ANALYTIC REVIEW

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Immune and Inflammatory Signaling Pathways in Exercise and Obesity

Abstract: *Over the last decades the combination of both a sedentary lifestyle and excessive food availability has led to a significant increase in the prevalence of obesity, which is increasingly recognized as an important risk factor for type 2 diabetes. Several lines of evidence exist demonstrating that expanded visceral adipose tissue produces several pro-inflammatory mediators that activate signaling pathways that contribute to the development of insulin resistance. Exercise training is an important lifestyle factor that is widely used as a tool for preventing and improving lifestyle-related obesity and insulin resistance. In this regard, exercise training is useful to increase energy expenditure thereby counteracting a positive energy balance. Exercise training is also able to attenuate the activation of several obesity-induced pathways of inflammation and oxidative stress. Thus, a better understanding of the molecular mechanisms and immune pathways in exercise, obesity, and diabetes can be extremely useful to exploit optimized lifestyle strategies to combat the increasing incidence of metabolic diseases.*

Keywords: adipocytes; physical activity; cytokines; fat tissue; insulin sensitivity

Obesity is a serious health issue dramatically increasing in an epidemic manner in most countries. Based on estimations from the World Health Organization, 2.3 billion adults will be overweight or obese by the year 2015.[1] The main etiologic factors for obesity are excess nutrition and physical inactivity, whereas rare genetic defects as etiologic factors are clearly less frequent, indicating that obesity is dependent on lifestyle and thus preventable in most instances. In recent years, it has become increasingly clear that the pathologic expansion of adipose tissue (AT) typical of obesity leads to the development of insulin resistance (IR)[2]—a common metabolic complication associated with obesity, which is characterized by an impaired glucose-uptake by insulin sensitive tissues (liver, skeletal muscle, AT). Insulin mediates glucose-uptake through activation of the insulin receptor, which causes phosphorylation of tyrosine residues of the downstream docking protein insulin receptor substrate 1 (IRS-1), subsequently leading to stimulation of a signaling pathway that causes translocation of glucose transporter type 4 (GLUT4) vesicles to the cell surface resulting in cellular glucose uptake.[3] IR is critical as it significantly increases the risk of developing several obesity-associated diseases, such as type 2 diabetes mellitus, dyslipidemia, hypertension, coronary heart disease, stroke, metabolic syndrome, nonalcoholic fatty liver disease, and certain cancers.[4-7] Development of systemic IR in obesity is explained by the fact that the expanded AT, particularly the visceral adipose tissue (VAT), is not simply a tissue functioning to store triacylglycerols in response to energy excess and to mobilize energy

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during periods of fasting. Rather, it is a endocrine organ secreting numerous hormone-like mediators, such as adipokines (leptin, resistin, adiponectin), cytokines (interleukin [IL]-6, IL-1β, tumor necrosis factor-α [TNFα]), and chemokines (monocyte chemoattractant protein-1 [MCP-1], macrophage inflammatory protein 1 [MIP1], regulated on activation normal T cell expressed and secreted [RANTES]), reactive oxygen species (ROS), as well as free fatty acids (FFA).[8] The circulating levels of leptin, resistin, IL-6, IL-1β, TNFα, MCP-1, ROS, and FFA increase as the adipocytes enlarge and the VAT expands, and which among other negatively affect insulin signaling thereby causing IR.[9] In addition, the VAT of obese subjects develops a localized inflammation due to accumulation of immune cells, in particular monocyte-derived macrophages, secreting also pro-inflammatory cytokines and chemotactic factors, which cause recruitment of further immune cells, thereby inducing a vicious cycle of chronic low-grade inflammation.[10] Due to secretion of pro-inflammatory cytokines from VAT into the circulation, the inflammation becomes systemic causing activation of diverse cellular inflammatory signaling pathways that converge in the activation of the serine kinases Jun N-terminal kinase (JNK)-1 and inhibitor of kB kinase (IKKβ) in insulin-sensitive tissues.[11] Both JNK-1 and IKKβ cause serine phosphorylation of IRS-1, a phosphorylation state associated with impaired insulin signaling and, thereby, systemic IR.[12]

Increased physical activity as achieved by exercise training is a reasonable approach to support diet-induced weight loss by simultaneously increasing energy expenditure and thereby counteracting a positive energy balance.[13] In addition, it has long been known that exercise training reduces low-grade inflammation and improves insulin sensitivity.[14-16] In this review, we provide an overview of the most striking obesity-induced changes in immune pathways and their impact on inflammatory signaling pathways mediating IR and the counterregulatory effects of exercise training on these pathways.

Obesity-Induced Changes in Adipose Tissue Physiology and Immune Pathways

The VAT is a multicellular tissue consisting of the adipocyte fraction, which consists of adipocytes and connective tissue with a dense network of capillaries, and the nonadipocyte fraction. The nonadipocyte fraction, also called stromal-vascular fraction, consists of extracellular matrix and different cells including pre-adipocytes, which are mesenchymal stem cells with the ability to differentiate into adipocytes, multipotent stem cells, fibroblasts, endothelial cells, and immune cells including macrophages, T cells, dendritic cells, natural killer cells, and eosinophils.[17,18] As described below, the pathologic expansion of the VAT during obesity development is accompanied by prominent morphological changes of the adipocyte and the nonadipocyte fraction, which together contribute to VAT inflammation and systemic IR typical for obesity.

Obesity-Induced Changes in the Adipocyte Fraction

Expansion of VAT occurs by increasing the volume of preexisting adipocytes (adipocyte hypertrophy) and/or by generating new small adipocytes (adipocyte hyperplasia) from pre-adipocytes. However, adipocyte hypertrophy, which is mediated by an increased storage of triacylglycerols by fully differentiated adipocytes leading to an enlargement of adipocytes, contributes most to the increase of AT mass during obesity.[19] Adipocyte hypertrophy is critical for developing obesity-related diseases since the adipocyte size is positively correlated with insulin concentrations, IR, and the risk of developing type 2 diabetes.[20-22] The crucial role of adipocyte size is further demonstrated by the finding that obese subjects with few large adipocytes are more insulin resistant than those having the same degree of adiposity and small adipocytes.[22] One reason for the detrimental effect of enlarged adipocytes on insulin action is that larger adipocytes have a higher basal and stimulated rate of lipolysis leading to chronically elevated circulating levels of FFA, which are known to mediate IR.[23] A second important reason is that enlarged adipocytes have an altered secretome, which is characterized by an increased secretion of pro-inflammatory adipokines (eg, leptin, resistin) and cytokines (TNFα, IL-6, IL-1β) and a decreased secretion of anti-inflammatory and insulin-sensitizing adipokines (eg, adiponectin).[8] This imbalance between pro- and anti-inflammatory mediators in blood causes IR in insulin-sensitive tissues and promotes a pro-inflammatory environment in VAT leading to immune cell infiltration, which exacerbates the inflammatory process. A further result of adipocyte enlargement during VAT expansion is the development of local hypoxic regions due to hypoperfusion of the expanded VAT and thus insufficient oxygen supply to the enlarged adipocytes.[24] The chronic hypoxia found in obese VAT causes hypoxic stress leading to activation of transcription factors that act as sensors of low oxygen tension, such as hypoxia-inducible factor-1α (HIF-1α),[25] which stimulates the expression of pro-inflammatory genes and inhibits expression of adiponectin. VAT inflammation is further promoted by the fact that hypertrophic adipocytes degenerate and subsequently die, which explains that adipocyte number remains remarkably stable during weight gain in adults, despite adipocyte hyperplasia from pre-adipocytes. Adipocyte death occurs via a process called pyroptosis involving activation of pro-caspase-1 within the nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome leading to the release of active caspase-1, which cleaves the cytokine precursors pro-IL-1β and pro-IL-18 into active IL-1β and IL-18, respectively.[26,27] The resulting adipocyte death via inflammasome

activation, thus, is inflammation-associated and contributes also to the local VAT inflammation and triggers the migration of monocyte-derived macrophages into VAT.[28] The majority of infiltrated macrophages localize around dead or dying adipocytes (identifiable by the lack of perilipin staining) thereby forming crown-like structures (CLS).[29] The CLS-associated macrophages phagocytose the cellular debris from dead adipocytes or their residual lipid droplets leading to a foam cell-like appearance. CLS formation in response to adipocyte death in obese subjects is an early event of VAT inflammation and highly correlated with IR.[30] Within weeks it can be observed that new adipocytes arising from pre-adipocytes of the stromal-vascular fraction populate the AT, a process called AT remodeling, which causes further AT expansion.[31] However, in the case of treatment of obese subjects with the thiazolidinedione group of insulin sensitizers this AT remodeling can be influenced in a beneficial manner, since thiazolidinediones cause the generation of small, insulin-sensitive adipocytes resulting in an improvement of systemic insulin sensitivity despite AT expansion.[32]

Obesity-Induced Changes in the Stromal-Vascular Fraction

Infiltration and accumulation of monocyte-derived macrophages in VAT triggered by inflammation-associated death of adipocytes is an early event during VAT expansion and weight gain in both humans and rodents.[10,28,33,34] Recruitment of monocytes from the circulation in obesity is mediated by chemokines, such as MCP-1 as well as macrophage inflammatory protein (MIP)1α, MIP1β, and regulated on activation normal T cell expressed and secreted (RANTES), released from enlarged adipocytes and resident VAT macrophages.[35] The MCP-1 ligand has high affinity for C-C motif receptors (CCR) 2 and 5 expressed on the cell surface of monocytes and binding of MCP-1 to CCR2 and CCR5 markedly stimulates monocyte infiltration into AT in different mouse models of obesity.[36-38] The VAT macrophages are phenotypically classified based on their activation/polarization state into "classically activated" pro-inflammatory M1 macrophages, which produce large amounts of pro-inflammatory cytokines (TNFα, IL-1β, IL-6) and generate ROS via inducible nitric oxide synthase, and "alternatively activated" non- or anti-inflammatory M2 macrophages, which have low pro-inflammatory cytokine expression and instead secrete high levels of anti-inflammatory cytokines, such as IL-10 and IL-1 receptor agonist.[39,40] Diet-induced obesity was found to induce a phenotypic switch in AT macrophage polarization from an M2-polarized state in lean animals that may protect adipocytes from inflammation to an M1 pro-inflammatory state that contributes to VAT inflammation and IR.[41] FFAs that are elevated in obesity exacerbate pro-inflammatory cytokine secretion from M1 macrophages through activation of TLR4 receptor, which induces IL-6, IL-1β, and MCP-1 expression in genetically and diet-induced obese mice.[42] Interestingly, lack of CCR5, but not CCR2, in mice causes a shift of the macrophage population toward M2 macrophages indicating that the chemokine CCR5 promotes VAT inflammation not only by recruiting macrophages into VAT but also by causing a shift to the pro-inflammatory M1 phenotype,[43] which favors the development of IR. The switch from M2 to M1 polarization of VAT macrophages is not irreversible but can be reverted by administration of thiazolidinediones to obese mice or by switching obese mice from a high-fat diet to a standard diet.[44,45]

Besides macrophages, several other immune cells in AT have been shown to play a role in obesity and AT inflammation-associated IR. In particular, significant alterations in the number of different subsets of T lymphocytes in VAT during obesity have been reported in different genetic and diet-induced mouse models of obesity. The number of $CD8^+$ T cells was shown to increase in VAT, which, in turn, initiates and propagates an inflammatory cascade involving secretion of pro-inflammatory cytokines and chemokines, thereby promoting accumulation of macrophages in AT of mice in response to a high-fat diet.[46] The finding that CD8-specific antibody treatment ameliorates preestablished AT inflammation in these mice indicates that $CD8^+$ T cells are also essential for maintenance of the inflammatory response.[46] In contrast, the number of $CD4^+$ regulatory T cells (T_{regs}) was reported to decrease in the expanding VAT of mice in response to a high-fat diet.[46,47] This also exacerbates AT inflammation, because T_{regs} secrete anti-inflammatory cytokines that inhibit macrophage migration and promote the noninflammatory M2 polarization state of macrophages.[47] The number of pro-inflammatory Th1 cells, which differentiate from mature $CD4^+$ T cells and produce mainly IFN-γ, increase in VAT of mice during obesity, which promotes VAT inflammation and systemic IR through activation of macrophages and antagonizing the anti-inflammatory properties of T_{regs} and Th2 cells.[48,49] Th2 cells, which also differentiate from mature $CD4^+$ T cells, are anti-inflammatory due to secretion of anti-inflammatory cytokines (IL-4, IL-13),[50] from which IL-4 induces the noninflammatory M2 polarization state of VAT macrophages. In addition, evidence was provided from different animal models of obesity that obesity causes changes in the number of mast cells,[51,52] eosinophils,[53,54] and natural killer T (NKT) cells.[55] However, the biological role of these obesity-induced changes in AT immune cell number and composition, for example, for VAT inflammation and IR, is yet largely undefined. Further outstanding questions originate from the fact that only few data are available with regard to the presence of specific immune cells in human obesity, because human AT, in particular VAT, is not readily available for clinical assessment. Thus, it remains to be demonstrated whether the AT immune cells found in obese mouse models are equivalently found in obese humans and whether they play similar roles.

Obesity-Induced Changes in Inflammatory Signaling Pathways

Despite activation of different intracellular signaling pathways, it is common to the inflammatory mediators secreted from adipocytes and immune cells that insulin sensitivity of target tissues is compromised through negatively interfering with insulin signaling. How insulin signaling in insulin-sensitive tissues (liver, skeletal muscle, AT) is impaired by these mediators in obesity is exemplified below for some of the most important representatives.

Leptin. Leptin is one of several adipokines secreted from adipocytes, and it has long been known that obese subjects have elevated circulating concentrations of leptin compared with normal-weight subjects.[56-58] In addition, circulating leptin levels were found to positively correlate with AT mass and adipocyte size.[9] Conversely, weight loss decreases circulating concentrations and/or AT expression of leptin in obese subjects.[58-60] Leptin exerts its effects in tissues by binding to the leptin receptor at the plasma membrane leading to recruitment and binding of cytoplasmic Janus tyrosin kinases (JAKs), which subsequently become transphosphorylated resulting in activation of signal transducer and activator of transcription (STAT)3 and STAT5.[61,62] Through this, acute-phase proteins and pro-inflammatory transcription factors, such as c-fos, c-jun, and activator protein (AP)-1, are induced.[61,62] Autophosphorylation of JAKs results also in activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway leading to stimulation of nuclear factor-κB (NF-κB).[61,62] Stimulation of NF-κB and other pro-inflammatory transcription factors (AP-1, STATs) is crucial for development of IR because these transcription factors causes upregulation of genes encoding pro-inflammatory mediators, like TNFα and IL-1β, which negatively interfere with insulin signaling.[63] In addition, NF-κB can be stimulated by leptin by activating p38 mitogen-activated protein kinase (MAPK) and stress-activated JNK-1.[64] Activation of JNK-1 directly impairs insulin signaling via serine phosphorylation of the IRS-1, the phosphorylation state of IRS-1, which is linked to IR and reduced glucose uptake.[12] JNK-1 inhibits insulin sensitivity also in a similar manner as NF-κB, AP-1, and STATs through stimulating transcription of pro-inflammatory genes.[12]

Adiponectin. Unlike leptin, the circulating concentrations of adiponectin, for which 3 major isoforms exist (low molecular weight, middle-molecular weight, and high molecular weight adiponectin), negatively correlate with adipocyte size in human subjects, for example, levels of adiponectin in the circulation are reduced in obese subjects compared with normal-weight subjects.[65] Reduced circulating levels of adiponectin, which is best reflected by the high molecular weight form,[66] in obesity are explained by inhibition of their secretion from cultured adipocytes by pro-inflammatory cytokines like TNFα and IL-6,[67] which are found at elevated levels in obese humans.[58,68,69] Conversely, weight loss, which is accompanied by an improvement of insulin sensitivity, induces AT adiponectin expression and/or increases blood levels of adiponectin in obese humans and genetically obese animals, indicating a beneficial role of adiponectin on insulin signaling.[70-72] The insulin sensitizing effect of adiponectin is mediated by binding of adiponectin to its receptors (AdipoR1 and AdipoR2) in target tissues, such as liver and skeletal muscle.[73] Activation of AdipoR1 and AdipoR2 in these tissues stimulates the peroxisome proliferator-activated receptor α(PPARα) pathway, which leads to increased fatty acid oxidation, and inhibition of the NF-κB pathway, which results in a reduced production of pro-inflammatory cytokines.[74] In addition, treatment of different myeloid cells with adiponectin was shown to increase the secretion of anti-inflammatory cytokines, like IL-10 and IL-1 receptor antagonist,[75] an effect that is expected to counteract the inhibitory effects of pro-inflammatory cytokines on insulin signaling. Moreover, studies in mice and rats revealed that the insulin sensitizing effect of adiponectin involves activation of the adenosine monophosphate-activated protein kinase (AMPK) pathway.[76,77] AMPK is a principal regulator of energy metabolism homeostasis, which stimulates glucose utilization in skeletal muscle and suppresses gluconeogenesis in the liver.[76] In addition, AMPK inhibits inflammatory responses induced by NF-κB activation as well as endoplasmic reticulum stress,[77] which occurs in different metabolic disorders including obesity and diabetes. In line with the antidiabetic effects of adiponectin, it was recently shown that AdipoR agonists also activate of AMPK and hepatic and muscle PPARα, ameliorate IR and glucose intolerance in mice fed a high-fat diet, but not in AdipoR1 and AdipoR2 double-knockout mice.[78]

FFA. It has long been known that circulating levels of FFA are chronically elevated in most obese subjects.[79] This is due to the fact that the expanded AT releases more FFA due to a stimulation of basal lipolysis in AT,[23] and once elevated, FFA inhibit the antilipolytic action of insulin further increasing FFA release from AT into the circulation.[80] Elevated FFA levels are considered to be a key factor in developing systemic IR and ultimately type 2 diabetes during obesity. This is based on the observation that pharmacological reduction of circulating FFA levels in obese and type 2 diabetic subjects improves peripheral insulin sensitivity.[81,82] Several mechanisms have been proposed to explain the IR-inducing effect of elevated FFA levels in obesity. Circulating FFA, in particular saturated fatty acids, impair insulin sensitivity through binding to the plasma membrane receptor toll-like receptor 4 (TLR4) in tissues of obese animals,[83] which leads to activation of signaling proteins, such as IKK, JNK, and MAPK, that negatively interfere with insulin signaling. The critical role of TLR4 for developing IR is shown by the fact

that TLR4 knockout mice are protected from IR and weight gain when fed a high-fat diet compared to wild-type controls.[84] In addition, acutely increasing plasma FFA levels in vivo was found to activate NF-κB in human skeletal muscle.[85] This effect leads to an increased expression of pro-inflammatory cytokines and elevated circulating levels of MCP-1 as shown in rats.[86] The latter chemokine acts as a chemotactic signal for immune cells promoting macrophage recruitment into the AT, thereby propagating the inflammatory process. Interestingly, elevated levels of FFA are also known to cause endoplasmic reticulum (ER) stress.[87] In line with this, ER stress is also found in the liver of obese mice, which have also elevated blood levels of FFA.[88] ER stress describes an imbalance between the folding capacity of the ER and the amount of proteins to be folded leading to accumulation of unfolded or misfolded proteins in the ER lumen.[89] As an adaptive response to this ER stress, the cell activates the so-called unfolded protein response (UPR), which is initiated by 3 ER stress sensors: inositol-requiring enzyme 1 (IRE1), RNA-dependent protein kinase (PKR)-like ER kinase (PERK), and activating transcription factor 6 (ATF6).[89] ER stress-induced UPR is proposed to mediate IR because IRE1 and PERK cause activation of JNK and IKKβ signaling pathways, which inhibit insulin signaling via serine phosphorylation of IRS-1 and activation of the pro-inflammatory transcription factors NF-κB and AP-1.[89] Noteworthy, it was recently shown that TLR4 deficiency protects against high-fat diet-induced ER stress and IR in the main organs for glucose and lipid metabolism (skeletal muscle, liver, AT) in mice, indicating that this receptor, which is activated by elevated FFA levels, is crucial for mediating the inhibitory effect of elevated FFA levels on insulin signaling.[90] It also has been proposed that the inhibitory effect of elevated FFA levels on insulin signaling is mediated by intracellular accumulation of toxic lipid derivatives in response to elevations of FFA levels,[91,92] for example, increased plasma FFA levels result in the accumulation of intramuscular long-chain fatty (LCFA) acyl-CoAs, diacylglycerol (DAG), triacylglycerol, and ceramide. Both LCFA-CoAs and DAG were shown to impair insulin signaling via activation of protein kinase C (PKC), which increases serine phosphorylation of IRS-1. A similar mechanism was found for ceramide, but ceramide was also shown to interact with Akt signaling.[91,92] Activation of Akt inhibits insulin signaling through stimulation of NF-κB.

Pro-Inflammatory Cytokines. A large number of studies have demonstrated that AT expression and/or circulating levels of pro-inflammatory cytokines, such as IL-6 and TNFα, are elevated in insulin-resistant overweight and obese subjects[58,68,69,93] and in animal models of obesity and IR.[94] In contrast, weight loss in overweight and obese subjects were accompanied by improvements in insulin sensitivity and decreases in circulating levels and/or AT expression of cytokines.[58,59,69,95-99] These observations suggest that pro-inflammatory mediators contribute to the obesity-induced impairment of IR. Pro-inflammatory cytokines mediate their actions through specific receptors located at the cell membrane (IL-1R, IL-6R, TNFR). On activation of these receptors different signaling cascades are stimulated that converge at the MAPK and the IKKβ pathway leading to JNK and NF-κB activation[100,101]) and through serine phosphorylation of IRS-1 to impairment of insulin signaling.[12] In addition, activation of NF-κB and other pro-inflammatory transcription factors, such as AP-1 and STAT3, by these cytokines promotes the production of further cytokines in a feed-forward manner, which also induces IR.

ROS. It has been shown that human obesity is positively correlated with systemic oxidative stress on the basis of urinary levels of 8-epi-prostaglandin F2α (8-epi-PGF2α)[102] and that systemic oxidative stress is associated with visceral fat accumulation, IR, and metabolic syndrome in humans.[103,104] Oxidative stress occurs when the production of ROS exceeds their clearance by the antioxidant defense system. Interestingly, the oxidative stress levels in obese mice were found to be elevated in plasma and AT but not in liver and skeletal muscle compared to control mice, indicating that the AT in obese individuals may represent a major source of ROS.[103] Production of ROS in AT is proposed to be the result of an increased expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a plasma membrane enzyme that converts molecular oxygen to superoxide radicals, and decreased antioxidant levels[103]; for example, the expression and activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) 1, and glutathione peroxidase (GPX) is known to be decreased in the AT of obese individuals.[105] ROS are known to activate several signaling pathways, which negatively affect insulin signaling, such as the MAPK, JNK, and NF-κB pathways. In addition, JNK activation potentiates oxidative stress through promoting ROS accumulation.[12]

Effect of Exercise on Obesity-Induced Changes in Adipose Tissue Physiology and Immune Pathways

Several studies provided evidence that physical activity exhibits multiple effects on obesity and VAT.[106] Observational studies demonstrated that there is an inverse relationship between measures of physical activity (total activity as well as exercise training) and measures of fat mass and distribution. Complementary, longitudinal studies revealed that exercise training programs are directly responsible for a reduction of body weight and favorable changes of body composition.[107] It is assumed that the exercise-induced increase of total energy expenditure and fat oxidation generates an energy deficit. The loss of body fat is mostly accompanied by the improvement of several obesity-related metabolic and cardiovascular disorders like IR, dyslipidemia, and hypertension.[108]

In light of the pathologic significance of VAT, it is of considerable interest whether exercise interventions are capable of preferentially targeting VAT. Numerous studies provided evidence for an exercise-induced reduction of VAT. Noteworthy, Ross et al[109] demonstrated that an endurance exercise-induced increase of energy expenditure by 700 kcal/day for 14 weeks was followed by a weight loss of 7.5 kg and a decrease in VAT of 52 cm^2 corresponding to a decrease of 6.9 cm^2 VAT fat per kg of weight loss in premenopausal women with abdominal obesity. Similarly, it was shown that overweight postmenopausal women, who exercised (treadmill walking, stationary bicycling, or strength training) at moderate-intensity 5 days/week for 12 months, lost about 8.5 cm^2 of VAT and 1.3 kg of body weight, corresponding to a ratio of 6.5 cm^2/kg of weight change.[110] In addition, a systematic review of human and clinical trials provided evidence that there is a dose–response relationship between amount of aerobic exercise, such as brisk walking, light jogging, or stationary ergometer usage, and reduction in VAT mass.[111] Regarding the physiological mechanisms of exercise-induced VAT reduction, it is known that exercise induces an increase in AT lipolysis, which is attributed to elevated catecholamine concentrations and a decrease in insulin concentrations. It is assumed that VAT is more responsive to adrenergic innervation than other AT depots and therefore benefits more from activity.[112]

Exercise-Induced Changes in the Adipocyte Fraction

Effects of exercise-induced reduction of VAT mass could be the result of both reduction in adipocyte size and adipocyte number. In this regard, cross-sectional studies revealed that exercise trained subjects exhibit smaller adipoctyes in the abdominal region than aged-matched sedentary controls.[113] Interventional studies suggest that exercise training directly reduces adipocyte cell size.[114] A reduction of adipocyte size might be a major reason for exercise effects on IR because smaller adipocytes have lower basal rates of lipolysis leading to a reduction of FFA levels in the circulation. Furthermore, smaller adipocytes are known to have a secretome, which is characterized by a reduced expression of pro-inflammatory adipokines (eg, leptin, resistin) and cytokines.[115] In contrast, results regarding the effect of exercise training on adipocyte cell number are inconsistent. These inconsistencies are probably partially due to methodological difficulties to determine adipocyte cell number with sufficient accuracy, because most studies estimated adipocyte cell number by calculating the body fat to adipocyte size ratio, which however does not take into account that adipocyte number and size is highly variable.[114] A more recent study, in which the adipocyte cell number was assessed from the DNA content of the fat pad, reported that endurance exercise training induced a significant reduction in cell number in VAT of high fat diet–fed rats.[116] Thus, while it is beyond question that exercise reduces adipocyte cell size, more studies are necessary to clarify whether exercise-induced reductions in VAT mass can be attributed also to a decrease of adipocyte number.

During exercise, fat oxidation steadily increases resulting in a breakdown of stored triacylglycerols.[117] In particular, moderate exercise up to intensities of 60% to 65% of VO_{2max} leads to an increased rate of appearance of FFA in the circulation.[118] It has been proposed that subcutaneous AT contributes most to this increase of circulating FFA levels during exercise, whereas the contribution of VAT to this exercise-induced increase of circulating FFA levels is less. Furthermore, intramuscular triacylglycerol stores provide fatty acids as energy substrates during exercise.[119] The release of fatty acids from VAT during exercise is influenced by the AT blood flow, the rate of lipolysis, and the rate of fatty acid re-esterification. Important mediators of these effects have been demonstrated to be elevated plasma levels of catecholamines and decreased plasma levels of insulin. In addition, the atrial natriuretic peptide (ANP), which is secreted during exercise,[120] has been shown to contribute to lipid mobilization during exercise. The increase of AT blood flow is assumed to attenuate hypoxic stress of adipocytes leading to a reduced translocation of HIF-1α into the nucleus. In this regard, it was demonstrated that endurance exercise training increased expression of AT vascular endothelial growth factor a (VEGFa), an important factor of tissue angiogenesis, and lowered AT lactate levels, an indicator of AT hypoxia, in obese rats. However, these effects are depot-specific and were only demonstrated in intra-abdominal AT.[121]

Exercise-Induced Changes in the Stromal-Vascular Fraction

Exercise training exhibits several direct and indirect effects on the stromal-vascular fraction in VAT. Regarding macrophages, it has been demonstrated that exercise training (treadmill running at 12-20 m/min, 60 min/day, 16 weeks) attenuated the infiltration and accumulation of inflammatory cells in AT of diet-induced in mice.[122] These authors also showed that exercise training affects the macrophage phenotype as demonstrated by a reduced expression of M1 macrophage markers and an increased expression of M2 markers, indicating that exercise training induces phenotypic switching from M1 macrophages to M2 macrophages in AT of obese mice.[122] These changes appear to result in a reduced inflammatory state of AT in general, because the reduction of inflammatory macrophages was accompanied by a decrease in the mRNA levels of several inflammatory genes including TNFα, MCP-1, and adhesion molecules in AT of diet-induced obese mice.[94] The exercise training–induced amelioration in inflammation status in VAT of diet-induced obese mice has been attributed to a decreased expression and activation of TLR4,[122,123] which promotes inflammatory gene expression and IR through activating IKKβ and JNK in diet-induced obesity.[124] In contrast to these data from animal studies, only few data are available from

human studies. For instance, the combination of exercise training (eg, walking, swimming, aerobics) and hypocaloric diet was reported to reduce macrophage infiltration and inflammatory gene expression in AT of severely obese subjects.[16] In addition, it has been shown that exercise training (aerobic and resistance) reduces also expression of pro-inflammatory genes involved in macrophage recruitment, including TLR4, TNFα, and IL-6 in skeletal muscle of obese humans.[125] Moreover, Roberts et al[126] demonstrated that male diabetic subjects exhibit a reduced expression of pro-inflammatory adhesion molecules, which also play a critical role for macrophage recruitment, after taking part in a daily walking exercise program.

Data about the effects of exercise training on the accumulation of other leukocyte subpopulations in VAT are limited. For both $CD4^+$ and $CD8^+$ lymphocytes, which are known to accumulate during obesity in AT, a reduction of cell number was observed after exercise training in mice.[122] However, the underlying mechanisms, the biological significance, and the transferability of data from animal studies to humans have yet to be clarified.

Exercise-Induced Changes in Inflammatory Signaling Pathways

Leptin. Several studies are available about the effects of endurance exercise training on leptin blood levels in humans. Interestingly, the decrease of leptin blood levels were regularly accompanied by a reduction of AT mass.[127,128] In line with this, in one study that reported no changes of blood leptin levels there was also no change in body AT mass.[129] Besides endurance training, it was also shown that blood leptin levels decreased after 6 months of strength training interventions in overweight inactive elderly persons, with the magnitude of leptin changes increasing with increasing intensity of resistance exercise.[130] Regarding the effect of exercise on leptin expression in AT, divergent results were obtained from human and animal studies. While no changes in AT leptin expression after 12 weeks of aerobic exercise training in humans have been reported,[131] treadmill running (5 days/week, 6 or 12 week, 40 min/day, 65% to 70% VO_{2max}) reduced the expression of leptin mRNA in VAT of obese mice.[132] One might speculate that the exercise-induced reduction of AT leptin expression in obese mice leads to a reduced stimulation of the JAK/STAT and NF-κB signaling pathways, thereby leading to a reduced inflammation.

Adiponectin. Several studies investigated the effects of exercise training on blood levels of adiponectin. While some studies found no changes, most others found increases in serum adiponectin after running training in obese or diabetic subjects.[16,130] In human studies, these increases were often accompanied by a reduction of body mass index or body fat. Since adiponectin exerts effects by activation of specific receptors, the effect of exercise was also investigated on AdipoR1 and AdipoR2 receptors. In this regard, it was shown that exercise training of obese mice is followed by an upregulation of adipoR1-2/APPL1 protein levels in liver, AT, and skeletal muscle.[133] These increases in receptor expression were accompanied by an improvement of insulin sensitivity. On binding to its receptors, adiponectin induces activation of the PPARα pathway, which is also known to be activated by exercise training.[134] PPARα activation is known to increase fatty acid oxidation and to inhibit NF-κB activation. Accordingly, the effect of exercise on adiponectin contributes, at least partially, to the insulin sensitizing and anti-inflammatory effect of exercise.

FFA. Besides serving as important energy substrates in skeletal muscle during exercise, FFA also activate several signaling pathways including MAPK and JNK during exercise.[135] Activation of MAPK and JNK signaling by FFA during endurance exercise in skeletal muscle has been shown to be transduced by the transmenbrane proteins TLR2 and TLR4,[136,137] which are activated by FFA by a yet unknown mechanism. Although TLR4 activation in response to chronically elevated FFA levels in obese subjects was shown to impair insulin action due to activation of MAPK and JNK,[83] it is expected that TLR activation by FFA during acute exercise does not impair peripheral insulin sensitivity. This assumption is based on the fact that TLR2/4 activation by the elevated FFA levels during exercise is an acute effect,[136] whereas plasma FFA levels decrease in the long term in response to chronic exercise due to a loss of body weight. In line with the divergent effect of FFA-induced TLR activation on insulin sensitivity between acute exercise and obesity, it was found that the increase of plasma FFA concentration during acute exercise was stronger in TLR2 knockout and TLR4 knockout mice than in wild-type mice, indicating that TLR2 and TLR4 also regulate metabolic functions in tissues or modify repartition of energy substrates during acute exercise.[136] However, most data are from animal studies or cell culture experiments and need to be interpreted with caution.

The elevated levels of FFA in obesity are also known to cause ER stress,[87] and it was shown that TLR4 is an important mediator of the effect of FFA on obesity-induced ER stress as evidenced from the finding that TLR4 deficiency protects against high fat diet–induced ER stress and IR in liver, muscle, and AT.[90] In contrast, da Luz et al[138] found that swimming training (1 hour per day, 5 days for week with 5% overload of the body weight for 8 weeks) leads to a reduction in ER stress in AT and liver of diet-induced obese rats, and an improvement of insulin signaling indicating again that FFA-induced TLR activation has different effects on insulin sensitivity between acute exercise and obesity.

To sum up, although TLR activation in response to elevations of plasma FFA levels can be observed during both obesity and acute bouts of exercise, the impact on insulin sensitivity is different between exercise and obesity because FFA levels are only acutely elevated during exercise, while they are chronically elevated in obesity.

Pro-Inflammatory Cytokines. It is well known that both acute bouts of exercise and exercise training influence the secretion of various adipokines, chemokines, and cytokines.[139] One of the most responsive cytokines to exercise is IL-6. Several studies presented evidence that IL-6 is expressed in muscle fibers during exercise,[140] and activates skeletal muscle AMPK and/or phosphatidylinositol-3-kinase (PI3K) to increase fat oxidation and glucose uptake. Moreover, IL-6 is released from contracting skeletal muscle to the circulation[141] and elicits metabolic effects in AT like stimulation of lipolysis and fat oxidation,[142] thereby improving the obesity-associated atherogenic lipid profile. Furthermore, IL-6 belongs to several anti-inflammatory cytokines, which are elevated in response to exercise training. In this regard, it has been speculated that the anti-inflammatory cytokine cascade leads to a suppression of TNFα production and thereby offers protection against TNFα-induced IR.[142]

The results about exercise-induced changes of TNFα expression and blood levels are conflicting. While Straczkowski et al[143] demonstrated a significant decrease of serum levels of TNFα and soluble fraction of TNFα receptor 2 in obese woman after 12 weeks of endurance training, Bruun et al[16] found no changes in AT expression of TNFα after several weeks of aerobic exercise training. In contrast, in obese mice it was shown that exercise training suppresses TNFα expression in AT.[132] Regarding IL-1β, it was reported that intense exercise training (treadmill running) over 7 weeks with weight loss reduces concentrations of IL-1β in AT of rats.[144] Similarly, swimming exercise was found to reduce IL-1β serum levels of diet-induced obese mice.[145] A recent systemic review has highlighted that acute and chronic exercise may elicit different responses on TNFα expression depending on the health status of subjects, exercise type, and exercise intensity.[146] On the one hand, there are some studies that demonstrated that the immune response to exercise differs in subjects with already increased baseline cytokine levels.[147,148] On the other hand, more needs to be understood about the nature of exercise that reduce inflammation in patients with chronic inflammatory diseases. In addition, the contribution of an exercise-induced reduction in visceral fat versus other exercise-induced anti-inflammatory mechanisms needs to be better understood.

ROS. Several studies demonstrated that acute exercise is accompanied by an increased generation of ROS. The sources of ROS during exercise include increased mitochondrial oxygen reduction, activation of xanthine oxidase, inflammation, and heme auto-oxidation.[149] Although increased ROS formation is widely considered to be detrimental due to causing oxidative damage to cellular lipids, proteins, and DNA, it is becoming increasingly clear that ROS also improve cellular protection mechanisms against ROS through upregulating antioxidant enzymes. In this regard, several studies reported that exercise training induces activities of antioxidant enzymes and the levels of cellular antioxidants in body fluids.[150] In muscle and AT, it was demonstrated for instance that both protein levels and activity of SOD, which reduces the superoxide radical to hydrogen peroxide (H_2O_2), are increased by exercise training. Likewise, in experimental animals it was demonstrated that exercise is able to increase expression of SOD in AT[151] and to prevent the increase in the levels of lipid peroxidation and protein oxidation after feeding a high-fat diet. In addition, it was shown that exercise training attenuates protein expression of the NADPH oxidase NOX2, a major source of ROS production in obese AT, in AT of rats.[151] Taken together, exercise training causes anti-oxidative effects in tissues by increasing resistance to oxidative stress, thereby decreasing the basal levels of oxidative damage.

Conclusion

An expanded VAT acts as an endocrine organ that secretes various substances that mostly negatively affect insulin signaling. Exercise is an important lifestyle factors that is known to affect fat metabolism, inflammation, and the oxidative capacity of an organism. Considering the data now reviewed, there is a cluster of evidence that exercise training affects several important mediators in these processes that might attenuate pathological consequences of obesity. However, further research is required to better understand the effects of exercise on complete signaling pathways. It would be also helpful to focus more properly on the type, duration, and intensity of exercise training to maximize the benefits in patients.

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