Modulation of pro-inflammatory status of visceral fat: a novel therapeutic perspective for cardiovascular disease

La prevalenza dell’obesità è aumentata nelle ultime decadi raggiungendo proporzioni epidemiologicamente significative nel mondo occidentale. È ampiamente riconosciuto che l’obesità rappresenta un fattore di rischio indipendente per le malattie metaboliche, quali insulino resistenza, diabete tipo 2, dislipidemia, e per le malattie cardiovascolari. Il tessuto adiposo è un organo dinamico la cui espansione nell’obesità porta al rilascio di lipidi aberranti e alla produzione di citochine che determinano la cosiddetta “chronic low grade inflammation”. Inoltre, l’età influenza l’omeostasi del tessuto adiposo con un significativo incremento età correlato della massa grassa e della redistribuzione del grasso corporeo con aumento del tessuto adiposo viscerale, una diminuzione del tessuto adiposo sottocutaneo ed un incremento dei depositi ectopici di grasso. Tutti questi cambiamenti età-correlati contribuiscono al peggioramento dello stato di salute nell’anziano. Numerosi studi sperimentali ed epidemiologici hanno mostrato che il link tra l’obesità, in particolare l’obesità viscerale, e le malattie cardiovascolari potrebbe essere rappresentato dallo stato pro-infiammatorio sistemico correlato all’obesità. A tal riguardo, differenti strategie sono state disegnate avendo come target l’infiammazione correlata all’obesità e, di conseguenza, la riduzione del rischio cardiovascolare. In questa review vengono illustrate le correnti strategie che potrebbero controllare il rischio cardiovascolare attraverso la modulazione dell’infiammazione.

Parole chiave: Obesità, Grasso viscerale, Infiammazione, Invecchiamento, Rischio cardiovascolare

VISCERAL OBESITY AND AGING

Normal aging is associated with a progressive increase in fat mass. It has been shown that adipose tissue (AT) accumulation with age is mainly distributed at visceral level. Aging is associated not only with visceral fat increase, but also with a decrease in subcutaneous fat in other regions of the body (abdomen, and in particular thigh, calves), with a simultaneous ectopic fat accumulation. Modifications in body fat distribution dramatically change the endocrine properties of AT, determining a dysregulation in adipokines production. Adipokines derived from visceral AT exert pro-inflammatory effects in a paracrine and/or autocrine matter on several systems representing a common ground for insulin resistance, metabolic syndrome and cardiovascular diseases’ (CVD) morbidity and mortality. These signals support an influx of pro-inflammatory leukocytes, especially type M1 macrophages, and might therefore contribute to the physiopathological consequences of obesity. AT-associated macrophages (ATM)
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are the most abundant leukocytes ranging from 10% in normal weight subjects to almost 50% in obese subjects. Interestingly, it seems that not only AT amount and distribution may change with age, but also the endocrine function of adipocytes as older adipocytes display reduced gene expression of adiponectin and leptin, and increased insulin resistance, when compared to young mature adipocytes. In a mouse model of aging, Lumeng et al. demonstrated that visceral AT presented a decreased anti-inflammatory macrophages (M2) infiltration, with a decreased ratio between anti-inflammatory and inflammatory (M2/M1) macrophages. All together these results suggest that AT in older ages presents a greater inflammatory profile with ATM polarization and lymphocytes expansion, that together with adipocytes dysregulation fuel a chronic low-grade AT inflammation that contributes to obesity-related comorbidities.

Visceral Obesity – One of the Major CVD Risk Factor in Old Individuals

Although age-related changes in AT distribution and quality have been clearly shown to be linked to higher risk of diabetes, dyslipidaemia and CVD in old subjects, the underlying mechanisms remain elusive. Nevertheless, accumulating evidence from animal studies proved that the pathogenesis of obesity-related CV dysfunction involves the development of a systemic, low-grade inflammatory state (Fig. 1). A strong association has been reported between visceral fat and several cytokines, such as interleukin-6 (IL-6), plasminogen activator inhibitor-I (PAI-1) and leptin which have been shown to be related also to endothelial dysfunction. In particular, the activation of inflammatory signalling by adipokines, like tumor necrosis factor alpha (TNFα), leptin, PAI-1, has been suggested to contribute...
to the development of CVD by stimulating the generation of endothelial adhesion molecules, proteases and other mediators, which may act locally or also enter the circulation in soluble form. The current evidence of the roles of various adipokines in the progression of CVD is resumed in Table 1. Leptin, the first adipokine identified, present circulating levels increasing as the accumulation of visceral AT increases. Moreover some studies proved a direct association between circulating leptin levels and the risk of coronary artery disease, while other experimental observations revealed that leptin determines an increased expression of PAI-1 and C-reactive protein (CRP) in human vascular endothelial cells. Leptin-deficient mice seem to be protected from atherosclerosis, despite a higher metabolic risk related to the development of a severe obesity phenotype. Visceral fat has also been shown to be negatively associated with adiponectin levels, whose protective effect on arteries has been well documented. For example, in macrophages and endothelial cells, adiponectin acts as a TNF-α suppressor and directly ameliorates endothelial dysfunction by increasing nitric oxide production, while in transgenic mice adiponectin over-expression exerts an improved lipid profile. Low levels of adiponectin were observed even in patients with coronary atherosclerosis and have been associated with biomarkers of insulin resistance, inflammation and endothelial dysfunction, which are independent risk factors for CVD. At a molecular level, the central player of this scenario seems to be the transcriptional factor NF-kB that activates a multitude of genes controlling immune cells adherence, diapedesis and accumulation, further contributing to the pro-inflammatory status central in CVD pathogenesis and progression. Further translational studies are needed in order to fully understand the mechanisms that link AT accumulation, its low-grade inflammation and the augmented CVD risk in elderly adults.

### OBESITY-TARGETED TREATMENT STRATEGIES FOR LOWERING CVD RISK IN THE ELDERLY

Weight loss is associated with CVD risk improvement, with consequent reduction in obesity-related morbidity and mortality rates, providing the rationale that interventions targeting even modest weight loss might reduce CVD risk.

### HEALTHY LIFE STYLE

It is broadly known that dietary patterns in old age are very important for health maintaining and for preventing disease-related complications, considering the complexity of age-related pathology and physiopathology. Nevertheless, managing overweight or obesity in elderly per-
sons should consider the fact that obese old subjects lose weight in similar proportion as young people, even though they start with less lean mass as a result of age-related changes in muscle mass and strength. Several studies investigated the benefits in old age of the adherence to various dietary recommendations, generally based on a Mediterranean Diet (MedDiet) type. For example, the EPIC-elderly study proved that people (age > 60 years) following a dietary pattern similar to MedDiet for 89 months presented a lower overall death rate. Furthermore, Anderson et al. showed that a dietary pattern high in low-fat dairy products, fruit, whole grains, poultry, fish and vegetables may be associated with greater insulin sensitivity and lower systemic inflammation in older adults.

Some studies demonstrated that physical activity may directly reduce inflammation, while others reported that the anti-inflammatory effects of physical activity can be only indirectly caused by reduced visceral adiposity and consequent reduction in fat-derived inflammatory adipokines. Moreover, it has been recently shown that in frail, obese older adults, lifestyle interventions associated with weight loss improved CVD risk factors, but continued improvement was only achieved when exercise training was added to dietary interventions.

Lately, there is a growing interest in caloric restriction (CR) in animal studies as a valid approach to enhance survival. The overall data from animal studies showed that the benefits of CR described a linear decline trend if plotted as a function of the age at onset. Furthermore, translating the results achieved in small rodents to human subjects, Speakman et al. sustained that late-onset CR seems unlikely to provide significant benefits in terms of increased lifespan.

**DIET SUPPLEMENTATION**

It has been shown that nutrients may also influence AT distribution and function, even if evidences are sparse and quite controversial with only a few studies performed in older subjects. In Table II, we presented the most commonly used types of dietary supplementations that have been proved to have an important effect in regulating AT-related inflammation. In the Framingham Heart Study, whole-grain intake was inversely associated with subcutaneous and visceral AT, while refined-grain was positively associated with both subcutaneous and visceral AT. Moreover, whole grain intake appeared to be inversely associated with markers of low-grade inflammation. This negative association between whole grains consumption and both the AT amount and distribution could be dependent on their capability to determine satiety by delaying gastric emptying, or on their effects on gut hormones, whilst anti-inflammatory activity may be explained by plant-specific constituents of fruit and vegetables such as phytochemicals. Indeed, several components present in fruit and vegetables seem to have anti-inflammatory effects on AT. In particular, some studies concentrated on the role of dietary polyphenols in the prevention of obesity and obesity-related diseases. It had been largely proved that commonly consumed polyphenols, including green tea catechins, epigallocatechin, resveratrol and curcumin, reduce viability of adipocytes and proliferation of preadipocytes, suppress adipocytes differentiation and triglyceride accumulation, stimulate lipolysis and fatty acid beta-oxidation, reducing inflammation. In particular, in vitro studies proved that resveratrol significantly reduced NF-kB activation and the expression and release of IL-6 and TNF-α in a co-culture model of macrophages and 3T3-L1 adipocytes. Moreover, in vivo studies demonstrated that resveratrol anti-obesity effect was mediated through stimulation of fat oxidation and inhibition of obesity-induced chronic inflammation as it down-regulated the expression of TNF-α, interferon-gamma (IFNγ) and IL-6 along with downstream signaling molecules. Although, a few clinical studies had been made, some demonstrated that resveratrol supplementation to patients resulted in an increased serum adiponectin and decreased PAI-1, by modulating various pathways involved in inflammation, cell migration and T-cell interaction signals. In vitro and in vivo studies demonstrated that polyunsaturated fatty acids (PUFAn-3) have beneficial effects on the inflammatory profile. The anti-inflammatory effects of PUFAn-3 appear to be mediated by the increase in adiponectin secretion by a PPAR-γ dependent pathway as well as by the decrease of pro-inflammatory cytokines such as TNF-α, IL-6, MCP-1. Moreover, it has been suggested that PUFAn-3 reduce the production of adipocytes-derived eicosanoids, which have pro-inflammatory actions. PUFAn-3 supplementation may determine a fat mass de-
Tab. II. Dietary Supplementation and the risk for CVD (from Calder et al., 2011 \(^{17}\) and Wang et al., 2014 \(^{18}\), mod.).

<table>
<thead>
<tr>
<th>Supplements</th>
<th>In vitro studies</th>
<th>In vivo studies</th>
<th>Clinical trials</th>
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<tbody>
<tr>
<td><strong>Green tea catechins</strong></td>
<td>↓ Inflammation ↓ Resistin in 3T3-L1 cells</td>
<td>↓ Weight, ↓ adiposity, ↓ cholesterol and TG ↓ Pro-inflammatory signals (TNF-α, TLR4) ↓ Pro-inflammatory cytokines (MCP-1, CRP, IL6) ↑ Adiponectin level and expression</td>
<td>Inconsistent outcome Modest weight loss ↓ Bioavailability ↓ Pro-inflammatory markers (TNF-α, IL-6, CRP) Multiple confounders (ethnicity, genetic effects, habitual caffeine, different intake)</td>
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<tr>
<td><strong>Resveratrol</strong></td>
<td>↓ TNFα-induced IL6 and PAI production in 3T3-L1 ↓ LPS-induced TNFα and IL6 production in 3T3-L1 ↓ Adipokines expressions ↑ TNFα-induced NF-kB activation</td>
<td>↓ Body weight and fat deposition in diet-induced obese rats ↓ TNFα in liver ↓ Pro-inflammatory cytokines (TNF-α, IFN-α, IFN-γ, IL-6) and triglycerides molecules (TLR2/4, MyD88, Tirap, TRIF, TRAF6, IRF5, p-IRF3, NF-κB) in leptin KO mice</td>
<td>↓ Inflammation (↑ hs-CRP, TNF-α, PAI-1, IL-6/IL-10 ratio, sICAM-1), ↑ Anti-inflammation (↑ IL-10) ↑ Adiponectin in subjects on statin and high CV risk (supplemented for 6 months) ↔ Blood pressure, resting energy expenditure, oxidation Rates of lipids, ectopic or visceral fat content, inflammatory and metabolic biomarkers in healthy obese</td>
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<tr>
<td><strong>Curcumin</strong></td>
<td>↓ NF-κB activation in 3T3-L1 cells ↓ Expression of IL-6, TNF-a and COX2 on 3T3-L1 cells ↓ Migration of macrophages in co-culture with 3T3 cells</td>
<td>↓ IFN-γ and IL-2 mRNA levels ↔ mRNA expression of TNF-α, IL-1β, IL-4, IL-5, IL-10, IL-12, IL-18, TGF-β on obese cats supplemented for 8 weeks ↓ Body weight and ↓ inflammatory markers in diet-induced obese mice ↓ NF-κB pathway ↓ Proinflammatory cytokines (↓ TNF, IL-6; ↑ IL-4) ↑ Insulin sensitivity (↓ fasting glucose and insulin, HOMA-IR) and ↑ Serum adiponectin in ob/ob mice</td>
<td>Improved lipid profile in healthy subjects supplemented for 30 days ↔ Serum lipid profile (TAG, total, LDL-C, HDL-C) in elderly healthy subjects supplemented for 6 months</td>
</tr>
<tr>
<td><strong>PUFA n3</strong></td>
<td>↑ Adiponectin, ↑ Inflammatory cytokine by inactivating NF-kB pathway</td>
<td>↓ Fat mass ↓ AT inflammation ↓ Macrophage infiltration ↓ NF-kB activation</td>
<td>↓ IL-18 in elderly subjects supplemented for 3 years ↓ Visceral adiposity ↓ CV risk</td>
</tr>
</tbody>
</table>

PUFA n3: polyunsaturated fatty acids n3; TNF-α: tumor necrosis factor alpha; IL: interleukin; MCP-1: monocyte chemotactic protein 1; TGF-β: transforming growth factor beta; IFN-α: interferon alpha; TLR4: Toll-like receptor 4; CRP: C reactive protein; LPS: lipopolisacharide; PAI-1: plasminogen activator inhibitor-1; CV: cardiovascular; TAG: triacilglicerol; LDL-C: low density lipoprotein cholesterol; HDL-C: high level density lipoprotein cholesterol; HOMA-IR: homeostasis assessment model- insulin resistance; AT: adipose tissue; TG: triglyceride; KO: knock out

ccline, as well as a decline in AT inflammation and macrophage infiltration \(^{19}\). Although the preliminary data are very encouraging, translational studies from animal observation to human clinical trials are needed to further confirm the anti-obesity benefits of specific dietary supplemendations in old adults and to set-up community interventions.

**NEW PHARMACOLOGICAL INTERVENTIONS IN OBESITY**

A limited number of medicine are approved at the present time as pharmacological intervention in obesity. Drug therapy had many setbacks over the past 20 years, basically because of serious adverse effects. In Table III we present the drugs currently approved by the US Food and Drug Association (FDA) for short or long-term obesity treatment \(^{20}\). The only obesity drug approved both in Europe and USA, for long-term therapy in conjunction with reduced-caloric diet, is Orlistat, a selective pancreatic lipase’s inhibitor that inhibits 30% of fat absorption. Orlistat appears to be modestly effective in promoting weight loss, as a recent meta-analysis showed, and averages greater weight reduction of 2,9% in patients who received orlistat as an adjunct to lifestyle modifications compared with placebo, with equivalent weight gain over time. Regarding the improvement in CVD risk factors, Orlistat treatment seems associated to a decrease in systolic and diastolic blood pressure, and in
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Serum levels of total cholesterol, LDL-cholesterol and glucose. Moreover, it seems that orlistat can be effective in older as in younger adults, with no significant increase in adverse effects. Among sympathomimetic drugs approved for short-term obesity treatment (12 weeks), phentermine, an appetite-suppressant, is the most often prescribed. It has been proven to lower significantly body weight and waist circumference, compared to the placebo group. As it may increase heart rate and blood pressure, it is not recommended for individuals with history of CVD, which narrows the indication in elderly patients. Moreover there is no data on its long-term efficacy and safety in older subjects. Lorcaserin, a serotonin type 2C receptor agonist, was approved by the US FDA in 2012 for long-term obesity therapy, in association with lifestyle intervention. The drug was associated with moderate weight loss after 2 years of treatment, while the data regarding CVD risk factors were inconclusive, as the initial improvement in cardiometabolic risk factors (glycemic and lipid levels) after 1 year of treatment, was no longer significantly maintained by the end of the study, reflecting maybe the effects of weight regain.

Phentermine/Topiramate extended-release (ER) medication was approved in 2012 for long-term obesity treatment and till this date there were only a few clinical trials that investigated its efficacy and safety. One of them, the CONQUER study, assessed also its impact on cardiometabolic risk factors. This clinical trial enrolled patients aged between 18 and 70 years old and the subgroup analyses of age proved that the combined therapy phentermine plus topiramte resulted in greater weight loss than placebo, with no age-related hazards. It was demonstrated that phentermine/topiramate ER treatment significantly decreased systolic and diastolic blood pressure and improved lipid and glycemic profile.

The latest drug approved by the FDA and introduced in the US is bupropion/naltrexone, a combination of a dopamine/noradrenaline reuptake inhibitor and an opioid antagonist. Although, initially rejected for lack of convincing CVD safety data, in 2014 this drug association was accepted by the US authorities, although there is a warning alert for suicide thoughts in long-term users. No data had been published so far about the safety of bupropion/naltrexone treatment in elderly subjects.

**BARIATRIC SURGERY**

Bariatric surgery (BS) represent an effective therapeutic option in accurately selected severely
obese patients, although it remains a demanding procedure, applicable only to a limited number of patients. Additionally, it has been demonstrated that BS is associated with restoration of normal serum profiles of adipokines and gut hormones. In a recent study, Vest et al. analysed 58 studies with a mean follow-up of 57.8 months after BS and demonstrated a substantial resolution or improvement of their baseline hypertension (62%), diabetes (73.2%) and hyperlipidaemia (65.2%), with a reduction in 10-year coronary heart disease Framingham risk score from 5.9% to 3.3%. In parallel, the same meta-analysis showed that among the 12 studies reporting the effect of BS on inflammatory status for CVD, CRP levels decreased by 73% after surgical procedure. Furthermore, after 6 months from BS, the registered weight loss in a group of clinically severely obese patients at a very high risk was especially effective in the reduction of CVD risk and related mortality. Recently it had been observed an increase in the number of bariatric procedures performed also in the elderly, reaching 10% of all bariatric operations in academic centres in the USA. Moreover, it seems that the in-hospital mortality in BS in the elderly has improved so much that it is now even lower than that of nonelderly BS patients. Although, older adults seem to experience less weight loss, it seems that the surgical intervention has potential benefits also for these patients, as it has been observed a significant improvement in hypertension, diabetes, and, to a lesser extent in dyslipidemia also in older patients undergoing BS; however no data is available regarding the changes in inflammatory profile in these studies. It should be noted that all these studies concern only short-term outcomes of BS in the elderly, so that long-term trials are needed to better evaluate the benefits of BS in aging obese patients.

CONCLUSIONS

Visceral obesity is a major risk factor for CVD, as it is associated with AT dysfunction, aberrant adipokines release and chronic low-grade inflammation. The most important ethiopathogenetic link between obesity and CVD has been shown to be represented by different adipokines, as leptin, adiponectin, TNF-alpha and other interleukins whose levels profoundly changes with AT expansion. Even a modest weight loss of 5-10% has been proved to ameliorate cardiometabolic risk factors and improve health outcomes. Understanding the molecular mechanisms linking visceral obesity, inflammation and CVD appears still to be essential to identify possible therapeutic strategies.

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