ESCOLA
SUPERIOR
DE SAÚDE
POLITÉCNICO
DO PORTO

P.PORTO

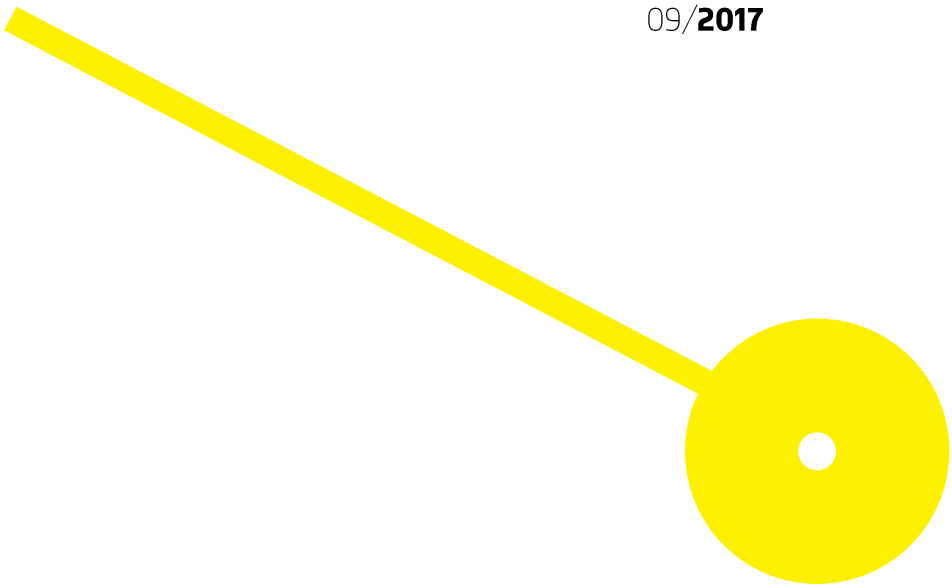
M

MSC DEGREE IN HEALTH BIOCHEMISTRY
BIOTECHNOLOGY

The effect of adipocyte secretome in bacterial growth within an hyperglicemic environment

Miguel Bronze

09/**2017**



ESCOLA
SUPERIOR
DE SAÚDE
POLITÉCNICO
DO PORTO

Miguel Bronze

The effect of adipocyte secretome in bacterial growth within an hyperglicemic environment

Dissertação submetida à Escola Superior de Saúde para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica em Saúde – Ramo em Biotecnologia, realizada sob a orientação do Professor Doutor Rúben Fernandes, Professor Adjunto com Agregação da Escola Superior de Saúde (ESS), Instituto Politécnico do Porto (P.PORTO)

Novembro, 2017

Acknowledgements

First of all I would like to thank the Escola Superior de Saúde - Politécnico do Porto for allowing me to work on their premises and providing me with the adequate material which allowed me to perform my research.

Here I present my appreciation to my university supervisor Rúben Fernandes, PhD for all his patience and tolerance, positive motivation, allowing me to finish this dissertation. I would also like to thank Cláudia Pereira for being an incredibly mentor and companion throughout the whole process.

A huge thank you to my loving family. For always being present, supporting my life choices and helping me overcome the challenges of becoming an emigrant. To my sister who was also at the time undertaking the same task as I and gave much needed support and motivation. To all my laboratory companions that made each day better, not only just with their presence but with insightful knowledge as well.

Thank you Jelena for always being there, and always believing in me.

Table of contents

Introduction

This project aims to check whether there is an influence on the bacterial growth due to the presence of the adipocyte's secretome by creating an in vitro model modulating obesity. Due to the rise of obesity all over the world, to discover a link between it and bacterial infections is crucial to better understand exactly what mechanisms hide behind it. Several different bacterial strains will be used to test the model. The strains to be used in this project were selected taking in consideration the most common infections, ranging from enterobacteria, gram positive and gram negative (*Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145, *Kebsiela pneumoniae* ATCC BAA-1705, *Salmonella enterica* ATCC 13076, *Proteus mirabilis* ATCC 25933, *Sthapylococcus aureus* ATCC 25923, *Sthapylococcus epidermis* ATCC 14990 and *Mycobacterium smegmatis* ATCC 19420). In order to mimic the adipocyte's secretome 3T3-L1 pre-adipocytes were selected to be grown and differentiated, which will later be incubated in serum-free DMEM, by harvesting the medium after a period of 24 hours a medium composed by DMEM and the 3T3-L1 adipocyte's is obtained. The bacterial strains will be grown in liquid M9 minimal medium and then placed in 96 wells microplates. Each strain will be placed within 3 different mediums, M9, to ensure the bacteria is alive, used as a control, DMEM and CM. With these last two mediums we will be able to verify the difference in growth caused by the adipocyte's secretome. The growth will be monitored through optical density, at a wavelength of 620nm during an expected period of 2 weeks, which may be adjusted impending on the results obtained.

I. State of the art

1.1 The Metabolic Syndrome

Epidemiological studies performed in the second half of the 20th century lead to the identification of several cardiovascular risk factors, including central obesity, insulin resistance, hyperinsulinaemia and hypertension. Additionally it was also found that certain cardiovascular risk factors tend to cluster, hence increasing the cardiovascular risk, this phenomenon became known as metabolic syndrome. (Cortez-Dias, Martins, Belo, Fiuza, & Investigadores do estudo, 2011)

The metabolic syndrome can be roughly defined as a cluster of various cardiovascular risks within an individual, being the core symptoms, central obesity, impaired glucose metabolism, athrogenic dyslipidemia (hypertriglyceridemia and/or low HDL cholesterol) and hypertension. However there are several clinical definitions available proposed by different scientific societies, which differ from each other by the threshold of each cardiovascular risk factor, the minimal number of cardiovascular risk factors required to be present and which ones are considered essential.

1.1.1 Its history

It was more than 80 years ago that the metabolic syndrome was first mentioned. A Swedish physician named Kylin described a clustering of hypertension, hypergycaemia and gout (Kylin, 1923). Vague in 1947 (Vague, Combes, Tramoni, & Angeletti, 1979) reported in an article that a certain type of obesity phenotype, upper body, male/type obesity, was associated with the metabolic abnormalities often found in type 2 diabetes and in cardiovascular disease. It was Reaven that 40 years after described the existence of a cluster of metabolic abnormalities, naming it “Syndrome X”, insulin resistance was the central pathophysiological feature, surprisingly not including obesity, a factor that has been linked with the metabolic syndrome in almost all subsequent reports. (Reaven, 1988).

The metabolic syndrome has been previously referred by other nominations such as The Deadly Quartet (Kaplan, 1989), Syndrome X (Reaven, 1988) and the

Insulin Resistance Syndrome (DeFronzo & Ferrannini, 1991). A number of different organizations formulated definitions in order to provide a tool for clinicians and researchers. All of these were concordant in the essential components of the metabolic syndrome, glucose intolerance, obesity, hypertension and dyslipidaemia, but all differing in the details.

The first organization to attempt a definition of the metabolic syndrome was the World Health Organization (WHO), who published it in 1999 (Alberti & Zimmet, 1998). The biological and physiological description of insulin resistance (measured by the euglycaemic camp) was crucial to the WHO definition which was presented as a working model, having the authors acknowledged that it should be improved in the light of new data. There were several limitations indicted to the new model, one of the most important being the measurement of insulin sensitivity through the use of the euglycaemic clamp, making it virtually impossible to use either in epidemiological studies or clinical practice.

The European Group for the Study of Insulin Resistance (EGIR) developed a modified version of the Who definition, identifying that the latter relied heavily on insulin resistance which rendered it too complex to apply in many settings, EGIR's definition relied on fasting insulin, leaving the euglycaemic clamp out of the equation (Syndrome & 2003). The EGIR believe that insulin resistance was the underlying cause of the metabolic syndrome, although restricting the use if the definition to those in whom it could be easily and reliably measured, excluding this way people with diabetes due to the beta-cell dysfunction making estimates of insulin resistance unreliable. In the EGIR definition it was also introduced waist circumference as the measure of adiposity (94 cm for men and 80 cm for women) as well as different parameters for the others components.

In 2001 the National Cholesterol Education Program of the USA introduced the ATP III definition. The overall goal was to achieve clinical utility, as this definition did not include a specific measure of insulin sensitivity as well as adopting an approach not so centered on glucose and treating all components with equal importance it was easily and routinely measured in most clinical and research settings, which made it popular. The ATP III still retained waist circumference as the measure of obesity, introducing higher cut points (102 cm for men and 88 cm for women) and did not incorporate inflammatory and

haemostatic variables as part of an extended definition, contrary to the WHO definition.

The American Association of Clinical Endocrinology (AACE) developed a modification of the ATP III definition believing that insulin resistance represented the core feature. The AACE presented four factors as the "identifying abnormalities" of the metabolic syndrome, these being elevated triglycerides, reduced HDL-C, elevated blood pressure, and elevated fasting and post load glucose. Many other factors were listed as factors that would increase the likelihood of the syndrome rather than key identifying abnormalities, such as obesity, diagnosis of hypertension, gestational diabetes or family history of diabetes, non-European ancestry, age over 40 years and sedentary lifestyle. By excluding obesity as a component, as in their belief central obesity was a contributory factor in the development of insulin resistance rather than a consequence, they faced many critics especially on the upcoming evidence that it is a major risk factor for type 2 diabetes and cardiovascular disease (Expert Panel on Detection, 2001).

In 2005 the International Diabetes Federation (IDF) decided to come forth with a new definition, where it requires someone to have central obesity, defined by wais circumference, plus any of the two following four factors: increased triglycerides or treatment thereof, reduced HDL cholesterol or treatment thereof, increased blood pressure or treatment thereof, raised fasting glucose or previously diagnosed diabetes. The IDF also introduced population specific cutpoints for the obesity requirement, accounting for the fact that different populations, ethnicities and nationalities have different distributions of norms for body weight and waist circumference, as well as recognizing that the relationship between these values and the risk for type 2 diabetes and cardiovascular disease differs in different populations. Despite visceral obesity is recognized as an important factor, the IDF definition was criticized for its emphasis on obesity rather than insulin resistance.

Also in 2005 the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) issue a statement revising the ATP III metabolic syndrome definition, where it keeps ATP III's criteria except for minor modifications. The reason was that the ATP III criteria are simple to use in a

clinical setting and have the advantage of avoiding emphasis on a single cause. In addition a large number of studies have been carried out to evaluate the ATP II criteria for the metabolic syndrome. This statement maintains continuity with the original ATP III definition mainly because no compelling reasons were found for making a change apart from the threshold for the elevated fasting glucose being changed from 110 to 100 mg/dL. This latter definition of the metabolic syndrome has been widely adopted worldwide (Lemieux et al., 2000).

The metabolic syndrome as always have his own share of disbelievers, as is the case of Edwin Gale, who in 2005 published an article entitled “The myth if the metabolic syndrome”, where he states that the metabolic syndrome is nothing but a fairy tale driven by the pharmaceutical companies in search of new medical conditions with exploitable treatments (Gale, 2005).

Table I - Definition of the metabolic syndrome by different institutions (T2DM - type 2 diabetes mellitus; WC - waist circumference; BMI -body mass index; TG -triglycerides; IGT - impaired glucose tolerance; IFG - impaired fasting glucose)

Clinical Measure	**WHO(1998)**	**EGIR**	**ATP III (2001)**	**AACE (2003)**	**IDF (2005)**	**AHA/NHLBI (2005)**
Insulin resistance	IGT, IFG, T2DM, or lowered insulin sensitivity **plus any 2 of the following**	Plasma insulin >75th percentile **plus any 2 of the following**	None, **but any 3 of the following 5 features**	IGT or IFG **plus any of the following based on clinical judgment**	None	IGT or IFG **plus any of the following based on clinical judgment**
Body weigh	Men: waist-to-hip ratio >0.90; women: waist-to-hip ratio> 0.85 and/or BMI >30 kg/m2	WC ≥ 94 cm in men or ≥ 80 cm in women	WC ≥ 102 cm in men or ≥ 88 cm in women	BMI ≥25 kg/m2	Increased WC (population specific) **plus any 2 of the following**	102 cm in men 88 cm in women
Lipid	TG ≥ 150 mg/dL and/or HDL-C <35 mg/dL in men or < 39 mg/dL in women	TG ≥ 150 mg/dL and/or HDL-C < 39 mg/dL in men or women	TG ≥ 150 mg/dL HDL-C <40 mg/dL in men or < 50 mg/dL in women	TG ≥ 150 mg/dL and HDL-C < 40 mg/dL in men or < 50 mg/dL in women	TG ≥ 150 mg/dL or on TG Rx HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on HDL-C Rx	≥ 150 mg/dL (1.7 mmol/L) or On drug treatment for elevated triglycerides <40 mg/dL (1.03 mmol/L) in men < 50 mg/dL (1.3 mmol/L) in women or On drug treatment for reduced HDL-C
Blood pressure	≥ 140/90 mm Hg	≥ 140/90 mm Hg or on hypertension Rx	≥ 130/85 mm Hg	≥ 130/85 mm Hg	≥ 130 mm Hg systolic or ≥ 85 mm Hg diastolic or on hypertension Rx	≥130/85 mm Hg systolic blood pressure or On antihypertensive drug treatment in a patient with a history of hypertension
Glucose	IGT, IFG, or T2DM	IGT or IFG (but not diabetes)	>110 mg/dL (includes diabetes)	IGT or IFG (but not diabetes)	≥ 100 mg/dL (includes diabetes)	≥100 mg/dL or On drug treatment for elevated glucose
Other	Microalbuminuria			Other features of insulin resistance		

1.1.2 The Metabolic Syndrome in the world

The metabolic syndrome has been increasing globally. It's estimated that 20-25% of the population carries this syndrome. Diverse epidemiologic studies have detect a great variability on the prevalence of the metabolic syndrome pending on the geographical area, sex and ethnicity, suggesting the importance of genetic and

environmental factors as well as the diagnose criteria that were applied (Alberti, Zimmet, & Shaw, 2006).

In the USA, data from the Third National Health and Nutrition Examination Survey ("Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report," 2002), demonstrated that 7% of the population aged between 20-29 years old, 42% of the population aged from 60-69 years old and 44% of the population aged higher than 70 years were diagnosed with the metabolic syndrome. This study surveyed 8814 adults residing in the USA, and was able to determine that the incidence rises with the age, especially above 40 years old. It was possible to verify significant variants according to ethnicity, being the metabolic syndrome more predominant between Hispanics (31,9%) rather than in Caucasians (23,8%) or Afro-Americans (21,6%). The same study was also able to demonstrate that the prevalence between the two sexes is identical, but it was noted that amongst the Caucasians there is a greater incidence in males while with Hispanic or Afro-Americans a greater incidence was noted on the females(Ford, Giles, & Dietz, 2002).

In the USA the metabolic syndrome prevalence has been stably rising, especially in adults, in part due to the clinics being more comfortable diagnosing it, but as well due to the prevalence of risk factors such as obesity (Ford, Giles, & Mokdad, 2004). Others studies suggest that the prevalence amongst western societies is high and displays a positive growth as a consequence of the obesity epidemic, especially in young people (Weiss et al., 2004).

In Europe, according to the DECODE study the prevalence of the metabolic syndrome is of 15,7% in men and 14,2% in women (Hu et al., 2004).

In Portugal there have been some regional studies (von Hafe, Lopes, Maciel, & Barros, 1998) (Santos, Lopes, & Barros, 2004) conducted regarding the incidence of the metabolic syndrome. In 2008 it was documented that the prevalence of obesity and excess weight in a sample of 8116 Portuguese, revealing that 39,4% are overweighed and 14,2% obese (do Carmo et al., 2008). The first study regarding the metabolic syndrome and how it relates to the cardiovascular disease amongst adults in health care (Fiuza, Cortez-Dias, Martins, Belo, & investigators,

2008) , with a sample number of 16856, concluded that the percentage of people affected by the syndrome is high, 27,5% . The study also enabled to conclude that the prevalence amongst women is higher, 28,7%, than in men, 26%. Regarding to geographical distribution, the metabolic syndrome prevalence exhibited significant regional differences, being more prevalent in Alentejo and Madeira and less in Algarve, Lisboa and Açores.

1.1.3 The Metabolic Syndrome and Obesity

Obesity is a fast growing problem that is reaching epidemic proportions worldwide. (James, Rigby, Leach, & International Obesity Task, 2004). There is a conventional agreement on the normal range of body weight values, evaluated as Body Mass Index (BMI $kg.m^{-2}$). It is stated that inside the BMI range between 18.5 and 25 the eventual health risks, due to body weight, are the lowest: this is the normal range. When the BMI is over 30 obesity is diagnosed. It is well known that single components of the cluster, that characterize the metabolic syndrome, are associated with visceral fat: this association does not depend on BMI. When the waist circumference is 102 cm or more in men or 88cm or more in women, the term abdominal obesity can be applied. The advantage of measuring waist circumference is that an excess abdominal fat is correlated more closely with the presence of metabolic risk factors than total body fat. The cut points for defining abdominal obesity are arbitrary. For susceptible individuals, lesser accumulations of abdominal fat can precipitate or aggravate metabolic risk factors. This is particularly so in certain populations; for example, in Asian populations lower waist circumference cut points have been identified to define abdominal obesity (Al-Gindan et al., 2015; Organization, 2000).

Our understanding of the relation between obesity and metabolic risk factors is growing rapidly. This understanding is based on the discovery of multiple products released from adipocytes. In the presence of obesity, these products are released in abnormal amounts. Each of these products has been implicated in the causation of one or another of the metabolic risk factors (Guerre-Millo, 2002). The main released adipokines such as FFA, inflammatory cytokines, PAI-1, adiponectin, leptin and resistin will be further descripted and main effects will also be described in detail in the next chapter.

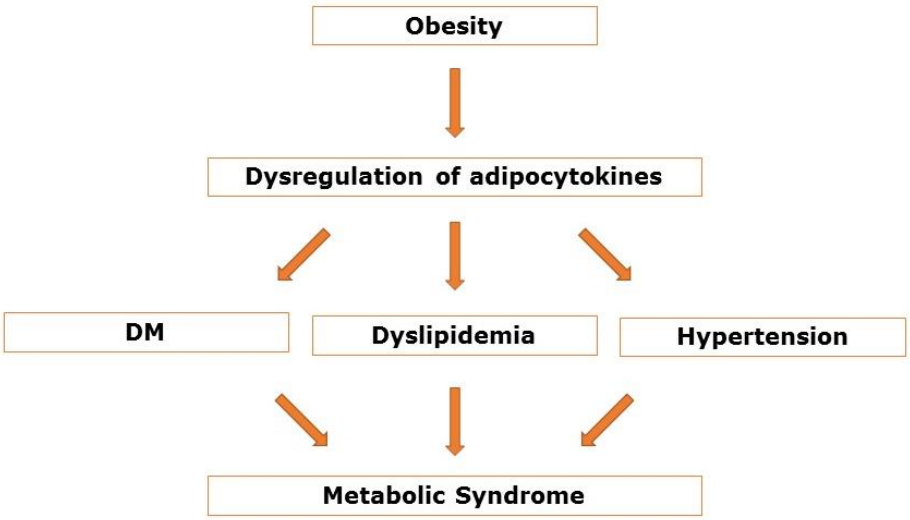


Figure 1 - Concept of the relation between obesity and the metabolic syndrome.

(DM- Diabetes mellitus)

1.1.4 The Metabolic Syndrome and Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes, 2004).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex path ways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or

the non ketotic hyperosmolar syndrome. Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (Expert Committee on the & Classification of Diabetes, 1997, 2003).

Several epidemiological studies have shown that the metabolic syndrome increases the probability to develop diabetes type 2 three to fourfold. The risk increases with the number of elements of the metabolic syndrome present within the individual. This has been shown using different definitions of the metabolic syndrome (Ford, 2005; Hanson, Imperatore, Bennett, & Knowler, 2002; Lakka et al., 2002; Meigs et al., 2006; Sattar et al., 2008; Wilson, D'Agostino, Parise, Sullivan, & Meigs, 2005).

1.1.5 The Metabolic Syndrome and Cardiovascular Diseases

There are several studies linking cardiovascular diseases to the metabolic syndrome, a report concluded that the metabolic syndrome could be responsible for 7% of all cause mortality and up to 17% of cardiovascular diseases (Ford, 2005). Another study also reported that the contribution of the metabolic syndrome to the risk of cardiovascular disease was 34% in men and 16% in women and the risk for coronary heart disease 29% in men and 8% in women (Wilson et al., 2005). The components of the metabolic syndrome that most contributed to the cardiovascular disease outcome were high blood pressure (33%) and low HDL cholesterol (25%). A meta-analysis which included 37 longitudinal studies concluded that there is a 78% increased risk for cardiovascular disease events and death in people with the metabolic syndrome (Gami et al., 2007). Insulin resistance is often clustered with classical cardiovascular risk factors such as lipid abnormalities, glucose intolerance and high blood pressure. Normal insulin signalling in cardiovascular tissues is conducted by two different pathways, a growth-factor-like pathway and one that is predominant in metabolic tissues (Nigro, Osman, Dart, & Little, 2006). Insulin resistance in cardiovascular tissues leads to the inhibition of the metabolic pathway, overstimulating the growth-factor-like pathway causing a decrease in glucose uptake therefore hampering the normal cardiac function (Ferrannini & Iozzo, 2006). Accumulation of abdominal fat increases cardiovascular risk moreover

visceral fat correlates with cardiovascular risk factors (Despres, Lemieux, & Prud'homme, 2001). Insulin resistance is possibly the most important factor linking abdominal visceral adiposity with cardiovascular risk. Impaired suppression of adipocyte lipolysis and elevated non-esterified fatty acids levels are associated with abdominal adiposity, leading to vascular endothelial dysfunction. Visceral adiposity is also correlated with increased levels of the pro-coagulant plasminogen activator inhibitor (PAI-1), low-grade inflammation, systolic blood pressure, quantitative and qualitative changes in lipoproteins such as small dense LDL as well as conveying a greater insulin resistance than subcutaneous fat (Kuk et al., 2006; Weisberg et al., 2003).

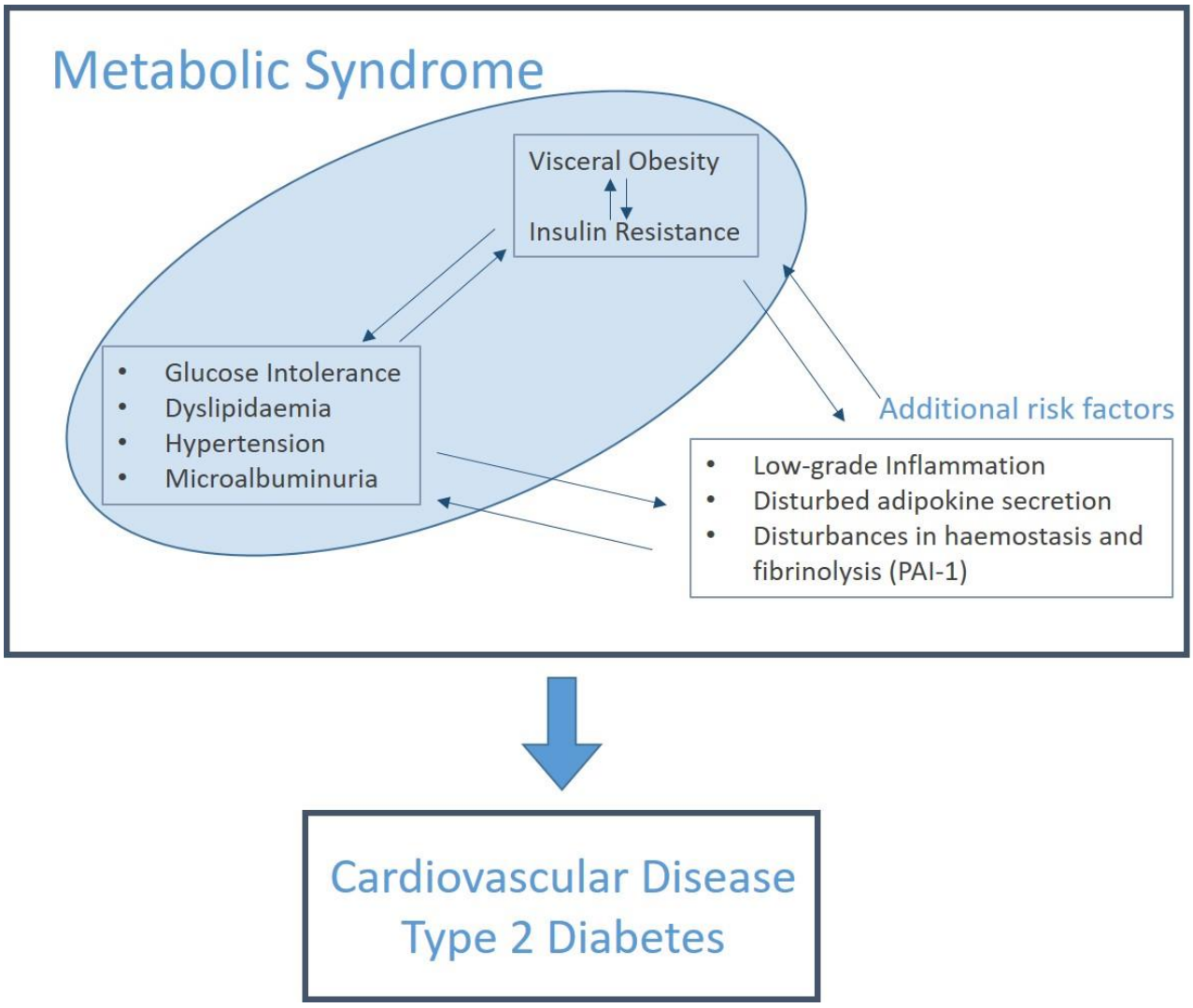


Figure 2 - Concept of the relationship between the metabolic syndrome and cardiovascular disease and type 2 diabetes

1.2 The Metabolic Syndrome and the Adipose Tissue

1.2.1 The Adipose Tissue as an Endocrine Organ

Over the past decade, adipose tissue has gained significant importance for its constitutional and functional complexity. Through the discovery of the ability to secrete hormones, great importance has been attributed to the role of adipose tissue (M. Coelho, Oliveira, & Fernandes, 2013). White adipose tissue may as well be the largest endocrine tissue in humans. Its ability to secrete numerous hormones, growth factors, enzymes, cytokines, complement factors and matrix proteins as well as expressing receptors for most of these factors that are implicated in the regulation of many processes including food intake, energy expenditure, metabolism homeostasis, immunity and blood pressure homeostasis, convey its pleiotropic nature. The secretory nature of the white adipose tissue has made it being seen as an extremely active endocrine tissue, being dynamically involved in cell function regulation through a complex network of endocrine, paracrine and autocrine signals that influence the response of many tissues, including hypothalamus, pancreas, liver, skeletal muscle, kidneys, endothelium, and the immune system, among others (Costa & Duarte, 2006; Matsuzawa, 2006).

1.2.2 Adipocytokines

In the table below the most relevant adipocytokines are listed along with a brief description of its effect.

Table II - Factors secreted by adipose tissue into the bloodstream and respective function/effect in their targets (M. Coelho et al., 2013). (TNFα – tumor necrosis factor α , IL-6 – interleukin-6, PAI-1 – plasminogen activator inhibitor 1, FFA – free fatty acids, ASP – acylation stimulating protein, VEGF – vascular endothelial growth factor, IGF-1 – insulin-like growth factor 1.)

Molecule	**Function/ effect**
Leptin	Signals to the brain about body fat stores. Regulation of appetite and energy expenditure. Wide variety of physiological functions
Adiponectin	Plays a protective role in the pathogenesis of type 2 diabetes and cardiovascular disease
Resistin	Hypothetical role in insulin resistance
TNF-α	Affects insulin receptor signaling, possible cause of the development of insulin resistance in obesit
IL-6	Pro-inflammatory, lipid and glucose metabolism, regulation of body weight
PAI-1	Inhibitor of the fibrinolytic system by inhibition of activation of plasminogen
Angiotensinogen	Precursor of angiotensin II; regulator of blood pressure and electrolyte homeostasis
FFA	Oxidized in tissues to produce local energy. Serve as a substrate for triglyceride and structural molecular synthesis. Involved in the development of insulin resistance
ASP	Influences the rate of triacylglycerol synthesis in adipose tissue
VEGF	Stimulation of angiogenesis
Adipsin	Potential relation between the complement pathway and adipose tissue metabolism
Glycerol	Structural component of the major classes of biological lipids and gluconeogenic precursor
IGF-1	Stimulates proliferation of a wide variety of cells and mediates many cells and many of the effects of growth hormone

1.2.2.1 Leptin

Leptin is an adipocyte-derived hormone belonging to the IL-6 family of cytokines. Leptin crosses the blood-brain barrier, and via reducing neuropeptide Y in the hypothalamus suppresses appetite and increases energy expenditure as well as increasing insulin sensitivity

in various tissues. It is primarily cleared from plasma by the kidney through glomerular filtration followed by proteolytic degradation in the renal tubules (Itoh, Suganami, Hachiya, & Ogawa, 2011; Wu et al., 2006). Adipose tissue and plasma leptin concentrations are dependent on the amount of energy stored as fat as well as the status of energy balance. Increased caloric intake and decreased expenditure result in increased adiposity, which results in increased leptin levels (Briley & Szczech, 2006; Laclaustra, Corella, & Ordovas, 2007). The leptin's short receptor (Ob-Ra) is expressed not only in the central nervous system, but also in some peripheral tissues and leptin has also been implicated in other roles, including modulation of the reward circuitry for feeding, glucose metabolism, lipid oxidation, substrate partitioning, and adipocyte apoptosis (Galic, Oakhill, & Steinberg, 2010; Serradeil-Le Gal et al., 1997).

1.2.2.2 Adiponectin

Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation. Adiponectin is exclusively secreted from adipose tissue (and also from the placenta in pregnancy(J. Chen et al., 2006)) into the bloodstream and is very abundant in plasma relative to many other hormones There is a strong negative correlation between plasma adiponectin concentration in humans and fat mass, with the exception of severe cases of undernutrition and in the newborn (Schraw, Wang, Halberg, Hawkins, & Scherer, 2008).Circulating adiponectin concentrations increase during caloric restriction in animals and humans, such as in patients with anorexia nervosa. This observation is surprising, given that adiponectin is produced by adipose tissue. However, a recent study suggests that adipose tissue within bone marrow, which increases during caloric restriction, contributes to elevated circulating adiponectin in this context (Cawthorn et al., 2014). Adiponectin circulates in the bloodstream under three different isoforms: a timer of low molecular weight (LMW); a hexamer of medium molecular weight (MMW); a multimeric high molecular weight (HMW) which is the biologically most active form regarding glucose homeostasis (Oh, Ciaraldi, & Henry, 2007), besides having been associated with a lower risk of diabetes (Zhu et al., 2010) and in recent studies positively associated with coronary artery disease unlike its LMW form (Rizza et al., 2010).

1.2.2.3 Resistin

Resistin also known as adipose tissue specific secretory factor is a cysteine rich adipose derived peptide hormone that is coded by the gene RETN in humans (Wang, Chu, Hemphill, & Elbein, 2002). Resistin levels are increased in both genetic and diet-induce forms of obesity as well as in both mice and human models of obesity and is decreased when the anti-diabetic drug rosiglitazone is taken. In mice with diet-induced obesity blood sugar and insulin action levels improved when anti-resistin antibody was administered (Guzik, Mangalat, & Korbut, 2006; Steppan et al., 2001). Resistin is also implied in diabetes and in the pathogenesis of diabetic complications (Guzik et al., 2006; Wasim et al., 2006). Resistin production is triggered by gonadal hormones, hyperglycemia, IL-6, inflammation and LPS (lipopolysaccharide) and when released in the fat tissue it acts on the adipocytes themselves leading to insulin resistance (Guzik et al., 2006). Resistin expression is found to be three times higher in pre-adipocytes when compared to mature adipocytes which could correlate it with a potential regulator of adipogenesis (McTernan et al., 2002; Steppan et al., 2001).

1.2.2.4 Tumor necrosis factor α

Tumor necrosis factor α (TNF-α) is a cytokine involved in systemic inflammation, mainly produce by macrophages within the adipose tissue although other cells such as adipocytes, lymphocytes, NK cells, neutrophils, mast cells, eosinophils and neurons have been reported of producing it as well (Gahring, Carlson, Kulmar, & Rogers, 1996; Weisberg et al., 2006). It was shown that TNF-α levels are elevated in the metabolic syndrome (Weiss et al., 2004) and that it takes part in insulin resistance within the adipose tissue (Hotamisligil, Arner, Caro, Atkinson, & Spiegelman, 1995). It was also reported that both in humans as in mice the quantity of macrophages within the adipose tissue correlates with fat mass and the high levels of TNF-α could be due to the increased infiltration of the adipose tissue by M1 macrophages who suffered a transformation from monocytes and infiltrated the adipose tissue from the bloodstream (Ouchi, Parker, Lugus, & Walsh, 2011; Schaffler & Scholmerich, 2010; Weisberg et al., 2006). TNF-α was the first adipose derived factor linking obesity, inflammation and diabetes. It has been shown that the mRNA expression levels of TNF-α in adipose tissue within obesity are strongly correlated with the pathogenesis of insulin resistance (Cai et al., 2005).

1.2.2.5 Inter Leukin-6 (IL-6)

Inter Leukin-6 (IL-6) is a pleiotropic cytokine produced by macrophages, T-cells and the adipose tissue who is responsible for 30% of the circulating IL-6. IL-6 concentrations are higher in visceral fat than in subcutaneous fat (Diamond & Eichler, 2002). Plasma IL-6 is positively correlated with obesity, insulin resistance, atherosclerosis, unstable angina, risk of coronary artery disease and predicts type 2 diabetes (Cai et al., 2005; Diamond & Eichler, 2002; Wisse, 2004). In general IL-6 increases glucose uptake, inhibits lipase lipoprotein and induces lipolysis (Cai et al., 2005). IL-6 also controls the hepatic acute-phase response and probably also controls CRP production in the liver. CRP is a strong marker for cardiovascular events, atherosclerosis (Danesh et al., 2004; Pepys & Hirschfield, 2003; Ridker & Morrow, 2003) and is found in elevated levels in the metabolic syndrome and visceral adiposity (Festa et al., 2000; Rexrode, Pradhan, Manson, Buring, & Ridker, 2003).

1.2.2.6 Plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1) is a protein coded by the human gene SERPINE1, also known as endothelial plasminogen activator inhibitor or serpin E1 (Eddy & Fogo, 2006). PAI-1 is mainly produced in the endothelial and vascular smooth muscle but a number of other cells also produce it such as platelets, mesangial cells, monocytes, hepatocytes, fibroblasts, macrophages and adipocytes (Correia & Haynes, 2006). Studies have shown that PAI-1 is upregulated mainly by cytokines such as TNF-α and growth factor-β but also by insulin, angiotensin II, glucocorticoids and some fatty acids, being downregulated by catecholamines (Correia & Haynes, 2006; Skurk & Hauner, 2004). The greater the adipose tissue mass and the greater the fat cell size are correlated with increased PAI-1 production as well as the fact that PAI-1 expression levels are increased in obesity and propositional to visceral adiposity, which led to believe it could be the link between abdominal obesity and the risk for cardiovascular diseases (Mertens & Van Gaal, 2005). PAI-1 can change the balance between fibrinolysis and fibrinogenesis leading to vascular architecture remodelling and the atherosclerotic process (Manolescu, Stoian, Atanasiu, Busu, & Lupescu, 2008). An altered function in the auto-/paracrine function or in the endocrine system within the fat cell disturb the fibrinolytic system raising the risk of cardiovascular disease (Skurk & Hauner, 2004).

1.2.2.7 Angiotensin

Angiotensin is a peptide hormone that makes part of the renin-angiotensin-aldosterone system (RAAS), being all the components expressed by the adipose tissue: angiotensinogen (AGT), renin, angiotensin I converting enzyme and angiotensin II type 1 receptor (Ahima, 2006). Angiotensin causes vasoconstriction leading to an increased blood pressure, it also stimulates the release of aldosterone, causing an increased sodium retention in the distal nephrons also leading to an increased blood pressure (Basso & Terragno, 2001). The adipose tissue angiotensinogen mRNA levels are regulated by nutrition, being decreased during fasting and increased during refeeding, which led to believe that it may regulate adipose tissue differentiation and growth. The RAAS peptides produced in the adipose tissue possibly act in the vasculature and distant targets, regulating blood pressure and cardiovascular responses in obese individuals (Carey et al., 2006). Angiotensin II has also been reported to have an effect on cardiovascular function, influencing hypertension and hemostasis (Touyz & Schiffrin, 1993).

1.2.2.8 Acylation stimulating protein (ASP)

ASP is produced by 3 proteins synthesized and secreted by the adipose tissue, C3, factor B and adiposin in a two-step process (Paglialunga et al., 2008). Plasma ASP levels are increased after meals, improving synthesis and storage of triglycerides, when ASP levels are decreased it leads to a rise in postprandial fatty acids levels, lower weight gain and decreased triglyceride synthesis (Manolescu et al., 2008). ASP influences positively lipogenesis through the translocation of glucose transporter (GLUT-4) in glycerol 3-phosphate and the activity of diacylglycerol acyltransferase (DGAT) (Cianflone, Xia, & Chen, 2003; Manolescu et al., 2008). It has been reported that ASP levels are increased in obesity, T2D and cardiovascular diseases while being reduced with exercise and weight loss. It was also found that ASP levels diminish with age and are higher in children. During an ASP-resistant state, similar to diabetes and cardiovascular diseases, there is a disturbed adipose tissue metabolism and dyslipidemia. (Cianflone, Lu, Smith, Yu, & Wang, 2005; Manolescu et al., 2008).

1.3 Microbial Strains

1.3.1 *Salmonella enterica*

Salmonella enterica is a rod-shaped, flagellated, facultative anaerobic, gram negative bacterium belonging to the genus *Salmonella* (Giannella, 1996). There are 7 subspecies and each subspecies has associated serovars that are distinguished by antigenic specificity, numbering over 2500(Murray PR, 2009). Humans are typically infected with Salmonella after consuming food or drinking water contaminated with bacteria and the transmission of most serovars uses the fecal-oral route (Gopinath, Carden, & Monack, 2012). Salmonella infections are typically classified into four categories: gastroenteritis, enteric fever, focal disease, and chronic carrier state. The infection may be localized to the gastrointestinal tract or may disseminate via the blood or lymphatic system. Focal salmonellosis is thought to be secondary to a brief episode of bacteremia after infection from the gastrointestinal tract. Patients with significant underlying conditions are at increased risk for the development of focal infection. This has been observed in patients with HIV, diabetes, and malignancy (Patrick et al., 2004).

1.3.2 *Escherichia coli*

Escherichia coli is gram negative, facultative anaerobic, rod-shaped, coliform bacterium, they are often found in the lower intestine of warm blooded organisms constituting part of the normal flora. The harmless strains can benefit its host by producing vitamin K and prevent pathogenic bacteria from colonizing the intestine. *Escherichia coli* is expelled into the environment within fecal matter being able to thrive in it matter for 3 days but having its numbers slowly declining afterwards (Russell & Jarvis, 2001; Singleton, 2005; Tenaillon, Skurnik, Picard, & Denamur, 2010). Virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and gram-negative pneumonia (Chung et al., 2017; Lim, Yoon, & Hovde, 2010).

1.3.3 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a gram negative, nonmotile, encapsulate, lactose fermenting, facultative anaerobic, rod-shaped bacterium. It can be found in the mouth, skin and intestines as part of the normal flora (Murray, Rosenthal, & Pfaller). It is naturally present in the soil, 30% of the strains being able to fix nitrate in anaerobic conditions (Sajidan et al., 2004). *Klebsiella* infections are mostly seen in people with a weakened immune system, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, glucocorticoid therapy, renal failure, and certain occupational exposures. The most common infection caused by *Klebsiella* bacteria outside the hospital is pneumonia in the form of bronchopneumonia and bronchitis. These patients have an increased tendency to develop lung abscess, cavitation, empyema, and pleural adhesions. It can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia and septicemia (Jung et al., 2012; Rashid & Ebringer, 2007).

1.3.4 *Proteus mirabilis*

Proteus mirabilis, a member of the *Enterobacteriaceae* family of Gram-negative facultative anaerobic rods, is widely distributed in water, soil and the human intestinal tract. P. mirabilis assumes alternate forms dependent upon the culture medium. When cultured in broth, these bacteria are fimbriated with a few polar flagella, whereas, when cultured on agar, *Proteus mirabilis* can differentiate into highly elongated hyperflagellated swarming cells (Armbruster & Mobley, 2012). Proteus mirabilis is the causative agent of approximately 3% of all opportunistic nosocomial infections including infections in the ear, eye, nose, throat, skin, respiratory tract, burns and wounds (Jacobsen, Stickler, Mobley, & Shirtliff, 2008). The isolation of anti-Proteus antibodies from patients with active rheumatoid arthritis implies that these organisms could be associated with this condition (Deighton, Gray, Bint, & Walker, 1992; Jensen, Haymond, Rizza, Cryer, & Miles, 1989). Proteus mirabilis is an occasional cause of urinary tract infection in the normal host, but it is also the causative agent of a higher percentage of patients with complicated urinary tracts. This includes

individuals with functional or anatomical abnormalities or chronic instrumentation, such as indwelling urinary catheters (Pellegrino, Scavone, Umpierrez, Maskell, & Zunino, 2013).

1.3.5 *Mycobacterium smegmatis*

Mycobacterium smegmatis is an acid-fast bacterial species in the phylum *Actinobacteria* and the genus *Mycobacterium*. It is 3.0 to 5.0 μm long with a bacillus shape and can be stained by Ziehl-Neelsen method and the auramine-rhodamine fluorescent method (Reyrat & Kahn, 2001). *M. smegmatis* was the first mycobacteria discovered after *Mycobacterium tuberculosis*, it was isolated from soil and water and was considered a non-pathogenic microbe for many years. Its role in human pathogenicity was later recognized, although being not so frequent it has been associated with obstructive bronchopulmonary disease, skin and soft tissue after trauma infections, surgery infections and joint infections. Sporadic cases of bacteraemia related to catheters, endocarditis, lymphadenitis and disseminated infection in immunocompromised patients (Halleran, Clamons, & Saha, 2015; Saffo & Ognjan, 2016; Shimizu et al., 2012).

1.3.6 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative, aerobic (and at times facultative anaerobic), bacillus with unipolar motility. It has been identified as an opportunistic pathogen of both humans and plants. P. aeruginosa is the type species of the genus Pseudomonas (Anzai, Kim, Park, Wakabayashi, & Oyaizu, 2000; Murray et al.). An opportunistic, nosocomial pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections. It is the most common cause of infections of burn injuries and of the outer ear (otitis externa), and is the most frequent colonizer of medical devices. Pseudomonas can be spread by equipment that gets contaminated and is not properly cleaned or on the hands of healthcare workers. Pseudomonas can, in rare circumstances, cause community-acquired pneumonias, as well as ventilator-associated pneumonias, being one of the most common agents isolated in several studies (Diekema et al., 1999; Fine et al., 1996; Prithiviraj et al., 2005).

1.3.7 *Staphylococcus aureus*

Staphylococcus aureus is a facultative anaerobic, rod-shaped, gram positive, non-motile, coccal bacterium also known as ”golden staph” or “oro staphina” due to the formation of

large, round yellow colonies. It is both a commensal bacterium and a human pathogen (Wertheim et al., 2005). Approximately 30% of the human population is colonized with *S. aureus*. Simultaneously, it is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections. Bacteremia is perhaps the best-described manifestation of *S. aureus* infection (Laupland et al., 2013). Age is a powerful determinant of *S. aureus* bacteremia incidence, among others such ethnicity, being HIV-infected, being an injection drugs user and hemodialysis patients, the latter ones due to the presence of an intravascular access device and inherited impairments of host defence including neutrophil dysfunction, iron overload and diabetes (Fitzgerald et al., 2011; Jeremiah et al., 2014; Zimakoff et al., 1996).

1.3.8 *Staphylococcus epidermidis*

Staphylococcus epidermidis is a gram positive, non-motile, facultative anaerobe commensal bacterium that colonizes the skin and mucous membranes of mammals and is the most prevalent staphylococcal species found in humans. It has been speculated that one human benefit of *S. epidermidis* colonization is inhibition of attachment of more virulent bacteria such as Staphylococcus aureus; however, as with the entire human microbiota, we are just beginning to understand these complex interactions (Blaser & Falkow, 2009; Lina et al., 2003). *S. epidermidis* has become the most common cause of primary bacteremia and infection of indwelling medical devices, particularly in immunocompromised individuals and neonates. Although sterile site S. epidermidis infections are known to occur, most infections are associated with a foreign body such as catheters or others biomaterials (Rogers, Fey, & Rupp, 2009). Infections by *S. epidermidis* are of a less acute and more long-lasting nature. In these infections, the main virulence mechanism of *S. epidermidis* is biofilm formation (Gotz, 2002). *S. epidermidis* also appears in high frequency in diabetic patients, often found in diabetic foot ulcers and foot osteomyelitis (Aragon-Sanchez, Lazaro-Martinez, Hernandez-Herrero, Quintana-Marrero, & Cabrera-Galvan, 2010; Galkowska et al., 2009).

II. Objectives

The aim of the present study is first to develop an in vitro model to study infection and bacterial growth under hyperglycaemic and high adiposity conditions and then to understand the bacterial growth within these adipocyte's secretome and hyperglycaemic environment.

1. Determine the bacterial growth curve under different mediums:
    1.1. Minimal medium;
    1.2. Dulbecco's Modified Eagle Medium (DMEM);
    1.3. Conditioned DMEM with mature 3T3-L1 secretome;
2. Analyse the influence of the different mediums on the bacterial growth;

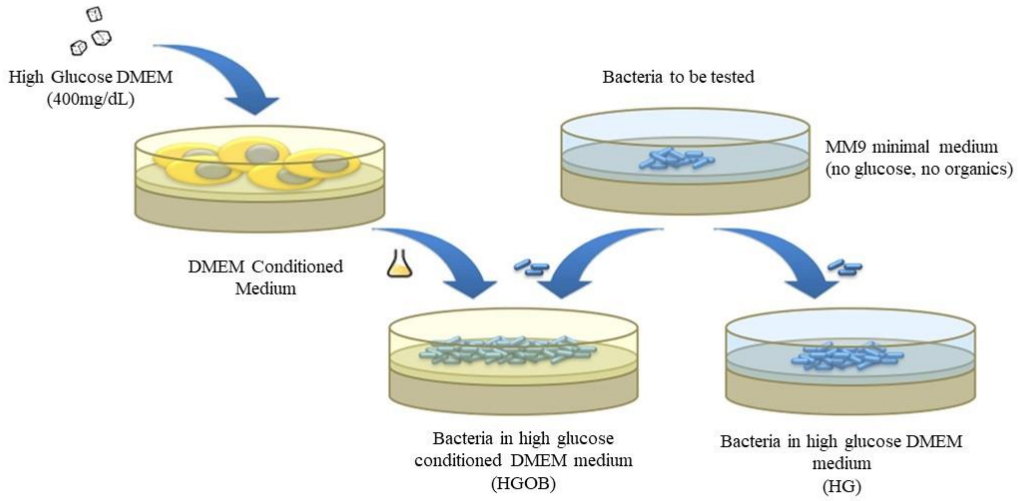


Figure 3 - In vitro model schematic

III. Material and Methods

3.1 Materials

3.1.1 Adipocyte differentiation and medium collection

3T3-L1 pre-adipocytes were harvested and allowed to reach confluence. After 2 days (day 0), the differentiation was initiated by addition of a hormonal mixture composed of 2 µM insulin (Sigma–Aldrich), 1 µM dexamethasone (Sigma– Aldrich) and 0.25 mM isobutylmethylxanthine (Fluka) in complete medium. Three days later (day 3), the induction medium was replaced by complete medium supplemented with insulin only. At day 7, cultures with a differentiation yield higher than 80% were washed with phosphate buffered saline (PBS) and incubated in serum-free DMEM. After 24–h (day 8), the conditioned medium was harvested from the adipocytes cultures, spun for 5 min at 300g and the supernatant was stored at −80°C for the subsequent treatments.

3.1.2 Adipocyte secretome profile characterization

The 3T3-L1 conditioned medium has been previously characterized in a previous experiment, as it can be seen illustrated in figure X the secretion patterns of numerous growth-factors, adipokines, cytokines and angiogenesis-related molecules (P. Coelho, Almeida, Prudencio, Fernandes, & Soares, 2016).

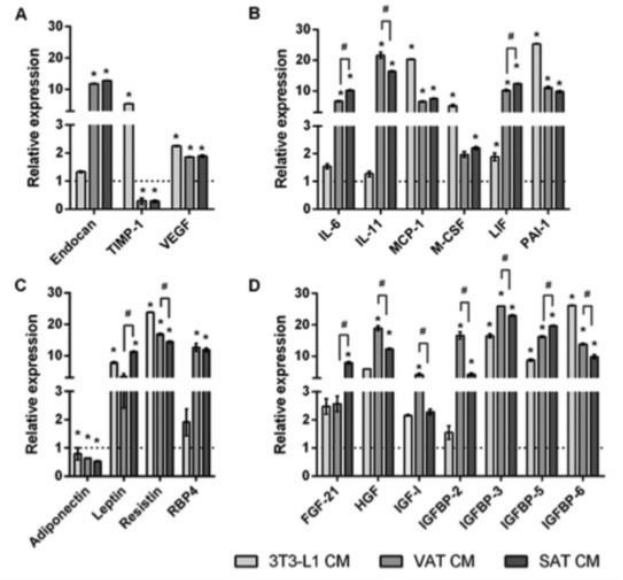


Figure 4 - Secretion profiles of 3T3-L1 cells and subcutaneous and visceral AT organ cultures. (A) Angiogenesis-related molecules. (B) Cytokines. (C) Feed/fasting control molecules. (D) Growth factors. (P. Coelho et al., 2016)

3.1.3 Microbial Strains

The microbial strains used in this project are:

Gram negative

Esherichia coli (ATCC 25922)

Pseudomonas aeruginosa (ATCC 10145)

Kebsiela pneumoniae (ATCC BAA-1705)

Salmonella enterica (ATCC 13076)

Proteus mirabilis (ATCC 2593)

Gram positive

Sthapylococcus aureus (ATCC 25923)

Sthapylococcus epidermis (ATCC 14990)

Mycobacteriaceae

Mycobacterium smegmatis (ATCC 19420)

3.1.3 Culture Mediums

MM9 minimal medium composition:

Na_2HPO_4 - 0.24 M

KH_2PO_4 - 0.11 M

NH_4Cl - 0.09 M

NaCl - 0.04 M

DMEM composition:

The DMEM medium used throughout this work was SIGMA-ALDRICH's D777 medium.

3.2 Methods

3.2.1 Determination of the bacterial growth curve

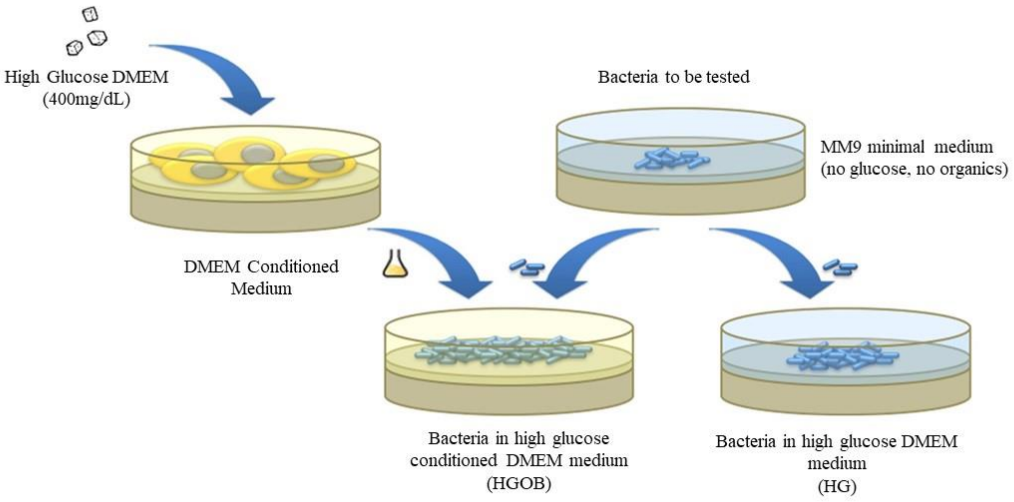


Figure 5 - In vitro model schematic

In order to determine the bacterial growth curves, bacteria were grown in petri dishes with tryptic soy agar (TSA) medium. Afterwards the bacteria were placed in test tubes with sterile water and set to an OD of 0,2 at 620 nm wavelength.

The bacteria were then inserted into 96 wells microplates (0,02 ml bacteria to 0,180 ml medium), each experiment includes 3 samples of each strain per different medium to be tested. The microplate was then placed inside the Thermo Scientific Multiskan Microplate Photometer, where it remain for 2 weeks, the microplate photometer was set for readings at 620nm and preprogramed to execute readings at each even hour.

3.2.2 Statistical analysis

The data collected from the assays will analysed in GraphPad Prism 6.0 (GraphPad Software Inc.), the results gathered will be compared using a 2way ANOVA test and it will be verified through the Tukey-Kramer multiple comparisons test method for significant difference between the HG and HGOB results.

IV. Results

4.1 Gram negative

4.1.1 Enterobacteriaceae

4.1.1.1 *Eschericia coli*

In picture 6 is depicted the growth curves of *Escherichia coli* in three different mediums, (i) control condition (C) which means bacteria grown only in minimal medium MM9, (ii) in the presence of a high glucose (400 mg/dL) medium (HG) which was DMEM, (iii) and conditioned medium of adipocytes (HGOB). This medium was the same glucose-enriched DMEM that was used to grow adipocytes (mature 3T3-L1). Thus, these adipocytes secrete several adipokines also known as adipocyte secretome. Therefore, this medium simulates hyperglycemia and obesity condition. This conditioned medium was enriched with 3T3-L1 secretome, for a period of 14 days as described in material and methods.

Regarding the HGOB growth curve, the bacteria begins in the logarithmic phase and reaches its peak growth within two days at a value of 1.43 of OD and starts declining until the fifth day where it stabilizes until the last measurement was taken. On the HG curve a logarithmical phase can be found until day 5, peaking at an OD of 1.41 where it then enters the stationary phase until the final day of measurements. The C growth curve exhibits minimal growth throughout all the period of the readings, apart from day 5 to 7 where the growth rate is slightly greater.

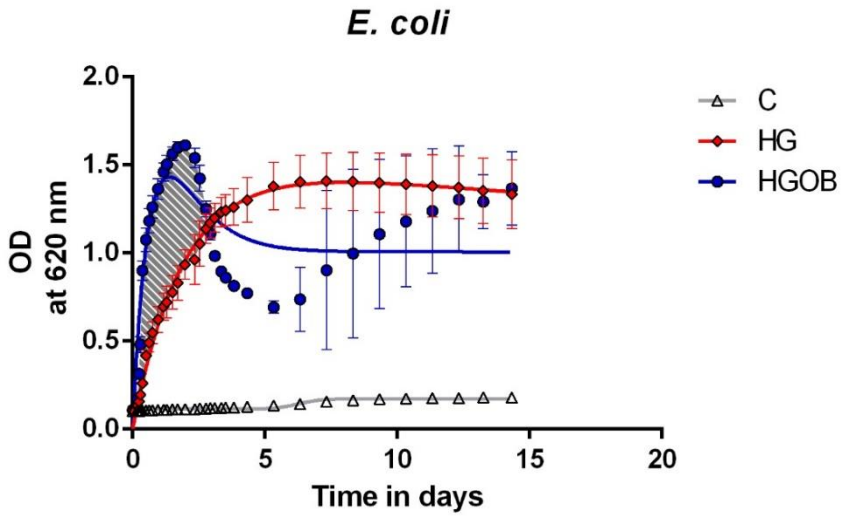


Figure 6 - Growth curve of *E. coli* in three different mediums for 15 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM with enriched with 3T3-L1 secretome)

4.1.1.2 *Klebsiella pneumoniae*

In picture 7 can be observed the growth curves of *Klebesiella pneumoniae* in three different mediums, (i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 14 days.

The *K. pneumoniae* within the HGOB reached the peak of the logarithmic phase on day 2 at an OD of 1.454 and then proceeded in the stationary phase until the end of measurements. *K. pneumoniae* within the HG demonstrate a similar curve although only reaching the stationary phase on day 7 at an OD of 1.356 and then maintaining on the stationary phase until the final day of measurements. The curve representing *K. pneumoniae* in C demonstrates clearly 3 phases, the latent until day 4, followed by the logarithmic phase until day 6 residing in the stationary phase at an OD of 0.7134 until the final day of measurements.

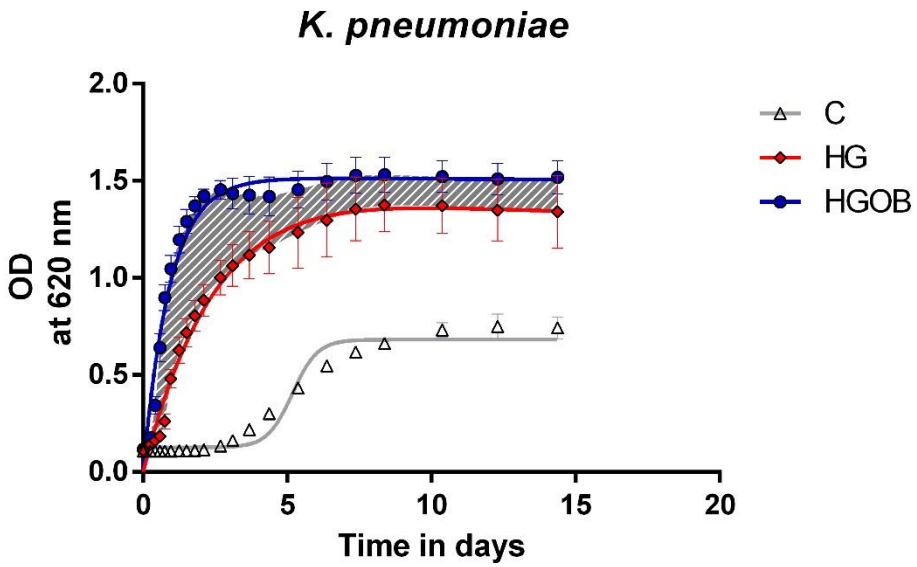


Figure 7 - Growth curve of *K. pneumoniae* in three different mediums for 15 days. (C-Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

4.1.1.3 *Salmonella enterica*

In picture 8 can be observed the growth curves of *Salmonella enterica* in three different mediums(i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 14 days.

The *S. enterica* growth curve in HGOB exhibits a high growth rate until day 3 where it stabilizes displaying a slight decrease throughout the remaining days, having peaked at an OD of 1.640. The growth curve of *S. enterica* in HG shows a steady increase from day 0 up until day 9, where the logarithmic phase stops at an OD of 1.350 and gives place to the stationary phase, entering the death phase on day 11. The *S. enterica* growth curve in C displays a slow and steady growth throughout all measurements having started at an OD of 0.105 and exhibiting an OD of 0.1935 on the final day of measurements.

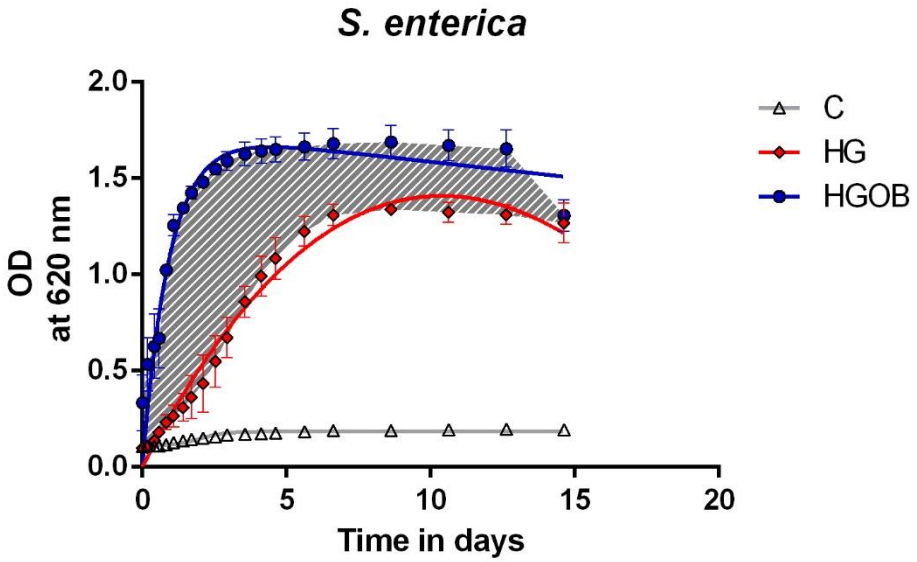


Figure 8 - Growth curve of *S. enterica* in three different mediums for 15 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

4.1.1.4 *Proteus mirabilis*

In picture 9 can be observed the growth curves of *Proteus Mirabilis* in three different mediums, (i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 14 days.

The curve representing the growth of *P. mirabilis* in HGOB finished the logarithmic phase reaching the third day at an OD of 1.604 proceeding to maintain in the stationary phase until the final day of measurements. The growth curve of *P. mirabilis* in HG starts in the logarithmic phase and reaches the stationary phase on day 5 at an OD of 1.602 where it settles in the stationary phase until the end of measurements. The beginning of the growth curve of *P. mirabilis* growing in C shows a lag phase that transitions into a logarithmic phase from day 2 until day 4 to then remain in the stationary phase until the final day of measurements.

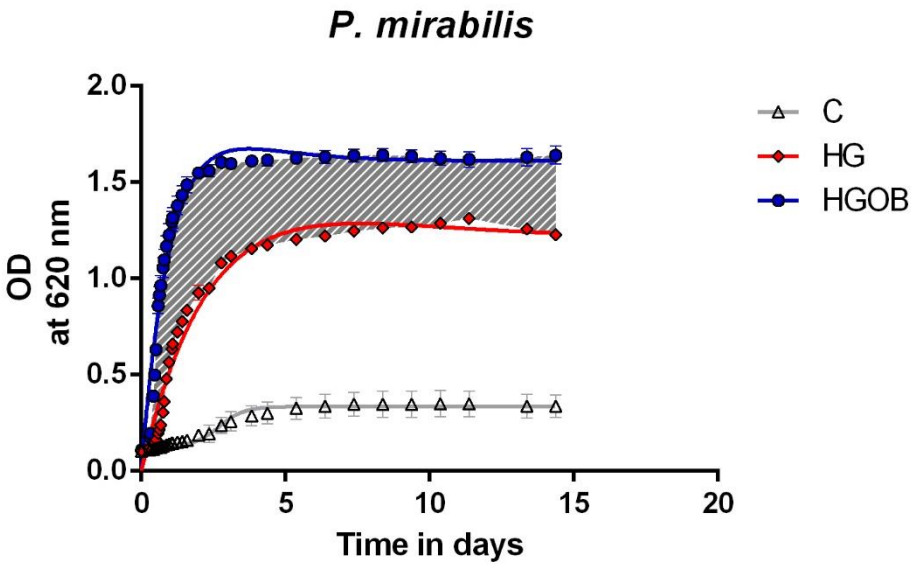


Figure 9 - Growth curve of *P. mirabilis* in three different mediums for 15 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

4.1.2 *Pseudomonadaceae*

4.1.2.1 *Pseudomonas aeruginosa*

In picture 10 can be observed the growth curves of *Pseudomonas aeroginosa* in three different mediums, (i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 16 days.

P. aeruginosa's growth curve in HGOB immediately enters the logarithmic phase that lasts until the beginning of day 2 and then proceeds in the stationary phase at an OD of approximately 0.400 until the final day of measurements. The growth curve of *P. aeruginosa* in HG presents a similar curve to the *P. aeruginosa* in HGOB, immediately entering the logarithmic phase until the end of day 1 and the proceeding in the stationary phase until the final day of measurements, at an OD of approximately 0.350. The curve describing the *P. aeruginosa*'s growth in C shows a visible lag phase until day 2 where then it enters the logarithmic phase and finally enters the stationary phase on day 7 where it remains until the final day of measurements.

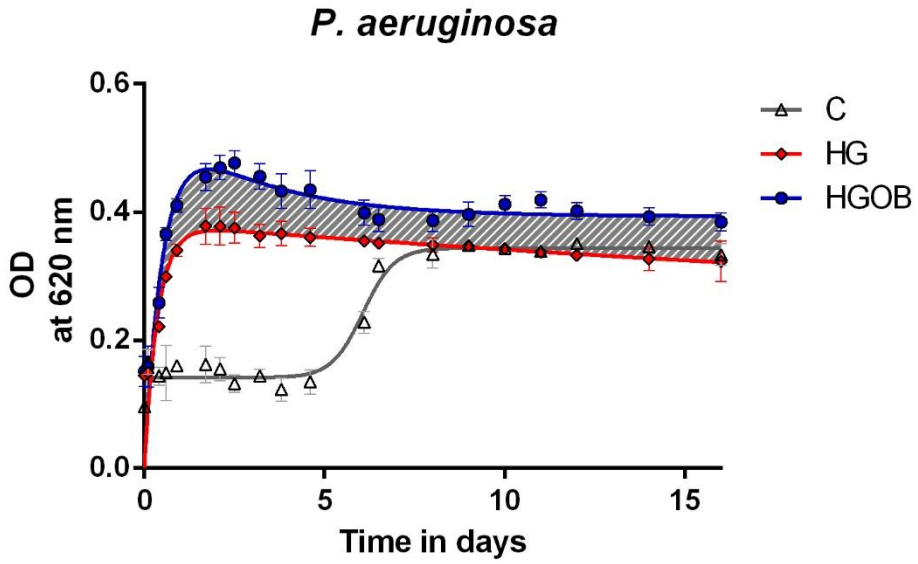


Figure 10 - Growth curve of *P. aeruginosa* in three different mediums for 16 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

4.2 Gram Positive

4.2.1 *Staphylococcaceae*

4.2.1.1 *Staphylococcus aureus*

In picture 11 can be observed the growth curves of *Staphylococcus aureus* in three different mediums, (i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 14 days.

S. aureus growing in HGOB depict a curve demonstrating a clear logarithmic phase ending on day 3 then proceeding to remain in the stationary phase until the final day of measurements at an OD of approximately 0.1350. The growth curve of *S. aureus* in HG displays a logarithmic phase that lasts until day 5, followed by the stationary phase that ends at day 10 and gives place to the death phase, having peaked at an OD of 0.840. The curve corresponding to *S. aureus* growing in C shows a slow and steady growth until day 8 where it then starts do decrease until the final day of measurements, reaching a top OD of 0.180.

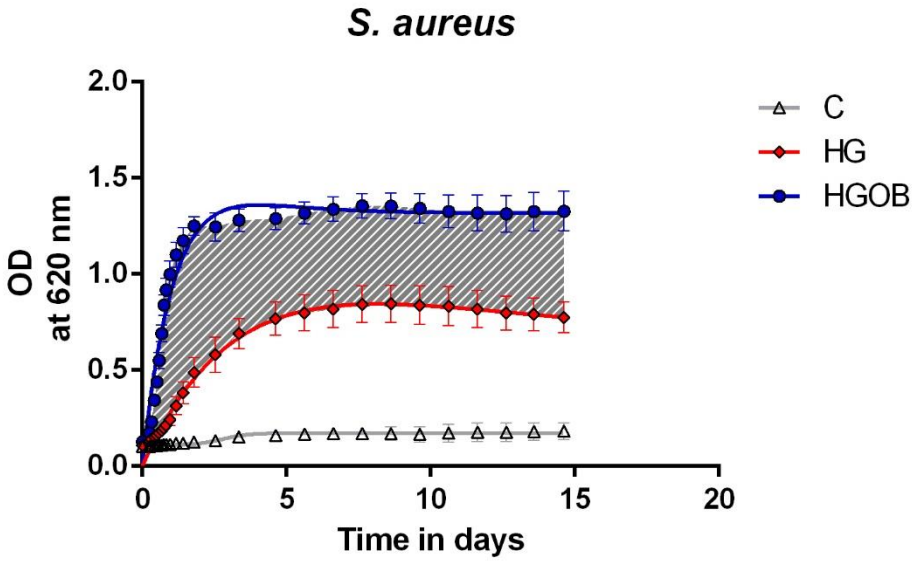


Fig 11 - Growth curve of *S. aureus* in three different mediums for 15 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

4.2.1.2 *Staphylococcus epidermidis*

In picture 12 can be observed the growth curves of *Staphylococcus epidermidis* in three different mediums, (i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 14 days.

The curve depicturing the growth of *S. epidermidis* in HGOB exhibits a logarithmic phase up to day 4 reaching an OD peak of 0.165, remains in the stationary phase for one day and then proceeds to enter the death phase until the final day of measurements. *S. aureus* growing in HG display a curve with a slow and steady growth throughout all assay, reaching up to a maximum OD of 0.670 on the final day of measurements. *S. aureus* growth curve in C displayed no growth throughout the experiment.

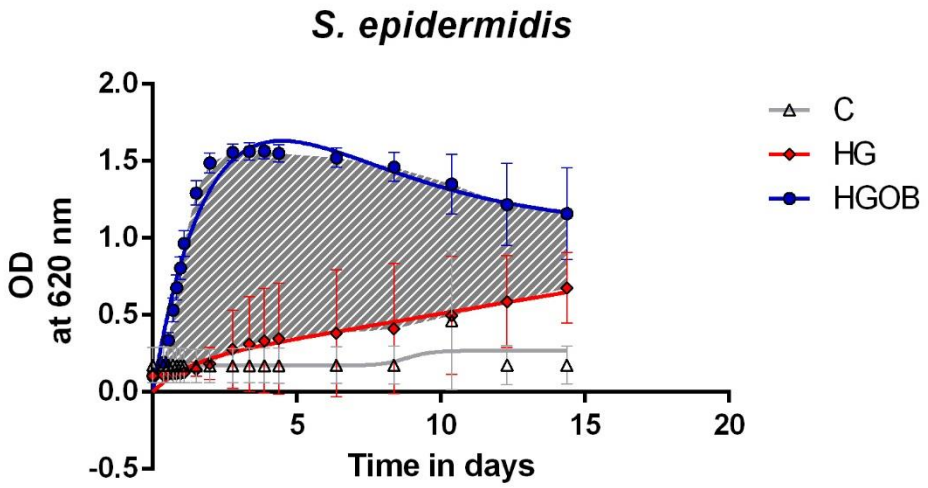


Figure 12 - Growth curve of *S. epidermidis* in three different mediums for 15 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

4.3 *Mycobacteriaceae*

4.3.1 *Mycobacterium smegmatis*

In picture 13 can be observed the growth curves of *Mycobacterium smegmatis* in three different mediums, (i) minimal medium (C), (ii) high glucose-enriched medium (HG) and (iii) high glucose- and adipocyte secretome-enriched medium (HGOB) for the duration of 6 days.

M. smegmatis' growth curve in HGOB exhibits a steady growth from day 0 barely changing its growth rate until the final day of measurements, where it peaked at an OD of 0.788. The growth curve of *M. smegmatis* in HG shows a rapid growth until it reaches an OD of 0.290 and then proceeds at a constant growth throughout all days of measurements reaching a maximum OD of 0.690. *M. smegmatis* grown in C has a growth curve that shows a slight growth that lasts until day 2 and then stabilizes at an OD of approximately of 0.280 until the final day of measurements.

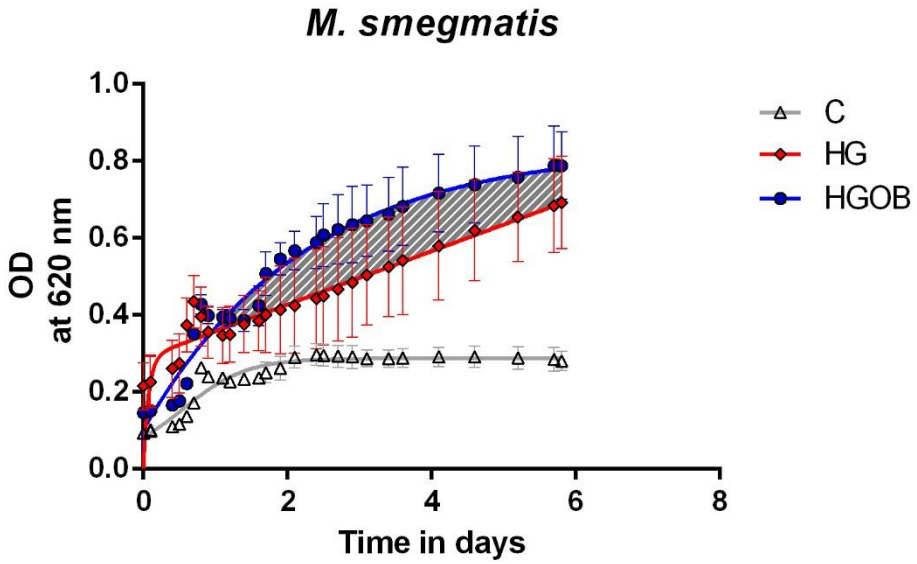


Figure 13 - Growth curve of *M. smegmatis* in three different mediums for 6 days. (C- Control medium; HG - High glucose DMEM; HGOB - High glucose DMEM enriched with 3T3-L1 secretome)

V. Discussion and Conclusion

Several studies on infectious diseases have drawn attention to the association between the obesity and infectious diseases as well as the association between type 2 diabetes and infectious diseases (Atreja & Kalra, 2015; Chita et al., 2017; Dhurandhar, Bailey, & Thomas, 2015; Hainer, Zamrazilova, Kunesova, Bendlova, & Aldhoon-Hainerova, 2015; Huttunen, Karppelin, & Syrjanen, 2013; Huttunen & Syrjanen, 2013; Lenherr et al., 2016; Malmartel & Ghasarossian, 2016; Tagliabue, Principi, Giavoli, & Esposito, 2016), what is left to disclosure is the correlation between people suffering both from type 2 diabetes and obesity, specifically central obesity.

The first great achievement of this work was the establishment of an *in vitro* model for the bacterial growth under a hyperglycaemia environment and a hyperglycaemic obese environment. The experiments, bacterial growth curves determination, took place for a period of two weeks approximately (15 days) in the three conditions previously described as control (C), high glucose (HG) and high glucose with the simulation of an obese environment (HGOB). In general, HG growth curves were must higher than control curves. Also, HGOB curves were higher than HG growth curves. All strains presented statistical significance when comparing the differences between growth curves of C and HG and for HGOB. Also, 6 out of 8 strains in the study were statistically different when comparing HG and HGOB mediums.

E. coli although not having displayed statistical significance for the duration of the whole assay, when only the first 5 days are taken into consideration that is no longer the case. The *E. coli* in HGOB displays a much sudden growth burst. When we take into account the medically important infections caused by this microorganism, it reveals to be of greater importance since the severity level of infections can be gauged within the first days. A study on antimicrobial resistance pattern in *E. coli* causing urinary tract infection (Niranjan & Malini, 2014) showed that the most common risk factor associated with multi drug resistance *E. coli* was diabetes mellitus (28.7% of patients). Another study regarding the clinical profile of urinary tract infections in diabetics and non-diabetics (Aswani, Chandrashekar, Shivashankara, & Pruthvi, 2014) demonstrated that *E.coli* was the most frequent uropathogen isolated. This pathogen was responsible for urinary tract infections in 60.2% and 65.3% of diabetic males and females respectively versus 50% and 51.4% of

non-diabetic males and females. This study suggests that diabetes raises the probability of acquiring *E.coli* infection.

K. pneumoniae is a strain that has been positively correlated with diabetes mellitus. A study regarding the role of diabetes mellitus in patients with bloodstream infections (Stoeckle, Kaech, Trampuz, & Zimmerli, 2008) came to the conclusion that besides bloodstream infection was more frequent in diabetics than in non-diabetics (25.8/1000 admissions vs. 5.8/1000 admissions, $p < 0.0001$) also suggested that *K. pneumoniae* was more frequent in diabetics than in non-diabetics (18% vs 5%, $p < 0.001$). A different study where the bacteriology of acute thoracic empyema was studied for the duration of ten years (K. Y. Chen, Hsueh, Liaw, Yang, & Luh, 2000) authors found *K. pneumoniae* to be the most frequently isolated sole pathogen of the study. Also, patients with *Klebsiella* empyema when compared with patients with non-*Klebsiella* empyema had a higher prevalence of diabetes mellitus (44.1% vs 15.3%, respectively; p ,0.001) demonstrating a strong correlation between diabetes and *K. pneumoniae.*

Literature can be very scarce relating *S. enterica* to either diabetes and/or obesity. Regardless there is a study concerning a new risk factor for *S. enteritidis* (Telzak, Greenberg, Budnick, Singh, & Blum, 1991) where a comparison between 75 cases with stool-confirmed salmonellosis and 80 asymptomatic culture-negative controls who had been served diets contaminated with *Salmonella.* As there was a differential exposure to *S. enteritidis* according to diet served the analyses were stratified according to diet. The cases were more likely than controls to be medication-dependent diabetics (adjusted OR = 3.1), and this relationship was evident for those with either high- or low-level exposure to a *Salmonella*-contaminated diet (OR = 2.4 and 3.5, respectively). Multivariate analysis also showed that diabetes was the only independent risk factor for infection after exposure to a *Salmonella*-contaminated meal (OR = 3.8, 95%CI = 1.4, 10.5).

A study aimed at identifying the factors associated with the isolation of multi drug resistant (MDR) *P. aeruginosa* (Tumbarello et al., 2011) correlated with non-MDR *P. aeruginosa*. In such study non-MDR *P. aeroginosa* cases were being more likely to have diabetes than the control counterparts. Another study regarding the effect of diabetes mellitus on chronic rhinosinusitis and sinus surgery outcome (Zhang et al., 2014) also showed interesting findings regarding *P. aeruginosa* infection. In the study of Zhang and collaborators patients with diabetes mellitus were significantly more likely to have *P. aeruginosa*

(26.32% vs 7.56%; p = 0.004). Moreover the same authors also concluded that the post-operative quality of life of diabetics patients is significantly worse.

S. aureus is able to manifest itself clinically in many different ways. A study regarding *S. aureus* infective endocarditis (Fowler et al., 2005) appoints diabetes has an associated characteristic of patients suffering from that condition, a different study mentions diabetes as a major risk factor for vertebral osteomyelitis caused by *S. aureus* (Bhavan et al., 2010) while a study concerning the incidence and risk factors of vertebral osteomyelitis mortality (Akiyama et al., 2013) shows that higher mortality rates are strongly associated with diabetes (OR, 2.37;P < 0.001). A systematic review on septic arthritis (Margaretten, Kohlwes, Moore, & Bent, 2007) included diabetes as one of the risk factors identified. According to (Peel et al., 2011) obesity is one of the major risk factors concerning *S. aureus* prosthetic joint infection. Therefore, all studies point out the importance of diabetic background to staphylococci infections.

In this study we chose *M. smegmatis* as a model for *Mycobacterium tuberculosis* due to the fact that *M. smegmatis* is a fast growing, non-pathogenic, biosafety level 1 strain. It shares more than 2000 homologous genes with *Mycobacterium tuberculosis* and has the same peculiar cell wall structure also being capable of oxidizing carbon monoxide aerobically. In the *M. smegmatis* assay the HGOB medium growth curve although having higher values than the HG medium growth curve it was not enough to attain statistical significance. Literature is able to provide us with very insightful information concerning diabetes and tuberculosis, in 2008 a random effects meta-analysis of cohort studies (Jeon & Murray, 2008) showed that diabetes mellitus was associated with an increased risk of tuberculosis regardless of study design and population (relative risk = 3.11, 95% CI 2.27–4.26). Evidence was also found that type two diabetes mellitus affects the treatment outcome of pulmonary tuberculosis (Alisjahbana et al., 2007), diabetic patients with tuberculosis had more symptoms, after 2 months the results of sputum microscopic examination was more often positive in diabetic patients (18.1% vs 10.0%) and after 6 months 22.2% of culture sputum specimens from diabetic patients were positive for *M. tuberculosis* (adjusted odd ratio (7.65, P = 0.004)). Also on the topic of treatment outcome a study (Baker et al., 2011) demonstrated diabetes to be associated with an increased risk of failure and death during tuberculosis treatment. Patients with diabetes have a risk ratio for the combined outcome of failure and death of 1.69 (95% CI, 1.36 to 2.12) as well as diabetes also being associated with an increased risk of relapse (RR, 3.89; 95% CI, 2.43 to 6.23).

Regarding *P. mirabilis* and *S. epidermidis* it was not possible to find literature where a cohort study was performed pairing diabetic or obese people with a control population where the rate of infection was the focus of the study. *P. mirabilis* is a bacteria known to cause urinary tract infections, between 1 to 10% of all urinary tract infections are caused by it (Schaffer & Pearson, 2015). There is previous evidence linking an increased risk of urinary tract infections both with diabetes and obesity. A study on the impact of obesity on urinary tract infection risk (Semins, Shore, Makary, Weiner, & Matlaga, 2012) concluded that within the obese population close to 20% females and 8% males were diagnosed with a UTI. Also, the same study demonstrated that the obese group were up to 2.5 times more likely to be diagnosed with an UTI then were the nonobese. A cohort study on the same subject but in children matched 41 819 obese patients by 41 819 nonobese patients showing that the female obese population has an increased risk of urinary tract infection by 45% compared with the nonobese female population (Grier, Kratimenos, Singh, Guaghan, & Koutroulis, 2016). A review of prevalence, diagnosis and management of urinary tract infections in patients with type 2 diabetes mellitus (Nitzan, Elias, Chazan, & Saliba, 2015) states that urinary tract infections are more common, more severe and carry worse outcomes in patients with type 2 diabetes mellitus. Gram-positive bacteria predominate in cases of joint prosthesis contamination, mainly *S. aureus* and *S. epidermidis, S. epidermidis* infections are also linked to the use of implanted medical devices like central venous catheters, prosthetic joints and heart valves, pacemakers, cardiac assist devices, cerebrospinal fluid shunts, and intraocular lenses (Rohde, Frankenberger, Zahringer, & Mack, 2010). From the main factors predisposing towards periprosthetic infection that have been cited in the literature we find obesity and diabetes mellitus amongst them. A study on the incidence, timing and predisposing factors for periprosthetic factors (Pulido, Ghanem, Joshi, Purtill, & Parvizi, 2008) identified morbid obesity as an independent predictor for periprosthetic joint infection. *S. epidermidis* was also found to be the most common causative organism (30%) of deep infection in a study that concluded diabetes to be predictive of a worse outcome respectively 30-day and 1-year mortality (Duckworth et al., 2012).

In the present thesis we have optimized an *in vitro* model for an hyperglycaemic environment and an *in vitro* model for an hyperglycaemic high adiposity environment. After having observed the effects that each had on the bacterial growth many paths could be taken from here. An interesting study to develop would be to identify each molecule present in the

adipocyte's secretome. Furthermore test each molecule individually in order to understand how it modulates the bacterial growth of each different bacterial strain. The spectrum of the strains tested could be broadened, allowing us to verify how different species react in the same medium. Given that the literature provided a limited number of cohort studies matching diabetics and or obese patients with non-diabetic and or obese patients, ongoing and future studies will provide us with larger datasets and allow us to make more extensive comparisons and understanding why there is a correlation between diabetes, obesity and infections. In particular regarding the adipocyte's secretome.

Bibliography

Ahima, R. S. (2006). Adipose tissue as an endocrine organ. *Obesity (Silver Spring), 14 Suppl 5*, 242S-249S. doi: 10.1038/oby.2006.317

Akiyama, T., Chikuda, H., Yasunaga, H., Horiguchi, H., Fushimi, K., & Saita, K. (2013). Incidence and risk factors for mortality of vertebral osteomyelitis: a retrospective analysis using the Japanese diagnosis procedure combination database. *BMJ Open, 3*(3). doi: 10.1136/bmjopen-2012-002412

Al-Gindan, Y. Y., Hankey, C. R., Govan, L., Gallagher, D., Heymsfield, S. B., & Lean, M. E. (2015). Derivation and validation of simple anthropometric equations to predict adipose tissue mass and total fat mass with MRI as the reference method. *Br J Nutr, 114*(11), 1852-1867. doi: 10.1017/S0007114515003670

Alberti, K. G., Zimmet, P., & Shaw, J. (2006). Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med, 23*(5), 469-480. doi: 10.1111/j.1464-5491.2006.01858.x

Alberti, K. G., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med, 15*(7), 539-553. doi: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

Alisjahbana, B., Sahiratmadja, E., Nelwan, E. J., Purwa, A. M., Ahmad, Y., Ottenhoff, T. H., . . . van Crevel, R. (2007). The effect of type 2 diabetes mellitus on the presentation and treatment response of pulmonary tuberculosis. *Clin Infect Dis, 45*(4), 428-435. doi: 10.1086/519841

American Diabetes, A. (2004). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 27 Suppl 1*, S5-S10.

Anzai, Y., Kim, H., Park, J. Y., Wakabayashi, H., & Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol, 50 Pt 4*, 1563-1589. doi: 10.1099/00207713-50-4-1563

Aragon-Sanchez, J., Lazaro-Martinez, J. L., Hernandez-Herrero, M. J., Quintana-Marrero, Y., & Cabrera-Galvan, J. J. (2010). Clinical significance of the isolation of Staphylococcus epidermidis from bone biopsy in diabetic foot osteomyelitis. *Diabet Foot Ankle, 1*. doi: 10.3402/dfa.v1i0.5418

Armbruster, C. E., & Mobley, H. L. T. (2012). Merging mythology and morphology: the multifaceted lifestyle of Proteus mirabilis. *Nat Rev Micro, 10*(11), 743-754.

Aswani, S. M., Chandrashekar, U., Shivashankara, K., & Pruthvi, B. (2014). Clinical profile of urinary tract infections in diabetics and non-diabetics. *Australas Med J, 7*(1), 29-34. doi: 10.4066/AMJ.2014.1906

Atreja, A., & Kalra, S. (2015). Infections in diabetes. *J Pak Med Assoc, 65*(9), 1028-1030.

Baker, M. A., Harries, A. D., Jeon, C. Y., Hart, J. E., Kapur, A., Lonnroth, K., . . . Murray, M. B. (2011). The impact of diabetes on tuberculosis treatment outcomes: a systematic review. *BMC Med, 9*, 81. doi: 10.1186/1741-7015-9-81

Basso, N., & Terragno, N. A. (2001). History about the discovery of the renin-angiotensin system. *Hypertension, 38*(6), 1246-1249.

Bhavan, K. P., Marschall, J., Olsen, M. A., Fraser, V. J., Wright, N. M., & Warren, D. K. (2010). The epidemiology of hematogenous vertebral osteomyelitis: a cohort study in a tertiary care hospital. *BMC Infect Dis, 10*, 158. doi: 10.1186/1471-2334-10-158

Blaser, M. J., & Falkow, S. (2009). What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol, 7*(12), 887-894. doi: 10.1038/nrmicro2245

Briley, L. P., & Szczech, L. A. (2006). Leptin and renal disease. *Semin Dial, 19*(1), 54-59. doi: 10.1111/j.1525-139X.2006.00119.x

Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., & Shoelson, S. E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med, 11*(2), 183-190. doi: 10.1038/nm1166

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., . . . Febbraio, M. A. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes, 55*(10), 2688-2697. doi: 10.2337/db05-1404

Cawthorn, W. P., Scheller, E. L., Learman, B. S., Parlee, S. D., Simon, B. R., Mori, H., . . . MacDougald, O. A. (2014). Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. *Cell Metab, 20*(2), 368-375. doi: 10.1016/j.cmet.2014.06.003

Chen, J., Tan, B., Karteris, E., Zervou, S., Digby, J., Hillhouse, E. W., . . . Randeva, H. S. (2006). Secretion of adiponectin by human placenta: differential modulation of adiponectin and its receptors by cytokines. *Diabetologia, 49*(6), 1292-1302. doi: 10.1007/s00125-006-0194-7

Chen, K. Y., Hsueh, P. R., Liaw, Y. S., Yang, P. C., & Luh, K. T. (2000). A 10-year experience with bacteriology of acute thoracic empyema: emphasis on Klebsiella pneumoniae in patients with diabetes mellitus. *Chest, 117*(6), 1685-1689.

Chita, T., Timar, B., Muntean, D., Baditoiu, L., Horhat, F., Hogea, E., . . . Licker, M. (2017). Urinary tract infections in Romanian patients with diabetes: prevalence, etiology, and risk factors. *Ther Clin Risk Manag, 13*, 1-7. doi: 10.2147/TCRM.S123226

Chung, Y. S., Hu, Y. S., Shin, S., Lim, S. K., Yang, S. J., Park, Y. H., & Park, K. T. (2017). Mechanisms of quinolone resistance in Escherichia coli isolated from companion animals, pet-owners, and non-pet-owners. *J Vet Sci*.

Cianflone, K., Lu, H., Smith, J., Yu, W., & Wang, H. (2005). Adiponectin, acylation stimulating protein and complement C3 are altered in obesity in very young children. *Clin Endocrinol (Oxf), 62*(5), 567-572. doi: 10.1111/j.1365-2265.2005.02260.x

Cianflone, K., Xia, Z., & Chen, L. Y. (2003). Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta, 1609*(2), 127-143.

Coelho, M., Oliveira, T., & Fernandes, R. (2013). Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci, 9*(2), 191-200. doi: 10.5114/aoms.2013.33181

Coelho, P., Almeida, J., Prudencio, C., Fernandes, R., & Soares, R. (2016). Effect of Adipocyte Secretome in Melanoma Progression and Vasculogenic Mimicry. *J Cell Biochem, 117*(7), 1697-1706. doi: 10.1002/jcb.25463

Correia, M. L., & Haynes, W. G. (2006). A role for plasminogen activator inhibitor-1 in obesity: from pie to PAI? *Arterioscler Thromb Vasc Biol, 26*(10), 2183-2185. doi: 10.1161/01.ATV.0000244018.24120.70

Cortez-Dias, N., Martins, S., Belo, A., Fiuza, M., & Investigadores do estudo, V. (2011). Comparison of definitions of metabolic syndrome in relation to risk for coronary artery disease and stroke. *Rev Port Cardiol, 30*(2), 139-169.

Costa, J. V., & Duarte, J. S. (2006). [Adipose tissue and adipokines]. *Acta Med Port, 19*(3), 251-256.

Danesh, J., Wheeler, J. G., Hirschfield, G. M., Eda, S., Eiriksdottir, G., Rumley, A., . . . Gudnason, V. (2004). C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med, 350*(14), 1387-1397. doi: 10.1056/NEJMoa032804

DeFronzo, R. A., & Ferrannini, E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care, 14*(3), 173-194.

Deighton, C. M., Gray, J. W., Bint, A. J., & Walker, D. J. (1992). Anti-Proteus antibodies in rheumatoid arthritis same-sexed sibships. *Br J Rheumatol, 31*(4), 241-245.

Despres, J. P., Lemieux, I., & Prud'homme, D. (2001). Treatment of obesity: need to focus on high risk abdominally obese patients. *BMJ, 322*(7288), 716-720.

Dhurandhar, N. V., Bailey, D., & Thomas, D. (2015). Interaction of obesity and infections. *Obes Rev, 16*(12), 1017-1029. doi: 10.1111/obr.12320

Diamond, F. B., Jr., & Eichler, D. C. (2002). Leptin and the adipocyte endocrine system. *Crit Rev Clin Lab Sci, 39*(4-5), 499-525. doi: 10.1080/10408360290795565

Diekema, D. J., Pfaller, M. A., Jones, R. N., Doern, G. V., Winokur, P. L., Gales, A. C., . . . Beach, M. (1999). Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. *Clin Infect Dis, 29*(3), 595-607.

do Carmo, I., Dos Santos, O., Camolas, J., Vieira, J., Carreira, M., Medina, L., . . . Galvao-Teles, A. (2008). Overweight and obesity in Portugal: national prevalence in 2003-2005. *Obes Rev, 9*(1), 11-19. doi: 10.1111/j.1467-789X.2007.00422.x

Duckworth, A. D., Phillips, S. A., Stone, O., Moran, M., Breusch, S. J., & Biant, L. C. (2012). Deep infection after hip fracture surgery: predictors of early mortality. *Injury, 43*(7), 1182-1186. doi: 10.1016/j.injury.2012.03.029

Eddy, A. A., & Fogo, A. B. (2006). Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. *J Am Soc Nephrol, 17*(11), 2999-3012. doi: 10.1681/ASN.2006050503

Expert Committee on the, D., & Classification of Diabetes, M. (1997). Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care, 20*(7), 1183-1197.

Expert Committee on the, D., & Classification of Diabetes, M. (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care, 26 Suppl 1*, S5-20.

Expert Panel on Detection, E., and Treatment of High Blood Cholesterol in Adults (2001). Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*, 2486-2497. doi: 10.1001/jama.285.19.2486

Ferrannini, E., & Iozzo, P. (2006). Is insulin resistance atherogenic? A review of the evidence. *Atheroscler Suppl, 7*(4), 5-10. doi: 10.1016/j.atherosclerosissup.2006.05.006

Festa, A., D'Agostino, R., Jr., Howard, G., Mykkanen, L., Tracy, R. P., & Haffner, S. M. (2000). Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation, 102*(1), 42-47.

Fine, M. J., Smith, M. A., Carson, C. A., Mutha, S. S., Sankey, S. S., Weissfeld, L. A., & Kapoor, W. N. (1996). Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis. *JAMA, 275*(2), 134-141.

Fitzgerald, S. F., O'Gorman, J., Morris-Downes, M. M., Crowley, R. K., Donlon, S., Bajwa, R., . . . Humphreys, H. (2011). A 12-year review of Staphylococcus aureus bloodstream infections in haemodialysis patients: more work to be done. *J Hosp Infect, 79*(3), 218-221. doi: 10.1016/j.jhin.2011.06.015

Fiuza, M., Cortez-Dias, N., Martins, S., Belo, A., & investigators, V. s. (2008). Metabolic syndrome in Portugal: prevalence and implications for cardiovascular risk--results from the VALSIM Study. *Rev Port Cardiol, 27*(12), 1495-1529.

Ford, E. S. (2005). Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. *Diabetes Care, 28*(7), 1769-1778.

Ford, E. S., Giles, W. H., & Dietz, W. H. (2002). Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA, 287*(3), 356-359.

Ford, E. S., Giles, W. H., & Mokdad, A. H. (2004). Increasing prevalence of the metabolic syndrome among u.s. Adults. *Diabetes Care, 27*(10), 2444-2449.

Fowler, V. G., Jr., Miro, J. M., Hoen, B., Cabell, C. H., Abrutyn, E., Rubinstein, E., . . . Investigators, I. C. E. (2005). Staphylococcus aureus endocarditis: a consequence of medical progress. *JAMA, 293*(24), 3012-3021. doi: 10.1001/jama.293.24.3012

Gahring, L. C., Carlson, N. G., Kulmar, R. A., & Rogers, S. W. (1996). Neuronal expression of tumor necrosis factor alpha in the murine brain. *Neuroimmunomodulation, 3*(5), 289-303.

Gale, E. A. (2005). The myth of the metabolic syndrome. *Diabetologia, 48*(9), 1679-1683. doi: 10.1007/s00125-005-1873-5

Galic, S., Oakhill, J. S., & Steinberg, G. R. (2010). Adipose tissue as an endocrine organ. *Mol Cell Endocrinol, 316*(2), 129-139. doi: 10.1016/j.mce.2009.08.018

Galkowska, H., Podbielska, A., Olszewski, W. L., Stelmach, E., Luczak, M., Rosinski, G., & Karnafel, W. (2009). Epidemiology and prevalence of methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis in patients with diabetic foot ulcers: focus on the differences between species isolated from individuals with ischemic vs. neuropathic foot ulcers. *Diabetes Res Clin Pract, 84*(2), 187-193. doi: 10.1016/j.diabres.2009.02.008

Gami, A. S., Witt, B. J., Howard, D. E., Erwin, P. J., Gami, L. A., Somers, V. K., & Montori, V. M. (2007). Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol, 49*(4), 403-414. doi: 10.1016/j.jacc.2006.09.032

Giannella, R. A. (1996). Salmonella. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). Galveston (TX).

Gopinath, S., Carden, S., & Monack, D. (2012). Shedding light on Salmonella carriers. *Trends Microbiol, 20*(7), 320-327. doi: 10.1016/j.tim.2012.04.004

Gotz, F. (2002). Staphylococcus and biofilms. *Mol Microbiol, 43*(6), 1367-1378.

Grier, W. R., Kratimenos, P., Singh, S., Guaghan, J. P., & Koutroulis, I. (2016). Obesity as a Risk Factor for Urinary Tract Infection in Children. *Clin Pediatr (Phila), 55*(10), 952-956. doi: 10.1177/0009922815617974

Guerre-Millo, M. (2002). Adipose tissue hormones. *J Endocrinol Invest, 25*(10), 855-861. doi: 10.1007/BF03344048

Guzik, T. J., Mangalat, D., & Korbut, R. (2006). Adipocytokines - novel link between inflammation and vascular function? *J Physiol Pharmacol, 57*(4), 505-528.

Hainer, V., Zamrazilova, H., Kunesova, M., Bendlova, B., & Aldhoon-Hainerova, I. (2015). Obesity and infection: reciprocal causality. *Physiol Res, 64 Suppl 2*, S105-119.

Halleran, A., Clamons, S., & Saha, M. (2015). Transcriptomic Characterization of an Infection of Mycobacterium smegmatis by the Cluster A4 Mycobacteriophage Kampy. *PLoS One, 10*(10), e0141100. doi: 10.1371/journal.pone.0141100

Hanson, R. L., Imperatore, G., Bennett, P. H., & Knowler, W. C. (2002). Components of the "metabolic syndrome" and incidence of type 2 diabetes. *Diabetes, 51*(10), 3120-3127.

Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., & Spiegelman, B. M. (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest, 95*(5), 2409-2415. doi: 10.1172/JCI117936

Hu, G., Qiao, Q., Tuomilehto, J., Balkau, B., Borch-Johnsen, K., Pyorala, K., & Group, D. S. (2004). Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch Intern Med, 164*(10), 1066-1076. doi: 10.1001/archinte.164.10.1066

Huttunen, R., Karppelin, M., & Syrjanen, J. (2013). Obesity and nosocomial infections. *J Hosp Infect, 85*(1), 8-16. doi: 10.1016/j.jhin.2013.06.012

Huttunen, R., & Syrjanen, J. (2013). Obesity and the risk and outcome of infection. *Int J Obes (Lond), 37*(3), 333-340. doi: 10.1038/ijo.2012.62

Itoh, M., Suganami, T., Hachiya, R., & Ogawa, Y. (2011). Adipose tissue remodeling as homeostatic inflammation. *Int J Inflam, 2011*, 720926. doi: 10.4061/2011/720926

Jacobsen, S. M., Stickler, D. J., Mobley, H. L., & Shirtliff, M. E. (2008). Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. *Clin Microbiol Rev, 21*(1), 26-59. doi: 10.1128/CMR.00019-07

James, P. T., Rigby, N., Leach, R., & International Obesity Task, F. (2004). The obesity epidemic, metabolic syndrome and future prevention strategies. *Eur J Cardiovasc Prev Rehabil, 11*(1), 3-8.

Jensen, M. D., Haymond, M. W., Rizza, R. A., Cryer, P. E., & Miles, J. M. (1989). Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest, 83*(4), 1168-1173. doi: 10.1172/JCI113997

Jeon, C. Y., & Murray, M. B. (2008). Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. *PLoS Med, 5*(7), e152. doi: 10.1371/journal.pmed.0050152

Jeremiah, C. J., Wills, C., Bayly, A., Perry, G. J., Davis, J. S., Tong, S. Y., & Currie, B. J. (2014). Vancomycin dosing nomogram for haemodialysis patients. *Nephrology (Carlton), 19*(8), 513-514. doi: 10.1111/nep.12270

Jung, Y., Lee, M. J., Sin, H. Y., Kim, N. H., Hwang, J. H., Park, J., . . . Oh, M. D. (2012). Differences in characteristics between healthcare-associated and community-acquired infection in community-onset Klebsiella pneumoniae bloodstream infection in Korea. *BMC Infect Dis, 12*, 239. doi: 10.1186/1471-2334-12-239

Kaplan, N. M. (1989). The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch Intern Med, 149*(7), 1514-1520.

Kuk, J. L., Katzmarzyk, P. T., Nichaman, M. Z., Church, T. S., Blair, S. N., & Ross, R. (2006). Visceral fat is an independent predictor of all-cause mortality in men. *Obesity (Silver Spring), 14*(2), 336-341. doi: 10.1038/oby.2006.43

Kylin, E. (1923). Studien ueber das Hypertonie-Hyperglyka "mie-Hyperurika" miesyndrom. *Zentralblatt fuer Innere Medizin,* (44), 105-127.

Laclaustra, M., Corella, D., & Ordovas, J. M. (2007). Metabolic syndrome pathophysiology: the role of adipose tissue. *Nutr Metab Cardiovasc Dis, 17*(2), 125-139. doi: 10.1016/j.numecd.2006.10.005

Lakka, H. M., Laaksonen, D. E., Lakka, T. A., Niskanen, L. K., Kumpusalo, E., Tuomilehto, J., & Salonen, J. T. (2002). The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA, 288*(21), 2709-2716.

Laupland, K. B., Lyytikainen, O., Sogaard, M., Kennedy, K. J., Knudsen, J. D., Ostergaard, C., . . . International Bacteremia Surveillance, C. (2013). The changing epidemiology of Staphylococcus aureus bloodstream infection: a multinational population-based surveillance study. *Clin Microbiol Infect, 19*(5), 465-471. doi: 10.1111/j.1469-0691.2012.03903.x

Lemieux, I., Pascot, A., Couillard, C., Lamarche, B., Tchernof, A., Almeras, N., . . . Despres, J. P. (2000). Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? *Circulation, 102*(2), 179-184.

Lenherr, S. M., Clemens, J. Q., Braffett, B. H., Cleary, P. A., Dunn, R. L., Hotaling, J. M., . . . Group, D. E. R. (2016). Glycemic Control and Urinary Tract Infections in Women with Type 1 Diabetes: Results from the DCCT/EDIC. *J Urol, 196*(4), 1129-1135. doi: 10.1016/j.juro.2016.04.071

Lim, J. Y., Yoon, J., & Hovde, C. J. (2010). A brief overview of Escherichia coli O157:H7 and its plasmid O157. *J Microbiol Biotechnol, 20*(1), 5-14.

Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J., & Vandenesch, F. (2003). Bacterial competition for human nasal cavity colonization: role of Staphylococcal agr alleles. *Appl Environ Microbiol, 69*(1), 18-23.

Malmartel, A., & Ghasarossian, C. (2016). Bacterial resistance in urinary tract infections in patients with diabetes matched with patients without diabetes. *J Diabetes Complications, 30*(4), 705-709. doi: 10.1016/j.jdiacomp.2016.01.005

Manolescu, B., Stoian, I., Atanasiu, V., Busu, C., & Lupescu, O. (2008). Review article: The role of adipose tissue in uraemia-related insulin resistance. *Nephrology (Carlton), 13*(7), 622-628. doi: 10.1111/j.1440-1797.2008.01022.x

Margaretten, M. E., Kohlwes, J., Moore, D., & Bent, S. (2007). Does this adult patient have septic arthritis? *JAMA, 297*(13), 1478-1488. doi: 10.1001/jama.297.13.1478

Matsuzawa, Y. (2006). The metabolic syndrome and adipocytokines. *FEBS Lett, 580*(12), 2917-2921. doi: 10.1016/j.febslet.2006.04.028

McTernan, P. G., McTernan, C. L., Chetty, R., Jenner, K., Fisher, F. M., Lauer, M. N., . . . Kumar, S. (2002). Increased resistin gene and protein expression in human abdominal adipose tissue. *J Clin Endocrinol Metab, 87*(5), 2407. doi: 10.1210/jcem.87.5.8627

Meigs, J. B., Wilson, P. W., Fox, C. S., Vasan, R. S., Nathan, D. M., Sullivan, L. M., & D'Agostino, R. B. (2006). Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. *J Clin Endocrinol Metab, 91*(8), 2906-2912. doi: 10.1210/jc.2006-0594

Mertens, I., & Van Gaal, L. F. (2005). Visceral fat as a determinant of fibrinolysis and hemostasis. *Semin Vasc Med, 5*(1), 48-55. doi: 10.1055/s-2005-871741

Murray PR, R. K., Pfaller MA. (2009). *Medical Microbiology (6th edition)*. Philadelphia: Elsevier.

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. *Medical microbiology* (8th edition. ed.).

Nigro, J., Osman, N., Dart, A. M., & Little, P. J. (2006). Insulin resistance and atherosclerosis. *Endocr Rev, 27*(3), 242-259. doi: 10.1210/er.2005-0007

Niranjan, V., & Malini, A. (2014). Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among inpatients. *Indian J Med Res, 139*(6), 945-948.

Nitzan, O., Elias, M., Chazan, B., & Saliba, W. (2015). Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management. *Diabetes Metab Syndr Obes, 8*, 129-136. doi: 10.2147/DMSO.S51792

Oh, D. K., Ciaraldi, T., & Henry, R. R. (2007). Adiponectin in health and disease. *Diabetes Obes Metab, 9*(3), 282-289. doi: 10.1111/j.1463-1326.2006.00610.x

Organization, W. H. (2000). *Obesity: preventing and managing the global epidemic*: World Health Organization.

Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nat Rev Immunol, 11*(2), 85-97. doi: 10.1038/nri2921

Paglialunga, S., Fisette, A., Yan, Y., Deshaies, Y., Brouillette, J. F., Pekna, M., & Cianflone, K. (2008). Acylation-stimulating protein deficiency and altered adipose tissue in alternative complement pathway knockout mice. *Am J Physiol Endocrinol Metab, 294*(3), E521-529. doi: 10.1152/ajpendo.00590.2007

Patrick, M. E., Adcock, P. M., Gomez, T. M., Altekruse, S. F., Holland, B. H., Tauxe, R. V., & Swerdlow, D. L. (2004). Salmonella enteritidis infections, United States, 1985-1999. *Emerg Infect Dis, 10*(1), 1-7. doi: 10.3201/eid1001.020572

Peel, T. N., Dowsey, M. M., Daffy, J. R., Stanley, P. A., Choong, P. F., & Buising, K. L. (2011). Risk factors for prosthetic hip and knee infections according to arthroplasty site. *J Hosp Infect, 79*(2), 129-133. doi: 10.1016/j.jhin.2011.06.001

Pellegrino, R., Scavone, P., Umpierrez, A., Maskell, D. J., & Zunino, P. (2013). Proteus mirabilis uroepithelial cell adhesin (UCA) fimbria plays a role in the colonization of the urinary tract. *Pathog Dis, 67*(2), 104-107. doi: 10.1111/2049-632X.12027

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. *J Clin Invest, 111*(12), 1805-1812. doi: 10.1172/JCI18921

Prithiviraj, B., Bais, H. P., Weir, T., Suresh, B., Najarro, E. H., Dayakar, B. V., . . . Vivanco, J. M. (2005). Down regulation of virulence factors of Pseudomonas aeruginosa by salicylic acid

attenuates its virulence on Arabidopsis thaliana and Caenorhabditis elegans. *Infect Immun, 73*(9), 5319-5328. doi: 10.1128/IAI.73.9.5319-5328.2005

Pulido, L., Ghanem, E., Joshi, A., Purtill, J. J., & Parvizi, J. (2008). Periprosthetic joint infection: the incidence, timing, and predisposing factors. *Clin Orthop Relat Res, 466*(7), 1710-1715. doi: 10.1007/s11999-008-0209-4

Rashid, T., & Ebringer, A. (2007). Ankylosing spondylitis is linked to Klebsiella--the evidence. *Clin Rheumatol, 26*(6), 858-864. doi: 10.1007/s10067-006-0488-7

Reaven, G. (1988). Role of insulin resistance in human disease. *Diabetes*(37), 1595-1607.

Rexrode, K. M., Pradhan, A., Manson, J. E., Buring, J. E., & Ridker, P. M. (2003). Relationship of total and abdominal adiposity with CRP and IL-6 in women. *Ann Epidemiol, 13*(10), 674-682.

Reyrat, J. M., & Kahn, D. (2001). Mycobacterium smegmatis: an absurd model for tuberculosis? *Trends Microbiol, 9*(10), 472-474.

Ridker, P. M., & Morrow, D. A. (2003). C-reactive protein, inflammation, and coronary risk. *Cardiol Clin, 21*(3), 315-325.

Rizza, S., Gigli, F., Galli, A., Micchelini, B., Lauro, D., Lauro, R., & Federici, M. (2010). Adiponectin isoforms in elderly patients with or without coronary artery disease. *J Am Geriatr Soc, 58*(4), 702-706. doi: 10.1111/j.1532-5415.2010.02773.x

Rogers, K. L., Fey, P. D., & Rupp, M. E. (2009). Coagulase-negative staphylococcal infections. *Infect Dis Clin North Am, 23*(1), 73-98. doi: 10.1016/j.idc.2008.10.001

Rohde, H., Frankenberger, S., Zahringer, U., & Mack, D. (2010). Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to Staphylococcus epidermidis biofilm formation and pathogenesis of biomaterial-associated infections. *Eur J Cell Biol, 89*(1), 103-111. doi: 10.1016/j.ejcb.2009.10.005

Russell, J. B., & Jarvis, G. N. (2001). Practical mechanisms for interrupting the oral-fecal lifecycle of Escherichia coli. *J Mol Microbiol Biotechnol, 3*(2), 265-272.

Saffo, Z., & Ognjan, A. (2016). Mycobacterium smegmatis infection of a prosthetic total knee arthroplasty. *IDCases, 5*, 80-82. doi: 10.1016/j.idcr.2016.07.007

Sajidan, A., Farouk, A., Greiner, R., Jungblut, P., Muller, E. C., & Borriss, R. (2004). Molecular and physiological characterisation of a 3-phytase from soil bacterium Klebsiella sp. ASR1. *Appl Microbiol Biotechnol, 65*(1), 110-118. doi: 10.1007/s00253-003-1530-1

Santos, A. C., Lopes, C., & Barros, H. (2004). Prevalence of metabolic syndrome in the city of Porto. *Rev Port Cardiol, 23*(1), 45-52.

Sattar, N., McConnachie, A., Shaper, A. G., Blauw, G. J., Buckley, B. M., de Craen, A. J., . . . Wannamethee, S. G. (2008). Can metabolic syndrome usefully predict cardiovascular disease and diabetes? Outcome data from two prospective studies. *Lancet, 371*(9628), 1927-1935. doi: 10.1016/S0140-6736(08)60602-9

Schaffer, J. N., & Pearson, M. M. (2015). Proteus mirabilis and Urinary Tract Infections. *Microbiol Spectr, 3*(5). doi: 10.1128/microbiolspec.UTI-0017-2013

Schaffler, A., & Scholmerich, J. (2010). Innate immunity and adipose tissue biology. *Trends Immunol, 31*(6), 228-235. doi: 10.1016/j.it.2010.03.001

Schraw, T., Wang, Z. V., Halberg, N., Hawkins, M., & Scherer, P. E. (2008). Plasma adiponectin complexes have distinct biochemical characteristics. *Endocrinology, 149*(5), 2270-2282. doi: 10.1210/en.2007-1561

Semins, M. J., Shore, A. D., Makary, M. A., Weiner, J., & Matlaga, B. R. (2012). The impact of obesity on urinary tract infection risk. *Urology, 79*(2), 266-269. doi: 10.1016/j.urology.2011.09.040

Serradeil-Le Gal, C., Raufaste, D., Brossard, G., Pouzet, B., Marty, E., Maffrand, J. P., & Le Fur, G. (1997). Characterization and localization of leptin receptors in the rat kidney. *FEBS Lett, 404*(2-3), 185-191.

Shimizu, F., Hatano, Y., Okamoto, O., Katagiri, K., Fujiwara, S., Sato, S., . . . Takahashi, K. (2012). Mycobacterium smegmatis soft tissue infection. *Int J Dermatol, 51*(12), 1518-1520. doi: 10.1111/j.1365-4632.2010.04835.x

Singleton, P. (2005). *Bacteria in Biology, Biotechnology and Medicine, 6th Edition*: Wiley.

Skurk, T., & Hauner, H. (2004). Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *Int J Obes Relat Metab Disord, 28*(11), 1357-1364. doi: 10.1038/sj.ijo.0802778

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., . . . Lazar, M. A. (2001). The hormone resistin links obesity to diabetes. *Nature, 409*(6818), 307-312. doi: 10.1038/35053000

Stoeckle, M., Kaech, C., Trampuz, A., & Zimmerli, W. (2008). The role of diabetes mellitus in patients with bloodstream infections. *Swiss Med Wkly, 138*(35-36), 512-519. doi: 2008/35/smw-12228

Syndrome, A. C. o. E. T. F. o. t. I. R., & (2003). American College of Endocrinology

Position Statement on the Insulin Resistance Syndrome. *Endocrine Practice, 9*, 236-252.

Tagliabue, C., Principi, N., Giavoli, C., & Esposito, S. (2016). Obesity: impact of infections and response to vaccines. *Eur J Clin Microbiol Infect Dis, 35*(3), 325-331. doi: 10.1007/s10096-015-2558-8

Telzak, E. E., Greenberg, M. S., Budnick, L. D., Singh, T., & Blum, S. (1991). Diabetes mellitus--a newly described risk factor for infection from Salmonella enteritidis. *J Infect Dis, 164*(3), 538-541.

Tenaillon, O., Skurnik, D., Picard, B., & Denamur, E. (2010). The population genetics of commensal Escherichia coli. *Nat Rev Microbiol, 8*(3), 207-217. doi: 10.1038/nrmicro2298

Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. (2002). *Circulation, 106*(25), 3143-3143.

Touyz, R. M., & Schiffrin, E. L. (1993). Effects of angiotensin II and endothelin-1 on platelet aggregation and cytosolic pH and free Ca2+ concentrations in essential hypertension. *Hypertension, 22*(6), 853-862.

Tumbarello, M., Repetto, E., Trecarichi, E. M., Bernardini, C., De Pascale, G., Parisini, A., . . . Bassetti, M. (2011). Multidrug-resistant Pseudomonas aeruginosa bloodstream infections: risk factors and mortality. *Epidemiol Infect, 139*(11), 1740-1749. doi: 10.1017/S0950268810003055

Vague, J., Combes, R., Tramoni, M., & Angeletti, S. (1979). Medical complications of Obesity. *Academic Press*.

von Hafe, P., Lopes, C., Maciel, M. J., & Barros, H. (1998). [The clustering of cardiovascular risk factors in the urban population of Porto]. *Acta Med Port, 11*(12), 1059-1064.

Wang, H., Chu, W. S., Hemphill, C., & Elbein, S. C. (2002). Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. *J Clin Endocrinol Metab, 87*(6), 2520-2524. doi: 10.1210/jcem.87.6.8528

Wasim, H., Al-Daghri, N. M., Chetty, R., McTernan, P. G., Barnett, A. H., & Kumar, S. (2006). Relationship of serum adiponectin and resistin to glucose intolerance and fat topography in South-Asians. *Cardiovasc Diabetol, 5*, 10. doi: 10.1186/1475-2840-5-10

Weisberg, S. P., Hunter, D., Huber, R., Lemieux, J., Slaymaker, S., Vaddi, K., . . . Ferrante, A. W., Jr. (2006). CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest, 116*(1), 115-124. doi: 10.1172/JCI24335

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest, 112*(12), 1796-1808. doi: 10.1172/JCI19246

Weiss, R., Dziura, J., Burgert, T. S., Tamborlane, W. V., Taksali, S. E., Yeckel, C. W., . . . Caprio, S. (2004). Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med, 350*(23), 2362-2374. doi: 10.1056/NEJMoa031049

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., & Nouwen, J. L. (2005). The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis, 5*(12), 751-762. doi: 10.1016/S1473-3099(05)70295-4

Wilson, P. W., D'Agostino, R. B., Parise, H., Sullivan, L., & Meigs, J. B. (2005). Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation, 112*(20), 3066-3072. doi: 10.1161/CIRCULATIONAHA.105.539528

Wisse, B. E. (2004). The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *J Am Soc Nephrol, 15*(11), 2792-2800. doi: 10.1097/01.ASN.0000141966.69934.21

Wu, Y., Liu, Z., Xiang, Z., Zeng, C., Chen, Z., Ma, X., & Li, L. (2006). Obesity-related glomerulopathy: insights from gene expression profiles of the glomeruli derived from renal biopsy samples. *Endocrinology, 147*(1), 44-50. doi: 10.1210/en.2005-0641

Zhang, Z., Adappa, N. D., Lautenbach, E., Chiu, A. G., Doghramji, L., Howland, T. J., . . . Palmer, J. N. (2014). The effect of diabetes mellitus on chronic rhinosinusitis and sinus surgery outcome. *Int Forum Allergy Rhinol, 4*(4), 315-320. doi: 10.1002/alr.21269

Zhu, N., Pankow, J. S., Ballantyne, C. M., Couper, D., Hoogeveen, R. C., Pereira, M., . . . Schmidt, M. I. (2010). High-molecular-weight adiponectin and the risk of type 2 diabetes in the ARIC study. *J Clin Endocrinol Metab, 95*(11), 5097-5104. doi: 10.1210/jc.2010-0716

Zimakoff, J., Bangsgaard Pedersen, F., Bergen, L., Baago-Nielsen, J., Daldorph, B., Espersen, F., . . . Tofte-Jensen, P. (1996). Staphylococcus aureus carriage and infections among patients in four haemo- and peritoneal-dialysis centres in Denmark. The Danish Study Group of Peritonitis in Dialysis (DASPID). *J Hosp Infect, 33*(4), 289-300.