and cardiovascular disease. Obesity leads to higher plasma lipid levels (*i.e.* cholesterol and triglycerides) and systemic inflammation. An exciting challenge within the dynamic field of immunometabolism is unraveling the molecular mechanisms connecting accumulation of adipose tissue, the subsequent increase in plasma lipids and systemic inflammation, and ultimately the development of type 2 diabetes and cardiovascular disease. A better understanding of these mechanisms will provide novel therapeutic targets and possibilities to improve treatment options.

To gain more insight into the role of the immune system in obesity, insulin resistance, dyslipidemia and atherosclerosis, we studied the effects of a bacterial infection and antibodies (IgG) on these parameters in several mouse models. We also investigated mediators of inflammation in blood and tissues of South Asians, a population with a particularly high risk of developing type 2 diabetes. Furthermore, we determined the potential of an anti-inflammatory compound and a fatty acid receptor agonist to reduce obesity and inflammation, and to ultimately improve glucose and lipid metabolism in mice.

From this thesis, various novel perceptions on the link between obesity, the immune system, and development of type 2 diabetes and cardiovascular disease have arisen which will be discussed and interpreted in this final chapter. Furthermore, therapeutic implications and future options for the management of metabolic disease will be addressed. The figure in this chapter is a graphical representation of the results obtained in this thesis (**Fig. 1**).

INFLAMMATION, LIPID METABOLISM AND ATHEROSCLEROSIS

Obesity is associated with high plasma levels of low density lipoprotein (LDL) cholesterol (1, 2) and inflammation (3, 4). Together with inflammation, cholesterol is a main risk factor for atherosclerosis as excess cholesterol initiates plaque formation in the arterial wall. Oxidized LDL (oxLDL) cholesterol is taken up by macrophages, upon which the macrophages turn into foam cells. Moreover, oxLDL triggers inflammation, e.*g.* by activating endothelial cells of the vessel wall to express adhesion molecules and chemokines such as the monocyte chemoattractant protein-1 (MCP-1), and thereby further enhancing plaque development (5-8). In **chapter 2**, we discovered that mycobacterial infection with BCG in *APOE*3-Leiden.CETP* (*E3L.CETP*) mice, despite overall immune activation, reduced plasma cholesterol levels and ultimately tended to reduce atherosclerosis development. This is a peculiar finding since alterations in levels of plasma lipids and plasma inflammatory mediators usually go hand in hand. As reviewed by Van Diepen *et al.* (7), various classes of lipid-lowering drugs have anti-inflammatory properties that are mainly attributable to the reduced presence of lipids, although lipid-lowering drugs also directly interfere with

inflammatory pathways. In turn, some anti-inflammatory drugs alter lipids levels, although the effects are ambiguous. For example, the inhibitor of NF-κB salicylate lowers plasma triglycerides in type 2 diabetes patients but not in individuals with pre-type 2 diabetes, and IL-6 signaling inhibitors raise plasma triglycerides in patients with rheumatoid arthritis. More investigations are needed to unravel the specific effects of anti-inflammatory drugs on lipid metabolism (7).

Even though inflammation and lipid levels usually go up or down together, other conditions exist in which an immune response is associated with lower lipid levels. Hepatitis C virus (HCV) infection causes hypolipidemia (9, 10), but the mechanism by which HCV deregulates lipid metabolism is different from what we think the mechanisms behind the cholesterol lowering effects of BCG are. While we observed that BCG increased cholesterol clearance and reduced intestinal cholesterol absorption, without evidence for reduced hepatic cholesterol synthesis, HCV interferes with host cholesterol metabolism by exploiting the lipoprotein machinery during replication in the hepatocyte (11, 12), resulting in lower synthesis of cholesterol (13). Despite the cholesterol-lowering effects of HCV infection, HCV infection was often reported to be associated with atherosclerosis (14, 15). One of the possible mechanisms mediating this link could be the activation of inflammatory processes and cytokine imbalance. It must be noted however that the results of studies describing the association between HCV infection and atherogenesis are not uniform and controversy still exists on the direction of the association (16). Knowing the relative contribution of inflammatory processes and lipid metabolism is a prerequisite to justify the development and application of anti-inflammatory drugs for the treatment of atherosclerosis, but lipid metabolism and inflammatory processes are so intertwined that it is nearly impossible to dissect what their relative contributions to atherogenesis are.

To accurately assess the relative contribution of cholesterol and inflammation in atherosclerosis development, superior mouse models than the currently available ones are needed. *E3L* and *E3L.CETP* mice express a mutation of the human *APOE*3* gene besides their endogenous apoE. This attenuates clearance of cholesterol-enriched lipoprotein remnants via the LDLR pathway, resulting in a humanized lipoprotein profile (17-19). Most studies performed in *E3L* and/or *E3L.CETP* mice to date investigated the effect of lipid level-modulating compounds on atherosclerosis (20-22). In most studies, the plasma total cholesterol exposure correlated with measures of the atherosclerotic lesion area (as in (17)), which underscores the high degree of dependence of atherogenesis on cholesterol levels in these models. Only few studies demonstrated a reducing effect of a compound on atherosclerosis beyond and independent of the reduction achieved by lowering of cholesterol by the compound alone in *E3L* mice (23, 24). These conclusions were drawn from an experimental set-up in which a separate cholesterol-fed control group was taken along with lower cholesterol intake that resulted in plasma cholesterol levels that were comparable to the cholesterol-fed group that was treated with the compound. In mice treated with rosuvastatin, the expression of MCP-1 and tumor necrosis factor (TNF) was lower than in the group with matched plasma cholesterol levels, and atherosclerosis was also lower than in the group with matched plasma cholesterol levels, indicating an additional

anti-inflammatory and inhibitory effect of rosuvastatin on atherogenesis (23). In another study, salicylate reduced hepatic NF-κB activity and macrophage content in plaques further than matched cholesterol feeding did, also pointing to an effect of salicylate independent of its cholesterol-lowering effect (24). Only one study in *E3L.CETP* mice showed increased rather than decreased inflammatory state without any effect on plasma lipid levels upon parasympathetic denervation of the spleen, evidenced by increased dendritic cells, B cells and T cells in the spleen, increased expression of inflammatory cytokines in the liver and in peritoneal leukocytes and increased levels of the cytokines IL-1β and IL-6 in the circulation. Nevertheless, this did not aggravate atherosclerosis development (25). Taken together, modulation of inflammation independently of lipid metabolism has hardly been studied in *E3L* and/or *E3L.CETP* mice and since many interventions modulate both lipids and inflammation, these models are not recommended if one wants to assess a pro- or anti-inflammatory effect on atherosclerosis development independent of alterations in lipid levels.

Apoe$^{-/-}$ and *Ldlr*$^{-/-}$ mice are the most widely used mouse models for atherosclerosis. Since these mice lack a functional hepatic ApoE-LDLR axis, the predominant route by which cholesterol-enriched lipoprotein remnants are cleared from the circulation (17), they do not respond to lipid-lowering therapies (26). *Apoe*$^{-/-}$ mice spontaneously develop atherosclerosis, even on a chow diet which does not contain cholesterol. In contrast, *Ldlr*$^{-/-}$ mice have a modest elevation of plasma cholesterol compared to wild-type mice and exhibit slow atherosclerosis development. Nevertheless, when fed a diet rich in cholesterol, these mice also have strongly elevated cholesterol and accelerated atherogenesis (26). ApoE not only mediates lipoprotein clearance, but also has anti-inflammatory and immunomodulatory properties. Compared to wild-type mice, *Apoe*$^{-/-}$ mice have higher expression of immunostimulatory cell surface molecules on macrophages, which results in enhanced T cell activation compared to wild-type mice (27). When stimulated with LPS, *Apoe*$^{-/-}$ mice show a higher upregulation of the pro-inflammatory cytokines TNF, IL-6, IL-12 and interferon-γ in liver and spleen compared to wild-type mice. This may also be explained by the ability of apoE to bind and inactivate LPS (28, 29). *Ldlr*$^{-/-}$*apobec-1*$^{-/-}$ mice, which also display high cholesterol levels, do not exhibit this inflated cytokine response upon LPS injection, indicating that the increase in pro-inflammatory cytokines in *Apoe*$^{-/-}$ mice upon LPS is independent of hypercholesterolemia (30). A mechanism explaining more inflammation in *Apoe*$^{-/-}$ mice is that lack of ApoE also reduces the uptake of apoptotic bodies by macrophages, resulting in more apoptotic bodies and macrophage recruitment in the liver, lungs, brain and possibly more organs compared to wild-type mice. In addition, the pro-inflammatory markers TNF and fibrinogen are higher in livers of *Apoe*$^{-/-}$ mice compared to wild-types. When comparing macrophage content between wild-type, *Apoe*$^{-/-}$ mice and *Ldlr*$^{-/-}$ mice, which have a similar lipoprotein profile as *Apoe*$^{-/-}$ mice, *Ldlr*$^{-/-}$ mice do not show increased macrophages in the lungs and only exhibit a ~1.5-fold upregulation of hepatic macrophages that does not reach significance compared to wild-type mice. In contrast, *Apoe*$^{-/-}$ mice have a ~3-fold higher macrophage content in both liver and lungs compared to wild-type mice, which equals ~2-fold increases compared to *Ldlr*$^{-/-}$

mice (31). The augmented immune activation and ectopic macrophage recruitment in *Apoe*$^{-/-}$ mice possibly contributes to atherosclerosis.

These differences in clearance of cholesterol-enriched lipoprotein remnants and inflammatory state between mouse models for atherosclerosis may explain why studies are sometimes conflicting. For example, BAT activation increases the selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT, resulting in the formation of cholesterol-enriched remnants. In *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, in which these remnants cannot be cleared by the liver, this leads to enhanced atherogenesis (17, 32). In contrast, BAT activation in E3L.CETP mice accelerates the hepatic clearance of these cholesterol-enriched remnants, resulting in protection from atherosclerosis development (17), an effect that may be expected relevant for most humans with an intact apoE-LDLr clearance pathway for lipoprotein remnants. As for the effect of BCG on atherosclerosis (**chapter 2**), different studies have been performed in different animal models. Subcutaneous BCG injections in rabbits of which plasma cholesterol levels were maintained within a certain range by varying the cholesterol content of the diet per rabbit revealed more atherogenesis upon BCG treatment (33). A study in which *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice were treated with subcutaneous injections of freeze-dried BCG showed less atherogenesis (34). Both of these studies justly concluded that the effects of BCG on atherogenesis went through immunomodulatory mechanisms since the cholesterol levels between BCG-treated and control mice were similar. The differences in outcome between the studies (more *vs.* less atherosclerosis) are possibly due to differences in immune responses of rabbits vs mice, the difference in BCG used (live attenuated *vs.* freeze-dried), the dosing and number of injections with BCG and the amount of cholesterol in the diet, since cholesterol is also a pro-inflammatory stimulus. If one would be interested to know whether the BCG-induced infection and associated inflammation we observed upon intravenous administration of live attenuated BCG, independently of its cholesterol-lowering effect, accelerates atherosclerosis, a new experiment would have to be done in which a group with matched low cholesterol levels is included. Alternatively, the experiment could be repeated in *Ldlr*$^{-/-}$ mice.

Sometimes, the differences between mouse models for atherosclerosis may help to gain mechanistic insight. Since *Ldlr*$^{-/-}$ mice lack hepatic clearance of cholesterol-enriched remnants and do not show immunological abnormalities like *Apoe*$^{-/-}$ mice do, *Ldlr*$^{-/-}$ might be the best model to study the effect of immunomodulatory effects independent of lipid metabolism on atherosclerosis. However, in the end we strive for models that are as comparable as possible to humans, with the ultimate aim to predict how a compound acts in man. In this light, rabbits and E3L.CETP mice are still the best option we currently have.

ADIPOSE TISSUE INFLAMMATION AND TYPE 2 DIABETES

Inflammation is also believed to be an important link between accumulation of adipose tissue and development of type 2 diabetes. White adipose tissue (WAT) harbours many types of immune cells, of which the numbers increase during obesity (35-39). The

chronic inflammation associated with obesity disturbs insulin signalling in the tissue, since inflammatory cytokines activate JNK and IKKβ signalling pathways. This results in inhibitory phosphorylation of insulin receptor substrate 1 and 2 (IRS1 and IRS2), proteins that transmit signals from the insulin receptor to intracellular signalling pathways (40, 41). This inhibition of insulin signalling induces insulin resistance in white adipose tissue and contributes to development of type 2 diabetes. Macrophages were the first and most abundant type of immune cells discovered to infiltrate obese WAT (36, 42), for which the majority of research on immune cells in adipose tissue focuses on macrophages. Adipose tissue macrophages fulfil conventional functions such as clearing cellular debris and taking part in tissue immune surveillance. Macrophages also have a lipid buffering function; during lipolysis (*e.g.* upon fasting or adrenergic activation), macrophages take up and store lipids released from adipocytes in order to ensure gradual lipid release into the circulation (43). Recent studies have also focused on the function of other immune cell types in WAT inflammation, such as B cells. B cells were first reported to be present in adipose tissue in 2005 (44), and were subsequently found to be recruited to adipose tissue during high-fat diet (HFD) feeding (45). Winer *et al.* (46) showed that IgG, an antibody produced by B cells that activates complement and binds Fc receptors (FcRs), promotes insulin resistance and glucose intolerance. In **chapter 3**, we confirm that adipose tissue B cells and IgG are more abundant in obese compared to lean adipose tissue (also see graphical representation in **Fig. 1** of this discussion). However, we showed that lack of FcγR and complement C3, the two pathways through which IgG signals, does not ameliorate the development of HFD-induced glucose intolerance. This implicates that presence of FcγR and complement C3 is not critical for the development obesity-associated glucose intolerance. When investigating adipose tissue inflammation, we found that mice lacking FcγR and complement C3 did not exhibit any or only marginal differences (*i.e.* increased number of macrophages in WAT only in mice lacking both FcγR and complement C3) in inflammation compared to controls. Perhaps, counter-regulatory mechanisms between different elements of the immune system prevent that effects on metabolic parameters are active in mice that lack FcγR or complement C3. Possibly, the number of natural killer cells or other immune cell types that we did not measure may be increased in adipose tissue, as has been shown in mice lacking B and T cells compared to wild-type mice fed a HFD (45). Counter-regulatory mechanisms within the immune system may stretch beyond up- or downregulation of entire immune cell populations, as elevated inflammatory cytokine responses can already compensate deficiency of others (47). Of note, our finding that deficiency of B cell-derived IgG downstream signalling pathways (*i.e.* FcγR and complement C3, **chapter 3**) does not ameliorate development of glucose intolerance is supported by the report that deficiency of B and T cells does not affect the onset of obesity and the state of insulin resistance in mice (45). Overall, the complexity and versatility of the immune system complicate the research into immune modulation of adipose tissue and metabolic disorders. Since removing B cells or B cell components without inducing compensatory effects by other constituents of the immune system is not possible, the precise role of B cells in WAT inflammation and insulin resistance remains obscure.

In **chapter 4**, our purpose was to gain insight into the inflammatory state of WAT (and also skeletal muscle and blood) in a population with a high risk of type 2 diabetes. Since South Asians have an exceptionally high risk to develop this disorder compared to white Caucasians (48), we compared transcriptomic levels of a large panel of inflammatory, immune-regulating and immune cell subset markers in blood, skeletal muscle and WAT of overweight, pre-diabetic South Asian and matched white Caucasian men. It is generally known that obesity is associated with the infiltration of several immune cell types in WAT including macrophages, T cells and B cells, which are held responsible for the induction of chronic low-grade inflammation and development of insulin resistance (41). For this reason, we had expected increased expression of markers for these cell types in WAT of South Asians compared to white Caucasians. Surprisingly, expression levels of the main markers for immune cell subsets such as CD3, CD4 (T cells), CD19 (B cells) and the monocyte/macrophage markers CD14, CD163 and CCL5 were comparable in WAT of South Asians and white Caucasians. Nevertheless, we revealed that South Asians have lower expression of interferon signalling genes in WAT than white Caucasians. These interferon signalling genes are transcribed by transcription factors called interferon regulatory factors (IRFs) (49). Interestingly, IRFs (IRF1-IRF9) were recently found to be expressed in adipose tissue, where they regulate adipogenesis (50). Subsequently, IRF4 was discovered to promote lipolysis and inhibit lipogenesis in adipocytes, indicating that this factor is involved in lipid handling within the adipose tissue. Moreover, lack of IRF4 increases adiposity in mice (51, 52). In contrast to IRF4, IRF3 protects from HFD-induced obesity (53), suggesting that the different IRFs have opposing functions which might be explained if they compete for the same co-factors. Together, these data imply that altered regulation of upstream transcription factors for interferon signalling such as IRF3 and IRF4 could, at least in part, underlie the higher abdominal adiposity in South Asians compared to white Caucasians (48, 54). Besides, links between interferon signalling and glucose metabolism exist. *Ifnβ1* overexpression maintains healthy glucose homeostasis upon HFD feeding of mice (55) whereas IRF4 deficiency and adipose tissue-specific knockout of *Ifnar1* deteriorate insulin resistance and glucose tolerance (52, 56). As South Asians are more glucose intolerant than white Caucasians (57), these data support the possibility of a link between the reduced interferon signaling in their WAT (**chapter 4** and **Fig. 1** of this discussion) and deteriorated glucose metabolism.

Obviously, interferon signaling may not be the only cause of the disadvantageous metabolic phenotype of South Asians. From literature it is known that South Asian newborns are characterized by elevated E-selectin and CRP levels in the cord blood, which suggests that endothelial dysfunction and enhanced inflammation are already present at birth in this population (58). Healthy lean adolescent South Asians have less BAT volume than matched white Caucasians (59), although this diminished BAT phenotype was not observed in the current study with overweight, pre-diabetic older South Asian and matched white Caucasian men (Boon & Hanssen, unpublished data). Nevertheless, the South Asians in the current study had reduced mitochondrial function as measured in skeletal muscle, which is probably the main reason for their lower energy expenditure compared to white Caucasians

(Boon & Hanssen, unpublished data). This finding matches the 'mitochondrial efficiency hypothesis' postulated by Bhopal and Rafnsson (60), who propose that differences in environmental stressors such as a cold environment for white Caucasians and a food shortage for South Asians have led to differences in mitochondrial coupling efficiency. As a result, white Caucasians produce relatively large amounts of heat during oxidative phosphorylation while in South Asians the conversion of energy to adenosine triphosphate (ATP) rather than to heat is maximised. The latter is very unfavourable in the current environment with abundance of food and reduced need to be physically active (60). It would be very exciting to assess whether evolutionary pressure led to differences in genes that contribute to mitochondrial function and inflammation in South Asians in genome-wide association studies in South Asians and white Caucasians. In addition, the relation between reduced interferon signalling in South Asians and their reduced mitochondrial function, BAT activity, energy expenditure and worsened glucose intolerance is an interesting field of future investigation.

THERAPEUTIC IMPLICATIONS

Obesity is associated with hyperlipidaemia (1, 2) and systemic inflammation (3, 4), which in turn are closely involved in the development of obesity-associated metabolic disorders (61, 62). In the first part of this discussion, lowering of cholesterol has already been passed in review and this is an effective strategy to reduce atherosclerosis and cardiovascular disease. In this section, additional strategies to constrain development of obesity-associated disorders by lowering plasma lipids and/or reducing inflammation to decrease development of atherosclerosis and insulin resistance will be discussed.

Triglyceride combustion by BAT and beige WAT

A promising therapeutic approach to reduce plasma triglyceride levels is to increase combustion of fatty acids by BAT. Activation of β3-adrenergic receptors on brown adipocytes is the most well-known and potent way to induce thermogenesis and lipid combustion (63, 64) and β3-adrenergic receptor agonists such as CL-316243 potently induce lipid uptake by BAT in mice (17). Many other compounds have also been identified to activate BAT. Among these, rimonabant (65), metformin (66), glucagon-like peptide-1 receptor activation (67) and salsalate (**chapter 5**) also reduce plasma triglycerides. We showed that salsalate does this by directly activating BAT (**Fig. 1**), evidenced by increased uncoupled respiration and lipolysis in brown adipocytes. Although it was long thought that salicylates exert their beneficial metabolic effects through activation of AMPK (68, 69), we found evidence for an alternative intracellular mechanism dependent on the PKA pathway. A later report by Smith *et al.* (70) confirms that AMPK does not mediate the beneficial effects of salicylates. Although they primarily focussed on glucose metabolism rather than triglycerides, they showed that mice lacking the AMPK subunit that salicylates interact with still experience beneficial effects on glucose metabolism upon salicylate treatment. Interestingly, the same

group also provided evidence for a mechanism by which salicylate induces mitochondrial uncoupling independent of UCP1, possibly explained by the protonophoric effects of salicylates, *i.e.* the ability to move protons across lipid bilayers (70). The potential of salsalate to directly induce mitochondrial uncoupling in primary hepatocytes (70) may also explain why salsalate prevents non-alcoholic steatohepatitis (71). If salsalate indeed functions as a mitochondrial uncoupler in any cell type, this also clarifies how salsalate increases energy expenditure in human subjects (72). Other protonophoric compounds are probably effective to lower plasma triglycerides by activating BAT too. However, systemic treatment with mitochondrial uncouplers can be very dangerous; the mitochondrial uncoupler 2,4-dinitrophenol (DNP) induces weight loss but is associated with side effects like hyperthermia, tachycardia and death (73-75). In order to safely induce weight loss by activating BAT with mitochondrial uncouplers, improved ways to specifically target BAT would be warranted first.

A novel approach to activate BAT is via GPR120, a free fatty acid receptor that is activated by medium and long chain fatty acids (76). GPR120 is highly expressed in WAT and BAT and others have shown that GPR120 mediates anti-inflammatory actions of ω-3 fatty acids (77-79), plays a role in adipocyte differentiation (80), and enhances glucose uptake, which contributes to improved insulin sensitivity (77). Lack of GPR120 leads to obesity, glucose intolerance and hepatic steatosis (81). Our incentive to study whether stimulation of GPR120 could activate BAT was the fact that *Gpr120* expression in BAT increases upon cold exposure (82), which indicates a role for GPR120 in thermogenesis. We found that lack of GPR120 increased fat mass and reduced energy expenditure, but did not have evident effects on triglyceride-rich lipoprotein turnover. Promisingly, injections with the GPR120 agonist TUG891 increased fat oxidation and reduced fat mass (**chapter 6**). While we were performing these experiments, others already published that the GPR120 agonist GW9508 activates BAT (**Fig. 1**) (83). Although these are encouraging results, future studies (which will soon be performed by Schilperoort *et al.*) will have to elucidate whether BAT activation by GPR120 agonists also leads to enhanced triglyceride-derived fatty acid uptake by BAT and consequently lower plasma lipids.

An alternative therapeutic approach is to induce the formation of beige adipocytes in WAT, because beige adipocytes probably also use triglycerides for nonshivering thermogenesis like classic brown adipocytes do. This is supported by the fact that fatty acid uptake in beige WAT upon β3-adrenergic stimulation increases while the WAT depots shrink (17). Beiging of WAT is typically induced by cold and catecholamine stimulation (17, 84, 85), but many other stimuli that promote a beige phenotype are rapidly being discovered and include pharmacological activation of β3-adrenergic receptors (17, 64), thyroid hormone (86), glucagon-like peptide 1 receptor activation (67), fibroblast growth factor 21 (87), and bone morphogenetic protein 7 (88). Furthermore, various components of the immune system have been implicated to promote beiging, such as alternatively activated macrophages (89, 90), eosinophils (91) and ILC2s (92, 93). Several cytokines were identified to be involved in the underlying mechanisms and recent evidence also points towards a prominent role for interferon regulatory factors in the regulation of beiging (52, 53). Thus,

genesis and activation of beige adipocytes is an alternative pharmacological strategy to combust lipids and thereby reduce lipid levels in the blood.

Although it was long thought that beige adipocytes are derived from $Myf5^+$ precursor cells whereas white adipocytes are not, the current model of the differentiation process seems more complex as $Myf5^+$ cells can also differentiate into white adipocytes. More specific factors that mark brown/beige adipogenesis (*e.g.* the transcription factor Ebf2) are presently being discovered (94). Gaining a better understanding of adipocyte development is a prerequisite for the development of compounds that promote beige adipocyte differentiation and is under investigation.

Cholesterol lowering strategies

Whether triglycerides are an independent risk factor for cardiovascular disease is still under debate (95), but cholesterol certainly is the main risk factor for atherosclerosis development (6). Therefore, cholesterol-lowering strategies are effective in battling atherosclerosis. In **chapter 2**, we found that mycobacterial BCG infection lowered cholesterol partly by increasing hepatic uptake of cholesterol. We speculate that the accumulation of mycobacteria we detected in the liver could be responsible for this increased cholesterol uptake (**Fig. 1** of this discussion), as mycobacteria use host cholesterol as an energy source (96-99). Since humans cannot use cholesterol as an energy source like triglycerides, the most effective strategy to lower cholesterol levels in humans is inhibition of HMG-CoA reductase, which decreases cholesterol synthesis in the liver. Other approaches are inhibition of intestinal cholesterol absorption and sequestration of bile acids. Bile acid sequestrants bind bile acids in the intestine, thereby preventing their reabsorption and leading to increased fecal excretion. This reduces the bile acid pool in the liver and thereby stimulates bile acid synthesis from cholesterol. Consequently, hepatic uptake of cholesterol is increased to replenish hepatic cholesterol levels and plasma cholesterol levels are lowered. The most recent addition to cholesterol lowering drugs are proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors. PCSK9 binds to the LDL receptor (mainly in the liver), resulting in intracellular degradation of the receptor and a subsequent decrease in LDL receptors on the plasma membrane. By preventing PCSK9 binding to LDL receptors with PCSK9 inhibitors, LDL receptor degradation is prevented, which results in increased LDL receptor expression and enhanced hepatic uptake of LDL from the circulation (100). As discussed above, BAT activation not only has the potential to reduce plasma triglyceride levels (101, 102), but activation of BAT by β3-adrenergic receptor stimulation in *E3L.CETP* mice also lowers cholesterol levels by indirectly accelerating the hepatic clearance of cholesterol-enriched remnants (17). Although (temporarily) introducing a cholesterol-consuming bacterium is far from optimal considering the pathogenic nature of *e.g. Mycobacterium tuberculosis* or *Mycobacterium leprae* (103), it would be neat to find a novel strategy to combust cholesterol in a similar way as BAT is able to burn triglycerides.

Anti-inflammatory strategies

Taking into account the wide spectrum of inflammatory cells and cytokines that are elevated in obesity, targeting a single cell, factor or cytokine might not be efficient to reduce systemic inflammation and ways to manage the overall metabolic inflammation should be considered. In this light, salicylates, such as salsalate, are favourable as they inhibit the master regulatory protein complex of inflammation NF-κB and thereby transcription of many inflammatory cytokines (104). Despite the fact that salicylates were shown to lower glucose levels in diabetic individuals more than 100 years ago (4, 105), and since then a large amount of research was dedicated to the role of inflammation in both insulin resistance and atherosclerosis, salicylates have not been applied in the clinic to treat these disorders yet. In **chapter 5**, we show that salsalate not only improves glucose metabolism, but also reduces inflammation in WAT (**Fig. 1** of this discussion). Although we did not demonstrate a causal relationship between the reduced adipose tissue inflammation and the improved glucose metabolism observed, it does confirm that the two effects go together. Promisingly, salicylate was also shown to reduce atherosclerosis partly by quenching inflammation. As described above, this was proven by showing that salicylate treatment reduces hepatic NF-κB activity and macrophage content in plaques further than low cholesterol feeding (to achieve similar plasma cholesterol levels in the salicylate-treated and the control group) did (24).

Since South Asians have reduced expression of type 1 interferon signalling in WAT (**chapter 4**) and a disadvantageous metabolic phenotype including obesity and insulin resistance (106, 107), one could speculate that anti-inflammatory type 1 interferons would qualify to treat adipose tissue inflammation and/or insulin resistance and atherosclerosis in this population. Interferons are currently used to treat viral infections (108) but have never been applied or tested to treat metabolic inflammation. Although more insight into the link between interferon signalling in adipose tissue and metabolic health would be useful, investigating whether interferon treatment would be effective to reduce metabolic disease in humans does not entail a high risk because interferons are approved drugs (108).

In summary, type 2 diabetes and atherosclerosis are both characterized by systemic inflammation and a decent amount of evidence suggests that targeting inflammation with pharmacological interventions may improve these metabolic disorders.

TRANSLATIONAL CHALLENGES

Large quantities of studies aimed at reducing the obesity epidemic and related morbidities are currently being performed in mice, which ultimately need to be translated towards the human situation. For instance, anti-inflammatory strategies to reduce atherosclerosis and insulin resistance may deserve faster implementation in the clinic if only we could show how and to which extent the immune system exactly contributes to these metabolic derangements in humans. The fact that lipid metabolism and inflammation are intertwined (7) complicates progress within this field of research. HMG-CoA reductase inhibitors

(*i.e.* statins) reduce C-reactive protein (CRP, a marker of inflammation) levels besides lowering LDL cholesterol in clinical trials (109-111). When added to statins, the cholesterol absorption inhibitor ezetimibe also reduces CRP (112). It was recently reported that PCSK9 is directly involved in promoting inflammatory processes (113, 114) and thereby contributes to atherosclerosis independent of cholesterol, which suggests that even PCSK9 inhibition may reduce inflammation (114). Comparative studies into the differential effects of statins, ezetimibe and PCSK9 inhibitors on cholesterol levels, inflammatory markers and cardiovascular outcome may shed more light upon the relative contribution of inflammation and cholesterol on cardiovascular disease in humans. It would also be interesting to take along assessment of the inflammatory phenotype of WAT and skeletal muscles more often in clinical studies as we did in **chapter 4**, to gain a better understanding of potential anti-inflammatory effects of promising compounds on these tissues.

With regards to lowering triglycerides by activating BAT and beige WAT, combusting lipids this way prevents obesity and atherosclerosis in mice, but the translational value of these promising results from different mouse models for the human situation is still uncertain. Interscapular classical BAT in mice does not regress with age whereas adult humans no longer possess this classical BAT depot. Another difficulty in extrapolating mouse data to the human is the fact that humans are usually situated in a thermoneutral zone (±25°C) due to the wearing of clothes, while mice are often housed at room temperature (18-22°C) at which BAT is active. Housing mice in their thermoneutral condition (30°C) would be more comparable to the human situation, which is not always acknowledged. Future studies BAT activation and beiging in mice should therefore preferably be performed at 30°C.

Interestingly, mechanisms of beiging of WAT display more similarities between mice and humans. Hormones, immune cells or cytokines that induce beige adipocytes in mice are also present in humans, suggesting that beiging via those mechanisms can also occur in humans. For example, presence of ILC2s in human adipose tissue has been confirmed, suggesting that the circuit of ILC2s, eosinophils, type 2 cytokines and anti-inflammatory macrophages that induce beiging in mice, is also operational in human beiging (115). Severe beiging of WAT is also seen in patients with pheochromocytoma, a catecholamine-secreting tumor. This condition indeed leads to increased energy expenditure (116). Together, these observations support a similar mechanism behind beiging in humans and in mice, although beiging capacity and its contribution to energy expenditure in humans still needs to be determined in detail.

Potential drugs that induce beiging of WAT in humans are currently being tested in the clinic (117). A possible target would be the β3-adrenergic receptor, although it is not specific for brown adipocytes as it is expressed in a variety of organs. Until recently, β-adrenergic receptor agonists had not been shown to have major effects on energy balance. In 2015, it was shown that a latest generation β-adrenergic receptor agonist, mirabegron (approved to treat the overactive bladder), activated BAT evidenced by increased [^{18}F]fluorodeoxyglucose (FDG) uptake, and increased energy expenditure in healthy male subjects (118). As for targeting inflammation, the effects of salsalate (**chapter 5**) are currently being investigated in clinical trials (119-122). Promisingly, some studies show that salsalate improves glycemic

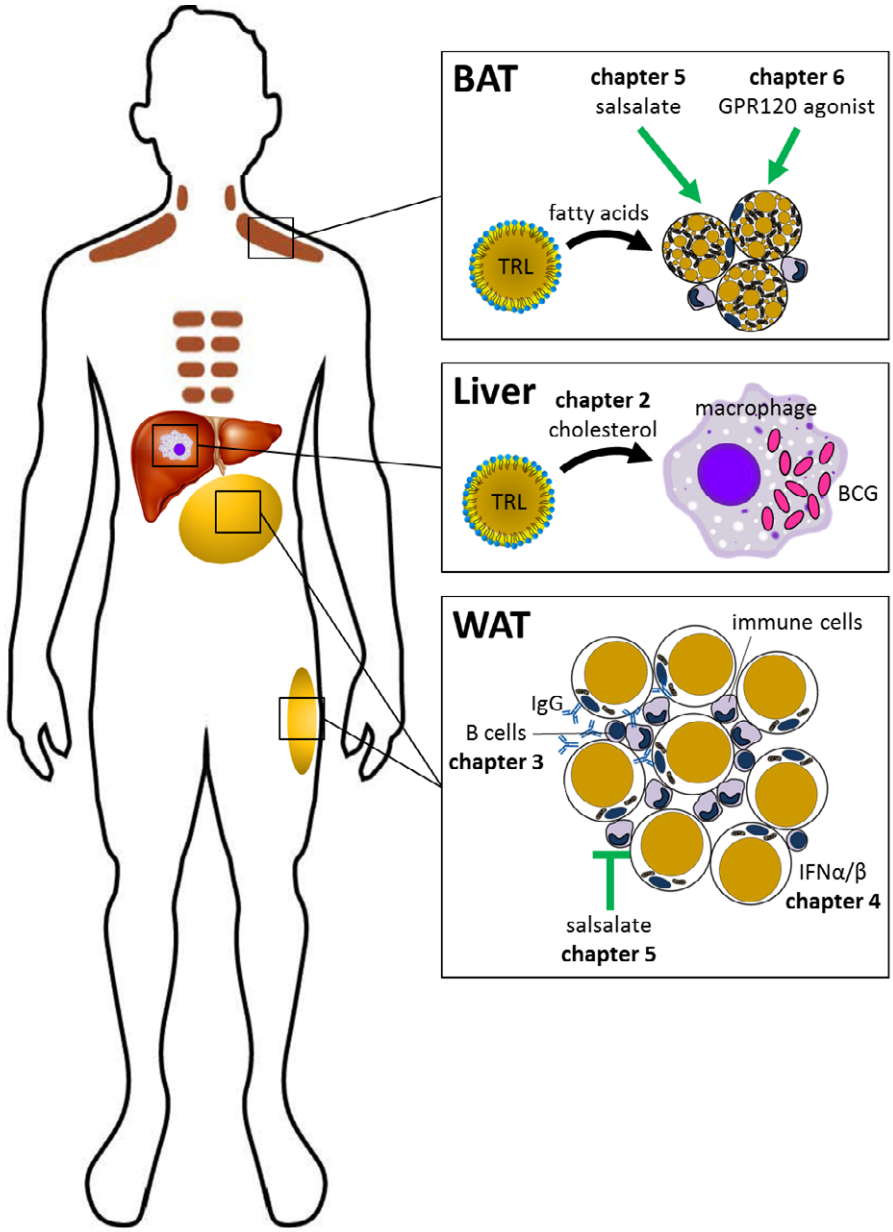

Figure 1. Immune modulation of adipose tissue and lipid metabolism. *See text for explanation. BAT, brown adipose tissue; BCG, Bacille-Calmette-Guérin; IFN, interferon; TRL, triglyceride-rich lipoprotein; WAT, white adipose tissue.*

control (123) and reduces systemic inflammation (119, 124, 125). However, salsalate did not reduce noncalcified coronary plaque volume in man (119), and thus the question remains whether reducing inflammation per se also reduces cardiovascular disease in humans.

Besides receptor-targeted drugs, diet and nutritional components can be considered as an alternative strategy to modulate thermogenesis and inflammation in humans. Although it is known that brown, beige and white adipocytes are fuelled by glucose and fatty acids, large knowledge gaps still exist. We still do not know whether different types of dietary fatty acids or carbohydrates elicit distinct effects on thermogenesis and inflammation. Whereas saturated fatty acids promote inflammation and are detrimental for metabolic health (126), n-3 fatty acids are anti-inflammatory and act beneficially (127). Whether dietary n-3 fatty acids activate BAT or promote beiging remains to be investigated. As for specific carbohydrates, hardly anything is known about their effects on BAT activity or beiging. Almost 30 years ago, Walgren *et al.* (128) showed that dietary carbohydrates increase noradrenaline turnover in heart and/or BAT of rats, unrelated to the type of carbohydrate (*i.e.* fructose, sucrose, dextrose, corn starch) (128). These promising data have not been followed up. Other nutritional components of interest may be amino acids. High plasma concentrations of branched-chain amino acids are found in obesity and type 2 diabetes, and they are associated with increased cardiovascular risk (129). In mice, dietary restriction for the amino acid methionine activates BAT (130). Together, this suggests that altered intake of amino acids could modulate metabolic health. Future research might shed light on the underexposed aspect of modulating inflammation and thermogenesis and ultimately metabolic health by nutritional components.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The immune system plays an important role in brown and white adipose tissue and lipid metabolism and mechanisms behind the interactions between immune cells, adipocytes and lipids are gradually being uncovered. These mechanisms could yield targets to develop novel therapeutic strategies to lower plasma lipid levels, attenuate adipose tissue and systemic inflammation, and ultimately reduce atherosclerosis and insulin resistance. Currently, the most favourable novel approach to eliminate triglycerides and possibly also cholesterol is by increasing combustion of fatty acids in BAT or beige adipocytes in WAT. Combining this with anti-inflammatory therapies to restrain systemic and adipose tissue inflammation will probably further improve metabolic outcome by uncoupling obesity from its associated disorders that are mediated by inflammation. Since the immune system and its functioning are very complex, targeting central mediators of inflammation (*i.e.* transcription factors or upstream regulators of main inflammatory pathways such as NF-κB or IRFs) are probably preferred.

In order to find novel and effective therapeutic targets, the challenge is to identify the metabolic crosstalk between (brown, beige and white) adipocytes and immune cells and the order of events that occur during obesity development. Immune modulation of adipose

tissue and lipid metabolism will not come down to an individual immune cell type and will involve significant crosstalk between different cell types. Important questions to further address include: What are the immune regulatory effector molecules that are secreted by adipocytes to attract or regulate immune cells? How are browning and the immune system regulated in obesity? And how do these processes change during aging? And last but not least, it is of great importance to translate these findings towards the clinic in favour of developing effective and safe treatment options for the patient.

REFERENCES

1. Klop B, Elte JW, Cabezas MC: Dyslipidemia in obesity: mechanisms and potential targets. Nutrients 2013;5:1218-1240
2. Nordestgaard BG: Triglyceride-Rich Lipoproteins and Atherosclerotic Cardiovascular Disease: New Insights From Epidemiology, Genetics, and Biology. Circulation research 2016;118:547-563
3. Hotamisligil GS: Inflammation and metabolic disorders. Nature 2006;444:860-867
4. Shoelson SE, Lee J, Goldfine AB: Inflammation and insulin resistance. The Journal of clinical investigation 2006;116:1793-1801
5. Getz GS, Reardon CA: The mutual interplay of lipid metabolism and the cells of the immune system in relation to atherosclerosis. Clinical lipidology 2014;9:657-671
6. Steinberg D: Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nature medicine 2002;8:1211-1217
7. van Diepen JA, Berbee JF, Havekes LM, Rensen PC: Interactions between inflammation and lipid metabolism: relevance for efficacy of anti-inflammatory drugs in the treatment of atherosclerosis. Atherosclerosis 2013;228:306-315
8. Schaftenaar F, Frodermann V, Kuiper J, Lutgens E: Atherosclerosis: the interplay between lipids and immune cells. Curr Opin Lipidol 2016;27:209-215
9. Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilleres O, Poupon R: Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. Journal of hepatology 2001;34:428-434
10. Miyazaki T, Honda A, Ikegami T, Saitoh Y, Hirayama T, Hara T, Doy M, Matsuzaki Y: Hepatitis C virus infection causes hypolipidemia regardless of hepatic damage or nutritional state: An epidemiological survey of a large Japanese cohort. Hepatology research : the official journal of the Japan Society of Hepatology 2011;41:530-541
11. Grassi G, Di Caprio G, Fimia GM, Ippolito G, Tripodi M, Alonzi T: Hepatitis C virus relies on lipoproteins for its life cycle. World journal of gastroenterology 2016;22:1953-1965
12. Aizawa Y, Seki N, Nagano T, Abe H: Chronic hepatitis C virus infection and lipoprotein metabolism. World journal of gastroenterology 2015;21:10299-10313
13. Clark PJ, Thompson AJ, Vock DM, Kratz LE, Tolun AA, Muir AJ, McHutchison JG, Subramanian M, Millington DM, Kelley RI, Patel K: Hepatitis C virus selectively perturbs the distal cholesterol synthesis pathway in a genotype-specific manner. Hepatology (Baltimore, Md) 2012;56:49-56
14. Huang H, Kang R, Zhao Z: Is hepatitis C associated with atherosclerotic burden? A systematic review and meta-analysis. PloS one 2014;9:e106376
15. Olubamwo OO, Onyeka IN, Miettola J, Kauhanen J, Tuomainen TP: Hepatitis C as a risk factor for carotid atherosclerosis – a systematic review. Clinical physiology and functional imaging 2016;36:249-260
16. Ishizaka N, Ishizaka Y, Yamkado M: Atherosclerosis as a possible extrahepatic manifestation of chronic hepatitis C virus infection. Clinical Medicine Insights Cardiology 2014;8:1-5
17. Berbée JF, Boon MR, Khedoe PP, Bartelt A, Schlein C, Worthmann A, Kooijman S, Hoeke G, Mol IM, John C, Jung C, Vazirpanah N, Brouwers LP, Gordts PL, Esko JD, Hiemstra PS, Havekes LM, Scheja L, Heeren J, Rensen PC: Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nature communications 2015;6:6356

18. Westerterp M, van der Hoogt CC, de Haan W, Offerman EH, Dallinga-Thie GM, Jukema JW, Havekes LM, Rensen PC: Cholesteryl ester transfer protein decreases high-density lipoprotein and severely aggravates atherosclerosis in APOE*3-Leiden mice. Arteriosclerosis, thrombosis, and vascular biology 2006;26:2552-2559
19. van den Maagdenberg AM, Hofker MH, Krimpenfort PJ, de Bruijn I, van Vlijmen B, van der Boom H, Havekes LM, Frants RR: Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. The Journal of biological chemistry 1993;268:10540-10545
20. Berbee JF, Wong MC, Wang Y, van der Hoorn JW, Khedoe PP, van Klinken JB, Mol IM, Hiemstra PS, Tsikas D, Romijn JA, Havekes LM, Princen HM, Rensen PC: Resveratrol protects against atherosclerosis, but does not add to the antiatherogenic effect of atorvastatin, in APOE*3-Leiden.CETP mice. The Journal of nutritional biochemistry 2013;24:1423-1430
21. Kuhnast S, Louwe MC, Heemskerk MM, Pieterman EJ, van Klinken JB, van den Berg SA, Smit JW, Havekes LM, Rensen PC, van der Hoorn JW, Princen HM, Jukema JW: Niacin Reduces Atherosclerosis Development in APOE*3Leiden. CETP Mice Mainly by Reducing NonHDL-Cholesterol. PloS one 2013;8:e66467
22. Kuhnast S, van der Tuin SJ, van der Hoorn JW, van Klinken JB, Simic B, Pieterman E, Havekes LM, Landmesser U, Luscher TF, Willems van Dijk K, Rensen PC, Jukema JW, Princen HM: Anacetrapib reduces progression of atherosclerosis, mainly by reducing non-HDL-cholesterol, improves lesion stability and adds to the beneficial effects of atorvastatin. European heart journal 2015;36:39-48
23. Kleemann R, Princen HM, Emeis JJ, Jukema JW, Fontijn RD, Horrevoets AJ, Kooistra T, Havekes LM: Rosuvastatin reduces atherosclerosis development beyond and independent of its plasma cholesterol-lowering effect in APOE*3-Leiden transgenic mice: evidence for antiinflammatory effects of rosuvastatin. Circulation 2003;108:1368-1374
24. de Vries-van der Weij J, Toet K, Zadelaar S, Wielinga PY, Kleemann R, Rensen PC, Kooistra T: Anti-inflammatory salicylate beneficially modulates pre-existing atherosclerosis through quenching of NF-kappaB activity and lowering of cholesterol. Atherosclerosis 2010;213:241-246
25. Kooijman S, Meurs I, van Beek L, Khedoe PP, Giezekamp A, Pike-Overzet K, Cailotto C, van der Vliet J, van Harmelen V, Boeckxstaens G, Berbee JF, Rensen PC: Splenic autonomic denervation increases inflammatory status but does not aggravate atherosclerotic lesion development. American journal of physiology Heart and circulatory physiology 2015;309:H646-654
26. Zadelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn J, Princen HM, Kooistra T: Mouse models for atherosclerosis and pharmaceutical modifiers. Arteriosclerosis, thrombosis, and vascular biology 2007;27:1706-1721
27. Tenger C, Zhou X: Apolipoprotein E modulates immune activation by acting on the antigen-presenting cell. Immunology 2003;109:392-397
28. Rensen PC, Oosten M, Bilt E, Eck M, Kuiper J, Berkel TJ: Human recombinant apolipoprotein E redirects lipopolysaccharide from Kupffer cells to liver parenchymal cells in rats In vivo. The Journal of clinical investigation 1997;99:2438-2445
29. Van Oosten M, Rensen PC, Van Amersfoort ES, Van Eck M, Van Dam AM, Breve JJ, Vogel T, Panet A, Van Berkel TJ, Kuiper J: Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality. A new therapeutic approach to treat gram-negative sepsis. The Journal of biological chemistry 2001;276:8820-8824
30. Ali K, Middleton M, Pure E, Rader DJ: Apolipoprotein E suppresses the type I

inflammatory response in vivo. Circulation research 2005;97:922-927

31. Grainger DJ, Reckless J, McKilligin E: Apolipoprotein E modulates clearance of apoptotic bodies in vitro and in vivo, resulting in a systemic proinflammatory state in apolipoprotein E-deficient mice. Journal of immunology (Baltimore, Md : 1950) 2004;173:6366-6375
32. Dong M, Yang X, Lim S, Cao Z, Honek J, Lu H, Zhang C, Seki T, Hosaka K, Wahlberg E, Yang J, Zhang L, Lanne T, Sun B, Li X, Liu Y, Zhang Y, Cao Y: Cold exposure promotes atherosclerotic plaque growth and instability via UCP1-dependent lipolysis. Cell Metab 2013;18:118-129
33. Lamb DJ, Eales LJ, Ferns GA: Immunization with bacillus Calmette-Guerin vaccine increases aortic atherosclerosis in the cholesterol-fed rabbit. Atherosclerosis 1999;143:105-113
34. Ovchinnikova OA, Berge N, Kang C, Urien C, Ketelhuth DF, Pottier J, Drouet L, Hansson GK, Marchal G, Back M, Schwartz-Cornil I, Lagranderie M: Mycobacterium bovis BCG killed by extended freeze-drying induces an immunoregulatory profile and protects against atherosclerosis. Journal of internal medicine 2014;275:49-58
35. Lumeng CN, Bodzin JL, Saltiel AR: Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation 2007;117:175-184
36. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr.: Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation 2003;112:1796-1808
37. Elgazar-Carmon V, Rudich A, Hadad N, Levy R: Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. Journal of lipid research 2008;49:1894-1903
38. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, Lu M, Li P, Yan Q, Zhu Y, Ofrecio J, Lin M, Brenner MB, Olefsky JM: Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nature medicine 2012;18:1407-1412
39. Wu D, Molofsky AB, Liang H-E, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM: Eosinophils Sustain Adipose Alternatively Activated Macrophages Associated with Glucose Homeostasis. Science 2011;332:243-247
40. Lumeng CN, Saltiel AR: Inflammatory links between obesity and metabolic disease. Journal of Clinical Investigation 2011;121:2111-2117
41. Chawla A, Nguyen KD, Goh YPS: Macrophage-mediated inflammation in metabolic disease. Nature Reviews Immunology 2011;11:738-749
42. van Beek L, van Klinken JB, Pronk AC, van Dam AD, Dirven E, Rensen PC, Koning F, Willems van Dijk K, van Harmelen V: The limited storage capacity of gonadal adipose tissue directs the development of metabolic disorders in male C57Bl/6J mice. Diabetologia 2015;58:1601-1609
43. Boutens L, Stienstra R: Adipose tissue macrophages: going off track during obesity. Diabetologia 2016;59:879-894
44. Caspar-Bauguil S, Cousin B, Galinier A, Segafredo C, Nibbelink M, Andre M, Casteilla L, Penicaud L: Adipose tissues as an ancestral immune organ: site-specific change in obesity. FEBS letters 2005;579:3487-3492
45. Duffaut C, Galitzky J, Lafontan M, Bouloumie A: Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochemical and biophysical research communications 2009;384:482-485
46. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch HM, Engleman EG: B cells promote insulin resistance through modulation of T cells and production

of pathogenic IgG antibodies. Nature medicine 2011;17:610-617

47. Mayer-Barber KD, Yan B: Clash of the Cytokine Titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses. Cellular & molecular immunology 2017;14:22-35
48. Raji A, Seely EW, Arky RA, Simonson DC: Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. The Journal of clinical endocrinology and metabolism 2001;86:5366-5371
49. Ivashkiv LB, Donlin LT: Regulation of type I interferon responses. Nature reviews Immunology 2014;14:36-49
50. Eguchi J, Yan QW, Schones DE, Kamal M, Hsu CH, Zhang MQ, Crawford GE, Rosen ED: Interferon regulatory factors are transcriptional regulators of adipogenesis. Cell metabolism 2008;7:86-94
51. Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, Dushay J, Estall JL, Klein U, Maratos-Flier E, Rosen ED: Transcriptional control of adipose lipid handling by IRF4. Cell metabolism 2011;13:249-259
52. Kong X, Banks A, Liu T, Kazak L, Rao RR, Cohen P, Wang X, Yu S, Lo JC, Tseng YH, Cypess AM, Xue R, Kleiner S, Kang S, Spiegelman BM, Rosen ED: IRF4 is a key thermogenic transcriptional partner of PGC-1alpha. Cell 2014;158:69-83
53. Kumari M, Wang X, Lantier L, Lyubetskaya A, Eguchi J, Kang S, Tenen D, Roh HC, Kong X, Kazak L, Ahmad R, Rosen ED: IRF3 promotes adipose inflammation and insulin resistance and represses browning. The Journal of clinical investigation 2016;126:2839-2854
54. Lear SA, Humphries KH, Kohli S, Birmingham CL: The use of BMI and waist circumference as surrogates of body fat differs by ethnicity. Obesity (Silver Spring, Md) 2007;15:2817-2824
55. Alsaggar M, Mills M, Liu D: Interferon beta overexpression attenuates adipose tissue inflammation and high-fat diet-induced obesity and maintains glucose homeostasis. Gene therapy 2016;24:60-66
56. Wieser V, Adolph TE, Grander C, Grabherr F, Enrich B, Moser P, Moschen AR, Kaser S, Tilg H: Adipose type I interferon signalling protects against metabolic dysfunction. Gut 2016; in press
57. McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG: Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. Circulation 1993;87:152-161
58. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C, Berbee JF, Middelkoop B, Rensen PC, Tamsma JT: E-selectin is elevated in cord blood of South Asian neonates compared with Caucasian neonates. The Journal of pediatrics 2012;160:844-848. e841
59. Bakker LE, Boon MR, van der Linden RA, Arias-Bouda LP, van Klinken JB, Smit F, Verberne HJ, Jukema JW, Tamsma JT, Havekes LM, van Marken Lichtenbelt WD, Jazet IM, Rensen PC: Brown adipose tissue volume in healthy lean south Asian adults compared with white Caucasians: a prospective, case-controlled observational study. The lancet Diabetes & endocrinology 2014;2:210-217
60. Bhopal RS, Rafnsson SB: Could mitochondrial efficiency explain the susceptibility to adiposity, metabolic syndrome, diabetes and cardiovascular diseases in South Asian populations? International journal of epidemiology 2009;38:1072-1081
61. Shoelson SE, Herrero L, Naaz A: Obesity, inflammation, and insulin resistance. Gastroenterology 2007;132:2169-2180
62. Legein B, Temmerman L, Biessen EA, Lutgens E: Inflammation and immune system interactions in atherosclerosis. Cellular and molecular life sciences : CMLS 2013;70:3847-3869

63. Cannon B, Nedergaard J: Brown adipose tissue: function and physiological significance. Physiological reviews 2004;84:277-359
64. Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elia E, Kessler SH, Kahn PA, English J, Chatman K, Trauger SA, Doria A, Kolodny GM: Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. Cell Metab 2015;21:33-38
65. Boon MR, Kooijman S, van Dam AD, Pelgrom LR, Berbée JF, Visseren CA, van Aggele RC, van den Hoek AM, Sips HC, Lombes M, Havekes LM, Tamsma JT, Guigas B, Meijer OC, Jukema JW, Rensen PC: Peripheral cannabinoid 1 receptor blockade activates brown adipose tissue and diminishes dyslipidemia and obesity. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2014;28:5361-5375
66. Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombes M, Princen HM, Havekes LM, Rensen PC, Guigas B: Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes 2014;63:880-891
67. Kooijman S, Wang Y, Parlevliet ET, Boon MR, Edelschaap D, Snaterse G, Pijl H, Romijn JA, Rensen PC: Central GLP-1 receptor signalling accelerates plasma clearance of triacylglycerol and glucose by activating brown adipose tissue in mice. Diabetologia 2015;58:2637-2646
68. Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard KJ, Kemp BE, Sakamoto K, Steinberg GR, Hardie DG: The ancient drug salicylate directly activates AMP-activated protein kinase. Science 2012;336:918-922
69. Steinberg GR, Dandapani M, Hardie DG: AMPK: mediating the metabolic effects of salicylate-based drugs? Trends in endocrinology and metabolism: TEM 2013;24:481-487
70. Smith BK, Ford RJ, Desjardins EM, Green AE, Hughes MC, Houde VP, Day EA, Marcinko K, Crane JD, Mottillo EP, Perry CG, Kemp BE, Tarnopolsky MA, Steinberg GR: Salsalate (Salicylate) Uncouples Mitochondria, Improves Glucose Homeostasis, and Reduces Liver Lipids Independent of AMPK-beta1. Diabetes 2016;65:3352-3361
71. Liang W, Verschuren L, Mulder P, van der Hoorn JW, Verheij J, van Dam AD, Boon MR, Princen HM, Havekes LM, Kleemann R, van den Hoek AM: Salsalate attenuates diet induced non-alcoholic steatohepatitis in mice by decreasing lipogenic and inflammatory processes. British journal of pharmacology 2015;172:5293-5305
72. Meex RC, Phielix E, Moonen-Kornips E, Schrauwen P, Hesselink MK: Stimulation of human whole-body energy expenditure by salsalate is fueled by higher lipid oxidation under fasting conditions and by higher oxidative glucose disposal under insulin-stimulated conditions. The Journal of clinical endocrinology and metabolism 2011;96:1415-1423
73. Zack F, Blaas V, Goos C, Rentsch D, Buttner A: Death within 44 days of 2,4-dinitrophenol intake. International journal of legal medicine 2016;130:1237-1241
74. Ost M, Keipert S, Klaus S: Targeted mitochondrial uncoupling beyond UCP1 – The fine line between death and metabolic health. Biochimie 2017;134:77-85
75. Grundlingh J, Dargan PI, El-Zanfaly M, Wood DM: 2,4-dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. Journal of medical toxicology : official journal of the American College of Medical Toxicology 2011;7:205-212
76. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G: Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nature medicine 2005;11:90-94

77. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky JM: GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 2010;142:687-698
78. Li X, Yu Y, Funk CD: Cyclooxygenase-2 induction in macrophages is modulated by docosahexaenoic acid via interactions with free fatty acid receptor 4 (FFA4). FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2013;27:4987-4997
79. Liu Y, Chen LY, Sokolowska M, Eberlein M, Alsaaty S, Martinez-Anton A, Logun C, Qi HY, Shelhamer JH: The fish oil ingredient, docosahexaenoic acid, activates cytosolic phospholipase A(2) via GPR120 receptor to produce prostaglandin E(2) and plays an anti-inflammatory role in macrophages. Immunology 2014;143:81-95
80. Gotoh C, Hong YH, Iga T, Hishikawa D, Suzuki Y, Song SH, Choi KC, Adachi T, Hirasawa A, Tsujimoto G, Sasaki S, Roh SG: The regulation of adipogenesis through GPR120. Biochemical and biophysical research communications 2007;354:591-597
81. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, Kimura I, Leloire A, Liu N, Iida K, Choquet H, Besnard P, Lecoeur C, Vivequin S, Ayukawa K, Takeuchi M, Ozawa K, Tauber M, Maffeis C, Morandi A, Buzzetti R, Elliott P, Pouta A, Jarvelin MR, Korner A, Kiess W, Pigeyre M, Caiazzo R, Van Hul W, Van Gaal L, Horber F, Balkau B, Levy-Marchal C, Rouskas K, Kouvatsi A, Hebebrand J, Hinney A, Scherag A, Pattou F, Meyre D, Koshimizu TA, Wolowczuk I, Tsujimoto G, Froguel P: Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature 2012;483:350-354
82. Rosell M, Kaforou M, Frontini A, Okolo A, Chan YW, Nikolopoulou E, Millership S, Fenech ME, MacIntyre D, Turner JO, Moore JD, Blackburn E, Gullick WJ, Cinti S, Montana G, Parker MG, Christian M: Brown and white adipose tissues: intrinsic differences in gene expression and response to cold exposure in mice. American journal of physiology Endocrinology and metabolism 2014;306:E945-964
83. Quesada-Lopez T, Cereijo R, Turatsinze JV, Planavila A, Cairo M, Gavalda-Navarro A, Peyrou M, Moure R, Iglesias R, Giralt M, Eizirik DL, Villarroya F: The lipid sensor GPR120 promotes brown fat activation and FGF21 release from adipocytes. Nature communications 2016;7:13479
84. Kuji I, Imabayashi E, Minagawa A, Matsuda H, Miyauchi T: Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. Annals of nuclear medicine 2008;22:231-235
85. Yamaga LY, Thom AF, Wagner J, Baroni RH, Hidal JT, Funari MG: The effect of catecholamines on the glucose uptake in brown adipose tissue demonstrated by (18)F-FDG PET/CT in a patient with adrenal pheochromocytoma. European journal of nuclear medicine and molecular imaging 2008;35:446-447
86. Lin JZ, Martagon AJ, Cimini SL, Gonzalez DD, Tinkey DW, Biter A, Baxter JD, Webb P, Gustafsson JA, Hartig SM, Phillips KJ: Pharmacological Activation of Thyroid Hormone Receptors Elicits a Functional Conversion of White to Brown Fat. Cell reports 2015;13:1528-1537
87. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonenkov A, Flier JS, Maratos-Flier E, Spiegelman BM: FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. Genes & development 2012;26:271-281
88. Boon MR, van den Berg SA, Wang Y, van den Bossche J, Karkampouna S, Bauwens M, De Saint-Hubert M, van der Horst G, Vukicevic S, de Winther MP, Havekes LM, Jukema JW, Tamsma JT, van der Pluijm G, van Dijk KW,

Rensen PC: BMP7 activates brown adipose tissue and reduces diet-induced obesity only at subthermoneutrality. PloS one 2013;8:e74083

89. Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM, Chawla A: Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 2011;480:104-108
90. Fabbiano S, Suarez-Zamorano N, Rigo D, Veyrat-Durebex C, Stevanovic Dokic A, Colin DJ, Trajkovski M: Caloric Restriction Leads to Browning of White Adipose Tissue through Type 2 Immune Signaling. Cell Metab 2016;24:434-446
91. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD, Chawla A: Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 2014;157:1292-1308
92. Lee MW, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, Yun K, Locksley RM, Chawla A: Activated type 2 innate lymphoid cells regulate beige fat biogenesis. Cell 2015;160:74-87
93. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D: Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 2015;519:242-246
94. Sanchez-Gurmaches J, Hung CM, Guertin DA: Emerging Complexities in Adipocyte Origins and Identity. Trends in cell biology 2016;26:313-326
95. Goldberg IJ, Eckel RH, McPherson R: Triglycerides and heart disease: still a hypothesis? Arteriosclerosis, thrombosis, and vascular biology 2011;31:1716-1725
96. Ouellet H, Johnston JB, de Montellano PR: Cholesterol catabolism as a therapeutic target in Mycobacterium tuberculosis. Trends in microbiology 2011;19:530-539
97. Wipperman MF, Sampson NS, Thomas ST: Pathogen roid rage: cholesterol utilization by Mycobacterium tuberculosis. Critical reviews in biochemistry and molecular biology 2014;49:269-293
98. Pandey AK, Sassetti CM: Mycobacterial persistence requires the utilization of host cholesterol. Proceedings of the National Academy of Sciences of the United States of America 2008;105:4376-4380
99. Lovewell RR, Sassetti CM, VanderVen BC: Chewing the fat: lipid metabolism and homeostasis during M. tuberculosis infection. Current opinion in microbiology 2016;29:30-36
100. Feingold KR, Grunfeld C: Cholesterol Lowering Drugs. In *Endotext* De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, Eds. South Dartmouth (MA), MDText.com, Inc., 2000
101. Khedoe PP, Hoeke G, Kooijman S, Dijk W, Buijs JT, Kersten S, Havekes LM, Hiemstra PS, Berbée JF, Boon MR, Rensen PC: Brown adipose tissue takes up plasma triglycerides mostly after lipolysis. Journal of lipid research 2015;56:51-59
102. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmuller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J: Brown adipose tissue activity controls triglyceride clearance. Nature medicine 2011;17:200-205
103. Mattos KA, Oliveira VC, Berredo-Pinho M, Amaral JJ, Antunes LC, Melo RC, Acosta CC, Moura DF, Olmo R, Han J, Rosa PS, Almeida PE, Finlay BB, Borchers CH, Sarno EN, Bozza PT, Atella GC, Pessolani MC: Mycobacterium leprae intracellular survival relies on cholesterol accumulation in infected macrophages: a potential target for new drugs for leprosy treatment. Cellular microbiology 2014;16:797-815

104. Shoelson SE, Lee J, Yuan M: Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2003;27 Suppl 3:S49-52
105. Williamson RT: On the Treatment of Glycosuria and Diabetes Mellitus with Sodium Salicylate. British medical journal 1901;1:760-762
106. McKeigue PM, Shah B, Marmot MG: Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. Lancet (London, England) 1991;337:382-386
107. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS, Simonson DC: Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. The Journal of clinical endocrinology and metabolism 2004;89:3965-3972
108. De Clercq E, Li G: Approved Antiviral Drugs over the Past 50 Years. Clinical microbiology reviews 2016;29:695-747
109. Ridker PM: Moving beyond JUPITER: will inhibiting inflammation reduce vascular event rates? Current atherosclerosis reports 2013;15:295
110. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ: Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. The New England journal of medicine 2008;359:2195-2207
111. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, Macfadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ: Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet (London, England) 2009;373:1175-1182
112. Bays HE, Neff D, Tomassini JE, Tershakovec AM: Ezetimibe: cholesterol lowering and beyond. Expert review of cardiovascular therapy 2008;6:447-470
113. Walley KR, Thain KR, Russell JA, Reilly MP, Meyer NJ, Ferguson JF, Christie JD, Nakada TA, Fjell CD, Thair SA, Cirstea MS, Boyd JH: PCSK9 is a critical regulator of the innate immune response and septic shock outcome. Science translational medicine 2014;6:258ra143
114. Cheng JM, Oemrawsingh RM, Garcia-Garcia HM, Boersma E, van Geuns RJ, Serruys PW, Kardys I, Akkerhuis KM: PCSK9 in relation to coronary plaque inflammation: Results of the ATHEROREMO-IVUS study. Atherosclerosis 2016;248:117-122
115. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D: Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 2015;519:242-246
116. Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, Cinti S: White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids 2013;1831:950-959
117. Giordano A, Frontini A, Cinti S: Convertible visceral fat as a therapeutic target to curb obesity. Nature Reviews Drug Discovery 2016;15:405-424
118. Cypess AM, Weiner LS, Roberts-Toler C, Elía EF, Kessler SH, Kahn PA, English J, Chatman K, Trauger SA, Doria A, Kolodny GM: Activation of Human Brown Adipose Tissue by a β3-Adrenergic Receptor Agonist. Cell metabolism 2015;21:33-38
119. Hauser TH, Salastekar N, Schaefer EJ, Desai

T, Goldfine HL, Fowler KM, Weber GM, Welty F, Clouse M, Shoelson SE, Goldfine AB: Effect of Targeting Inflammation With Salsalate: The TINSAL-CVD Randomized Clinical Trial on Progression of Coronary Plaque in Overweight and Obese Patients Using Statins. JAMA cardiology 2016;1:413-423

120. Ariel D, Kim SH, Liu A, Abbasi F, Lamendola CA, Grove K, Tomasso V, Reaven GM: Salsalate-induced changes in lipid, lipoprotein, and apoprotein concentrations in overweight or obese, insulin-resistant, nondiabetic individuals. Journal of clinical lipidology 2015;9:658-663
121. Penesova A, Koska J, Ortega E, Bunt JC, Bogardus C, de Courten B: Salsalate has no effect on insulin secretion but decreases insulin clearance: a randomized, placebo-controlled trial in subjects without diabetes. Diabetes, obesity & metabolism 2015;17:608-612
122. Barzilay JI, Jablonski KA, Fonseca V, Shoelson SE, Goldfine AB, Strauch C, Monnier VM: The impact of salsalate treatment on serum levels of advanced glycation end products in type 2 diabetes. Diabetes care 2014;37:1083-1091
123. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE: The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Annals of internal medicine 2010;152:346-357
124. Goldfine AB, Fonseca V, Jablonski KA, Chen YD, Tipton L, Staten MA, Shoelson SE: Salicylate (salsalate) in patients with type 2 diabetes: a randomized trial. Annals of internal medicine 2013;159:1-12
125. Goldfine AB, Conlin PR, Halperin F, Koska J, Permana P, Schwenke D, Shoelson SE, Reaven PD: A randomised trial of salsalate for insulin resistance and cardiovascular risk factors in persons with abnormal glucose tolerance. Diabetologia 2013;56:714-723
126. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS: TLR4 links innate immunity and fatty acid – induced insulin resistance. The Journal of clinical investigation 2006;116:3015-3025
127. Lee JY, Plakidas A, Lee WH, Heikkinen A, Chanmugam P, Bray G, Hwang DH: Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. Journal of lipid research 2003;44:479-486
128. Walgren MC, Young JB, Kaufman LN, Landsberg L: The effects of various carbohydrates on sympathetic activity in heart and interscapular brown adipose tissue of the rat. Metabolism 1987;36:585-594
129. Bifari F, Nisoli E: Branched-chain amino acids differently modulate catabolic and anabolic states in mammals: a pharmacological point of view. British journal of pharmacology 2016; in press
130. Wanders D, Burk DH, Cortez CC, Van NT, Stone KP, Baker M, Mendoza T, Mynatt RL, Gettys TW: UCP1 is an essential mediator of the effects of methionine restriction on energy balance but not insulin sensitivity. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2015;29:2603-2615