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# Role of obesity in modulating the immune system

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**ROLE OF OBESITY IN MODULATING THE IMMUNE SYSTEM**

by

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B.A., Bentley University, 1996

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# **ROLE OF OBESITY IN MODULATING THE IMMUNE SYSTEM**

**ROMAN FAYNGERSH**

## **ABSTRACT**

**INTRODUCTION:** Diet induced obesity (DIO) is a major driving force responsible for low-grade inflammation mediated immune system decline. Impaired immune defenses lead to a number of chronic diseases and ultimately to an increased mortality.

**DISCUSSION:** Over half a billion people worldwide are considered overweight or obese. It has been estimated that \$190 billion dollars was spent in the US on obesity-related healthcare costs just in 2005. Lower productivity, lost wages, higher insurance costs, and an increased strain on the healthcare system as a whole, are the hallmarks of the obesity epidemic. Considerable body of epidemiologic evidence implicates DIO as the major cause of numerous pathologies. The obese population doesn't just suffer increased mortality from chronic conditions such as, cardiovascular disease, pulmonary diseases, Type 2 diabetes, various cancers, hyperlipidemia, hypertension, non-alcoholic fatty liver disease (NAFLD), renal failure, osteoarthritis and many other slow-onset diseases. Obese individuals also have increased mortality for more acute conditions such as N1H1 influenza virus, allergic diseases, and post-surgical complications while also lowering the efficacy for vaccinations and *Helicobacter pylori* eradication therapies. Today scientists recognize adipose tissue as the largest endocrine organ in the human body, releasing a myriad of paracrine and endocrine molecular factors. During DIO these

adipocytokines induce a proinflammatory switch in the adipose tissue machinery, initiating chronic low-grade inflammation. Sensing an ongoing attack the immune system responds trying to maintain homeostasis. DIO however, initiates a positive feedback loop, which perpetuates inflammation and further decimates immune system's capacity to resist threats and to restore order.

**CONCLUSION:** While the basic obesity-inflammation-disease road map has been outlined, many questions remain. Multiple areas of immunometabolism and meta inflammation require deeper understanding, but two key recommendations for future studies stand out. First, since it is easier to prevent obesity than to reverse it, attention should be focused on elucidating the endocrine role of foodstuff. Second, to find cures for chronic conditions of the ever growing obese population, scientists must elucidate the mechanism of obesity-induced inflammation's function in diminishing immune system's capacity.

## TABLE OF CONTENTS

TITLE.....	i
COPYRIGHT PAGE.....	ii
READER APPROVAL PAGE.....	iii
ABSTRACT.....	iv
TABLE OF CONTENTS.....	vi
LIST OF ABBREVIATIONS.....	ix
INTRODUCTION .....	1
PUBLISHED STUDIES .....	5
Epidemiological Studies .....	5
Obesity and H1N1.....	5
Obesity and High Blood Pressure .....	9
Obesity and Urinary Tract Infections .....	10
Obesity and Pancreatic cancer .....	11
Obesity and Myelodysplastic Syndrome .....	11
Obesity and Asthma.....	12
Obesity and Helicobacter Pylori .....	12
Obesity and Liver Disease .....	13
Obesity and Transplant Surgery.....	15

Obesity and Inflammation.....	16
Obesity and Other Diseases .....	16
Events that turn on Diet Induced Obesity.....	17
Food as a hormone .....	17
Fat as an Endocrine Organ.....	22
Lean Versus Obese Adipose Tissue.....	25
ATMs, M1 vs. M2 profile.....	26
Hyperplasia .....	29
Hypertrophy .....	30
Adipose Cell Growth .....	33
Lipotoxicity.....	37
Hypoxia.....	38
Obesity, Inflammation and an Impaired Immunity.....	43
Innate Immunity.....	44
Receptors.....	46
White Blood Cells.....	50
Humoral factors .....	54
Interleukin-17A.....	55
Hormones.....	57
Hormones as cytokines .....	58
Hormones and Adaptive Immunity.....	59

CONCLUSION.....	62
BIBLIOGRAPHY.....	63
VITA.....	114

## LIST OF ABBREVIATIONS

25(OH)D	25-hydroxycholecalciferol or 25-hydroxyvitamin D
AFLD	alcoholic fatty liver disease
ANG	Angiopoietin
Angptl4	Angiopoietin-like 4
APO	Apolipoprotein
AT	adipose tissue
ATM	adipose tissue macrophages
BDNF	Brain-derived neurotrophic factor
BMI	Body Mass Index
CAM-1	cell adhesion molecule
CCL-2	chemokine ligand 2 or MCP-1
CCR2	C-C chemokine receptor type 2
CD4+	subset of T cells
CD8+	subset of T cells
C-JUN	jun proto-oncogene
CLR	C-type lectin receptors
CLSs	crown-like structures
COPD	Chronic obstructive pulmonary disease
COX-2	Cyclooxygenase 2
CpG-DNA	C-phosphate-G DNA sequence
CRP	C-reactive protein

CVD	cardiovascular disease
DHA	Docosahexaenoic acid
DIO	Diet Induced Obesity
DNA	Deoxyribonucleic Acid
dsRNA	Double-stranded RNA
EF	ectopic fat
EPA	Eicosapentaenoic acid
FFA	free fatty acids
GHSR	growth hormone secretagogue receptor
GI	gastro-intestinal
GLP-1	glucagon-like peptide-1
GLUT	Glucose transporter
GM-CSF	Granulocyte-macrophage colony-stimulating factor
H. pylori	Helicobacter pylori
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis B virus
HFD	high fat diet
HIF-1 $\alpha$	Hypoxia-inducible factor 1-alpha
ICAM-1	Intercellular Adhesion Molecule 1
ICU	Intensive Care Unit
IFN $\gamma$	Interferon gamma

IGF	Insulin-like growth factor
IGFBP-1	Insulin-like growth factor-binding protein 1
I- $\kappa$ B	inhibitor- $\kappa$ B
IL	Interleukin
iNOS	inducible nitric oxide synthase
LCN-2	Lipocalin-2
LD	lipid droplets
LDL	Low-density lipoprotein
LOX-1	lectin-type oxidized LDL receptor 1 or OLR1
LPS	lipopolysaccharide
LXR	liver X receptors
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein 1 or CCL-2
MDS	Myelodysplastic Syndrome
MHC	major histocompatibility complex
MIF	migration inhibitory factor
MIP-1 $\alpha$	Macrophage Inflammatory Proteins 1 alpha
MMP2	Matrix metalloproteinase 2
MOF	multiple organ failure
mRNA	Messenger RNA
mTOR	mechanistic target of rapamycin

MyD88	Myeloid differentiation primary response gene
n-3 PUFA	n-3 polyunsaturated fatty acids
NAFLD	Non-alcoholic fatty liver disease
NDOs	non-digestible oligosaccharides
NEFA	non-esterified fatty acids
NF- $\kappa$ B	nuclear factor kappa beta
NF- $\kappa$ $\beta$	nuclear factor kappa $\beta$
NHANES	National Health and Nutrition Examination Survey
NHGRI	National Human Genome Research Institute
NIH	National Institute of Health
NLR	Nod-like receptors
NO	nitric oxide
Nos2	Nitric oxide synthase
O <sub>2</sub>	molecular oxygen
OLR1	Oxidized low-density lipoprotein receptor 1 or LOX-1
OMN	obese but metabolically normal
PAI-1	Plasminogen activator inhibitor-1
PAMPs	pathogen-associated molecular patterns
PERK	phosphorylation by the endoplasmic reticulum kinase
PLA2	Phospholipase A2
Po <sub>2</sub>	oxygen partial pressure
PPAR	Peroxisome proliferator-activated receptors

PRR	pattern recognizing receptors
RANTES	regulated on activation normal T cell expressed and secreted
RLR	Rig-1-like receptors
RNA	Ribonucleic acid
ROR $\gamma$ $\tau$	Retinoid-related orphan receptor gamma tau
ROS	reactive oxygen species
RXR	retinoid X receptor
SAT	subcutaneous adipose tissue
SCFA	short-chain fatty acids
SREBP-1c	sterol regulatory element binding protein-1c
STAT	signal transducer and activator of transcription
SVF	stromal vascular fraction
T2D	type 2 diabetes
TAG	triglyceride
TCR	T cell receptor
TGF	transforming growth factor
Th17	subset of T cells
TLR	Toll-like receptors
TNF- $\alpha$	tumor necrosis factor alpha
TRAS	transplant renal artery stenosis
Treg	regulatory T cell
UTI	Urinary tract infections

VAT	visceral adipose tissue
VCAM-1	Vascular cell adhesion protein 1
VEGF	Vascular endothelial growth factor
WAT	white adipose tissue
WBC	white blood cells
WHO	World Health Organization
WNT5a	wingless-type MMTV integration site family, member 5A

## INTRODUCTION

Even ancient Greeks knew that man's diet is the key to his health the diseases that afflict us. Hippocrates could not have known how far ahead of his time he was when 2400 years ago he stated: "Let food be thy medicine and medicine be thy food". Diet has an immense impact on our health, yet there is no scientific consensus on the underlying links between what we eat and the infections and the diseases that afflict us. The question this thesis attempts to address is the role obesity plays in modulating diseases via its interactions with the immune system. In the past two decades there has been an enormous amount of research linking Diet Induced Obesity (DIO) with many pathophysiologies. While scientists have yet to uncover the "smoking gun", the obesity epidemic is progressing at a brisk rate. Between 1980 and 2008 the worldwide number of obese (BMI>30 kg/m<sup>2</sup>) individuals has nearly doubled to more than half a billion people (WHO |, n.d.). That is equivalent to the population of North and Central America combined ("The World Bank. Population, total | Data Table.," n.d.). For the first time in history the worldwide number of overweight people has surpassed the number of underweight individuals (Finucane et al., 12). Excess body weight is implicated as a risk factor for mortality and morbidity for nearly 3 million people annually (WHO, n.d.). One third of the world's population (34.3%) has a body mass index (BMI) of 25 kg/m<sup>2</sup> or greater (WHO, n.d.), classifying them as overweight, obese or extremely obese according to the NIH guidelines ("NIH. NHLBI. Obesity Guidelines. Body Mass Index Table 1.," n.d.). Surprisingly, it does not take many years of overnutrition, to markedly

increase the size of the individual's adipose tissue. A recent study by Gupta et al. demonstrated that a caloric excess, even in the short term, results in a significant increase in body fat and visceral adipose tissue volume (Gupta et al., 2013). During this study, healthy, young, mean age 28.4 years old, volunteers were overfed for eight weeks, resulting in a 30% increase in subcutaneous adipose tissue and an even more astounding 103% increase in visceral adipose tissue. Also noted was an average BMI increase of 2.5 points, as well as an increase in a litany of important biomarkers of inflammation and cardiovascular disease (Gupta et al., 2013).

The effect of overnutrition on multiple organ systems has its roots in the *hominids'* evolutionary machinery. Our ancestors, who had to survive through constant food scarcity, under the evolution exerted pressure, became highly efficient at extracting and storing food derived energy. Over 40 years ago, James Neel - a preeminent geneticist, suggested that

“a ‘thrifty genotype’ had evolved to protect human populations from starvation facilitating quick release of insulin, efficient conversion of sugar and storage of excess energy as body fat during the rare times of food abundance” (Neel, 1962).

Biological and cultural changes, such as increased physical activities, expanding brain size and fertility requirements created selection pressures and necessitated the switch to a more energetically dense foodstuff. Further brain size increase might have been facilitated by invention of agriculture and the introduction of cooking, making food more digestible, allowing easier access to nutrients and calories (Aiello & Wheeler,

1995). As brain development involves phospholipids-associated organization of synapses, it is not unreasonable to consider that genes promoting greater energy efficiency may have co-evolved with those influencing evolution of the large human brain (Erren & Erren, 2004). The next developmental step was brought about by the advent of modern agriculture, the discovery of electricity and the invention of refrigeration. In evolutionary terms the switch from a permanent caloric deficit to a caloric abundance, happened instantaneously. Human's inability to rapidly reverse millennia of evolutionary selection and turn down the metabolic machinery to accompany the new energetic homeostasis is leading to what World Health Organization (WHO) is calling a 'globesity' epidemic (WHOa, n.d.).

Current scientific consensus points to the most basic cause of obesity as the energy intake that exceeds energy expenditures. The incoming calories are converted and stored as energy in the form of white adipose tissues (WAT). The unique structure of unilocular adipocyte, the building block of WAT, is responsible for its uncanny ability to greatly increase in size during times of caloric abundance. Along with other organelles, adipocytes contain intracellular lipid droplets (LDs) and while LDs are ubiquitous and are present in all types of eukaryotic cells, they have a very different structure in the white adipose tissue. Non-adipocyte cells usually contain many tiny LDs, typically with a diameter of less than 1  $\mu\text{m}$ , while WAT adipocytes coalesce TAGs into one massive LD, that is in 100+  $\mu\text{m}$  range and occupies 95% of the intracellular space (Fujimoto & Parton, 2011; Suzuki et al., 2011; Brasaemle & Wolins, 2011). It is interesting to note that while increasing adipose cell size 10 fold, from 20 to 200  $\mu\text{m}$ , increases cells' lipid content by a

factor of a 1,000 (Jo et al., 2012). Superbly adopted and primed by evolution to store excess energy the results of the previously mentioned overfeeding study by Gupta et al. shouldn't be surprising, where the subcutaneous adipose cells significantly increased in size, from 0.596(0.91) to 0.919(0.1)  $\mu$ L (Gupta et al., 2013). In a certain sense the fat cell is a perfect storage medium to accumulate potential energy.

Modern industrial societies have achieved the ultimate foraging strategy by maximizing energy intake and minimizing physical effort and energy expenditure, but the tradeoff is a decline in nutritional health. Obesity is linked to diseases of cardiovascular, renal, pulmonary and endocrine systems, as well as, type2 diabetes, insulin intolerance, osteoarthritis, asthma, immune paralysis, periodontitis, cognitive decline and multiple other ailments (Xu et al., 2003; Ciesla et al., 2006; Kelly et al., 2008; DeFronzo, 2010; Lönn et al., 2010; Suvan et al., 2011; Barrette & Schwertani, 2012; Kim et al., 2011; Jämsen et al., 2012; Cazzola et al., 2013; Gupta et al., 2013; Liu et al., 2013; Mancuso, 2013; Yau et al., 2014). Considering the far reaching implications of obesity, the large amount of research scientists are generating should not be surprising. This thesis will attempt to elucidate the impact of diet induced obesity on humans, by evaluating whether overweight individuals immune system fairs worse, same, or better than in their lean counterparts and secondly to suggest possible mechanisms leading to inflammation and to the disease state.

## **PUBLISHED STUDIES**

### **Epidemiological Studies**

Epidemiological and observational studies are the best available tools to track and test hypothesis of viral/bacterial infections in a population. Aggregation of this information offers a glimpse into the effects an infectious load has on the overweight and obese demographic. There has also been considerable scrutiny of the animal models and numerous *in vitro* experiments in the past decade, all in an effort to elucidate a link between the immune system, the myriad of obesity influenced diseases and the positive energy balance of the overweight and obese individuals.

### **Obesity and H1N1**

In 2009 a novel flu virus appeared in Mexico and The United States, presenting a perfect opportunity for the scientist to observe and study infectious load's impact on the different strata of the population. Since then, there have been multiple studies reporting obesity to be an independent risk factor for increased morbidity and mortality following the 2009 influenza A (H1N1) virus. In the early spring of 2009 the first reports of H1N1 surfaced and by August of 2010 almost 500,000 laboratory confirmed cases of the pandemic influenza infection have been recorded, with over 18,000 deaths worldwide (Miller et al., 2010; WHOd,2009).

A noteworthy review was conducted following the 2009 H1N1 flu outbreak, looking at six cross-sectional studies. This meta-analysis has shown that for patients

hospitalized for 2009 H1N1 influenza viral infection, obesity doubled the risk of critical care unit admission and/or death, with a much stronger associations for morbidly obese individuals (Fezeu et al., 2011). In this meta-review Fezeu and colleagues included studies which published obesity data among hospitalized participants having pandemic influenza A (H1N1) infection and who were either admitted to the intensive care unit or died. These inclusion criteria identified 149 articles in PubMed, with a total of six eligible studies to be included in this review. In all, 3059 subjects were hospitalized for influenza A (H1N1) viral infection. Morbidly obese H1N1 patients, BMI index  $\geq 40 \text{ kg/m}^2$ , were twice as likely to be admitted to ICU or die (OR 2.01,  $P < 0.002$ ) and obese patients, BMI index  $\geq 30 \text{ kg/m}^2$ , had an over two fold non-significant increased likelihood risk of ICU admission or death (OR 2.14,  $P < 0.07$ ) (Fezeu et al., 2011). Obese patients were compared to the control group of lean individuals hospitalized with the same H1N1 viral infection. The limitations of this study centered on a limited patient data, leading to an inability to distinguish associations by age class. Authors were not able to adjust for baseline conditions such as respiratory functions, because these were not available in the pooled data and authors did not have access to the individual patient data.

Another prospective cohort study by Viasus et al. conducted between June and November of 2009, tried to identify factors associated with composite outcome of intensive care unit admission or in-hospital mortality, among adults with confirmed pandemic influenza A (H1N1) virus infection. Scientists found that morbidly obese patients (BMI  $> 40 \text{ kg/m}^2$ ) had a higher risk of severe disease (OR, 6.74; 95% CI, 2.25-20.19) (Viasus et al., 2011). A California study conducted in the November of 2009

concluded that more than half (52%) of patients hospitalized with influenza (H1N1) virus, were obese ( $\text{BMI} \geq 30\text{kg/m}^2$ ), and more than a third (40%) were morbidly obese ( $\text{BMI} > 40\text{kg/m}^2$ ). Percentages are even higher for the obese patients in the fatal cases, 66% of obese and 50% of morbidly obese patients (Louie, 2009). As a reference point, according to the results from the 2009–2010 National Health and Nutrition Examination Survey (NHANES), the percent of US adults who were obese is thirty five and morbidly obese is six (Fryar et al., 2012).

A study by Van Kerkhove et al. looked at the information compiled by the WHO from the surveillance programs of the 2009 flue pandemic supplied by its member states, trying to assist policy makers in determining especially vulnerable risk groups for targeted preventive measures. Looking through 70,000 laboratory confirmed H1N1 influenza cases, among other risk factors, the study found obesity to be a major contributor to the three levels of severity of illness: hospitalization, admissions to ICU, and fatalities. The proportion of patients with obesity increased with increasing disease severity, and the pooled OR for death given hospitalization for obesity ( $\text{BMI} \geq 30\text{kg/m}^2$ ) was 2.9. Available data from the two member states supplying the necessary data, indicated the risk of death associated with morbid obesity ( $\text{BMI} > 40\text{kg/m}^2$ ) was increased ( $\text{RR} = 36.3$ ) relative to the general population. One of the limitations of this study was its inability to get more granular patient data, as many countries were not willing to have their country-specific data compared with others (Van Kerkhove et al., 2011).

Two compelling studies looked at the worldwide deaths associated with the influenza A H1N1 pandemic, extrapolated from the pooled WHO country data. First, a study published in 2012 by Darmwood et al, estimated a total of 151,700 – 575,400 pulmonary and cardiovascular deaths worldwide associated with the influenza virus (Dawood et al., 2012). And in the second, published in the fall of 2013, Simonsen et al. estimated that there was a 300,000 – 400,000 all-cause mortality rate in the under 65-yr-old cohort and the respiratory specific mortality that was approximately ten times higher than the WHO laboratory confirmed census data (Simonsen et al., 2013). Overall these numbers are significantly (8 – 31 and 16 – 22 times) higher, respectively, than those results reported in the WHO statistics. These studies used distinct methodology and applied different statistical modeling techniques, which only strengthen their combined assertions of a higher than reported real influenza associated death toll. Researchers did not present comorbidities stratified data, but given the numerous studies showing an epidemiologic link between obesity and H1N1 influenza virus, increased severity of influenza virus outcomes in the overweight population is all but guaranteed.

These data clearly indicate that the overweight and obese population (BMI > 25 kg/m<sup>2</sup>) was at a higher risk for contracting the influenza pandemic (H1N1) virus, than the non-overweight patients (BMI < 25 kg/m<sup>2</sup>). A possible explanation of the link between obesity and severe outcomes is the prevalence of the preexisting conditions in this subset of the population. There could have been direct causation (flu overwhelmingly strains the pulmonary systems), indirect causation via other risk factors (cardiovascular disease, asthma, diabetes, renal disease, GI disorders, etc.), or a non-causal association (genetic,

dietary). To establish these links researchers require much more granular patient records, which are not made available for the broad epidemiologic studies.

It appears that besides humans, obese mice were also susceptible to the same 2009 influenza virus. O'Brien et al. demonstrated that both genetically and diet-induced obese animals, independent of the viral strain, were more susceptible to developing severe infection (O'Brien et al., 2011). Even prior to the 2009 influenza epidemic, scientists knew that obese mice had an altered immune response and were more likely to die from exposure to the influenza virus. In 2007 a study by Smith et al. indicated that high BMI impairs immunological response to the influenza infection. Even postinfection, obese mice had a 6.6 times higher mortality rate (Smith et al., 2007).

Unfortunately pleiotropic actions of obesity are not limited to the influenza infections - copious epidemiologic data implicates obesity in a myriad of acute and chronic conditions.

### **Obesity and High Blood Pressure**

In January of 2013, the Obesity Society and the American Society of Hypertension published a position paper to inform physicians of the association between obesity and high blood pressure. The authors concluded that obesity-related hypertension is an important public health issue worthy of an extensive review (Landsberg et al., 2013). The link between obesity and high blood pressure was demonstrated as far back as the early 1960s, in the Framingham Heart Study, and today it is estimated that at least

75% of the incidence of hypertension is tied directly to obesity (Kannel et al., 1967; Lloyd-Jones et al., 2009). A 2004 study looked at 1999-2000 NHANES data, and concluded that prevalence of hypertension for the normal weight individuals (BMI < 25 kg/m<sup>2</sup>) is 15.3%, while the overweight and the obese populations have a 42.5% and a 27.8% prevalence of hypertension, respectively (Wang, 2004). A more recent study examining the 2007-2010 NHANES data indicated that the odds of high blood pressure were about 1.6 times higher among overweight individuals (BMI ≥ 25 kg/m<sup>2</sup> to < 30 kg/m<sup>2</sup>), and 3.0 times higher for obese individuals ( BMI ≥ 30kg/m<sup>2</sup>) as compared to individuals of normal weight (BMI < 25 kg/m<sup>2</sup>) (Ostchega et al., 2012).

### **Obesity and Urinary Tract Infections**

Obesity has been implicated in increased risk of urinary tract infections (UTI). A study published in 2011 evaluated records of over ninety five thousand patients diagnosed with UTI. Researchers concluded that as a group, the obese were up to 2.5 times more prone to urinary tract infections (Semins et al., 2012) while another looked into association between UTI and obesity, and whether this association is independent of diabetes mellitus and vitamin D or 25(OH)D levels (Saliba et al., 2013). Research showed that in males, obesity was independently associated with UTI, and in females, while it was independent, the association was confined to the group with a BMI ≥ 50kg/m<sup>2</sup> and was not statistically significant (p=0.187).

## **Obesity and Pancreatic cancer**

Pancreatic cancer is one of the deadliest cancers, with a very low five year survival rate, estimated to be 6% (Bracci, 2012). A recent overview of the epidemiologic data, considered latest evidence and previously conducted reviews, concluded that obesity is a modifiable risk factor that is directly associated with the increased risk of pancreatic cancer compared to non-obese patients. Bracci suggested that diabetes, a common complication arising from obesity, maybe the pathway linking BMI and pancreatic cancer (Bracci, 2012). One other paper alluded to a link between obesity and pancreatic cancer through diabetes and that aggressive treatment of diabetes might reduce the risk of cancer among individuals with pancreatogenic diabetes (Andersen, 2013). Additionally, Gukovsky et al. considered how inflammation mediates the pathogenic effects of defective autophagy and obesity in pancreatic cancer patients. Gukovsky concluded that obesity, amplifies the severity of pancreatitis, and creates the environment that promotes the development and progression of pancreatic ductal adenocarcinoma (Gukovsky et al., 2013).

## **Obesity and Myelodysplastic Syndrome**

Ma et al. correlated high BMI to a risk of the development of preleukemia. The study concluded that obesity is a risk factor for Myelodysplastic Syndrome (MDS), with a more than 2-fold increased risk for the obese ( $BMI > 40 \text{ kg/m}^2$ ) and a significant

positive correlation between BMI and MDS for all overweight, obese and morbidly obese individuals (P for trend < 0.001) (Ma et al., 2009).

### **Obesity and Asthma**

The association between asthma and obesity is ambiguous, nevertheless, there is substantial epidemiologic evidence linking them together. A systematic literature review concluded that not only is obesity associated with asthma, but it also increases the severity of the disease and lowers response to treatment (Ali & Ulrik, n.d.). Another review by Liu et al. examined the correlation between asthma and prevalence of obesity in children, looking at published literature between 1966 and 2011. Out of 35 included studies, 27 reported a positive correlation between obesity and asthmatic symptoms. Authors also point to accumulating evidence suggesting an obesity asthma connection through non-allergic pathways (Liu et al., 2013). A study evaluated Italian adults, examining the link between obesity, asthma and smoking. Scientists found increased prevalence of asthma in overweight and obese individuals regardless of their smoking status (Cazzola et al., n.d.).

### **Obesity and Helicobacter Pylori**

Scientists looked at the eradication rates of *Helicobacter pylori* (*H. pylori*) in obese patients, prior to undergoing bariatric surgery. In 55% of overweight/obese patients

(BMI  $\geq$  25 kg/m<sup>2</sup>) the treatment was successful, versus an 85.4% success rate for the control group ( $p < 0.005$ ). Authors stated that the 30.4% difference between treatment groups was not only statistically significant ( $p = 0.0059$ ) but was also clinically important (Abdullahi et al., 2008). In an interesting side note, a more recent study with respect to the difference in the rate of *H. pylori* eradication between Portuguese obese patients from 2006 and 2010 concluded, that the overall efficacy of the antibiotic treatment has gone down below the current medical consensus, mainly due to the bacteria developing drug resistance. It seems that the first-line clarithromycin-based Maastricht III consensus eradication is no longer viable in bariatric patient (Cerqueira et al., 2013).

### **Obesity and Liver Disease**

Liver disease is a growing clinical burden and is a rising health concern in both pediatrics and adult care. Population-based studies have indicated obesity as the chief risk factor for pediatric NAFLD (non-alcoholic fatty liver disease), while others point to the link between BMI and prevalence of NAFLD in adults and progression of chronic hepatitis B and C virus as well as hepatocellular carcinoma (HBV, HCV and HCC, respectively) (Neuschwander-Tetri & Caldwell, 2003; Loannou et al., 2005; Chen et al., 2008; Wong et al., 2009; Everhart et al., 2009; Liu et al., 2010; Wang et al., 2010; Berzigotti et al., 2011; Lee et al., 2011; Armstrong et al., 2012; Wiegand & Berg, 2013; Giorgio et al., 2013). The most common causes are alcoholic and non-alcoholic fatty liver disease (AFLD & NAFLD), and viral hepatitis infections (“European Liver Transplant

Registry - ELTR,” n.d.). The initial hepatic necro-inflammation progresses to hepatic fibrosis, which evolves into cirrhosis and can ultimately morph into hepato-cellular carcinoma (HCC). German autopsy studies have revealed presence of fatty liver pathology in 70% of overweight and 35% of normal weight individuals (Wiegand & Berg, 2013). Cirrhosis and HCC due to chronic HBV and HCV infections are among the leading causes for the liver transplantation in the developed countries (Adam et al., 2003; Wiesner et al., 2003; Sugawara & Makuuchi, 2006). In Europe they have accounted for almost 40% of liver transplants in the past twenty years (“European Liver Transplant Registry - ELTR,” n.d.). In US the numbers are even more alarming. At the end of 2011, almost 80% of patients awaiting liver transplant had liver pathologies due to non-cholestatic cirrhosis, metabolic diseases or malignant neoplasms (“Scientific Registry of Transplant Recipients. Table 9.1C. Waiting List Patient Characteristics at Year-End Liver Waiting List. All Waitlist Patients, 2002-2011,” n.d.). Obesity is often associated with cryptogenic liver diseases and is a noteworthy factor in progression and response to anti-viral treatments for hepatitis (Chen et al., 2008; Berzigotti et al., 2011). A 2011 study showed that, increased body mass, as measured by BMI, is a strong and an independent ( $p=0.02$ ) predictor of decompensation in patients with compensated cirrhosis, as well as a positive correlation trend between BMI and the development of decompensation (Berzigotti et al., 2011). Authors also made an interesting epidemiologic observation, two thirds of their patients were overweight or obese, a proportion similar to the general population. Another 2011 study looked at the impact of BMI and viral load on liver histology in individuals with hepatitis B antigen-negative chronic infections. Findings

from this study suggest a synergistic effect of BMI and hepatitis B virus replication on disease progression, and are both independent of all well-established concomitant factors (Lee et al., 2011). The overarching theme is the link between infectious liver pathologies, NAFLD and BMI, as obesity's link to the liver disease is coming into focus through chronic inflammatory processes.

### **Obesity and Transplant Surgery**

There have been studies concerned with wound complications following transplant surgeries. A study completed in Poland found that donor's BMI was an important factor in post pancreas-kidney transplantation surgical complications (Ziaja et al., 2011). Another study uncovered a connection between post surgery wound complications not only in overweight patients, but also in patients with a history of significant weight loss, over 10kg (Kuo et al., 2012). Authors conjectured that the adverse outcomes are related to the changes of the abdominal panniculus. BMI over 30 kg/m<sup>2</sup> is a risk factor for transplant renal artery stenosis (TRAS), this is one of the reasons medical centers take extreme caution when considering transplantation in obese patients (Kamali et al., 2010).

## **Obesity and Inflammation**

A few international studies have looked at the link between obesity and white blood cell (WBCs) counts, the premise here is the interconnectedness of BMI, inflammation and immune response. An epidemiological study looked at WBC counts in obese females. They reported a highly significant, direct correlation between increasing BMI and platelet count ( $p=0.009$ ), leukocytes ( $p<0.000$ ) and neutrophils ( $p=0.001$ ). Researchers did caution that not all innate immunity cells measured in this study showed correlation with the BMI (basophils, eosinophils, monocytes, NK cells) (Al-Sufyani & Mahassni, 2011). Other studies have confirmed the trend line of increased WBC count with increased BMI (Sekitani et al., 2010; Chae et al., 2013; Marzullo et al., 2014).

## **Obesity and Other Diseases**

There have been a number of studies pointing to obesity as a factor in increased prevalence of diseases. Obesity has been linked to increases in allergic diseases in children (Kusunoki et al., 2008; Visness et al., 2009; Murray et al., 2011). There has been research indicating that obese patients have a depressed cytokine response to blunt injury, remained in metabolic acidosis longer than normal BMI patients, are more prone to post-resuscitation multiple organ failure (MOF), and have increased post-injury morbidity (Ciesla et al., 2006; Winfield, Delano, Lottenberg, et al., 2010; Winfield, Delano, Dixon,

et al., 2010; Nelson et al., 2012; Winfield et al., 2012). An Australian population-based prospective cohort study indicated that obesity is partly associated with 50%-60% greater risk of pertussis notification (Liu et al., 2012). Another epidemiologic study found that overweight and obese pregnant women have a greater risk of developing complications pre and postpartum, versus their normal weight counterparts. Neonates of women with BMI > 25 kg/m<sup>2</sup> have a higher chance of developing complications (El-Gilany & Hammad, 2010). While this study does not prove direct causative relationship between obesity and adverse maternal and perinatal risks, it does add to the existing evidence suggesting a possible link.

Considering its impact and the overwhelming epidemiologic data suggesting a correlation between obesity and various diseases and infections, the question becomes, why are overweight and obese individuals more vulnerable?

### **Events that turn on Diet Induced Obesity.**

#### ***Food as a hormone***

To begin addressing this dilemma we need to consider the adipose tissue. Every human being walking the earth has adipose tissue, however, not every human possessing adipose cells becomes overweight or develops obesity and its deleterious effects. Dr. Francis Collins, Director of the National Institutes of Health (NIH) and former Director of the National Human Genome Research Institute (NHGRI) succinctly stated: “Genetics loads the gun and environment pulls the trigger”. Present scientific consensus points to

two common pathways leading to obesity, most likely there is a third pathway which is the combination of the two. First do we become obese by inheriting genes resulting in anomalies in the metabolic machinery that lead to energetic imbalance and the storage of overabundance of consumed calories. Second is improper diet, leading to the changes in the underlying cellular machinery a key factor in obesity development. This second pathway is aptly named Diet Induced Obesity (DIO), as it turns on the obesity switch epigenetically and is presently under increased scrutiny for it is an easily modifiable risk factor across multiple demographics. This view is supported by the growing body of evidence suggesting that food acts as a hormone and that WAT is the biggest endocrine organ in the human body. Although food isn't produced in the body, but is rather externally sourced, its components are carried throughout the body by blood and act as signaling molecules analogously to the endogenously produced hormones (Hirai et al., 2010; Ryan & Seeley, 2013). In broad terms, hormones influence target tissues by acting on cell-surface receptors, activating cellular cascades that alter activity of the intracellular pathways or via nuclear receptors that control gene transcription.

An example of such interaction comes from omega-3 fatty acids (n-3 PUFA), found in fish, certain vegetables, nuts and grains. Epidemiological studies have long touted cardioprotective properties of omega-3 fatty acids, but simple biochemistry cannot explain why n-3 PUFA should lead to benefits compared to other fatty acids. Omega-3s are known to increase arrhythmic thresholds, decrease blood pressure, improve arterial and endothelial function, reduce platelet aggregation, favorably affect autonomic tone and reduce inflammation (Kromhout et al., 2011). Among its many targets, omega-3 fatty

acids could down-regulate the activity of nuclear factor (NF)- $\kappa$ B, which is a key player in the inflammatory response and is partially responsible in the pathogenesis of cardiovascular disease (CVD). NF- $\kappa$ B is sequestered in the cytoplasm bound to the protein inhibitor- $\kappa$ B (I- $\kappa$ B). In response to inflammatory stimuli (cytokines, lipopolysaccharide (LPS), viruses) I- $\kappa$ B is phosphorylated and is released, thereby freeing NF- $\kappa$ B, which in turn translocates into the nucleus. In the nucleus nuclear factor- $\kappa$ B modulates expression of genes responsible for the inflammatory signaling pathways (upregulation of Interleukin (IL)-1 $\beta$ , IL-2, IL-6, IL-12, tumor necrosis factor alpha (TNF- $\alpha$ ), GM-CSF (Granulocyte-macrophage colony-stimulating factor), MCP-1 (monocyte chemoattractant protein 1), MIP-1 $\alpha$  (Macrophage Inflammatory Proteins 1 alpha), iNOS (inducible nitric oxide synthase), COX-2 (Cyclooxygenase 2), PLA2 (Phospholipase A2), ICAM-1 (Intercellular Adhesion Molecule 1), VCAM-1 (Vascular cell adhesion protein 1), etc.) (Adkins & Kelley, 2010; “Gilmore, Thomas (n.d.). Boston University Biology Department. "NF- $\kappa$ B Target Genes"). Omega-3 fatty acids inhibit phosphorylation of inhibitor- $\kappa$ B (I- $\kappa$ B), thereby halting release and translocation of the NF- $\kappa$ B and its ability to induce transcription of proinflammatory factors (Zhao et al., 2004). Another recent study using n-3 PUFA, is emphasizing Th17 cells and IL-17A connection to DIO inflammation. Monk et al. hypothesized that n-3 PUFA, would decrease the expression of inflammatory genes and lower the number of circulating inflammatory immune cells in the concurrent obesity and colitis model (Monk et al., 2012). The decrease in inflammatory markers was noted in many inflammatory cytokines (MCP-1, IFN $\gamma$ (Interferon gamma), IL-6, IL17F and IL-21), but more importantly, his group saw a

considerable decline in mRNA (Messenger RNA) expression of the Th17 cell master transcription factor (ROR $\gamma$  $\tau$ ) and key inflammatory factors (IL-6, IL-17A, IL-17F, IL-21, IL-23 and IFN $\gamma$ ) (Monk et al., 2012). These results expand our understanding of the ways food derived molecules suppress Th1/Th17 cells and proinflammatory adipose tissue macrophages (ATMs), via reconfiguring the inflammatory gene expression.

Peroxisome proliferator-activated receptors (PPAR), which includes multiple isoforms ( $\alpha$ ,  $\gamma$ ,  $\delta$ ), are a group of ligand-regulated nuclear receptors that form heterodimers, along with retinoid X receptor (RXR), and bind to the promoter region of the target genes involved in lipid metabolism and inflammation. Activation of PPAR $\alpha$  and PPAR $\delta$  has the ability to inhibit NF-k $\beta$  activation, leading to decreased expression of the proinflammatory factors IL-1 $\beta$ , IL-6 and TNF- $\alpha$  (Evans et al., 2004; Touyz & Schiffrin, 2006; Álvarez-Guardia et al., 2011). PPARs upregulate I $\kappa$ B- $\alpha$  which binds to NF-k $\beta$ , and prevents binding to the genes' promoter region (Winther et al., 2005). Eicosapentaenoic acid (EPA) and docosahexaenoic acid(DHA), both omega-3 fatty acids, are known to be PPAR $\alpha$ / $\delta$  agonists. Results of a recent computational study via molecular dynamics simulation, confirmed very high affinity binding of DHA to PPARs and RXR, elucidating DHAs mechanisms of action (Gani & Sylte, 2008). There are other food borne compounds that perform similar functions. A compound found in tomatoes, lycopene being another example of food turned into a hormone. A study found it to be an agonist of PPAR $\gamma$  and as has been discussed earlier, it also inhibits NF-k $\beta$  and its inflammatory consequences (Palozza et al., 2011).

Other fatty acids act directly on endogenous hormones. For example, once activated, ghrelin, a stomach derived hormone, increases appetite, food intake and weight by binding to its receptor, growth hormone secretagogue receptor (GHSR). However, in order to be activated, a fatty acid side chain needs to be attached to ghrelin and various side chains direct different outcomes (Al Massadi et al., 2011).

Another example of food acting as a hormone by regulating endogenous activity of the gut microbiome and inducing signaling cascades is food derived non-digestible oligosaccharides (NDOs). Various types of NDOs naturally occur in milk, honey, fruits and vegetables such as onion, asparagus, artichoke, chicory, leek, garlic, yacon, salsify, sugar beet, banana, tomato, soybean as well as grains, rye, barley and wheat (Rivero et al., 2001; Mussatto & Mancilha, 2007). Of interest is the fact that many NDOs are found in the human breast milk, which for millennia was the only nutritional source for newborn's postnatal development (Rijnierse et al., 2011; Marcobal & Sonnenburg, 2012). Commensalistic gut bacteria metabolize and ferment NDOs into short-chain fatty acids (SCFA), mainly propionate, butyrate, acetate and L-lactate. In adults these SCFAs bind to and activate cell-surface receptors, free fatty acid receptor 2, 3 are expressed on enteroendocrine L cells that produce the incretin hormone glucagon-like peptide-1 (GLP-1) (Ryan & Seeley, 2013).

Not to be overlooked, the currently ubiquitous carbohydrate-rich foods and sucrose loaded drinks are capable of creating a hormonal response as well. These nutrients potentiate a robust insulin release and over a prolonged period of time produce hyperinsulinemia which upregulates expression of the lipogenic transcription factor

SREBP-1c (sterol regulatory element binding protein-1c) and its target enzymes.

Furthermore, the abundant glucose provides substrate for de novo lipogenesis and as such, the excessive calories contain all of the necessary components to get stored as triacylglycerol.

These studies suggest direct mechanisms whereby food derived molecules, are not simply processed to generate energy, but also act in a hormonal capacity to control the expression of pro and anti-inflammatory factors that regulate physiological activity.

### ***Fat as an Endocrine Organ***

It turns out that food is not the only culprit in the etiology of DIO puzzle – white adipose tissue itself is part of the problem. Over the last decade researchers have uncovered numerous molecular factors through which adipose tissues exerts systemic impact on energy homeostasis and projects significant physiologic influence on health and disease states. The long-standing belief that the energy storage is adipocytes' main function has fallen by the wayside as scientists have documented a plethora of WAT's paracrine and endocrine secretions, recognizing fat tissues as the largest endocrine organ.

Not that long ago, in 1953, Gordon Kennedy proposed that the regulation of food intake and energy homeostasis is under influence of circulating factors, exerting central negative feedback control (Kennedy, 1953). Support for this theory came in 1972, when studies of genetically obese and diabetic mice confirmed the presence of such molecular factors (Johnson & Hirsch, 1972). In the 1980s researchers showed that adipose tissue secretes multiple factors affecting control over energy homeostasis, and whose

production is affected by metabolic dysregulation. These signaling molecules remained elusive until 1994, when Zhang et al. was able to sequence the obese gene and its protein product, leptin ( Zhang et al., 1994). Through the 1990s researchers confirmed the validity of Kennedy's original hypothesis, along the way solidifying general acceptance of the adipose tissue as an endocrine organ, by identifying additional secretory factors, termed adipokines. By 2004 scientific community recognized over 75 distinct adipokines secreted by the adipocytes and as technology advanced this number grew (Trayhurn & Wood, 2004). Researchers have also discovered secretions of inflammatory cytokines from the non-adipose cells in the adipose tissue, the stromal vascular fraction (SVF). These include preadipocytes, endothelial cells, fibroblasts, mast cells, eosinophils, B cells, T cells and mature macrophages, among others. Even though many endocrine factors are not produced directly by the adipocytes, estimated 14 – 19% of adipose tissues' cytokines originate in the SVF, as their expression and secretion is enriched by the paracrine signaling initiated by the adipose tissues itself (Peinado et al., 2012). In a positive feedback loop, SVF's paracrine signals *themselves* exert control over the secretion of adipocyte-derived adipokines (Peinado et al., 2012). Of the vascular fraction's leukocytes, macrophages are the most abundant cells, ranging from 10% in lean AT to almost 50% in obese AT (Weisberg et al., 2003).

In line with the complexity of these interactions Chaldakov and his colleagues, coined a new term to describe this intertwined concept – neuroadipology, tying together multiple sub-disciplines such as neuroendocrinology, neuroimmunology, neurogastroenterology with neurobiology (Chaldakov et al., 2010). One of the reasons

investigators have been kept at bay is the complexity of the necessary analysis and the difficulty in pinpointing with any certainty the origins of specific molecules (Pardo et al., 2012). One of the earlier gene-expression profiling studies found that 40% of genes expressed in the adipose tissue were novel (Okamoto et al., 2006). A more current paper, profiled proteins released by the primary human adipocyte, counted 347 distinct proteins, 44 of which are novel adipokines (Lehr et al., 2012). In the same paper scientists note that up to this point researchers have uncovered over 700 different, mostly distinct, secretory components potentially released by the adipose tissues (Lehr et al., 2012). This number is bound to grow as new and novel methods of measuring are invented, along with improvements in the analysis and the equipment sensitivities.

The factors discovered through adipoproteomic analysis fall into many categories, cytokines, chemokines, growth factors, enzymes, free fatty acids, steroid hormones, prostaglandins, endocannabinoids, extracellular matrix proteins, lipid droplet-associated proteins and anti-inflammatory lipid mediators such as resolvins, protectins, lipoxins and neuroprotectin D (Chaldakov et al., 2010). Much of the latest research has concentrated on a few key adipose tissue derived proteins implicated in obesity and chronic low-grade inflammation that leads to a variety of disease states. Particular attention has been paid to adiponectin, leptin, resistin, visfatin, interleukin 6 (IL-6), interleukin 10 (IL-10), monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor alpha (TNF- $\alpha$ ) (Halberg et al., 2008; Harwood Jr., 2012). These signaling and mediator molecules establish an auto-paracrine communication cross link between adipose tissues and the other organs and tissues, such as liver, skeletal muscles, central nervous system,

cardiovascular system and the pancreas. The cross-talk is bidirectional, as organ systems themselves release factors and cytokines that exert adipocentric influence. Certain cardiokines and myokines, from the heart and from the skeletal muscles, respectively, have been shown to interact with the adipose tissue itself (Rosen & Spiegelman, 2006; Romacho et al., 2014). While these mechanisms of action have not been clearly elucidated, there is a growing stream of evidence supporting the existence of these connections (Ouchi et al., 2011).

It is interesting to note that even *in utero* during fetal development the nascent adipose tissue exerts endocrine control over the development of many organ systems. A number of expressed factors have been noted to act in an endocrine manner, factors such as IL-1, -1A, -1B, -4, -5, -6, -8, -12, -15, IGF-I (Insulin-like growth factor I), -II, leptin, IGFBP-1 (Insulin-like growth factor-binding protein 1), -2, -3, -4, -5, APO-E (Apolipoprotein E), APO-A1, APO-A2, APO-R1, TGB-beta, adipsin, BDNF (Brain-derived neurotrophic factor), TNF- $\alpha$ , adiponectin, ANG-2 (Angiopoietin-2), PAI-1 (Plasminogen activator inhibitor-1) (Hausman et al., 2006). During the postnatal and neonatal development, the list of secreted adipokines expands further (Hausman et al., 2006; Poulos, Hausman, & Hausman, 2010)

During obesity, paralleling adipose tissue expansion, the secreted amounts of the above mentioned factors swell, simultaneously changing their profile.

### ***Lean Versus Obese Adipose Tissue.***

Another twist in the saga of food exerting hormone-like effects leading to inflammation is the changeover in the expressed factors based on the internal disturbances of the adipose tissue. Current consensus that adipose tissue can and does act in an endocrine capacity raises the question of the underlying mechanisms. What prompts the production and secretion of factors that lead to the state of inflammation? To address it, first we must look at the changes occurring in a healthy adipose tissue once the long term energy balance becomes positive.

### ***ATMs, M1 vs. M2 profile***

In 2003, two seminal works published findings that obesity induces macrophage infiltration in adipose tissues of mice, as well as humans (Weisberg et al., 2003; Xu et al., 2003). These studies shed light on the role of macrophage-induced inflammation component of diet induced obesity. Amongst the numerous types of macrophages, two subsets of the adipose tissue macrophages are believed to wield the most influence. In the obese AT, M1, also known as the classically activated, is the predominant phenotype, but in the lean adipose tissues, M2 or alternatively activated macrophages, is the principal phenotype (Lumeng et al., 2008). As will be detailed in the next paragraph, the principal differentiators between the two are the gene expression patterns, with M1 expressing proinflammatory factors, while M2 produces high levels of anti-inflammatory secretions.

Currently, there is considerable evidence indicating that both subtypes of ATMs exist synchronously, in the lean as well as in the obese AT, but in immensely different quantities (Lumeng et al., 2007; Prieur et al., 2011). Lean AT contains about 10%

resident macrophages in anti-inflammatory, M2 polarization (Osborn & Olefsky, 2012). During obesity macrophage number increases significantly, up to as much as 50-60% of adipose tissue stromal fraction, with over 90% of these ATMs becoming proinflammatory, M1 polarization (Osborn & Olefsky, 2012; Sorisky et al., 2013). Unlike M1 ATMs, large number of which is recruited into obese AT, M2 ATMs are evenly distributed in adipose tissue under both obese and non-obese conditions (Lumeng et al., 2008). And as the expansion takes place, the influx of M1 polarized cells grows exponentially causing the inversion of M2/M1 ratio, shifting the non-inflammatory milieu maintained by M2 macrophages toward the pro-inflammatory state (Lumeng et al., 2008; Prieur et al., 2011).

The M2 type macrophages exert anti-inflammatory effects and have been shown to be responsible for the tissue repair, remodeling and angiogenesis, increased insulin sensitivity, upregulation and expression of IL-10, IL-1 receptor antagonist and arginase-1. The M2 ATMs have also been found to secrete catecholamines to induce thermogenesis in brown adipose tissue and lipolysis in white adipose tissue (Nguyen et al., 2011). To protect from metabolic deterioration during lipolysis, ATMs temper extracellular increase in free fatty acids, thereby protecting the primary function of the adipose tissues (Kosteli et al., 2010). As an organism becomes increasingly obese there is a shift to a more proinflammatory polarization, along with considerable inflow of M1 macrophages into AT. M1 ATMs secrete TNF- $\alpha$ , CD11c, CCR2 (C-C chemokine receptor type 2), IL-6 and WNT5a (wingless-type MMTV integration site family, member 5A), produce inducible nitric oxide synthase (iNOS) and reactive oxygen species

(ROS), cause insulin resistance and are generally thought to be responsible for inducing a number of inflammatory pathways leading to a state of chronic low-grade inflammation (Gordon, 2007; Galic et al., 2010; Ouchi et al., 2011; Johnson et al., 2012; Cao, 2014).

Despite their apparent importance in regulating adipose tissue inflammatory responses, the crucial mechanism responsible for switching from M2 to M1 polarization remains poorly understood. In 2007 Saltiel et al. put forth a model describing the polarization switch from M2 to M1 phenotype as AT transforms from lean to obese. Examining lean/obese mice, his team hypothesized and were able to prove through flow cytometry and gene expression analysis, that positive energy balance leads to AT growth and under these conditions there is increased production of monocyte chemoattractants, followed by recruitment of inflammatory type CCR2<sup>+</sup> monocytes to AT, where they differentiate into M1 polarized ATMs (Lumeng et al., 2007). Almost 90% of M1 ATMs in the obese AT, originate from these recruited monocytes and are not converted from the resident M2 population *in situ*, as others have hypothesized ( Lumeng et al., 2008; Prieur et al., 2011).

## *Hyperplasia*

As the positive energy balance becomes chronic, the calories are converted and stored in the adipocytes, which from a metabolic vantage point is the most favorable, harking back to the ‘thrifty genotype’ theory (Neel, 1962). Adipose tissue is able to grow via two mechanisms: hyperplasia - increase in the number of adipose cells, and hypertrophy - growth of the existing adipose cells in size. Hyperplasia or recruitment and differentiation of new fat cells, is less common in adults and usually stops during puberty (Drolet et al., 2007; Spalding et al., 2008; Virtue & Vidal-Puig, 2008; Gustafson et al., 2009). Evidence suggests that both lean and obese adipose tissue is in a constant state of remodeling, where old and sick adipocytes die, while adipose progenitor cells differentiate to take their place (Spalding et al., 2008). While at its core this is the physiologic hyperplasia, the process does not increase the total number of adipocytes and as such does not increase adipose tissue’s total lipid buffering and storing capacity. The limited number of studies concerned with hyperplasia; either have not looked at or have not been able to determine whether the new adipocytes came from the already committed, or preprogrammed progenitor cells, or whether they are a newly differentiated adipose tissue derived stem cells. It has been a considerable challenge to pinpoint precisely the origins of preadipocytes as well, and while many believe that preadipocytes originate from the stromal vascular fraction (SVF) of adipose tissue, there are some who think that they originate from the circulating bone marrow progenitor cells (Majka et al., 2011).

When hyperplasia does occur, it leads to a state known as “healthy obese” or “obese but metabolically normal (OMN)” (Stefan et al., 2008; Unger & Scherer, 2010;

Samocha-Bonet et al., 2012). Depending on the varying published articles, obesity in adults ranges from 6% to 40%. Despite their increased BMI or high percent body fat, they seem to have a better long term prognosis, showing 30% to 50% lower risk for mortality and morbidity, than their “typically” obese counterparts (Stefan et al., 2008; Ortega et al., 2013). There is however a compelling study that disputes these findings, stating that once fitness is accounted for as a confounder, any type of obesity has an increased risk for all-cause mortality (Kuk & Ardern, 2009). The underlying assertion for the OMN subjects is that hyperplasia does not elicit the proinflammatory polarization of the ATMs and adipocytokines. Additional research is required as most studies concentrate on subjects with pronounced disease, thus there is sparse evidence concerning hyperplasia in healthy adults who are in the process of gaining weight, or in the OMN population.

One more thought-provoking point requires consideration when discussing hyperplasia studies in an animal model, where the efforts of many researchers are concentrated. Since hyperplasia takes place in the young, it is of great importance to consider the age of the animals. Trying to decipher the transition timing in the animal model from the “child” and the “teenage” stages into adulthood can be very challenging and skew the results, as one study has hinted at in its findings (Jo et al., 2009).

### ***Hypertrophy***

Hypertrophy, or increasing size of the adipose cells, is a more prototypical type of obesity (Kai Sun, Kusminski, & Scherer, 2011). The concept of limited expandability of

subcutaneous adipose tissue (SAT), the main free fatty acids (FFA) storage compartment in a healthy organism, supports the theory of lipid storage overflow (Virtue & Vidal-Puig, 2008; Tsatsoulis et al., 2013). When subcutaneous fat storage depots exhaust their capacity to store lipids and are unable to expand further, the incoming triglycerides are diverted into ectopic depositions in non-adipose organs, such as liver, heart, muscles and pancreas, collectively termed visceral adipose tissue (VAT), or ectopic fat (EF) (Virtue & Vidal-Puig, 2008). Visceral fat surrounds the inner organs in the abdominal cavity, but more poignantly it accumulates inside these organs altering their metabolic processing. This certainly agrees with the accumulating evidence concerning body fat distribution and its influence on disease and inflammation, with VAT being more important than SAT, in the etiology of metabolic syndrome, as well as other diseases like T2D, CVD and various cancers (Unger & Scherer, 2010; Sacks & Fain, 2011). There is even evidence to suggest that there are intrinsic cell-autonomous differences between VAT and SAT cells, possibly accounting for their divergent metabolic functions (Tran et al., 2008). In 1983 a group of Japanese scientists developed a method using CT scans for measuring and distinguishing fat deposits, and has since followed up with studies indicating that accumulation of VAT abets development of metabolic disorders (Matsuzawa et al., 2011). More recently, with the development and advancement of new radiological techniques, similar findings have been confirmed by others (Bentham Science Publisher, 2006; DeFronzo, 2010; Gastaldelli & Basta, 2010; Sacks & Fain, 2011; Sironi et al., 2012; Snel et al., 2012). Many of the same studies see ectopic fat as a predictive marker of developing cardiovascular disease, insulin resistance and Type 2 Diabetes (Preis et al.,

2010; Tadros et al., 2010; Morelli et al., 2013). A number of additional studies have linked increased ectopic fat to an increase in the mediastinal, as well as in the total fat (Sacks & Fain, 2011; Sironi et al., 2012; Morelli et al., 2013).

Of note is a 2013 study, contradicting the generally accepted pathological role of hypertrophic AT, reporting that in 55 obese patients undergoing bariatric surgery and omentectomy, the increase in major omentum was due largely to hyperplasia and not hypertrophy (Arner et al., 2013). The contradictory findings were based on regression analysis of cell samples taken from the patients and while the study seems to be pertinent, its limitations are crippling. The small sample size, inclusion of only nine obese men and zero lean subject of either sex, raise questions of clinical significance of these findings. More importantly this study failed to examine the life-cycle time frame at which omental adipose cells were extracted. This key metric would indicate whether these cells are being differentiated into mature adipocytes, therefore undergoing hyperplasia, to take place of the necrotic mature adipocytes that have gone past their physiologic expansion limit – the end point of hypertrophic growth. A similar study of women undergoing abdominal hysterectomy came to an inverse conclusion (Drolet et al., 2007). Authors looked at patients' subcutaneous and omental AT to determine whether adipocytes expressed depot-specific transcription factors involved in lipid metabolism. They also considered gene expression patterns along with computed tomography body fat distribution and compartment specific adipose cell size. One of their main findings was a bit counterintuitive. Researchers discovered that hyperplasia is predominant in the subcutaneous fat cells (SAT), while hypertrophy was visible in both compartments,

omental and subcutaneous (SAT) (Drolet et al., 2007). It is relevant to mention the inclusion of obese and lean subjects in this study, which might better explain hyperplasia in SAT.

### ***Adipose Cell Growth***

For years some scientists have argued that sustaining an appropriate amount of adipose tissue is crucial for optimal health (Wood et al., 2009). In light of this argument, a proper physiologic response to a chronic caloric abundance would be expansion of SAT that encompasses both, increase in the number of adipocytes as well as a minimal increase in their size. Conversely, a pathologic response leads to hypertrophy, without any meaningful increase in the total quantity of adipose cells. Some have suggested that the breakdown of the expansion process is a series of cycles of adipocyte hyperplasia, followed by hypertrophy and hypoplasia, leading to *the general* degradation of adipose tissue function, exhaustion of adipocyte progenitor pool, followed by impaired expandability of SAT (White & Tchoukalova, 2012). Gustafson et al. has stated that inappropriate signaling of progenitor cell's differentiation pathways leads to hypertrophic expansion (Gustafson et al., 2013). However, as long as there is production of new adipocytes, lipid accumulation is controlled and the energy homeostasis is maintained. The ability to recruit new adipocytes therefore, prevents build-up of ectopic lipids, followed by inflammation and a switch to a pro-inflammatory milieu. As many studies have pointed out, expansion of AT during caloric surplus is instrumental in preventing inappropriate adipose cell enlargement (Blüher, 2009; Christine et al., 2009; White &

Tchoukalova, 2012). Some scientists even suggest that adipogenesis conveys organism wide protection and delays, rather than causes MS instigated by overnutrition (Christine et al., 2009; Unger & Scherer, 2010).

Understanding adipocyte life cycle becomes paramount, as the knowledge of this process offers a glimpse into the events that initiate AT inflammation. However, studying adipose cell turnover has been a considerable challenge. While generating new adipocytes from adult human mesenchymal stem cells and pre-adipocytes *in vitro* is possible, it was unclear, until very recently, if and how this happened *in vivo* (Cinti et al., 2005). Using methods adapted to studying AT in animals, such as incorporation of labeled nucleotides, is not transferable to humans due to its possible toxicity. Performing multiple time-lapsed biopsies to examine cell proliferation in humans is unethical and overly invasive. Looking for molecular markers associated with AT cell division might give clues of mitotic activity, but cannot provide information regarding the fate of scions of that cell. These issues limit researchers' ability to study cells that do not proliferate further or express mitotic markers themselves, for example, adipocytes or neurons. In 2004 a novel approach for testing *in vivo* adipogenesis was developed, via incorporation of a stable isotope deuterium into adipocyte DNA (Deoxyribonucleic Acid) (Strawford et al., 2004). More recently scientists refined this approach making it more accurate by purifying the isolated adipocytes from other potential contaminants (White & Tchoukalova, 2012). Their approach shows results comparable with the only other *in vivo* adipocyte turnover measuring method. Which was developed by Spalding et al. via

incorporation of atmospheric  $^{14}\text{C}$  in adipocyte DNA, left over from the 1960s above ground nuclear bomb tests (Spalding et al., 2008).

As both of these studies agree, in an adult, there is a continuous turnover of adipocytes averaging about 10% per year of the total AT cell count (Spalding et al., 2008; Arner et al., 2010; White & Tchoukalova, 2012). Thus due to necrosis and apoptosis, the number of adipocytes is in a constant state of flux forcing continuous regeneration of new adipocytes. Obese individuals turnover larger quantity of these cells than their lean counterparts do, with the difference between the two population of  $\approx 0.5 \times 10^{10}$  cells, with an estimated half-life of an adipocyte of about 8.3 years (Spalding et al., 2008). In contrast to the bulk of the studies, Spalding et al. approached AT changes from a different angle, he considered changes during the weight loss stage and not during the weight gain. His team showed that adipocyte number is tightly controlled and not influenced by the energy balance. They found no significant change in the number of adipocytes two years post bariatric surgery followed by an extreme caloric restriction diet, even though BMI and fat cell volume decreased (Spalding et al., 2008). Of interest are the findings from other research indicating that individuals with highly enlarged adipocytes have a lower cell turnover rate and that obese have fewer available preadipocytes (Tchoukalova et al., 2007; Arner et al., 2010). And yet another study confirms these assertions, reporting that in obesity there is a reduction in the number of adipose progenitor cells (Oñate et al., 2012). Collectively, evidence suggests that regardless of whether the adipose tissue is expanding or contracting the number of adipocytes is fairly constant. More importantly, these findings imply that at any given

time the quantity of AT cells lingering in the necrotic or apoptotic state is higher in obese individuals not only in absolute terms, but also in relative terms (*on percentage basis*). Simply stated this means that obese have a higher percentage of adipocytes in the proinflammatory state. Although scientists recognize that during obesity hypertrophy predominates, there is still some debate as to the role of hyperplasia and a possible cyclicity of the expanding AT.

As adipocytes grow past their physiologic limit, they become dysfunctional and are tagged for removal from AT, similar to a pathogen or any other dead cell. Macrophages are superbly suited for these tasks and have been shown to aggregate into crown-like structures (CLSs) around necrotic adipocytes (Tchoukalova et al., 2007; Osborn & Olefsky, 2012). While scientist speculated that adipocyte necrosis drives the inflammatory response, it turned out to be simply and association. A couple of recent studies argue that DIO induced adipocyte necrosis is an intrinsic cellular process, not triggered by ATM accumulation, which arrived in the inflamed regions prior to any detectable early stage adipocyte necrosis (Li et al., 2010; Feng et al., 2011). Both studies argue that adipocyte necrosis is disassociated from inflammation, but it is also possible that our current detection techniques are not of sufficient sensitivity. Henceforth, the question of whether inflammation triggers necrosis or necrosis initiates inflammation remains outstanding (Osborn & Olefsky, 2012).

## ***Lipotoxicity***

A healthy adipose organ can and does buffer daily lipid swings by controlling the postprandial serum concentration of non-esterified fatty acids (NEFA), the so called 'fatty acid flux'. Serum FFA and triglyceride levels are never constant, swinging up and down throughout the day. Adipose tissue's ability to effectively buffer the upswings protects the lipid-intolerant organs from lipotoxic damage. Studies have shown that in obese individuals with dysfunctional adipose tissue, inability to appropriately take up lipids, leads to lipotoxicity which is an important pathogenic driver in etiology of metabolic syndrome and a switch over to a pro-inflammatory adipocytokine profile (Frayn, 2002; Schaffer, 2003; Unger, 2005; Virtue & Vidal-Puig, 2008; Unger & Scherer, 2010; DeFronzo, 2010; Snel et al., 2012; Kusminski et al., 2009; Estadella et al., 2013). It has also been suggested that hypertrophic expansion reduces effectiveness of the AT storage capacity and as a consequence, fatty acids are redirected into EF storage (Sorisky et al., 2013). It is important to note that only when SAT's ability to store and oxidize excess fatty acids is impaired and lipids spill over into EF, does the body succumb to deleterious effects of overnutrition. Until such a point, expansion of adipose tissue is considered to be a protective mechanism, helping the organism cope with a positive energy balance (Kusminski et al., 2009; Unger & Scherer, 2010). Some even think that ectopic fat accumulation can be seen initially as a protective mechanism against lipotoxicity (Morelli et al., 2013).

## ***Hypoxia***

At the cellular level adipose tissue is heterogeneous, with adipocytes comprising 60% of the total cell content, while the other 40% is made up of multitude of cells collectively known as SVF (Wood et al., 2009). Adipocytes are able to expand up to  $\approx 20$  fold in diameter and several thousandfold in volume (Jernås et al., 2006). Generally, WAT is considered to be poorly vascularized and in 2002 scientists demonstrated that without angiogenesis adipose tissues are not able to expand (Rupnick et al., 2002; Virtanen et al., 2002; Kabon et al., 2004; Fleischmann et al., 2005). Such extreme volumetric expansion, even when accompanied by angiogenesis, creates areas devoid of blood supply.  $O_2$  diffusion distances have been measured at 100-200  $\mu\text{m}$ , but in some cases  $O_2$  pressure may be close to zero at just 100  $\mu\text{m}$  from the capillaries (Folkman et al., 2000; Gatenby & Gillies, 2004; Brahim-Horn & Pouyssegur, 2007). Thus large adipocytes, which can grow to be 150-200  $\mu\text{m}$  in diameter (Skurk et al., 2007), exceed physical limitations of oxygen diffusion creating environments conducive to expression of inflammatory genes and altered adipokine expression (Gatenby & Gillies, 2004; Brahim-Horn & Pouyssegur, 2007; Wood et al., 2009; Trayhurn, 2013). Researchers have documented intriguing dichotomies between adipose tissues of obese and lean subject. In lean subjects blood flow to adipose tissue increases postprandially, but it does not change in the obese (Karpe et al., 2002; Kabon et al., 2004; Goossens et al., 2011). In obese the adipocyte size is increased in SAT relative to VAT, but there is no change in size in the lean subjects (O'Rourke et al., 2011). An important marker of tissue oxygen perfusion is the capillary density and the obese VAT and SAT had reduced density

compared to the lean VAT and SAT. Also, in obese adipose tissue, there is no difference in density between VAT and SAT, while the lean adipose tissue, shows greater capillary density in VAT than in SAT (Pasarica et al., 2008; O'Rourke et al., 2011; Spencer et al., 2011). But the lower capillary density in obese adipose tissue coincides with larger vessels, indicating that the body is trying to deliver O<sub>2</sub> to the undernourished adipocytes (O'Rourke et al., 2011; Spencer et al., 2011).

Besides blood flow and capillary density data, research shows direct evidence of hypoxia in the human adipose tissue (Virtanen et al., 2002). In a study of subjects during surgery, Po<sub>2</sub> in the upper arm SAT was lower in the obese than in the lean patients (Kabon et al., 2004). Another study employing O<sub>2</sub> electrode as a sensor, indicated lower Po<sub>2</sub> in AT of overweight and obese versus lean patients (Pasarica et al., 2008). Moreover, there is an inverse relationship between percent body fat and Po<sub>2</sub>, along with increasing accumulation of ATMs as the O<sub>2</sub> levels decline (Pasarica et al., 2008). There is additional evidence of existence of a direct dose-dependent relationship between O<sub>2</sub> perfusion and several adipokines, both, in terms of gene expression and manufacture of mature proteins (Wood et al., 2011). Adipocytes appear to be very sensitive to the smallest changes in the oxygen pressure even within the normal physiologic range, constantly titrating their metabolic function (Trayhurn, 2013). Clinical studies support these findings, pointing out that hypertrophy spawns areas of local hypoxia at the earliest stages of expansion (Trayhurn & Wood, 2004; Fujisaka et al., 2013). Collectively, scientists suggest that many inflammatory factors are upregulated by hypoxia and the underlying interdependency of adipose tissue on O<sub>2</sub> tension leads to an increase in ATM

accumulation, changeover in gene transcription, expression of proinflammatory proteins and recruitment of inflammatory factors.

From published studies it is evident that obese adipose tissues are infiltrated with M1 polarity macrophages and as Rausch et al. confirmed via flow cytometry and immunohistochemistry, hypoxic adipose tissue is colocalized with M1 polarity macrophages (Rausch et al., 2007). It has been thought for some time that hypoxia was partially responsible for the influx of proinflammatory macrophages, but only recently scientist illuminated the possible mechanism implicating hypoxia as the inducer of the changeover to the M1 polarity ATMs, possibly via HIF-1 $\alpha$  (Hypoxia-inducible factor 1-alpha) transcription factor (Fujisaka et al., 2013). But Fujisaka et al. observed and cautioned that while M1 ATMs became considerably more hypoxic than M2 ATMs, at least in SAT, hypoxia alone was not a sufficient driver of the increased influx of the M1 ATMs (Fujisaka et al., 2013). Influx of ATMs may be an effect rather than the causation of inflammation in the hypoxic area, perhaps macrophages arrive to remove dead, necrotic, apoptotic adipocytes and/or scavenge for the already released lipid droplets, linking hypoxia to the organism's survival as it struggles to intercede in hypertrophic expansion (Cinti et al., 2005; Wood et al., 2009; Miyake & Yamasaki, 2012). Realization that there is another, hypoxia-independent pathway, which also facilitates recruitment of M1 ATMs, presents significant challenges in deciphering the M1 engagement mechanism (Fujisaka et al., 2013). Indeed, leptin has been shown to be macrophage chemoattractant, along with MCP-1 (Curat et al., 2004; Lilja et al., 2012). It seems that triggers responsible for the recruitment of the macrophages remain an enigma.

Hypoxic changes in AT lead to inflammation through multiple pathways. Hypoxia has been shown to inhibit differentiation of preadipocytes into adipocytes, controlled via the downregulation of the expression of the nuclear transcription factor PPAR $\gamma$  through hypoxic inducible factor (HIF)-1 (Yun et al., 2002; Kim et al., 2005; Zhou et al., 2005; Lin et al., 2006; Semenza, 2011; Shin et al., 2012). HIF is a master regulator of the cellular response to hypoxia, which has evolved over the millennia of evolution to expeditiously restore oxygen flow. The alpha subunit of the HIF-1 heterodimer is thought to be the molecular O<sub>2</sub> sensor (Semenza, 1998; Poitz et al., 2014). It is stabilized by the low oxygen pressure, allowing the binding of HIF-1 transcription factor to its domain, inducing transcription of over seventy genes. These genes are involved in inflammation, angiogenesis, apoptosis, cellular stress and glucose metabolism amongst other processes (Semenza, 2003; Sun et al., 2013). It has also been noted that in mature adipocytes expression of PPAR $\gamma$  is diminished as well (Hosogai et al., 2007; El-Gilany & Hammad, 2010) Reduction in the expression of PPAR $\gamma$  and inability to differentiate new adipocytes in a hypoxic environment could be an effective mechanism to control the runaway growth of the adipose tissue, and is consistent with the earlier described theory supporting the concept that the number of adipocytes stays nearly constant in the adulthood (Spalding et al., 2008; Virtue & Vidal-Puig, 2008; Gustafson et al., 2009). NF-k $\beta$ , another pertinent transcription factor is also affected by the low oxygen tension. NF-k $\beta$  has been shown to mediate inflammatory signaling pathways associated with TNF- $\alpha$  and HIF-1a, and is thought to be attempting to inhibit apoptosis to allow the cell to survive the perceived intermittent hypoxic stress (Rius et al., 2008;

Taylor, 2008). Furthermore, hypoxia induces activation of NF- $\kappa$ B inside macrophages (Rius et al., 2008). Preadipocytes themselves are known to secrete multiple inflammatory adipokines, leptin however, not being one of them, but in the hypoxic environment preadipocytes have been shown to switch on leptin production (Wang et al., 2008). Quantitatively, produced leptin does not significantly raise circulating levels of the hormone, it does however, play a local paracrine role recruiting macrophages along with other inflammatory factors to the locally hypoxic adipose tissue.

As previously indicated, a large portion of the adipose tissue is comprised of the stromal vascular fraction (SVF) and a low  $P_{O_2}$  has been shown to induce secretion of crucial cytokines and chemokines from these cells. Factors such as IL-6, IL-1 $\beta$ , MIF-1, TNF- $\alpha$ , CCL-2 (MCP-1), MMP2 (matrix metalloproteinase 2), MMP9, Angptl4 (Angiopoietin-like 4), PAI-1, leptin, as well as GLUT1, PERK (phosphorylation by the endoplasmic reticulum kinase) and c-JUN transcription factors flood the area or are produced in the hypoxic adipose tissues (Wood et al., 2009; O'Rourke et al., 2011; Pérez et al., 2010). M1 macrophages themselves have been shown to express proinflammatory genes, including, Tnf, Il6, Il1b and Nos2 (Fujisaka et al., 2013). Although some of the infiltrating cytokines and transcription factors are anti-inflammatory, such as IL-10, NF- $\kappa$ B, macrophage migration inhibitory factor (MIF), they are unable to cope and ultimately lose the battle of the inflamed and expanding adipose tissue. A number of angiogenic factors are also upregulated during hypoxia, such as VEGF (Vascular endothelial growth factor), leptin and apelin, in a classical response to the low oxygen tension, in an attempt to restore blood flow through capillary network growth (Trayhurn, 2013). But just like

the anti-inflammatory stimuli, the angiogenic catalysts are no match for the expanding adipose tissue.

### **Obesity, Inflammation and an Impaired Immunity**

Clearly there is merit in the argument that in order to survive organisms depend on two key processes, storing energy and fighting infections. Ostensibly the molecular and cellular pathways seem to be unrelated - AT being charged with energy stores, while infections are the domain of the immune system. At its core the connections remain elusive, but the association is likely attributable to the way AT influences immunologic functions. In all likelihood, these poorly understood connections work in concert and as the research progresses, a new term - immunometabolism, has been coined to describe the intersection of two seemingly different disciplines of immunology and metabolism (Mathis & Shoelson, 2011; Schipper et al., 2012).

DIO forces a change in the AT-resident immune cells phenotype and numbers (O'Rourke et al., 2011). Obesity itself provides danger signals and bacterial activators that trigger inflammatory cascades and prompt the ATM polarization switch, triggering adipose tissue inflammation. Moreover, there is a reduction in AT-resident regulatory T cells and the IL-4 producing eosinophils, while inflammatory cell such as IFN- $\gamma$ <sup>+</sup> Th1 cells and CD8<sup>+</sup> T cells proliferate and multiply. These changes lead to an archetypal Th1 inflammatory response, but in DIO instance it results in a long term low-grade inflammation and insulin resistance. As countless epidemiologic studies indicate, AT

inflammation and insulin resistance underlie a large portion of comorbidities in the obese population. As obesity triggers chronic low grade inflammation, it alters the overall immune system homeostasis. Comparing lean and obese subjects Nieman et al. noticed multiple discrepancies in leukocyte numbers, subset counts, phagocytic and oxidative burst activity of monocytes, as well as impaired lymphocyte proliferation to polyclonal stimulation (Nieman et al., 1999). In obese versus their lean counterparts, circulating mononuclear cells display a pro-inflammatory polarization (Ghanim et al., 2004). Obesity has also been implicated in advancing thymic aging and reducing T-cell variability, apparently harming immune system's surveillance capabilities (Yang et al., 2009). Impaired immune cell activation is a common mark of Type 2 diabetes, a prevailing complication following onset of obesity (Geerlings & Hoepelman, 1999). Farooqi and his colleagues have noted, that individuals with genetic mutations that impede bodies leptin synthesizing abilities, become morbidly obese and present weakened immune defenses (Farooqi et al., 2002). These are but a few examples of the far reaching consequences of obesity. The mechanisms responsible for the altered immune state are multifactorial, but the key to understanding inflammation-disease link is in understanding how the energy rich environment of DIO impacts immune cell function.

### ***Innate Immunity***

Innate immunity is a set of predetermined humoral and cellular factors that have developed to sense physiologic abnormalities, trigger responses and bring the whole system back into equilibrium. Interestingly the immune responses are triggered not only

by the exogenous, but endogenous stimuli as well. Microbes, viruses and bacteria are the typical stressors of the immune system, warranting a rather acute inflammatory response. But beyond the initial reaction, innate immune system is tasked with resolving inflammation, repairing damaged tissues and activating adaptive immune system to prepare memory for future confrontations. The difficulty in teasing out individual relationships to attribute causation of various responses lies in their overlapping nature. For example, post workout repair of damaged skeletal muscles is intimately linked to circulating monocytes that induce myogenesis (Arnold et al., 2007). Monocytes patrol blood vessels and rapidly magnify the inflammatory response in case of damage or infection, followed by induction of an innate immune response (Auffray et al., 2007). Another example of such duality is liver X receptors (LXRs), that control inflammation as well as lipid metabolism (Hong et al., 2011). This control mechanism might be related to the high energy demands of the immune cells while generating reactive oxygen species, executing phagocytosis, directing cell migration and producing inflammatory cytokines. Interestingly, certain hormones that are dysregulated in DIO impair leukocyte function and leptin receptor (Mancuso et al., 2004; Baumgartl et al., 2006). The list of such examples is extensive, but I will focus on effectors of innate immunity in the context of DIO.

For many years scientists have known that DIO is marked by a state of chronic low-grade inflammation (Yudkin et al., 1999; Festa et al., 2001; Bulló et al., 2003; Engström et al., 2003; Yudkin, 2003; Fenton et al., 2009; Symonds et al., 2009; Maury & Brichard, 2010; Esser et al., 2014). Intrinsic inflammation is a normal physiologic

response to tissue expansion (Kosteli et al., 2010). Why that might be becomes clearer upon examination of the immune cells that are collocated with adipocytes within the AT, and are either summoned or secreted by the AT. An abundance of evidence points to a considerable number of key metabolic regulators that also play crucial roles in modulating inflammation (Lumeng, 2013). Moreover, adipocytes express receptors for microbial ligands, factors released upon tissue damage, inflammatory factors and the so-called danger signals (Schäffler & Schölmerich, 2010). In obesity numerous molecular sensors are primed to react to exogenous factors. These inflammatory pathways are established to guard against infections and are the backbone of the innate immunity's ability to sense these dangers. Unique chemical structures present in bacterial complexes (e.g. peptidoglycan, LPS, endotoxins), collectively termed pathogen-associated molecular patterns (PAMPs), also include peptides, proteins, carbohydrates, lipoproteins and nucleic acid species (e.g. CpG-DNA, dsRNA) (Zhang & Mosser, 2008; Kumar et al., 2011). These PAMPs are identified by a set of mammalian pattern recognizing receptors (PRRs) that are triggered during the inflammatory response to DIO. PRRs are typically grouped into receptor classes: Toll-like receptors (TLRs), Nod-like receptors (NLRs), C-type lectin receptors (CLRs), and Rig-1-like receptors (RLRs). Evidence suggests that TLRs, NLRs, and CLRs partake in DIO inflammation.

### ***Receptors***

Free fatty acids have the ability to activate many TLRs, which in turn activate NF- $\kappa$ B and production of ceramide along with its anti-inflammatory effects (Holland et

al., 2011). TLR4 plays a large role in sepsis and has the ability to activate virtually every type of leukocyte. Experimental evidence suggests that TLR4s are linked to obesity via increase in circulating LPS. The source of these LPS is gut microbiota, which responds quickly to dietary inputs and shortly after a high fat meal are capable of releasing enough LPSs to activate monocytes and endothelial cells (Manco et al., 2010; Rabot et al., 2010; Tilg & Kaser, 2011). Studies of obese mice show abnormally high levels of LPS translocating from the gut, resulting in increased circulating levels of LPS (Cani et al., 2007). Additional studies have pointed out that TLR4 knockout mice are virtually immune to insulin resistance during a high fat diet (Davis et al., 2008). TLR4 expressed on adipocytes and hepatocytes are capable of modifying insulin sensitivity via MyD88 (myeloid differentiation primary response gene) dependent pathway, while TLR4 on skeletal muscle fibers can regulate substrate utilization (Davis et al., 2009; Raetzsch et al., 2009; Frisard et al., 2010). A study of obese humans indicated that levels of LPS correlate with AT inflammation and insulin resistance (Sabeti et al., 2009).

TLR2s are also capable of sensing dietary fatty acids and are activated by saturated, but not polyunsaturated fatty acids (Lee et al., 2004). Studies dealing with a loss of TLR2 function in murine models of obesity, show protection from DIO-activated insulin resistance (Ehnes et al., 2010; Himes & Smith, 2010; Davis et al., 2011). Additional benefits of a TLR2 knockout model is a decrease in ATM accumulation and a decrease in pro-inflammatory cytokine production within adipose tissue (Lumeng, 2013).

Besides reconnaissance of extracellular threats there is constant surveillance of the cytoplasm for danger signals, this job falls to Nod-like receptor's (NLR). In

partnership with other PRRs, NLRs are activated by various pathogens (e.g. Bacterial toxins, viral RNA, fungal glycoproteins) and other immune activators (asbestos, amyloid  $\beta$ ) (Davis et al., 2011). There is evidence to indicate that the NLR functionality overlaps with obesity and is activated to negatively influence insulin resistance (Lumeng, 2013). NLRP3 forms a structure known as an inflammasome and is activated to translate danger signals into the production of proinflammatory cytokines (IL-1 $\beta$ , IL-18) via Caspase-1 (Wen et al., 2012; Lumeng, 2013). This mechanism is especially active in macrophages playing a leading role in gut inflammation and infectious responses (Davis et al., 2011). Additional research indicates that NLRP3 might be an activator of NF- $\kappa$ B signaling, play an indirect role in leukocyte inflammation and apoptosis, gout, Type2 diabetes and neuroinflammation during Alzheimer's disease (Haneklaus et al., 2013; Heneka et al., 2013; Lumeng, 2013). In DIO inflammasomes are activated by ceramides, saturated fatty acids and ROS, thereby adversely impacting insulin receptor signaling (Lukens et al., 2011; Wen et al., 2011). Of interest are the NLRP3 knockout mice, as they have diminished markers of visceral fat activated M1 ATMs and a surge in anti-inflammatory M2 ATM gene expression, suggesting inflammasomes' involvement in ATM regulation (Lumeng, 2013).

Dendritic cells and macrophages are antigen presenting cells that express C-type lectin receptors (CLRs). Together with TLR activation signals, CLRs mediate antigen internalization, processing and surface presentation to improve T cells activation. Over a 1000 various CLR extracellular domains, combined with a large variety of intracellular signaling domains have been uncovered. This multitude of combinations of

intra/extracellular domains result in antigen-receptor binding that drives immune system activation, while at other times allows inhibition of inflammatory signals (Lumeng, 2013). Extensive studies have been conducted on a CLR termed oxidized low-density lipoprotein receptor 1 (LOX-1 or OLR1) found on endothelial cells, macrophages, platelets, and smooth muscle cells and which is partly responsible for endocytosis of oxidized LDL (Li & Mehta, 2000). LOX-1 is expressed ubiquitously in adipose tissues and atherosclerotic lesions where it induces macrophage and endothelial cell activation via NF- $\kappa$ B and MAP kinase pathways (Li & Mehta, 2000). Elevated LOX-1 expression has been noted in obese rodent models as well as in obese humans, leading to extensive vascular inflammation (Inoue et al., 2005). High levels of AT LOX-1 parallel BMI and degree of insulin resistance (Brinkley et al., 2008; Kelly et al., 2008; Rasouli et al., 2009). LOX-1 acts as a metabolic switch mediating onset of inflammation in DIO.

Widely expressed TNF- $\alpha$  receptors and Toll-like receptors (TLRs) by adipocytes, underscore the bidirectionality of the immunometabolic cross-talk (Cawthorn & Sethi, 2008). Previously discussed concept of AT lipid spillover activates a multitude of ATM mediated inflammatory cascades via TLRs, ER-stress mediators and NLRP3 inflammasome-mediated pathways (Erbay et al., 2009; Vandanmagsar et al., 2011; Wen et al., 2012). Lilja et al. noted that inflammation and adipogenesis are closely associated processes, with adipocytes and inflammatory cells sharing receptors and secreting pro-inflammatory factors such as TNF- $\alpha$ , IL-1 $\beta$ , MCP-1 and TGF- $\beta$  (Lilja et al., 2012). Authors hypothesized and subsequently elucidated how inflammation appears fundamental to the process of neo-adipogenesis, via the macrophage-derived MCP-1

protein and recruitment of bone-marrow derived progenitors along with TNF- $\alpha$ , LCN-2 (Lipocalin-2) and IL-1 $\beta$  (Lilja et al., 2012). Additional evidence indicates that adipocyte precursor cells and macrophages share similar functions (Cousin et al., 1999; Charrière et al., 2003). Furthermore, adipokines and cytokines secreted by the ATMs are involved in the three crucial steps of AT growth and stability: adipogenesis, angiogenesis, and matrix and scaffold remodeling (Lilja et al., 2012). Preadipocytes were also shown to secrete MCP-1 and MIP-1 $\alpha$ , factors known to be released by macrophages (Menghini et al., 2005).

### ***White Blood Cells***

Numerous systemic signals and dynamic cellular events drive obesogenic changes. In addition to the above implicated receptors, a number of innate immune cellular effectors play an important role in the inflammation during obesity. From our previous discussion it is clear that, macrophages are involved in multiple innate immune system initiations of responses to inflammation, and its resolution. Robbins et al. showed that inflammatory monocytes, Ly-6c<sup>hi</sup> in mice and CD16<sup>+</sup> in humans, generate pro-inflammatory macrophages (Robbins & Swirski, 2010). It has also been noted that obesity increases circulating inflammatory monocytes in mice (Tsou et al., 2007). In obese human diabetic patients CD16<sup>+</sup> monocytes are increased, but they decline coincidentally with surgical weight loss and improvements in vascular inflammation (Poitou et al., 2011). These findings promote the theory that bone marrow and splenic derived monocyte pools are ultimately responsible for the circulating inflammatory

monocytes that drive inflammation during obesity. The exact process of the ATMs changing polarization from the anti-inflammatory M2 to inflammatory M1 profile remains unclear, but monocyte recruitment concurrent with inflammatory signaling are clearly important factors (Weisberg et al., 2006; Westcott et al., 2009).

NK cells are lymphocytes derived from common lymphoid progenitor, along with B and T cells. There is research to suggest that during human obesity AT NK cells are a considerable source of inflammatory cytokines such as IFN $\gamma$  and can proliferate in omental fat along with obesity (O'Rourke et al., 2011).

Natural killer T (NKT) cells are a group of T cells that express markers of NK cells. Interest in NKT cells was piqued because of their ability to recognize lipids and glycolipids presented by CD1d receptor on antigen presenting cells, rather than peptide-MHC (major histocompatibility) complexes. Link to metabolism comes from the fact that human omental fat contains large numbers of NKT cells along with CD1d<sup>+</sup> cells (Lynch et al., 2009). Upon activation these NKT cells produce Th1 (IFN $\gamma$ ), Th2 (IL-4) factors and decrease in number with DIO (Lumeng, 2013). Even though the exact mechanism is still unknown, the NKT cells are inhibited in the liver with the progression of DIO (Syn et al., 2010; Mantell et al., 2011). Ohmura et al. noted that despite gaining the same amount of weight, NKT cells deficient mice are protected from insulin resistance with DIO and exhibit reduction in ATM infiltration (Ohmura et al., 2010).

Recent evidence suggests that inflamed adipose tissue is being invaded not only by proinflammatory ATMs but also by T cells, neutrophils, natural killer cells and dendritic cells brought about by AT secretion of adipokines and chemokines (J. Liu et al.,

2009; Moro et al., 2010; Nishimura et al., 2009). Wu et al. has shown that in DIO mice, AT is not only infiltrated by T cells, but there was also an increase in the expression of T-cell chemoattractant RANTES (Wu et al., 2007). Another recent study shows that in mice, AT is exhibiting specific TCR rearrangements, indicating that clonal T cell populations are entering AT (Lumeng et al., 2009). This indicates that antigens in adipose tissue might be communicating with components of the adaptive immunity. TCR changes along with the extensive Type 1 ATM infiltration and Th1 cytokine secretion result in subsequent insulin resistance in AT and chronic DIO inflammation (Lumeng et al., 2009). As mentioned previously, the current scientific consensus states that Type 1, proinflammatory ATMs are the driving force behind inflammation during DIO. An intriguing study by Nishimura et al. challenges this assertion. His team looked at the way T cell populations change with increasing obesity. They noted an increase in the ratio of CD8<sup>+</sup> to CD4<sup>+</sup> T cells in adipose tissue, weeks prior to the typical onset of macrophage infiltration (Nishimura et al., 2009). Additional research by two different scientific groups came to the same conclusion, although looking at different parts of this transition (Feuerer et al., 2009; Winer et al., 2009). Nishimura and his colleagues showed that in a DIO model of obesity, number of CD8<sup>+</sup> effector T cells infiltrating epididymal adipose tissue increased, but the number of CD4<sup>+</sup> helper and Treg cells declined (Nishimura et al., 2009). They were also able to show that the declining population of CD8<sup>+</sup> T cells lowered ATM infiltration and AT inflammation, as well as alleviate systemic insulin resistance (Nishimura et al., 2009). Their research points to the possibility that CD8<sup>+</sup> T cells and adipocytes cooperate in recruitment of ATMs to the adipose tissue. On the

whole these data suggests that obesity alters properties of the adipose tissue T cells prior to the arrival of the macrophages, but more importantly, that CD8<sup>+</sup> T cells play an essential role in initiation and propagation of the AT inflammatory processes.

The importance of mast cells in the context of obesity became evident when Liu et al. studied mast cell deficient mice. HFD (high fat diet) induced obesity curtailed angiogenesis, but this had no impact on these mice most likely due to the mast cell induced expression of IL-6 and IFN $\gamma$  (Liu et al., 2009).

Eosinophils in partnership with mast cells react to increases in IgE and control mechanisms of allergic inflammation. Surprisingly these cells have been found in AT and are thought to regulate metabolism. Eotaxin, a potent eosinophil chemoattractants, is elevated in AT and serum of obese humans and animals, suggesting an inflammatory activation of allergic innate immune responses in DIO (Vasudevan et al., 2006; Lumeng, 2013). Eosinophils which are present in AT decline with DIO and eosinophils deficient mice exhibit weight gain, as well as dampened insulin response, pointing to their protective role during the progression of obesity. This is thought to work via eosinophils secretion of IL-4 and IL-13, helping maintain alternatively activated ATM M2 polarization (Wu et al., 2011).

Typical innate immune responses are marked by an early neutrophils infiltration of inflamed tissues (Mantovani et al., 2011; Schipper et al., 2012). Neutrophils are able to communicate with multiple components of both innate and adaptive immunity and are linked to macrophage immune function (Gordy et al., 2011). There is a link between neutrophils and obesity, their counts are associated with BMI, waist circumference and

total AT in female teenagers (Kim & Park, 2008). Elgazar-Carmon et al. showed that during a short period of HFD, large numbers of neutrophils move into the fat tissues (Elgazar-Carmon et al., 2008). Elgazar-Carmon et al. suggest systemic neutrophils activation in obesity and short-term infiltration of AT, at the onset of inflammation during DIO (Elgazar-Carmon et al., 2008).

### ***Humoral factors***

Collaborating with the cellular components of the innate immunity are humoral factors, a number of which play a large role during inflammation. These include cytokines and chemokines, including innate inflammatory molecules such as C-reactive protein (CRP), defensins and complement components. Inflammatory cytokines amplify immune response and trigger leukocyte activation. In DIO most of these cytokines may emanate from the AT, largely from the stromal vascular fraction cells within AT, and are coupled with the AT growth and expansion. Complement system is another humoral component of the immune system, consisting of many different proteins that work together to “assist”, or complement, the antibody activity. Muscari et al. showed that core complement components C2, C3 and C4 are elevated in obese subjects, especially in AT (Muscari et al., 2007). The association is exceptionally strong for serum C3 levels with measures of insulin resistance, demonstrating yet another link between obesity and disease (van Greevenbroek et al., 2011). As far back as 1995 Muscari et al. showed association between complement and cardiovascular disease (Muscari et al., 1995). Later on, Bhatia and colleagues, using animal models demonstrated that the classical

complement pathway which is important in atherogenesis, is tied to protection from atherosclerotic plaque formation (Bhatia et al., 2007). It has also been known that platelet activation leads to activation and propagation of the complement system. Anfossi et al. has described causes of platelet dysfunction in central obesity, leading to increased risks of major cardiovascular events (Anfossi et al., 2009). Various genome-wide studies support the role complement system plays in obesity. Schadt et al. discovered that C3a receptor (C3a1) regulates visceral fat mass in mice (Schadt et al., 2005). Mamane et al. later showed that during obesity, C3a1 knockout mice are protected from insulin resistance and DIO caused inflammation (Mamane et al., 2009). It is thought that C3a1 knockout mice are able to suppress ATM infiltration of adipose tissue and show a direct link between complement and pro-inflammatory ATMs.

Of interest is research that indicates that adipocytes are very similar to immune cells (T cells, macrophages, dendritic cells, etc.) in several features such as phagocytic properties, complement activation, and production of inflammatory mediators to pathogen sensing (Dixit, 2008; MacLaren et al., 2008; Neda Rasouli & Kern, 2008; Procaccini et al., 2013).

### ***Interleukin-17A***

Adipose cells and innate immunity are part of an intricate network, in which during DIO, the chronic low-degree inflammatory state is able to affect acute inflammation (Galgani & Matarese, 2010). The implicated culprit of this link is IL-17A, it is produced in especially high amounts by activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells and has

been found in eosinophils, neutrophils, and human blood monocytes (Kolls & Lindén, 2004). It is a well-known inflammatory cytokine able to induce secretion of chemokines, growth factors (GM-CSF, IL-6), and adhesion molecules (ICAM-1) leading to accumulation, activation and migration of neutrophils and monocytes to the inflammatory site (Kolls & Lindén, 2004). IL-17A links adaptive and natural immune responses allowing T cells to control inflammatory responses induced by innate immunity (Galgani & Matarese, 2010). Pini and Fantuzzi et al. showed that in obese mice, during an induced peritonitis, a common model of acute inflammation, neutrophils produced high amounts of this cytokine (Pini & Fantuzzi, 2010). They noted significant increase in IL-17A mRNA expression in AT of obese mice versus their lean controls. But there was no increase of IL-17A production in spleen cells or in CD4<sup>+</sup> T cells in the peritoneal fluid. Results of their study indicate that in their mouse model, obesity enhanced production of IL-17A (Pini & Fantuzzi, 2010). The deeper implication that requires further exploration is the possibility that AT induced Th 17-like response may be able to induce the break of self-tolerance in some conditions activating autoimmunity. A link between IL-17A, NLRP3 inflammasome, development of airway hyperreactivity (AHR), a key asthma feature, and obesity has been established by Kim et al. In a study of DIO mice scientists showed development of AHR dependent on IL-17A produced by ILC3 lymphoid cells and NLRP3 inflammasome (Kim et al., 2014). Ever present during obesity pro-inflammatory ATMs induced production of IL-1 $\beta$  which expanded the number of ILC3 cells. Authors hypothesized that since ILC3 cells are typically found in the bronchoalveolar lavage fluid of people suffering from asthma, DIO-associated asthma is

advanced by inflammation mediated via NLRP3, IL-1B and ILC3 cells producing IL-17A cytokine (Kim et al., 2014).

### ***Hormones***

So far emphasis has been on cellular gateways and immune system factors that affect, or are affected by obesity-induced inflammation. Another distinct class of factors intertwined in this system is hormones, which have had an enormous body of research dedicated to the discussion of their roles in obesity, inflammation and disease. With leptin and adiponectin taking central role, these two have been mentioned in nearly every DIO related scientific paper to date. In the context of this thesis their role in modulation of inflammation and immune system control will be discussed.

Although leptin is produced by many cell types (e.g. stomach, skeletal muscles, bone marrow, placenta), it is primarily made by the white adipose tissue and its levels directly correlate with body fat mass and adipocyte size (Friedman & Halaas, 1998; Cava & Matarese, 2004). It is often referred to as a “satiety hormone”, as it controls body mass, specifically fat stores, and energy expenditures, exercising control over hypothalamic arcuate nucleus, midbrain and brainstem neurons. Leptin functions chiefly via a long arc, not impacting immediate meals, but rather balancing the ratio of total food consumed relative to the amount of expended energy (Friedman & Halaas, 1998). Genetic abnormalities resulting in impaired leptin or its receptor production cause excessive food intake, weight gain and general obese phenotype (Chua et al., 1996). Initially these observations lead to the conclusion that leptin should abrogate DIO changes as it might

slow down fat storage. But the leptin paradox, also known as leptin resistance, has forced scientists to question its role, as observational studies have shown that most individuals with DIO have high levels of circulating leptin. Insert **218 Fig 2**.

Adiponectin is mainly produced by mature adipocytes in WAT, but is also found in skeletal muscles, cardiac myocytes and endothelial cells. It has two distinct forms that act in different locals in the body (skeletal muscles and liver). Adiponectin is considered an anti-inflammatory, anti-apoptotic agent with pro-angiogenic properties and its levels inversely correlate with insulin resistance, weight gain and visceral obesity (Maeda et al., 2002; Kusminski & Scherer, 2009; Shetty et al., 2009). Adiponectin's receptors are expressed on most human monocytes, a large number of B cells and NK cells, but only on a small percentage of T cells (Pang & Narendran, 2008). Even though surface receptor expression is limited, new research indicates that adiponectin may be a negative regulator of T cells (Wilk et al., 2011). Adiponectin predominantly acts to counteract leptin's influence and exerts control over the liver, its specific target organ (Yamauchi et al., 2003). In skeletal muscles it stimulates fatty acids oxidation and glucose uptake. It is also capable of helping ameliorate insulin resistance, by enhancing insulin signaling and increasing glucose uptake in myocytes via induction of expression of GLUT-4 transporter (Ceddia et al., 2005).

### ***Hormones as cytokines***

Leptin, adiponectin and other adipocytokines not only function as hormones controlling energy homeostasis and regulating neuroendocrine functions, but also act as

cytokines, are capable of modulating immune functions and inflammatory processes (Procaccini et al., 2012; Procaccini et al., 2013). Leptin modulates a number of pivotal pro-inflammatory processes; (i) it stimulates innate immune responses, prompts the production of key inflammatory factors (e.g. IL-1, IL-6, IL-12, TNF), (ii) it is able to activate neutrophils chemotaxis, (iii) it stimulates production of reactive oxygen species (ROS), and (iv) it promotes activation and phagocytosis by monocytes/macrophages and their production of leukotriene B<sub>4</sub> (LTB<sub>4</sub>), cyclooxygenase 2 (COX-2) and nitric oxide (NO) (Carbone et al., 2012). In the innate immune system specifically, leptin activates NK cells, promoting NK-cell cytotoxicity through activation of signal transducer and activator of transcription 3 (STAT3) and IL-2 (Zhao et al., 2003). In comparison, adiponectin acts mainly as an anti-inflammatory agent. Studies show adiponectin inhibiting NF- $\kappa$ B activation, leading to anti-inflammatory effects on endothelial cells (Ouchi et al., 1999). It also inhibits TNF-induced vascular cell adhesion molecule-1 (VCAM-1) expression, endothelial-leukocyte adhesion molecule-1 (E-selectin), as well as the expression of intracellular adhesion molecule-1 (ICAM-1) (Ouchi & Walsh, 2007). In human monocytes, macrophages and dendritic cells, it induces secretion of anti-inflammatory cytokines, such as IL-10 and a receptor antagonist IL-1RA, and inhibits production of INF- $\gamma$  (Wolf et al., 2004).

### ***Hormones and Adaptive Immunity***

Both hormones and cytokines partake in the modulation of the adaptive immunity; leptin however, has more research elucidating its effects. In human adaptive

immunity leptin has been shown to promote proliferation and secretion of IL-2 from naive T cells (CD45RA<sup>+</sup>). Lord et al. in a study of ob/ob mice, noted that leptin deficiency is associated with immunosuppression and thymic atrophy, both actions of the adaptive immunity (Lord et al., 1998). Leptin also acts as a negative signal in the regulation of the expansion of the human regulatory T cells (Treg), specifically, naturally occurring Foxp3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup> cells, which are involved in the prevention of autoimmune diseases (De Rosa et al., 2007). In the same study De Rosa and colleagues noted that human Treg cells produce leptin and express large quantity of leptin receptor. Procaccini et al. noted that leptin, via activation of mTOR (mechanistic target of rapamycin), inhibits rapamycin-induced proliferation of Tregs (De Rosa et al., 2007). It was further noted by Kim et al. that mTOR kinase regulates varying aspects of helper T cell differentiation (Kim et al., 2002). Cumulative research on the leptin-mTOR axis suggests that this pathway might control immune system tolerance via cellular energy status and metabolic signaling in Treg cells (Carbone et al., 2012). As has been already mentioned, adiponectin is thought to be a negative T cell and NK cell regulator. In an additional study by Kim et al. adiponectin was found to suppress IL-2-enhanced cytotoxic activity of NK cells (Kim et al., 2006). Tsang and colleagues showed that dendritic cells treated by adiponectin had a lower production of IL-12p40, a chemoattractant for macrophages, and a declining expression of CD80, CD86 and histocompatibility complex class II (MHCII), all three proteins partake in T cell activation (Cooper & Khader, 2007; Tsang et al., 2011). These data indicate that immune response is partially mediated by adiponectin's ability to alter dendritic cell functions.

Furthermore, adiponectin may exercise some control over Treg cell homeostasis, which was shown by the same group. During an experiment with co-cultures of T cells and adiponectin-treated dendritic cells, a reduction was noted in T cells proliferation and IL-2 production, along with an increase in CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells (Tsang et al., 2011).

## CONCLUSION

In the past two decades the scientific community has grown extensively, discovering numerous factors and elucidating a myriad of pathophysiological processes. Hence the terms immunometabolism and meta-inflammation have been coined, but they are deceptively neat, serving as only shorthand terms ascribed to a wide array of cellular processes. While there are many unresolved questions connecting DIO to inflammation, immunity and ultimately infections and diseases, the basic road map has been outlined. Diet induced obesity leads to chronic low-grade inflammation, which in turn impairs body's defenses, rendering the immune system incapable of differentiating between exogenous and endogenous threats, leaving the host exposed. As the immune system's capabilities decline infections have an easier time penetrating the body and causing harm. The obese organism becomes its own poisonous pill. Instead of providing nourishment to the body, food, exerting hormonal control, activates inflammatory cascades, and initiates a positive feedback loop which negatively affects the body and future health (Weisberg et al., 2003; Curat et al., 2004; Lilja et al., 2012).

Today the scientific community is facing more questions than answers, but within these mysteries future opportunities to treat and prevent obesity-associated morbidities are apparent. The progress will not be quick and will inevitably consume vast sums of money, as well as time and effort by physicians, scientists, politicians and ordinary people, who ultimately bear the burden of disease diagnoses and treatment.

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