Health Research Institute of Santiago de Compostela (IDIS)

Neurology Department – University Clinical Hospital

Clinical Neurosciences Research Laboratory (LINC)

Clinical, molecular and cellular study of the obesity paradox in acute ischemic stroke.

Estudio clínico, molecular y celular de la paradoja de la obesidad en el ictus isquémico agudo.

PhD Thesis

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O **Prof. Dr. José Castillo Sánchez**, Director Científico do Instituto de Investigación Sanitaria de Santiago de Compostela, e o **Dr. Tomás Sobrino Moreiras**, Investigador Miguel Servet no Instituto de Investigación Sanitaria de Santiago de Compostela, fan constar:

que a presente memoria, titulada: ***"Clinical, molecular and cellular study of the obesity paradox in acute ischemic stroke"*** foi realizada baixo a súa supervisión polo Licenciado en Medicina, **D. Emilio Francisco Rodríguez Castro**, e que considerando que reúne os requisitos pertinentes autorizan a súa defensa como Tese de Doutoramento ante o tribunal correspondente na Universidade de Santiago de Compostela.

Santiago de Compostela, setembro de 2017.

Os directores:

Prof. Dr. José Castillo Sánchez   Dr. Tomás Sobrino Moreiras

O doutorando:

D. Emilio Francisco Rodríguez Castro

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ABBREVIATIONS

α-MSH: α-melanocyte stimulating hormone

AA: arachidonic acid

AGRP: agouti-related protein

AHA/ASA: American Heart Association/American Stroke Association

ALG2: apoptosis-linked gene 2

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANGPTL2: angiopoietin-like protein 2

AP-1: activator protein-1

APC: antigen presenter cells

APSCS: Asia Pacific Cohort Studies Collaboration

ASCs: adipose tissue-derived stem cells

ASPECTS: Alberta Stroke Program Early CT Score

ATBC: Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

ATP: adenosine triphosphate

BBB: blood brain barrier

BDNF: brain-derived neurotrophic factor

BLT1: leukotriene B4 receptor

BM-MSCs: bone marrow-derived mesenchymal stem cells

BMI: body mass index

CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

CBF: cerebral blood flow

CCK: cholecystokinin

CCR2: C-C chemokine receptor type 2

CCR3: C-C chemokine receptor type 3

CCR4: C-C chemokine receptor type 4

CCR5: C-C chemokine receptor type 5

CI: confidence interval

CNS: central nervous system

CO_2: carbon dioxide

COX: cyclooxygenase

COX-2: cycloxygenase-2

CPP: cerebral perfusion pressure

CRP: C reactive protein

CSF-1: colony stimulating factor-1

CT: computerized tomography

CX_3CL1: chemokine CX_3C motif ligand1

CX_3CR1: chemokine CX_3C motif receptor 1

CXCR4: C-X-C chemokine receptor type 4

DALYs: disability-adjusted life-years

DAMPs: damage-associated molecular patterns

DBP: diastolic blood pressure

DEXA: dual-energy X-ray absorptiometry

eNOS: endothelial nitric oxide synthase

EPCs: endothelial progenitor cells

FFA: free fatty acids

GABA: γ-Aminobutyric acid

GDNF: glial cell-derived neurotrophic factor

GFAP: glial fibrillary acid protein

GLP1: glucagon-like peptide 1

GM-CSF: granulocyte-macrophage colony-stimulating factor

HbA1c: glycated haemoglobin

HC: hip circumference

HDL: high density lipoproteins

HGF: hepatocyte growth factor

HFD: high-fat diet

HMGB1: high mobility group protein B1

hsCRP: high sensitivity CRP

ICAM-1: intracellular adhesion molecule-1

ICAM-2: intracellular adhesion molecule-2

IDO: indoleamine 2,3-dioxygenase

IGF-1: insulin-like growth factor 1

IL-1: interleukin-1

IL-1β: interleukin-1β

IL-1ra: interleukin-1 receptor antagonist

IL-4: interleukin-4

IL-5: interleukin-5

IL-6: interleukin-6

IL-8: interleukin-8

IL-10: interleukin-10

IL-12: interleukin-12

IL-13: interleukin-13

IL-17: interleukin-17

IL-18: interleukin-18

IL-20: interleukin-20

IL-23: interleukin-23

IMT: intima-media thickness

INF-γ: interferon-γ

iNOS: inducible nitric oxide synthase

IRAK: IL-1R-associated kinase

IRS-1: insulin receptor substrate-1

LDL: low density lipoproteins

LOX: lipoxygenase

Lp(a): lipoprotein(a)

LPS: lipopolysaccharide

LTB4: leukotriene B4

MadCAM-1: mucosal vascular addressin cell adhesion adhesion molecule-1

MAO: monoamine oxidase

MAP2: microtubule-associated

MAPK: mitogen-activated protein kinase

MBL: mannose-binding lectin

MCAO: middle cerebral artery occlusion

MCP-1: monocyte chemoattractant protein-1

MHC: major histocompatibility complex

MIP-1α: macrophage inflammatory protein-1α

MIP-1β: macrophage inflammatory protein-1β

MMPs: matrix metalloproteinases

mRS: modified Rankin Scale

MPO: myeloperoxidase

MRI: magnetic resonance imaging

MSCs: mesenchymal stem cells

NIHSS: National Institute of Health Stroke Scale

NF-κB: nuclear factor κ-B

NMDA: N-Methyl-D-aspartate

nNOS: neuronal nitric oxide synthase

NO: nitric oxide

NOS: nitric oxide synthase

NOX: NADPH oxidase

NPY: neuropeptide Y

OECD: Organization for Economic Co-Operation and Development

OCSP: Oxfordshire Community Stroke Project

OR: odds ratio

Ox-LDL: oxidized low-density lipoprotein

PAMPs: pathogen-associated molecular patterns

PECAM-1: platelet endothelial cell adhesion molecule-1

PLA_2: phospholipase A_2

POMC: pro-opiomelanocortin

PPARγ: peroxisome proliferator-activated receptor γ

PYY_{3-36}: peptide YY_{3-36}

RBP4: retinol binding protein 4

ROS: reactive oxygen species

SBP: systolic blood pressure

SD: standard deviation

SFA: saturated fatty acids

SFRP5: secreted frizzled-related protein 5

sTNFR: soluble TNF-α receptor

TGF-β: transforming growth factor-β

TGF- β_1: transforming growth factor-β_1

Th: helper T cell

Th1: helper T cell type-1

Th2: helper T cell type-2

Th17: helper T cell type-17

TLR: Toll-like receptor

TLR2M: TLR2 expression on monocytes

TLR2N: TLR2 expression on neutrophils

TLR4M: TLR4 expression on monocytes

TLR4N: TLR4 expression on neutrophils

TNF-α: tumor necrosis factor-α

TNFR: TNF-α receptor

TOAST: Trial of ORG 10172 in Acute Stroke Treatment

TRAF6: TNFR-activated factor-6

Treg: regulatory T cell

UCP1: uncoupling protein 1

USA: United States of America

VCAM-1: vascular cellular adhesion molecule-1

VEGF: vascular endothelial growth factor

WC: waist circumference

WHO: World Health Organization

Abbreviations

WHR: waist-to-hip ratio

YLL: years of life lost

YLD: years lost due to disability

INTRODUCTION

1. ISCHEMIC STROKE

1.1. STROKE DEFINITION

For more than two millennia, "apoplexy" was the word used to define acute neurological deficits non-related to traumatic injuries. The first reference we have of the use of the term "stroke" in medicine dates back to 1689 in the text “A Physico-Medical Essay Concerning the Late frequencies of Apoplexies” by William Cole[1].

In the 1970s The World Health Organization (WHO) defined stroke as rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin[2]. This classic definition does not account for the advances that have been made in recent decades about our knowledge of stroke and its mimics, pathology, timing and neuroimaging. With the aim to update and formalize the definition the AHA/ASA published an expert consensus document in 2013[1]. According to this new statement the term "stroke" should be broadly used to refer to "CNS infarction", "ischemic stroke", "silent CNS infarction", "intracerebral hemorrhage", "stroke caused by intracerebral hemorrhage", "silent cerebral hemorrhage", "subarachnoid hemorrhage", "stroke caused by subarachnoid hemorrhage", "stroke caused by cerebral venous thrombosis" and, finally, "stroke, not otherwise specified" which is defined as an episode of acute neurological dysfunction presumed to be caused by ischemia or hemorrhage, persisting for 24 hours or more or until death, but without sufficient evidence to be classified as one of the above.

Therefore, strokes are either ischemic or hemorrhagic. Hemorrhagic stroke is due to a blood extravasation to the parenchyma, the ventricles or the subarachnoid space. Ischemic stroke is, according to the new definition, an episode of neurological dysfunction caused by focal cerebral, spinal or retinal infarction. And CNS infarction is brain, spinal cord or retinal cell death attributable to ischemia, based on: 1) pathological, imaging, or other objective evidence of CNS focal ischemic injury in a defined vascular distribution; or 2) clinical evidence of CNS ischemic injury based on symptoms persisting 24 hours or more or until death, and other etiologies excluded. Brain ischemia is the consequence of the cerebral blood flow (CBF) downfall that leads to metabolic and biochemical alterations and, therefore, to cell necrosis and CNS dysfunction[3]. When the episode of neurological dysfunction caused by ischemia is transient and without acute infarction it is called transient ischemic attack[4].

1.2. STROKE EPIDEMIOLOGY

Stroke is a major global health problem. Although in the past two decades age-adjusted mortality rates for stroke have decreased, the aging population implies that absolute number of people who have strokes annually, as well as related deaths and DALYs (disability-adjusted life years) lost, is increasing. With the most recent data from the Global Burden of Disease Study it was estimated that, worldwide in the year 2010, 16.9 million people had a first stroke[5], of which 11.6 million events were ischemic and 5.3 million events were hemorrhagic[6]. Among all the causes of death stroke is the third (11.1% of the total worldwide deaths) after cancer and ischemic heart disease, and ischemic stroke is responsible of approximately half[7].

YLL (years of life lost) and DALYs (YLL plus YLD –years lost due to disability–) are two useful epidemiological tools when assessing the burden of a disease. Stroke is the third leading cause of YLL both globally, in Western Europe and in Spain[8]. Respect to DALYs, a composite measure of both morbidity and mortality, stroke is the third cause globally and in Western Europe, and the forth in Spain[9].

In 2010, a population-based study of stroke survivors demonstrated that 5 years after the event 71% patients experienced mild neurologic impairment, 22.5% had cognitive impairment indicative of dementia, 20% had experienced a recurrent stroke, almost 15% were institutionalized, and 29.6% had symptoms suggesting depression[10].

Recent data from Spain is provided by the IBERICTUS study[11]. The annual incidence for stroke in this study was 187 per 100000 (202 per 100000 men and 173 per 100000 women). Among all cerebrovascular events the 81% were ischemic strokes and the 19% were hemorrhagic strokes. The in-hospital mortality was 11%.

The costs of stroke represent about 2-4% of total health-care funds, and more than 4% of direct costs in industrialised countries[12]. The CONOCES study[13] of patients admitted to stroke units in Spain estimated an average cost per patient/year of €27711. Direct healthcare costs accounted €8491 per patient/year (68.8% due to inpatient costs) and non-healthcare costs accounted €18643 per patient/year.

1.3. ISCHEMIC STROKE RISK FACTORS

As noted above, stroke includes distinct entities with different pathophysiology and therefore different risk factors. For this work, we will focus on ischemic stroke risk factors, which can be categorized in two classes: non-modifiable, those hereditary or natural markers we cannot act on; and modifiable, those lifestyle and environment features we can change[14–16].

An analysis from the Global Burden of Disease Study[17] shows that in a worldwide ranking of risk factors by number of DALYs attributable to stroke, high systolic blood pressure (SBP), diet low in fruits, high body-mass index, diet high in sodium and smoking stand at the top five in that order. This study estimates that more than 90% of the worldwide stroke burden is attributable to modifiable risk factors, and get control of behavioural and metabolic risk factors could prevent more than 75% of the global stroke burden.

1.3.1. Non-modifiable risk factors

Age: it is the most important non-modifiable risk factor. People over 45 years-old suffer 95% of strokes, and about two-thirds occur in those older than 65. The stroke rate more than doubles for every 10 years over 55.

Sex: the risk of stroke in men is 19% higher than women. This slight excess of risk occurs mainly among the middle to old-aged, and is not present in the young or very elderly.[18]

Race: Hispanics and Blacks have a higher incidence of stroke than Whites, and particularly a greater proportion of intracranial atherosclerotic strokes.

Genetic and hereditary factors: different studies have demonstrated an increased risk of stroke within families after adjusting for other risk factors. There are also rare monogenic disorders that predispose to stroke like CADASIL or Fabry's disease.

1.3.2. Modifiable risk factors

Hypertension: it is the most important modifiable risk factor for ischemic stroke. Hypertension is present in almost a quarter of the adult population and about 50% of the people over 65 years and it is associated with an increased risk of all subtypes of ischemic strokes. It has been shown that this risk is dependent on age, with a decrease in the odds ratio (OR) from 4 to 1 between 50 and 90 years old.

Cardiac diseases: the risk of stroke is increased in some cardiac diseases such as atrial fibrillation (one in six strokes takes place in patients with this arrhythmia[19]), coronary artery disease (within two weeks after an acute myocardial infarction the risk of stroke accounts for a 5%), valvulopathies (mitral stenosis, infectious and non-infectious endocarditides, and prosthetic heart valves), patent foramen ovale (especially in younger patients without another explanation), and left ventricular hypertrophy.

Diabetes mellitus: population studies have reported an increase in relative risks from 1.5 to 3 in diabetic patients[20]. Pathology studies have demonstrated the presence of small cerebral infarcts and predominantly lacunes in basal ganglia, pons, thalamus and cerebellum. An association has also been found with extracranial carotid and basilar artery occlusion.

Smoking: the risk is dose-dependent, with twice the relative risk in smokers of more than 40 cigarettes/day than those who smoke less than 10/day. After two years of smoking cessation the risk reduction is significant and after 5 it reaches the level of a non-smoker[21].

Physical inactivity: different population studies have shown a reduction in stroke risk with physical activity.

Obesity: the association between obesity and ischemic stroke will be discussed in depth further.

Hyperlipidaemia: most studies have found a positive correlation between total cholesterol levels and risk of ischemic stroke[22]. In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study[23] the risk of ischemic stroke was raised at concentrations higher than 271 mg/dL. The Asia Pacific Cohort Studies Collaboration (APSCS) found that each 38.7 mg/dL higher level of total cholesterol was associated with 25% increased risk of ischemic stroke[24]. It is noteworthy the strong association between elevated serum concentrations of lipoprotein(a) (Lp(a)), which could have thrombogenic and pro-atherogenic effects by blocking plasminogen actions, with increased risk of stroke[14]. Regarding high density lipoprotein (HDL) levels, no consistent association has been found with ischemic stroke, which contrasts to inverse correlation demonstrated with the risk of coronary heart disease[25]. Epidemiological studies have shown contradictory associations between ischemic stroke and triglycerides[22]. In the Emerging Risk Factors Collaboration meta-analysis, authors found no association[25].

Diet and nutrition: a high consumption of fruit, vegetables, nuts, whole grains and olive oil, with a moderate consumption of fish and a low consumption of red meat is associated with a lower risk of ischemic stroke.

Chronic inflammation: A cohort study which took place in the United Kingdom showed that the risk of vascular diseases is increased among a range of organ-specific and multisystemic chronic inflammatory disorders[26]. It has been postulated that the releasing of pro-inflammatory cytokines into systemic circulation results in endothelial dysfunction and atherosclerosis. White blood cell count has also been associated with cerebrovascular and cardiovascular diseases through atherosclerosis progression[27].

Sleep-disordered breathing and obstructive sleep apnea, psychosocial stress and depression, and air pollution have also been associated with a higher risk of ischemic stroke.

1.4. ISCHEMIC STROKE PHYSIOPATHOLOGY

Brain ischemia is the result of a fall in CBF below the threshold necessary to maintain the proper functioning of nervous system[28]. This drop is consequence of occlusion of a particular artery due to embolic or hemodynamic mechanisms and leads to a metabolic and biochemical cascade, both neuronal and glial, which ultimately results in cell death[29].

1.4.1. The concept of ischemic penumbra

When there is a blockage of a vessel, a blood perfusion gradient takes place in the affected vascular territory, which leads to a severe ischemia in the core and a less intense ischemia at the periphery **(figure 1)**. In the core, where the CBF is below the critical level for the survival of the cells, the necrosis is very quick[30]. In the periphery, the mild fall in the CBF leads to an impairment on neuron functions that results in electrophysiological silence and neurological symptoms but with minimal metabolic activity that preserves its structural integrity at least temporarily. This is called the ischemic penumbra area[31]. In this area tissue is damaged, autoregulation mechanisms are altered, reactivity to carbon dioxide (CO_2) is partially maintained, synaptic transmission and ATP content are normal and there is a decrease in glucose content. The penumbra area has the potential for functional recovery if the blood flow is restored, but irreversible damage will expand from core if reperfusion is insufficient, depending on the interaction

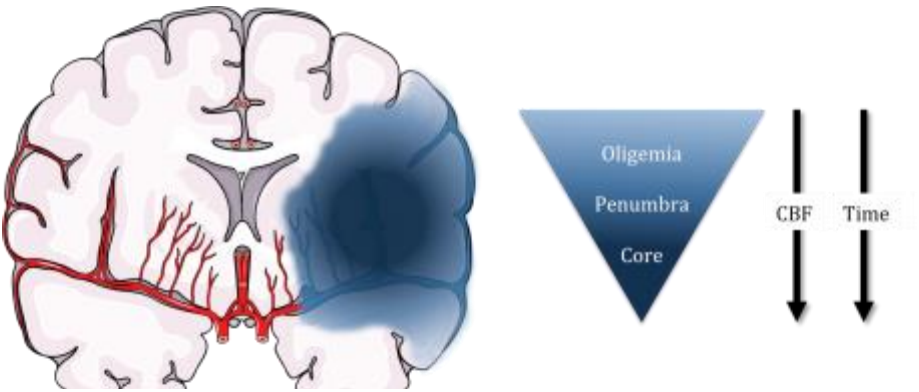


Figure 1. The concept of ischemic penumbra. After a vessel occlusion, a blood perfusion gradient takes place, which leads to a severe ischemia in the core and a less intense ischemia at the periphery (penumbra and oligemic areas). If the CBF restoration is not fast enough, the reperfusion may not stop the metabolic and biochemical cascade, so irreversible damage will expand from core.

of severity and duration of ischemia[32]. Surrounding the penumbra area, there is a slightly hypoperfused area known as oligemic area, where tissue is also threatened but where neuron functions and metabolic activities are still preserved. Therefore, reperfusion would be the basis of acute treatment of stroke, but if the CBF restoration is not fast enough the reperfusion may not stop the metabolic and biochemical cascade and it may even aggravate the damage[33].

1.4.2. Factors related to brain ischemia

There are several factors that will modulate the appearance and evolution of damage: hypoxia, hypoglycaemia, CBF, collateral circulation and temperature[28].

To cause acute brain injury, hypoxia needs the presence of simultaneous ischemia or acidosis[34]. Its effects are aggravated by hypotension. If it extends over time irreversible brain damage occurs with preference for grey matter and watershed areas[35,36].

Severe hypoglycaemia can cause functional and structural brain disorders. When the cerebral glycogen and glucose reserves are depleted, the brain starts to consume other substances and structural damage appears with selective cortical neuron necrosis and glial preservation[37].

CBF depends on the gradient of cerebral perfusion pressure (CPP) and vascular resistances[38]. CPP is defined as the difference between the average pressure of the cerebral arteries and intracranial and venous pressures[39]. In normal conditions, the regulation of CBF is independent of wide variations in blood pressure due to a autoregulatory mechanism of brain circulation[29]. However, in brain ischemic tissue the autoregulation disappears and the CPP becomes dependent on blood pressure, so that a fall in the blood pressure cannot be compensated by a decrease in vascular resistances[40].

Collateral circulation protects the brain against ischemia. The main anastomoses are between both common carotid arteries, between external carotid artery and vertebral artery, between external carotid artery and intracranial circulation through ophthalmic artery, between intracranial arteries through Willis polygon, and through Heubner's leptomeningeal anastomoses. CPP is the most important factor in the regulation of the collaterals function after ischemia. The drop in blood pressure reduces the collateral circulation to the ischemic tissue and increases the infarct size[38].

The links between stroke and temperature will be expanded in depth below.

1.4.2.1. Temperature and ischemic stroke

The hypothalamus is the responsible of regulating body temperature, which needs integrated autonomic, endocrine and skeletomotor responses. When body temperature rises, hypothalamus induces sweating and skin vasodilatation to maintain temperature in the normal range. However, certain pathological disorders induce an increase of body temperature which exceeds the thermoregulatory capacity of hypothalamus resulting in hyperthermia[41].

There is a robust relationship between body and cerebral temperature and the evolution of acute cerebral infarct[42]. In the first days after ischemic stroke hyperthermia emerges in up to 60.8% of patients[43]. In most recent published works, frequency of hyperthermia tends to be lower due to the improved attention to patients with stroke. Treatment of hyperthermia, independently from its origin, has been found to improve patient comfort and short and long-term outcome. In the following lines we will discuss different aspects about this issue.

1.4.2.1.1. Etiology of hyperthermia in stroke

According to its origin hyperthermia can be divided in two groups: central (or neurogenic) and systemic hyperthermia.

Neurogenic hyperthermia can be the consequence of the direct damage on the thermoregulation center of the hypothalamus or of the pontine centers[44], as a result of large infarcts or strategically located ischemic lesions in those areas. Neurogenic hyperthermia also occurs as a consequence of inflammatory cascade, with increased pro-inflammatory cytokines and leukocytes around the infarcted tissue, resulting in hypothalamic stimulation[45–47].

Usually, systemic hyperthermia is associated with respiratory and urinary infections. Most studies have demonstrated infections in approximately half of hyperthermic stroke patients[43,48,49]. This can occur as a result of the immunosuppression induced by cerebral infarction[50] .

On synthesis, earliest hyperthermia can be associated to a neurogenic response as a consequence of acute phase reaction, whereas the late can be related with infections.

1.4.2.1.2. Brain versus body temperature

In healthy subjects, the temperature of the brain is approximately 1.5°C higher than rectal[51]. In stroke patients with hyperthermia, such differences can increase, which suggests that the incidence of brain hyperthermia is higher than reflected in observational studies, in which only body temperature is registered.

Today we know that at the infarcted area the temperature is higher than in contralateral hemisphere, and the increase in brain temperature precedes the increase of body temperature[52]. Temperature is also higher in the ischemic penumbra than in the infarct core[52,53]. However, contrary to the clear association between systemic hyperthermia and poor outcome, such relationship has not been found for brain temperature.

1.4.2.1.3. Hyperthermia and poor prognosis in stroke

The effects of hyperthermia are clearly deleterious on animal models[54], resulting in increased infarct volumes[55–57]. In humans, results are also consistent with poor prognosis. In 1994, Castillo et al.[58] showed for the first time, in a prospective study, that hyperthermia was an independent predictor of mortality and morbidity at 6 months in patients with ischemic stroke. A work of the same group found that for each 1°C of axillary temperature increase at inclusion, the relative risk of early neurological deterioration was 9.2 (CI 95%, 4 to 21)[59]. Castillo et al.[43] also showed that the influence of hyperthermia is greater in the earlier phases of stroke. Later, more studies confirmed the association between hyperthermia in ischemic stroke and higher mortality, more severe neurological deficits, and worse outcome[60–62].

Two meta-analyses assessing the influence of hyperthermia have been published. Hajat et al.[63], with the data from 3790 stroke patients, found a 9% increase of morbidity and 1% increase in mortality in patients with hyperthermia. Greer et al.[64], analysed the data from 14431 patients with stroke and other brain injuries and found a relative risk of 1.5 for mortality and 2.2 for bad outcome. Data of 5305 patients in acute stroke trials from the

Virtual International Stroke Trials Archive[49] confirmed that hyperthermia was associated with poor clinical outcome, but in this case, the later the hyperthermia occurs within the first week, the worse the prognosis.

1.4.2.1.4. Mechanisms of ischemic damage by hyperthermia

Campos et al.[42] postulate that in the early phases of ischemic stroke, locally at the peri-infarct area, metabolic dysfunction and inflammatory response take place, inducing an increment in brain temperature within that region. Moreover, systemic hyperthermia can also contribute to the damage. As we noted above, early systemic hyperthermia can be the consequence of large or strategic infarcts, but this only explains the association between temperature and bad outcome in less than 4% of the cases[52]. In later phases, systemic hyperthermia, usually due to infections, promotes excitotoxicity, inflammation, metabolic alterations, oxidative stress, blood brain barrier (BBB) damage and protein degradation, resulting in depolarization from the peri-infarct zone, increase of the ischemic lesion, and neurological deterioration[65].

In experimental models of ischemia, it has been demonstrated that glutamate release is higher in hyperthermic rats compared to normothermic animals[66], which could result in more peri-infarct depolarizations and higher infarct volumes[67]. Castillo et al.[68] found that glutamate levels in CSF were significantly higher in patients with hyperthermia compared to normothermic ones, and the association between stroke worsening and higher infarct sizes in patients with hyperthermia was dependent on the glutamate effect.

The inflammatory cascade, with pyrogenic activity, has a key role in stroke physiopathology. In a prospective study with 229 patients with ischemic stroke, hyperthermia was associated with higher levels of IL-6, TNF-α and ICAM-1, higher infarct volumes and poor outcome at 3 months[69]. There was also a significant correlation between those pro-inflammatory markers and poor outcome.

The inflammation could promote an increase of temperature in the infarct region, mainly in the peri-infarct zone where there is an increase in metabolic activity. Such increase may lead to a depletion of limited energy supplies, therefore increasing the conversion of ischemic viable tissue to infarction[52].

BBB disruption is also present in animals with hyperthermia[70]. Chaperones need ATP and other energy sources for protein restitution, and this is compromised in hyperthermia[71].

1.4.2.1.5. Anti-hyperthermic treatment in ischemic stroke

The objectives of treating hyperthermia are to alleviate patients' distress, reduce metabolic demand, reduce morbidity, improve cognitive alteration and prevent seizures[72].

Common antipyretic drugs, such as aspirin, paracetamol, metamizol and ibuprofen, have been tested in ischemic stroke. Data from 423 patients of 5 clinical trials have shown no efficacy on reduction of death or dependency[73]. In the PAIS study[74], which included 1400 patients with ischemic stroke with less than 12 hours from the onset, patients who received daily dose of 6 g of paracetamol showed a better outcome, but this was not statistically significant. A post-hoc analysis demonstrated a statistically significant better outcome in patients who did not received fibrinolytic treatment, more important in patients with temperatures higher than 37°C.

It has been proposed to be reasonable to treat patients with temperature higher than 37°C with high doses of paracetamol, or even with therapeutic protocols with paracetamol, followed by metamizol, cool compresses and refrigerated saline[75].

1.4.3. Molecular aspects of brain ischemia

Brain ischemia leads to a sequence of molecular phenomena that starts with the energetic failure related to the interruption of the oxidative phosphorylation and the deficit in the ATP production (**figure 2**)[76].

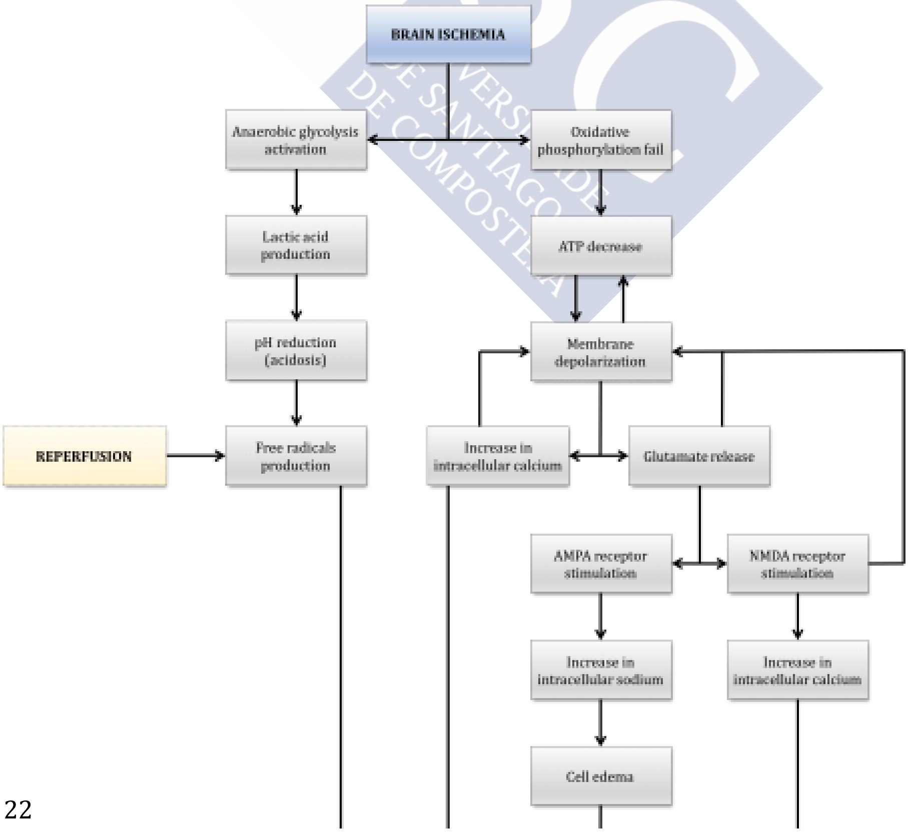


Figure 2. Molecular aspects in the early stages of brain ischemia.

The ischemia leads to the activation of anaerobic glycolysis with the production of lactic acid and the intra and extracellular pH reduction[77,78]. As a result, the acidosis aggravates brain damage due to the production of free radicals[79]. During ischemia and especially during reperfusion, these free radicals like nitric oxide (NO) are generated. Such molecules are highly reactive and the biochemical characteristics of nervous system (high lipid concentration and high energetic requirements) make it particularly sensible to injury by free radicals. Reactive oxygen species (ROS) are produced in the initial moments by the metabolism of the arachidonic acid and the neuronal NO synthase (nNOS). At intermediate stages, free oxygen radicals are provided by the infiltration of neutrophils to the ischemic area. At late stages, synthesis and activation of the inducible NO synthase (iNOS) and the cycloxygenase-2 (COX-2) are involved[80,81].

Because of the energetic failure, the neurons and the glia are unable to maintain the membrane polarization. This determines the opening of the voltage-dependent calcium channels and the receptor-operated calcium channels. As a result, the intracellular calcium concentration increases leading to a sudden membrane depolarization[82]. This intense depolarization determines the release of great amounts of glutamate and other excitatory amino acids[83–86]. The hyperexcitation leads to peri-infarct depolarization which increases the energy expenditure especially when the membrane tries to repolarize[87,88]. Moreover, glutamate stimulates ionotropic (AMPA and NMDA) and metabotropic receptors. Through AMPA stimulation it increases the intracellular sodium concentration that causes cell edema, and through NMDA stimulation it causes a high increase in intracellular calcium concentration that leads to the activation of the ischemic cascade that determines cell death.

In physiological conditions, the astrocytes are responsible of the glutamate reuptake via different transporters. For this purpose, these transporters use sodium membrane gradient. Inside the astrocyte the glutamate is transformed into glutamine for new neurotransmitters synthesis[89]. During ischemia, the reuptake of glutamate falls due to astrocyte edema leading to an increase in its extracellular concentration and therefore aggravating the damage[90].

The rise in intracellular calcium concentration along with acidosis and peri-infarct depolarization determines the beginning of the damage. Later, inflammation and activation of apoptosis phenomena contribute to increase the injury[91,92].

Brain ischemia leads also to gene activation and expression. Among them, the induction of heat shock protein (HSP) genes[93], the activation of pro-inflammatory cytokine and adhesion molecule related genes[94], the induction of iNOS and COX-2 genes[80], the induction of apoptosis related genes[95] and the induction of growth factors genes[96,97].

1.4.4. Cell death in brain ischemia: necrosis and apoptosis

Cell death in brain ischemia can occur in two ways[76]. The most common is necrosis. It is a consequence of acute energetic failure, with loss of cell morphology and lysis leading to inflammatory processes[98,99]. On the other hand, apoptosis or programmed cell death can take place. In this case, intracellular mechanisms (such as the induction of pro-apoptotic genes and factors like nuclear factor κ-B (NF-κB)[100] or apoptosis-linked gene 2 (ALG2)[101]) which require energy are activated and determine a regulated cell degradation that finally is phagocyted without leading to inflammation[92,102,103]. Necrosis is preponderant on the hyperacute phase and apoptosis is characteristic of the next stages. Nevertheless, in the acute phase of brain ischemia both death types occur because the necrotic process can lead to the activation of proteolytic enzymes of the apoptotic way, the caspases.

1.4.5. Physiopathology of edema in brain ischemia

The edema that occurs during stroke aggravates the ischemic damage. This edema is the result of the accumulation of liquid into the cells (cytotoxic edema), into the cellular interstice (vasogenic edema), or both[104].

As Glia and neurons are more sensible to ischemia than endothelium, at the first moment the edema is cytotoxic and it occurs due to cell permeability disorder and increase in intracellular osmolarity.

The persistence of the ischemia determines BBB deterioration and, therefore, the appearance of the vasogenic edema which starts with the activation of an inflammatory response at the microcirculation that leads to the release of cytokines like interleukine-1β (IL-1β) and tumor necrosis factor-α (TNF-α). These cytokines activate a second inflammatory response with the release of IL-6 and IL-8, which participate in the development of acute phase reactants, like fever, C reactive protein (CRP) and fibrinogen, and the release of adhesins, molecules that lead to leukocyte aggregation and its adherence to the vessel wall. Moreover, this process continues with the release of matrix metalloproteinases (MMPs), proteolytic enzymes that participate in the extracellular matrix remodelling. This phenomenon leads to the accumulation of liquid in the interstice due to the protein extravasation to the brain parenchyma through the damaged BBB[105].

1.5. ISCHEMIC STROKE AND INFLAMMATION

Although inflammation has traditionally been considered as a mere reaction of damaged brain tissue, it plays a key role in the pathophysiology of ischemic stroke[106]. There is a very active interaction between the central nervous system and the immune system[107].

Several studies have shown that systemic inflammation is related to the stroke risk[26,27,108]. On the other hand, we know today that elements of both innate and adaptive immunity are involved in all phases of the ischemic cascade during and after the event[106]. Signals released during cerebral ischemia activate innate immunity components and promote a pro-inflammatory response that contributes to tissue damage. Likewise, these processes stimulate a potentially harmful adaptive immune response directed against antigens.

After focal cerebral ischemia onset, an intense inflammatory reaction characterized by peripheral leukocyte migration into the brain parenchyma and activation of microglia takes place[109–113]. The stop in CBF results in energy depletion and neuron necrosis, which triggers inflammatory cell activation and infiltration. Reperfusion of the occluded vessel, results in the generation of ROS either by reperfusion with oxygenated blood or production within brain and immune cells. Then, ischemic cells are stimulated by ROS to secrete inflammatory cytokines and chemokines that cause an upregulation of adhesion molecules in the cerebral vasculature and peripheral leukocyte recruitment. Once activated, inflammatory cells are able to release more cytokines, MMPs, NO and more ROS, which increase cell damage and determine the disruption of the BBB and extracellular matrix[114,115]. BBB breakdown contributes to secondary ischemic damage by allowing serum elements and blood to enter the brain[116,117]. This results in post-ischemic inflammation, involving activation of microglia and brain infiltration of peripheral inflammatory cells enhancing the damage[117,118].

Moreover, ischemic tissue itself contributes to systemic immunosuppression through the autonomic nervous system which favours the development of infections and, therefore, affects morbidity and survival of patients with stroke[119,120].

Furthermore, in recent years it has been observed that these inflammatory processes may also have a beneficial effect through their role in the resolution of dead cells and the initiation of repairing mechanisms[121].

1.5.1. Innate immunity and adaptive immunity

1.5.1.1. Innate immune system and stroke

Innate immunity provides the early response against microbes and products of injured cells[107]. It does not require prior exposure to antigens to be activated[121]. It is constituted by chemical and physical "walls", cellular components (monocytes, macrophages, neutrophils and natural killer cells) and different inflammatory mediators (cytokines, chemokines, etc.). On the other hand, the induction of adaptive immunity will require some signals provided by the innate immunity system to facilitate the expansion of antigen-specific T- and B-lymphocytes.

Acute ischemia results in destruction on neural cells. This leads to the release of damage-associated molecular patterns (DAMPs) to the extracellular environment which triggers the inflammatory cascade[122,123]. Neurons are very vulnerable to ischemia and rapidly release DAMPs that activate microglia[106]. DAMPs include high mobility group protein B1 (HMGB1), uric acid, heat shock proteins, ATP, S100 proteins, heparan sulphate, DNA, and RNA[107]. Different studies have shown that the release of DAMPs is associated with either beneficial or deleterious effects[124–126].

Unlike adaptive immunity, in which T and B cells can recognize a huge number of potential antigens, innate immune system cells must recognize antigens through a predetermined subset of germline-encoded receptors. Thus, these cells focus on a few highly-conserved structures expressed by large groups of microorganisms. The conserved structural patterns are called pathogen-associated molecular patterns (PAMPs) and the receptors of the immune system that recognize these structures are called pattern recognition receptors[121]. These receptors include RIG-1-like receptors, NOD-like receptors, C-type lectin receptors, AIM2-like receptors and Toll-like receptors (TLRs)[127]. TLRs family is the best characterized subtype of pattern recognition receptors in mammals (this family will be reviewed in depth later). All of them determine the activation of different signalling pathways, such as NF-κB, mitogen-activated protein kinase (MAPK) and type 1 interferon pathways, resulting in the upregulation of pro-inflammatory cytokines, chemokines, ROS, and costimulatory signals, and the activation and clonal expansion of antigen-specific T cells[127,128].

As previously noted, several cells such as monocytes, macrophages and neutrophils are part of the innate immunity, and their role is stroke physiopathology will be discussed later, as other innate immune components.

1.5.1.2. Adaptive immune system and stroke

Adaptive immunity provides a more effective defense mechanism than does innate immunity[107]. The adaptive response acts after initial innate immunity response. It is a slower response with a high specificity for a larger number of antigens. It is able to remember previous exposure to an antigen and start a faster response when a new exposure occurs. B and T cells and the products secreted by these cells (such as antibodies or cytokines), antigen-presenting cells, and effector cells are the main components of adaptive immunity. This response is initiated by recognition of antigens by lymphocytes, which results in proliferating and differentiating into effector cells that can have cytotoxic or cytoprotective effects. The role of these cells in stroke will be discussed later.

1.5.2. Immune components

1.5.2.1. Inflammatory cell response to ischemia

Peripheral leukocyte counts are increased during acute ischemic stroke[129], and an epidemiological correlation has been shown between the risk of vascular diseases and the leukocyte counts[130].

In the ischemic brain, inflammation is characterized by the accumulation of inflammatory cells (blood-derived leukocytes and resident microglia) and mediators[121]. Leukocytes adhere to vessel walls after stroke onset, resulting in the migration and accumulation into ischemic tissue through the engagement of adhesion molecules on leukocytes with ligands on endothelial cells. This leads to the release of pro-inflammatory mediators which determine secondary injury of potentially salvageable tissue within the ischemic penumbra area[111].

1.5.2.1.1. Neutrophils and lymphocytes

Neutrophils are secretory and phagocytic cells of the innate immune system which carry different types of cytoplasmic granules and secretory vesicles[106]. They are the first leukocyte subtype recruited to the ischemic tissue[111]. Under physiological conditions, neutrophils circulate and do not interact with endothelial cells, whereas lymphocytes and monocytes pass through capillary walls constantly. After brain ischemia neutrophils adhere to the cerebral endothelium and transmigrate into the tissue[131]. It starts 6 to

12 hours after ischemia onset, the level of neutrophils accumulated in the tissue stands until 6 to 9 days, and then decreases[132]. These cells potentiate ischemic injury. Among the major pro-inflammatory molecules stored they carry iNOS, NADPH oxidase (NOX), myeloperoxidase (MPO), MMP8, MMP9, elastase and cathepsins. Vesicle and granule exocytosis is induced after receptor engagement, e.g. binding to E-selectin on endothelial cells, or after IL-8 stimulation[133]. It has been shown that depletion and inhibition of neutrophil infiltration leads to a reduction in infarct volume[134–136] and an improved neurological outcome[132].

Lymphocytes are key cells in innate and adaptive immune responses[106]. The concentration of these cells increases in peripheral blood after neutrophils following MCAO (middle cerebral artery occlusion) in rats[137]. There are two main types of lymphocytes, B and T cells. B-lymphocytes determine humoral immune responses, characterized by the production of antibodies that attack and neutralize specific antigens. T-lymphocytes, which can be divided into CD4+ and CD8+ based on the expression of surface markers, are involved in cellular immunity, responsible for the suppression of extraneous antigens by a cytotoxic cellular response. T cell levels in ischemic brain rise 24 hours after ischemia onset and reach the peak at the third day[138]. T cells are the most relevant lymphocytes involved in inflammatory response following ischemic brain damage and there are several subtypes of these cells involved in stroke physiopathology.

Central to adaptive immunity is antigen presentation. Hours following stroke, antigen-presenting cells (APC) such as dendritic cells and macrophages process and exhibit antigens complexed with major histocompatibility complex (MHC) to lymphocytes[106]. Among these antigens are brain products as myelin basic protein, glial fibrillary acidic protein (GFAP) and S100 protein[139]. CD4+ T cells recognize antigens presented through MHC class II and displayed on the APC surface, which results in T cell activation. Once activated, most CD4+ T cells become helper T cells (Th) that coordinate and modulate immune responses[140]. Th cells include effector (such as Th1, Th2 and Th17 cells) and regulatory cells (Treg cells). Depending on the molecular signals present in their environment, different subtypes of Th cells can develop. Once CD4+ T cells go through the BBB, microglia stimulates them to turn into different T cell subtypes, mainly Th1 and Th2. Th type 1 (Th1) effector cells secrete pro-inflammatory cytokines such as IL-12, interferon-γ (IFN-γ) and TNF-α, and stimulate innate and T-cell induced immune responses leading to cytotoxicity. Therefore, Th1 cells may play a key role in the pathogenesis of stroke[138,140]. In the other hand, Th2 cells secrete anti-inflammatory cytokines such as IL-4, IL-5, IL-10, 1L-13 and therefore may play a protective role[138,140]. Th17 cells secrete IL-17, participate in autoimmunity, but have not been implicated in cerebral ischemic damage[141]. Regulatory T cells (Treg) induce immunosuppression by producing IL-10 and TGF-β[140]. Treg cells are critical for immune system homeostasis by counterbalancing the destructive effects of excessive inflammation and may play a protective role in cerebral ischemia[106]. Depletion of these cells in mice considerably increased infarct size and behavioural deficits after brain ischemia[142]. These effects did not become evident until 3-7 days after MCAO.

Cytotoxic T cells (CD8+) express a T cell receptor that binds the antigen presented through MHC class I. After clonal expansion, activated CD8+ T cells patrol the internal environment in search of somatic cells expressing the antigens against they were sensitized, leading to apoptosis of those cells. Under inflammatory conditions, MHC class I may be expressed on any nervous system cell, depending on the intensity of the pro-inflammatory stimulus through IFN-γ and TNF-α. Thus, following brain ischemia, cytotoxic T cells may play a major role mediating inflammatory damage in the CNS[138].

An experimental study showed that the ablation of perforin, which mediates the key mechanisms of CD8+ T cell cytotoxicity, improved stroke outcome[143].

γδT cells are a subset of effector lymphocytes particularly present in mucous membranes[144]. These cells recognize non-peptide antigens and react to danger signals produced by stressed cells. γδT cells can exert different functions depending on the context, such as cytolysis, antigen presentation, immunoregulation and production of growth factors[145]. In brain ischemia this subtype of T cells has been implicated in both cytotoxicity and protective immunomodulation[106].

About B cells, regulatory B cells were reported to have beneficial effects on the ischemic brain as early as 24-48 hours after MCAO[146]. Lack of B cells increased infiltration of several leukocyte subpopulations into the brain, and reduced their functional activation. Conversely, transfer of B cells intro the brain reduced infarct size and production of inflammatory cytokines by peripheral T cells.

1.5.2.1.2. Monocytes, macrophages and microglia

Monocytes are multifunctional innate immune cells with crucial roles in the regulation of inflammation and tissue repair[107,147]. They represent the mononuclear phagocyte system. Monocytes circulate in the blood, bone marrow, and spleen and do not proliferate in a steady state. They can migrate to tissues and differentiate to macrophages during infection and inflammation. 12 to 24 hours after the ischemia onset, monocytes accumulate in the injured brain and rapidly differentiate to pro-inflammatory macrophages capable of aggressive phagocytosis[121].

Macrophages are resident phagocytic cells in lymphoid and non-lymphoid tissues. They are involved in the tissue homeostasis through the clearance of apoptotic cells and the production of growth factors, but they are also equipped with pathogen recognition receptors that make them efficient at phagocytosis and induce production of inflammatory cytokines.

We can differentiate two subtypes of macrophages, M1 (classic) and M2 (alternative). M2 are the usual residents of our organs, they derive from CCR2- (C-C chemokine receptor type 2, the monocyte chemoattractant protein-1 -MCP-1- receptor, a key chemokine that regulates migration and infiltration of monocytes/macrophages) monocytes[148], participate in tissue homeostasis, promote Th2 responses[147] and segregate anti-inflammatory cytokines like IL-4, IL-10, IL-13 and TGF-β[149,150]. On the other hand, M1 derive from CCR2+ monocytes[148], they support Th1 responses[147], have pro-inflammatory activity and release IL-1β, IL-6, TNF-α, NO and ROS[151].

There are macrophages confined in the "glia limitans", the space between the vascular basement membrane and the brain surface, which are continuously replaced by hematogenous precursors[152,153]. During brain ischemia, these perivascular macrophages release cytokines that drive the infiltration of inflammatory cells to the brain[154].

<u>Microglia</u> represent the CNS resident subset of macrophages, the innate immune cells of the brain[152]. They have a key role as resident immunocompetent and phagocytic CNS cells[155] and serve as scavenger cells in case of ischemia, infection, neurodegeneration or trauma[156,157].

When cerebral ischemia occurs, microglia are activated through Toll-like receptor (TLR) pathway[158], transform into phagocytes virtually indistinguishable from circulating macrophages and release several cytokines. Studies in rodent have shown that, along with macrophages, they are the principal CNS source of cytokines such as IL-1β, TNF-α and TGF-β[159], Studies in different experimental models of ischemia have demonstrated that microglia inhibition may have beneficial effects with the reduction of infarct volume and improvement of neurological deficit scores[160–162].

Although microglia contribute to post-ischemic inflammation and tissue damage, they also contribute to the resolution of inflammation, are involved in clearing of dead and dying cells and participate in tissue repairing. This may be related to the fact that, similar to macrophages, microglia exist in two different subtypes, M1 and M2[163]. On activation, microglia take on the M1 phenotype and release pro-inflammatory cytokines like IL-1β, TNF-α and ROS[106,164]. On the other hand, microglia can take on M2 phenotype which contribute to the resolution of inflammation and tissue repair by producing anti-inflammatory cytokines like IL-10 and TGF-β, as well as growth factors like IGF-1 and glial cell line-derived neurotrophic factor[106,164].

1.5.2.1.3. Astrocytes

Whereas astrocytes play an important role for the maintenance of neurons, its activation after brain ischemia could be dangerous. Due to this activation the GFAP increases and reactive gliosis associated with characteristic structural and functional changes takes place[165].

Astrocytes express different types of inflammatory mediators[166]. These cells are involved in brain inflammation by expressing MHC, developing Th2 anti-inflammatory immune responses, suppressing the expression of pro-inflammatory IL-12, and secreting pro-inflammatory factors such as MCP-1[166], iNOS[167], and tumor necrosis factor-like weak inducer of apoptosis (TWEAK) (a member of the tumor necrosis factor superfamily, which blockade in astrocytes markedly reduced infarct volume in a murine model of stroke [168]).

1.5.2.1.4. Endothelial cells

Endothelial cells are responsible of vascular integrity maintenance. In physiological conditions, the endothelium promotes blood flow through its anticoagulant and antithrombotic role.

After ischemia, endothelial dysfunction takes place resulting in the loss of the homeostatic properties of endothelium, which leads to increased adhesiveness and endothelial permeability to leukocytes and platelets[169], Thus, it changes its anticoagulant properties to other procoagulant. As a result of the endothelial damage, monocytes and lipids accumulate in the injured tissue. The liver releases acute phase reactants such as CRP and fibrinogen due to the inflammatory response. CRP induces mononuclear cells to release tissue factor, which initiates the coagulation, activates the complement pathway and neutralizes platelet-activating factor[170]. Fibrinogen is transformed into fibrin using thrombin at the end of the coagulation cascade.

1.5.2.2. Adhesion molecules

Brain parenchyma infiltration by leukocytes through the endothelium involves rolling, adhesion and transendothelial migration of these cells[121,171]. Therefore, adhesion molecules in leukocytes and endothelial cells contribute to cerebral damage. Those molecules are classified into: selectins, the superfamily of immunoglobulins and integrins.

1.5.2.2.1. Selectins

Selectins are the glycoproteins responsible for the initial interaction between leukocytes and endothelial cells in the periphery of the infarct.

Three kinds of selectins have been identified: E-selectin, P-selectin and L-selectin. They are expressed on the outer cell membrane after cell activation by different molecules such as thrombin or histamine. E- and P-selectins are involved in leukocyte rolling and recruitment during the early phases of activation. L- selectin guides the unstimulated leukocytes[172].

The role of E- and P-selectins in stroke has been evaluated in different experimental models. Their upregulation promotes ischemic inflammatory responses and increases brain damage and infarct volumes[173,174], whereas its deficiency or blockade is associated with improved neurological outcome[173,175].

The role of L-selectin in brain ischemia is less clear, it does not appear to significantly influence stroke outcome in experimental studies[176].

1.5.2.2.2. Immunoglobulin superfamily

Five are the members of immunoglobulin superfamily: intracellular adhesion molecule-1 (ICAM-1), intracellular adhesion molecule-2 (ICAM-2), vascular cellular adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1) and mucosal vascular addressin cell adhesion molecule-1 (MadCAM-1). Activated endothelial cells express all of them. These molecules mediate the adhesion of leukocytes to endothelia, and set up stronger links than selectins[177].

ICAM-1 is constitutively expressed, and its expression in cerebral microvascular endothelial cells is increased by IL-1β, TNF-α and lipopolysaccharide (LPS)[178]. There is evidence that indicates its role in brain ischemia. In experimental studies it has been shown increased expression of this adhesin in the first hours after stroke onset, which precedes leukocyte infiltration[179]. Blocking ICAM-1[180] and knocking-out its gene[181] are associated with better outcome and smaller infarcts in experimental models of stroke. Clinical studies in patients with ischemic stroke have also demonstrated a rise in serum levels of ICAM-1[182] but a clinical trial with anti-

ICAM-1 antibody has failed to show improvement in outcome and, indeed, may significantly worsen it[183].

The role of VCAM-1 in stroke is controversial. Some authors have described an increase in VCAM-1 mRNA after brain ischemia[184] but others have failed to observe significant changes[185]. Treatment with anti-VCAM-1 antibodies has failed to show any effect on stroke outcome[186] suggesting that VCAM-1 may not play a significant role in brain ischemia.

Little is known about the role in stroke of the other members of this family.

1.5.2.2.3. Integrins

Integrins are adhesion molecules that consist of heterodimeric membrane glycoproteins, with a common β subunit and a variable α subunit, that participate in cell-cell and cell-extracellular matrix interactions[187]. There are three subfamilies of β-subunits, denoted $\beta_{1\text{-}3}$. β_2 integrins (CD18) are the ones involved in leukocyte cell adhesion and can be combined with one of the three distinct α chains (CD11a, CD11b, CD11c). Leukocyte integrins are activated by chemokines, cytokines, and other chemoattractants. In order to bind leukocytes to activated endothelium, integrins need to be able to recognize endothelial cell adhesion molecules, so they must be expressed on the cell surface. Although leukocyte rolling is mediated primarily by P-selectin and E-selectin[188], the resulting attachment to the vascular endothelium requires the expression of ICAM-1 in endothelial cells, and the interaction with the leukocyte integrin CD11b/CD18[189].

In vitro studies have shown that hypoxia causes an increase in neutrophil CD11b expression, and that the damage is reduced with the downregulation of neutrophil CD11b[190]. Moreover, in *in vivo* experimental studies, reduction of infarct volumes and apoptosis, associated with decreased accumulation of neutrophils were observed after administration of anti-CD11b or anti-CD18 monoclonal antibodies[191,192]. Despite these results, treatment with antibodies against CD11/CD18 in acute stroke patients showed negative results[193,194].

1.5.2.3. Inflammatory mediators

1.5.2.3.1. Cytokines

Cytokines are small glycoproteins that play an important role as activators of adhesion molecules[121]. Different brain cells such as neurons, microglia, astrocytes and endothelial cells are able to release cytokines. There are also peripheral cells such as monocytes, T lymphocytes, natural killer cells, and neutrophils responsible of the production and secretion of cytokines that might participate in inflammation on CNS. Cytokines are upregulated in the brain due to several stimuli like ischemia.

IL-1, IL-6, TNF-α, IL-10, TGF-β are the most studied cytokines related to inflammation in stroke. The role of these and some other cytokines will be discussed in the following lines. Their inflammatory behaviour is synthesized in **table 1**.

PRO-INFLAMMATORY CYTOKINES	ANTI-INFLAMMATORY CYTOKINES
IL-1	IL-4
IL-6	IL-10
IL-8	IL-13
IL-20	TGF-β

TNF-α

IL-1 has: two isoforms, IL-1α and IL-1β, the latter being the best investigated in stroke; two receptors, IL-1R1 and IL-1R2, while only the former is involved in signal transduction[195], IL-1R2 and both soluble receptors sIL-1R1 and sIL-R2 act as decoy receptors[196,197]; and an endogenous inhibitor, IL-1 receptor antagonist (IL-1ra)[158].

IL-1β mRNA is expressed in the brain after excitotoxicity[198] and stimulation with LPS[199]. Within 15 to 30 minutes following ischemia[200] its expression rises and a few hours later the protein levels increases and remain elevated for up to 4 days[201,202]. Several experimental studies have

Table 1. The inflammatory behaviour of cytokines in ischemic stroke.

found a correlation between the increase in the levels of IL-1β after ischemia and worsening of the infarct. Intraventricular injection of IL-1β increased brain damage during ischemia in rats[203]. IL-1β deficient mice presented smaller infarcts in comparison with wild-type mice[204]. Treatment with IL-1ra reduced infarct size[205] and IL-1ra deficient mice exhibited an increase in ischemic damage[206], all of which suggests that endogenous IL-1ra is neuroprotective in brain ischemia. Moreover, inactivating or knocking out the IL-1R1 decreased the extent of damage and preserved neurological function[207]. Despite importance shown in experimental studies, IL-1β is generally not observed at elevated levels in serum or plasma from stroke patients[208–210], which is likely due to its localized role.

High circulating levels of IL-1β elevate IL-6, a pro-inflammatory cytokine that is upregulated in brain ischemia[211]. IL-6 exerts its function through binding to the IL-6R, which allows to the formation of gp130/gp130 homodimers thereby initiating intracellular signaling[212]. The soluble form of IL-6R (sIL-6R) has no decoy properties, unlike sIL-1R and sTNFR.

Experimental studies in rats have shown increased levels of IL-6 as soon as 3 hours and to peak 12 hours after MCAO[213]. In stroke patients it has been also demonstrated an elevation of its serum levels during the first week[214,215]. IL-6 may have detrimental effects in cerebral ischemia. Raised plasma concentrations of this cytokine are a powerful predictor of early neurological deterioration[215] and are associated with greater infarct volume[216] and bad outcome[217]. Moreover, the association between IL-6 and early neurological worsening is not influenced by the initial size, topography, or mechanism of the ischemic stroke[218]. Furthermore, it has been shown that concentrations of IL-6 higher than 5 pg/mL, determined within the first three months after an ischemic stroke event (in patients with no anticoagulant treatment), predicts a 29-fold higher risk of vascular disease recurrence[219]. However, some studies have shown a potential beneficial effect of IL-6 during ischemia. In an experimental study with mice, IL-6 appeared to act as a key regulator of body temperature and an endogenous neuroprotectant in cerebral ischemia[220]. In the same study the authors found an anti-apoptotic and neuroprotective effect when administering recombinant human IL-6 to a cell culture. Related to this potential neuroprotective effect and contrary to previous reports in stroke patients, Sotgiu et al[221] found that IL-6 serum level inversely correlated with both final neurological impairment and infarct size.

TNF-α is likely the most studied cytokine in experimental stroke[213]. There are two TNF-α receptors, TNFR-I and TNFR-II. Both can be cleaved from the cell surface as soluble TNF-α receptors, sTNFR-I and sTNFR-II, acting as decoy molecules for TNF-α[222,223].

This cytokine is upregulated in the brain after ischemia and it can both exacerbate and counteract infarct evolution in animal models. Induction of TNF-α mRNA has been shown in ischemic cortex after ischemia in rats[224] and its upregulation is proportional to IL-1[225] and IL-6[226] upregulation. Initial increases are detected 1 to 3 hours after the ischemia onset[224] and have a second peak at 24 to 36 hours[227,228]. Clinical studies have confirmed that TNF-α is upregulated in the brain of patients with acute ischemic stroke[229] and appears sequentially in the core and peri-infarct areas before it is detected in the contralateral hemisphere and other remote cerebral areas[230]. Increased serum concentrations have also been found in ischemic stroke patients[218,231] and this elevation is associated with early neurologic deterioration and poor functional outcome in patients suffering lacunar strokes[215]. There are several studies about the neurotoxicity of TNF-α, but its role is still controversial.[232] Some of these studies suggest a potential deleterious effect of TNF-α in experimental models. Inhibition and blockade of TNF-α reduces ischemic brain damage[233,234], while administration of recombinant TNF-α worsens ischemic injury[235]. However, TNF-α may also have a neuroprotective function under certain circumstances, and the causes of this different behaviour might be found in the different TNF-α signaling pathway. Larger infarcts have been detected in studies with TNF-α receptor-deficient mice (mainly in TNFR-I knockout)[236–239]. Thus TNFR-I, besides a role in normal neuronal function, may have a role for neuroprotection after experimental stroke. On the other side, as soluble TNF-α receptors act as decoy molecules, increasing the cleavage of TNFR-I may be an important step in reducing the activation of TNFR-I-mediated apoptotic signaling, as it has been shown[240]. Finally, TNF-α may have a neuroprotective role through ischemic preconditioning. High plasma levels of this cytokine are associated with human cerebrovascular ischemic tolerance[241] and the upregulation of both TNF-α receptors after brain ischemia has been shown[236,242]. The reduction in TNFR-I expression through the administration of TNFR-I antisense oligodeoxynucleotide inhibits the ischemic preconditioning-induced protective effect, suggesting that TNFR-I upregulation is implicated in the phenomenon of ischemic tolerance[243].

IL-10 is a cytokine with anti-inflammatory properties. IL-10 acts by inhibiting IL-1 and TNF-α, and also by suppressing cytokine receptor expression and receptor activation. It is synthesized in the CNS and is upregulated in experimental stroke[244]. Patients with reduced production of this cytokine have an increased risk of stroke, which suggests a protective role of IL-10[245]. Moreover, it has been shown that low plasma levels of IL-10 are independently associated with clinical worsening[246]. Experimental models of cerebral ischemia have supported this neuroprotective role. Exogenous administration[247] and gene transfer of IL-10[248] seem to have beneficial effects in models of cerebral ischemia[249].

TGF-β is another cytokine with potential anti-inflammatory properties. This cytokine is produced by M2 type macrophages and microglia[250]. TGF-β mRNA expression is increased in ischemic tissues 1 to 6 hours after ischemic onset in rodents[251], and remain elevated up to 15 days[252]. Overexpression of TGF-β resulted in mouse brain protection from ischemic stroke and reduction of the inflammatory response[253]. Another study showed that cultured neurons may be protected from ischemia-like insults by microglia-secreted TGF-β[254]. Since TGF-β is prominently expressed in the recovery phase of some CNS diseases, it may contribute to the recovery of ischemic stroke[255].

Other cytokines known to participate in stroke inflammatory processes are IL-4, IL-8, IL-13 and IL-20.

Thymus cells and T lymphocytes produce IL-4. It has anti-inflammatory activity through a negative feedback that reduces cytokine production[246]. In ischemic stroke IL-4 inhibitory function is less important than IL-10[256,257].

IL-8 is the main cytokine responsible in neutrophils chemotaxis. It is produced by monocytes, fibroblasts and endothelial cells and promotes integrin expression, improving leukocyte adhesion and activation, producing free radicals and increasing capillary permeability[258]. Some studies have shown high levels of IL-8 in stroke, which activate leukocytes in an early stage[259].

IL-13 is expressed in activated T lymphocytes and M2 type macrophages. It acts similarly to IL-4 inhibiting pro-inflammatory cytokines production[260].

IL-20 is induced when IL-1β modulates p38 MAPK and the NF-κB pathway. IL-20 in turn induces the production of IL-6. Inhibition of IL-20 significantly ameliorated the brain ischemic infarction in rats following MCAO[261].

1.5.2.3.2. Chemokines

Chemokines are cytokines that promote neutrophils and macrophages migration toward the source of the chemokine. Therefore, they play a key role in cellular communication and inflammatory cell recruitment. Chemokines increase leukocyte infiltration after cerebral ischemia increasing the damage[115]. Levels of chemokines such as MCP-1, IL-8, macrophage inflammatory protein-1α (MIP-1α) and macrophage inflammatory protein-1β (MIP-1β) are increased in animal models of ischemia and stroke patients[158,262], and its inhibition or deficiency is associated with reduced damage[263]. In particular, MCP-1 is a potent chemoattractant of monocytes and its expression induces an increment of monocyte infiltration in cerebral parenchyma after ischemia. A significant increase in MCP-1 levels in serum[264] and CSF[265] has been found in patients with acute ischemic stroke. Mice without the chemokine receptor CCR2 (which binds MCP-1) are protected against ischemia-reperfusion injury[266].

Chemokines also participate in the disruption of the BBB. The addition of MCP-1 increases 17-fold the permeability of the BBB in an *in vitro* model, suggesting their implication in the opening of the barrier in cerebral ischemia[267].

It has also been proposed that chemokines could have a key role in homing stem cells to injured regions and could also be involved in marrow-derived stromal cell migration into the ischemic brain[268].

1.5.2.3.3. Arachidonic acid metabolites

Arachidonic acid (AA) metabolites are potent mediators that contribute to post-ischemic brain inflammation and circulatory disorders[269]. During immune cell activation, the AA cascade is initiated via the release of phospholipase A_2 (PLA_2)[270]. In line with the damaging role of this pathway, the deficit of PLA_2 is associated with smaller infarcts, less brain edema, and fewer neurological deficits in mice models of ischemic stroke[271].

AA is metabolized through cyclooxygenase (COX) or lipoxygenase (LOX) pathways. Little is known about the role of the lipoxygenase pathway in brain ischemia, and the studies are controversial[158].

Relative to COX pathway, there are two isoforms of the enzyme: COX-1[272] and COX-2[273]. It has been shown that COX-1 deficient mice have increased vulnerability to focal brain ischemia, although COX-1 inhibition increased the number of healthy neurons in the hippocampus in transient global ischemia[274]. COX-2 is upregulated 12 to 24 hours after ischemia[275], and it is expressed in neurons and vascular cells on the border of the ischemic territory[81] COX-2 inhibitor treatment has been demonstrated to improve neurological outcome after cerebral ischemia[81,276], COX-2 deficient mice have reduced injury after NMDA exposure[277], and COX-2 overexpression is associated with exacerbation of brain damage[278].

1.5.2.3.4. Nitric oxide and nitric oxide synthase

NO is a gas that acts as a signaling molecule involved in different processes such as host defense, neuronal communication, and regulation of the vessel tone[121]. This gas diffuses into cells and cell membranes where it reacts with different molecules. NO is synthesized by NO synthase (NOS). Three isoforms of NOS have been described: endothelial NOS (eNOS), mainly localized in endothelial cells; neuronal NOS (nNOS), found in particular groups of neurons; and inducible NOS (iNOS), induced during pathological states and associated with inflammation[279,280]. eNOS and nNOS are constitutively expressed and are regulated by intracellular calcium. iNOS is inducible, and it is not regulated by intracellular calcium.

Although its presence at normal levels is important, NO may cause DNA damage in cerebral ischemia due to the formation of peroxynitrite[280]. Where and when it is expressed will determine whether the effects of this molecule are beneficial or harmful[281]. After ischemia onset the NO produced by eNOS is beneficial because it induces vasodilatation and limits blood flow reduction[282]. However, when ischemia has developed, NO produced by iNOS contributes to brain damage[281]. It has been shown in experimental models that iNOS expression is harmful and its inhibition leads to a decrease in the infarct volumes and reduced neurological deficits[283,284]. Moreover, knockout mice for iNOS gene submitted to MCAO have smaller infarcts than wild-type mice[283]. Therefore these studies showed the detrimental role of iNOS in brain ischemia.

1.5.2.3.5. Reactive oxygen species

Several enzyme systems are involved in the generation of ROS by inflammatory cells[158]. COX, xanthine dehydrogenase, xanthine oxidase and NOX generate superoxide. MPO and monoamine oxidase (MAO) generate hypochlorous acid and hydrogen peroxide.

Among all the oxidants generated in the brain parenchyma after ischemia, superoxide anion is a major one, which aggravates injury to ischemic brain by direct damage or by reacting with NO to generate peroxynitrite[285]. NOX is a major source of inflammatory cell generated superoxide. The most studied NOX is NOX2. A work with mice deficient in the gp91 subunit of NOX2 showed smaller infarcts than wild-type[286]. Another study in brain ischemia models showed that microglia potentiates injury to the BBB through the production of superoxide by NOX2[287].

MPO is linked to inflammation and is present in neutrophils and monocytes. MPO has been documented in both permanent and transient MCAO[263]. However, MPO may have a beneficial role in brain ischemia. MPO deficient mice had increased infarct size[288] and also increased products of nitrosylation within the ischemic brain, suggesting that MPO's protective effect may be due to its ability to scavenge nitrotyrosine (a product of peroxynitrite reactions) in the presence of glutathione.

1.5.2.3.6. Matrix metalloproteinases

MMPs are proteolytic enzymes that participate in the remodelling of the extracellular matrix and in the degradation of all its components. Expression of MMPs in the adult brain is very low to undetectable, but many MMPs are upregulated in response to injury[289]. Neurons, astrocytes, microglia, and endothelial cells have all been shown to express MMPs after injury.

MMP-2 and MMP-9 have been implicated in brain ischemia[290]. Both IL-6 and TNF-α are able to express MMP-9. In brain and serum from patients with acute ischemic stroke elevated MMP-9 levels have been detected[291], which had been claimed to be responsible for the rupture of the BBB, resulting in the appearance of vasogenic edema and facilitation of hemorrhagic transformation of ischemic lesions[289,292]. The inhibition or deficit of MMP-2 or MMP-9 in experimental models of ischemia is associated with a reduction of infarct size and brain edema[293,294].

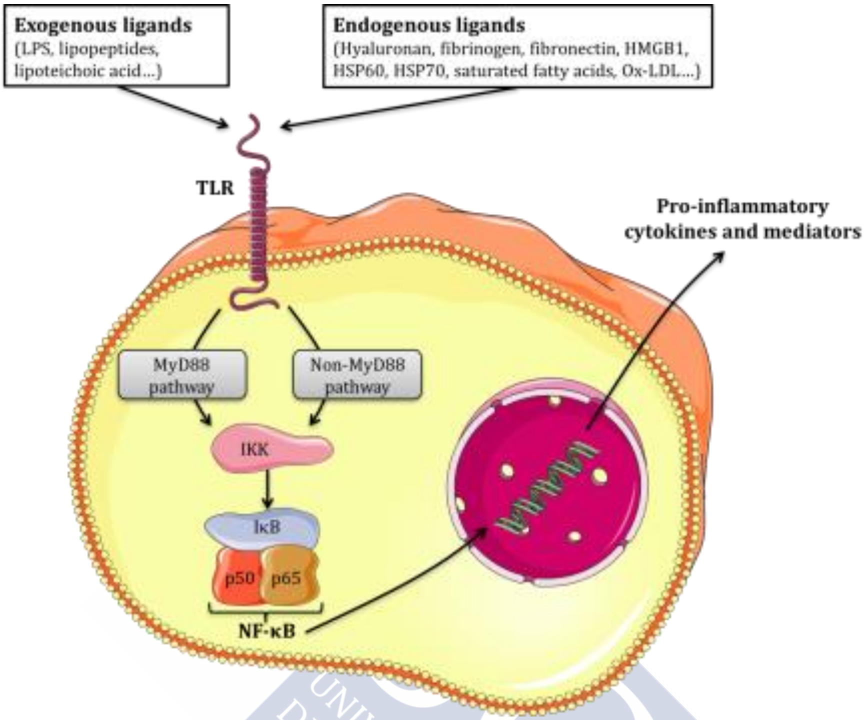
On the other side, it seems that MMPs elevation may play a potential beneficial role in later phases of ischemia when it may be related to plasticity and recovery. MMP-9 is related to different growth factors involved in angiogenesis such as vascular endothelial growth factor (VEGF). Indeed, the inhibition of MMP-9 in rats is associated with increased ischemic injury and reduced VEGF signaling 14 days after MCAO[295].

1.5.2.3.7. Toll-like receptors

TLRs are a key component of innate immune response and are critical in establishing adaptive immunity[296]. TLRs are expressed by several immune cells such as macrophages, dendritic cells, B cells and some subtypes of T cells as well as non-immune cells such as epithelial cells, fibroblasts or adipocytes[296,297]. Although the exact number of TLR subtypes may vary among different mammalian species, it is likely that most have 10 to 15 TLRs[298]. The role of TLRs as initiators of the innate immune response relies on their ability to recognize the highly-conserved structures expressed by large groups of microorganisms previously mentioned and known as PAMPs, such as LPS (also referred to as endotoxin), lipopeptides, or lipoteichoic acid among others. TLRs also detect endogenous ligands that might signal other dangerous conditions, such as the presence of degradation products from macromolecules, products from proteolytic cascades, intracellular components of broken cells and products from genes that are activated by inflammation, such as hyaluronan, fibrinogen, fibronectin, HMGB1, HSP60, HSP70, saturated fatty acids (SFA), oxidized low density lipoprotein (Ox-LDL), and others[297,299–305]. Every TLR subtype recognizes different ligands.

Recognition triggers a signalling cascade that culminates in the activation of NF-κB transcription factor, which leads to the transcription of pro-inflammatory and immunomodulatory factors, which initiate both innate and adaptive immunity (**figure 3**)[299]. Most TLRs are membrane glycoproteins, when an exogenous or endogenous ligand interacts with their extracellular domain the TLR pathway is activated. The exception are TLR3, -7, -8, and -9, which reside mainly in endosomes[305]. This receptor has an adaptor protein named MyD88[296], which is involved in downstream signaling of all TLRs with the exception of TLR3[306]. TLR4 can activate both MyD88-dependent and non-MyD88-dependent signaling pathways[305]. Almost all pathways converge on the activation of the IKK complex which induces the phosphorylation of the IκB proteins, leading to their degradation[296]. The degradation of IκB protein allows the translocation of the liberated NF-κB dimers to the nucleus where they activate the transcription of several genes such as IL-1, TNF-α, IL-6, iNOS, COX-2, ICAM-1, VCAM-1 or MCP-1[121,304]. This process starts inflammatory response[307].

Figure 3. TLR signaling and NF-κB activation.



Several studies have analysed the role of TLRs in stroke. In the CNS, they are mainly located on glial cells including microglia, astrocytes, and oligodendrocytes[308]. Microglia and astrocytes express a wide repertoire of TLRs, and they both could produce pro-inflammatory cytokines when TLRs are attached with their corresponding ligands. However, oligodendrocytes can express a little repertoire of TLRs such as TLR2 and TLR3. Neurons express TLR2, TLR4 and TLR9. Furthermore, other cells from the immune system express TLRs in the CNS, such as monocytes or neutrophils.

Among TLRs, TLR2 and TLR4 are found to be the most important in the pathological progression of cerebral injury due to ischemia and reperfusion. Those receptors have a key role in the physiopathology of stroke as they bind endogenous ligands released after ischemia such as HSP60, HSP70, HMGB1 and fibronectin. The release of all of them increases in the first hours after ischemia onset leading to the expression of pro-inflammatory cues through TLR2 and TLR4 pathways. The importance of these receptors was highlighted in several studies. Expression of TLR2 and TLR4 in neurons have been shown to be upregulated after brain ischemia in experimental models[309]. Studies performed in TLR4 knockout mice showed smaller infarct sizes and improved neurologic test scores in comparison with wild-type[310,311]. However, results found for TLR2 knockout mice are controversial, some studies showed smaller infarcts compared to wild-type mice[298,312] and other suggest that TLR2 could be neuroprotective[313]. Similarly, clinical studies showed consistent findings for TLR4 and controversial results for TLR2 studies. TLR4 expression in peripheral monocytes correlates with stroke severity[314] and poor outcome[315]. Nevertheless, one of those studies showed that, although TLR2 monocyte expression in stroke patients was elevated compared to controls, it does not correlate with outcome[315]. TLR4 expression in neutrophils is associated with poor prognosis and higher infarcts[316]. This study neither found differences in the prognosis depending on the TLR2 expression of neutrophils. However, another study with patients suffering ischemic stroke showed that both TLR2 and TLR4 were independently associated with poor outcome and correlated with higher serum levels of IL-1β, IL-6, TNF-α, and VCAM-1, and that TLR4 was independently associated to lesion volume[317]. In the same study it was found that cultured cells treated with serum from those patients showed a strong inflammatory response that was blocked when TLR2 and TLR4 or cellular fibronectin or HSP60 were blocked. Not only TLR2 and TLR4 have been studied in ischemic stroke. Brea et al.[318], in a model of brain ischemia, found a positive correlation between the expression of TLR8 and infarct volumes, and a positive association between the expression of both TLR8 and TLR7 and poor outcome, which were also correlated with levels of IL-1β and IL-6.

However, apart from their role in tissue damage, recent evidences have pointed out that TLRs may be involved in homeostasis and regeneration. After tissue injury, intracellular and extracellular products provide endogenous ligands to TLRs, which stimulate the innate immune system through such receptors leading to the expression of a variety of genes involved in tissue regeneration[319]. These genes are responsible for the expression of chemokines that induce infiltration of neutrophils and macrophages that remove necrotic cells, the expression of metalloproteinases that promote extracellular matrix remodelling, and the release of cytokines such as IL-6 or TNF-α and different growth factors that initiate cell proliferation and angiogenesis.

Some examples in different tissues are described in the following lines. In colon[320,321] and lung[322], activation of TLR2 and TLR4 is important for epithelial maintenance and barrier function. TLR signaling is required for liver regeneration after hepatectomy, in part through the expression of genes associated with cell replication in hepatocytes[323]. Experimental data have shown that the recognition of endogenous RNA by TLR3 is necessary for the expression of repair genes and the function of tight junctions in the program of skin barrier repair after ultraviolet damage[324]. TLR2 and TLR4-agonistic DAMPs drive kidney regeneration by enhancing the clonal expansion and differentiation of renal progenitor cells and accelerating tubule re-epithelialization from surviving tubular epithelial cells respectively[325]. In a mice model of muscle ischemia-reperfusion injury, Donndorf et al.[326] showed that c-kit^{+} bone marrow stem cells require TLR signaling to interact with endothelium. In such study authors found that local ischemia-reperfusion injury induced the interaction of stem cells with endothelium, which was impaired in TRL2 and TLR4 knockout animals.

Regarding nervous system, several studies have shown the potential effects of TLR activation in regeneration. During Wallerian degeneration after peripheral nerve injury, macrophages and Schwann cells are involved in the clearance of myelin debris and proteins that inhibit axonal regeneration. Axonal degeneration products activate TLRs in Schwann cells initiating the innate immune response leading to macrophage recruitment[327,328]. In a model of peripheral nerve injury, TLR2 and TLR4 knockout mice exhibit impaired debris clearance, axonal regeneration, and recovery of sensory and motor functions[329]. In the same study, animals injected with TLR2 and TLR4 ligands (zymosan and LPS) at the site of nerve lesion experienced faster clearance of the degenerating myelin and recovery. TLRs seem to be also involved in CNS repair. As we noted before, after CNS injury (e.g. stroke) one of the key functions of microglia is the phagocytosis of dying cells and cellular debris, which is required to recovery. In line with this, it has been shown that TLR4 mediates microglial clearance of degenerating axons, enabling a more permissive environment for outgrowth of axons[330]. Glezer et al.[331] found also that intracerebral infusion of LPS in mice models of demyelination induces the proliferation of oligodendrocyte progenitor cells, less accumulation of myelin debris, and accelerated remyelination. This was not observed in TLR4-deficient animals. Similarly, different studies assessed the effect of intraperitoneal administration of TLR ligands (including LPS) on spinal cord regeneration in animal models, and found accelerated clearance of myelin debris[332] and increased proliferation of local progenitor cells at spinal cord[333]. Moreover, the local administration of LPS after spinal cord injury in an animal model was associated higher expression of glial cell-derived neurotrophic factor (GDNF) and improved locomotor function[334]. In another study, the intravitreal administration of Pam_3Cys, a TLR2 selective agonist, induced macrophage infiltration, the upregulation of ciliary neurotrophic factor and GFAP in retinal astrocytes, and the switch from mature retinal ganglion cells to an active regenerative state that resulted in stronger axon regeneration in the injured optic nerve[335].

One of the key points in the stimulation of tissue repair by TLRs signaling may be their role in promoting angiogenesis[336]. It has been found that the stimulation of TLR2[337,338] and TLR4[339,340] mediates the upregulation of VEGF. Moreover, some studies have shown that TLR2 and TLR4 activation may be required for adequate vessel formation. Groot et al.[341], in a mice model of femoral artery occlusion, illustrated that TLR2 and TLR4 expression is necessary for collateral artery growth. In another study, Xu et al.[342] found that TLR2 signaling induces endothelial cell migration and permeability in vitro, and promotes angiogenesis and long-term recovery in a mouse model of hindlimb ischemia, which may be associated with the production of cytokines such as TNF-α and IL-6. It has been shown that the stimulation of TLR2 and TLR6 in endothelial cells induces the release of high levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), which exhibits angiogenic properties and has been demonstrated to mobilize bone marrow–derived progenitor cells into the peripheral circulation with potential implications for vasculogenesis[343]. In the last years, different studies have described the role of HMGB1 in angiogenesis[344]. As we noted before, this protein, an endogenous ligand of TLRs, is released after brain ischemia. To promote angiogenesis, HMGB1 signals through TLR4 and activate endothelial cells, progenitor cells and the secretion of other proangiogenic cytokines. Although it has been shown that, in the early phase of brain ischemia, HMGB1 triggers an inflammatory cascade through TLR4 resulting in increased tissue damage, in the late phase, HMGB1 produced by reactive astrocytes stimulates neurovascular remodelling by activating endothelial progenitor cells.

1.5.2.4. Transcriptional regulation of inflammation

The activation of several transcription factors such as NF-κB, MAPK and activator protein-1 (AP-1) is involved in the physiopathology of stroke. Some of these factors have a key role in inflammatory responses[158].

NF-κB, as noted above, participates in the regulation of inflammation through the gene transcription of pro-inflammatory cytokines and mediators[345]. However, its role in stroke is controversial[346]. Rodents deficient in NF-κB's p50 subunit are protected from experimental stroke[347], suggesting a death-promoting role of NF-κB in ischemia. Similar results were found by inhibiting NF-κB activation[348]. On the other hand, Han et al.[349] found that NF-κB activation is associated with the anti-inflammatory effect of mild hypothermia following experimental stroke. Moreover, the inhibition with diethyldithiocarbamate was associated with enhanced neuronal DNA fragmentation and larger infarct sizes, suggesting a beneficial role[350]. The explanation for these discrepancies is not clear, but could be related to the cell type in which NF-κB is activated, the experimental model studied, or a lack of specificity of pharmacological inhibitors[158].

MAPK is involved in transducing stress-related signals by a cascade of intracellular kinase phosphorylation and transcription factor activation that regulate inflammatory gene production[351]. There have been three documented interlinked signaling pathways during cerebral ischemia: the stress-activated protein kinases/c-Jun N-terminal kinases (SAPK/JN), the p38 MAPKs, and the extracellular signal regulated kinases (ERKs)[352–354].

AP-1 is a heterodimer compound of bZIP transcription factors (such as c-Jun and JunD), activating transcription factor 2 (ATF2) and c-FOS. The JNK/SAPK cascade mediates the upstream activation of AP-1 components.

Inhibition of p38 MAP kinase results in the attenuation of c-fos and c-jun mRNA and AP-1 DNA binding by LPS, and subsequently leads to neuroprotection in cerebral ischemia[355].

2. OBESITY

2.1. OBESITY DEFINITION

Obesity is typically defined as an excessive fat accumulation in adipose tissue, where the undesirable positive energy balance that results in weight gain is the underlying problem[356]. But this definition simplifies a problem of much greater magnitude. Nowadays it is considered as a complex, multifactorial and preventable disease, with important metabolic manifestations and potential consequences in terms of morbidity, disability and quality of life[357–359]. The fact of defining obesity as a disease tries to emphasize the need to change the way medical community faces this global health problem. At the present moment, it is essential to answer to important needs in terms of prevention and treatment in both adults and children.

2.2. OBESITY EPIDEMIOLOGY

Obesity has become a global pandemic[360]. Over the past decades, the prevalence of overweight and obesity has increased dramatically all around the world, with several differences between countries and regions[361].

An estimate from the WHO[359] indicates that, in 2014, about 39% of the world's adult population were overweight and about 13% were obese. This accounts for more than 1.9 billion adults overweight or obese. The prevalence in children has also reached alarming levels all over the world. The WHO estimates that over 41 million children under 5 years were overweight or obese at 2014. The analysis of the Global Burden of Disease 2013 Study showed that the global prevalence of overweight and obesity has increased a 27.5% for adults and a 47.1% for children from 1980 to 2013[361]. The proportion of adults with a body mass index (BMI) higher than 25 kg/m^2

increased from 28.8% in 1980 to 36.9% in 2013 for men, and from 29.8% to 38% for women. The rising has taken place in both developed and developing countries. The rate of increase has been higher between 1992 and 2002, but it appears to have attenuated over the last decade, particularly in developed regions.

In developed countries, the peak age for overweight and obesity is around 55 years in men and over 60 for women. At these peaks, about two in three men are overweight and one out of four are obese, and two in three women are overweight and one out of three are obese. For developing countries, the age patterns are similar but the levels are much lower. Nonetheless, 64% of the world's obese people live in developing countries.

The first indications that obesity was taking on epidemic proportions came from USA and Europe populations[357]. Nowadays, at USA, over 72% of adults are either overweight or obese, and about 35% are obese[362]. In the same study, among USA youth (< 20 years), over 32% were overweight or obese, and 17% were obese. European obesity rates differ markedly among countries and studies, ranging from 4% to 28.3% for men and from 6.2% to 36.5% for women[363]. In central, eastern, and southern regions of Europe, prevalence rates are higher than in the western and northern regions. Among the countries with the higher proportions of obesity, Spain and Italy are at leading rates for both sexes. Childhood obesity prevalence varies also between European countries: 19.3% to 49% boys and 18.4% to 42.5% girls were overweight or obese; 6% to 26.6% boys and 4.6 to 17.3% girls were obese. Similarly to adults, there appears to be a north-south gradient with the highest level of overweight found in southern European countries[364].

It has also been shown that mean WC has increased progressively in late 19th century and early 20th [365,366]. In the USA, the mean WC increased from 1960-1962 to 1999-2000, 9.9 cm in men and 23.2 cm in women[365].

These trends were found not only in obese patients, but also in underweight and normal weight ones. Moreover, whereas in men the increase in WC in the early 20th century was very close to what would be expected due to the increase in mean BMI, in women most of the increase was found to be independent of changes in BMI[367]. The same trends were observed in children and adolescents in the USA, with greatly increasing in mean WC, WHR and prevalence of abdominal obesity in the past decades[368].

2.3. OBESITY PATHOGENESIS AND RISK FACTORS

Obesity is the consequence of an energy imbalance between calories consumed and calories expended[357]. When energy intake is greater than energy expenditure a positive energy balance occurs, resulting in an increase in body fat stores and a weight gain (**figure 4**)[356]. This imbalance is the result of biological, behavioural, socioeconomic and environmental factors. The rapid increase in the prevalence of obesity in the last century is secondary to several "obesogenic changes" such as economic growth, growing availability of abundant, inexpensive and nutrient-poor food, industrialization, mechanized transportation and urbanization[357]. However, not everyone experience the same body fat accumulation. Individual behaviours in response to hereditary and particular socioeconomic and sociocultural factors play a dominant role in preventing obesity.

The responsible for energy balance or homeostasis is a system that involves different brain circuits, making adjustments to food intake in response to circulating signals (**figure 5**)[369]. The best-known signal is leptin, an adipocyte derived hormone that circulates in direct proportion to body fat stores, and acts on key neurons to reduce food intake. Similarly, the pancreatic hormone insulin circulates in proportion to body fat and acts in the brain to reduce food intake. Beyond those adiposity negative feedback signals, some gut peptides such as peptide YY_{3-36} (PYY_{3-36}), glucagon-like

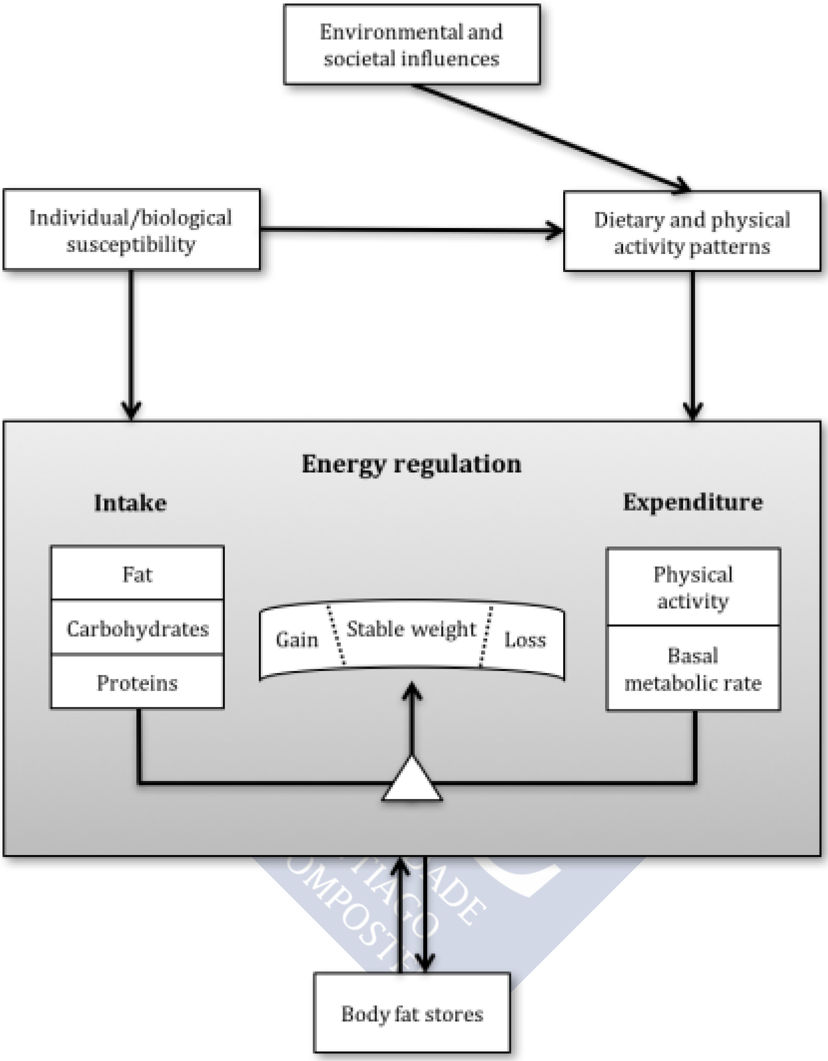


Figure 4. Energy balance. Modified from WHO[356].

peptide 1 (GLP1) and cholecystokinin (CCK), play a key role in the perception of satiety and contribute to the termination of individual meals. The adiposity negative feedback, through leptin and insulin, reduces food intake in part by increasing brain responsiveness to satiety signals. Moreover, leptin reduces food intake through the modulation of brain areas that are associated with reward in response to food-related stimuli.

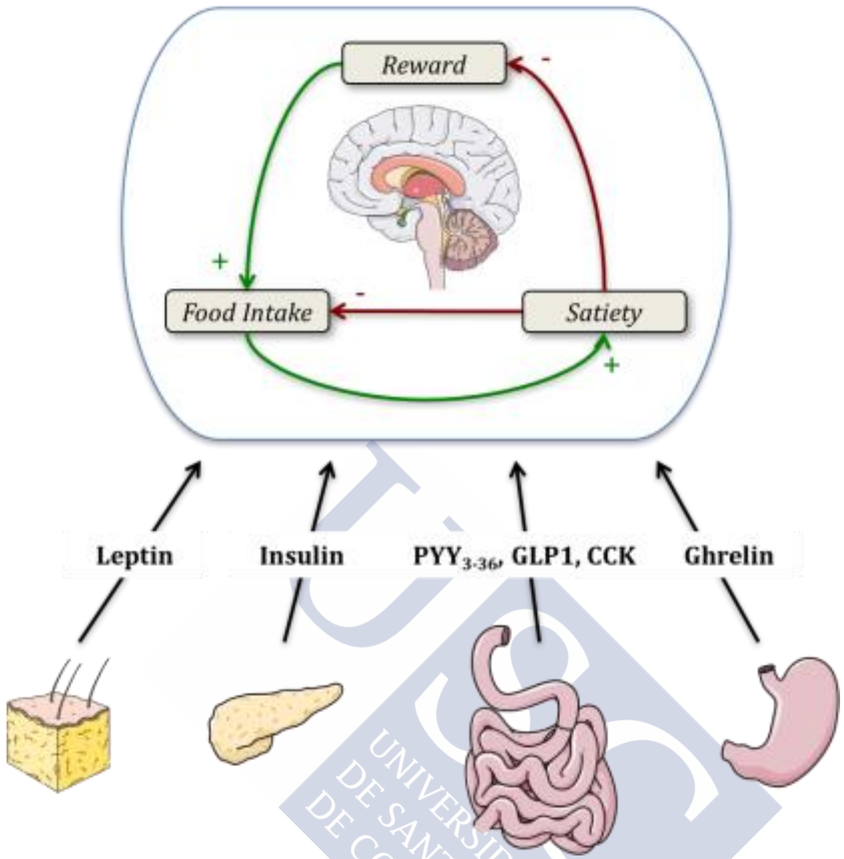


Figure 5. The energy homeostasis system.

The best-studied neuronal subpopulations involved in energy homeostasis are AGRP and POMC neurons, located in the arcuate nucleus of hypothalamus (**figure 6**)[369]. AGRP cells include neurons that co-express neuropeptide Y (NPY), agouti-related protein (AGRP) and γ-aminobutyric acid (GABA). Those cells stimulate feeding and are inhibited by leptin and insulin, whereas they are activated by ghrelin, a gastric hormone secreted before meal onset. POMC neurons express pro-opiomelanocortin (POMC) and release α-melanocyte stimulating hormone (α-MSH), which inhibits food intake. Unlike AGRP neurons, POMC cells are stimulated by leptin and are involved in long-term control of feeding. In obese animals and humans, the capacity of leptin to reduce food intake and body weight is limited[369]. Its ability to cross the BBB and its capacity to activate neuronal leptin receptor signaling are impaired. Although the cause of obesity-associated leptin resistance is unknown, inflammation, gliosis and injury affecting hypothalamic neurons may have a role.

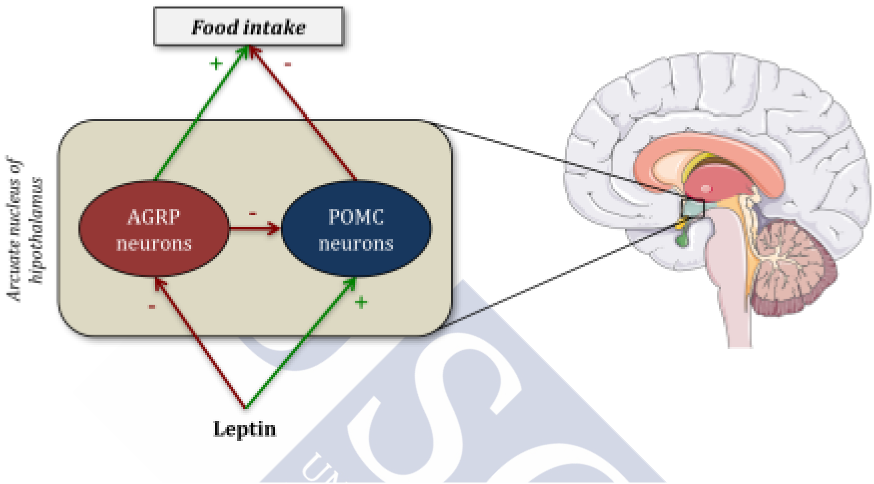


Figure 6. The involvement of leptin and hypothalamus in the homeostatic regulation of food intake (a simplification).

2.3.1. Genetics

Genetic is the only non-modifiable risk factor implicated in obesity. There have been discovered about 60 common genetic markers implicated in the susceptibility to obesity[357,370,371]. However, a genome wide association study showed that 32 most common genetic variants explained just 1.45% of the inter-individual variation in BMI[370]. In the same study, they estimated that the difference in BMI between individuals with a high genetic-susceptibility score and those with a low score was 2.73 kg/m^2, which accounts for a difference of 6.99 kg in 160 cm adults and 8.85 kg in 180 cm adults. Therefore, although genetics play a role in obesity, these little changes in BMI and the rise in obesity prevalence in the last century suggests that other factors are of greater importance[357]. Moreover, parental habits and lifestyle and also pre- and perinatal management exert an impact on the obesity risk through different mechanisms such as epigenetic.

2.3.2. Individual behaviours

2.3.2.1. Diet

Attempts to explain the great increases in obesity in the last decades have focused on several contributors among which are the increase in calories intake due to high energy density diet and increases in portion sizes[361]. Nowadays, caloric restriction remains the main way of most popular and clinical weight-loss approaches[357]. Beyond caloric restriction, dietary patterns and diet quality have also been studied. However, evidence from clinical trials has showed that caloric restriction, regardless of dietary patterns, is related to better weight outcomes. So that, it appears to be that adhering to a diet, more than the type of healthy diet, has an impact on weight loss.

2.3.2.1. Physical activity

Personal behaviours such as low physical activity, adoption of sedentary lifestyle and screen time have also been associated with development of obesity. Combined with diet, these behaviours have synergistic effects on every one's ability to maintain a healthy body weight[357]. Evidence supports that moderate intensity physical activity between 150 and 250 minutes per week is effective to prevent weight gain, and improves weight loss when associated with moderate diet restriction[372]. Greater amounts of physical activity (>250 minutes per week) are associated with clinically significant weight loss and contribute to weight maintenance after loss.

2.3.3. Socioeconomic factors: income and education

The role of income in obesity risk has shifted in the last century[357]. At the mid 20th century in USA and Europe, the wealthier an individual was, the more likely to be overweight. In the last decades this link has flipped, likely due to the abundance of cheap and high energy density food, combined with changing in sociocultural norms. Nowadays, in USA it is poverty that direct correlates with excess body fat. However, this trend reverses in men of some countries of the Organization for Economic Co-Operation and Development (OECD), where lower income was associated with more favourable weight status, maybe due to low-paying jobs typically related to more physically demanding work performed usually by men instead of women[373]. Furthermore, in the OECD studied countries, an inverse relation has been found between obesity and education, with higher levels of BMI associated with less educated population.

2.3.4. Environmental risk factors

2.3.4.1. The built environment

It has been studied the relationship between weight status and neighbourhood characteristics such as the presence of fast food restaurants, supermarkets, parks, transportation or walkability[357]. Nowadays, the evidence focus on the primacy of diet-related built environments over those associated with physical activity. Healthy food environments, characterized by availability of produce or presence of supermarkets over convenience stores or fast food restaurants, play a more important role than the presence of neighbourhood physical activity or recreational spaces, which have been related with increased physical activity and higher energy expenditure levels.

2.3.4.2. Environmental pathogens: virus, gut microbiome and social networks

There is evidence that shows that obesity may be attributable to infection, or that obesity itself may be a contagion[357].

Human adenovirus 36 has been found to have a direct effect on adipose tissue regulating the proliferation and differentiation of adult adipose tissue-derived stem cells and other adipogenic progenitors, resulting in an increase in the number of fat cells in animal models. It has been also shown that this virus may contribute to the obesity development and weight gain in humans[374].

Recent experimental evidence has focused on the importance of the gut bacteria, the gut microbiome[357]. In 2006, it was published in Nature that there are two main groups of bacteria in the human gut, the proportion of which differs between obese and lean people, and changes with weight loss due to low-calorie diet[375]. It has been shown that gut hormones could potentially regulate appetite via the called "Gut-Brain-Axis"[376]. The question if gut populations are responsible of weight gain or consequence of diet consumed remains a key one[377].

A new concept is the importance of social networks, real and virtual, as risk factors for developing obesity[357]. A study published in 2007 with data form Framingham Heart Study, showed that obesity may spread in social networks in a quantifiable and discernible pattern that depends on the nature of social ties[378]. For example, the individual's risk of becoming obese increases by 57% if a friend became obese.

2.4. OBESITY MEASUREMENTS AND CLASSIFICATION

The fact of classifying obesity is of key importance for several reasons[356]. It allows making useful comparisons of weight status within and among populations, helps to identify people with higher risks of morbidity and mortality, makes it possible to identify priorities for intervention at different levels, and provides a strong basis for the evaluation of interventions.

2.4.1. The body mass index

The most widely extended criteria for classifying obesity in adults is the body mass index (BMI), a simple index of weigh-for-height devised in the 19th century by the Belgian statiscian Adolphe Quetelet. It is defined as the weight in kilograms divided by the square of the height in metres (**figure 7**) [356].

$$\boxed{\mathbf{BMI = weight\ (kg)/\ height^2\ (m^2)}}$$

Figure 7. The BMI formula.

BMI provides the most useful, although crude, population-level measure of obesity[356]. It helps to estimate the prevalence of obesity in different populations and the risks associated with it due to the robust nature of the measurements and the widespread routine inclusion of weights and heights in clinical and population health surveys.

Nowadays, the most widely used classification is based on WHO cut-off points (**table 2**), which defines obesity as a BMI ≥ 30.

CATEGORY	BMI
Underweight	<18.50
Normal	18.50-24.99
Overweight	25.00-29.99
Obese class I	30.00-34.99
Obese class II	35.00-39.99
Obese class III	≥40.00

Table 2. Classification of adults according to WHO BMI cut-off points.

This classification is based on the relation between BMI and mortality[356]. There has been much controversy about the association between obesity and mortality. Several studies have found a U- or J-shaped association, showing higher mortality rates at both the upper and lower weight ranges, whereas some have shown a linear increase in mortality with increasing weight, and others have even reported no clear association. Nevertheless, it is generally accepted today that, whatever the shape of the curve, it appears that the lowest mortality risk is associated with a BMI between 18.5 and 25 (**figure 8**).

The BMI has some limiting aspects. Although it can commonly be assumed that people with a BMI ≥ 30 have an excess of fat mass, this index does not distinguish between weight associated with muscle or with fat[356].

The value of BMI depends on the body build and proportion, so a given BMI may not correspond to the same degree of adiposity across different populations. Therefore, it has been proposed that lower BMI cut-off points may apply for some ethnic groups such as Asian populations[379]. Moreover, there have been shown statistically significant associations between BMI and the percentage of body fat dependent on age and sex[380]. The proportion of body fat mass increases with age up to 60-65 years[381,382]. Due to the decrease on height and lean mass, older people show higher body fat percentage than younger people for the same BMI value. Women have shown to have higher percentage of body fat than men for the same BMI value, throughout the entire adult life[383]. Thus, differences in body fat percentage and its relation with this index can affect the BMI values considered healthy.

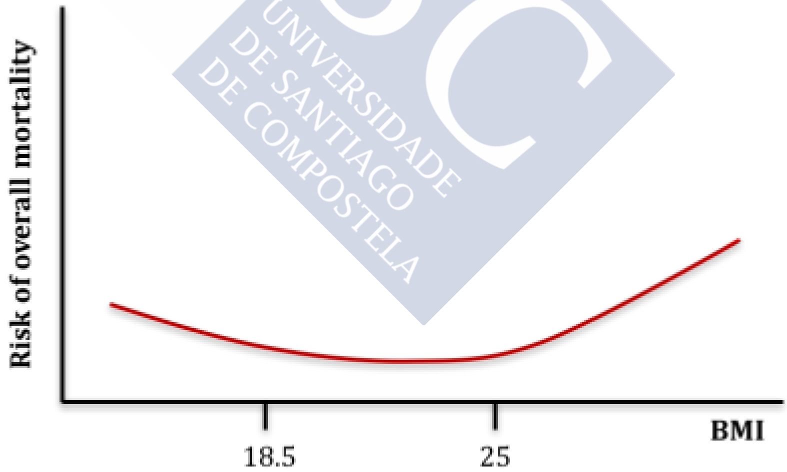


Figure 8. Relationship between BMI and overall mortality (an approximation).

2.4.2. Measures of abdominal obesity

On the other hand, BMI does not take into account the variations in body fat distribution. This is not a trivial limitation since individuals with excess fat in the intra-abdominal depots are at particular high risk for vascular diseases, independent of BMI[384,385]. Abdominal fat is thought to be mainly visceral, metabolically active fat surrounding the organs, and is associated with metabolic dysregulation, predisposing patients to vascular disease and related comorbidities[386]. Abdominal fat may vary dramatically for a given range of total body fat or BMI[356]. Moreover, men have on average twice the amount of abdominal fat than premenopausal women for any amount of total body fat[387]. Thus, other measurements that evaluate the abdominal fat accumulation could be useful to analyse the risks related to this depot.

Waist-to-hip ratio (WHR) and waist circumference (WC) were proposed as additional measures of body fat distribution[388]. Both bring information about the amount of abdominal fat, can be measured more precisely than skin folds, correlate with BMI (the level of this association varies among studies) and may be better predictors of vascular diseases than this index. WHR, defined as waist circumference divided by hip circumference (HC), provides an index of both subcutaneous and intra-abdominal adipose tissue[356]. A high value of this ratio (≥0.90 in men, ≥ 0.85

INDICATOR	CUT OFF POINTS	RISK OF METABOLIC COMPLICATIONS
WC	>94 cm (men) >80 cm (women)	Increased
WC	>102 cm (men) >88 cm (women)	Substantially increased
WHR	≥0.90 (men) ≥0.85 (women)	Substantially increased

Table 3. WHO cut off points and risk of metabolic complications. Modified from WHO[388].

in women; **table 3**) is associated with a substantially increased risk of metabolic complications[388]. However, today is though that WC may provide a simple and more practical correlate of abdominal fat distribution and associated ill health[356,389]. WC is not only an approximate measure of intra-abdominal fat mass[390] but also of total body fat[391]. The risks associated with a particular WC differ between populations so different cut off points are needed. The recommended cut off points for European are 94 cm (men) and 80 cm (women) for increased risk of metabolic complications, and 102 cm (men) and 88 cm (women) for substantially increased risk (**table 3**)[388].

2.4.3. Additional tools for the assessment of obesity. The dual-energy X-ray absorptiometry

In addition to the anthropometric methods described above, there are several other tools useful for the evaluation of body composition and anatomical distribution of fat both in clinical practice and research[356]. The most common are described in **table 4**.

MEASUREMENT TOOLS	
BODY COMPOSITION	**ANATOMICAL DISTRIBUTION OF FAT**
Underwater weighing	Computerized tomography
Isope dilution	Ultrasound
Bioelectrical impedance	Magnetic resonance imaging
Skinfold thickness	
Dual-energy X-ray absorptiometry	

Table 4. Obesity measurement tools.

Among several methods for studying human body composition, dual-energy X-ray absorptiometry (DEXA)(**figure 9**) is one of the most commonly used clinical standards[392]. This method can accurately measure body composition with high-precision, low X-ray exposure (between 1 and 10% of a chest radiograph)[393], and short-scanning time[394]. The energy source produces photons at two different energy levels, 40 and 70 keV, which pass through tissues and attenuate at rates related to its elemental composition. Bone is rich in highly attenuating minerals, calcium and phosphorous, and is readily distinguished from soft tissues[392]. The unique elemental profiles of bone, fat, and non-bone lean tissue allow for visualization and separate analysis of each tissue type. Thereby, DEXA helps to determine body fat distribution, provides a direct and precise measure of abdominal visceral fat, and could be a better measure of adiposity than BMI[394].

2.4.4. Classification in children

Adult BMI changes very slowly with age, so age-independent cut off points can be used to grade obesity[356]. However, classifying obesity in children and adolescents is complicated because height and body composition change greatly as they develop, and it varies also between boys

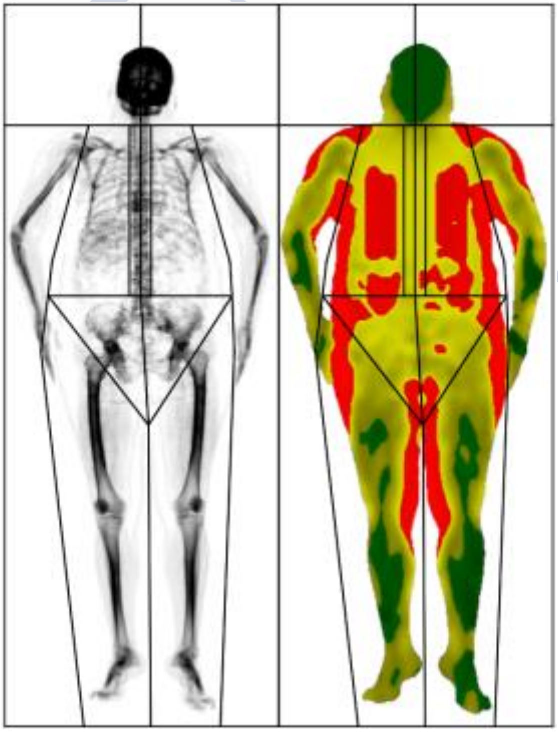


Figure 9. Dual-energy X-ray absorptiometry of an obese patient with stroke. Red: > 60% of fat. Yellow: 25-60% of fat. Green: < 25% of fat.

and girls due to different sexual development and maturation[357]. Moreover, the age of onset of puberty and the rates of fat accumulation vary internationally[356].

The WHO Child Growth Standards[395] is the most widely used classification system for children from birth to 5 years old. In this system, overweight is defined as weight-for-height greater than 2 standard deviations the WHO Child Growth Standards median, and obesity is weight-for-height greater than 3 standard deviations above the WHO Child Growth Standards median. In 2007, the WHO published updated growth references for individuals aged 5 to 19 years[396]. With these new references, overweight is BMI-for-age greater than 1 standard deviation above the WHO Growth Reference median, and obesity is BMI-for-age greater than 2 standard deviations above the WHO Growth Reference median.

2.5. OBESITY CONSEQUENCES

2.5.1. Overall mortality and disability

An analysis for the Global Burden of Disease Study, estimated that overweight and obesity caused, at the year 2010, 3.4 million deaths, the 3.9% of YLL and the 3.8% DALYs globally[397]. Recently, an USA study has suggested that as a consequence of the significant rise in the prevalence of obesity and its complications, life expectancy could level off or even decline within the first half of this century[398].

2.5.2. Comorbidities

Type 2 diabetes mellitus: Compared to normal weight people, overweight and obese have over 3 and 7 times more risk of developing type 2 diabetes mellitus, respectively[399]. Excess weight in childhood and adolescence are also associated with increased risk of later diabetes in adult life[400]. Even without other metabolic dysregulation, obesity itself raises diabetes risk[357].

Dyslipidaemia: obese individuals are characterized by the presence of high plasma levels of triglycerides, low HDL-cholesterol concentrations and raised LDL-apo B levels[356]. This profile is most common in obese patients with high amounts of intra-abdominal fat, which is also associated with higher proportion of dense and small LDL particles. However, a large proportion of these particles cannot be identified by the measurement of LDL-cholesterol since these cholesterol levels are frequently in normal range in obese individuals. Thus, an elevated ratio of LDL-apoB to LDL-cholesterol is a better indicator of dense and small LDL particle levels.

Hypertension: it is present in more than one-third of obese adults, twice the prevalence in those with a BMI below 25 kg/m^2. It has been estimated that every 10% increase in body weight is accompanied by an increase in 6 mmHg of SBP and 4 mmHg of diastolic blood pressure (DBP)[401].

Vascular diseases: besides their typical antecedents (diabetes, dyslipidaemia and hypertension), high body fat levels are a well-known risk factor for ischemic stroke and coronary heart disease[357]. It has also been shown that obesity in childhood and adolescence is associated with higher risk of vascular diseases in adulthood[400]. Its association with stroke will be discussed in depth further.

Pulmonary diseases: obesity impairs respiratory function and structure[356]. The accumulation of adipose tissue in and around the ribs, abdomen and diaphragm results in thoracic cage stiffness and, therefore, in an increased work of breathing and hypoxemia. The risk of obstructive sleep apnoea is also increased in obesity. More than 10% of obese people suffer sleep apnoea, and from 65% to 75% people with this syndrome are obese.

Gallbladder disease: obese individuals have 3 to 4 times more risk of gallstones formation compared to non-obese, and this risk is higher when excess fat is intra-abdominal[356]. Acute and chronic cholecystitis is also more frequent in obese people.

Cancer: an study in the USA has estimated that obesity may contribute to about 6% of all cancers (4% in men, 7% in women)[402]. Evidence has shown that overweight and obesity are associated with increased risk of cancers of oesophagus, colon, pancreas, gallbladder, liver, postmenopausal breast, endometrium, ovary, prostate, kidney, and leukaemia[357].

Trauma: a recent meta-analysis of obesity in trauma care showed that obesity was associated with increased risk of mortality, longer stays in the intensive care unit and higher rates of complication, despite equivalent injury severity[403].

Infection: obesity is an established risk factor for surgical-site infections, nosocomial infections, periodontitis and skin infections[404]. The underlying mechanism may be an impaired immune response[357]. Recent data have also shown lower vaccine efficacy in obese individuals, when vaccination to hepatitis B virus[405].

Mental health: obesity has been found to be associated with functional and anatomical changes in the human brain[357]. In elderly subjects, obesity was shown to be associated with atrophy in the frontal lobes, anterior cingulate gyrus, hippocampus and thalamus[406]. In children and adolescents, obesity was associated with poorer performance on inhibitory control tasks, and altered function and diminished volume of orbitofrontal cortex[407]. It has been also shown that being obese in midlife increases significantly the risk of Alzheimer's disease and any type of dementia when compared to normal weight individuals[408].

2.5.3. Economic costs

Obesity is associated with excess in healthcare costs. In the USA, it has been estimated that obese men, when compared to non-obese, incur in additional $1152 per year in medical costs, attributable to hospitalizations and drugs, whereas women incur over double that of obese men[409]. Therefore, treating obesity and related conditions represents about $190 billion per year of healthcare costs, the 21% of USA healthcare expenditure. Moreover, an analysis realized in the USA showed that medical, pharmacy, sick leave, workers' compensation costs and absent days were higher for patients with higher BMI[410]. In Europe, a recent review of healthcare costs studies in Western countries estimated excess spending of about €117 to €1873 per person when comparing obese to non-obese patients[411]. Approximately 23% of medication costs were attributable to overweight or obesity. The estimates showed that 2.1% to 4.7% of total health care costs and 2.8% of total hospital costs were due to overweight and obesity. Total (direct and indirect) costs accounted for 0.47% to 0.61% of gross domestic product.

3. THE OBESITY PARADOX

As noted above, obesity has classically been considered a poor health indicator, a risk factor for vascular diseases, associated with several comorbidities and high mortality rates. However, in the last decade, a growing number of studies have shown that obesity may be associated with improved prognosis and lower mortality rates in several diseases. This phenomenon is known as the "obesity paradox". Although this term was first introduced in 2002[412] in a study about the outcome after percutaneous coronary intervention, the phenomenon was described for the first time in patients undergoing hemodialysis in the year 1999[413]. On that study the authors found that excess body weight patients had higher survival rates than normal weight patients. Since then, many studies have shown the potential benefits of obesity in different diseases such as coronary heart disease[414–416], heart failure[417–422], atrial fibrillation[423–426], chronic obstructive pulmonary disease[427], chronic kidney disease[428], or rheumatoid arthritis[429]. In recent years, obesity has also been linked with reduced rates of morbidity and mortality after stroke[430]. Nonetheless, there is now a great controversy affecting different aspects of vascular disease. In the following lines we will describe the evidence for and against this association and the possible mechanisms.

3.1. ASSOCIATION BETWEEN OBESITY AND POOR PROGNOSIS

3.1.1. Epidemiological evidences

Obesity has been found to be a risk factor for stroke and other vascular diseases in different populations[431–440]. Although this effect may be at least partly attributable to other risk factors consequence of excess body fat, some studies have shown that obesity is independently associated with stroke[438,439].

Nonetheless, other studies have failed to show an independent association after adjusting for confounding factors when obesity is assessed by BMI[441]. Thus, it has been proposed that BMI may not be the best anthropometric predictor for vascular risk.

First, as previously noted, statistically significant associations between BMI and the percentage of body fat have been shown to be dependent on age and sex[380]. Moreover, the presence of concomitant diseases may lead to an unintentional weight loss acting as confounders, so that, at least in developed countries, a higher BMI in elderly people may be associated with a better health condition. Secondly, and again as noted above, other measurements that evaluate the abdominal fat accumulation could be more useful when assessing the vascular risk. Several studies have shown that WHR or WC are potent and may be better predictors of ischemic stroke risk, independent of other risk factors and even BMI[442–444].

This applies not only to the risk of vascular events but also to the prognosis after them. Although the "obesity paradox" concept arises from many studies that have found a positive correlation between BMI and good prognosis in different disorders, others have shown that mortality is directly associated with adiposity when assessed by central adiposity instead of BMI in vascular diseases[445]. Indeed, some authors have even found that after acute coronary syndrome the combination of high WC and low or normal BMI confers the higher mortality rates[446,447].

3.1.2. Association with vascular risk factors

The risk associated with obesity significantly increases by exposure to other vascular risk factors such as hypertension, hyperlipidaemia, diabetes, and especially smoking[432]. Obesity is indeed associated with such risk factors[448] but also with atrial fibrillation[449]. Several studies have shown epidemiological and mechanistic associations between excessive body fat and this emboligenic arrhythmia that establish a pathophysiological cause-effect relationship[449]. However, it is important to highlight that, as we noted before, an obesity paradox has also been found for outcomes in atrial fibrillation. In a systematic review and meta-analysis, Proietti et al.[426] describe the finding of the paradox for cardiovascular death and all-cause death in randomized trial cohorts of atrial fibrillation and for stroke/systemic embolic event and major bleeding in the randomized trials of novel oral anticoagulants for stroke prevention in atrial fibrillation. However, observational studies fail to show this relationship after statistical adjustments.

Obesity is part of the metabolic syndrome[448]. The elimination of the metabolic syndrome results in a 19% reduction in overall stroke, a 30% reduction of stroke in women, and a 4% reduction of stroke in men[450]. Among the aspects that are part of the metabolic syndrome, insulin resistance seems to be the pivotal pathophysiological contributor the development of vascular risk factors[451]. Insulin resistance promotes impaired glucose homeostasis, endothelial dysfunction (the first event in atherosclerosis), intimal thickening, vascular inflammation, and leads hemostatic abnormalities with prothrombotic effects both in the endothelium and the fibrinolytic system[452]. This is of key importance because of the long-recognized relationship of obesity, particularly visceral, to insulin resistance. In humans, the expansion of visceral fat depot is associated with an increased risk of insulin resistance, whereas the increase in subcutaneous adipose tissue mass decreases the risk of insulin resistance[453]. The mechanisms by which visceral obesity leads to insulin resistance appear to be related to fat accumulation in liver. This may be consequence of excess fatty acids draining from visceral adipose depot into the portal vein. This excess lipid accumulation may result in impaired insulin signalling through cell autonomous mechanisms, or through an inflammatory response (issue that will be discussed further). Contrary, the storage of fat in subcutaneous depots may decrease the risk of insulin resistance through the prevention of accumulation of fat in visceral adipose tissue, liver, and skeletal muscle.

3.1.3. Response to thrombolytic treatment

In a series of 304 ischemic stroke treated with intravenous thrombolysis, obesity was an independent predictor of mortality and unfavourable outcome[454]. This may be related to a higher risk of in-hospital complications, an insufficient dose of alteplase (because the maximum dose is limited at 90mg), and the fact that obesity has been shown to be associated with a prothrombotic state (obese patients have higher levels of serum fibrinogen, factor VII, von Willebrand factor and plasminogen activator inhibitor antigen)[455,456].

3.1.4. Experimental ischemic models

Preclinical studies in rodents have shown that obesity is associated with worst outcomes after experimental cerebral ischemia[457]. This has been studied both in genetic models of obesity (defective leptin hormone or defective leptin receptor) and models of diet-induced obesity (high-fat diet), and also with different mechanisms of brain ischemia such as transient and permanent MCAO, common artery ligation and exposure to a low-oxygen environment. These animal models experience higher infarct volumes and neurological deficits in comparison with controls. Obese rodents show increased BBB permeability, cerebral edema, and incidence of hemorrhagic transformation. Evidences from experimental studies suggest that obesity intensifies inflammatory damage of cerebral microvasculature. Obese rodents show increased post-stroke vascular ICAM-1 expression, which may mediate neutrophil recruitment (increased in cerebral vessels and ischemic parenchyma in obese rodents), and therefore contribute to the release of MMP-9 (increased in ischemic hemispheres of obese rodents), which is involved in BBB breakdown.

3.1.5. The low-grade inflammatory response

In last years, it has been demonstrated that obesity is associated to other vascular risk factors through a disturbance of the immune and inflammatory response. The excessive white adipose tissue generates a chronic low-grade inflammatory response, known as "metainflammation"[458]. This inflammatory response involves many components of the classical inflammatory response and includes systemic increases in circulating inflammatory cytokines and acute phase proteins (e.g., CRP, IL-6, TNF-α, MCP-1)[459–461], recruitment of leukocytes, and activation of tissue leukocytes[458]. Both innate and adaptive immunity are involved, but it has been proposed that innate immunity triggers the response and has a main role. This may have a key importance as post-stroke peripheral immune response is increased in obesity models, which show higher serum levels of IL-6, MCP-1[462], or C-X-C motif chemokine ligand 1 (CXCL-1)[463]. The metainflammation may modify stroke outcome by modulating CNS inflammation. In the next lines we will discuss the role of the different components of this phenomenon.

3.1.5.1. Immune cells

Adipose tissue is composed by two different entities[464], adipocytes and the interadipocytar stromal-vascular fraction formed by extracellular matrix with dispersed fibroblasts, preadipocytes, endothelial and immune cells. Adipose tissue resident immune cells include almost every type of immune cell, playing an important role in homeostasis under non-obese conditions[465]. However, excessive fat accumulation results in changes in amount and function of different immune cells. According to their lineage, among myeloid line we will discuss about macrophages, dendritic cells, mast cells and granulocytes (such as neutrophils and eosinophils), and among lymphoid line we will describe the role of innate lymphoid type 2 cells, T cells, and B cells.

3.1.5.1.1. Macrophages

Macrophages are the largest subpopulation of immune cells in adipose tissue. The infiltration of adipose tissue by macrophages along with its altered function and anatomical localization is considered the culprit of obesity-related inflammation[464].

In rodent models it has been demonstrated that obesity is associated with an increase in macrophage infiltration of adipose tissue, the greater amount of cytokines derived from adipose tissue are released by macrophages[466,467], and, indeed, after an extended period of weight loss the adipose tissue macrophage content decreases[468]. Similar results have been found in humans. The percentage of macrophages in human adipose tissue increases in obese individuals[469,470], specially in visceral depot, and the surgery-induced weight loss is associated with an improvement of the inflammatory profile and a reduction of the number of macrophages in adipose tissue[471].

Most macrophages infiltrating obese adipose tissue come mainly from systemic circulation[467]. However, a small fraction of those macrophages may originate from local preadipocytes, which have the potential to be very efficiently and rapidly converted into macrophages, and thus comprise one of the primary cellular initiators of adipose tissue inflammation[472].

The mechanism of macrophage recruitment is not known at all and it involves complex chemotactic pathways. Obesity and high-fat diet (HFD) are associated with the expression of pro-inflammatory genes in adipose tissue, including the increase in production of chemokines such as MIP-1β and MCP-1[473–476], which bind respectively to CCR5 and CCR2 receptors on macrophages stimulating their migration. Both chemokines are also involved in atherosclerosis by modulating the recruitment of these cells[477]. MCP-1 has been deeply studied in obesity (**figure 10**). Adipocytes are the main source of this chemokine at adipose tissue[474,475], and these cells also produce CSF-1 (colony stimulating factor-1)[478], the primary regulator of macrophage differentiation and survival, thus creating a permissive microenvironment for the maturation of macrophages in adipose tissue[467]. Overexpression of MCP-1 results in adipose tissue macrophage infiltration, insulin resistance and liver steatosis, whereas deletion of MCP-1 or CCR2 macrophages leads to a reduction in macrophage infiltration and improves insulin sensitivity[479,480]. It has also been shown that visceral adipose tissue macrophages themselves express MCP-1[470]. Other chemotactic pathways such as chemokine CX_3C motif ligand1/chemokine CX_3C motif receptor 1 (CX_3CL1/CX_3CR1) and leukotriene B4/leukotriene B4 receptor (LTB4/BLT1) have been found to be involved[481].

Obesity alters not only the proportion of macrophages in adipose tissue, but also their function and anatomical distribution[464]. As previously noted, macrophages can be divided in two subpopulations, the classical "pro-inflammatory" phenotype M1, and the alternative "anti-inflammatory" phenotype M2. In lean individuals, adipose tissue macrophages are polarized toward an M2 state derived from CCR2- monocytes, localize to stromal-vascular fraction[482], release anti-inflammatory cytokines and perform homeostatic functions[483]. The maintenance of adipose tissue M2 macrophages requires the releasing of IL-4 and IL-13, whose major sources are eosinophils[484] and innate lymphoid type 2 cells respectively[485]. Obesity results in a switch from the anti-inflammatory M2 phenotype to pro-

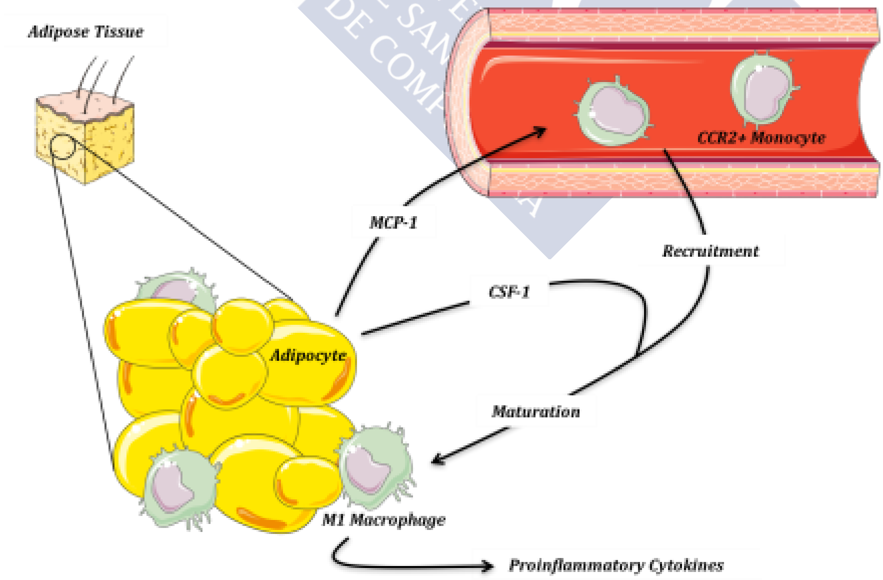


Figure 10. Macrophage recruitment to adipose tissue in obesity.

inflammatory M1 phenotype. With diet-induced obesity, on the one hand, the number of interstitial adipose tissue M2 macrophages decreases, and on the other hand, CCR2+ monocytes are recruited from circulation, differentiate to pro-inflammatory M1 macrophages, and localize surrounding necrotic adipocytes[482] (during obesity, adipose tissue mass increases by hyperplasia and hypertrophy, the latter is associated with stress signaling pathways in adipocytes that can lead to cell death)[483]. The polarization state of adipose tissue macrophages has been found to affect insulin sensitivity. In animal models, higher amounts of adipose tissue M1 macrophages and higher M1/M2 ratios are associated with insulin resistance[486], and the ablation of M1 macrophages normalizes insulin sensitivity[487].

TLRs, free fatty acids (FFA), and adipokines have also been identified to participate in the M2/M1 shift. As previously noted, LPS and saturated FFA can activate TLRs. In TLR4 deficient mice, HFD was associated with reduced macrophage content and reduced M1 polarization compared to control mice[488]. HFD was also associated with higher LPS circulating levels in mice as a result of LPS translocation from the gut, and liver insulin resistance was detected in LPS-infused mice[489]. Contrary, polyunsaturated fatty acids can promote infiltration of alternatively activated macrophages into adipose tissue through the activation of peroxisome proliferator-activated receptor γ (PPARγ)[490]. Finally, it has been shown that the adipokine adiponectin promotes the switch to an M2 anti-inflammatory phenotype in cultured murine and human macrophages[491].

3.1.5.1.2. Dendritic cells

Dendritic cells are the primary APCs of the immune system. They enable the transition from innate to adaptive immunity by presenting antigens via MHC to T cells[492]. In rodents, HFD induces an increase in adipose tissue dendritic cells[493,494] and these cells may be involved in adipose tissue macrophage infiltration[493]. In obese humans, it has also been shown that dendritic cells accumulate in adipose tissue and its levels correlate with BMI[494]. In obese mice, most of dendritic cells isolated from adipose tissue support the differentiation of Th17 and Th1 cells, whereas most of dendritic cells isolated from lean mice support the differentiation of Treg cells. Moreover, dendritic cells from obese individuals can induce *in vitro* differentiation of Th17 cells. The imbalance between anti-inflammatory Treg cells and pro-inflammatory Th17 and Th1 cells may result in M1 polarization of adipose tissue macrophages[464].

3.1.5.1.3. Mast cells

Mast cells are involved in allergic and anaphylactic reactions, but increasing evidence implicates them in inflammatory diseases. Once activated, mast cells secrete vasoactive and pro-inflammatory mediators such as histamine, serotonin and different cytokines[495]. It has been demonstrated that the number of mast cells in obese mice and humans is increased compared to their lean counterparts[496]. Moreover, genetically induced deficiency of mast cells or their pharmacological stabilization, in the context of mice fed with Western diet, results in reduction of body weight gain, of levels of inflammatory markers, and of adipose tissue macrophage infiltration, while mast cell reconstitution is associated with insulin resistance.

3.1.5.1.4. Neutrophils

It has been considered that neutrophils might be one of the initial mediators of this inflammatory process. After three days of HFD, visceral adipose tissue shows an important increase in neutrophils content[497,498]. However, studies are controversial about the persistence of the infiltration, with one reporting a decrease after the first week[497] and other showing persistence up to 90 days of HFD[498]. A potential chemoattractant for neutrophil infiltration into adipose tissue is IL-8, which was found to be secreted by visceral adipose tissue[499], and is increased in serum of obese subjects[500]. Neutrophils secrete several proteases, such as neutrophil elastase, which can promote inflammatory responses through TLR4. Genetic deletion or pharmacological inhibition of neutrophil elastase results in a reduction in inflammatory markers and M1 macrophage infiltration of adipose tissue, and an increase in insulin sensitivity[498].

3.1.5.1.5. Eosinophils

Classically, eosinophils have been known to be responsible of allergic reactions and defense to parasitic infections[501]. Recently, it has been shown that eosinophils are involved in anti-inflammatory responses, release anti-inflammatory cytokines (IL-4, IL-13) and stimulate Th2 cell differentiation[502]. As noted before, these cells are the main source of IL-4 in adipose tissue and they participate also in the secretion of IL-13, which are both necessary for the maintenance of alternative M2 polarization[484]. Moreover, mice fed with HFD showed a decrease in the number of eosinophils in adipose tissue compared to their lean counterparts. In eosinophil-deficient mice, after HFD it was found a higher increase in total body fat and reduced insulin sensitivity, compared to wild-type, whereas helminth-induced adipose eosinophilia enhanced insulin sensitivity and was associated with a decrease in adipose macrophages.

3.1.5.1.6. Innate lymphoid type 2 cells

Innate lymphoid type 2 cells are the main source of IL-5 and IL-13 in visceral adipose tissue. IL-5 is essential for the maintenance of eosinophils, and, as previously noted, IL-13 is necessary for the maintenance of M2 macrophages. It has been shown that deletion of innate lymphoid type 2 cells results in significant reductions in visceral adipose tissue eosinophils and alternative activated macrophages[485].

3.1.5.1.7. T cells

As previously noted, T cells can be divided into CD4+ and CD8+ based on the expression of surface markers. Once activated, most of CD4+ T cells become helper T cells (Th) that act coordinating and modulating immune responses. Th cells include effector (such as Th1, Th2 and Th17 cells) and regulatory cells (Treg cells). These Th cells produce different cytokines: Th1 (IFN-γ, TNF-α, IL-12), Th2 (IL-4, IL-5, IL-10, IL-13), Th17 (IL-17), Treg (IL-10, TGF-β). CD8+ are considered cytotoxic immune cells, which produce a variety of cytolitic substances.

T cells represent the second largest immune cell population in adipose tissue after macrophages, and obesity is associated with an increase in the total number and changes in the proportions of different T cells subtypes[464].

In visceral adipose tissue, the absolute number and the proportion of Th1 cells is increased in obese mice and humans compared to their lean counterparts[503]. The number of Th2 remains static, resulting in a shift in Th1/Th2 ratio toward the pro-inflammatory Th1 phenotype, which may contribute to the M1 polarization. However, in subcutaneous adipose tissue, the number of Th1 cells is smaller than in visceral adipose tissue both in mice fed with normal chow diet and HFD. The expression of IFN-γ, a Th1 prototype cytokine, is increased in adipose tissue of diet-induced obese rodents, and T cells extracted from fat tissue produce higher amounts of this cytokine than the ones extracted from lean animals[504]. When stimulated with IFN-γ, adipocytes secrete inflammatory mediators such as the T cell chemoattractants IP-10 and MIG, as well as the monocyte chemoattractant

MCP-1. Moreover, obese IFN-γ deficient animals had reduced expression of inflammatory cytokines, decreased adipose tissue inflammatory cell accumulation and better insulin sensitivity.

Th17 cells are typically involved in pro-inflammatory functions, and secrete IL-17 and IL-23[464]. IL-17 induces expression of several chemokines (e.g., MCP-1, IP-10), and also cytokines such as IL-6 in preadipocytes, which has been found to induce insulin resistance[505]. However, in white adipose tissue, it has been found that the main source of IL-17 is γδT cells. IL-17 deficiency enhances glucose tolerance and insulin sensitivity in young mice. On the other side, IL-17 deficient mice are more susceptible to accumulating adipose tissue mass than wild-type mice, maybe due to the fact that IL-17 impairs adipocyte differentiation. However, the protection from metabolic disorders in IL-17 knockout mice is lost on the development of age-associated obesity.

Treg cells are suppressors of inflammatory reactions, release IL-10, and counteract pro-inflammatory Th1 and Th17 responses. Obesity is associated with a decrease in the number of Treg cells at visceral adipose tissue[506,507]. Moreover, Treg cell depletion is associated with insulin resistance[506], whereas its restitution is associated with improved insulin sensitivity[503].

The number of CD8+ T lymphocytes in adipose tissue is increased in obesity[508,509]. The depletion of CD8+ T cells results in reduction of macrophage infiltration and inflammatory markers, and improved insulin sensitivity, whereas their adoptive transfer lead to increased macrophage infiltration and insulin resistance[508].

3.1.5.1.8. B cells

It has been shown that HFD in mice induces early accumulation of B cells in visceral adipose tissue, where they promote CD8+ T cell and Th1 activation and pro-inflammatory cytokine production, which results in M1 polarization and insulin resistance[510]. In this process it seems to be involved the production of B-cell derived IgG2c antibodies, which are elevated in obese mice and their transfer to lean mice leads to adipose tissue inflammation and insulin resistance. Mice treated with a B cell-depleting CD20 antibody showed improved insulin sensitivity and reduced levels of inflammatory markers such as IFN-γ and TNF-α.

3.1.5.2. Toll-like receptors

We previously talked about stroke and TLRs, pattern recognition receptors with a key role in innate immune responses[305]. Besides their role in ischemic stroke, TLRs appear also to contribute to the chronic inflammatory state of obesity and insulin resistance (**figure 11**). As previously noted, TLRs are expressed in several cell types such as macrophages and adipocytes, being TLR2 and TLR4 the most studied ones. These receptors recognize exogenous ligands such as LPS, and some endogenous ligands such as SFA and Ox-LDL, which results in the activation of NF-κB and subsequently in the release of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, and MCP-1.

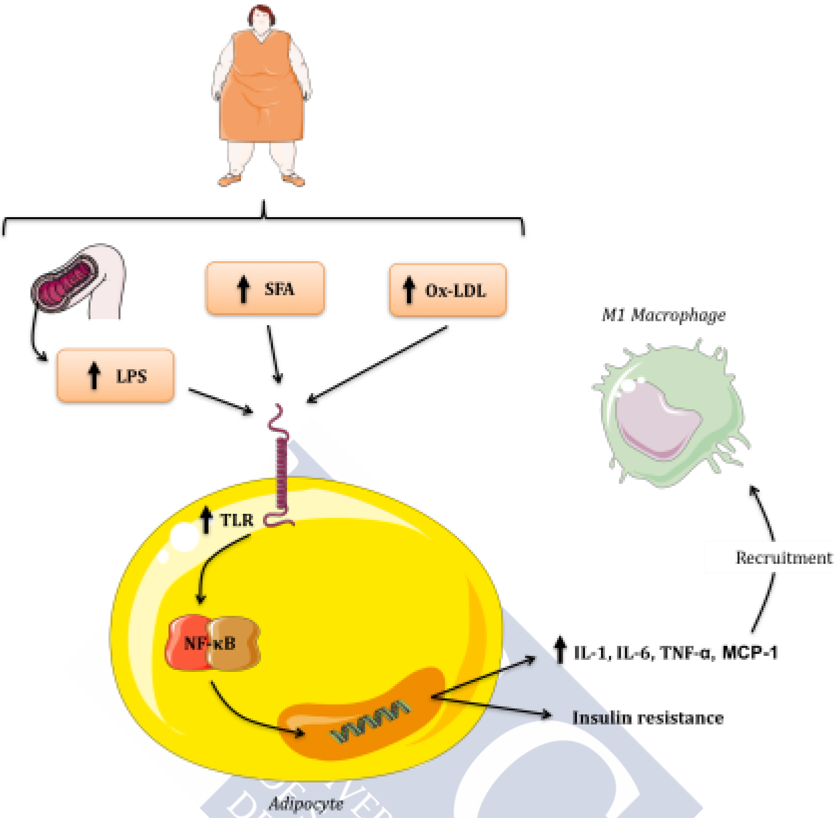


Figure 11. TLRs and obesity.

Many studies have described, both in humans and animal models, the increased expression of TLRs in adipose tissue of obese subjects[305], predominantly in visceral depot, and both in macrophages and adipocytes[297]. This increased expression seems to be involved in the metabolic syndrome associated with obesity. In animal models, mice fed with HFD show increased expression of TLR2 in adipocytes from visceral depots compared to subcutaneous depots, and FFA increases TNF-α through TLR2 signaling[511]. The inhibition[512] or gene deletion[513] of TLR2 improves insulin sensitivity in mice models, possibly preventing the phosphorylation of IRS-1 (insulin receptor substrate-1)[512]. The expression of TLR4 is also increased in adipose tissue of obese mice, and its activation with LPS or FFA results in the expression of pro-inflammatory cytokines by adipocytes and insulin resistance[514]. On the other side, TLR4 knockout mice show better insulin sensitivity[297,488] and reduced adipose tissue inflammatory markers and macrophage content[488]. Similar findings have been described in humans, where enhanced expression of TL2 and TLR4 has been found both in adipose tissue and peripheral monocytes of obese individuals[305]. A recent work showed that expression of TLR4 in visceral fat of obese subjects is higher compared to lean controls[515]. Another study showed increased expression of TLR2 and TLR4 both in peripheral blood mononuclear cells and adipose tissue compared with lean individuals, and the expression was higher in patients with type 2 diabetes. In the same study, the authors found higher levels of IL-6 and TNF-α in obese and overweight subjects, which correlated with the TLRs expression[516].

As we said before, SFA are TLRs ligands. Obesity is associated with higher circulating FFA, due to increased release from adipose tissue among other causes[297]. Fatty acids from diet can be categorized into SFA (e.g., palmitate, the major FFA circulating in humans), monounsaturated fatty acids, and polyunsaturated fatty acids[517]. A diet rich in SFA is associated with obesity, insulin resistance and vascular disease, whereas a diet rich in unsaturated fatty acids inversely correlates with metabolic syndrome and inflammatory markers[518–521]. It has been demonstrated that SFA activate TLR4 signaling in adipocytes and macrophages[297], and this may be due to structural similarities between SFA and LPS[517,522]. Contrary, unsaturated fatty acids are involved in anti-inflammatory functions, inhibit inflammatory responses depending of SFA[297], improve insulin sensitivity, and promote macrophage alternative activation through PPARs[490,522].

The classical ligand of TLRs, LPS, is also increased in obesity and metabolic syndrome[305]. This is of key importance since obesity is associated with changes in gut microbiome, which results in increased gut permeability and, therefore, in endotoxemia (high LPS circulating levels). Ox-LDL, another TLR ligand, is increased in obesity and metabolic syndrome too[523].

3.1.5.3. Adipokines

Adipocytes release an important variety of mediators among which are cytokines such as IL-6 and TNF-α, chemokines such as MCP-1[524], but also characteristic adipokines from adipose tissue such as leptin or adiponectin, among others. Although some adipokines display pro-inflammatory behaviours and others anti-inflammatory, the functions of these cytokines will be discussed in this chapter for better understanding. Their inflammatory behaviour is synthetized in **table 5**.

<table>
<tr><th>PRO-INFLAMMATORY ADIPOKINES</th><th>ANTI-INFLAMMATORY ADIPOKINES</th></tr>
<tr><td>Leptin</td><td>Adiponectin</td></tr>
<tr><td>Resistin</td><td>Omentin</td></tr>
<tr><td>Chemerin</td><td>Vaspin</td></tr>
<tr><td>Visfatin</td><td>SFRP5</td></tr>
<tr><td>RBP4</td><td></td></tr>
<tr><td>ANGPTL2</td><td></td></tr>
<tr><td colspan="2">Apelin</td></tr>
<tr><td colspan="2">Lipocalin 2</td></tr>
</table>

Table 5. Adipokines and their inflammatory behaviour.

3.1.5.3.1. Leptin

Leptin is a hormone almost exclusively secreted by adipocytes, and its levels increase in direct relation with white adipose tissue mass[525]. As noted above, it regulates energy homeostasis through its anorexigenic properties and stimulating energy expenditure due to its action at the hypothalamus[526]. However, although its levels are increased in obese individuals, it has hardly any impact to regulate energy homeostasis in these patients, which is known as leptin resistance in obesity[527]. Moreover, it has been shown that leptin resistance may contribute to the reduction of lipid oxidation in insulin-sensitive organs, resulting in lipids accumulation and insulin resistance.

Leptin is also involved in immune system regulation, it has mainly pro-inflammatory properties, and its receptor is expressed by almost every immune cell[528]. It activates monocyte proliferation and production of IL-6 and TNF-α[529], promotes the production of CC-chemokine ligands by macrophages[530], stimulates T cell proliferation, promotes Th1 responses (polarization to Th1 phenotype and IFN-γ production)[531], and induces B cells to secrete IL-6, TNF-α, but also IL-10[532]. Moreover, leptin levels increase in response to LPS administration[533]. Leptin also regulates TLRs expression, but studies are controversial. Leptin has been demonstrated to increase TLR2 expression in human monocytes, but not TLR4 expression[534]. Contrary, it had been previously shown that adipocytes and preadipocytes from knockout mice for leptin or leptin receptor have an up-regulation of TLR1 to -9 expression, and preincubation of preadipocytes with leptin resulted in a decrease in IL-6 production after LPS stimulation in knockout mice[535].

It has been shown that leptin exerts several pro-atherogenic effects: promotes endothelial dysfunction, pro-inflammatory responses, platelet aggregation and migration, and hypertrophy and proliferation of smooth muscle cells from the vascular wall[536]. It has been found that plasma leptin levels are independently associated with higher intima-media thickness (IMT) of common carotid artery[537]. However, experimental models suggest that leptin may have a neuroprotective role in cerebral ischemia. In a mice model, authors showed that after brain ischemia/reperfusion injury, the administration of leptin increases the expression of activated Akt (also known as protein kinase B, a serine/threonine protein kinase) in the ischemic brain, which successively reduces the levels of LDH and the lactic acid/pyruvate ratio and increases brain glucose uptake and ATP levels[538]. This phenomenon results in the repair of the brain energy deficit, and therefore reduces the infarct volume, brain edema, and neurologic deficits. A recent study demonstrated that leptin treatment induces neurogenesis and angiogenesis after stroke in rodents, and results in better functional outcome[539]. On the other hand, observational studies reveal contradictory results. Some studies have shown that higher circulating leptin levels increase risk of vascular disease, such as ischemic coronary disease, stroke or both, even when adjusting for confounders such as obesity[540–547], whereas others have not found such association[548–553], or even have revealed a protective role in coronary disease[554,555].

3.1.5.3.2. Adiponectin

Adiponectin, the most abundant adipose-specific adipokine[556], is a cytokine with anti-inflammatory properties. Adiponectin levels are reduced in obese individuals[557] and negatively correlate with visceral fat accumulation[558–560]. The correlation with subcutaneous adipose depot is not so clear, with studies showing positive[559], negative[558], and no correlation[560]. Adiponectin levels positively correlate with insulin sensitivity[561,562], are lower in type 2 diabetes patients[557], and the treatment with this cytokine enhances insulin sensitivity in animal models[563].

Adiponectin exerts its anti-inflammatory and metabolic actions through its receptors: AdipoR1, expressed ubiquitously, and AdipoR2, expressed mainly in the liver[564]. The expression of both receptors is reduced in obesity and insulin resistance[565,566]. Disruption of AdipoR1 and AdipoR2, which leads to reduced activation of AMPK and decreased activity of PPARα signaling pathways respectively, results in increased tissue triglyceride content, inflammation, oxidative stress, and, thus, insulin resistance[566]. The binding of adiponectin to AdipoR1 inhibits TLR2 and TLR4-mediated NF-κB activation in mouse macrophages[567], whereas the expression of TRL2 in adipocytes is associated with downregulation of AdipoR1 and AdipoR2[568]. Moreover, this adipokine stimulates the production of IL-10 by macrophages[569], promotes LPS tolerance in these subtype of immune cells reducing the production of pro-inflammatory cytokines such as TNF-α[570,571], and induces polarization to an M2 phenotype[491]. It has also been shown that adiponectin supresses IFN-γ production and T cell activity due to alteration in dendritic cells function[572]. Finally, the levels of adiponectin decrease with TNF-α treatment[573].

Adiponectin exerts anti-atherogenic functions and hypoadiponectinemia is associated with atherosclerosis[574]. Adiponectin increases NO production and participates in endothelium-dependent vasodilatation[575], attenuates ROS production in endothelial cells, and reduces vascular smooth muscle cell proliferation and migration[576]. It has been demonstrated that subjects with lower levels of this cytokine have increased carotid IMT[577]. In a mice model of carotid arterial injury, adiponectin deficiency results in enhanced thrombus formation and platelet aggregation[578]. A preclinical study in a rat model of cerebral ischemia showed that the administration of adiponectin was associated with a reduction on infarct size and neurological deficits, possibly due to its anti-inflammatory actions blocking NF-κB and inhibiting production of IL-1, IL-8 and TNF-α[579]. Another experimental model of brain ischemia revealed that adiponectin may exert neuroprotective effects through an eNOS-dependent mechanism, ameliorating CBF[580]. Similarly to leptin, observational studies have shown contradictory results in vascular diseases. Although some studies reported higher risk of stroke or even poorer prognosis in patients with lower levels of this adipokine[545,581–583], several showed no association[543,547,549,552,584,585], or even higher levels in stroke patients[551].

3.1.5.3.3. Resistin

Resistin, a pro-inflammatory cytokine, is involved in insulin resistance and its levels are elevated in obesity[586]. In mice, resistin is mainly secreted by adipocytes[587], whereas in humans it is mainly secreted by macrophages and monocytes[588], thought it has been recently demonstrated to be also secreted by human adipocytes[589].

Pro-inflammatory cytokines such as IL-1, IL6 and TNF-α stimulate the expression of resistin[590]. Moreover, resistin promotes the expression of such cytokines via NF-κB pathway[590], and it has been demonstrated that resisting may exert this effect by binding to TLR4[591].

This adipokine has been shown to contribute to atherosclerosis[592]. Resistin increases proliferation and migration of human endothelial cells and vascular smooth muscle cells, inhibits eNOS, promotes the expression of adhesion molecules, acts as a modulator for macrophage to foam cell transformation, and induces a prothrombotic phenotype. However, contradictory results have been found related to carotid IMT, while one study showed positive correlation[593], another showed an inverse correlation which disappeared after adjustments[594]. Although less investigated than leptin and adiponectin, some studies have assessed the relationship between resistin and vascular diseases. It has been found independently higher risk of myocardial infarction[595] and poorer prognosis in patients with higher levels of this adipokine[596]. On the former, authors did not find association with the risk of ischemic stroke, whereas one study found a positive correlation with the risk of stroke in postmenopausal women[549], and another, a positive correlation with the severity of stroke in women[597].

3.1.5.3.4. Apelin

Apelin is a peptide released by adipocytes and other cells of white adipose tissue, and organs such as kidney and heart[598]. Its levels are higher in obese subjects and individuals with insulin resistance.

Nowadays, its role is not clear. On the one hand, apelin increases serum adiponectin and decreases that of leptin[599], and exerts anti-atherogenic effects reducing macrophage infiltration on the vascular wall and chemokines and pro-inflammatory cytokines secretion[600]. On the other, some pro-inflammatory and pro-atherogenic effects have been described since it positively correlates with TNF-α levels[601] and its administration increases the levels of ICAM-1, VCAM-1 and MCP-1 via NF-κB pathway[602].

In experimental models of cerebral ischemia, different peptides of apelin have shown a potential neuroprotective effect reducing infarct volumes and even improving neurological deficits[603–605], and it has been suggested that this may be due to inhibition of inflammation[606]. However, no differences have been found in plasma levels of apelin between ischemic stroke patients and healthy controls in a case-control study[607].

3.1.5.3.5. Omentin

Omentin, an adipokine with anti-inflammatory and anti-atherogenic properties, is mainly expressed by visceral fat[608]. Its levels inversely correlate with obesity and insulin resistance[609,610], and directly with serum HDL and adiponectin[609]. Omentin improves insulin sensitivity. The addition of omentin promotes glucose uptake in human adipocytes[611].

This adipokine induces eNOS leading to endothelium-dependent vasorelaxation[612], attenuates arterial calcification[613], and inhibits TNF-α-induced expression of adhesion molecules in endothelial cells via blocking NF-κB pathway[614]. Circulating levels of omentin are independently and negatively correlated with carotid IMT[615,616]. Conversely, an observational study has found that higher levels of omentin were significantly associated with a higher risk of stroke in metabolically healthy subjects, but no with the risk of myocardial infarction[617]. A previous study has shown higher risk of cardiovascular events in patients with higher levels of omentin[618].

3.1.5.3.6. Chemerin

Chemerin is an adipocyte-secreted protein with pro-inflammatory properties[619], which was firstly identified as a chemotactic factor for macrophages and dendritic cells[620].

Evidence suggests the involvement of this adipokine in obesity and metabolic syndrome. Administration of chemerin exacerbates glucose intolerance, lowers serum insulin levels, and decreases tissue glucose uptake in obese and diabetic mice[621]. In nondiabetic subjects, chemerin levels positively correlate with BMI, fasting serum glucose, and circulating triglycerides[622]. Chemerin activates NF-κB pathway and induces insulin resistance in human skeletal muscle cells[623]. Moreover, the levels of chemerin positively correlate with TNF-α[601], and TNF-α treatment of adipocytes increases chemerin levels[624].

Chemerin may also contribute to atherosclerosis. It has been shown that chemerin can rapidly stimulate the adhesion of macrophages to fibronectin and VCAM-1[625]. Another study reported that chemerin is associated with arterial stiffness, as represented by the brachial ankle pulse wave velocity, but not with carotid IMT[626]. A recent study with Asian population found that chemerin is a risk factor for stroke and carotid artery plaque instability[627].

3.1.5.3.7. Visfatin

Visfatin was first described as a growth factor for pre-B-cells expressed in several tissues[628]. Serum visfatin levels are elevated in patients with obesity and type 2 diabetes and positively correlate with insulin resistance[629–631]. This adipokine exerts pro-inflammatory activities. In monocytes it induces the production of IL-1, IL-6 and TNF-α[632].

Moreover, visfatin stimulates the production of VEGF and MMPs by endothelial cells[633], and vascular smooth muscle cell proliferation[634], thus it is involved in angiogenesis and atherogenesis. Visfatin has been also found to have a role in plaque destabilization, since its gene is markedly enhanced in carotid plaques from symptomatic compared with asymptomatic subjects, and its expression is localized in areas rich in lipid-loaded macrophages[635]. In animal models of cerebral ischemia, it has been suggested that visfatin could have a protective role through enhanced energy metabolism[636]. However, observational studies have revealed that visfatin is an independent predictor of ischemic stroke[607], and may help in the prediction of long-term outcomes in these patients[637].

3.1.5.3.8. Vaspin

Vaspin in secreted by adipocytes from white adipose tissue, hypothalamus, pancreatic islets, and skin[527]. This adipokine enhances insulin sensitivity and its levels are increased in obesity and type 2 diabetes patients. In ischemic stroke patients there have been found lower levels of vaspin when compared to controls[607].

3.1.5.3.9. Retinol binding protein 4

Retinol binding protein 4 (RBP4) was firstly described as a hepatocyte-synthesized protein that is responsible for the transport of vitamin A in the body[638]. It is also expressed by macrophages[639] and adipocytes and contributes to insulin resistance[640]. Circulating levels of RBP4 are increased in obesity and insulin resistance, and correlate with levels of inflammatory factors such as CRP and IL-6[641]. It has been found higher circulating levels of this adipokine in patients with stroke compared to controls[642].

3.1.5.3.10. Lipocalin 2

Lipocalin 2 is highly expressed by adipocytes[643]. Experimental studies show controversial findings, as it seems to have pro- and anti-inflammatory effects. It is induced by inflammatory factors via NF-κB pathway[644]. Circulating levels of lipocalin 2 are positively correlated with adiposity, insulin resistance and CRP levels[645]. And while some studies reveal improved insulin sensitivity in lipocalin 2 deficient mice[646], others show that molecular disruption of this adipokine results in insulin resistance and increased expression of pro-inflammatory factors[647], or even that lipocalin 2 administration suppresses LPS-induced cytokine production[648].

In an experimental model of brain ischemia, lipocalin 2 deficient mice showed lower infarct volumes, neurologic scores, and inflammatory mediator expression than wild-type animals, suggesting that lipocalin 2 may contribute to neuronal cell death[649]. Another experimental study also showed the potential neurotoxic effect of this adipokine in cerebral ischemia[650]. In ischemic stroke patients, higher plasma levels of lipocalin 2 were associated with a worse clinical outcome and post-stroke infections[651].

3.1.5.3.11. Secreted frizzled-related protein 5

Secreted frizzled-related protein 5 (SFRP5) is highly expressed in adipocytes and it has anti-inflammatory properties[652]. SFRP5 deficient mice develop glucose intolerance and show infiltration of activated macrophages in adipose tissue, whereas adenovirus-mediated delivery of SFRP5 to mice models enhances glucose tolerance. Plasma levels of SFRP5 are lower in Chinese patients with type 2 diabetes and obesity compared to controls, and negatively correlate with BMI, insulin resistance, and IL-6[653].

In a mice model of heart ischemia/reperfusion, SFRP5 knockout mice displayed larger infarct sizes, with greater macrophage infiltration and greater pro-inflammatory cytokine and chemokine gene expression at ischemic lesions, and diminished cardiac function when compared to wild-type mice, suggesting that SFRP5 antagonizes inflammatory responses after ischemia/reperfusion in the heart[654].

3.1.5.3.12. Angiopoietin-like protein 2

Angiopoietin-like protein 2 (ANGPTL2) is a pro-inflammatory cytokine secreted by adipose tissue[655]. Its circulating levels closely correlate to adiposity, insulin resistance, and inflammation in both mice and humans.

In a MCAO mice model, ANGPTL2 knockout mice showed decreased neurological deficits, infarct volumes, and levels of IL-1 and TNF-α, relative to wild-type mice[656]. Authors found that bone marrow-derived macrophages secreting ANGPTL2 significantly contribute to acute brain injury. An observational study showed that serum ANGPTL2 levels in patients with type 2 diabetes were independently associated with vascular events (vascular death, myocardial infarction or stroke)[657].

3.2. ASSOCIATION BETWEEN OBESITY AND GOOD PROGNOSIS

3.2.1. Epidemiological evidences

Observational studies have evidenced that excessive body weight may be associated with a better prognosis after ischemic stroke.

In 2008, Olsen et al.[658] reported the results of a Danish study with more than 21000 patients with stroke in whom BMI was recorded. They found that poststroke mortality was inversely related to BMI, with higher mortality rates in overweight and obese patients compared to normal-weight and underweight patients. Since then, several studies have found similar results.

In 2009, Towfighi et al.[659] described that the effect of obesity on mortality after stroke was age-dependent. Overweight and obese patients under 70 years displayed higher mortality rates than their normal-weight counterparts, whereas overweight and obese ones above 70 had lower mortality rates than normal-weight.

Later, in 2011, Vemmos et al.[660] found higher early (1 week) and long-term (10 years) survival rates among overweight and obese patients when compared to normal-weight patients. Few days after admission, in spite of the same severity of neurological deficit, obese patients demonstrated lower rates of brain edema and mass effect. On admission, obese patients were more frequently taking antihypertensive drugs that affect the renin-angiotensin system, which has been demonstrated to be associated with reduced severity in ischemic stroke[661,662]. However, after adjusting for this treatment, the association between BMI and mortality persisted.

Kim et al.[663], in more than 34000 patients from the Korean Stroke Registry, showed an inverse association between obesity and mortality, which was evident at 90 days after stroke and became significant 1 year after stroke onset. Similar results were found in a prospective Iranian study with negative correlation between BMI and mortality[664]. A study in the USA revealed a U-shaped relationship between BMI and mortality after acute ischemic stroke, with the lowest mortality risk among patients with an approximate BMI of 35 kg/m^2, while those above and below had higher mortality rates[665]. A recent study showed that, in addition to lower mortality risk among obese subjects with stroke, a weight loss of more than 3 kg at the outpatient visit was associated with increased mortality risk[666]. However, it seems that there is a difference between intentional and unintentional weight loss, as it has been studied in cardiac pathologies[667]. Intentional weight loss results in a loss of body fat that has been associated with potential benefits[668,669], whereas unintentional weight loss is associated with sarcopenia and loss of lean mass due to occult diseases[670].

Andersen et al.[671], with the data from almost 30000 Danish patients, found lower mortality rates among overweight and obese patients and also a reduced risk of readmission for recurrent stroke in obese ones compared to normal weight patients. Barba et al.[672] found similar results in a retrospective study made in Spain with more than 200000 patients with stroke, showing reduced in-hospital mortality and reduced early readmittance in obese patients. Doehner et al.[673] described also an inverse relationship between BMI and mortality, recurrent stroke, and functional impairment after ischemic stroke. Another study of Andersen et al.[674] confirmed that obese and overweight patients had experienced fewer previous strokes than normal weight patients. Ovbiagele et al.[675], in a study with more than 20000 patients, showed that overweight and obese patients had lower risk of major vascular event, but failed to find a significant association between obesity and stroke recurrence.

On the other side, in 2011 Ryu et al.[676], in a prospective study with almost 1600 patients, found an inverse correlation between BMI and long-term mortality that disappeared after adjusting for initial neurological severity, except for underweight patients. In 2014, Dehlendorff, Andersen and Olsen[677], the same authors that previously had found results that supported the paradox, published a new study with more than 70000 patients from the Danish Stroke Register, which contradicted previous findings. They found that stroke severity was related to BMI, with patients with the most severe strokes having the lowest BMI. After adjusting for initial severity, they found no differences in the risk of death in different groups of BMI. A recent study by Kim et al. showed a similar phenomenon[678].

In terms of functional outcome, some studies have also found a potential positive role of excess body weight. The results from the TEMPiS trial[673] revealed that functional outcomes followed the same inverse patter of association with body weight. Overweight and obese patients had a lower risk of institutional care and dependency than patients with normal weight or underweight. An study in Korean population showed that stroke patients who were extremely obese, at 6 months from stroke onset, performed activities of daily living better than did patients with a normal BMI, but just in the group above 65 years old[679]. Another study in Japanese patients with stroke who were admitted from convalescent rehabilitation wards found that obesity was independently and positively correlated with the functional independence measure gain[680]. Interestingly, weight loss after stroke appears to be associated with unfavourable functional outcomes[681,682]. On the contrary, other studies have found an inverse correlation between BMI and functional recovery[683–685].

Moreover, the obesity paradox in ischemic stroke seems not to be limited to prognosis. In a Korean study with 365 stroke patients, it has also been found that the risk of hemorrhagic transformation decreases significantly in obese patients[686].

3.2.2. Obesity and less severe strokes

As previously noted, in some studies the association of BMI and prognosis disappears after adjusting for the initial severity of stroke[676–678]. In these cases, patients with higher BMI had less severe strokes. One proposed explanation is the possible association of obesity with small-vessel disease. Ryu et al.[676] showed that small-vessel occlusion was the most frequent cause of stroke in patients with high BMI, whereas cardioembolism was the predominant etiology in patients with low BMI. In the Hisayama study[687], which included Japanese population with stroke, BMI was positively and independently correlated with the occurrence of lacunar infarction, but just in women. Another Japanese study showed that abdominal obesity was significantly associated with silent lacunar infarcts[688]. Similarly, in a Korean population with stroke, obese patients were significantly linked to the stroke subtype of small vessel occlusion, whereas lean patients were more likely to have strokes due to cardioembolism[678]. However, it is noteworthy that those studies were done in Asian populations, where the different aetiologies of stroke vary in frequency with respect to Western countries. The ARIC study[689], which included USA black and white patients with stroke, showed that every stroke subtype (lacunar, nonlacunar thrombotic, and cardioembolic stroke) were all significantly and positively associated with different obesity measures. However, although WHR, WC and BMI were significantly higher in patients with nonlacunar and cardioembolic stroke as compared to subjects who did not develop ischemic stroke, just WHR, and not WC or BMI, was significantly higher in patients with lacunar stroke.

3.2.3. Obesity and better therapeutic control

It has been suggested that obese subjects, as they are supposed to have more vascular risk factors and develop vascular diseases, receive earlier and more optimal and aggressive treatments than lean individuals.

Some works have detected this phenomenon. In 2007, a study with more than 130000 patients admitted because of coronary disease showed that patients with higher BMI were more frequently treated with aspirin, beta blockers, inhibitors of the renin-angiotensin system, and lipid-lowering therapy[690]. Later, more studies in coronary heart disease have shown that obese patients, when compared to normal-weight ones, are on more optimal medical treatment after the event[691,692], or even are more likely to undergo revascularization procedures[693].

3.2.4. Malnutrition, underweight, weight loss and poor prognosis

It is a well-established fact the association between malnutrition[694–696] and underweight[658,663–665,671,673,676] with poor prognosis in stroke. The FOOD trial[695], a multicentric study with more than 3000 patients with stroke, showed that undernourished patients were significantly more likely to be dead or dependent at 6 months (OR, 2.08; 95% confidence interval [CI], 1.50 to 2.88). Previously, Dávalos et al.[694] reported that malnutrition after 1 week independently increased the risk of poor outcome assessed after 1 month (OR, 3.5; 95% CI, 1.2 to 10.2). Similarly, a thesis done in our department[696] demonstrated that more than a half patients with stroke admitted to our hospital were at risk of malnutrition, which increased by 6 times the risk of poor functional prognosis at 3 months.

As noted above, weight loss after stroke is associated with poor prognosis[666,681,682]. Weight loss in this context is the consequence of a global negative caloric and nitrogen balance[697]. It takes place a catabolic/anabolic imbalance, where catabolism increases and anabolic stimulation fails. Not only impaired feeding and inactivity contribute to this phenomenon, but also factors such as neuroendocrine sympathetic activation, fever, appetite dysregulation, cytokines, and oxygen-free radical accumulation. After stroke, the stress response results in local and systemic sympathetic activation, which promotes the catabolic stimulation and therefore insulin resistance and increased degradation of protein and lipid energy stores. Moreover, the release of TNF-α is one of the main mechanisms responsible for muscle loss due to its catabolic effects. All of these factors contribute to tissue wasting of both muscle and fat tissue.

Obese subjects may have a higher metabolic reserve, which may help to counterbalance the deleterious effects of cachexia and inflammation[667].

3.2.5. Obesity and anti-inflammatory molecular factors

TNF-α is secreted by adipocytes and its expression in fat tissue is increased in obesity[698]. There are two TNF-α receptors, TNFR-I and TNFR-II, and both are expressed in human adipose tissue[699]. Both can be cleaved from the cell surface as soluble TNF-α receptors (sTNFR), which are present in the circulation and act as antagonists by inhibiting the ligand-binding cell surface receptor. Although some studies have shown that the serum concentration of both soluble receptors positively correlates with obesity[700], others have shown no differences in TNFR-I levels between obese and lean patients[700–702]. Therefore, the production of soluble TNF-α receptors by adipose tissue may promote anti-inflammatory effects by inhibiting TNF-α actions[703].

It has been shown, in *in vitro* and *in vivo* models of endotoxemia, that lipoproteins can modulate LPS activity, by binding to this molecule in direct proportion to their cholesterol content[704]. LDL are the lipoproteins with the higher proportion of cholesterol[705]. Therefore, by binding to LPS through the formation of micelles, LDL and other lipoproteins can inhibit the pro-inflammatory effects of endotoxin. Many studies have evaluated the association between cholesterol and prognosis on different diseases showing that cholesterol levels may correlate positively with prognosis. It has been found that higher cholesterol levels are associated with improved survival among patients with heart failure[706]. A prospective study conducted in Japan, which included more than 12000 patients, revealed that low cholesterol was related to higher mortality rates in patients with hemorrhagic stroke, heart failure, and cancer[707]. Koton et al.[708], in a study with patients with a first-ever ischemic stroke, showed that low cholesterol was associated with increased stroke severity and poor functional outcome, independently of the previous use of statins. In the same study, they found increased risk of mortality among patients with low cholesterol levels and no previous use of statins. Later, Markaki et al. showed also higher mortality rates related to low cholesterol levels after ischemic stroke[709]. These results are controversial, as classical studies have demonstrated reductions in mortality risk in patients receiving statin treatment[710]. It has been suggested that beneficial effects of statins may be more related with endothelial dysfunction improvement, anti-inflammatory properties, anti-thrombotic and anti-proliferative actions, and apoptosis reduction[711]. In this sense, statins have been found to: reduce the levels of ox-LDL[712], a ligand of TLRs, an independent predictor of poor prognosis after ischemic stroke; inhibit the reduction in eNOS expression induced by ox-LDL, independently of lipoprotein lowering[713]; inhibit the activation of NF-κB and chemokine expression[714]; reduce serum CRP and pro-inflammatory cytokine levels[711].

3.2.6. White adipose tissue as source of progenitor cells

In the past years it has been demonstrated the importance of stem cells mobilization from endogenous depots in the evolution of several diseases[715]. Also after stroke, the stress response that takes place in CNS contributes to stem cells mobilization to the brain via cytokine production[716].

Stem cells can be categorized into three classes, totipotent, pluripotent, and multipotent[717]. Totipotent cells can differentiate into any cell type required for fetal development. Pluripotent cells are able to differentiate into any of the three main types of body tissues, ectoderm, mesoderm, and endoderm, but cannot develop into a fetus. Finally, multipotent cells, which derive from pluripotent cells, can differentiate into a restricted number of cell types.

A population of these multipotent cells, endothelial progenitor cells (EPCs), are involved in vascular endothelium repair and angiogenesis, either directly, recruited by cytokines to injury sites, or through trophic effects mediated by VEGF[718]. Those cells are considered markers of vascular risk and endothelial function, their number inversely correlate with vascular risk factors such as smoking, dyslipidaemia, diabetes mellitus and hypertension[719]. The increase in EPCs has been associated with reduction in mortality and better prognosis in different vascular diseases[720–722]. In a work published in 2010, severely obese showed higher flow-mediated dilation compared to obese and normal-weight subjects, lower carotid IMT than obese individuals and similar to normal-weight, and higher EPCs than obese[723]. Authors suggested that severely obese subjects might be protected from atherosclerosis, due to a greater mobilization of EPCs.

Mesenchymal stem cells (MSCs) are another well-known subtype of multipotent cells[717]. Those are a heterogeneous population of non-hematopoietic cells, present in several connective tissues, and capable of differentiation into mesenchymal tissues such as bone, cartilage, adipose tissue, muscle, and marrow strome[724,725]. Bone marrow-derived MSCs (BM-MCSs), which have been suggested to be able to differentiate into neuronal and glial cells, have been used for the treatment of different vascular diseases including stroke, with positive findings[726]. It has been shown that

MSCs may be mobilized and migrate following chemokine gradients in response to different pathological situations promoting the repairing of injured tissues and recovery[717,727–730]. Moreover, Bellows et al.[731] found that obese humans show increased circulating levels of MSCs, whereas they were not able to detect circulating MSCs at healthy lean individuals. Several chemokine receptors such as CCR2, CCR3, CCR4, CCR5 or CXCR4 have been described in MSCs as potential effector molecules that are activated to induce migration[732]. In response to inflammatory signals such as TNF-α, the expression of some of these chemokine receptors on MSCs is upregulated, just as happens with different MMPs, which have also been reported to be involved in MSC migration as they favour cell locomotion and tissue reconstitution by breaking down the extracellular matrix. This suggests that the mobilization of MSCs and their subsequent homing to damaged tissues may depend on the systemic and local inflammatory state.

White adipose tissue represents an important source of progenitor cells[730]. In addition to adipocytes and immune cells, this depot has a class of MSCs known as adipose tissue-derived stem cells (ASCs). These cells are located at stromal vascular fraction of both visceral and subcutaneous white adipose tissue, where most of progenitor cells are ASCs[733,734]. In the last years, ASCs have become an object of attention as a promising source for cell therapy. These cells display some important advantages that make them specially interesting: similar to MSCs, ASCs do not express HLA-DR (of MHC class II), which makes them less immunogenic than other cell types; but, in addition, ASCs are easier to obtain and much more abundant[735]. Adipose tissue contains a largely greater proportion of stem cells than bone marrow (5% vs. 0.01%)[736], in a single liposuction procedure just one millilitre of fat is enough to produce 250000 ASCs in a single passage[735]. Moreover, it has also been demonstrated that, like MSCs, native and transplanted ASCs can be mobilized from white adipose tissue and migrate in response to a specific immune stimulus[737,738].

Several studies have assessed the potential benefits of ASCs in different neurological diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease or stroke[739], and even in non-neurological diseases such as other vascular diseases[740]. Although the differentiation into various cell lineages has been suggested, the mechanisms underlying the positive effects found in these studies are mainly related, on the one hand, to neurorepair and neuroprotection via angiogenesis, synaptogenesis, gliogenesis, and neurogenesis, through the secretion of several growth factors such as brain-derived neurotrophic factor (BDNF), GDNF, VEGF, hepatocyte growth factor (HGF), or IGF-1; and, on the other hand, to their immunomodulatory actions, inhibiting the expression of pro-inflammatory cytokines and participating in the secretion of anti-inflammatory cytokines[739].

With regard to stroke, most studies have shown beneficial effects after ASCs therapy in experimental models[741]. Similar to other diseases, transdifferentiation, immunomodulation, and stimulation of brain repair response, have been proposed as the responsible mechanisms (**figure 12**).

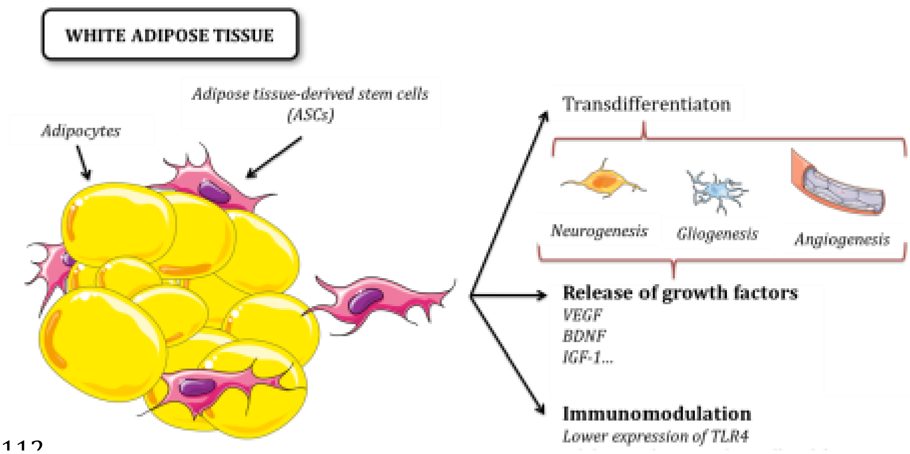


Figure 12. Potential mechanisms for ASCs therapy in stroke.

First, Kang et al.[742] found that injected ASCs into the lateral ventricle of the rat brain migrated to the injured cortex after cerebral ischemia, some of them expressed microtubule-associated protein 2 (MAP2), a neuron marker, and GPAP, an astrocyte marker, and their transplantation was associated with improved functional deficits. Another study, in a hemorrhagic stroke experimental model, showed that intravenous administered ASCs attenuated neurological deficits, were found in perihematomal areas and expressed von Willebrand factor and endothelial barrier antigen, both endothelial markers[743]. However, studies showed little survival of ASCs in brain after transplantation, and further works have shown benefits in spite of no implantation in damaged tissues[744]. Therefore, cell integration may not be enough to explain functional recovery after ASCs therapy, or even not necessary[741].

ASCs are able to modulate the brain repair response through different growth factors and brain repair-associated markers. These cells secrete several growth factors such as VEGF, HGF, TGF-beta, BDNF and IGF-I[745]. In 2011, Ikegame et al.[746] in a mice model of ischemia, showed that intravenous administration of ASCs was associated with reduction in infarct volume and edema, and higher expression of VEGF, HGF and angiopoietin-1, despite injected cells were not detected in the brain sections after 24 hours of administration. In another work, Gutiérrez-Fernández et al.[747] found that intravenous administration of ASCs was associated with better functional recovery after 14 days of permanent MCAO in mice, despite the lack of a reduction in infarct volume or the absence of migration or implantation by these cells. Animals receiving this treatment showed decreased cell death, increased cell proliferation, and significantly augmented expression of neurogenesis, oligodendrogenesis, synaptogenesis and angiogenesis markers in the brain such as neurofilament (NF), oligodendrocyte transcription factor (Olig-2), synaptophysin (SYP) and VEGF. The latter is one of the most studied trophic factors related to ASC. The administration of VEGF in a model of transient MCAO was associated with a reduction in infarct volume and brain edema[748]. Regarding ASC, it has been demonstrated that hypoxia activates the receptor tyrosine kinases in the membrane of ASCs resulting in the expression of VEGF, and VEGF itself stimulates ASCs[749]. Several studies have shown the contribution of ASCs to angiogenesis in damaged tissues and its relation with the expression of VEGF[750,751]. It has even been suggested that obesity might influence cancer risk and progression through ASCs and their pro-angiogenic properties, promoting the formation of new blood vessels, needed for the expansion of the tumor[730].

Finally, ASCs exert important immunomodulatory functions that may modulate inflammation in stroke. For example, in a murine model of cerebral ischemia, the intravenous administration of these cells was associated with a lower expression of IL-18 and TLR4, and improved functional outcome[752]. But immunomodulation by ASCs is much more extensive: ASCs inhibit peripheral blood mononuclear cells proliferation; TNF-α induces ASCs production of prostaglandin-E2, which has immunosuppressive effects; IFN-γ induces ASCs production of indoleamine 2,3-dioxygenase (IDO), which inhibits T cell proliferation (remember that T cells produce both TNF-α and IFN-γ); ASCs decrease the number of Th17 cells; and upregulate the expression of IL-10 by Treg cells, which supresses the autoreactivity of T cells and inhibits Th1 and Th17 responses[735].

Stem cell therapy is a novel and promising treatment for many diseases, including several neurological entities such as stroke. In this context, ASCs represent a largely accessible an abundant source. Many clinical trials have been performed with ASCs in many different diseases with positive results[753]. At the moment, three clinical trials with ASCs therapy in stroke patients are in process[754–756].

3.3. THE BROWN-ADIPOSE TISSUE

To combat cold and food shortage we have white and brown adipose tissue: while white helps offset fluctuations in energy availability, brown keeps us warm[757]. Just recently, at least in some areas of the world, problems associated with cold and hunger have been overcome. However, sedentary lifestyle and excessive calories intake have led to the emergence of new dangers: obesity and related health problems. In this context, the functions of brown adipose tissue are of special interest.

Brown adipose tissue is only present in mammals[757]. In humans, it is especially abundant in newborns and it largely diminishes with increasing age. In 2009, three articles confirmed the existence of functional brown adipose tissue in human adults[758–760]. Nowadays we know that it represents 2-5% and 0.05-0.1% of body weight in children and adults respectively[757]. Its predominant locations are cervical, axillary, perirenal and adrenal, along the large blood vessels and trachea, and as finger-like projections following intercostal arteries (**figure 13**). It is highly vascularized and innervated by sympathetic nervous system, and unlike white adipose tissue it is

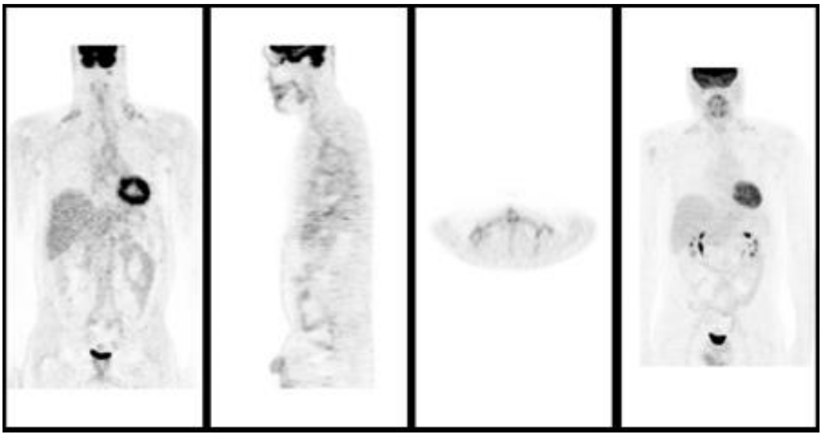


Figure 13. Reactivation of brown adipose tissue in body PET. Hyperuptake of 18F-fluodeoxyglucose at latero-cervical and supraclavicular areas.

multiloculated, lipids accumulate as droplets surrounded by numerous mitochondria[761]. These mitochondria express uncoupling protein 1 (UCP1), which enables brown adipose tissue to produce heat by uncoupling cellular respiration from ATP production (**figure 14**)[761,762]. This is known as adaptive thermogenesis, and is positively regulated by the sympathetic nervous system that leads to a cascade of intracellular events ending in activation of UCP-1[763].

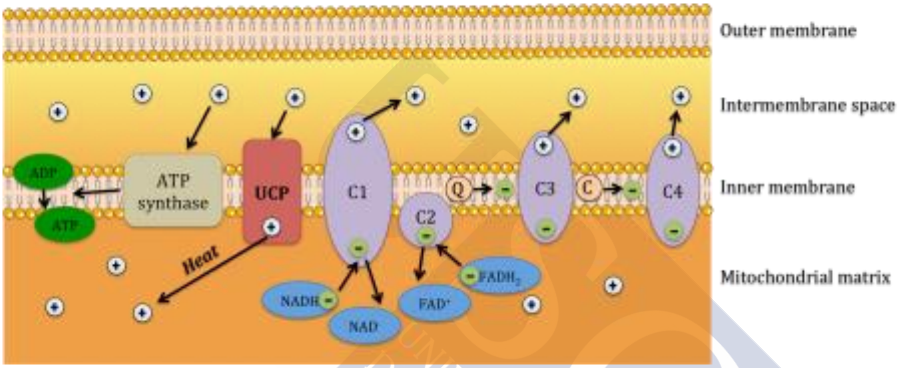


Figure 14. UCP produces heat by uncoupling cellular respiration from ATP production. Modified from Andrews et al.[762].

It has been proposed a dual role for brown adipose tissue, thermoregulatory thermogenesis and metaboloregulated thermogenesis[757]. The former consists in the production of heat in response to a cold environment. The second, also referred as diet-induced thermogenesis, occurs in response to diet as a defense mechanism against a caloric load, implies an induction of extra energy expenditure under conditions of energy abundance, probably with the aim to maintain body weight[761]. The existence and significance of diet-induced thermogenesis and its relation to brown adipose tissue have been debated for long time, and nowadays it remains controversial. Experimental models showed that, in wild-type mice, HFD increased norepinephrine-induced thermogenesis, confirming the presence of diet-induced thermogenesis, and this is not present in UCP1 ablated mice, thus demonstrating that diet-induced thermogenesis depends on UCP1[764]. Moreover, in thermoneutrality conditions (similar to the conditions under which most humans normally live), ablation of UCP1 induces obesity in mice, even if mice were fed with control diet, and it highly increases obesity induced by HFD. Authors suggest that UCP1 activity can exert a key role in the development of obesity in mice and possibly in humans, so that the brown adipose tissue depots may significantly vary between humans, to the point that body weight may be regulated, at least in part, by the amount of UCP1. In 2014, Sugimoto et al.[765] showed that in mice fed with HFD the treatment with miglitol (an anti-diabetic drug that reduces obesity in humans and rodents) significantly reduced body weight, white adipose tissue, and insulin resistance, increased brown adipose tissue temperature, and enhanced expression of UCP1, so that they attribute the anti-obesity effect of this drug to increased energy expenditure by up-regulating UCP1 in brown adipose tissue.

The activity and quantity of brown adipose tissue in human adults are inversely correlated with BMI[759,760,766]. It has also been shown that UCP expression in adipose tissue is lower in obese subjects, and recently, Sakamato et al.[767] found that infiltration of adipose tissue by M1 macrophages and TNF-α administration supress the UCP1 induction, suggesting that inflammation promotes reduction of energy expenditure in adipose tissues. Moreover, it has been shown that abdominal fat mass, total body fat mass, and body mass index, did not vary with aging in brown adipose tissue-positive subjects, whereas increased with age in brown adipose tissue-negative subjects, suggesting a potential protective role of brown adipose tissue presence in age-associated development of obesity in humans[768]. Therefore, the cold response in lean subjects produces higher amounts of energy, while obese individuals display a reduced response and a higher insulation capacity. This lower activity of brown adipose tissue in obese subjects inhibits the energetic transformation and may predispose to develop more obesity, which may explain the difference in body weight gain in overfeeding studies[769].

We previously noted that hyperthermia is one of the most potent predictors of poor prognosis in acute ischemic stroke[42]. As in the acute phase of stroke the stress results in the activation of local and systemic sympathetic responses, and since brown adipose tissue thermogenesis is triggered by the activation of sympathetic nervous system[763], it could be postulated that, obese subjects with low expression of UCP1, are at lower risk to brain damage due to hyperthermia than lean subjects who express more UCP1. However, on the other hand, fatty acids are an important stimulator of UCP1[770], and obesity is associated with higher circulating fatty acids[297]. In addition, the sympathetic discharge that occurs after stroke can lead to increased lipolysis, resulting in higher circulating levels of fatty acids, which could be even higher in obesity due to larger depots. Therefore, it is also possible to found a higher degree of hyperthermia in obese subjects in the acute phase of stroke as a consequence of these facts.

JUSTIFICATION

Stroke is a major global health problem. It is a leading cause of death and disability, and is responsible of high health care costs. The aging population implies that absolute number of people who suffer strokes annually, as well as DALYs lost, is increasing. Among all the causes of death, stroke is the third globally and ischemic stroke is responsible of approximately half[7]. Respect to DALYs, stroke is the third cause[9]. The costs of stroke represent about 2-4% of total health-care funds, and more than 4% of direct costs in industrialised countries[12].

In the pathophysiology of ischemic stroke aspects such as temperature and inflammation are of special importance. Respect to inflammation, elements of both innate and adaptive immunity are involved in all phases of the ischemic cascade during and after the event[106]. Signals released during cerebral ischemia activate innate immunity components, such as TLRs, and promote a pro-inflammatory response that contributes to tissue damage. Likewise, these processes stimulate a potentially harmful adaptive immune response directed against antigens. However, apart from their role in tissue damage, recent evidences have pointed out that innate immunity through TLRs stimulation may be involved in tissue repair and regeneration[319].

Moreover, there is a robust relationship between body temperature and the evolution of acute cerebral infarct[42]. In the first days after ischemic stroke hyperthermia emerges in more than a half patients[43]. The effects of hyperthermia are clearly deleterious on animal models[54–57] and humans[43,49,58–64], so that it is one of the most powerful predictors of poor prognosis in acute cerebral ischemia. First, the intense release of cytokines or neuro-excitatory amino acids in infarcted areas can induce a systemic hyperthermia[52], and, secondly, systemic hyperthermia promotes inflammatory mechanisms, metabolic dysfunction, excitotoxicity, oxidative stress, BBB alteration and protein degradation, resulting in an increase of the ischemic lesion[65].

Obesity, the undesirable consequence of a positive energy balance[356], is a rising public health problem that has reached pandemic proportions in the last decades[360]. It has traditionally been considered an indicator of poor health, associated with vascular risk factors such as type 2 diabetes mellitus[399], dyslipidaemia[356] or hypertension[401], and a recognized risk factor for vascular diseases by itself[357,400], including stroke. An analysis for the Global Burden of Disease Study, estimated that overweight and obesity caused, at the year 2010, 3.4 million deaths, the 3.9% of YLL and the 3.8% of DALYs globally[397]. Obesity is associated with excess in healthcare costs. In Europe, a recent review of healthcare costs studies in Western countries estimated excess spending of about €117 to €1,873 per person when comparing obese to non-obese patients[411].

However, it has recently emerged the concept of "obesity paradox", a term used to describe the unexpected improved prognosis and lower mortality rates found in several diseases in patients with excessive body weight[412–425,427–430]. Regarding stroke, contradictory findings have been reported. Several authors have found an inverse correlation between excessive body weight and mortality in stroke patients[658,660,663,664,671–673]. Other studies have shown that this phenomenon takes place only in elderly populations[659], or showed an U-shaped relationship with the lowest mortality among patients with BMI of 35 kg/m^2 [665]. Contrary, some authors have found that the paradox disappears after adjusting for the initial severity of stroke[676–678]. In regard to functional outcomes, studies are also controversial, some have shown a positive correlation between better prognosis and body weight[673,679,680], whereas other authors have found a poorer prognosis[683–685], Therefore, nowadays there are still doubts as to whether or not this paradox actually exists in stroke patients, and in case it is a real phenomenon, which are the mechanisms that explain such association?

Obesity generates a low-grade inflammatory response known as "metainflammation"[458], which associates excess body weight to the rest of vascular risk factors, and where different elements such as immune cells, cytokines, chemokines, adipokines or TLRs are involved. Furthermore, some experimental studies have shown that post-stroke peripheral immune response is increased in obesity models[462]. This immune activation might exert a determinant role since, as we noted above, the pro-inflammatory response could contribute to tissue damage. However, despite this potentially increased inflammatory reaction, several authors have shown that obese patients experience a better outcome after stroke. The way obesity influences the inflammatory response in ischemic stroke patients is not known and might provide answers on how obesity affects the prognosis.

On the other hand, white adipose tissue represents an important and abundant source of progenitor cells[771], most of which are ASCs[733,734]. It has been demonstrated the importance of stem cells mobilization from endogenous depots in the evolution of several diseases[715], and some authors have shown that obesity may promote the mobilization of progenitor cells[723,731]. Moreover, apart from transdifferentiation, neurorepair and neuroprotection, one of the proposed main mechanisms for the potential therapeutic properties of ASCs in stroke is immunomodulation[741]. Therefore, progenitor cells could have a major role in the outcome of obese patients after stroke.

Finally, another of the issues involved could be the temperature. Brown adipose tissue is involved in the production of heat through adaptive thermogenesis via UCP1 activation. It is the main responsible for this process and the amount of this tissue and the expression of such protein in adults are inversely related to BMI. As in the acute phase of stroke the stress results in the activation of local and systemic sympathetic responses, and since brown adipose tissue thermogenesis is triggered by the activation of sympathetic nervous system[763], it could be postulated that, obese subjects with low expression of UCP1, are at lower risk to brain damage due to hyperthermia than lean subjects who express more UCP1. However, on the other hand, fatty acids are an important stimulator of UCP1[770], and obesity is associated with higher circulating fatty acids[297]. In addition, the sympathetic discharge that occurs after stroke can lead to increased lipolysis, resulting in higher circulating levels of fatty acids, which could be even higher in obesity due to larger depots. Therefore, it is also possible to found a higher degree of hyperthermia in obese subjects in the acute phase of stroke as a consequence of these facts. These potential differences in body temperature regarding BMI in the acute phase of stroke, could determine, in turn, differences in the prognosis depending on body weight.

HYPOTHESIS

- After ischemic stroke, the outcome of obese patients (BMI $\geq$ 30 kg/m^2) is not worse than that of non-obese patients (BMI < 30 kg/m^2).
- Obese patients counteract their morbidity through increased expression of anti-inflammatory cytokines.

OBJECTIVES

PRIMARY OBJECTIVES

- To compare the clinical characteristics and evolution of two groups of patients, obese (BMI ≥ 30 kg/m^2) and non-obese (BMI < 30 mg/m^2).
- To determine the inflammatory balance (anti-inflammatory cytokines/pro-inflammatory cytokines) in both groups.

SECONDARY OBJECTIVES

- To study the influence of obesity in infarct volume.
- To analyse the role of anthropometric characteristics in ischemic stroke.
- To determine if obesity affects body temperature in the acute phase of ischemic stroke, and how temperature modifies prognosis depending on body weight.
- To evaluate the cell and molecular profiles in both groups of patients.

MATERIALS AND METHODS

1. STUDY DESIGN

A case-control study was performed, with the prospective inclusion of patients diagnosed of a first-ever ischemic stroke within 24 hours from the symptom onset. Patients were categorized into two groups: cases, the obese patients (BMI ≥ 30 kg/m^2); and controls, the non-obese patients (BMI < 30 kg/m^2). The inclusion was consecutive with a 1:1 proportion; one control was included after each case.

We recorded clinical, anthropometric, DEXA, neuroimaging and laboratory variables in an anonymous database.

The Local Ethical Committee of the Estructura Organizativa de Gestión Integrada de Santiago de Compostela approved the protocol. Every patient gave signed informed consent before inclusion in the study. If patients were not able to sign, relatives gave signed informed consent.

2. PATIENT SELECTION

Patients who met every inclusion criteria and none of exclusion criteria (listed below) were prospectively included.

INCLUSION CRITERIA:

1. Patients admitted to the Stroke Unit due to a first-ever ischemic stroke within 24 hours from the symptom onset.

EXCLUSION CRITERIA:

1. Chronic inflammatory disease.
2. Cancer.
3. Severe systemic disease which determines a life expectancy lower than 6 months.
4. Infectious disease in the last 15 days.

5. Continuous anti-inflammatory drugs intake in the last 15 days.

3. PATIENT MANAGEMENT

All the patients were admitted to the Stroke Unit of our Hospital. The neurologists of the Unit conducted the diagnostic and therapeutic management of patients according to the guidelines for stroke management of our Hospital[772] and AHA/ASA guidelines updates[773–776].

4. PATIENT EVALUATION

Patients were evaluated during hospital stay and at 3 months after stroke onset.

4.1. CLINICAL VARIABLES

The clinical variables collected were:

- Demographic variables:

- Age.
- Sex.

- Previous medical history:

- Hypertension.
- Diabetes mellitus.
- Dyslipidaemia.
- Hyperuricemia.
- Atrial fibrillation.
- Peripheral arterial disease.
- Ischemic heart disease.
- Obstructive sleep apnea.

- Smoking.
- Excessive alcohol consumption.

- Vital signs:

- SBP and DBP at the basal moment.
- Axillary temperature at the basal moment and every 6 hours during the first 48 hours.

- Anthropometric variables:

- Height.
- Body weight at the basal moment and at 3 months after stroke.
- BMI at the basal moment and at 3 months after stroke.
- WC at the basal moment and at 3 months after stroke.
- HC at the basal moment and at 3 months after stroke.
- WHR at the basal moment and at 3 months after stroke.

- Safety variables:

- Presence or not of any infection during hospitalization.

- Variables related to stroke:

- Treatment with intravenous tissue plasminogen activator (intravenous fibrinolysis), endovascular treatment, or none.
- To evaluate the severity of neurologic impairments the National Institute of Health Stroke Scale (NIHSS)[777–780] was used (**see appendix 1**). NIHSS was assessed at admission, at 24 hours, at 48 hours, on the 7th day, at discharge and at 3 months.

- After diagnostic evaluation and based on the most likely etiology, stroke was classified according to the TOAST criteria[781] (**see appendix 2**).

- Functional outcome:

- To evaluate functional disability and dependence the modified Rankin Scale (mRS)[782] was used (**see appendix 3**). mRS was assessed previous to stroke, at discharge and at 3 months.

4.2. NEUROIMAGING VARIABLES

Patients underwent brain CT at admission, between 4th and 7th day and at 3 months after stroke. The variables collected were:

- The Alberta Stroke Program Early CT Score (ASPECTS)[783] value at admission, a standard CT examination with a reproducible grading system to assess early ischemic changes.
- Infarct volume in the brain CT between 4th and 7th day and at 3 months after stroke.
- Presence or not of hemorrhagic transformation in the 4th to 7th day brain CT.

4.3. DUAL-ENERGY X-RAY ABSORPTIOMETRY (DEXA) VARIABLES

During the first 2 weeks after stroke, a selection of non-obese and obese patients (5 and 11 respectively) underwent DEXA. The selected patients were those who were in adequate conditions to cooperate and to tolerate the test and agreed to perform it. The variables collected were:

- Bone mineral content.
- Bone mineral density.
- The amount of lean body mass.
- The amount of body fat.
- The percentage of body fat.
- The percentage of android fat distribution.
- The percentage of gynoid fat distribution.
- Android fat/gynoid fat ratio.
- The percentage of fat in arms.
- The percentage of fat in legs.
- The percentage of fat in trunk.
- Arms and legs fat/trunk fat ratio.
- Legs fat/total body fat ratio.
- Trunk fat/total body fat ratio.
- Visceral fat volume.
- Visceral fat mass.

4.4. LABORATORY VARIABLES

Blood samples were extracted for routine laboratory tests, molecular study and cell study.

- Routine laboratory tests (at the basal moment, blood sample was obtained for blood count, coagulation tests, and basic biochemistry tests; during the first 24 hours of hospitalization a second blood sample was obtained for more complete coagulation tests and biochemistry test):

- Leukocytes count.
- Platelets count.
- Fibrinogen.

- hsCRP.
- Total cholesterol.
- HDL-cholesterol.
- LDL-cholesterol.
- Triglycerides.
- Blood glucose.
- Glycated haemoglobin (HbA1c) (it was also collected at 3 months after stroke).

- Molecular study (blood samples were obtained at the basal moment, at 72 hours from stroke onset, and at the 7th day or at discharge, according to cell study):

- IL-6.
- IL-10
- TNFR-I.
- TNFR-II.
- MCP-1.
- MIP-1β.
- Leptin.
- Adiponectin.
- VEGF.

- Cell study (blood samples were obtained at the basal moment, at 72 hours from stroke onset, and at the 7th day or at discharge, according to previous published investigations of our laboratory in progenitor cells[784,785]):

- The expression of TLR2 and TLR4 in circulating neutrophils and monocytes.
- The levels of circulating ASCs.

5. METHODS OF COLLECTION AND DEFINITION OF VARIABLES

5.1. CLINICAL VARIABLES

- Vital signs:

- Axillary temperature: a monitoring probe was used to measure temperature; if not possible, a digital thermometer was used.

- Anthropometric variables:

- Height: it was measured during hospitalization if patient was able to stand upright; if not, height was obtained from Primary Care registries; if not present, patient or his family were asked for it; if not known, height was estimated using the Chumlea et al.[786] formulas for non-Hispanic white men and women based in body measurements (**figure 15**).

Men stature (cm) = 78.31 + (1.94 x knee height (cm)) – (0.14 x age)

Women stature (cm) = 82.21 + (1.85 x knee height (cm)) – (0.21 x age)

Figure 15. Chumlea et al. formulas for the estimate of height in non-Hispanic white men and women[786]. Knee height was measured with patient in the supine position with the non-paretic leg at an angle of 90° with knee and ankle using a caliper, positioning the fixed part in the plantar surface of the heel and the movable part on the head of the patella.

- Body weight: it was measured with an electric scale if patient (dressed with underwear or completely nude) was able to stand on it; if not, patient or his family were asked for last known body weight not earlier than last month; if not known, it was obtained from last month Primary Care registries; if not present, body weight was estimated using the Chumlea et al.[787] formulas for men and women based in body measurements (**figure 16**).

Men body weight (kg) = (0.98 x calf circumference (cm)) + (1.16 x knee height(cm)) + (1.73 x arm circumference (cm)) + (0.37 x subscapular skinfold thickness (mm)) – 81.69

Women body weight (kg) = (1.27 x calf circumference (cm)) + (0.87 x knee height (cm)) + (0.98 x arm circumference (cm)) + (0.4 x subscapular skinfold thickness (mm)) – 62.35

Figure 16. Chumlea et al. formulas for estimate of body weight in men and women.[787] Circumferences were measured with a flexible and inelastic measure tape. Calf circumference was measured at the maximun circumference of the calf muscle of the non-paretic leg. Arm circumference was measured at the midpoint between the acromion and the olecranon of the non-paretic arm. Subscapular skinfold thickness was measured with the patient in right lateral decubitus, by holding the skin and subcutaneous adipose tissue fold in an imaginary line which joins the inferior angle of the scapula and the left elbow; the caliper was positioned perpendicular to the fold below the inferior angle of the scapula.

- Body weight difference at 3 months, defined as: body weight at the basal moment – body weight at 3 months after stroke.
- Percentage difference in body weight at 3 months, defined as: (body weight at the basal moment – body weight at 3 months after stroke)/body weight at the basal moment x 100.

- BMI, defined as: weight (kg)/height^2 (m^2); patients were classified according to WHO BMI cut-off points[356] mentioned before.
- BMI difference at 3 months, defined as: BMI at the basal moment – BMI at 3 months after stroke.
- Percentage difference in BMI at 3 months, defined as: (BMI at the basal moment – BMI at 3 months after stroke)/BMI at the basal moment x 100.
- WC: it was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest, and parallel to the floor at the level at which the measurement is made, with a flexible and inelastic measure tape[388].
- Percentage difference in WC at 3 months, defined as: (WC at the basal moment – WC at 3 months)/WC at the basal moment x 100.
- HC: it was measured around the widest portion of the buttocks, and parallel to the floor at the level at which the measurement is made, with a flexible and inelastic measure tape[388].
- Percentage difference in HC at 3 months, defined as: (HC at the basal moment – HC at 3 months)/HC at the basal moment x 100.
- WHR, defined as: WC/HC.
- Percentage difference in WHR at 3 months, defined as: (WHR at the basal moment – WHR at 3 months after stroke)/WHR at the basal moment x 100.

- Variables related to stroke:

- Early neurological deterioration, defined as: the increase of 4 points or more in NIHSS assessment between NIHSS at admission and any other NIHSS evaluation during the first 48 hours.
- Clinical improvement at 3 months, defined as: (NIHSS at admission – NIHSS at 3 months)/NIHSS at admission x 100). If the patient was dead at 3 months after stroke, a clinical improvement at 3 months of –100% was established.

- Functional outcome:

- Functional outcome at 3 months was dichotomized into good outcome (mRS ≤ 2) and bad outcome (mRS > 2).

5.2. NEUROIMAGING VARIABLES

- Infarct volume: it is expressed in cm^3 and it was calculated according to the formula 0.5 x a x b x c, where "a" and "b" represent the largest perpendicular diameters and "c" the thickness of the cut.
- Volume improvement at 3 months, defined as: (infarct volume between 4th and 7th day – infarct volume at 3 months after stroke)/infarct volume between 4th and 7th day x 100.

5.3. DUAL-ENERGY X-RAY ABSORPTIOMETRY (DEXA) VARIABLES

- The percentage of android fat distribution: the android region was defined as the region from pelvis cut (lower boundary) to above the pelvis cut by 20 % of the distance between pelvis cut line and neck cut line (upper boundary), and is totally enclosed by the trunk region (**figure 17**)[788].

- The percentage of gynoid fat distribution: the gynoid region includes the hips and upper thighs, and overlaps both the leg and trunk regions; the upper demarcation is the pelvis cut at a distance of 1.5 times the android height; the total height of the gynoid region is 2 times the height of the android region (**figure 17**)[788].
- The percentage of fat in trunk: the trunk region includes the neck, chest, abdominal, and pelvic areas; its upper perimeter is the inferior edge of the chin and the lower borders intersect the middle of the femoral necks without touching the brim of the pelvis (**figure 17**)[788].

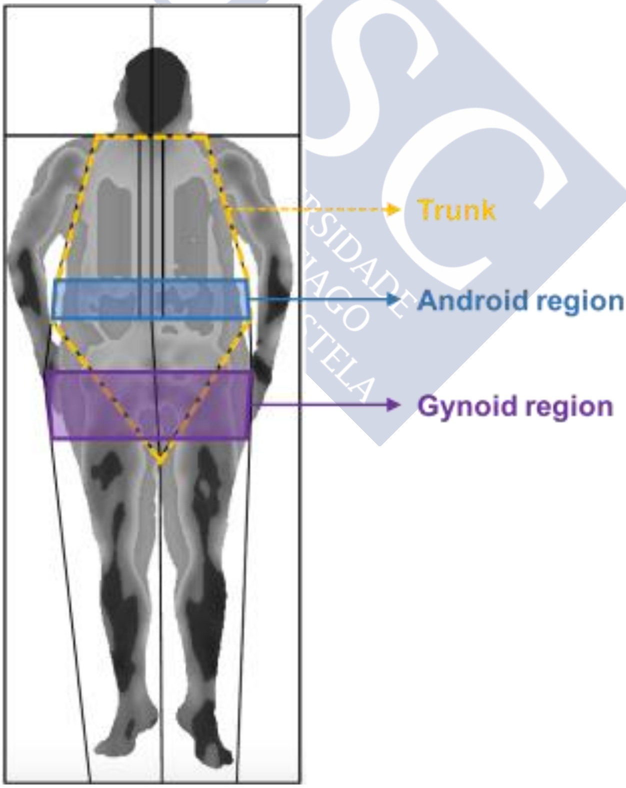


Figure 17. DEXA regions: trunk, android and gynoid regions.

5.4. LABORATORY VARIABLES

- Routine laboratory tests:

- HbA1c values at 3 months after stroke were collected from Primary Care registries.
- HbA1c difference at 3 months, defined as: HbA1c at the basal moment – HbA1c at 3 months.

- Molecular study:

- Blood samples were collected in chemistry test tubes at admission, 72 hours and day 7, centrifuged at 3000g for 15 minutes, and immediately frozen and stored at -80 ºC. Serum levels of MCP-1, MIP-1β and VEGF were measured using a multiplex Melliplex® Map Kit (EMD Millipore Corporation, Billerica, MA, USA) following the manufacturer instructions. On the other hand, serum levels of TNFR-I (Abcam, Cambridge, UK), TNFR-II (Assay Biotech, Sunnyvale, CA, USA) as well as Leptin (Abnova Corporation, Taipei, Taiwan), and Adiponectin (Proteintech Group, Manchester, UK) were measured using commercial ELISA kits following manufacturer instructions. On the other hand, IL-6 and IL-10 were measured by using an immunodiagnostic IMMULITE 1000 System (Siemens Healthcare Global, Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation (CV) for all molecular markers were < 8%. Determinations were performed in a laboratory blinded to clinical and neuroimaging data.
- To determine the balance between anti- and pro-inflammatory cytokines, we defined the anti/pro-

inflammatory index according to the following formula: IL-10 on admission decile/IL-6 on admission decile.

- Cell study:

- TLR2 and TLR4 expression analysis: TLR2 and 4 expression analyses were performed by flow cytometry in blood samples, withdrawn from all patients, collected in EDTA-anticoagulated tubes at admission, 72 hours and day 7. For the expression analysis of TLR2 and TLR4, monocytes, lymphocytes and neutrophils were separated by their forward and side scattering signal characteristics (**figure 18**). FITC-TLR2 antibody (IMMUNOSTEP, Salamanca, Spain) and PE-TLR4 antibody (IMMUNOSTEP, Salamanca, Spain) were used to quantify TLR expression. Samples were analyzed on a FACSAria IIu flow cytometer (BD Biosciences, NJ, USA). Cell fluorescence was measured immediately after staining, and data were analyzed with the use of FACSDiva software (BD Biosciences, NJ, USA). Mean expression of TLR2 and TLR4 in monocytes and neutrophils was analyzed and expressed as AFU (arbitrary fluorescence units). Determinations were performed in a laboratory blinded to clinical and neuroimaging data.
- Quantification of circulating progenitor cells in peripheral blood by Flow Cytometry: circulating progenitor cell levels were measured by flow cytometry in blood samples obtained at admission, 72 hours and at day 7. Blood samples were processed within 1-2 hours after collection by a single researcher who had no knowledge of the patients' clinical or radiological results. In brief, circulating progenitor cells were

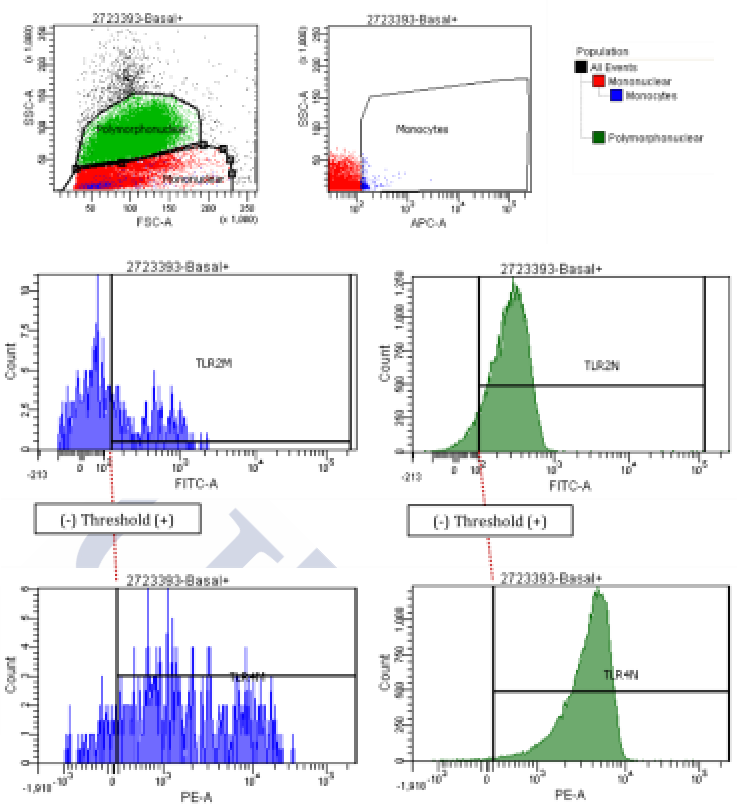


Figure 18. Determination of TLR2 and TLR4 expression in neutrophils and monocytes by flow cutometry. For the expression analysis of TLR2 and TLR4, monocytes and lymphocytes (mononuclear), and neutrophils (polymorphonuclear) were separated by their forward and side scattering signal characteristics. FITC-TLR2 antibody and PE-TLR4 antibody were used to quantify TLR expression.

analyzed for the expression of specific surface antigens using direct flow cytometry (BD FACSAria IIu, BD, Franklin Lakes, NJ, USA). Cells were labelled with FITC-conjugated anti-CD34 (BD, Franklin Lakes, NJ, USA), PerCP-conjugated anti-CD45 (Immunostep, Salamanca, Spain) and APC-conjugated anti-CD31 (Immunostep, Salamanca, Spain) monoclonal antibodies (**figure 19**). We considered circulating progenitor cells as CD34+/CD45-/CD31- staining cells in the mononuclear cell fraction, according to literature[730]. In all analyses, 2.5×10^5 events were acquired, scored using a FACSAria IIu analyzer (BD, Franklin Lakes, NJ, USA), and processed using the PC FACSDiva software program (BD, Franklin Lakes, NJ, USA). Cell count was always expressed per 2.5×10^5 events.

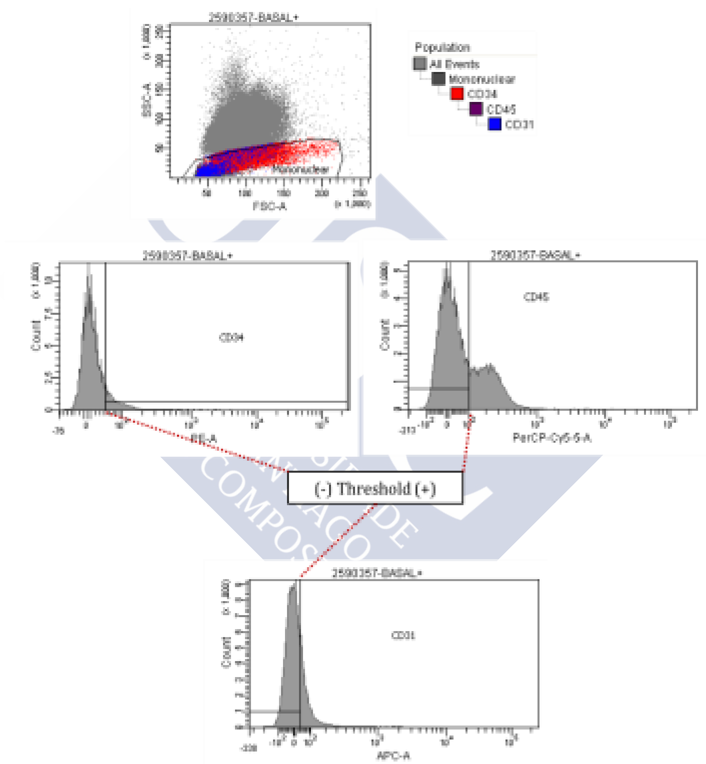


Figure 19. Quantification of progenitor cells by flow cytometry. Cells were labelled with FITC-conjugated anti-CD34, PerCP-conjugated anti-CD45 and APC-conjugated anti-CD31 monoclonal antibodies. We considered circulating progenitor cells as CD34+/CD45-/CD31- staining cells in the mononuclear cell fraction

6. STATISTICAL METHOD

6.1. SAMPLE SIZE

The study was designed with the hypothesis that baseline IL-10 levels in the obesity group would be 30% higher compared to levels in non-obese patients. The program EPIDAT version 4.2 (www.sergas.es/saude-publica/EPIDAT) was used to estimate the sample size. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in bilateral contrast, it would be necessary to include 42 patients in each group. A loss to follow-up rate of 10% was estimated.

6.2. STATISTICAL ANALYSIS

The statistical analysis was performed using the statistic program IBM®SPSS® statistics v.20 for Mac.

To identify the variables that followed a normal distribution, the Kolmogorov-Smirnov test was used. Continuous variables with normal distribution were expressed as mean (SD) and those variables not normally distributed were expressed as median [quartiles].

Proportions between groups were compared by chi-square test. Student's T test was used to compare continuous variables with normal distribution between 2 groups. In case of variables with non-normal distribution, Mann-Whitney U test was used to compare 2 groups. In case of more than 3 groups, variables were compared using ANOVA test.

To evaluate the association between two continuous variables, Pearson correlation coefficient (r) was performed. In order to assess the influence of the different variables in functional outcome at 3 months, logistic regression analysis were performed. The results were expressed as OR with corresponding 95% confidence intervals (95% CI).

Values of p below 0.05 were considered to be statistically significant in all tests.

RESULTS

1. DESCRIPTIVE ANALYSIS

1.1. PATIENT SAMPLE

Over a period of 33 months (from January 2014 to September 2016), a total of 616 patients with a first-ever ischemic stroke within 24 hours from the symptom onset were admitted to the Stroke Unit of Neurology Department at Hospital Clínico of Santiago de Compostela. According to the previously established criteria, 516 were excluded: 39 were included in clinical trials, 23 initially refused to participate in the study, 41 had chronic inflammatory disease, 11 had cancer, 9 had severe systemic disease, 11 suffered infectious disease in the last 15 days, and 24 due to continuous anti-inflammatory drugs intake in the last 15 days, and 358 because of the consecutive (one control for each case) character of the inclusion. After the initial inclusion, 2 patients in the control group were later excluded at the request of their families. Finally, 98 patients who fulfilled all the inclusion criteria and none of the exclusion criteria were consecutively included, 48 patients in the control group (non-obese) and 50 in the case group (obese).

1.2. DESCRIPTIVE ANALYSIS OF THE SAMPLE

The mean age or the patients was 69.3 ± 14.6 years. The sex distribution was 45 women (45.9%) and 53 men (54.1%).

Regarding anthropometric characteristics, the mean height was 1.60 ± 0.01 m, the mean weight at the basal moment was 77.1 ± 16.9 kg, and the mean BMI at the basal moment was 29.8 ± 5.3 kg/m^2. Among controls, 20 (20.4%) were normal weight patients and 28 (28.6%) were overweight patients. Among cases, 31 (31.6%) were in the obese class I group, 16 (16.3%) in the obese class II group, and 3 (3.1%) in the obese class III group. At the basal moment, the mean WC was 102.1 ± 13.9 cm, the mean HC was 101.9 ± 10.8 cm, and the mean WHR was 1.00 ± 0.09.

The median previous mRS was 0 [0, 1]. The median NIHSS at admission was 9 [3, 15]. Intravenous fibrinolytic treatment was administered to 34 patients (34.7%), while 3 patients (3.1%) received endovascular treatment. The median ASPECTS value at admission was 10 [10, 10]. The mean infarct volume in the brain CT between 4th and 7th day was 61.5 ± 103.6 mL. According to TOAST criteria, 9 patients (9.2%) had a stroke attributable to large-artery atherosclerosis, 38 (38.8%) had cardioembolic strokes, 9 (9.2%) had lacunar strokes, 1 (1%) had a stroke of other cause (arterial dissection), and 41 (41.8%) had strokes of undetermined etiology.

Three months after stroke, the median mRS was 2 [1, 4], 53 patients (55.2%) had experienced good outcome, and 8 (8.3%) had died.

2. PRIMARY OBJECTIVES

2.1. COMPARATIVE ANALYSIS OF THE CLINICAL CHARACTERISTICS AND EVOLUTION

First, we compared the baseline characteristics between control and obese patients as shown in **table 6**.

No differences with respect to age and sex were found.

Regarding medical history, it should be noted that: atrial fibrillation was more frequent in obese patients (46% vs. 22.9%, p = 0.016); and, although we did not find any other statistically significant difference, the prevalences of dyslipidaemia (50% vs. 37.5%, p = 0.213) and obstructive sleep apnea (12% vs. 2.1%, p = 0.057) were higher in the obese group, and there were more smokers in the control group (29.2% vs. 16%, p = 0.118). There were no differences on previous mRS.

There were no differences in blood pressure at baseline.

	Non-obese n = 48	Obese n = 50	p
Age, years	70.1 ± 15.3	69.5 ± 14.2	0.834
Women, %	41.7	50.0	0.408
Hypertension, %	47.9	56.0	0.423
Diabetes mellitus, %	31.2	26.0	0.565
Dyslipidaemia, %	37.5	50.0	0.213
Hyperuricemia, %	10.4	14.0	0.589
Atrial fibrillation, %	22.9	46.0	0.016
Peripheral artery disease, %	2.1	4.0	0.582
Ischemic heart disease, %	12.5	8.0	0.462
Obstructive sleep apnea, %	2.1	12.0	0.057
Smoking, %	29.2	16.0	0.118
Excessive alcohol consumption, %	14.6	14.0	0.934
Previous mRS	0 [0, 1]	0 [0, 1]	0.553
SBP, mmHg	154.5 ± 24.3	149.2 ± 24.2	0.082
DBP, mmHg	81.8 ± 15.9	82.9 ± 15.2	0.831
Leukocytes, x 10^3/mL	8.1 ± 2.9	10.4 ± 2.1	< 0.001
Platelets, x 10^3/mL	227.7 ± 85.6	224.7 ± 71.2	0.850
Fibrinogen, mg/dL	405.9 ± 89.8	461.5 ± 86.9	0.005
hsCRP, mg/dL	1.6 ± 2.4	2.5 ± 2.6	0.051
Total cholesterol, mg/dL	169.6 ± 38.0	173.6 ± 40.6	0.994
HDL-cholesterol, mg/dL	43.9 ± 14.5	39.1 ± 10.0	0.024
LDL-cholesterol, mg/dL	101.2 ± 32.0	105.0 ± 36.1	0.940
Triglycerides, mg/dL	112.6 ± 55.3	141.7 ± 79.8	0.056
Blood glucose, mg/dL	137.1 ± 58.3	156.9 ± 72.9	0.363
HbA1c, %	6.1 ± 1.2	6.8 ± 1.7	0.112
NIHSS at admission	8 [2, 16]	10 [3, 14]	0.101
Intravenous fibrinolysis, %	35.4	34	0.883

Table 6. Comparative analysis of the baseline characteristics between non-obese and obese patients.

Leukocyte count (10.4 ± 2.1 x10^3/mL vs. 8.1 ± 2.9 x10^3/mL, p < 0.001), fibrinogen (461.5 ± 86.9 mg/dL vs. 405.9 ± 89.8 mg/dL, p = 0.005), and hsCRP (2.5 ± 2.6 mg/dL vs. 1.6 ± 2.4 mg/dL, p = 0.051) were higher in obese patients. No differences were found in total cholesterol and LDL-cholesterol levels, but HDL-cholesterol levels were lower (39.1 ± 10 mg/dL vs. 43.9 ± 14.5 mg/dL, p = 0.024) and triglyceride levels were higher (141.7 ± 79.8 mg/dL vs. 112.6 ± 55.3 mg/dL, p = 0.056) in obese patients compared to non-obese. Blood glucose levels (156.9 ± 72.9 mg/dL vs. 137.1 ± 58.3 mg/dL, p = 0.363) and HbA1c (6.8 ± 1.7% vs. 6.1 ± 1.2%, p = 0.112) were slightly higher in obese patients but not statistically significant.

No significant differences in NIHSS at admission between obese and control patients were found (10 [3, 14] vs. 8 [2, 16], p = 0.101) (**figure 22a**). There were no differences according to recanalization treatment between groups.

After the analysis of baseline characteristics, we compared the follow-up data between both groups of patients as shown in **table 7**.

	Non-obese n = 48	Obese n = 50	p
Difference in HbA1c at 3 months, %	0.2 ± 0.8	1.6 ± 1.7	0.210
TOAST			0.249
Atherothrombotic, %	14.6	4.0	
Cardioembolic, %	31.2	46.0	
Lacunar, %	10.4	8.0	
Undetermined, %	43.8	40.0	
Others	0	2.0	
NIHSS at 24 h	4 [1, 10]	4 [1, 13]	0.647
NIHSS at 48 h	2 [0, 9]	3 [1, 12]	0.802
NIHSS at 7th day/discharge	2 [0, 8]	2 [0, 8]	0.991
NIHSS at 3 months	0 [0, 4]	1 [0, 2]	0.693
Early neurologic deterioration, %	6.2	2.0	0.293
Clinical improvement at 3 months, %	41.4 ± 79.6	62.4 ± 54.1	0.141
mRS at discharge	3 [1, 4]	3 [1, 5]	0.882
mRS at 3 months	2 [1, 4]	2 [1, 3]	0.508
Good functional outcome, %	53.2	57.1	0.697
Mortality at 3 months, %	10.6	6.1	0.424
Infections during hospitalization, %	16.7	28.0	0.179
Hemorrhagic transformation, %	12.5	30	0.035

Table 7. Comparative analysis of the follow-up data between non-obese and obese patients.

A higher reduction in HbA1c was found in obese patients, although it did not reach statistical significance (**figure 20**).

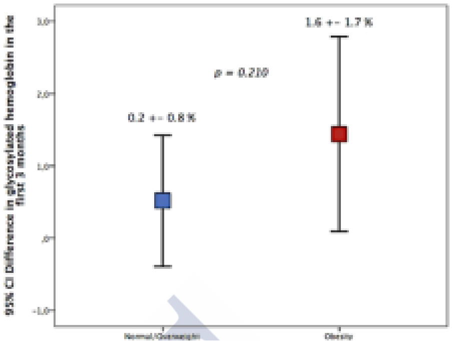


Figure 20. Comparative analysis of HbA1c reduction at 3 months between non-obese and obese patients.

No significant differences according to TOAST classification were found among groups (p = 0.249).

There were no differences in NIHSS at 24 hours, at 48 hours, at 7th day or at discharge, and at 3 months between groups (**figures 21 and 22**),

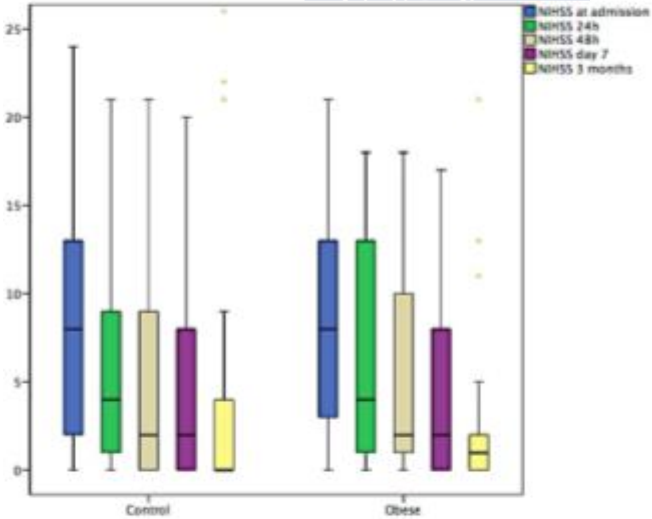


Figure 21. NIHSS (at 24h, at 48h, at 7th day, and at 3 months) in non-obese and obese patients.

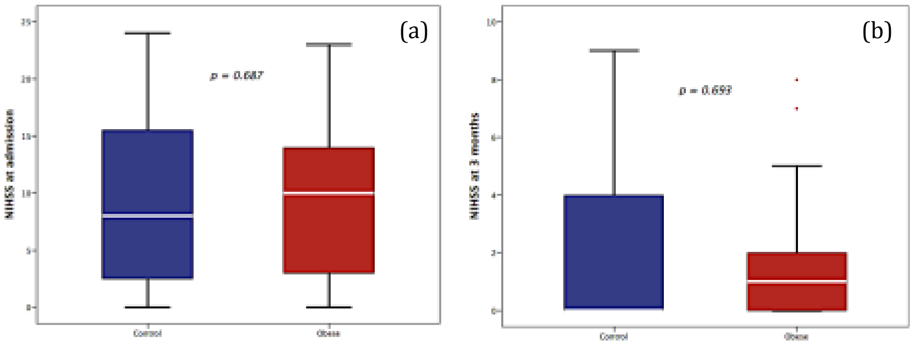


Figure 22. Comparative analysis of NIHSS (at admission (a) and at 3 months (b)) between non-obese and obese patients.

nor in the percentage of patients that suffered early neurological deterioration. The clinical improvement at 3 months was higher in obese patients (62.4 ± 54.1% vs. 41.4 ± 79.6%, p = 0.141), but it did not reach statistical significance (**figure 23a**). We performed an ANOVA test to compare the clinical improvement at 3 months in the different groups of BMI. Although there were no statistical differences (p = 0.672), there was a tendency favouring higher BMI (**figure 23b**).

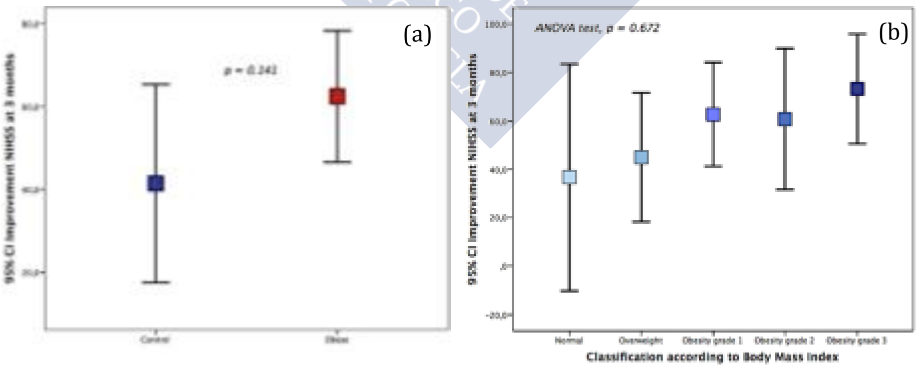


Figure 23. Comparative analysis of clinical improvement at 3 months between non-obese and obese patients (a). ANOVA test comparing clinical improvement between different groups of BMI (b).

Regarding functional outcome, we found no differences in mRS at discharge (3 [1, 4] vs. 3 [1, 5], p = 0.882) and at 3 months (2 [1, 4] vs. 2 [1, 3], p = 0.508), in good functional outcome at 3 months (53.2% vs. 57.1%, p= 0.697), and in mortality at 3 months (6.1% vs. 10.6%, 0.424) when comparing control and obese patients. In the **figure 24** the scores of mRS at 3 months in both groups are shown.

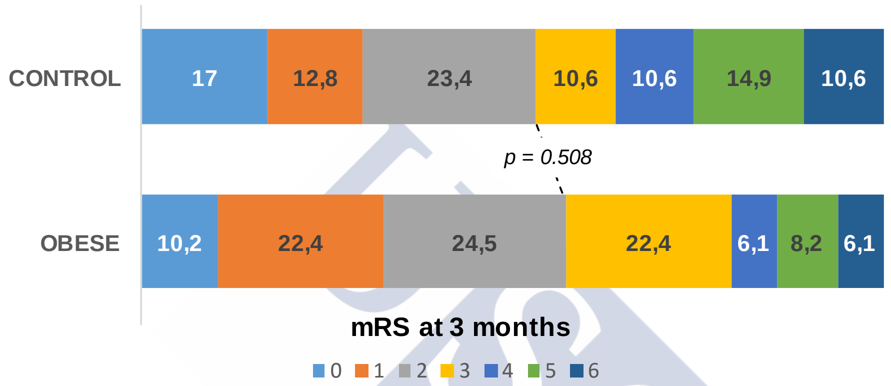


Figure 24. Comparison in mRS at 3 months between non-obese (control) and obese patients

Although not statistically significant, infections during hospitalization were more frequent in the obese group (28% vs. 16.7%, p = 0.179). The percentage of patients that exhibited hemorrhagic transformation was significantly higher in the obese group (30% vs. 12.5%, p = 0.035).

Finally, to assess the prognostic value of atrial fibrillation, difference in HbA1c at 3 months, basal leukocyte count, basal fibrinogen, HDL-cholesterol and hemorrhagic transformation in obese and non-obese patients, a non-adjusted analysis was performed. The dependent variable was bad outcome (mRS > 2) at 3 months. The ORs are shown in **table 8**. Atrial fibrillation was significantly associated with bad outcome but only in non-obese patients. Hemorrhagic transformation was associated with bad outcome in both groups of patients.

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
Atrial fibrillation	5.19	1.94 – 1.83	3.22	0.96 – 14.09
Difference in HbA1c at 3 months	0.12	0.01 – 47.01	3.36	0.61 – 18.24
Leukocytes	1.95	0.78 – 2.16	1.95	0.84 – 1.44
Fibrinogen	1.00	0.99 – 1.01	1.00	0.99 – 1.01
HDL-cholesterol	0.97	0.94 – 1.02	1.01	0.96 – 1.07
Hemorrhagic transformation	5.46	1.39 – 21.28	7.06	1.76 – 65.98

Table 8. Non-adjusted logistic regression model of the prognostic value of several clinical variables in non-obese and obese patients.

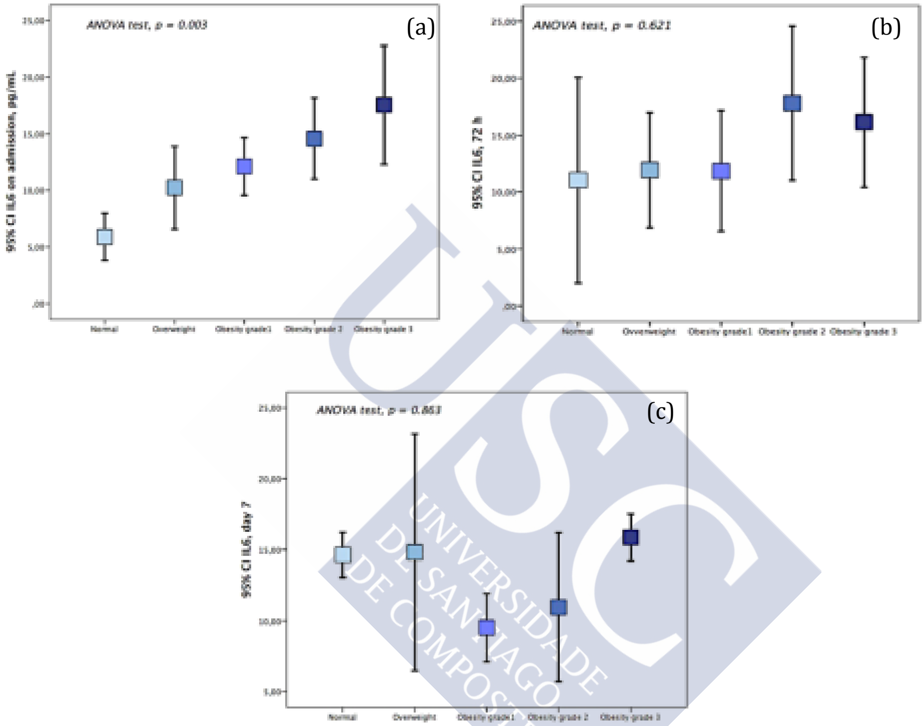
2.2. COMPARATIVE ANALYSIS OF THE INFLAMMATORY BALANCE

First, to assess the inflammatory profile of obese and non-obese patients, we analysed the levels of IL-6 and IL-10 (**table 9**).

	Non-obese	Obese	p
IL-6 admission, pg/mL	10.4 ± 9.3	14.1 ± 6.7	0.002
IL-6 72h, pg/mL	12.9 ± 16.4	12.2 ± 8.1	0.394
IL-6 7th day/discharge, pg/mL	15.2 ± 14.8	10.9 ± 5.2	0.115
IL-10 admission, pg/mL	2.6 ± 1.4	7.1 ± 2.1	< 0.001
IL-10 72h, pg/mL	2.1 ± 0.9	7.8 ± 2.1	< 0.001
IL-10 7th day/discharge, pg/mL	2.3 ± 1.1	8.4 ± 1.6	< 0.001

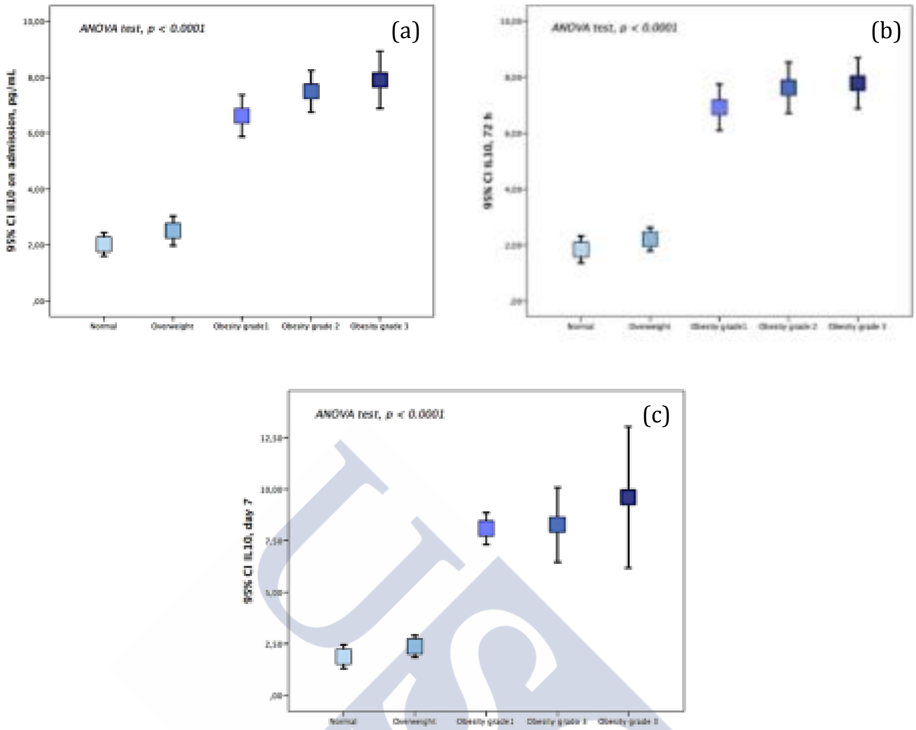
Table 9. Comparative analysis of IL-6 and IL-10 levels (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

On admission, the levels of IL-6 were significantly lower in controls compared to obese patients (10.4 ± 9.3 pg/mL vs. 14.1 ± 6.7 pg/mL, p = 0.002). However, at 72 hours and at 7th day/discharge the levels of IL-6 increased in non-obese while they decreased in obese, although the difference between groups did not reach statistical significance. We performed ANOVA tests to compare the levels of IL-6 at the 3 sampling moments in the different groups of BMI. On admission, the levels of this



cytokine increased with BMI (p = 0.003) (**figure 25a**). No correlation with BMI was found at 72 hours (p = 0.621) (**figure 25b**) and at 7th day/discharge (p = 0.863) (**figure 25c**).

The levels of IL-10 were significantly lower in non-obese compared to obese patients at the 3 sampling moments. We performed ANOVA tests to



compare the levels of IL-10 at the 3 sampling moments in the different groups of BMI. We found a significant and positive correlation with BMI on admission (p < 0.001) (**figure 26a**), at 72 hours (p < 0.001) (**figure 26b**), and at 7th day/discharge (p < 0.001) (**figure 26c**).

ANOVA test, p < 0.001

ANOVA test, p < 0.001

To assess the different prognostic value of IL-6 and IL-10 in obese and non-obese patients, a non-adjusted analysis was performed. The dependent variable was bad outcome at 3 months. No significant associations were found (**table 10**).

ANOVA test, p < 0.001

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
IL-6 admission	[illegible]	0.98 – 1.15	1.07	0.97 – 1.17
IL-10 admission	1.44	0.81 – 2.56	0.96	0.85 – 1.46
IL-10 72h	1.12	0.53 – 2.37	0.96	0.93 – 1.99

IL-10 7th/discharge	2.36	0.87 – 6.36	0.77	0.45 – 7.33

Table 10. Non-adjusted logistic regression model of the prognostic value of IL-6 and IL-10 in non-obese and obese patients.

After the analysis of IL-6 and IL-10 levels and, as mentioned above, to determine the balance between anti- and pro-inflammatory cytokines we defined the anti/pro-inflammatory index according to the following formula: IL-10 on admission decile/IL-6 on admission decile.

The analysis showed that the values of anti/pro-inflammatory index were higher in obese compared to non-obese patients (1.3 ± 0.5 vs. 1.1 ± 0.8, p = 0.203) (**figure 27a**), although not statistically significant. An ANOVA test was performed to compare the anti/pro-inflammatory index in the different groups of BMI. We found a non-statistically significant trend with higher values in overweight and obese patients (**figure 27b**).

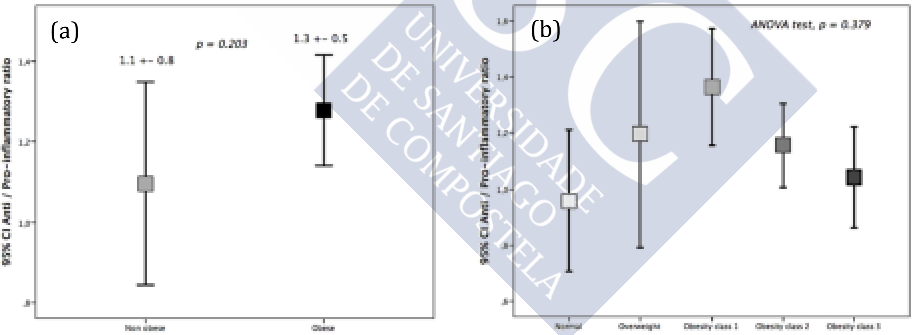


Figure 26. Comparative analysis of the anti/pro-inflammatory index between non-obese an obese patients (a). ANOVA test comparing the values of this index between different groups of BMI (b).

Then, we analyzed the association of this index with outcome and we found that it was higher in patients with good outcome (mRS ≤ 2) compared to patients with bad outcome (mRS > 2) (1.3 ± 0.8 vs. 1.0 ± 0.6, p = 0.037) (**figure 28**).

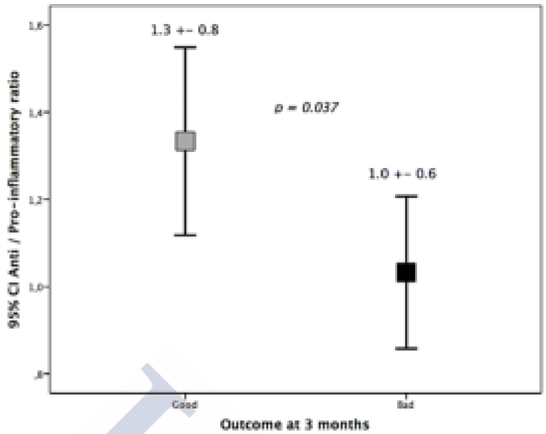


Figure 27. Comparative analysis of the anti/pro-inflammatory index between groups of good and bad outcome.

Finally, to assess the prognostic value of the anti/pro-inflammatory index in obese and non-obese patients, a non-adjusted analysis was performed. The dependent variable was bad outcome at 3 months. Although a trend favouring good outcome was found in both groups, the associations were not significant (**table 11**).

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
Anti/pro-inflammatory index	0.53	0.24 – 1.21	0.41	0.11 – 1.51

Table 11. Non-adjusted logistic regression model of the prognostic value of the anti/pro-inflammatory index in non-obese and obese patients.

3. SECONDARY OBJECTIVES

3.1. COMPARATIVE ANALYSIS OF INFARCT VOLUMES

Regarding baseline neuroimaging characteristics, we did not find differences between obese and non-obese patients in the ASPECTS value at admission CT (10 [9, 10] vs. 10 [10, 10], p = 0.061), nor in the infarct volume between 4th and 7th day (61.5 ± 104.7 cm³ vs. 61.4 ± 103.4 cm³, p = 0.994) (**table 12**) (**figure 29a**).

	Non-obese n = 48	Obese n = 50	p
ASPECTS	10 [10, 10]	10 [9, 10]	0.061
Volume 4th-7th day, cm³	61.4 ± 103.4	61.5 ± 104.7	0.994

Table 12. Comparative analysis of baseline neuroimaging characteristics between non-obese and obese patients.

In the follow-up, no significant differences between non-obese and obese patients in infarct volume at 3 months were found (38.5 ± 68.5 cm³ vs. 46.5 ± 86.3 cm³, p = 0.664) (**table 13**)(**figure 29a**). There were no significant differences in volume improvement at 3 months (52.7 ± 32.5% vs. 45.6 ± 23.1%, p = 0.300) (**figure 29b**).

	Non-obese n = 48	Obese n = 50	p
Volume at 3 months, cm³	38.5 ± 68.5	46.5 ± 86.3	0.664
Volume improvement, %	52.7 ± 32.5	45.6 ± 23.1	0.300

Table 13. Comparative analysis of the follow-up neuroimaging characteristics between non-obese and obese patients.

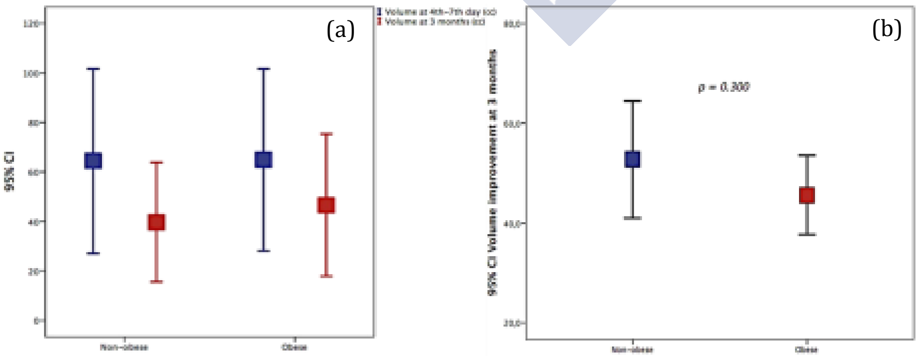


Figure 28. Comparative analysis of infarct volume at baseline and at 3 months between non-obese and obese patients (a). Comparative analysis of volume improvement at 3 months between non-obese and obese patients (b).

3.2. ANTHROPOMETRIC STUDY

We decided to make a more comprehensive analysis of the anthropometric characteristics. The baseline data of obese and control patients are shown in **table 14**.

	Non-obese n = 48	Obese n = 50	p
Height, m	1.59 ± 0.08	1.61 ± 0.10	0.417
Weight, kg	65.0 ± 9.4	88.7 ± 14.1	< 0.001
BMI, kg/m^2	25.4 ± 2.5	34.1 ± 3.6	< 0.001
WC, cm	91.6 ± 8.6	112.1 ± 10.2	< 0.001
HC, cm	94.8 ± 6.8	108.8 ± 9.4	< 0.001
WHR	0.97 ± 0.08	1.03 ± 0.08	0.001

Table 14. Comparative analysis of anthropometric characteristics between non-obese and obese patients.

The comparative analysis of the anthropometric follow-up data between obese and control patients is shown in **table 15**.

	Non-obese n = 48	Obese n = 50	p
Weight difference, kg	0.9 ± 5.6	5.7 ± 9.1	0.007
Weight difference, %	1.2 ± 8.6	6.6 ± 10.1	0.013
Difference in BMI, kg/m^2	0.5 ± 2.3	2.3 ± 3.6	0.008
Difference in BMI, %	1.5 ± 9.1	6.7 ± 10.1	0.020
Difference in WC, %	-0.7 ± 6.3	4.7 ± 8.2	0.002
Difference in HC, %	-1.3 ± 5.6	1.4 ± 6.1	0.041
Difference in WHR, %	0.5 ± 5.1	3.3 ± 6.4	0.038

Table 15. Comparative analysis of anthropometric follow-up data between non-obese and obese patients.

After 3 months there was a reduction in body weight in both groups, which was higher in obese compared to non-obese patients (5.7 ± 9.1 kg vs. 0.9 ± 5.6 kg, $p = 0.007$) (**figure 30**).

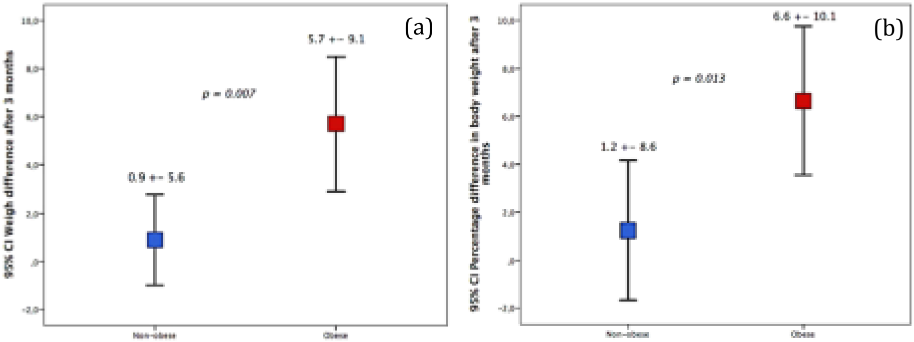


Figure 29. Comparative analysis of reduction in body weight after 3 months between non-obese and obese patients. Absolute values (a) and percentages (b).

Similar findings as for body weight were detected for BMI (**figure 31**).

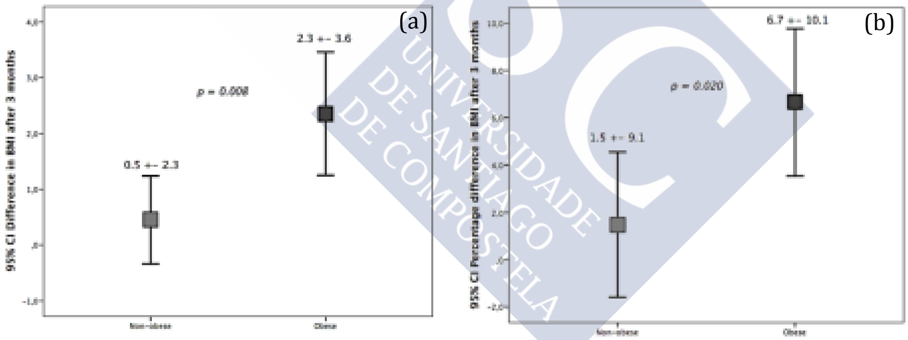


Figure 30. Comparative analysis of reduction in BMI after 3 months between non-obese and obese patients. Absolute values (a) and percentages (b).

However, WC and HC decreased only in obese patients, while these measures increased slightly in the non-obese group (**figure 32**).

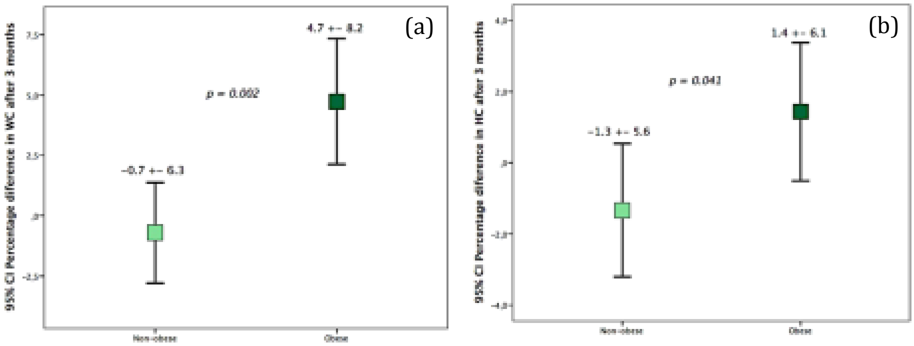


Figure 31. Comparative analysis of changes in WC (a) and HC (b) after 3 months between non-obese and obese patients.

We also found that the reduction of WC in obese patients was significantly higher compared to the reduction in HC (**figure 33**).

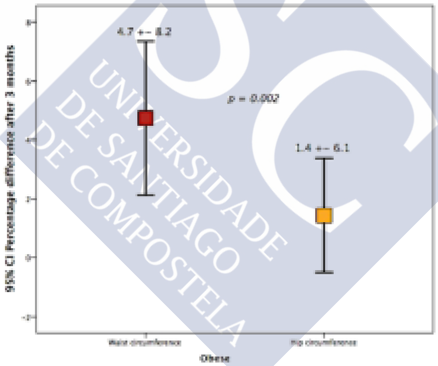


Figure 32. Comparative analysis of the reduction in WC and HC after 3 months in obese patients.

The reduction in WHR was higher in obese patients than in non-obese (**figure 34**).

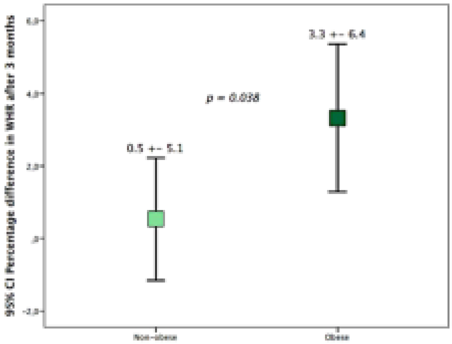


Figure 33. Comparative analysis of the reduction of WHR after 3 months in non-obese and obese patients.

Then, we analysed the association between anthropometric follow-up data and outcome. The comparative analysis is shown in **table 16**.

	Good outcome	Bad outcome	p
Weight difference, kg	1.7 ± 6.7	6.8 ± 9.1	0.006
Weight difference, %	2.0 ± 7.6	8.2 ± 11.9	0.007
Difference in BMI, kg/m^2	0.7 ± 2.6	2.8 ± 3.7	0.004
Difference in BMI, %	2.0 ± 7.7	8.4 ± 12.2	0.006
Difference in WC, %	1.3 ± 6.3	3.4 ± 9.8	0.246
Difference in HC, %	0.03 ± 5.4	0.2 ± 6.9	0.895
Difference in WHR, %	1.2 ± 5.8	3.4 ± 5.9	0.122

Table 16. Comparative analysis of the anthropometric follow-up data between good and bad outcome groups.

Reductions in body weight (**figure 35**) and BMI (**figure 36**) were statistically higher in the bad outcome group.

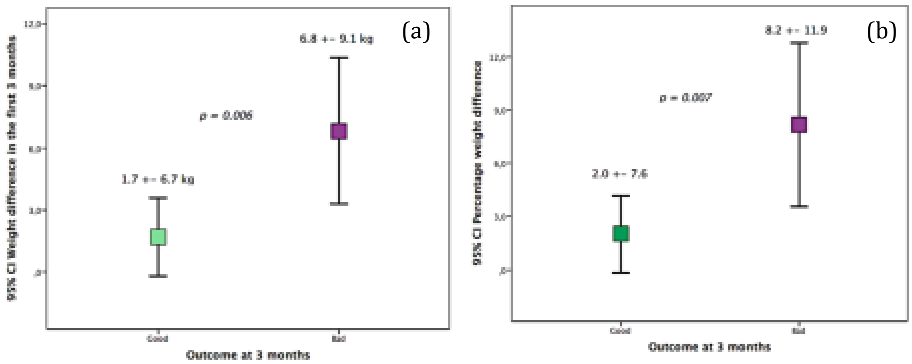


Figure 35. Comparative analysis of reduction in body weight after 3 months between good and bad outcome groups. Absolute values (a) and percentages (b).

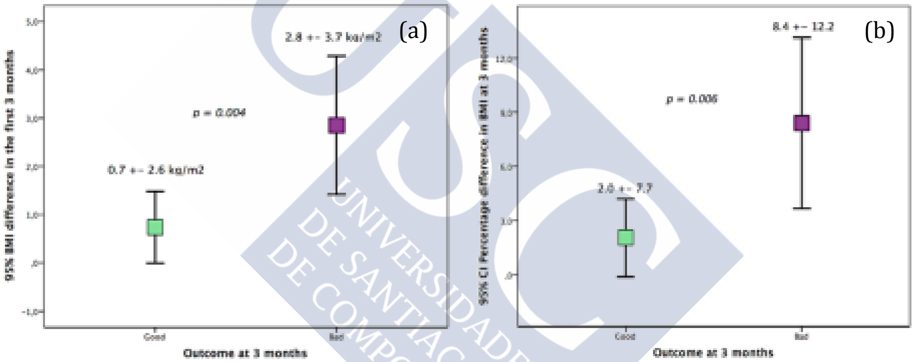


Figure 34. Comparative analysis of reduction in BMI after 3 months between good and bad outcome groups. Absolute values (a) and percentages (b).

Patients with bad outcome also experienced a higher reduction in WC (**figure 37a**) and WHR (**figure 37c**), although it did not reach statistical significance. There were no differences in evolution of HC (**figure 37b**).

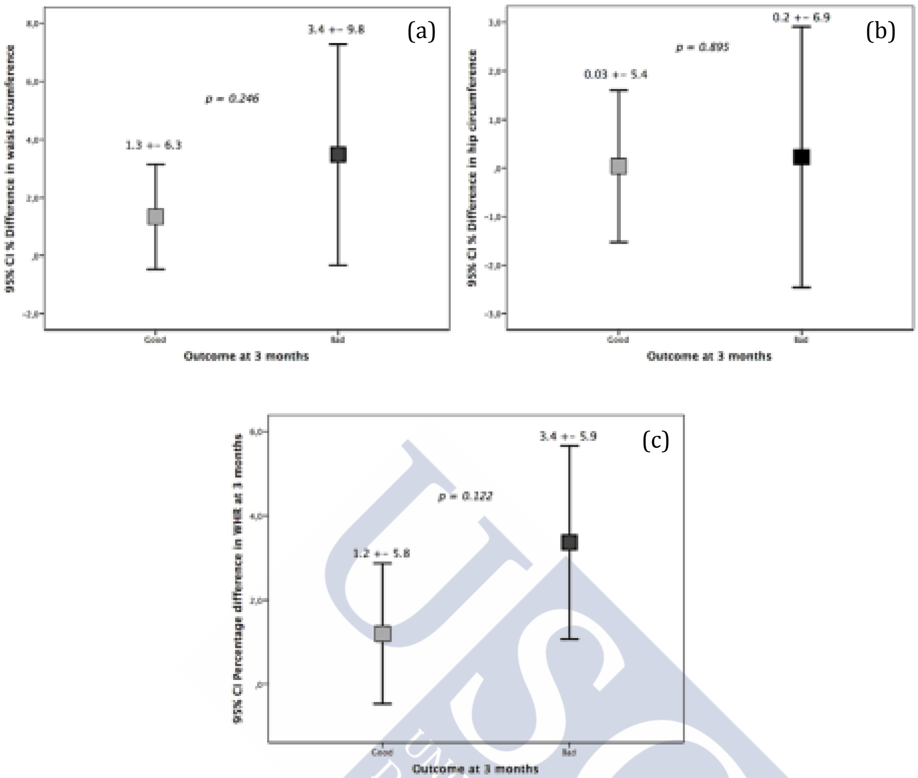


Figure 36. Comparative analysis of changes in WC (a), HC (b), WHR (c), between good and bad outcome groups.

Finally, to assess the prognostic value of anthropometric follow-up data in obese and non-obese patients, a non-adjusted analysis was performed as shown in **table 17.** Bad outcome at 3 months was selected as dependent variable. In obese patients, reductions in body weight, BMI and WC were significantly associated with bad outcome at 3 months. No significant associations were found for non-obese patients.

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
Weight difference	1.04	0.92 – 1.18	1.12	1.02 – 1.22
% Weight difference	1.02	0.94 – 1.11	1.12	1.03 – 1.22
Difference in BMI	1.12	0.83 – 1.52	1.34	1.07– 1.67
% Difference in BMI	1.03	0.95 – 1.11	1.12	1.03 – 1.22
% Difference in WC	0.95	0.85 – 1.06	1.10	1.01 – 1.20
% Difference in HC	0.89	0.78 – 1.02	1.10	0.98 – 1.25
% Difference in WHR	1.07	0.92 – 1.23	1.07	0.96 – 1.19

Table 17. Non-adjusted logistic regression model of the prognostic value of anthropometric follow-up data in non-obese and obese patients.

3.2.1. SUBSTUDY IN PATIENTS WITH DEXA

With the aim of analysing accurately body fat distribution, a selection of 16 non-obese and obese patients (5 and 11 respectively) underwent DEXA. The comparative study is shown in **table 18**.

	Non-obese n = 5	Obese n = 11	p
Age, years	54.4 ± 20.0	63.6 ± 13.4	0.290
Women, n	2	6	0.590
Bone mineral content, g	2379.8 ± 316.8	2451.8 ± 680.4	0.827
Bone mineral density, g/cm^2	1.14 ± 0.07	1.12 ± 0.17	0.744
Lean body mass, g	37720.8 ± 5381.5	48374.0 ± 10026.7	0.045
Body fat, g	21486.0 ± 2478.7	35828.4 ± 8271.9	< 0.001
Body fat, %	35.2 ± 4.3	43.2 ± 7.7	0.020
Android fat distribution, %	38.6 ± 4.1	52.3 ± 7.4	< 0.001
Gynoid fat distribution, %	35.7 ± 6.1	41.9 ± 9.6	0.209
Android fat/gynoid fat ratio	1.1 ± 0.2	1.3 ± 0.2	0.087
Fat in arms, %	35.1 ± 9.5	39.2 ± 10.6	0.482
Fat in legs, %	34.2 ± 7.4	38.1 ± 9.6	0.441
Fat in trunk, %	36.9 ± 3.3	49.1 ± 7.6	0.001
Trunk fat/total body fat ratio	0.6 ± 0.1	0.6 ± 0.1	0.130
Legs fat/total body fat ratio	0.3 ± 0.1	0.3 ± 0.1	0.369
Arms and legs fat/trunk fat ratio	0.8 ± 0.3	0.6 ± 0.1	0.124
Visceral fat volume, cm^3	1088.6 ± 849.8	2692.4 ± 896.8	0.005
Visceral fat mass, g	1027.0 ± 801.5	2540.1 ± 845.9	0.005

Table 18. Comparative analysis of DEXA characteristics between non-obese and obese patients.

No significant differences between age and sex were found among groups. In the obese group, the amount of lean body mass, the amount of body fat and the percentage of body fat were higher compared to control group. The visceral fat volume, the visceral fat mass, the percentage of fat in trunk, the percentage of android fat distribution and the android fat/gynoid fat ratio were higher in obese patients compared to non-obese patients, although the latter did not reach statistical significance.

Among these patients, we carried an analysis about body fat distribution and severity of neurologic impairments. In relation to the percentage of android fat distribution and the percentage of trunk fat, patients were dichotomized according to whether they had a percentage of such fat distribution lower than 45% or equal and higher than 45%. In relation to visceral fat mass, patients where dichotomized according to whether they had an amount of visceral fat lower than 2000 g or 2000 g and more. There were no significant differences in stroke severity in relation to percentage of android fat distribution (p = 0.443) (**figure 38a**), percentage of trunk fat (p = 0.791) (**figure 38b**), and visceral fat mass (p = 0.631) (**figure 38c**).

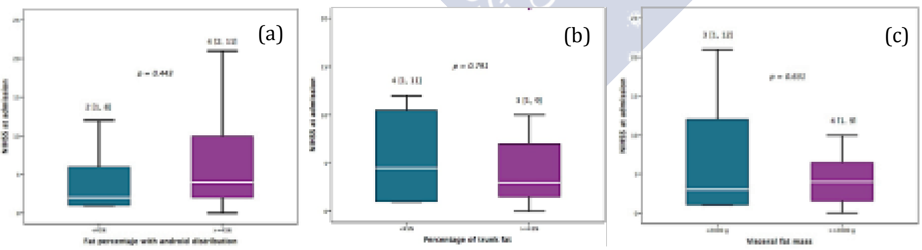


Figure 37. Comparative analysis of fat distribution and severity of neurological impairments (fat percentage with android distribution (a), percentage of trunk fat (b), visceral fat mass (c)).

Finally, we performed an analysis about body fat distribution and functional outcome. There were no significant differences in functional outcome in relation to percentage of android fat distribution (p = 0.864) (**figure 39a**), percentage of trunk fat (p = 0.294) (**figure 39b**), and visceral fat mass (p = 0.243) (**figure 39c**).

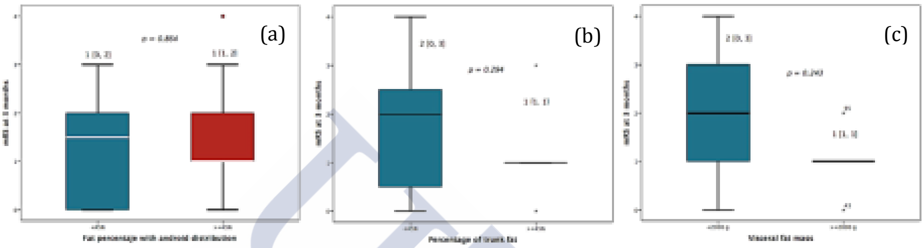


Figure 38. Comparative analysis of fat distribution and functional outcome (fat percentage with android distribution (a), percentage of trunk fat (b), visceral fat mass (c)).

3.3. COMPARATIVE ANALYSIS OF TEMPERATURE

To assess the variations in body temperature among groups and its influence in prognosis, we registered axillary temperature every 6 hours for the first 48 hours.

In **figure 40**, we can see mean and maximum body temperatures in both groups during the first 48 hours. These figures show a trend indicating higher mean and maximum temperatures in obese compared to non-obese patients.

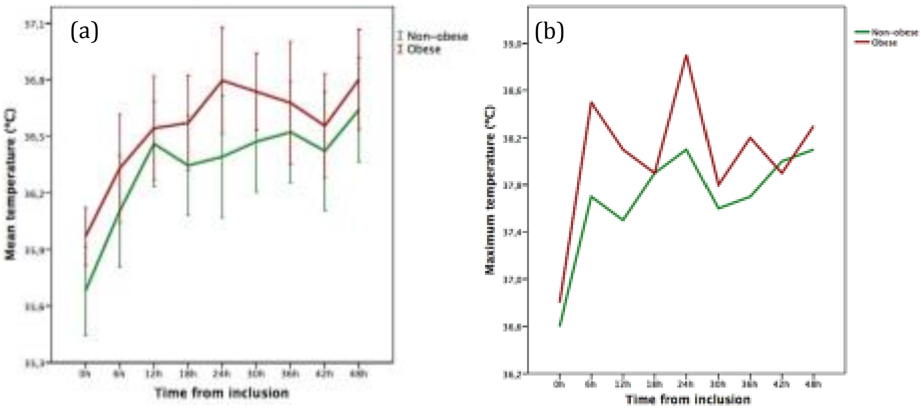


Figure 39. Comparison of mean (a) and maximum (b) temperatures during the first 48h between non-obese and obese patients.

To perform a comparative analysis of body temperature between groups, we selected baseline axillary temperature, maximum temperature within the first 24 hours, maximum temperature within 24 to 48 hours, and presence of hyperthermia in the first 24 hours (defined as an axillary temperature equal or higher to 37.5°C) (**table 19**).

	Non-obese	Obese	p
Baseline axillary temperature, °C	35.6 ± 0.6	35.9 ± 0.5	0.033
Maximum temperature 24 h, °C	36.7 ± 0.6	37.0 ± 0.6	0.033
Maximum temperature 24-48 h, °C	36.8 ± 0.6	37.4 ± 0.6	0.233
Hyperthermia 24 h, %	12.5	28	0.057

Table 19. Comparative analysis of body temperature between non-obese and obese patients.

Axillary temperature at baseline was slightly but statistically higher in obese patients (**figure 41a**). The maximum temperatures during the first 24 hours (**figure 41b**) and during 24 to 48 hours were higher in obese compared to control patients, but no statistical significance was found for the latter (**figure 41c**). The presence of hyperthermia in the first 24 hours, was higher in obese compared to non-obese patients, although it did not reach statistical significance.

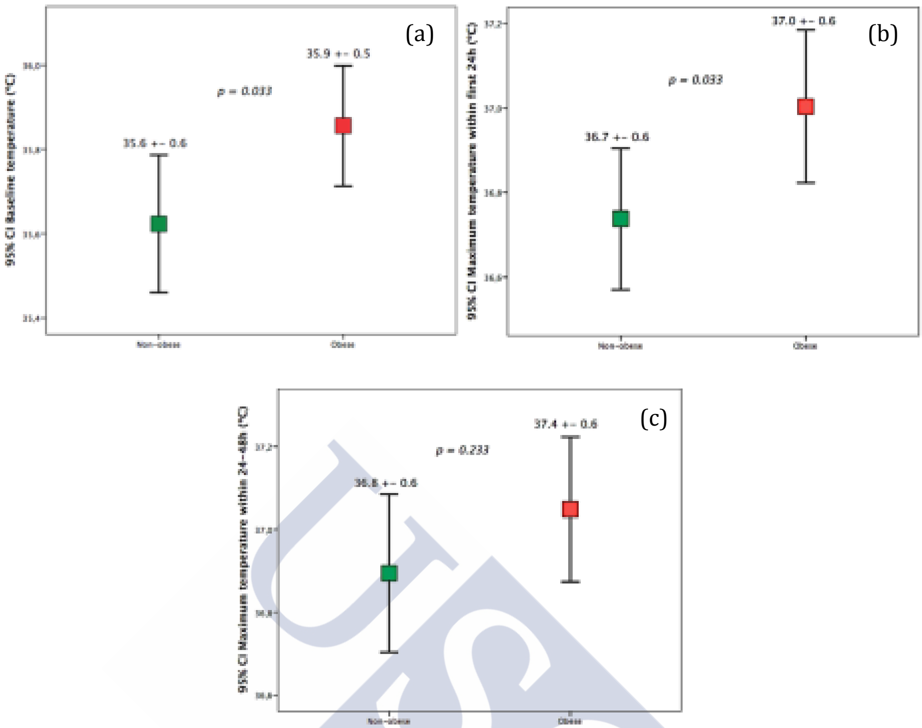


Figure 40. Comparative analysis of baseline axillary temperature (a), maximum temperature within 24h (b) and maximum temperature within 24-48h (c) between non-obese and obese patients.

Then, we conducted a comparative analysis of body temperature between patients with good outcome and bad outcome at 3 months in relation to BMI (**table 20**).

	Non-obese			Obese		
	Good outcome	Bad outcome	p	Good outcome	Bad outcome	p
Baseline axillary temperature, °C	35.6 ± 0.5	35.7 ± 0.6	0.405	35.8 ± 0.5	36.0 ± 0.4	0.346
Maximum temperature 24 h, °C	36.5 ± 0.4	37.0 ± 0.6	0.001	36.8 ± 0.6	37.2 ± 0.6	0.031

Table 20. Comparative analysis of temperatures between groups of good and bad outcome, for non-obese and obese patients respectively.

The analysis showed that, although there were no differences in baseline temperature in prognostic groups, the maximum temperature during the first 24 hours was higher in the patients with bad outcome for both non-obese (**figure 42a**) and obese patients (**figure 42b**). However, it appears that the influence of temperature in prognosis was higher for non-obese compared to obese patients.

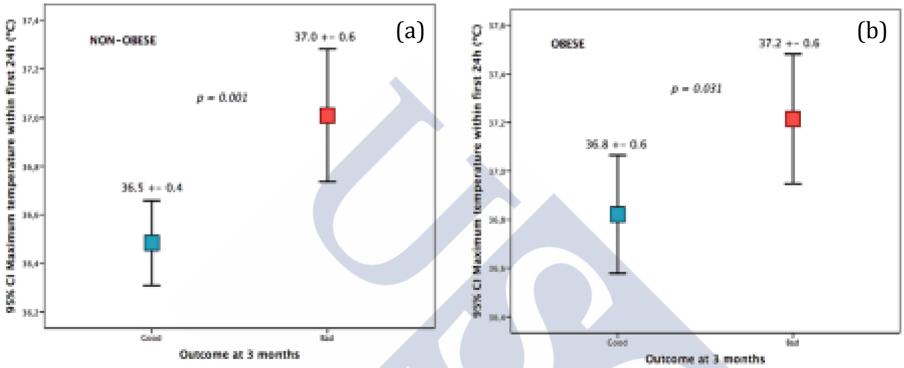


Figure 41. Comparative analysis of maximum temperature within the first 24h between good and bad outcome groups, for non-obese (a) and obese (b) patients.

To assess the prognostic value of temperature in obese and non-obese patients, a non-adjusted analysis was performed. The dependent variable was bad outcome at 3 months. The ORs are shown in **table 21**. Only maximum temperature during the first 24 hours was significantly associated with bad outcome in both groups of patients and the power of the association was higher for non-obese patients.

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
Baseline axillary temperature, °C	1.57	0.55 – 4.52	1.87	0.52 – 6.76
Maximum temperature 24 h, °C	6.78	1.82 – 25.23	2.94	1.06 – 8.20

Table 21. Non-adjusted logistic regression model of the prognostic value of baseline axillary temperature and maximum temperature within the first 24h in non-obese and obese patients.

Finally, we conducted a multivariate analysis adjusted by the presence of infections during hospitalization. The dependent variable was bad outcome at 3 months. The ORs are shown in **table 22**. In this case, maximum temperature during the first 24 hours was independently associated with bad outcome only in non-obese patients.

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
Baseline axillary temperature, °C	1.24	0.39 – 3.94	2.35	0.58 – 9.58
Maximum temperature 24 h, °C	7.62	1.4 – 40.96	2.36	0.80 – 7.02

Table 22. Logistic regression model of the prognostic value of baseline axillary temperature and maximum temperature within the first 24h in non-obese and obese patients, adjusted by the presence of infections during hospitalization.

3.4. COMPARATIVE ANALYSIS OF CELL AND MOLECULAR PROFILES

3.4.1. PROGENITOR CELLS

We analysed the levels of circulating progenitor cells (CD34+/CD45-/CD31-) in both groups of patients. The comparative study is shown in **table 23** and **figure 43**. The levels of these cells were higher in obese compared to non-obese patients.

	Non-obese	Obese	p
CD34+/CD45-/CD31- on admission, /250.000 lymphocytes	1035.5 ± 2416.1	2232.5 ± 2716.5	0.226
CD34+/CD45-/CD31- 72h, /250.000 lymphocytes	662.9 ± 1436.2	2359.5 ± 2601.6	0.034
CD34+/CD45-/CD31- 7th day/discharge, /250.000 lymphocytes	947.2 ± 1656.8	2388.6 ± 2795.8	0.029

Table 23. Comparative analysis of the levels of circulating progenitor cells (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

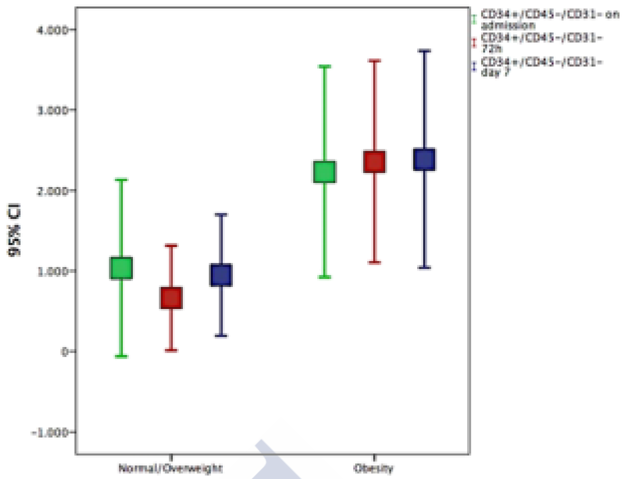


Figure 42. Comparative analysis of the levels of circulating progenitor cells (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

We performed an analysis of frequencies (**figure 44**) in which we can see that the most common number of circulating cells was higher in the obesity group for every sample.

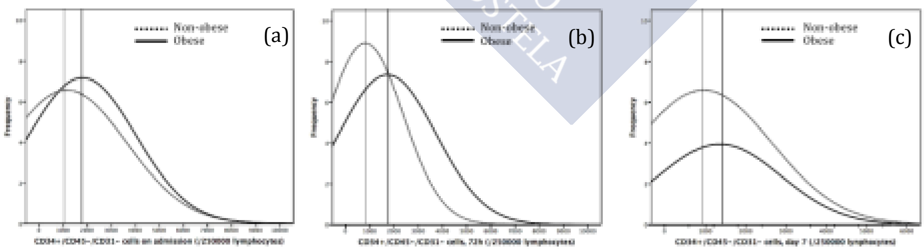


Figure 43. Analysis of frequencies of circulating progenitor cells at admission (a), 72h (b) and 7th day/discharge (c), comparing non-obese and obese patients.

3.4.2. GROWTH FACTORS: VEGF

The serum levels of VEGF in obese and non-obese patients are shown in **table 24** and **figure 45**. There were no differences.

	Non-obese	Obese	p
VEGF admission, pg/mL	582.3 ± 822.8	529.5 ± 497.1	0.706
VEGF 72h, pg/mL	392.4 ± 495.2	439.8 ± 435.7	0.959
VEGF 7th day/discharge, pg/mL	471.4 ± 603.7	400.2 ± 364.2	0.852

Table 24. Comparative analysis of serum VEGF levels (at admission, 72h, and 7th day/discharge) between non-obese and obese patients.

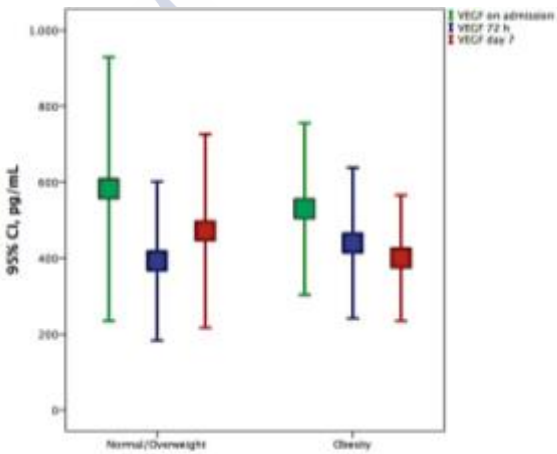


Figure 44. Comparative analysis of serum VEGF levels (at admission, 72h and 7th day/discharge), between non-obese and obese patients.

3.4.3. INNATE IMMUNITY: TLR2 AND TLR4

The comparative analysis of TLR2 expression on monocytes (TLR2M), TLR2 expression on neutrophils (TLR2N), TLR4 expression on monocytes (TLR4M), and TLR4 expression on neutrophils (TLR4N) between obese and non-obese patients is shown in **table 25** and **figure 46**. The expression of TLRs was lower in obese patients, although it only reached statistical significance for monocyte TLR4 expression on admission.

	Non-obese	Obese	p
TLR2M admission, UAF	802.3 ± 547.7	581.4 ± 424.7	0.110
TLR2M 72h, UAF	729.6 ± 495.9	645.1 ± 442.7	0.834
TLR2M 7th day/discharge, UAF	1142.6 ± 927.9	812.9 ± 759.1	0.279
TLR2N admission, UAF	495.1 ± 255.3	390.2 ± 211.1	0.725
TLR2N 72 h, UAF	437.6 ± 301.2	390.2 ± 226.6	0.849
TLR2N 7th day/discharge, UAF	476.2 ± 283.2	433.7 ± 334.2	0.573
TLR4M admission, UAF	4538.8 ± 3390.8	3051.6 ± 3666.8	0.033
TLR4M 72h, UAF	4861.7 ± 4900.1	3419.3 ± 3396.4	0.250
TLR4M 7th day/discharge, UAF	5403.6 ± 4036.3	5338.6 ± 8742.4	0.900
TLR4N admission, UAF	2543.5 ± 2346.0	1870.1 ± 2214.6	0.130
TLR4N 72 h, UAF	2407.7 ± 2034.3	1873.9 ± 2151.2	0.166
TLR4N 7th day/discharge, UAF	2664.9 ± 2365.8	2374.6 ± 2590.9	0.546

Table 25. Comparative analysis of TLR2 and TLR4 expression on monocytes and neutrophils (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

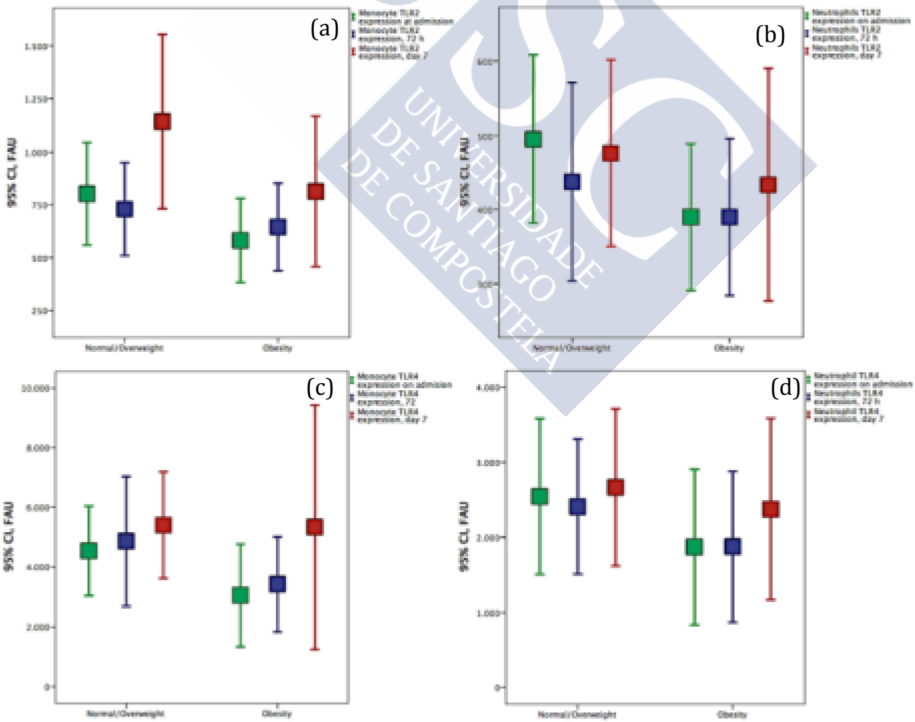


Figure 45. Comparative analysis of TLR2 expression on monocytes (a) and neutrophils, and TLR4 expression on monocytes (c) and neutrophils (d) (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

3.4.4. INFLAMMATORY MEDIATORS: TNFR-I and -II, MCP-1 AND MIP-1β

The comparative analysis of TNFR-I and TNFR-II and the chemokines MCP-1 and MIP-1β is shown in **table 26.**

	Non-obese	Obese	p
TNFR-I admission, ng/mL	7.3 ± 3.8	4.4 ± 2.3	< 0.001
TNFR-I 72h, ng/mL	7.4 ± 3.7	4.9 ± 2.8	0.002
TNFR-I 7th day/discharge, ng/mL	6.9 ± 2.3	5.4 ± 2.1	0.047
TNFR-II admission, pg/mL	364.2 ± 210.1	884.5 ± 375.9	< 0.001
TNFR-II 72h, pg/mL	385.0 ± 247.2	843.7 ± 418.6	< 0.001
TNFR-II 7th day/discharge, pg/mL	436.7 ± 468.1	878.2 ± 457.3	< 0.001
MCP-1 admission, pg/mL	1109.1 ± 563.0	1004.6 ± 485.3	0.321
MCP-1 72h, pg/mL	923.4 ± 369.7	905.6 ± 609.4	0.420
MCP-1 7th day/discharge, pg/mL	1052.3 ± 296.8	854.1 ± 384.7	0.082
MCP-1β admission, pg/mL	62.6 ± 67.8	42.9 ± 53.6	0.220
MCP-1β 72h, pg/mL	34.9 ± 16.7	37.7 ± 39.6	0.772
MCP-1β 7th day/discharge, pg/mL	44.8 ± 27.6	37.7 ± 39.6	0.824

Table 26. Comparative analysis of the serum levels of TNFR-I and -II, MCP-1 and MIP-1β (at admission, 72h and 7th day/discharge)between non-obese and obese patients.

Levels of TNFR-I were significantly lower in obese patients (**figure 47a**), whereas levels of TNFR-II were significantly higher in obese patients (**figure 47b**). We performed ANOVA tests to compare the TNFR-I and -II levels on admission in the different groups of BMI. The levels of TNFR-I were lower in the upper groups of BMI (**figure 48a**) whereas levels of TNFR-II (**figure 48b**) were higher in the upper.

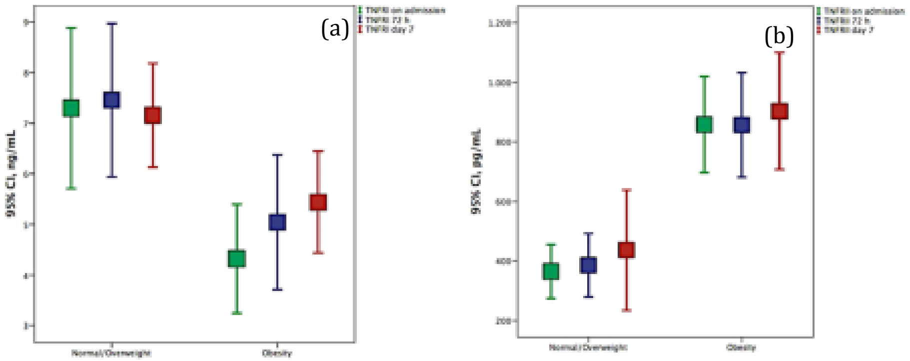


Figure 47. Comparative analysis of serum levels of TNFR-I (a) and TNFR-II (b) (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

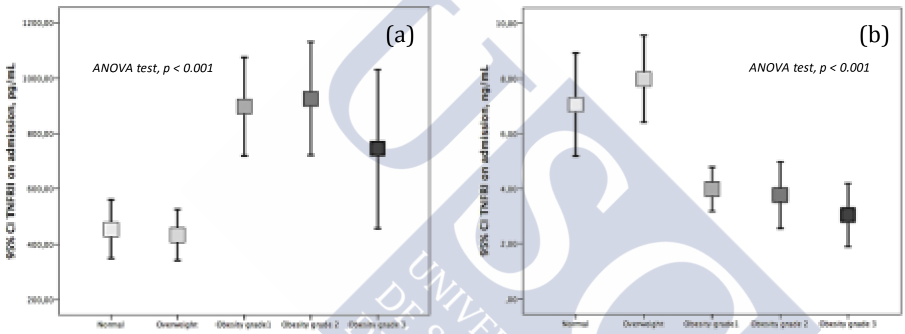


Figure 46. ANOVA tests comparing serum levels of TNFR-I (a) and TNFR-II (b) at admission between different groups of BMI.

Although the levels of MCP-1 (**figure 49a**) and MIP-1β (**figure 49b**) were slightly lower in obese patients, this was not significant.

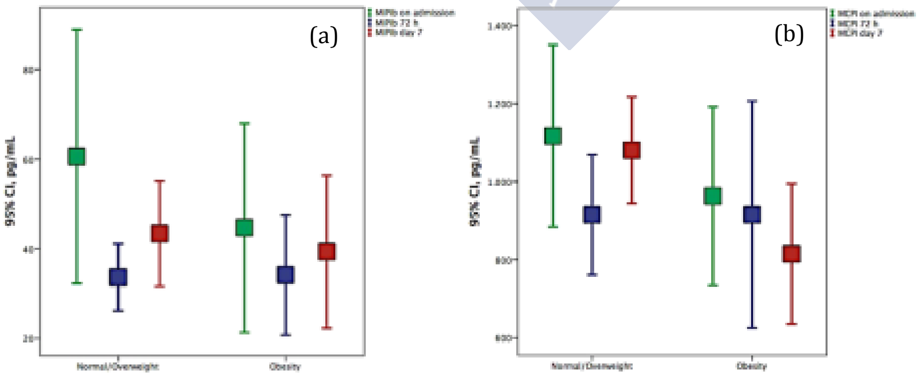


Figure 48. Comparative analysis of the serum levels of MCP-1 (a) and MIP-1β (b) (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

Finally, to assess the prognostic value of TNFR-I and -II in obese and non-obese patients, a non-adjusted analysis was performed (**table 27**). Bad outcome at 3 months was selected as dependent variable. No significant associations were found between TNFRs and outcome in any group of patients.

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
TNFR-I admission	0.99	0.84 – 1.16	1.28	0.95 - 1.74
TNFR-I 72h	1.18	0.96 – 1.44	1.26	0.98 - 1.59
TNFR-I 7th day/discharge	0.95	0.76 – 1.18	1.19	0.94 - 1.73
TNFR-II admission	0.99	0.99 – 1.00	1.66	0.85 - 2.15
TNFR-II 72h	0.99	0.99 – 1.00	1.74	0.82 - 2.25
TNFR-II 7th day/discharge	0.98	0.97 – 1.00	2.02	0.74 - 3.15

Table 27. Non-adjusted logistic regression model of the prognostic value of TNFR-I and TNFR-II levels, in non-obese and obese patients.

3.4.5. ADIPOKINES: LEPTIN AND ADIPONECTIN

The comparative analysis of leptin and adiponectin in obese and non-obese patients is shown in **table 28**.

	Non-obese	Obese	p
Leptin admission, ng/mL	21.9 ± 19.1	65.1 ± 32.3	< 0.001
Leptin 72h, ng/mL	28.0 ± 28.6	62.6 ± 33.2	< 0.001
Leptin 7th day, ng/mL	25.5 ± 29.3	60.1 ± 43.4	0.001
Adiponectin admission, µg/mL	8.9 ± 3.7	11.6 ± 10.1	0.557
Adiponectin 72h, µg/mL	9.0 ± 3.8	11.4 ± 7.4	0.130
Adiponectin 7th day, µg/mL	9.4 ± 6.2	10.6 ± 4.3	0.408

Table 28. Comparative analysis of serum levels of leptin and adiponectin (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

The expression of leptin was significantly higher in obese patients (**figure 50a**). We performed an ANOVA test to compare the leptin levels on admission in the different groups of BMI, and we found higher levels of this

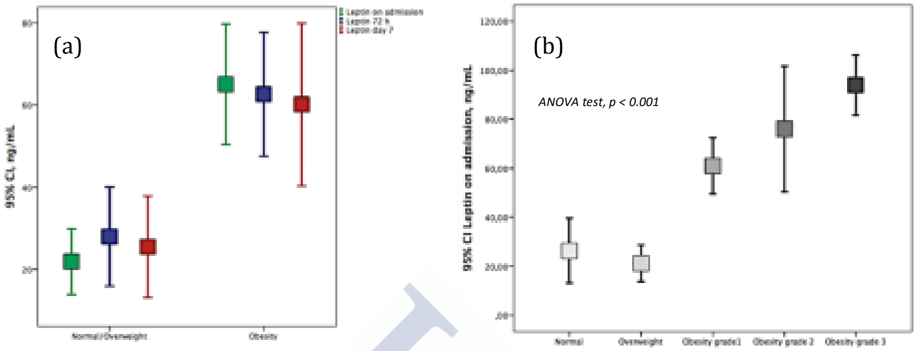


Figure 49. (a) Comparative analysis of serum levels of leptin (at admission, 72h and 7th day/discharge) between non-obese and obese-patients. (b) ANOVA test comparing leptin levels on admission between different groups of BMI.

adipokine with higher BMI (**figure 50b**).

The expression of adiponectin, although slightly higher in obese compared to non-obese patients, it did not reach statistical significance (**figure 51**).

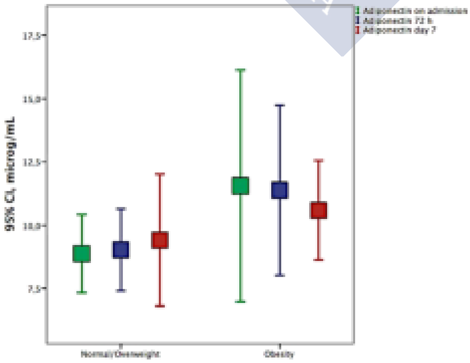


Figure 50. Comparative analysis of serum levels of adiponectin (at admission, 72h and 7th day/discharge) between non-obese and obese patients.

Finally, to assess the prognostic value of leptin in obese and non-obese patients, a non-adjusted analysis was performed (**table 29**). Bad outcome at 3 months was selected as dependent variable. No significant associations were found between leptin and outcome in any group of patients.

	Non-obese		Obese	
	OR	95% CI	OR	95% CI
Leptin admission	0.99	0.84 - 1.16	1.28	0.95 - 1.74
Leptin 72h	1.18	0.96 - 1.44	1.26	0.91 - 1.59
Leptin 7th day	0.95	0.76 - 1.18	1.19	0.96 - 1.73

Table 29. Non-adjusted logistic regression model of the prognostic value of leptin levels, in non-obese and obese patients.

DISCUSSION

In this prospective study, we have analysed the evolution of obese and non-obese patients after a first-ever ischemic stroke. We have used BMI to classify our patients because it is the most widely used system and it is validated worldwide[356]. We have set the cut-off point of comparison in 30 kg/m^2, given that it is from that point onward that obesity is defined, and where the highest increments in morbidity and mortality are traditionally found at population level. We have shown that functional outcome does not vary significantly depending on BMI, thus, obese do not evolve worse than non-obese (normal weight and overweight) patients. What is more, we have found a trend favouring a greater recovery of neurological impairments as BMI increases. On the other hand, from an index defined by us in relation to IL-6 and IL-10 levels, we have found that, compared to the control group, obese patients show an inflammatory balance favouring anti-inflammation that may counteract the pro-inflammatory response in the acute phase of ischemic stroke.

A total of 98 patients in a period of 33 months were included. Regarding their baseline characteristics, the apparently low mean height (1.60 m) of our patients could draw our attention, however, we must take into account two considerations: the first is the mean age of our patients (69.3 years), since data from the Instituto Nacional de Estadística de España (Spanish Statistical Office)[789] show that the mean height of the Spanish population over 65 years is 1.62 m; the second is that the sample was obtained in Galicia, a region whose population is traditionally shorter than the rest of Spain[790]. It is also important to note that patients were previously independent with a median mRS of 0 [0, 1], and that TOAST subtypes were similar to the data of our Unit[791].

One of the most remarkable aspects of our sample is that the baseline characteristics of both groups (obese and non-obese) were very similar, which makes them comparable. We did not find differences in sex or age, either in relation to their previous dependency situation or to the most of their medical history. As expected, the frequencies of obstructive sleep apnoea, hypertension and dyslipidaemia were higher in obese patients (although the latter two were not significant). In relation to dyslipidaemia, there were no differences in total cholesterol or LDL-cholesterol levels, and we found lower HDL-cholesterol and higher triglycerides levels in the obese ones. The fact that there were no differences in total cholesterol and LDL-cholesterol levels is not surprising since, firstly, many of these patients were already taking lipid-lowering treatments and, secondly and as previously noted, excessive intra-abdominal fat accumulation is typically associated with a higher proportion of small, dense LDL particles, and a large proportion of these particles cannot be identified by the measurement of total or LDL-cholesterol levels because these cholesterol levels are frequently in the normal range in obese individuals[356]. There was no difference in the previous history of diabetes mellitus, although blood glucose and HbA1c at admission were slightly higher. It is remarkable the higher frequency of atrial fibrillation in obese and of smoking in non-obese patients. Several studies have shown that BMI is independently related to the risk of atrial fibrillation[449]. Impaired diastolic function observed in obese patients and pericardial fat depots have been proposed as potential mechanisms. Paracrine effects of pericardial fat, through inflammatory mediators and adipokines may be involved in the atrial remodelling process. Animal models have pointed out that leptin signalling participates in atrial fibrosis and atrial fibrillation, and epidemiological studies have shown that adiponectin levels are independently associated with atrial fibrillation. Regarding smoking habit, Rásky et al.[792] at the year 1996 showed for the first time that smoking was inversely correlated with BMI. One of the main mechanisms that could explain this fact is that nicotine directly activates POMC neurons in the arcuate nucleus of the hypothalamus via $\alpha 3\beta 4$ nicotinic acetylcholine receptors, powerfully suppressing appetite and so weight gain[793].

In relation to evolution during hospitalization, it is important to note that obese suffered almost twice as many infections as non-obese patients. This finding agrees with previous literature which indicates that obesity is an established risk factor for nosocomial and post-procedure infections[404]. As some authors have suggested, the responsible mechanism could be the disruption of the immune response in obese patients[357].

Focusing on stroke, its characteristics and evolution, we should start by pointing out that, although there were no significant differences in TOAST subtypes between groups, we found more cardioembolic strokes in obese patients and more atherothrombotic strokes in non-obese patients, which could be explained by the higher frequencies of atrial fibrillation in the first group and smokers in the second group. On the other hand, obese subjects had more than twice as many hemorrhagic transformations as non-obese (30% vs. 12.5%), which, as expected, was associated with worse prognosis in both groups of patients. Although Kim et al.[686] found that the risk of hemorrhagic transformation decreased significantly with obesity, our finding is in line with that previously observed in animal models[457]. Different mechanisms could explain this phenomenon. The first one is that obese patients had a higher frequency of cardioembolic strokes, which are associated with an increased risk of hemorrhagic transformation[794]. In our study, hemorrhagic transformation was also more frequent in patients with such subtype of stroke (22.2% in atherothrombotic strokes, 34.2% in cardioembolic strokes, 0% in lacunar strokes, 17.1% in strokes of undetermined etiology, 0% in strokes of other etiologies). Nonetheless, if we select only those patients with cardioembolic strokes, we see that even in that subgroup obese patients had a higher risk of hemorrhagic transformation (**figure 52**). For this reason, we suspect that there are more mechanisms involved. As we will discuss later, obese patients may develop a more intense inflammatory response in the first moments after stroke onset. In the introduction, we already described that experimental studies suggest that obesity intensifies inflammatory damage of cerebral microvasculature. Obese rodents show increased poststroke adhesion molecules, which leads to neutrophil recruitment, and therefore contributes to the release of MMP-

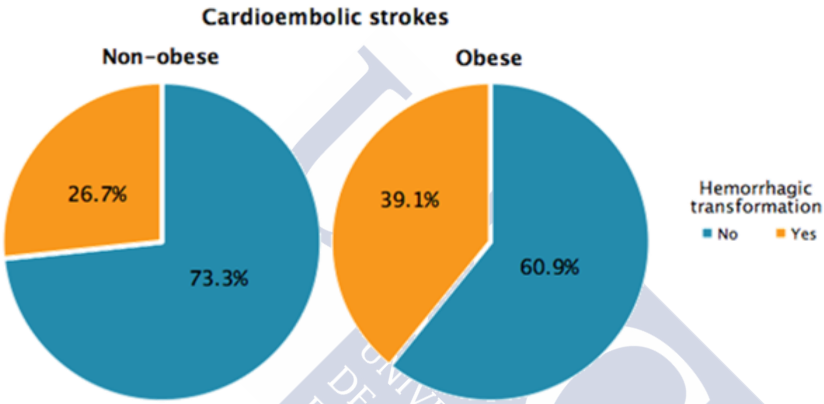


Figure 51. Frequency of hemorrhagic transformation in patients with cardioembolic strokes, in non-obese and obese groups.

9, which is involved in BBB breakdown[457]. Therefore, it is very likely that such intense inflammatory response will contribute to the BBB damage resulting in an increased risk of hemorrhagic transformation.

Another important consideration when comparing obese and non-obese patients is the fact that the severity of neurological impairments assessed by NIHSS at admission was similar for both groups. Moreover, infarct volumes were also similar, despite experimental studies have shown that obesity animal models experienced higher infarct volumes[457]. In the follow-up, we observed that there was a trend (albeit not statistically significant) in favour of a greater recovery of neurological impairments in obese when comparing with non-obese patients, although we did not find significant differences in the reduction of infarct volumes. In **figure 22b** we clearly see the direct correlation between BMI and greater improvement in neurological impairments. Despite this trend, there were no differences regarding functional outcome at 3 months assessed by mRS. On the other hand, and though the mortality was lower in the obese group, the differences are minimal and we do not give special important to this finding in in our sample. Therefore, with these results we can affirm that obese patients do not evolve worse than non-obese patients. In this sense, in a recent work by Sun et al.[795], no differences were found either in terms of prognosis. We should remember that in the last years, several studies have shown lower mortality rates and even better functional outcome in obese patients[658,660,663–665,671,672]. Although we did not observe the latter in our study, we found a trend favouring the recovery of neurological impairments as BMI increases. To the best of our knowledge, our study is the first to analyse and show this trend by using a scale specifically designed for this evaluation, the NIHSS.

There are several key aspects. Among them, this improvement took place even though obese patients showed some data typically associated with bad outcome such as: the presence of a higher frequency of infections during hospitalization, a greater number of cardioembolic strokes, and a greater proportion of hemorrhagic transformations. Another and very important consideration is that these differences in clinical improvement are not justified by the severity of stroke at admission or by the age of the patients, since in our sample both the median NIHSS at admission and the mean age were similar for obese and non-obese patients. This goes against some recent studies that have described that obese patients exhibit a better post-stroke evolution because they have milder strokes or are younger than non-obese patients[676–678]. We must also remember that one of the justifications for the obesity paradox was the fact, subscribed by some authors, that obese patients present a higher relative proportion of lacunar strokes, which are associated with a better prognosis than cardioembolic strokes, more frequent in lean patients. However, and as we have just mentioned, in our study we found even the opposite, a higher proportion of atrial fibrillation and cardioembolic strokes in obese patients compared to non-obese, with a similar percentage of lacunar strokes. So, in theory, it was logical to expect a worse outcome in the obesity group. It is possible that in this disparity might influence the fact that such studies were carried out in Asian populations, where the different aetiologies of stroke vary in frequency with respect to Western countries.

We decided to perform a more comprehensive analysis about the anthropometric characteristics, their post-stroke variations, and whether such variations influence the patient's evolution. As expected, obese patients showed higher values of WC, HC and WHR at admission. After 3 months of follow-up, we found a decrease in body weight in both groups, a mean of 0.9 kg and 5.7 kg in non-obese and obese patients respectively, therefore, it was more striking in the latter group. When explaining these variations in body weight, other data from the analysis should be considered. Firstly, in non-obese patients, despite the weight loss, WC and HC values increased rather than decreased. As the circumferences of compartments with great proportion of adipose tissue increased, this makes us suspect that weight loss could be mainly due to lean mass loss. On the contrary, body weight decrease in obese subjects was accompanied by a significant reduction in WC (4.7%) and smaller in HC (1.4%), which suggests an important loss of fat mass. We do not know the exact reason for these discrepancies between the two groups, but there might be different explanations. The first and simplest is the voluntary reduction of body weight because of a better control of vascular risk factors, that is, with diet. This would explain why it was greater in those patients in whom there is an excess of body fat, who, theoretically, need it, and who arise from more extreme situations in which reductions would be more remarkable. In support of this point we have the lowering of HbA1c levels at 3 months, also higher in obese patients. On the other hand, as noted in the introduction, following ischemic stroke it takes place a catabolic/anabolic imbalance that leads to tissue wasting of both fat and muscle tissue manifestating in weight loss[697]. After stroke, the stress results in the activation of local and systemic sympathetic responses, which promotes the catabolic stimulation and increased degradation of protein and lipid stores. Among the molecules released there are catecholamines and natriuretic peptides, which have been shown to exert strong lipolytic signaling. It is possible that obese patients, due to the chronic low-grade inflammation, a higher inflammatory response in the acute phase (we found higher levels of leukocytes, fibrinogen and hsCRP in blood samples), and the release of a greater amount of pro-inflammatory cytokines (as shown in animal models[463]), experience a powerful sympathetic discharge which results in a higher lipolysis with a greater reduction in adipose tissue depots.

We observed that there was a higher reduction in body weight and WC (albeit the latter was not significant) in patients with bad outcome. Previous studies have reported the association between weight loss and higher rates of mortality and dependence[666,681,682]. Although some studies in cardiac pathologies have shown contrary results[668,669], it is highly probable that the key is whether the weight loss is voluntary or involuntary[667]. Jonsson et al.[682] found that reduction in body weight was higher in patients with more severe strokes. In those patients, weight loss could be the consequence of sarcopenia and loss of lean mass due to occult diseases, more marked catabolic/anabolic imbalance, and prolonged immobilization, or even a loss of lean and fat mass due to difficulties for alimentation and malnutrition[670,697]. In this sense, we found a significant correlation between the severity of neurological impairments (NIHSS at 48 hours) and weight loss (**figure 53**). However, in the non-adjusted analysis of the prognostic value of those variables, only in obese patients the weight loss and WC reduction were significantly associated with bad outcome. Although it is possible that this statistical finding is conditioned by the major reduction of these measurements in the obese sample, we cannot discard the theory that

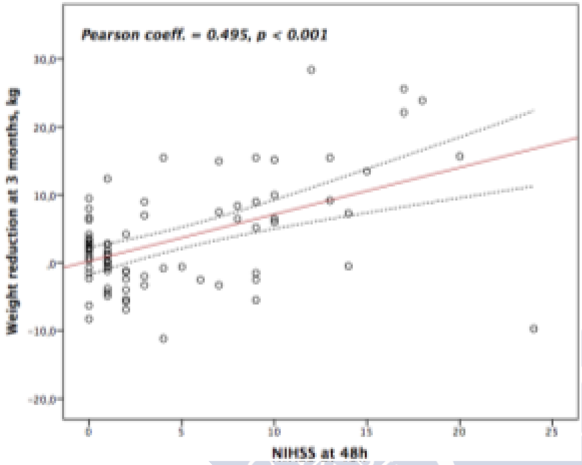


Figure 52. Correlation between weight reduction 3 months after stroke and NIHSS at 48h.

the loss of part of that adipose tissue excess determines a loss of its hypothetical beneficial effects and this has repercussions in the prognosis. It is important to remember that, even though weight loss is associated with a worse prognosis and this loss is greater in obese patients, in that group the outcome was not worse.

In order to better understand the distribution of body adipose tissue in stroke patients and its influence, we performed a DEXA study in a sample of patients. Although we were not able to demonstrate any relationship between the distribution of body fat and the severity and prognosis of stroke, we encountered and interesting finding: the abdominal distribution of adipose tissue predominated in both obese and non-obese patients. The two groups showed a higher proportion of trunk fat respect to total body fat, as well as a predominance of android fat distribution respect to gynoid distribution despite women were the 50% of such sample. Moreover, in obese patients all abdominal fat parameters (the percentage of android fat distribution, the proportion of trunk fat, and the visceral fat) were remarkably high. After these findings, we decided to analyse how many patients in the entire study sample had WC and WHR values above the limits associated with high risk of metabolic complications (reference values at **table 3**), an indirect measure of excessive abdominal fat. We observed again that even among the non-obese group, there were many patients with high values of WC and WHR (**table 29**). Therefore, it seems that abdominal distribution of excess adipose tissue is what characterizes obesity in patients with ischemic stroke. Considering that individuals with excess fat in the intra-abdominal depots are at particular risk for vascular diseases independent of BMI[384,385], these findings seem to be the expected ones.

	Non-obese	Obese
Patients with WC associated to increased risk of metabolic complications, %	66	100
Patients with WC associated to substantially increased risk of metabolic complications, %	23.4	95.9
Patients with WHR associated to substantially increased risk of metabolic complications, %	78.7	100

Table 30. Frequency of patients with values of WC and WHR associated to higher risk of metabolic complications, among non-obese and obese patients.

As we discussed in the introduction, inflammation could play a key role in obese patients. Excessive white adipose tissue results in a low-grade inflammatory response, known as metainflammation, which results, among other things, in a systemic elevation of acute phase reactants and pro-inflammatory cytokines[458–461]. In this line, we found higher levels of leukocytes, fibrinogen, and hsCRP in blood samples of obese patients compared to non-obese, which suggests the presence of a more powerful inflammatory response in this group of patients during the acute phase of stroke[106]. Many studies have shown that the release of pro-inflammatory cues has potentially harmful effects that contribute to tissue damage and negatively influence post-stroke outcome[132,134–136,203–207,215–218]. Thus, if there is a more intense inflammatory response in relation to obesity, one could expect that it would negatively influence the evolution and outcome of obese subjects. However, we observed that obese patients do not evolve worse, but even experience a greater recovery of neurological impairments. In addition, we did not find a significant association between such inflammatory markers in peripheral blood and prognosis of those patients. It is possible that obese patients, chronically exposed to low-grade inflammation, exhibit a certain tolerance to intense inflammatory responses in different types of diseases such as stroke. Our hypothesis, which could complement that theory, is that obesity counterbalances the inflammatory response in some way, counteracting to some extent the deleterious effects of pro-inflammatory signals.

In order to study this, we analysed the serum levels of IL-6 and IL-10, pro- and anti-inflammatory cytokines respectively. At the time of admission, the levels of IL-6 were significantly higher in obese compared to non-obese patients. We observed a direct and clear correlation between its levels and BMI. This finding agrees with animal obesity models where higher serum levels of IL-6 are found in the acute phase of stroke[462], and supports the suspect that the immune response after stroke is increased in obese patients. Another interesting finding is that levels of this cytokine raised over the first week in non-obese patients, whereas they evolved in the opposite direction in obese patients. Thus, we propose that an anti-inflammatory stream is boosted in this group of patients following the first moments of stroke, and which counterbalances the pro-inflammatory response. This is where IL-10 comes in. We detected that serum levels of this cytokine at admission were much higher in obese patients, and slightly increased over the first week in this group. The levels of IL-10, as an indirect expression of that anti-inflammatory stream, could explain how obesity counteracts the powerful inflammatory response exhibited by these patients and thereby justify the decline in IL-6 levels during the first days following stroke. With the aim of indirectly assessing the balance between the two opposing responses, we created a ratio, the "anti/pro-inflammatory index", which relates the serum levels IL-10 and IL-6. As we defend, this index could represent the "battle" between both streams. Then, we observed two very interesting findings: on the one hand, the values of this index formed a curve in relation to BMI that reaches its peak in patients with obesity grade I; on the other hand, its values were higher in patients with good outcome.

Therefore, this supports the fact that, in obese patients, excess adipose tissue could enhance an anti-inflammatory response that counteracts the release of pro-inflammatory cues. Since this index is associated with good outcome, this could be one of the reasons why such patients do not evolve worse despite they probably have a more powerful baseline and hyperacute inflammatory response, and, thus, it could also contribute to their clinical improvement. Though the peak of this index is reached in obesity grade I and descends afterwards, levels of IL-10 were also elevated in obese patients and slightly increased with BMI in this group, so it does not invalidate the hypothesis. Nonetheless, it is true that we cannot discard that too high amounts of white adipose tissue could lead to an even more intense pro-inflammatory response potentially more difficult to counterbalance (we must remember that IL-6 levels continued to rise as BMI increased). Even so, clinical improvement increased with BMI also in the top grades, thus, there might be other mechanisms not related to inflammation, or that do not result in a modification of the relationships between the serum levels of these cytokines.

To further investigate the possible mechanisms that modulate the post-stroke evolution of obese patients, involved or not in the inflammatory response, we decided to complete the study by analysing different cellular and molecular profiles.

As we already described in introduction, white adipose tissue represents an important source of progenitor cells[730]. This depot has a class of MSCs known as ASCs, defined by CD34+/CD45-/CD31- markers according to literature[730]. We analysed the circulating levels of such progenitor cells in both groups and we found that levels were significantly higher in obese patients during the first week. In this sense, Biasucci et at.[723] in 2010, and Bellows et al.[731] in 2011, first described that levels of circulating EPCs and MSCs were higher in severely obese and obese individuals respectively, showing that obesity promotes mobilization of progenitor cells. Moreover, it must be pointed out that the stress response that takes place in CNS after stroke contributes to stem cell mobilization to the brain via cytokine production[716]. MSCs may be mobilized and migrate following chemokine gradients promoting the repairing of injured tissues and recovery[717,727–730]. In response to inflammatory signals, the expression of chemokine receptors in MSCs is upregulated, just as happens with different MMPs, which have also been reported to be involved in MSC migration as they favour cell locomotion and tissue reconstitution by breaking down the extracellular matrix[732]. Furthermore, it has also been demonstrated that, like MSCs, native and transplanted ASCs can be mobilized from white adipose tissue and migrate in response to a specific immune stimulus[737,738]. Then, the chronic low-grade inflammatory state and the enhanced hyperacute inflammatory response in obese patients, could promote a higher mobilization of progenitor cells and their subsequent homing to damaged tissues.

Therefore, this represents a very important finding due to the potential beneficial effects of progenitor cells. As we noted before, although the differentiation into several cell lineages has been suggested, the positive mechanisms are mainly related to neurorepair and neuroprotection, and to immunomodulation[739]. These progenitor cells contribute to angiogenesis, synaptogenesis, gliogenesis, and neurogenesis, through the secretion of several growth factors such as VEGF[745]. Contrary to reported by Ikegame et al.[746], who showed that intravenous administration of ASCs in a mice model of ischemia was associated with reduction in infarct volume and higher expression of VEGF, we did not find differences in the evolution of infarct volumes nor in the serum levels of VEGF between obese and non-obese patients although the former had higher circulating levels of progenitor cells. Nonetheless, we did find a greater clinical improvement, as demonstrated by Gutiérrez-Fernández et al.[747] in another experimental study, who found that intravenous administration of those cells was associated with better functional recovery, despite no reduction in infarct volume. Despite there were no differences between obese and non-obese subjects regarding VEGF levels, we found a slight correlation (not statistically significant) between circulating progenitor cells levels and serum VEGF levels in obese patients (Pearsson coeff.: 0.353, $p = 0.085$). It is quite possible that we did not find differences in levels of this growth factor due to the fact that the analysis were performed in serum, whereas experimental models detected higher concentrations in *in vitro* and brain tissue studies. On the other hand, regarding their immunomodulatory actions, ASCs are able to inhibit the expression of pro-inflammatory cytokines and participate in the secretion of anti-inflammatory cytokines. Among other actions, these progenitor cells upregulate the expression of IL-10 by Treg cells, which supresses the autoreactivity of T cells and inhibits Th1 and Th17 responses[735]. This could be partially responsible of the higher levels of IL-10 in obese compared to non-obese patients. Therefore, one explanation for the way obesity counterbalances the inflammatory response and contributes to clinical improvement after stroke could be the immunomodulatory potential of progenitor cells. Further studies would be necessary to confirm the involvement of ASCs in this mechanism, but it seems to be a promising hypothesis.

Following this line, we analysed the implication of innate immunity through the expression of TLRs. First of all, me must mention some already noted aspects of these receptors. Its activation triggers a signalling cascade that culminates in the transcription of pro-inflammatory and immunomodulatory factors such as TNF-α, IL-6 or MCP-1[121,304]. In acute ischemic stroke, TLR2 and TLR4 expression correlates with higher serum levels of different cytokines such as IL-6[317], and contributes to pathological progression of cerebral injury[298,310–312,316]. Moreover, besides their role in ischemic stroke, TLRs appear also to contribute to the chronic inflammatory state of obesity. Many studies have described the increased expression of TLR2 and TLR4 in adipose tissue[297,305] and peripheral blood mononuclear cells[516] of obese compared to lean individuals, and higher levels of IL-6 in these subjects which correlate with TLRs expression[516]. Surprisingly, in our sample of patients with ischemic stroke, we detected a lower expression of TLRs in obese patients compared to non-obese ones, although it did not reach statistical significance. It is possible that the anti-inflammatory stream that counterbalances the immune response in obese patients following stroke is responsible for limiting the expression of these receptors. Furthermore, we cannot discard that this finding might be partially responsible for the reduction in IL-6 levels observed over the first week in these patients. Hence, given the potentially harmful effects of TLRs in the acute phase of ischemic stroke, the limitation of their expression may also explain why obese patients recover better.

Other molecules analysed were the soluble forms of TNF-α receptor (TNFR-I and TNFR-II), and the chemokines MCP-1 and MIP-1β. TNFR-I and TNFR-II are expressed in human adipose tissue and both can be cleaved from the cell surface as soluble TNF-α receptors and act as antagonists by inhibiting the ligand-binding cell surface receptor[699]. Consequently, the production of soluble TNF-α receptors by adipose tissue may promote anti-inflammatory effects by inhibiting TNF-α actions[703]. The analysis showed significantly higher levels of TNFR-I in non-obese patients and higher levels of TNFR-II in obese patients. Despite previous studies have reported the direct correlation between serum TNFR-II levels and obesity, to date, all published studies have shown that either there are no differences in TNFR-I levels between lean and obese patients[700–702], or they are also higher in obese patients[699]. This discrepancy is difficult to explain and requires further study. It should be noted that our analysis was carried out in the acute phase of stroke and that it has been proposed that there is a significant correlation between the release of soluble TNFR-I and circulating TNF-α concentrations which suggests that this production may be regulated by the ligand itself[700]. One plausible explanation might be that the anti-inflammatory counterpart in obese patients limits the expression of TNF-α and, subsequently, the release of TNFR-I in the acute phase of stroke. Finally, and despite the anti-inflammatory potential of the soluble forms of these receptors, we did not found a significant association between their levels and outcome. The analysis of chemokines MCP-1 and MIP-1β did not show significant differences between groups, although we observed that their levels were slightly lower in obese patients. This contradicts what was previously reported in experimental models of cerebral ischemia in which obese animals exhibited increased MCP-1 levels[462]. However, it is in line with the observations of the rest of this study regarding the modulation of the inflammatory response in obese patients.

The last molecular analysis we carried out was that of adipokines, leptin and adiponectin. As expected, given that their levels raise in direct relation with white adipose tissue mass[525], serum leptin levels were markedly higher in obese subjects. Although this is not a striking finding, it is interesting to remember that leptin is involved in the regulation of the immune system and stimulates the secretion not only of IL-6 but also of IL-10[532], which, as we have seen, is substantially increased in obese individuals of our sample. So, despite it has been classically related to pro-inflammatory actions, we do not discard its involvement in potentially anti-inflammatory effects in acute phase of stroke via the releasing of IL-10. Furthermore, experimental models of ischemia have suggested that leptin may promote neurogenesis and angiogenesis[539], and exert neuroprotective actions through a repair of the brain energy deficit[538] contributing to the reduction of neurological impairments. However, in our study were not able to demonstrate an association between its levels and outcome. Adiponectin, a cytokine with anti-inflammatory properties, is the most abundant adipose-specific adipokine[556]. Unlike leptin, its levels are reduced in obese individuals[557] and negatively correlate with visceral fat accumulation[558–560]. In spite of this, in our sample we found slightly higher levels of this adipokine in obese patients compared to controls, albeit the differences were not significant. This discrepancy is difficult to explain, since although some studies have shown a positive correlation with subcutaneous adipose depot[559], abdominal adipose tissue clearly predominates in our patients. On the other hand, due to the potential anti-inflammatory effects of adiponectin through, inter alia, the inhibition of TLR2 and TLR4[567] and the stimulation of

IL-10 production[569], its expression could be of special importance in the inflammatory counterbalance of obese patients. In fact, treatment with this adipokine in animal models of ischemia has demonstrated neuroprotective effects and a reduction in neurological impairments[579,580]. Then, we cannot exclude the implication of leptin and adiponectin in the clinical improvement shown by obese patients of our study.

Finally, since hyperthermia is one of the most potent predictors of poor outcome in acute ischemic stroke[42], we decided to analyse if there was any relationship between body temperature, BMI, and the evolution of our patients. The first thing that stands out in this analysis is the presence of higher temperatures in the early phases of stroke in obese patients when compared to controls. One of the explanations for this increase might be the direct damage on the thermoregulation center of the hypothalamus or of the pontine centers as a result of large infarcts[44], however, this seems unlikely since infarct volumes were virtually identical in both groups. Another justification might be the fact that infections during hospitalization were more frequent in obese patients. In this sense, we must remember that, in general, earliest hyperthermia, which we observed in our patients, is usually associated to a neurogenic response as a consequence of acute phase reaction, whereas the late is related with infections[42]. In any case, with the aim to rule out that such differences were due to the disparity in the number of infections, we carried out another analysis exclusively selecting those patients who did not suffer infections. We can see how, for at least the first 24-36 hours, trends are maintained for mean (**figure 54a**) and maximum (**figure 54b**) temperatures respectively. In the comparative analysis, similar results were found for baseline temperature, (**figure 55a**) maximum temperature during the first 24 hours (**figure 55b**), and maximum temperature during 24 to 48 hours (**figure 55c**), although differences were less significant. This data rule out that, at least in the early stages, the

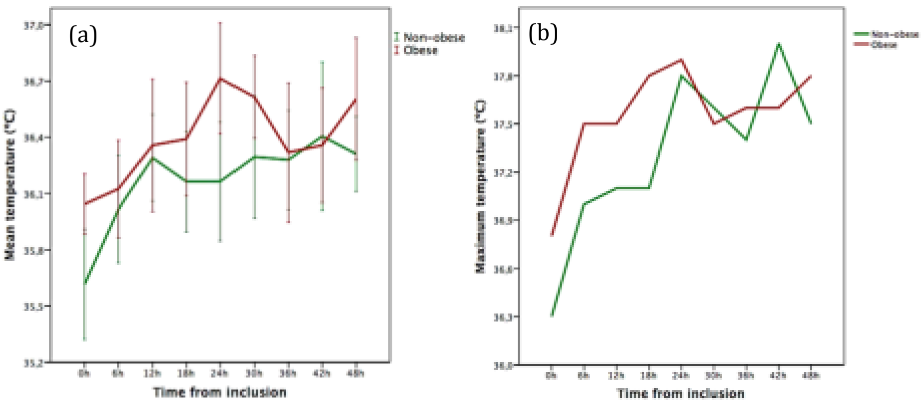


Figure 54. Comparison of mean (a) and maximum (b) temperatures during the first 48h between non-obese and obese groups, only in those patients who did not suffer any infection during hospitalization.

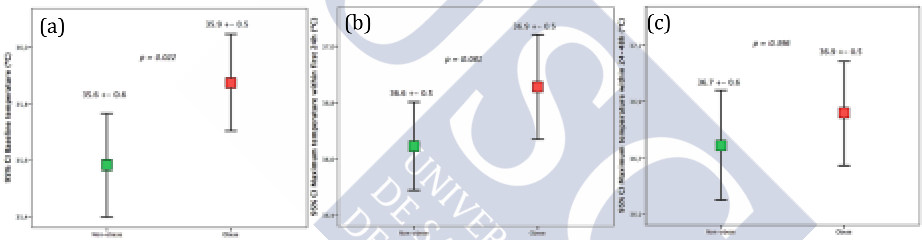


Figure 54. Comparative analysis of baseline axillary temperature (a), maximum temperature within 24h (b) and maximum temperature within 24-48h (c) between non-obese and obese patients, only in those patients who did not suffer any infection during hospitalization.

temperature differences were largely justified by the frequency of infections. Therefore, our hypothesis is that, the metainflammation and the potent inflammatory response in the early phases of stroke in obese patients are responsible for this temperature increase in the first hours after onset. Nowadays, we know that neurogenic hyperthermia can occur as a consequence of inflammatory cascade with pyrogenic activity, leading to increased pro-inflammatory cytokines and leukocytes around the infarcted tissue, resulting in hypothalamic stimulation[45-47]. We also propose that

UCPs, mitochondrial proteins that release heat by uncoupling cellular respiration from ATP production[762], might play an important role in the hyperthermia of obese patients. In ischemic stroke, the stress results in the activation of local and systemic sympathetic response and, as previously mentioned, UCP thermogenesis is triggered by the sympathetic system[763]. It could be postulated that the sympathetic response which could be enhanced in obese subjects due to the intense inflammatory reaction in the acute phase of stroke, might determine the increase in body temperature in these patients. Furthermore, obesity is associated with higher depots and higher circulating fatty acids[297] and, as we noted in introduction, fatty acids are an important stimulator of UCP1[770]. The potentially enhanced sympathetic discharge associated to obesity can lead to increased lipolysis, resulting in higher circulating levels of fatty acids, which could stimulate UCPs resulting in increased body temperature. Finally, if we remember the role of UCP1 activity in the regulation of body weight, we cannot discard that its possible higher activation in the acute phase of stroke in obese patients contributes to the higher reduction in body weight exhibited by these individuals.

In the analysis about the prognostic value, we found that maximum temperature in the first 24 hours was associated with bad outcome in both groups of patients, however, after adjusting for the presence of infections during hospitalization, only in the group of non-obese patients remained statistically significant. It seems that obese patients, despite having higher temperatures in the early stages of stroke, they better tolerate hyperthermia. Although we do not know the reasons for this phenomenon and other specifically designed studies would be necessary, we suspect that, at least in part, the anti-inflammatory counterpart associated to this type of patients could be involved. If we look again at the **figure 54** we can see that from 24-36 hours, differences almost disappear or become minimal. Thus, it is possible that against the intense inflammatory response of the hyperacute phase, obese patients experience an anti-inflammatory response, inferred through the IL-10/IL-6 index, that counteracts the phenomenons that lead to hyperthermia and subsequently tissue damage.

Before we finish, we must recognize some limitations. The main one is that, despite this was a prospective study in which the leading characteristics of both groups were comparable, the size of the sample was small. It is very probable that this fact has determined that several of the trends we have found they have not reached statistical significance. Nonetheless, it should be noted that most of these trends described are clear. Another limitation is the use of BMI to assess excess body fat and to classify patients. Although it is the most widely used index and it can generally be assumed that people with a BMI ≥ 30 have an excess of fat mass, this index does not distinguish between weight associated with muscle or with fat[356], and there have been shown statistically significant associations between BMI and the percentage of body fat dependent on age and sex[380]. Regarding the anthropometric characteristics, their evolution, and their association with outcome, we did not registered aspects such as immobilization, alimentation difficulties, or nutritional status (through, for example, albumin/prealbumin levels) which would have provided more data to explain the variations in these variables and their consequences. Concerning the substudy with DEXA, its validity is limited because there were included very few patients. In addition, it might have been interesting to carry out a DEXA follow-up study to adequately assess the variations in body compartments. On the other hand, to assess the inflammatory balance we employed an index that relates IL-6 and IL-10 levels, which has not been previously validated, and we do not discard that the use of other cytokines or even a combination of some of them would have been more useful or precise. When characterizing circulating ASCs we have employed the markers CD34+/CD45-/CD31- according to literature[730], however, recent studies have pointed out that the use of a greater number of markers may be desirable in order to precisely differentiate them from other types of progenitor cells[796]. In any case, what is unquestionable is that the number of circulating progenitor cells in the acute phase of stroke in increased in obese patients. With regard to the measurement of temperatures we should explain that, although we tried to ensure that they were always performed by the monitoring probe, some patients, due to care needs, could not be monitored for the entire time during the first 48 hours.

In summary, in line with what has been published in recent years regarding the so-called obesity paradox, and unlike what we might intuitively think, obese patients do not show a worse outcome after ischemic stroke, but also seem to experience a greater recovery of neurological impairments. All this, despite having several factors strongly associated with bad outcome such as: elevated serum markers of inflammation, a higher temperature in the acute phase, a higher proportion of cardioembolic strokes, an increased frequency of hemorrhagic transformations, and a higher risk of infections during hospitalization; furthermore, bearing in mind that both groups were comparable in terms of age, severity of stroke at admission, and infarct volumes. We postulate that obesity can counterbalance, to some extent, the inflammatory reaction responsible for the deleterious effects of the mentioned factors of poor prognosis, through an anti-inflammatory stream enhanced in the first moments of stroke. Thus, the finding of higher values of the IL-10/IL-6 index would indirectly confirm this anti-inflammatory counterpart associated with excess adipose tissue. On the other hand, and given that white adipose tissue is a source of progenitor cells, the finding that obesity is associated with a greater mobilization of these cells in the acute phase of stroke is of key importance due to their potentially beneficial effects through neurorepair, neuroprotection and immunomodulation. In the present work, we have tried to explore the connection between obesity and ischemic stroke, two major global health problems with severe economic and clinical consequences. Further studies are needed to analyse in depth the mechanisms involved in the relationships between adipose tissue, inflammation and progenitor cells, not only in stroke, but also in many other different pathologies.

CONCLUSIONS

1. The outcome of obese patients after ischemic stroke is not worse than non-obese patients. Even though they show several factors strongly associated with bad outcome such as: elevated serum markers of inflammation, a higher temperature in the acute phase, a higher proportion of cardioembolic strokes, an increased frequency of hemorrhagic transformations, and a higher risk of infections during hospitalization.

2. There is a trend favouring a greater improvement of neurological impairments as BMI increases.

3. The finding of higher values of the IL-10/IL-6 index suggests that obesity may counterbalance the inflammatory response after ischemic stroke through an anti-inflammatory stream.

4. There are no differences in infarct volumes between obese and non-obese patients.

5. The abdominal distribution of adipose tissue is what characterizes ischemic stroke patients.

6. Obese patients exhibit a higher reduction in body weight after ischemic stroke than non-obese patients.

7. Body weight loss after ischemic stroke is associated with bad outcome.

8. The body temperature in the first stages after ischemic stroke onset is higher in obese patients. Despite this fact, obese tolerate better hyperthermia than non-obese patients.

9. Obesity is associated with a greater mobilization of progenitor cells in the acute phase of ischemic stroke.

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APPENDIX

1. NATIONAL INSTITUTE OF HEALTH STROKE SCALE (NIHSS) Modified from[780]

1a. Level of consciousness:

0 = Alert; keenly responsive.

1 = Not alert; but arousable by minor stimulation to obey, answer, or respond.

2 = Not alert; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped).

3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.

1b. LOC questions (the patient is asked for month and his/her age):

0 = Answers both questions correctly.

1= Answers one question correctly.

2 = Answers neither question correctly.

1c. LOC commands (the patient is asked to open and close the eyes and then to grip and release the non-paretic hand):

0 - Performs both tasks correctly.

1 = Performs one task correctly.

2 = Performs neither task correctly.

2. Best gaze (only horizontal eye movements will be tested):

0 = Normal.

1 = Partial gaze palsy; gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present.

2 = Forced deviation, or total gaze paresis not overcome by the oculocephalic manoeuvre.

3. Visual gaze (upper and lower quadrants of the visual fields are tested by confrontation, using finger counting or visual threat, as appropriate):

0 = No visual loss.

1 = Partial hemianopia.

2 = Complete hemianopia.

3 = Bilateral hemianopia (blind including cortical blindness).

4. Facial palsy (ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes):

0 = Normal symmetrical movements.

1 = Minor paralysis (flattened nasolabial fold, asymmetry on smiling).

2 = Partial paralysis (total or near-total paralysis of lower face).

3 = Complete paralysis of one or both sides (absence of facial movement in the upper and lower face).

5. Motor arm (5a left arm, 5b right arm; the limb is placed in the appropriate position: extend the arms – palms down – 90 degrees – if sitting – or 45 degrees – if supine –; drift is scored if the arm falls before 10 seconds):

0 = No drift; limb holds 90 (or 45) degrees for full 10 seconds.

1 = Drift; limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support.

2 = Some effort against gravity; limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity.

3 = No effort against gravity; limb falls.

4 = No movement.

UN = Amputation or joint fusion.

6. Motor leg (6a left leg; 6b right leg; the limb is placed in the appropriate position: hold the leg at 30 degrees – always tested supine–; drift is scored if the leg falls before 5 seconds):

0 = No drift; leg holds 30-degree position for full 5 seconds.

1 = Drift; leg falls by the end of the 5-second period but does not hit bed.

2 = Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity.

3 = No effort against gravity; leg falls to bed immediately.

4 = No movement.

UN = Amputation or joint fusion.

7. Limb ataxia (this item is aimed at finding evidence of a unilateral cerebellar lesion):

0 = Absent.

1 = Present in one limb.

2 = Present in two limbs.

8. Sensory (sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient.):

0 = Normal; no sensory loss.

1 = Mild-to-moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched.

2 = Severe to total sensory loss; patient is not aware of being touched in the face, arm, and leg.

9. Best language (a great deal of information about comprehension will be obtained during the preceding sections of the examination; for this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences; comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam):

0 = No aphasia; normal.

1 = Mild-to-moderate aphasia; some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response.

2 = Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response.

3 = Mute, global aphasia; no usable speech or auditory comprehension.

11. Dysarthria (If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list; if the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated; only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice; do not tell the patient why he or she is being tested):

0 = Normal.

1 = Mild-to-moderate dysarthria; patient slurs at least some words and, at worst, can be understood with some difficulty.

2 = Severe dysarthria; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric.

UN = Intubated or another physical barrier.

11. Extinction and inattention (formerly neglect):

0 = No abnormality.

1 = Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities.

2 = Profound hemi-inattention or extinction to more than one modality; does not recognize own hand or orients to only one side of space.

2. ISCHEMIC STROKE CLASSIFICATION

There are two main systems for stroke classification, the TOAST (Trial of ORG 10172 in Acute Stroke Treatment)[781] criteria and the OCSP classification (Oxfordshire Community Stroke Project)[797].

TOAST criteria

The aim of the TOAST criteria is to identify the most likely etiology based on clinical findings and complementary tests. It categorizes ischemic stroke in 5 subtypes:

i. Large-artery atherosclerosis (atherothrombotic): patients with clinical and neuroimaging findings of either significant (>50%) stenosis or occlusion of a major brain artery or branch cortical artery, presumably due to atherosclerosis. Supportive evidence by duplex imaging or arteriography of a stenosis of greater than 50% of an appropriate intracranial or extracranial artery is needed. Diagnostic studies should exclude potential sources of cardiogenic embolism.

ii. Cardioembolism: patients with arterial occlusions presumably due to an embolus arising in the heart. At least one cardiac source for an embolus must be identified for a possible or probable diagnosis of cardioembolic stroke. Potential large-artery atherosclerotic sources of thrombosis or embolism should be eliminated.

iii. Small-artery occlusion (lacune): patients with lacunar syndrome (pure motor, pure sensory, sensorimotor, ataxic hemiparesis, dysarthria-clumsy hand) and normal CT/MRI examination or a relevant brain stem or subcortical demonstrated hemispheric lesion with a diameter of less than 1.5 cm. Potential cardiac sources for embolism should be absent, and evaluation of the large extracranial arteries should not demonstrate a stenosis of greater than 50% in an ipsilateral artery.

iv. Acute stroke of other etiology: patients with rare causes of stroke, such as nonatherosclerotic vasculopathies, hypercoagulable states, or hematologic disorders. Patients in this group should have clinical and CT or MRI findings of an acute ischemic stroke, regardless of size or location. Diagnostic studies such as blood tests or arteriography should reveal one of these unusual causes of stroke. Cardiac sources of embolism and large-artery atherosclerosis should be excluded by other studies

v. Stroke of undetermined etiology: patients with no likely etiology determined despite an extensive evaluation, or with no cause found but with incomplete evaluation and patients with two or more potential causes.

OCSP classification

The OCSP classification relies just in clinical findings to categorize stroke in 4 subtypes according to the extent and the localization of the infarct:

i. Lacunar infarct (LACI): patients with lacunar syndrome. Patients with more restricted deficits than faciobrachial and brachiocrural involvement are not included.

ii. Total anterior circulation infarct (TACI): patients with the combination of higher cerebral dysfunction (aphasia, acalculia, visuospatial disorder...), homonymous visual field defect and ipsilateral motor and/or sensory deficit of at least two areas of the face, arm and leg. If the conscious level is impaired and formal testing of higher cerebral function or the visual fields is not possible, a deficit is assumed.

iii. Partial anterior circulation infarct (PACI): patients with only two of three components of the TACI syndrome, with higher cerebral dysfunction alone, or with motor/sensory deficit more restricted than those classified as LACI.

iv. Posterior circulation infarct (POCI): patients with any of the following: ipsilateral cranial nerve palsy with contralateral motor and/or sensory deficit; bilateral motor and/or sensory deficit; disorder of conjugate eye movement; cerebellar dysfunction without ipsilateral long-tract deficit; or isolated homonymous visual field detect.

3. THE MODIFIED RANKIN SCALE (mRS)[782]

- 0 = No symptoms at all.
- 1 = No significant disability despite symptoms: able to carry out all usual duties and activities.
- 2 = Slight disability: unable to carry all previous activities but able to look after own affairs without assistance.
- 3 = Moderate disability: requiring some help, but able to walk without assistance.
- 4 = Moderately severe disability: unable to walk without assistance, and unable to attend to own bodily needs without assistance.
- 5 = Severe disability: bedridden, incontinent, and requiring constant nursing care and attention.
- 6 = Dead.

SUMMARY

Stroke is a major global health problem. It can be either ischaemic or hemorrhagic[1]. Ischemic stroke is an episode of neurological dysfunction caused by focal cerebral, spinal or retinal infarction, that is, CNS cell death attributable to ischemia[3]. Brain ischemia is the result of a fall in CBF below the threshold necessary to maintain the proper functioning of nervous system[28]. This drop is consequence of occlusion of a particular artery due to embolic or hemodynamic mechanisms and leads to a metabolic and biochemical cascade, both neuronal and glial, which ultimately results in cell death[29].

It is a leading cause of death and disability, and it is responsible of high health care costs. The aging population implies that absolute number of people who have strokes annually, as well as DALYs lost, is increasing. Among all the causes of death, stroke is the third globally and ischemic stroke is responsible of approximately half[7]. Respect to DALYs, stroke is the third cause[9]. The costs of stroke represent about 2-4% of total health-care funds, and more than 4% of direct costs in industrialised countries[12].

In the pathophysiology of ischemic stroke aspects such as temperature and inflammation are of special importance. Regarding inflammation, although it has traditionally been considered as a mere reaction of damaged brain tissue, it plays a key role in the pathophysiology of ischemic stroke[106]. There is a very active interaction between the central nervous system and the immune system[107]. Elements of both innate and adaptive immunity are involved in all phases of the ischemic cascade during and after the event.[106] After focal cerebral ischemia onset, an intense inflammatory reaction characterized by peripheral leukocyte migration into the brain parenchyma and activation of microglia takes place[109–113]. The stop in CBF results in energy depletion and neuron necrosis. The destruction on neural cells leads to the release of damage-associated molecular patterns

(DAMPs) to the extracellular environment and activate innate immunity components, such as TLRs, which triggers the pro-inflammatory cascade[122,123]. Ischemic cells are stimulated to secrete inflammatory cytokines (IL-1, IL-6, TNF-α…) and chemokines (MCP-1…) that cause an upregulation of adhesion molecules in the cerebral vasculature and peripheral leukocyte recruitment. Once activated, inflammatory cells are able to release more cytokines, MMPs, NO and more ROS, which increase cell damage and determine the disruption of the BBB and extracellular matrix[114,115]. BBB breakdown contributes to secondary ischemic damage by allowing serum elements and blood to enter the brain[116,117]. This results in post-ischemic inflammation, involving activation of microglia and brain infiltration of peripheral inflammatory cells enhancing the damage[117,118].

There is also a robust relationship between body temperature and the evolution of acute cerebral infarct[42]. In the first days after ischemic stroke hyperthermia emerges in more than a half patients[43]. The effects of hyperthermia are clearly deleterious on animal models[54–57] and humans[43,49,58–64], so that it is one of the most powerful predictors of poor prognosis in acute cerebral ischemia. First, the intense release of cytokines or neuro-excitatory amino acids in infarcted areas can induce a systemic hyperthermia[52], and, secondly, systemic hyperthermia promotes inflammatory mechanisms, metabolic dysfunction, excitotoxicity, oxidative stress, BBB alteration and protein degradation, resulting in an increase of the ischemic lesion[65].

The other face of this work is obesity, typically defined as an excessive fat accumulation in adipose tissue, consequence of an undesirable positive energy balance[356]. Nowadays it is also considered as a complex, multifactorial and preventable disease, with important metabolic manifestations and potential consequences in terms of morbidity, disability and quality of life[357–359].

Obesity has become a global pandemic[360]. associated with excess healthcare costs[411]. The global prevalence of overweight and obesity has increased a 27.5% for adults and a 47.1% for children from 1980 to 2013[361]. An analysis for the Global Burden of Disease Study, estimated that overweight and obesity caused, at the year 2010, 3.4 million deaths, the 3.9% of YLL and the 3.8% of DALYs globally[397].

Although It has traditionally been considered an indicator of poor health, it has recently emerged the concept of "obesity paradox" to describe the unexpected improved prognosis and lower mortality rates found in several diseases in patients with excessive body weight[412–425,427–430]. Regarding stroke, contradictory findings have been reported. Several authors have found an inverse correlation between excessive body weight and mortality in stroke patients[658,660,663,664,671–673]. Contrary, some authors have found that the paradox disappears after adjusting for the initial severity of stroke[676–678]. In regard to functional outcomes, studies are also controversial, some have shown a positive correlation between better prognosis and body weight[673,679,680], whereas other authors have found a poorer prognosis[683–685]. Therefore, nowadays there are still doubts as to whether or not this paradox actually exists in stroke patients, and in case it is a real phenomenon, which are mechanisms that explain such association?

Obesity generates a low-grade inflammatory response known as "metainflammation"[458], which associates excess body weight to the rest of vascular risk factors, and where different elements such as immune cells, cytokines, chemokines, adipokines or TLRs are involved. Furthermore, some experimental studies have shown that post-stroke peripheral immune response is increased in obesity models[462]. This immune activation might exert a determinant role since, as we noted above, the pro-inflammatory response could contribute to tissue damage. However, despite this potentially increased inflammatory reaction, several authors have shown that obese patients experience a better outcome after stroke. The way obesity influences the inflammatory response in ischemic stroke patients is not known and might provide answers on how obesity affects the prognosis.

On the other hand, white adipose tissue represents an important and abundant source of progenitor cells[771], most of which are ASCs[733,734]. It has been demonstrated the importance of stem cells mobilization from endogenous depots in the evolution of several diseases[715], and some authors have shown that obesity may promote the mobilization of progenitor cells[723,731]. Moreover, apart from transdifferentiation, neurorepair and neuroprotection, one of the proposed main mechanisms for the potential therapeutic properties of ASCs in stroke is immunomodulation[741]. Therefore, progenitor cells could have a major role in the outcome of obese patients after stroke.

Finally, another of the issues involved could be the temperature. Brown adipose tissue is involved in the production of heat through adaptive thermogenesis via UCP1 activation. It is the main responsible for this process and the amount of this tissue and the expression of such protein in adults are inversely related to BMI. As in the acute phase of stroke the stress results in the activation of local and systemic sympathetic responses, and since brown adipose tissue thermogenesis is triggered by the activation of sympathetic nervous system[763], it could be postulated that obese subjects, with low expression of UCP1, are at lower risk to brain damage due to hyperthermia than lean subjects who express more UCP1. However, on the other hand, fatty acids are an important stimulator of UCP1[770], and obesity is associated with higher circulating fatty acids[297]. In addition, the sympathetic discharge that occurs after stroke can lead to increased lipolysis, resulting in higher circulating levels of fatty acids, which could be even higher in obesity due to larger depots. Therefore, it is also possible to found a higher degree of hyperthermia in obese subjects in the acute phase of stroke as a consequence of these facts. These potential differences in body temperature regarding BMI in the acute phase of stroke, could determine, in turn, differences in the prognosis depending on body weight.

With this background, we decided to perform a clinical, molecular and cellular study about the association between obesity and acute ischemic stroke.

The hypothesis were that:

- After ischemic stroke, the outcome of obese patients (BMI ≥ 30 kg/m^2) is not worse than that of non-obese patients (BMI < 30 kg/m^2).
- Obese patients counteract their morbidity through increased expression of anti-inflammatory cytokines.

The objectives were:

- Primary:
    - To compare the clinical characteristics and evolution of two groups of patients, obese (BMI ≥ 30 kg/m^2) and non-obese (BMI < 30 mg/m^2).
    - To determine the inflammatory balance (pro-inflammatory cytokines/anti-inflammatory cytokines) in both groups.

- Secondary:
    - To study the influence of obesity in infarct volume.

- To analyse the role of anthropometric characteristics in ischemic stroke.
- To determine if obesity affects body temperature in the acute phase of ischemic stroke, and how temperature modifies prognosis depending on body weight.
- To evaluate the cell and molecular profiles in both groups of patients.

For this purpose, we performed a case-control study. The two groups were defined as follows: cases, the obese patients (BMI ≥ 30 kg/m^2); controls, the non-obese patients (BMI < 30 kg/m^2). The inclusion was consecutive with a 1:1 proportion. The inclusion criterion was: admission to the Stroke Unit due to a first-ever ischemic stroke within 24 hours from the symptom onset. The exclusion criteria were: chronic inflammatory disease, cancer, severe systemic disease which determines a life expectancy lower than 6 months, infectious disease in the last 15 days, and continuous anti-inflammatory drugs intake in the last 15 days.

Patients were evaluated during hospitalization and at 3 months after stroke for clinical, anthropometric and neuroimaging variables. In a selection of patients, DEXA was performed during the first 2 weeks after stroke to assess the distribution of body fat. Blood samples for laboratory tests were obtained at admission, 72 hours, and at day 7 or at discharge. In such samples, the serum levels of IL-6, IL-10, VEGF, TNFR-I, TNFR-II, MCP-1, MIP-1β, leptin and adiponectin, as well as the expression of TLR2 and TLR4 in neutrophils and monocytes, and the circulating levels of progenitor cells (CD34+/CD45-/CD31-), were analysed. All these variables were recorded in an anonymous database

Over a period of 33 months, a total of 98 patients who fulfilled all the inclusion criteria and none of the exclusion criteria were consecutively included, 48 patients in the control group (non-obese) and 50 in the case group (obese). Both groups were very similar in terms of clinical baseline characteristics, although the presence of atrial fibrillation was higher in obese patients (46% vs. 22.9%, $p = 0.016$). In routine laboratory tests, obesity was associated with higher levels of serum inflammatory markers (leukocytes count, fibrinogen and hsCRP).

Obese patients exhibited a higher proportion of cardioembolic strokes (46% vs. 31.2%), more hemorrhagic transformations (30% vs. 12.5%, $p = 0.035$), and an increased risk of infections during hospitalization (28% vs. 16.7%, $p = 0.179$). We found no significant differences in the severity of neurological impairments (assessed by NIHSS) at admission and during the follow-up between groups. However, obesity was associated with a greater clinical improvement at 3 months (defined as: (NIHSS at admission – NIHSS at 3 months after stroke)/NIHSS at admission x 100), although it was not statistically significant ($62.4 \pm 54.1\%$ vs. $41.4 \pm 79.6\%$, $p = 0.141$). An ANOVA test showed a trend favouring a greater improvement of neurological impairments as BMI increases. Finally, there were no differences in functional outcome (assessed by mRS) at 3 months.

To evaluate the hypothesis that obesity counterbalances the inflammatory response after stroke, we assessed the inflammatory profile of both groups of patients by analysing the levels of IL-6 and IL-10, pro- and anti-inflammatory cytokines respectively. On admission, the levels of IL-6 were significantly lower in controls compared to obese patients (10.4 ± 9.3 pg/mL vs. 14.1 ± 6.7 pg/mL, $p = 0.002$). However, at 72 hours and at 7th day/discharge the levels of IL-6 increased in non-obese while they decreased in obese patients. On the other hand, serum levels of IL-10 at admission were much higher in obese patients (7.1 ± 2.1 pg/mL vs. 2.6 ± 1.4 pg/mL, $p < 0.001$), and slightly increased over the first week in this group. With the aim of indirectly assessing the balance between the two opposing responses (anti- and pro-inflammatory), we created a ratio, the "anti/pro-inflammatory index", which relates the serum levels IL-10 and IL-6. As we defend, this index could represent the "battle" between both streams. First, we observed that values of this index were higher in obese patients (1.3 ± 0.5 vs. 1.1 ± 0.8, $p = 0.203$) and form a curve in relation to BMI that reaches its peak in patients with obesity grade I; secondly, its values were higher in patients with good outcome. Therefore, this supports the fact that, in obese patients, excess adipose tissue could enhance an anti-inflammatory response that counteracts the release of pro-inflammatory cues. Since this index is associated with good outcome, this could be one of the reasons why such patients do not evolve worse despite they probably have a more powerful baseline and hyperacute inflammatory response, and, thus, it could also contribute to their clinical improvement.

Infarct volumes were assessed by brain CT during the first week after stroke and at 3 months. The comparative analysis showed no differences between obese and non-obese patients for volumes between 4th and 7th day (61.5 ± 104.7 cm^3 vs. 61.4 ± 103.4 cm^3, $p = 0.994$) and at 3 months (38.5 ± 68.5 cm^3 vs. 46.5 ± 86.3 cm^3, $p = 0.664$), nor for volume improvement at 3 months (defined as: (volume between 4th and 7th day – volume at 3 months after stroke)/volume between 4th and 7th day x 100) (52.7 ± 32.5% vs. 45.6 ± 23.1%, $p = 0.300$).

We decided to perform a more comprehensive analysis about the anthropometric characteristics, their post-stroke variations, and whether such variations influence the patient's evolution. After 3 months of follow-up, there was a reduction in body weight in both groups, which was higher in obese compared to non-obese patients (5.7 ± 9.1 kg vs. 0.9 ± 5.6 kg, $p = 0.007$). In non-obese patients, despite the weight loss, WC and HC values increased rather than decreased. As the circumferences of compartments with great proportion of adipose tissue increased, this makes us suspect that weight loss could be mainly due to lean mass loss. On the contrary, body weight decrease in obese subjects was accompanied by a significant reduction in WC and smaller in HC. which suggests an important loss of fat mass. On the other hand, body weight loss was higher in the group of patients that experienced bad outcome (6.8 ± 9.1 kg vs. 1.7 ± 6.7 kg, $p = 0.006$). In relation to this finding, we observed that there was positive correlation between body weight reduction and the severity of neurological impairments assessed by NIHSS at 48 hours (Pearson coeff. = 0.454, $p < 0.001$). Weight loss could be the consequence of sarcopenia and loss of lean mass due to occult diseases, more marked catabolic/anabolic imbalance, and prolonged immobilization, or even a loss of lean and fat mass due to difficulties for alimentation and malnutrition. Finally, in order to better understand the distribution of body adipose tissue in stroke patients and its influence, we performed a DEXA study in a sample of patients. Although we were not able to demonstrate any relationship between the distribution of body fat and the severity and prognosis of stroke, we found that abdominal distribution of adipose tissue predominated in both obese and non-obese patients with ischemic stroke.

Since hyperthermia is one of the most potent predictors of poor outcome in acute ischemic stroke[42], we decided to analyse if there was any relationship between body temperature, BMI, and the evolution of our patients. The comparative analysis showed that baseline axillary temperature (35.9 ± 0.5°C vs. 35.6 ± 0.6°C, $p = 0.033$), maximum temperature within the first 24 hours (37.0 ± 0.6°C vs. 36.7 ± 0.6°C, $p = 0.033$), maximum temperature within 24 to 48 hours (37.4 ± 0.6°C vs. 36.8 ± 0.6°C, $p = 0.233$), and presence of hyperthermia in the first 24 hours (defined as an axillary temperature equal or higher to 37.5°C)(28% vs. 12.5%, p =

0.057) were higher in obese compared to non-obese patients, although it was statistically significant only for the first two. In the non-adjusted logistic regression model only maximum temperature during the first 24 hours was associated with bad outcome in non-obese (OR 6.78, 95% CI 1.82 – 25.23) and obese patients (OR 2.94, 95% CI 1.06 – 8.20). Finally, we conducted a multivariate analysis adjusted by the presence of infections during hospitalization. In this case, maximum temperature during the first 24 hours was independently associated with bad outcome only in non-obese patients (OR 7.62, 95% CI 1.4 – 40.96).

To further investigate the possible mechanisms that modulate the post-stroke evolution of obese patients, involved or not in the inflammatory response, we completed the study by analysing different cellular and molecular profiles. Obesity was associated with higher levels of circulating progenitor cells at admission (2232.5 ± 2716.5/250000 lymphocytes vs. 1035.5 ± 2416.1/250000 lymphocytes, p = 0.226), 72 hours (2359.5 ± 2601.6/250000 lymphocytes vs. 662.9 ± 1436.2/250000 lymphocytes, p = 0.034), and at 7th day/discharge (2388.6 ± 2795.8/250000 lymphocytes vs. 947.2 ± 1656.8/250000 lymphocytes, p = 0.029). We found no differences in VEGF serum levels between non-obese and obese patients. The expression of TLRs was lower in obese patients, although it only reached statistical significance for monocyte TLR4 expression on admission. Levels of TNFR-I were significantly lower in obese patients, whereas levels of TNFR-II were significantly higher in obese patients. Although the levels of MCP-1 and MIP-1β were slightly lower in obese patients, this was not significant. The expression of leptin was significantly higher in obese patients. The expression of adiponectin, although slightly higher in obese compared to non-obese patients, it did not reach statistical significance. No statistically significant associations with outcome were found for these molecules among groups.

In summary, in line with what has been published in recent years regarding the so-called obesity paradox, and unlike what we might intuitively think, obese patients do not show a worse outcome after ischemic stroke, but also seem to experience a greater recovery of neurological impairments. All this, despite having several factors strongly associated with bad outcome such as: elevated serum markers of inflammation, a higher temperature in the acute phase, a higher proportion of cardioembolic strokes, an increased frequency of hemorrhagic transformations, and a higher risk of infections during hospitalization; furthermore, bearing in mind that both groups were comparable in terms of age, severity of stroke at admission, and infarct volumes. We postulate that obesity can counterbalance, to some extent, the inflammatory reaction responsible for the deleterious effects of the mentioned factors of poor prognosis, through an anti-inflammatory stream enhanced in the first moments of stroke. Thus, the finding of higher values of IL-10/IL-6 index would indirectly confirm this anti-inflammatory counterpart associated with excess adipose tissue. On the other hand, and given that white adipose tissue is a source of progenitor cells, the finding that obesity is associated with a greater mobilization of these cells in the acute phase of stroke is of key importance due to their potentially beneficial effects through neurorepair, neuroprotection and immunomodulation. In the present work, we have tried to explore the connection between obesity and ischemic stroke, two major global health problems with severe economic and clinical consequences. Further studies are needed to analyse in depth the mechanisms involved in the relationships between adipose tissue, inflammation and progenitor cells, not only in stroke, but also in many other different pathologies.

Finally, the conclusions were:

1. The outcome of obese patients after ischemic stroke is not worse

than non-obese patients. Even though they show several factors strongly associated with bad outcome such as: elevated serum markers of inflammation, a higher temperature in the acute phase, a higher proportion of cardioembolic strokes, an increased frequency of hemorrhagic transformations, and a higher risk of infections during hospitalization.

2. There is a trend favouring a greater improvement of neurological impairments as BMI increases.

3. The finding of higher values of the IL-10/IL-6 index suggests that obesity may counterbalance the inflammatory response after ischemic stroke through an anti-inflammatory stream.

4. There are no differences in infarct volumes between obese and non-obese patients.

5. The abdominal distribution of adipose tissue is what characterizes ischemic stroke patients.

6. Obese patients exhibit a higher reduction in body weight after ischemic stroke than non-obese patients.

7. Body weight loss after ischemic stroke is associated with bad outcome.

8. The body temperature in the first stages after ischemic stroke onset is higher in obese patients. Despite this fact, obese tolerate better hyperthermia than non-obese patients.

9. Obesity is associated with a greater mobilization of progenitor cells in the acute phase of ischemic stroke.

RESUMEN

El ictus es un importante problema de salud global. Puede ser tanto isquémico como hemorrágico[1]. El ictus isquémico se define como un episodio de disfunción neurológica debido a un infarto a nivel cerebral, medular o retiniano, es decir, muerte celular a nivel del sistema nervioso central atribuible a isquemia[3]. La isquemia cerebral es el resultado de una caída en el flujo sanguíneo cerebral por debajo del límite necesario para mantener el correcto funcionamiento del sistema nervioso[28]. Esta caída es consecuencia de la oclusion de una arteria debido a mecanismos embólicos o hemodinámicos y da lugar a una cascada metabólica y bioquímica, tanto neuronal como glial, que finalmente resulta en la muerte celular[29].

Es una de las principales causas de muerte y discapacidad, y es responsable de elevados gastos en atención médica. El envejecimiento de la población implica que tanto el número absoluto de pacientes con ictus como la pérdida de años de vida ajustados por discapacidad, están aumentando. Entre todas las causas de muerte, el ictus es la tercera a nivel mundial y el ictus isquémico es responsable de la mitad aproximadamente[7]. En relación con los años de vida ajustados por discapacidad, el ictus es la tercera causa[9]. Los gastos en esta patología representan entre el 2 y el 4% de los costes sanitarios y más de un 4% de costes directos en los países industrializados[12].

En la fisiopatología del ictus isquémico, aspectos como la temperatura o la inflamación son de especial importancia. En relación con esta última, aunque ha sido considerada tradicionalmente como una mera reacción del tejido cerebral dañado, hoy sabemos que juega un papel clave[106]. Hay una interacción muy activa entre el sistema nervioso central y el sistema inmune[107]. Elementos tanto de la inmunidad innata como de la adaptativa están involucrados en todas las fases de la cascada isquémica durante y después del evento[106]. Tras el inicio de la isquemia cerebral focal, tienen lugar una intensa reacción inflamatoria caracterizada por la migración de leucocitos periféricos al parénquima cerebral y la activación de la microglía[109–113]. Al detenerse el flujo sanguíneo cerebral, se produce una pérdida energética y necrosis neuronal. La destrucción de las células nerviosas da lugar a la liberación de patrones moleculares asociados a daño al espacio extracelular, activándose diferentes componentes del sistema inmune, entre los que se encuentran los TLRs, lo cual inicia la cascada inflamatoria[122,123]. Las células isquémicas son estimuladas para segregar citoquinas inflamatorias (IL-6, IL-10, TNF-α…) y quimioquinas (MCP-1…), lo cual da lugar a la activación de las moléculas de adhesión a los vasos cerebrales y al reclutamiento de leucocitos periféricos. Una vez activadas, las células inflamatorias son capaces de liberar más citoquinas, metaloproteasas de matriz, óxido nítrico y más especies reactivas de oxígeno, aumentando así el daño celular y determinando la ruptura de la barrera hematoencefálica y la matriz extracelular[114,115]. La ruptura de la barrera contribuye al daño isquémico secundario al permitir que elementos del suero y la sangre entren en el cerebro[116,117]. Esto tiene como resultado la inflamación post-isquémica, que implica a la activación de la microglía y la infiltración del tejido cerebral por células inflamatorias lo cual potencia el daño[117,118].

Existe además una relación muy robusta entre la temperatura corporal y la evolución de la lesión isquémica[42]. En los primeros días tras el ictus aparece hipertermia en más de la mitad de los pacientes[43]. Sus efectos son claramente perjudiciales en modelos animales[54–57] y en humanos[43,49,58–64], por lo que se trata de una de los más potentes predictores de mal pronóstico en la isquemia cerebral aguda. En primer lugar, la intensa liberación de citoquinas o aminoácidos neuroexcitadores en las áreas infartadas puede inducir hipertermia sistémica[52] y, en segundo lugar, la hipertermia sistémica estimula mecanismos inflamatorios, disfunción metabólica, excitotoxicidad, estrés oxidativo, alteración de la barrera hematoencefálica y degradación de proteínas, dando lugar a un aumento de la lesión isquémica[65].

La otra faceta de este trabajo es la obesidad, definida clásicamente como un exceso de acúmulo de tejido adiposo, consecuencia de un indeseable balance energético positivo[356]. Hoy en día se considera además una enfermedad compleja, multifactorial y prevenible, con importantes manifestaciones metabólicas y potenciales consecuencias en términos de morbilidad, discapacidad y calidad de vida[357–359].

La obesidad se ha convertido en una pandemia global[360], asociada a grandes costes sanitarios[411]. La prevalencia global del sobrepeso y la obesidad ha aumentado un 27.5% en los adultos y un 47.1% en los niños desde 1980 a 2013[361]. Un análisis del Global Burden of Disease Study, estimó que el sobrepeso y la obesidad causaron en el año 2010 3.4 millones de muertes a nivel mundial, el 3.9% de los años de vida perdidos y el 3.8% de los años de vida ajustados por disapacidad[397]. Aunque ha sido considerada tradicionalmente un indicador de mala salud, recientemente ha surgido el concepto de "paradoja de la obesidad" para describir el inesperado hallazgo de un mejor pronóstico y unos menores índices de mortalidad en diferentes enfermedades en aquellos pacientes con exceso de peso corporal[412–425,427–430]. En lo que se refiere al ictus, se han descrito hallazgos contradictorios. Numerosos autores han encontrado una correlación inversa entre el exceso de peso corporal y la mortalidad de estos pacientes[658,660,663,664,671–673]. Sin embargo, otros han señalado que la paradoja desaparece cuando se ajusta por la gravedad inicial del ictus[676–678]. Los estudios muestran también resultados controvertidos acerca del pronóstico funcional, mientras algunos han observado una correlación positiva entre un mejor pronóstico y el peso corporal[673,679,680], otros autores han encontrado la asociación inversa[683–685]. Por tanto, hoy en día existen todavía dudas acerca de si dicha paradoja existe o no realmente en los pacientes con ictus, y de cuáles podrían ser los mecanismos implicados.

La obesidad genera una respuesta inflamatoria de bajo grado conocida como "metainflamación"[458], que asocia el exceso de peso corporal con el resto de los factores de riesgo vascular y en la cual diferentes elementos como células inmunes, citoquinas, quimioquinas, adipoquinas o TLRs, están implicados. Además, algunos estudios experimentales han mostrado que la respuesta inmune tras el ictus está aumentada en los modelos de obesidad[462]. Esta activación inmune podría ejercer un papel determinante ya que, como señalamos previamente, la respuesta pro-inflamatoria contribuye al daño tisular. Sin embargo, a pesar de esta potencialmente aumentada reacción inflamatoria, numerosos autores han señalado que los pacientes obesos experimentan un mejor pronóstico tras el ictus. El modo en el que la obesidad modula la respuesta inflamatoria en los pacientes con ictus isquémico no es conocido y podría ofrecer respuestas a cómo esta entidad afecta al pronóstico.

Por otro lado, el tejido adiposo blanco representa una importante y abundante fuente de células progenitoras[771], la mayor parte de las cuales son las denominadas células madre derivadas de adipocitos[733,734]. Se ha demostrado la importancia de la movilización de células madre a partir de depósitos endógenas en la evolución de numerosas enfermedades[715], y algunos autores han señalado que la obesidad podría estimular la movilización de células progenitoras[723,731]. Además, aparte de la transdiferenciación, la neurorreparación y la neuroproteción, uno de los principales mecanismos que explicarían el potencial terapéutico de las células madre derivadas de adipocitos en el ictus es la inmunomodulación[741]. Por tanto, estas células progenitoras podrían tener un papel principal en la recuperación de los pacientes con ictus.

Por último, otro de los aspectos implicados podría ser la temperatura. El tejido adiposo pardo está implicado en la producción de calor a través de la termogénesis adaptativa mediante la activación de UCP1. Es el principal responsable de este proceso y la cantidad de este tejido y la expresión de dicha proteína guardan una relación inversa con el índice de masa corporal. Ya que en la fase aguda del ictus el estrés provoca la activación de respuestas simpáticas a nivel local y sistémico y, puesto que la termogénesis dependiente del tejido adiposo pardo es activada por el sistema nervioso simpático[763], se podría postular que los sujetos obesos, con menor expresión de UCP1, tienen menor riesgo de daño cerebral por hipertermia que los sujetos delgados, que expresan más UCP1. Sin embargo y por otro lado, los ácidos grasos son un importante estimulador de UCP1[770], y la obesidad se asocia a niveles elevados en suero de estas moléculas[297]. Además, la descarga simpática que ocurre tras el ictus puede dar lugar a un aumento de la lipólisis, lo cual llevaría a un incremento de los niveles circulantes de ácidos grasos, que serían incluso más altos en los pacientes obesos debido a que presentan mayores depósitos. De este modo, es posible que encontremos temperaturas más elevadas en la fase aguda del ictus en los pacientes obesos. Estas potenciales diferencias en la temperatura corporal podrían determinar, a su vez, diferencias en el pronóstico en relación con el peso corporal.

Con estos antecedentes decidimos llevar a cabo un estudio clínico, molecular y celular acerca de la asociación entre la obesidad y el ictus isquémico agudo.

Las hipótesis fueron que:

- Tras el ictus isquémico, el pronóstico de los pacientes obesos (índice de masa corporal ≥ 30 kg/m^2) no es peor que el de los pacientes no obesos (índice de masa corporal < 30 kg/m^2).
- Los pacientes obesos contrabalancean su morbilidad a través de

una expresión incrementada de citoquinas antiinflamatorias.

Los objetivos fueron:

- Primarios:
    - Comparar las características clínicas y la evolución de dos grupos de pacientes, obesos (índice de masa corporal ≥ 30 kg/m²) y no obesos (índice de masa corporal < 30 kg/m²).
    - Determinar el balance inflamatorio (citoquinas proinflamatorias/antiinflamatorias) en ambos grupos.
- Secundarios:
    - Estudiar la influencia de la obesidad en el volumen de infarto.
    - Analizar el papel de las características antropométricas en el ictus isquémico.
    - Determinar si la obesidad afecta a la temperatura corporal en la fase aguda del ictus isquémico, y cómo la temperatura modifica el pronóstico dependiendo del peso corporal.
    - Evaluar los perfiles celulares y moleculares en ambos grupos de pacientes.

Para este propósito diseñamos un estudio de casos y controles. Ambos grupos se definieron de la siguiente forma: casos, los pacientes obesos (índice de masa corporal ≥ 30 kg/m²); controles, los pacientes no obesos (índice de masa corporal < 30 kg/m²). La inclusión fue consecutiva con una proporción 1:1. El criterio de inclusión fue: ingreso en la Unidad de Ictus debido a un primer ictus isquémico de menos de 24 horas de evolución.

Los criterios de exclusión fueron: enfermedad inflamatoria crónica, cáncer, enfermedad sistémica grave, enfermedad infecciosa en los últimos 15 días, toma continua de antiinflamatorios en los últimos 15 días.

Los pacientes fueron evaluados durante la hospitalización y a los 3 meses tras el ictus para la recogida de variables clínicas, antropométricas y de neuroimagen. Se llevó a cabo un estudio mediante DEXA en una muestra de pacientes durante las primeras 2 semanas tras el ictus para evaluar la distribución de la grasa corporal. Las muestras de sangre para los análisis de laboratorio se extrajeron en el momento del ingreso, a las 72 horas y al 7º día o al alta. En dichas muestras, se analizaron los niveles séricos de IL-6, IL-10, VEGF, TNFR-I, TNFR-II, MCP-1, MIP-1β, leptina y adiponectina, así como la expresión de TLR2 y TLR4 en neutrófilos y monocitos, y los niveles circulantes de células progenitoras (C34+/CD45-/CD31-). Todas estas variables se registraron en una base de datos anónima.

En un período de 33 meses, se incluyeron de forma consecutiva un total de 98 pacientes que cumplieron todos los criterios de inclusión y ninguno de exclusión, 48 en el grupo control (no obesos) y 50 en el grupo de casos (obesos). Ambos grupos presentaron características clínicas basales muy similares, aunque la presencia de fibrilación auricular fue mayor en los pacientes obesos (46% vs. 22.9%, $p = 0.016$). En los análisis de rutina, la obesidad se asoció a niveles séricos más elevados de marcadores de inflamación (recuento de leucocitos, fibrinógeno y proteína C reactiva ultrasensible).

Los pacientes obesos mostraron una mayor proporción de infartos cardioembólicos (46% vs. 31.2%), un mayor número de transformaciones hemorrágicas (30% vs. 12.5%, $p = 0.035$) y un riesgo aumentado de infecciones durante el ingreso (28% vs. 16.7%, $p = 0.179$). No encontramos diferencias significativas entre ambos grupos en la gravedad del déficit neurológico (evaluada mediante la NIHSS) al ingreso ni durante el seguimiento. Sin embargo, la obesidad se asoció a una mayor recuperación clínica a los 3 meses (definida como: (NIHSS al ingreso – NIHSS a los 3 meses tras el ictus)/NIHSS al ingreso x 100), aunque esta diferencia no alcanzó significación estadística (62.4 ± 54.1% vs. 41.4 ± 79.6%, $p = 0.141$). Un test ANOVA mostró una tendencia en favor de una mayor recuperación del déficit neurológico a medida que el índice de masa corporal aumenta. Finalmente, no hubo diferencias en el pronóstico funcional (evaluado mediante mRS) a los 3 meses.

Para analizar la hipótesis de que la obesidad contrabalancea la respuesta inflamatoria tras el ictus, evaluamos el perfil inflamatorio de ambos grupos de pacientes estudiando los niveles séricos de IL-6 e IL-10, citoquinas pro y antiinflamatorias respectivamente. Al ingreso, los niveles de IL-6 fueron significativamente más bajos en los controles comparado con los pacientes obesos (10.4 ± 9.3 pg/mL vs. 14.1 ± 6.7 pg/mL, $p = 0.002$). Sin embargo, a las 72 horas y al 7º día/alta los niveles de IL-6 aumentaron en los no obesos mientras que disminuyeron en los obesos. Por otro lado, los niveles séricos de IL-10 al ingreso fueron mucho mayores en los pacientes obesos (7.1 ± 2.1 pg/mL vs. 2.6 ± 1.4 pg/mL, $p < 0.001$) y aumentaron ligeramente a lo largo de la primera semana en este grupo. Con el objetivo de evaluar de forma indirecta el balance entre las dos respuestas opuestas, anti y proinflamatoria, creamos el "índice anti/proinflamatorio", el cual relaciona los niveles séricos de IL-10 e IL-6. Como defendemos, este índice podría representar la "lucha" que existe entre estas dos corrientes. En primer lugar, observamos que los valores de este índice fueron mayores entre los pacientes obesos (1.3 ± 0.5 vs. 1.1 ± 0.8, $p = 0.203$) y formaban una curva en relación con el índice de masa corporal que alcanza su pico en los pacientes con obesidad de grado I. En segundo lugar, los pacientes con buen pronóstico mostraron valores más altos. Por tanto, esto apoya el hecho de que, en los pacientes obesos, el exceso de tejido adiposo podría potenciar una respuesta antiinflamatoria que contrarreste la liberación de señales proinflamatorias. Dado que este índice se asocia a buen pronóstico, esta podría ser una de las razones por las cuáles dichos pacientes no evolucionan peor a pesar de tener probablemente una respuesta inflamatoria basal e hiperaguda más potente, y podría contribuir de este modo a su recuperación clínica.

Los volúmenes de infarto fueron evaluados mediante tomografía computarizada durante la primera semana tras el ictus y a los 3 meses. El análisis comparativo no mostró diferencias entre pacientes obesos y no obesos para los volúmenes del 4º al 7º día (61.5 ± 104.7 cm^3 vs. 61.4 ± 103.4 cm^3, $p = 0.994$) o a los 3 meses (38.5 ± 68.5 cm^3 vs. 46.5 ± 86.3 cm^3, $p = 0.664$), ni para la mejoría del volumen a los 3 meses (definida como: (volumen entre el 4º y el 7º día – volumen a los 3 meses tras el ictus)/volumen entre el 4º y el 7º día x 100)(52.7 ± 32.5% vs. 45.6 ± 23.1%, $p = 0.300$).

Decidimos llevar a cabo un análisis más exhaustivo acerca de las características antropométricas, sus variaciones tras el ictus y la posibilidad de que dichos cambios influyeran en la evolución de los pacientes. Tras 3 meses de seguimiento, hubo un descenso en el peso corporal en ambos grupos, pero mayor en los pacientes obesos (5.7 ± 9.1 kg vs. 0.9 ± 5.6 kg, $p = 0.007$). En los pacientes no obesos, a pesar de la pérdida de peso, los valores de la circunferencia de cintura y de la circunferencia de cadera aumentaron en lugar de descender. Ya que las circunferencias de compartimentos con gran proporción de tejido adiposo aumentaron, esto nos hace pensar que la pérdida de peso en estos pacientes pudo ser sobre todo a expensas de pérdida de masa magra. Por el contrario, el descenso de peso de los sujetos obesos se acompañó de una reducción significativa de la circunferencia de cintura y algo menor de la circunferencia de cadera, lo que sugiere una importante pérdida de tejido adiposo. Por otro lado, la pérdida de peso fue mayor en el grupo de pacientes que experimentaron mal pronóstico (6.8 ± 9.1 kg vs. 1.7 ± 6.7 kg, $p = 0.006$). En relación con este hallazgo, observamos una correlación positiva entre la pérdida de peso corporal y la gravedad del déficit neurológico a las 48 horas evaluado mediante la NIHSS (Pearson coeff. = 0.454, $p < 0.001$). La pérdida de peso podría ser consecuencia de sarcopenia y pérdida de masa magra por enfermedades ocultas, un desequilibrio catabólico/anabólico más marcado y la inmovilización prolongada, o incluso una pérdida tanto de masa magra como masa grasa debido a dificultades para la alimentación y malnutrición. Finalmente, para entender mejor la distribución del tejido adiposo en los pacientes con ictus y su influencia, realizamos un subestudio mediante DEXA en una selección de pacientes. Aunque no hemos sido capaces de demostrar ninguna relación entre la distribución de la grasa corporal y la gravedad o el pronóstico del ictus, hemos observado que, tanto en los pacientes obesos como en los no obesos con ictus isquémico, predominó la distribución abdominal del tejido adiposo.

Dado que la hipertermia es uno de los predictores más potentes de mal pronóstico en el ictus isquémico agudo[42], decidimos analizar si existe alguna relación entre la temperatura corporal, el índice de masa corporal y la evolución de los pacientes. El análisis comparativo mostró que la temperatura axilar basal (35.9 ± 0.5°C vs. 35.6 ± 0.6°C, $p = 0.033$), la temperatura máxima en las primeras 24 horas (37.0 ± 0.6°C vs. 36.7 ± 0.6°C, $p = 0.033$), la temperatura máxima entre las primeras 24 y 48 horas (37.4 ± 0.6°C vs. 36.8 ± 0.6°C, $p = 0.233$) y la presencia de hipertermia en las primeras 24 horas (definida como una temperatura axilar mayor o igual de 37.5°C)(28% vs. 12.5%, $p = 0.057$) fueron mayores en los pacientes obesos comparado con los no obesos, aunque solo alcanzó significación estadística en las dos primeras. En el modelo de regresión logística no ajustado, solo la temperatura máxima en las primeras 24 horas se asoció de forma significativa a mal pronóstico en los no obesos (OR 6.78, 95% CI 1.82 – 25.23) y en los obesos (OR 2.94, 95% CI 1.06 – 8.20). Finalmente, realizamos un análisis multivariado ajustado por la presencia de infecciones durante el ingreso. En este caso, la temperatura máxima en las primeras 24 horas se asoció de forma independiente a mal pronóstico solamente en los pacientes no obesos (OR 7.62, 95% CI 1.4 – 40.96).

Para investigar en mayor profundidad los posibles mecanismos, implicados o no en la respuesta inflamatoria, que modulan la evolución tras el ictus en los pacientes obesos, completamos el estudio con un análisis de diferentes perfiles celulares y moleculares. La obesidad se asoció a niveles más altos de células progenitoras circulantes en el momento del ingreso (2232.5 ± 2716.5/250000 linfocitos vs. 1035.5 ± 2416.1/250000 linfocitos, p = 0.226), a las 72 horas (2359.5 ± 2601.6/250000 linfocitos vs. 662.9 ± 1436.2/250000 linfocitos, p = 0.034), y al 7º día/alta (2388.6 ± 2795.8/250000 linfocitos vs. 947.2 ± 1656.8/250000 linfocitos, p = 0.029). No encontramos diferencias en los niveles séricos de VEGF entre obesos y no obesos. La expresión de TLRs fue menor en los pacientes obesos, aunque solo alcanzó significación estadística para la expresión de TLR4 en monocitos al ingreso. Los niveles de TNFR-I fueron significativamente más bajos en los sujetos obesos, mientras los de TNFR-II fueron significativamente más altos en ese grupo de pacientes comparado con los controles. Aunque los niveles de MCP-1 y MIP-1β fueron ligeramente más bajos en los pacientes obesos, esto no fue significativo. Los niveles de leptina fueron significativamente mayores en los pacientes obesos. Los niveles de adiponectina fueron ligeramente mayores en los obesos respecto a los no obesos, pero no alcanzó significación estadística. No encontramos asociaciones estadísticamente significativas con el pronóstico para los niveles de estas moléculas en ambos grupos de pacientes.

En resumen, en línea con lo que se ha venido publicando en los últimos años en relación con la llamada paradoja de la obesidad, y contrariamente a lo que intuitivamente se podría pensar, los pacientes obesos no muestran un peor pronóstico tras el ictus isquémico, de hecho, parece que experimentan una mayor recuperación de las secuelas neurológicas. Todo ello, a pesar de presentar numerosos factores asociados de forma consistente con mal pronóstico como son: niveles séricos elevados de marcadores de inflamación, una temperatura corporal más elevada en la fase aguda, una mayor proporción de ictus de origen cardioembólico, una mayor frecuencia de transformaciones hemorrágicas y un mayor riesgo de infecciones durante el ingreso. Además, hay que tener en cuenta que ambos grupos, obesos y no obesos, fueron comparables en términos de edad, gravedad inicial del ictus y volúmenes de infarto. Nosotros postulamos que la obesidad puede contrabalancear, hasta cierto punto, la reacción inflamatoria responsable de los efectos perjudiciales de los mencionados factores de mal pronóstico, a través de una "corriente" antiinflamatoria potenciada en las primeras fases del ictus. De esta forma, el hallazgo de valores más altos del índice IL-10/IL-6 confirmaría de forma indirecta esta contrapartida antiinflamatoria asociada con el exceso de tejido adiposo. Por otro lado, y dado que el tejido adiposo es una fuente de células progenitoras, el hallazgo de que la obesidad se asocia con una mayor movilización de estas células en la fase aguda del ictus podría ser clave debido a sus potenciales efectos beneficiosos a través de la neurorreparación, neuroprotección e inmunomodulación. En el presente trabajo hemos tratado de explorar la conexión entre la obesidad y el ictus isquémico, dos importantes problemas de salud a nivel global con graves consecuencias clínicas y económicas. Se necesitan más estudios para analizar en profundidad los mecanismos implicados en la relación entre el tejido adiposo, la inflamación y las células progenitoras, no solo en el ictus isquémico, sino también en muchas otras patologías.

Finalmente, las conclusiones fueron:

1. El pronóstico de los pacientes obesos tras el ictus isquémico no es peor que el de los no obesos. Y ello a pesar de que los primeros presentan factores asociados de forma consistente a mal pronóstico como son: niveles séricos elevados de marcadores de inflamación, una temperatura corporal más elevada en la fase aguda, una mayor proporción de ictus de origen cardioembólico, una mayor frecuencia de transformaciones hemorrágicas y un mayor riesgo de infecciones durante el ingreso.

2. Existe una tendencia a favor de una mayor recuperación del déficit neurológico a medida que aumenta el índice de masa corporal.

3. El hallazgo de valores más altos del índice IL-10/IL-6 sugiere que la obesidad podría contrabalancear la respuesta inflamatoria tras el ictus isquémico a través de una "corriente" antiinflamatoria.

4. No hay diferencias en los volúmenes de infarto entre pacientes obesos y no obesos.

5. La distribución abdominal del tejido adiposo es la que caracteriza a los pacientes con ictus isquémico.

6. Los pacientes obesos experimentan una mayor reducción del peso corporal tras el ictus isquémico que los pacientes no obesos.

7. La pérdida de peso tras el ictus isquémico se asocia a mal pronóstico.

8. La temperatura corporal en las primeras fases tras el ictus isquémico es mayor en los pacientes obesos. A pesar de ello, los obesos toleran mejor la hipertermia que los no obesos.

9. La obesidad se asocia con una mayor movilización de células

progenitoras en la fase aguda del ictus isquémico.