CHARLES UNIVERSITY IN PRAGUE

FIRST FACULTY OF MEDICINE

Biomedicine

Biochemisty and Pathobiochemistry

Effects of obesity on the course of Trypanosoma cruzi infection

Doctoral Dissertation

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Prague/New York, February 2016

Univerzita Karlova v Praze

1. lékařská fakulta

Biomedicína

Biochemie a Patobiochemie

Účinky obezity v průběhu infekce Trypanosoma cruzi

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Declaration:

I, Wunnie Brima, hereby declare that this doctoral thesis titled 'Effects of obesity on the course of *Trypanosoma Cruzi* infection' has been compiled by me under the supervision of Prof. MUDr. Tomáš Zima DrSc. MBA and Prof. Jesse Roth, MD, FACP.

This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. I also undertake that any quotation or paraphrase from published or unpublished work of another person has been duly acknowledged.

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Identifikační záznam:

BRIMA, Wunnie. Účinky obezity v průběhu infekce *Trypanosoma cruzi*. [Effects of obesity on the course of *Trypanosoma Cruzi* infection]. Praha/New York, 2016. Počet stran - 96.

Disertační práce. Univerzita Karlova v Praze, 1. lékařská fakulta; Ústav lékařské biochemie a laboratorní diagnostiky 1.LF UK v Praze,

The Feinstein Institute for Medical Research, NSLIJ Health Systém, NY. Školitel Tomáš Zima, školitel konzultant Jesse Roth.

DEDICATION

This thesis is dedicated to my late parents, Prince Brima and Mariatu Rose Conteh. You did not only raise and nurture me but you also taught me the importance of hard work and dedication. Although you did not live to see this day, your words of inspiration and encouragement in pursuit of excellence linger on.

This work is also dedicated to my wife, Tufikameni Brima, who has always loved me unconditionally and has given me unwavering support through the seemingly endless years.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Prof. MUDr. Tomáš Zima, DrSc., MBA, for the opportunity to pursue my Ph.D study and research at the Department of Biochemistry, 1.LF UK v Praze. Thank you for your guidance and support through the years.

I am very fortunate to have met Prof. Jesse Roth, MD., FACP and his wife, Susan shortly upon arrival to the United States. Dr Roth, thank you for giving me a home in your lab at the Feinstein Institute. I am grateful for your guidance, support and the opportunities you have afforded me. Your wisdom, mentorship, patience and encouragement have been immensely helpful in moving my project forward. I fondly remember our meetings on weekends at the Albert Einstein College of Medicine library to review my thesis.

I would also like to thank committee members of my PhD study, including the late prof. MUDr. Jiří Kraml, DrSc. A special thank you to Prof. MUDr. Stanislav Štípek, DrSc., the current head of the committee; and for giving me the opportunity to tutor biochemistry practical sessions in the Department of Biochemistry, 1.LF UK. Thank you MUDr. Jan Platenik, MUDr Jan Krtil and Mgr. Richard Buchal for your support and for teaching me scientific research techniques and bench skills pertaining to laboratory work. I remember my time spent with you doing experiments, going out for sushi and in the summer, grilling. I would also like to recognize other members of Platenik's lab especially Alena, Kveta, Adrianna, Juraj; you have all contributed to the progress I have made.

I performed part of my graduate work at the Feinstein Institute and at the Albert Einstein College of Medicine, New York. I need to thank many people there for their contributions to my success. I thank Dr Louis Weiss and Dr Herbert Tanowitz for their guidance and support and for giving me the opportunity to perform my experiments in the laboratories at Einstein. They demonstrated a sincere interest in my work and were always ready to answer questions. They also provided me with many insightful suggestions. Special thanks go to Dr Jyothi Nagajyothi for helping with my experiments; her knowledge and insights have been of great help. I am also indebted to Yang Fen Ma for expert assistance with tissue culture, Dazhi Zhao, Betsy Herold, Ashley Huber, Huan Huang, Regina Kuliawat, Irwin Kurland, Jeffrey E. Pessin, Gary J. Schwartz, Robin Sgueglia, Hardik Shah, Henry Shikani, Daniel Stein, Licheng Wu and Rama Yakubu for their assistance and support.

I thank my fellow lab mates at the Feinstein Institute for helping with my experiments and thesis: Daniel J. Eden, Syed F. Mehdi, Michelle Bravo, Mohammad M. Wiese, Priya Patel, Mark Westlake, Hafiz Basit Mahboob, and Meghan Dancho. Their excitement and willingness to work with me made the completion of this research an enjoyable experience.

Last but not the least, I would like to thank my family and friends: my wife, Tufikameni Brima, my brother, Foday Conteh, Susan Roth, and my uncle, Dauda Brima Sallu. Thank you for your words of encouragement and your support. Special thanks to my friend Imani Kondo for helping with Czech language translation, Jakub Otahal and family, and Jana Dudova and family for material and emotional supports.

ABSTRACT

Effects of obesity on the course of *Trypanosoma cruzi* infection.

Keywords: Obesity, metabolic syndrome, high fat diet, obesity paradox, mortality, *Trypanosoma cruzi*, Chagas disease, American trypanosomiasis

Obesity is very widespread and detrimental to health. Obesity brings with it many changes including heightened immune function, and a higher prevalence of major cardiovascular disorders, cancer, diabetes, and Alzheimer disease. Obesity is also associated with shortened lifespan. The detrimental effects of obesity are linked to the "metabolic syndrome", a broad range of changes in metabolic processes and immune function.

As a first approximation, we agree with this formulation but we will then proceed to document some of its weaknesses. (i) Crude mortality rates increase with increasing body mass index (BMI) but as the BMI approaches the normal range, mortality rates reverse (the now classic "J-shaped curve") so that individuals with reduced BMI have elevated mortality. (ii) A multiplicity of medical and surgical conditions have been reported where short term and medium term outcomes are better for overweight patients. These conditions are placed under the heading of "obesity paradox". (iii) The medical community has introduced a binary system for the metabolic syndrome ---- *yes*, patient has it or *no*, the patient does not have it, despite the fact that all of the changes that are considered components of the metabolic syndrome are continuous variables.

Our work is focused on sharpening focus and improving understanding of these three weaknesses in the conceptualization of obesity as a medical problem. Using historical perspective, we have hypothesized that the metabolic syndrome is evolutionarily ancient. In addition to the well-accepted harm associated with late-in-life disorders, we propose that throughout human history, the so-called syndrome has benefited younger individuals by enhancing host defenses against infectious diseases. We posit that the major benefits of the metabolic syndrome accrue in controlling the widespread potentially ravaging infections that the body cannot self-cure, including tuberculosis and (the subject of the thesis) *Trypanosomiasis cruzi* also known as Chagas disease or American trypanosomiasis. Infection with *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease, results in chronic infection that leads to cardiomyopathy with increased mortality and morbidity in endemic regions.

In support of our hypothesis that the consequences of the metabolic syndrome may be positive or negative, depending on age and on life events such as infectious diseases, we induced the metabolic syndrome in CD-1 mice by high fat feeding prior to infection with *T. cruzi*. The lethality of *Trypanosoma cruzi* infection was reduced from 55% to 20%.

In a companion study, our group found that a high-fat diet (HFD) protected mice from *Trypanosoma cruzi*-induced myocardial damage and significantly reduced post-infection mortality during acute *Trypanosoma cruzi* infection.

ABSTRAKT

Účinky obezity v průběhu infekce Trypanosoma cruzi

Obezita je velmi rozšířený a škodí zdraví. Obezita s sebou přináší mnoho změn, včetně zvýšené imunitní funkce, a vyšší prevalenci závažných kardiovaskulárních onemocnění, rakovinu, diabetes a Alzheimerovy choroby. Obezita je také spojena se zkrácenou životností. Škodlivé účinky obezity jsou spojeny s "metabolického syndromu", široký rozsah změn v metabolických procesech a imunitní funkce.

Jako první aproximaci, budeme souhlasit s touto formulací, ale pak budeme pokračovat zdokumentovat některé jeho nedostatky. (i) Hrubé úmrtnost zvyšuje se zvyšujícím se 'body mass index' (BMI), ale jak se blíží BMI normální rozsah, úmrtnost reverzní (dnes již klasickou "J-křivku ve tvaru písmene") tak, že jedinci se sníženou BMI mají zvýšené úmrtnosti. (ii) Velké množství lékařských a chirurgických případů byly zaznamenány, kde krátkodobé a střednědobé výsledky jsou lepší pro pacienty s nadváhou. Tyto podmínky jsou umístěny pod hlavičkou "obezity paradoxu". (iii) lékařská obec zavedla binární systém pro metabolický syndrom ---- *Ano*, pacient má, nebo *Ne*, pacient nemá ji, a to navzdory skutečnosti, že všechny změny, které jsou považovány za složky metabolického syndromu jsou spojité proměnné.

Naše práce je zaměřena na ostření zaměření a lepší pochopení těchto tří slabiny v pojímání obezity jako lékařský problém. Použití historickou perspektivu, jsme předpokládali, že metabolický syndrom je evolučně starobylý. Kromě dobře přijímán újmou v souvislosti s pozdně životni poruchy, navrhujeme, že v celé historii lidstvo, tento syndrom má benefit u mladších jedinců tím, že zvýrazníte přirozenou ochranu proti infekčním nemocem. Jsme předpokládají, že hlavní benefit metabolického syndromu narůstat při kontrole rozšířené potenciálně devastující infekce, které tělo nedokáže samo se ovládat, včetně tuberkulózy a (předmět práce) trypanosomiázu cruzi také známý jako Chagasova choroba nebo amerického trypanosomiáze. Infekce *Trypanosoma cruzi*, parazitickým prvokem, který způsobuje, Chagasova choroba, vede k chronické infekce, která vede ke kardiomyopatii s zvýšené úmrtnosti a nemocnosti v endemických oblastech.

Na podporu naší hypotézy, že důsledky metabolického syndromu může být pozitivní nebo negativní, v závislosti na věku a na životními událostmi, jako infekčních nemocí, indukovali jsme metabolicky syndrom u CD-1 myší krmeni vysokým obsahem tuku před infekcí *T. cruzi*. Úmrtnost z infekce *Trypanosoma cruzi* se snížil ze 55% na 20%.

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List of Abbreviation

AACE	American Association of Clinical Endocrinologists
AHA	American Heart Association
ATP III	Adult Treatment Panel III
BAT	Brown adipose tissue
BMI	Body mass index
BMP	Bone morphogenetic protein
CDC	Center for disease control
CRP	C-reactive protein
DMEM	Dulbecco's modified Eagle's medium
EGIR	The European Group for the study of Insulin Resistance
EPSTI1	Epithelial stromal interaction 1
fa/fa	Diabetic Zucker fatty rats
FGF21	Fibroblast growth factor 21
FIZZ	Found in inflammatory zone
GDI	Guanosine diphosphate dissociation inhibitor
GLUT2	Glucose transporter 2
GnRH	Gonadotropin-releasing hormone
HDL	High-density lipoprotein
HDL-C	High density lipoprotein cholesterol
HFD	High fat diet
HGF	Hepatocyte growth factor
HsCRP	High sensitivity C-reactive protein
IDF	International Diabetes Federation
IGF	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-1b	Interleukin 1 beta

IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
INF –y	Gamma interferon
IRS-2	Insulin receptor substrate-2
KSSO	Korean Society for the Study of Obesity
$LepR^{db/db}$	Leptin receptor-deficient mice
$Lep^{ob/ob}$	Leptin-deficient mice
MC4-R	Melanocortin 4 receptor
MCP-1	Monocyte chemoattractant protein 1
MIF	Macrophage migration inhibitory factor
NCEP ATP III	National Cholesterol Education Programme Adult Treatment Panel III
NGF	Nerve growth factor
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung, and Blood Institute
NK	Natural killer
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator inhibitor-1
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PGE2	Prostaglandin E2
PPAR-α	Peroxisome proliferator-activated receptor alpha
RD	Regular diet
Rx	Receiving treatment
SAA 1 & 2	Serum amyloid A proteins 1 & 2
STZ	Streptozotocin
sWAT	Subcutaneous white adipose tissue

T2DM	Type 2 diabetes mellitus
TBX1	T-box1
TCF21	Transcription factor 21
T. cruzi	Trypanosoma cruzi
TGs	Triglycerides
TGF-b1	Transforming growth factor-beta 1
TLRs	Toll-like receptors
TMEM26	Transmembrane protein 26
TNF-a	Tumor necrosis factor alpha
UCP1	Uncoupling protein 1
USA	United State of America
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein
WAT	White adipose tissue
WC	Waist circumference
WHO	World Health Organization
ZDF	Diabetic Zucker fatty rats
ZIC1	Zinc finger protein of the cerebellum 1

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1.0 CHAPTER 1: LITERATURE REVIEW

1.1 Overview of obesity and the metabolic syndrome

1.1.1 Definition

Obesity is a result of chronic positive energy balance associated with excess fat storage resulting from energy intake exceeding energy expenditure. Excess fat deposition in adipose tissues is associated with chronic low-grade inflammation and insulin resistance. The metabolic syndrome, defined by a constellation of interconnected physiological, biochemical, clinical, and metabolic factors, produces immune activation and metabolic alterations that promote complications of obesity and diseases of later life, including myocardial infarction, stroke, diabetes, Alzheimer disease and cancer.

Currently the most widely used criteria for definition of the metabolic syndrome are from the World Health Organization (WHO)[1], the International Diabetes Federation (IDF)[2], the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III)[3], American Association of Clinical Endocrinologists (AACE) [4], and the European Group for the Study of Insulin Resistance (EGIR)[5]. However, it is to be noted that the features of the metabolic syndrome are a continuum and not a simple on/off switch.

Clinical measures	**WHO (1998)**	**EGIR (1999)**	**ATPIII (2001)**	**AACE (2003)**	**IDF (2005)**
Insulin resistance	Impaired glucose tolerance (IGT), Impaired fasting glucose (IFG), Type 2 diabetes mellitus (T2DM), or lowered insulin	Plasma insulin >75th percentile plus any 2 of the features below	None, but any 3 of the following 5 features below	IGT or IFG plus any of the following features below based on clinical judgment	None

	sensitivity plus any 2 of the features below				
Body weight	Men: waist-to-hip ratio >0.90; women: waist-to-hip ratio>0.85 and/or BMI > 30 kg/m^2	Waist circumference (WC) ≥94 cm in men or ≥80 cm in women	WC ≥102 cm in men or ≥88 cm in women	BMI ≥ 25 kg/m^2	Increased WC (population specific) plus any 2 of the following features below
Lipids	Triglycerides (TGs) ≥150 mg/dL and/or high density lipoprotein cholesterol (HDL-C) <35 mg/dL in men or <39 mg/dL in women	TGs ≥150 mg/dL and/or HDL-C < 39 mg/dL in men or women	TGs ≥150 mg/dL HDL-C < 40 mg/dL in men or < 50 mg/dL in women	TGs ≥150 mg/dL and HDL-C < 40 mg/dL in men or < 50 mg/dL in women	TGs ≥150 mg/dL or on TGs Rx. HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on HDL-C Rx
Blood pressure	≥140/90 mm Hg	≥140/90 mm Hg or on hypertension Rx	≥130/85 mm Hg	≥130/85 mm Hg	≥130 mm Hg systolic or ≥85 mm Hg diastolic or on hypertension Rx
Glucose	IGT, IFG, or T2DM	IGT or IFG (but not diabetes)	>110 mg/dL (includes diabetes)	IGT or IFG (but not diabetes)	≥100 mg/dL (includes diabetes)
Other	Microalbuminuria: Urinary excretion rate of >20 mg/min or albumin: creatinine ratio of >30 mg/g			Other features of insulin resistance	

Table 1: Diagnostic criteria proposed for the clinical diagnosis of the metabolic syndrome- Adapted from Jaspinder Kaur [6]

1.1.2 Historical background

In his 1988 Banting Lecture, GM Reaven described “a cluster of risk factors for diabetes and cardiovascular disease” and named it “Syndrome X” [7]. However, the history dates back to the early twentieth century. During the First World War in Vienna, Austria, two physicians, Karl Hitzenberger and Martin Richter-Quittner discussed the interdependence of metabolic and vascular hypertension, as well as the relationship between blood pressure and diabetes mellitus [8, 9]. At about the same time, a Swede, Eskil Kylin, and a Spaniard, Gregorio Maran published independently two papers under the same title: ‘Hypertension and diabetes mellitus’ where they described the coexistence of hypertension and diabetes mellitus of adults and proposed common mechanisms for the development of these disorders [10, 11].

Later in 1947, Vague described that visceral obesity was commonly associated with development of diabetes, hypertension, gout and atherosclerosis[12]. Following this, Avogaro and Crepaldi presented an abstract in 1965 at the European Association for the Study of Diabetes annual meeting which again described a syndrome (termed ‘plurimetabolic syndrome’) that comprised hypertension, hyperglycemia, and obesity[13].

In 1988, after several years of research on the resistance to insulin-mediated glucose uptake, Reaven hypothesized that insulin resistance is the common denominator for a group of disorders consisting of impaired glucose tolerance (IGT), hyperinsulinemia, high levels of very low-density lipoprotein (VLDL)-triglycerides, low levels of high-density lipoprotein (HDL) cholesterol and hypertension. Reaven named this group of disorders ‘syndrome X’, in an attempt to stress its unknown aspects. In 1989, Kaplan added central obesity as one of the components of this disorder in addition to insulin resistance, hyperlipidemia and hypertension and he named this cluster ‘the deadly quartet’, to point out its great importance for the development of cardiovascular disease.

Several groups have attempted to develop a unified diagnostic criteria for the diagnosis of the metabolic syndrome[14]. The first attempt was made by a World Health Organization (WHO) diabetes group in 1998 to provide a definition of the metabolic syndrome. Other groups such as the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program Adult Treatment Panel (NCEP/ATP), the American Association of Clinical

Endocrinologists (AACE) and the International Diabetes Federation (IDF) have proposed their version of a definition of the metabolic syndrome. One of the major unresolved issues for defining the syndrome is that of the appropriate waist circumference. The primary difference between NCEP/ATP and IDF definitions is that waist-circumference cut points for Whites, Blacks, and Hispanics is higher in NCEP/ATP than in IDF.

Population	Organization	Male, cm	Female, cm
Europid	IDF	≥94	≥80
Caucasian	WHO	≥94 (increased risk)	≥80 (increased risk)
		≥102 (higher risk)	≥88 (higher risk)
United States	AHA/NHLBI (ATP III)	≥102	≥88
Canada	Health Canada	≥102	≥88
European	European Cardiovascular Societies	≥102	≥88
Asian	IDF /WHO	≥90	≥80
Korean	KSSO	≥90	≥85
Japanese	Japanese Obesity Society	≥85	≥90
China	Cooperative Task Force	≥85	≥80
Middle East, Mediterranean, Sub-Saharan African	IDF	≥94	≥80
Ethnic Central and South American	IDF	≥90	≥80

Table 2: Current Recommended Waist Circumference Thresholds for Abdominal Obesity. Adapted from[15] IDF- International Diabetes Federation; WHO- World Health Organization; AHA - American Heart Association; NHLBI- National Heart, Lung, and Blood Institute; ATP III- Adult Treatment Panel III; KSSO- Korean Society for the Study of Obesity.

1.1.3 Epidemiology

The increasing prevalence of the metabolic syndrome parallels the rise in the prevalence of obesity. Worldwide prevalence of the metabolic syndrome ranges between 10% to as much as 84%, depending on the region, composition (sex, age, race, and ethnicity) of the population studied, and the criteria used to define the metabolic syndrome [16, 17]. The prevalence of the metabolic syndrome in Japan ranges between 7.8% and 14.9% [18], and 44% among obese adolescents in the United States [19]. In the subpopulation with diabetes, prevalence of the metabolic syndrome was estimated to be 80% [18]. The IDF estimates that one-quarter of the world's adult population has the metabolic syndrome[2]. Higher socioeconomic status, sedentary

lifestyle, and high body mass index (BMI) were found to be significantly associated with the metabolic syndrome.

In a study that was done in Ontario, Canada[20], approximately 60,000 non-diabetic men and women, ages 30 and up were followed for up to 12.8 years for diabetes incidence. The median duration of follow-up was six years. The incidence of diabetes increased with increased body mass index in each ethnic group, as expected. South Asians became diabetic at BMI values that were the lowest followed by the Chinese, the Black, and the Whites.

In the National Health and Nutrition Examination Survey (NHANES), the observed prevalence of the metabolic syndrome was 5% among the subjects of normal weight, 22% among the overweight, and 60% among the obese [21]. Palaniappan et al. noted that each 11 cm increase in waist circumference (WC) is associated with an adjusted 80% increased risk of developing the metabolic syndrome within 5 years [22].The prevalence of the metabolic syndrome was further noted to increase with age (10% in individuals aged 20–29, 20% in individuals aged 40–49, and 45% in individuals aged 60–69)[23].

Ethnicity	**Body Mass Index**		
	24 kg/m^2	26 kg/m^2	30 kg/m^2
White	2.6	5.0	11
Black	6.0	11	25
Chinese	7.5	14	29
South Asian	11	19	38

Table 3: The incidence of diabetes with increased BMI in different ethnic groups. Adapted from [20]. The table shows the incidence rate of diabetes per 1,000 person-years at each level of BMI.

1.1.4 Adipose Tissue and the Metabolic Syndrome

The metabolic syndrome is a state of chronic low grade inflammation. This has been linked to the release of cytokines by adipose tissue [24-26]. Adipose tissue is a heterogeneous mix of

adipocytes, stromal preadipocytes, immune cells, and endothelium, and can respond to alterations in nutrient excess through adipocytes hyperplasia and hypertrophy [27]. Adipose tissues, in addition to being a major organ for energy storage is also considered to be an endocrine and paracrine organ. It plays an integral role in energy metabolism, neuroendocrine function, and immune function. Adipose tissue is sub-classified into white adipose tissue (WAT), brown adipose tissue (BAT) and beige adipocytes. Fat in some depots promotes inflammation more than fat in other sites. The term visceral fat, as currently used by the scientific community, is a misnomer as it refers to mesenteric or omental fat. Fat deposits around visceral organs, such as epicardial fat and perinephric fat, are currently referred to as ectopic fat or metastatic fat by the scientific community. Subcutaneous fat is more energy efficient, wasting fewer calories on immune related processes. Visceral or ectopic fats deposits are highly inflammatory fat deposits when compared to subcutaneous fat deposits[28].

Several authors have noted a linkage between adipose tissue and immune function; the immune system is a major user of energy (it consumes about 15% of resting metabolic rate) and extremely malnourished individuals have been shown to have impaired immune function. Adipose tissue secretes into the general circulation interleukins and adipokines (such as IL-6, adipsin, TNF alpha, leptin, monocyte chemoattractant protein-1 et al.) that are predominantly involved with immune functions [29].

1.1.4.1 White adipose tissue (WAT)

WAT is the main site of metabolic energy storage in the form of triacylglycerols (TGs) and are found mainly in subcutaneous fat deposits, mesentery, and visceral organs in the abdomen and thorax. WAT is made of adipocytes as well as macrophages, fibroblasts, endothelial cells and adipocyte precursor cells held together by highly vascularized and innervated connective tissue. WAT also secretes inflammatory cytokines such as IL-6 and TNF-alpha and is involved in local and systemic inflammatory processes. WAT also regulates appetite and energy metabolism, including glucose and lipid metabolism.

1.1.4.2 Brown adipose tissue (BAT)

Adipocytes in BAT are UCP1 (uncoupling protein 1) positive, polygonal shaped adipocytes with numerous large mitochondria. They constitute a specialized thermogenic organ that burns substrates to produce heat during cold or high-calorie diet exposure. BAT can be found during the entire lifespan in hibernators and some small mammals such as mice and rats. It is found in small amounts in newborn humans and at the base of the neck in adult humans. Its major function is heat generation to prevent hypothermia, especially in young infants and in adults exposed to cold.

UCP1, a fatty acid anion/H+ symporter, uncouples mitochondrial oxidative phosphorylation by bypassing the electrochemical gradient across the inner mitochondrial membrane from ATP synthase and thereby dissipates energy as heat. This uncoupling enables BAT to transform energy stored as TGs into heat [30, 31]. In addition to thermogenesis, BAT also has an autocrine/paracrine function by secreting complement factor D (adipsin), fibroblast growth factor 2 (FGF2), nerve growth factor (NGF) and VEGF. BAT is activated by cold, β adrenoreceptor agonists, thyroid hormone, FGF21, thiazolidinediones, bone morphogenic protein (BMP), and natriuretic peptide [32].

1.1.4.3 Beige or Brite adipocytes

Brite fat cells are WAT expressing thermogenic genes [UCP1 on chromosome 4 and PGC-1α on chromosome 20] under specific circumstances (such as exposure to cold weather). This so called 'browning of WAT' has not been fully elucidated. Beige cells have characteristics of both WAT and BAT [33]. Subcutaneous WAT is particularly prone to browning. The number of brite adipocytes within WAT depots increases after chronic treatment with β-adrenergic receptor (βAR) activators and like BAT, it is activated by cold, thiazolidinediones, natriuretic peptide, FGF21, irisin, and catecholamines [32].

Characteristic	White adipocytes	Brite adipocytes	Brown adipocytes
Location	Subcutaneous (sWAT), mesenteric, retroperitoneal, perigonadal, omental	Within inguinal WAT, other sWAT?	Interscapular, perirenal, axillary, paravertebral
Morphology	Unilocular/large lipid droplets	Multilocular/multiple small lipid droplets	Multilocular/multiple small lipid droplets
Origin	Adipoblast (*Myf5*$^-$)	Adipoblast (*Myf5*$^-$)	Myogenic precursor (*Myf5*$^+$)
Function	Energy storage as triacylglycerols	Increase energy expenditure	Thermogenesis
UCP1 level	Nearly undetectable	Basal: low Stimulated: high	High
Mitochondria content	Low	Medium	High
Correlation with insulin resistance and obesity	Positive	Inverse	Inverse
Characteristic markers	TCF21	CD137, TMEM26, TBX1, EPSTI1	ZIC1
Activators	High caloric intake (HFD, sugar), low physical activity	Cold, thiazolidinediones, natriuretic peptide, FGF21, irisin, catecholamines, β-adrenergic receptor agonists	Cold, βAR, thyroid hormone, FGF21, thiazolidinediones, BMP7, BMP8B, natriuretic peptide

Table 4: Main characteristics of white, brite and brown adipocytes. Adapted from [32]. sWAT- subcutaneous white adipose tissue; TCF21- transcription factor 21; TMEM26 -transmembrane protein 26; TBX1-T-box1; EPSTI1- epithelial stromal interaction 1; ZIC1- zinc finger protein of the cerebellum 1; FGF21- fibroblast growth factor 21;BMP- bone morphogenetic protein.

1.1.5 Cytokines and Adipokines

Obesity is associated with increased adiposity and stimulation of immune functions. It induces a complex immune response involving innate and adaptive immune cell types [34]. Adiposity, as seen in obesity, results in more blood vessels, more connective tissue fibroblasts, and macrophages. Increased circulating levels of pro-inflammatory cytokines in obese humans is caused by enhanced secretion of interleukins (IL-1b, IL-6, IL-8, IL-10) and inflammatory cytokines (leptin, adiponectin, haptoglobin, NGF, PAI-1, MCP-1, MIF, VEGF, HGF, TGF-beta 1, cathepsin S, resistin, CRP, SAA 1 & 2, TNF-alpha, PGE2) in adipose tissue by adipocytes and non fat cells (macrophages, fibroblasts, mast cells, leukocytes etc).

1.1.5.1 Leptin

Leptin, a 16-kDa polypeptide containing 167 amino acids with structural homology to cytokines, is a multifunctional protein secreted primarily by adipocytes. Secretion of leptin by adipocytes is

in direct proportion to adipose tissue mass. Its central function is energy homeostasis by repressing food intake and promoting energy expenditure via hypothalamic pathways. Other functions of leptin include regulation of the neuroendocrine axis, inflammatory response (leptin activates macrophages via the mTOR pathway) and bone mass. Levels of plasma leptin increase during the development of obesity and obesity-induced hyperleptinemia improves survival and immune response in sepsis [35]. Leptin resistance is a fundamental finding in obesity. In times of caloric restriction and weight loss, there is a decline in leptin levels. This decline is associated with adaptive physiological responses to starvation (for example, increased appetite and decreased energy expenditure). Interestingly, acute cessation of food intake leads to a very rapid fall in plasma leptin to levels far below those predicted by the BMI alone [36].

1.1.5.2 Adiponectin

Adiponectin is a 30-kDa multifunctional polypeptide secreted by adipocytes. It is considered to be an anti-inflammatory molecule. Unlike leptin, plasma levels of adiponectin are decreased in obesity. Adiponectin enhances fatty acid oxidation, facilitates glucose transport in muscles, increases insulin sensitivity and inhibits hepatic gluconeogenesis [14, 37]. Adiponectin is an anti-inflammatory cytokine. It protects against chronic inflammation by suppressing TNF alpha–mediated inflammatory changes. It also inhibits monocyte adhesion and macrophage transformation to foam cells [38]. Adiponectin improves hepatic lipid metabolism and opposes hepatic steatosis [39]. It also targets the pancreas and protects beta cells from apoptosis [40].

1.1.5.3 Monocyte chemoattractant protein-1 (MCP-1/CCL2)

Monocyte chemoattractant protein-1 (MCP-1/CCL2), a 13-kDa polypeptide, is a member of the C-C chemokine family and a potent chemotactic factor for monocytes [41]. Macrophages and monocytes are the major source of MCP-1 [42, 43]. MCP-1 regulates the migration and infiltration of monocytes, memory T lymphocytes, and natural killer (NK) cells. Obesity is associated with increased adipose tissue infiltration by macrophages [44], and MCP-1 levels have been shown to be elevated in obese rodents [45].

1.1.5.4 Resistin

Resistin, a 12-kDa protein, belongs to a family of four proteins referred to as FIZZ proteins (FIZZ for "found in inflammatory zone"). It is mainly secreted by macrophages and it exerts pro-inflammatory effects on smooth muscle cells. It also plays a role in glucose metabolism by decreasing the expression of insulin receptor substrate IRS-2.

1.1.5.5 TNF-alpha

TNFα, a 26-kDa transmembrane protein, is a pro-inflammatory cytokine primarily released by macrophages. It plays a role in the insulin resistance of obesity. Plasma TNFα is positively associated with the body weight and triglycerides (TGs). A negative association between plasma TNFα and high density lipoprotein–cholesterol (HDL-C) has been noted [46].

1.1.5.6 IL-1 beta

IL-1beta is a pro inflammatory cytokine produced by macrophages; this process is caspase-1-dependent. In humans, IL-1beta is released by adipose tissue but primarily from the non-fat cells, and the release is enhanced in obesity. It enhances the activation of T cells in response to antigen and induces expression of interferon gamma by T cells. IL-1beta is implicated in the development of obesity-associated insulin resistance [47].

1.1.5.7 IL 6

Circulating levels of IL-6 are elevated in obesity. IL-6 is a pluripotent cytokine that is produced and secreted by adipose tissues and is positively associated with BMI and insulin resistance [48]. Secretion of IL-6 is up to 3 times greater in visceral adipose tissues when compared to subcutaneous adipose tissue [36]. It is a primary inducer of the acute phase response in liver and acts in synergy with TNF alpha and IL-1beta in many immunological processes, including T cell activation.

Hormones	Increased/ Decreased	Function
Leptin	Increases	Encoded by the obesity (ob) gene and secreted by white adipocytes. Leptin is a satiety hormone, providing negative feedback to the hypothalamus to control appetite and energy expenditure. Decrease glucagon synthesis and secretion. Decrease hepatic glucose production. Increase Insulin hepatic extraction. Decrease lipogenesis. Increase lipolysis. Decrease sensitivity of pituitary gland to GnRH.
Adiponectin	Decreases	Anti-atherogenic and anti-tumor action. Adiponectin mediates insulin-sensitizing effects through binding to its receptors (AdipoR1 and AdipoR2), leading to activation of adenosine monophosphate dependent kinase, PPAR-α, and most probably other yet-unidentified signaling pathways. Adiponectin suppress the action of inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha). It also tempers natural killer cell function and other immune regulatory molecules.
Resistin	Increases	Pro-inflammatory atherogenic cytokine. It also plays a role in glucose metabolism by decreasing the expression of IRS-2 (Insulin receptor substrate)
Chemerin	Increases	Chemerin, also known as Retinoic Acid Receptor Responder Protein 2, is secreted from mature

		adipocytes. A critical function of chemerin is to regulate adipogenesis, and may also play an important role in macrophage infiltration into adipose tissue. Chemerin levels are increased in obesity and dyslipidemia. It appears to play a significant role in the development of insulin resistance and in glucose- and lipid homeostasis.
Omentin	Decreases	It exerts insulin-sensitizing actions and has anti-inflammatory, anti-atherogenic, anti-cardiovascular disease and anti-diabetic properties. Regarding its effects in the cardiovascular system, omentin causes vasodilatation of blood vessels and mitigates C-reactive protein-induced angiogenesis.
Fibroblast growth factor 21	Increases	Human fibroblast growth factor 21 (FGF21) is a circulating protein that belongs to the human FGF superfamily. It is produced mainly in the liver but also in other tissues, such as white adipose tissue, skeletal muscle and pancreas. FGF21 plays a role in glucose and lipid metabolism regulation. It stimulates glucose uptake in mature adipocytes by increasing the expression of glucose transporter-1
IL-6	Increases	IL-6 is released by adipocytes and other tissues and has been implicated in regulating insulin signaling in peripheral tissues, by promoting insulin-dependent hepatic glycogen synthesis and glucose uptake in adipocytes. It is an inducer of the acute phase response in liver and acts in synergy with TNF-alpha and IL-1-beta

Plasminogen activator inhibitor-1	Increases	Plasminogen activator inhibitor-1, a serine protease inhibitor, is secreted from adipocytes, and the vascular endothelium. It exerts its effects by inhibiting the tissue plasminogen activator

Table 5: The characterization of hormones and cytokines that play a role in metabolic syndrome

1.1.6 The obesity paradox

Obesity and the metabolic syndrome are considered a major risk factor for cardiovascular and other diseases and thus have a negative reputation as a major detriment to health and longevity. Emerging positive outcomes in studies on obesity and the metabolic syndrome are grouped by the biomedical community under the rubric of "obesity paradox". The obesity paradox refers to the inverse relationship between body fat composition, (defined by the BMI), and all-cause mortality[49]. Several studies have observed that obesity, despite it being a major risk factor in the development of cardiovascular and other diseases, can be protective and is associated with greater survival in individuals with certain diseases including heart failure, infections, sepsis, thromboembolism, end stage renal disease (ESRD), and sterile injury. To date, there are over 800 articles on the obesity paradox in pubmed.org.

The obesity paradox is observed in several medical conditions, non-infectious and infectious. It has been observed that overweight and obese patients with coronary artery disease undergoing percutaneous coronary intervention have better outcome compared with their normal-weight counterparts[50]. The obesity paradox has also been observed in other non-infectious conditions such as in patients with end stage renal disease undergoing dialysis, patients who underwent cardiac and non-cardiac surgeries, critically ill patients in intensive care units, and osteoporosis [49-51].

Several studies have shown improved outcomes in obese individuals with infectious diseases including tuberculosis, sepsis, pneumonia, and Chagas disease [49, 52-54]. These studies suggest

that adiposity provides an "immuno-metabolic shield" that benefits individuals in their struggles with infectious diseases.

Non-surgical populations	**Surgical populations**
-Acute coronary syndromes -Percutaneous coronary interventions -Coronary artery disease -Chronic atrial fibrillation -Chronic heart failure -Chronic obstructive pulmonary disease -Chronic kidney disease -Maintenance dialysis -Rheumatoid arthritis -Acquired immunodeficiency -Intensive care unit patients -Hospitalized patients	-Peripheral arterial disease -Abdominal aortic aneurysm -Pancreaticoduodenectomy -Gastrectomy -Arthroplasty -Coronary artery bypass grafting -Left ventricular assist device placement

Table 6: Populations showing the obesity paradox. Adapted from [49].

1.2 Experimental murine models of the metabolic syndrome

1.2.1 Relevance of animal models

In light of the growing obesity epidemic, there is a need to develop animal models of the metabolic syndrome to help determine the pathophysiologic basis for the syndrome. Mouse models are the most commonly used; spontaneously occurring obese mouse strains have been used for several decades and high-fat feeding studies require only months to induce metabolic syndrome. However, no animal model mimics all the features of the metabolic syndrome. Animal models of metabolic syndrome differ from the human disease in many different aspects such as hypertension, which in obese mice is inconsistent. In contrast to normal human lipoprotein profile, the normal mouse lipoprotein profiles have primarily HDL, which is atheroprotective. Obesity and insulin resistance, however, generally coincide in mouse models of metabolic syndrome.

1.2.2 Dietary models of the metabolic syndrome

So called "visceral obesity" is one of the essential components of the metabolic syndrome. Diet provides a source of key nutrients that is essential for growth and development. The modern day 'western diet' is high in calories and rich in fat and carbohydrates such as fructose and sucrose. Combinations of carbohydrate such as fructose and sucrose, and fat have been used in mice to mimic the metabolic syndrome. Dietary model shows high nutrient diet induces adipocyte secretion and beta cell changes in GLUT2 expression that leads to hyperinsulinemia and metabolic syndrome [55].

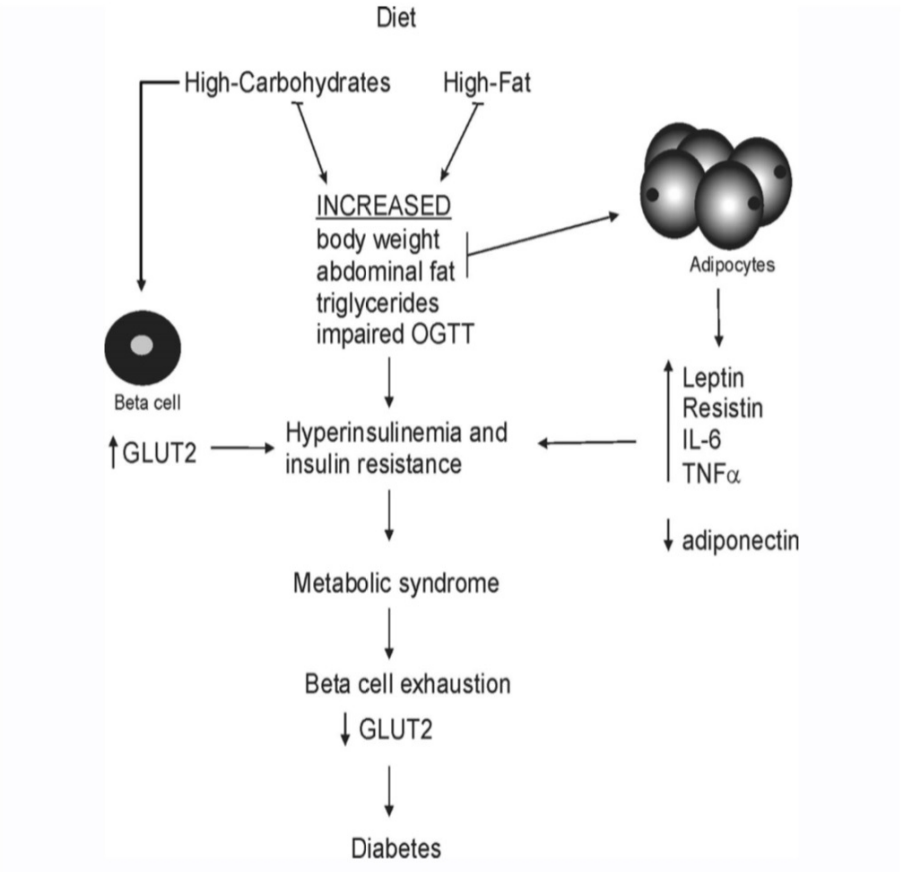

Figure 1: High nutrient diet induced adipocyte secretion and beta cell changes. Adapted from [55].

1.2.2.1 High-Fat Diet (HFD) model

High-fat diet models have been used in rodents for many decades to mimic the features of the metabolic syndrome, i.e. obesity, dyslipidemia and insulin resistance. High-fat diet models mimic most of the symptoms of human metabolic syndrome in mice. However, it is worth noting that the human diet causing metabolic syndrome and its associated complications, is more complex than a simple high-fat diet.

The fraction of fats used ranges between 20% and 60% kcal and are either animal-derived fats, such as lard or beef tallow, or plant based such as olive or coconut oil [56].

High-fat diet mice showed increased body weight compared to standard chow-fed controls [57, 58]. Sutherland et al found that the increase in body weight was significant after two weeks of high-fat feeding [57]. Weight gain is accompanied by increases in adipose tissue mass (that may

trigger inflammation through adipokines) [59-61], increased free fatty acids, cholesterol, and triglycerides.

Long-term feeding with high fat diet induces moderate hyperglycemia, impaired glucose tolerance and insulin resistance [62, 63]; This may be accompanied by fatty liver [64], renal fat accumulation, and albuminuria [65].

The source and amount of fat used varies, depending on the model being used. Lard, coconut oil and olive oil have 42% kcal energy content; these have been shown to increase body weight, plasma triglyceride and free fatty acid concentrations, plasma insulin concentrations and deposition of liver triglycerides. They also caused hepatic steatosis with no signs of inflammation and fibrosis [56]. Beef tallow has 40% kcal energy; it increases plasma insulin and leptin concentrations with increased plasma lipid concentrations and hepatic steatosis [66]. High fat diet may also induce cardiac hypertrophy, cardiac fibrosis, myocardial necrosis, and endothelial dysfunction [56, 67-69].

1.2.2.2 High-Carbohydrate Diets

Metabolic syndrome in mice can be induced by high carbohydrate diets [47, 70-72]. Fructose and sucrose are widely used. Fructose is a commonly used ingredient in western diets [73, 74]. The main sources of fructose in the diet are sucrose, high-fructose corn syrup, fruits and honey. During metabolism, fructose bypasses the rate-limiting step that is catalyzed by phosphofructokinase, thus leading to uncontrolled supply of carbon for lipogenesis in liver [75]. Unlike glucose, high-fructose feeding to mice induces the development of features of the metabolic syndrome: high blood pressure, insulin resistance, impaired glucose tolerance, dyslipidemia and hepatic steatosis with microvesicular and macrovesicular changes [76, 77]. Fructose feeding induces ventricular dilatation, ventricular hypertrophy, and decreased ventricular contractile function [78].

Unlike glucose, fructose does not cause insulin secretion from pancreatic β-cells; this may be due to the absence of GLUT5 fructose transporter on pancreatic β-cells [76]. Fructose also lacks the ability to stimulate the secretion of leptin [76] but has the ability to activate *de novo* lipogenesis in the liver [79].

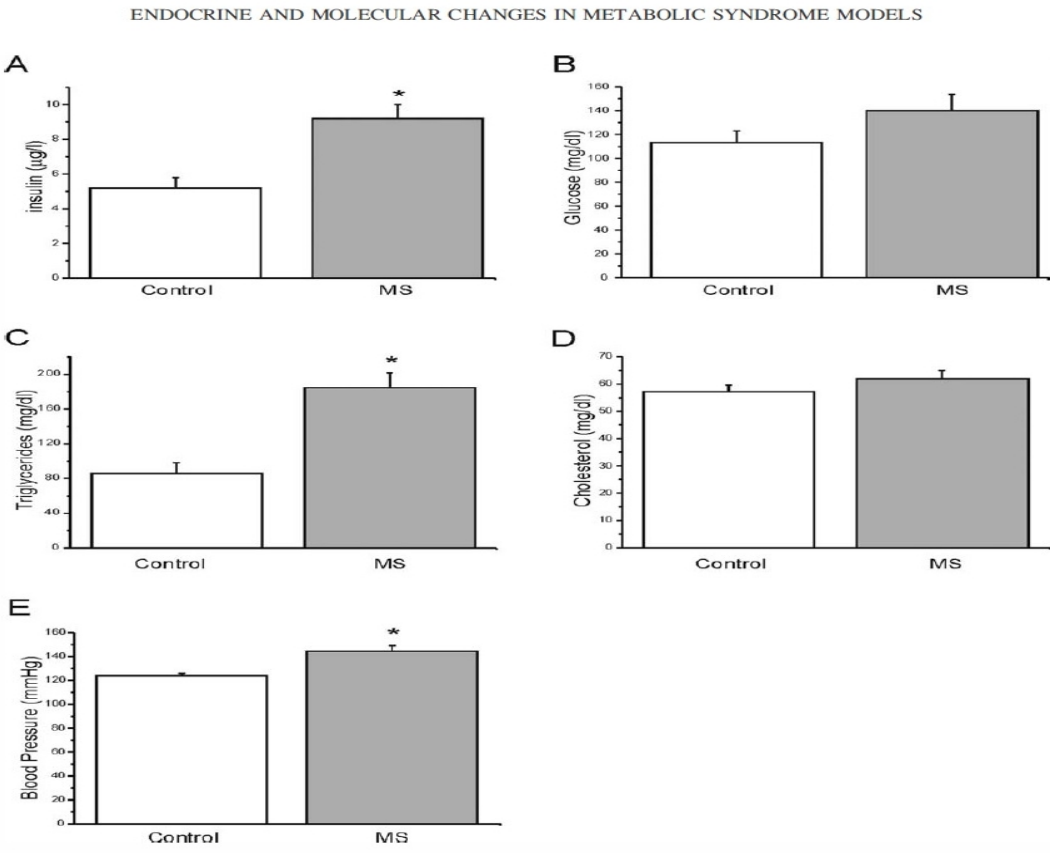

Figure 2: Changes in plasma glucose, insulin, cholesterol and triglycerides after 8 weeks sucrose treatment. Adapted from [55]. Metabolic status after 8 weeks of sucrose treatment; white (control) and gray (metabolic syndrome rats). Plasma insulin (A), glucose (B), cholesterol (C), and triglycerides levels (D) and systolic blood pressure (E). Data are expressed as mean +/- SE, n = 30 rats. *P =0.001 with respect to control group.

1.2.2.3 Combined High-Fat and High-Carbohydrate Diet

A diet high in carbohydrates and fat, sometimes called a “cafeteria diet” is similar to human diets and is probably best suited to study the human metabolic syndrome. When used in mice, it induces most of the features present in human metabolic syndrome. Different combinations and amounts of carbohydrates [such as fructose and sucrose] and fats have been used in different studies [80-83]. Mice on high-sucrose (10-30% kcal), and high-fat (20-40% kcal) diet develop features of the metabolic syndrome. They have increased body weight, abdominal fat deposition, hyperinsulinemia, hyperglycemia, hyperleptinemia, hepatic steatosis and increased hepatic lipogenic enzymes [84-86].

1.2.3 Genetic models

Genetic models of the metabolic syndrome can be useful in evaluating specific molecular mechanisms in development of obesity in mice, however the metabolic syndrome in humans is not a monogenic disorder.

1.2.3.1 Leptin-deficient mouse model

$Lep^{ob/ob}$ (C57BL/6J-ob/ob) model was one of the first genetic models used for the study of diabetes [87]. These mice arose from a spontaneous nonsense mutation of the leptin gene at codon 105 that results in a complete loss of leptin. This autosomal recessive monogenic mutation in the leptin gene on chromosome 6 leads to hyperphagia, reduced energy expenditure, extreme obesity, hyperinsulinemia and hyperglycemia after 4 weeks of age [88]. Leptin deficient mice have increased body weight; up to 4 times that of their lean littermates [88, 89] and their growth curves do not plateau even at 12 months of age [90].

$Lep^{ob/ob}$ mice have elevated plasma cholesterol levels; with elevation of HDL and a unique lipoprotein referred to as LDL/HDL1 rather than VLDL or LDL. They are protected from diet-induced atherosclerosis [91]. These mice can develop cardiac complications such as left ventricular hypertrophy with decreased cardiac function at 24 weeks of age [92] and cardiac fibrosis after 20 weeks of age [93]. However, unlike humans with metabolic syndrome, these mice do not develop high blood pressure [89].

1.2.3.2 Leptin receptor-deficient mouse model

$LepR^{db/db}$ (C57BL/KsJ-db/db) model is caused by an autosomal recessive mutation in the leptin receptor gene on chromosome 4 [94]. $LepR^{db/db}$ mice have a metabolic profile that is very similar to that of $Lep^{ob/ob}$ mice; both are obese, hyperinsulinemic, hyperglycemic and hyperlipidemic. However, the main difference is in the levels of circulating leptins. $LepR^{db/db}$ mice have elevated circulating leptin concentrations that are proportional to their degree of adiposity. On the other hand, $Lep^{ob/ob}$ mice lack circulating leptin. $LepR^{db/db}$ mice have higher body weights, increased plasma concentrations of triglycerides, total cholesterol and nonesterified fatty acids than their lean littermates after 6-13 weeks of age [95].

These mice also have vascular endothelial dysfunction at 12 weeks of age [95] and hepatic steatosis after 20 weeks of age [96].

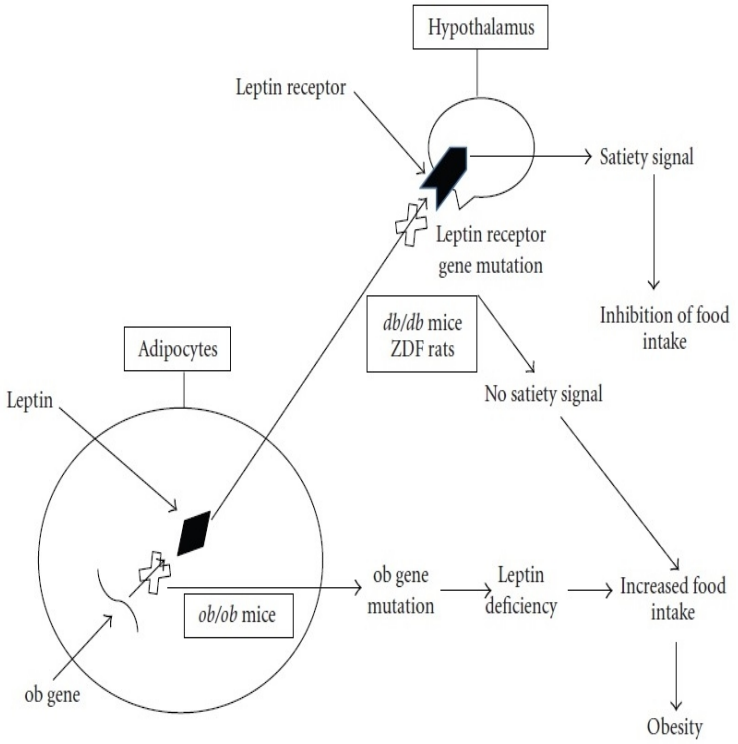

Figure 3: Mechanism of the actions of leptin including the effects of leptin deficiency or leptin receptor deficiency. Adapted from [97]

1.2.3.3 Zucker rats (ZDF)

Diabetic Zucker fatty rats (ZDF) (*fa/fa*) harbor a missense mutation (adenine to cytosine at nucleotide 806) in the gene coding the leptin receptor. This mutation results in a Gln269Pro substitution in the extracellular domain (glutamine to proline) [98] that is associated with reduced binding of leptin to its surface receptor and the development of leptin resistance, which in brain leads to hyperphagia and obesity.

Features of the metabolic syndrome are usually manifested by ZDF rats after 12 weeks of age. Hyperglycemia occurs after 13–15 weeks of age [99]; hyperinsulinemia and hypertriglyceridemia appear after 12–14 weeks of age along with cardiac dysfunction [100]. Serum cholesterol concentrations are approximately 2.5 times higher with increased hepatic triglyceride deposition

at 20 weeks of age [101]. Other features manifested by ZDF rats include increased serum markers of inflammation such as TNF-α and IL-1β after 26 weeks of age [102], albuminuria at age 31 weeks and glomerular fibrosis after 47 weeks [103].

1.2.3.4 Melanocortin 4 receptor (MC4-R) null mouse model

The central melanocortin systém mediates some of the actions of leptin and plays a crucial role in the central regulation of energy homeostasis. Melanocortin 4 receptor (MC4-R) is expressed in a number of nuclei in the rodent brain and these are associated with autonomic and neuroendocrine pathways [104]. *MC4-R* gene mutation is a common monogenic cause of obesity including behavioral obesity syndrome characterized by hyperphagia, hyperglycemia, hyperinsulinemia, and hypometabolism [105-108]. Despite their profound obesity in adulthood, the MC4-R-deficient animals do not have hypertension [109].

1.2.4 Pharmacological manipulation

1.2.4.1 Streptozotocin model

Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3- nitrosoureido)-D-glucopyranose), synthesized by Streptomycetes achromogenes, is a structural analogue of glucose that enters pancreatic beta cells via the GLUT2 transporter [110] and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus. Classically, STZ caused selective necrosis of pancreatic β cells leading to the destruction of β cells, hypoinsulinemia and hyperglycemia. Now it is recognized that depending on the dose, streptozotocin (STZ) can induce diabetes in 2 ways. High doses, single injection of STZ targets β cells by its alkylating property corresponding to that of cytotoxic nitrosourea compounds while at low doses, given in multiple exposures, STZ causes immune and inflammatory reactions [111]. Depending on the animal strain, dose, route of drug administration, and the period in which STZ is administered in rats, severe diabetes with blood glucose over 200 mg/dL [112-118] or mild diabetes with blood glucose between 120 and 200 mg/dL are generated [119-122]. In one study by Sinzato et al, type 2 diabetes was induced with a low-dose (70mg/kg) streptozotocin given on day 5 of life, thereby producing moderate hyperglycemia in

adult rats with decreased HDL-cholesterol concentrations; no other lipid abnormalities or oxidative enzyme changes were noted [121]. Another study, by Sharma A. K. et al, found that after a dose of 90mg/kg STZ given on day 2 of life, insulin resistance, an approximate doubling of serum C-reactive peptide and TNF-α were produced in rats at 14 weeks of age [123].

As shown above, these changes following injection of streptozotocin in the neonatal period are insufficient to define the signs and features of the metabolic syndrome, as seen in humans; in this model, there was no report of obesity and hypertension. STZ induced mice do not show the diverse characteristics of the metabolic syndrome and therefore they are not a suitable model for this syndrome in humans; in contrast to human metabolic syndrome, STZ-induced diabetic rats are hypoinsulinemic [124], do not gain weight and are usually hypotensive [97].

1.3 Chagas disease

1.3.1 Definition, Etiology and Epidemiology of Chagas disease

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness named after Carlos Ribeiro Justiniano Chagas, a Brazilian doctor, who described the disease in 1909.

It is caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi)*. It is found mainly in endemic areas of Latin American, where it is mostly vector-borne transmitted to humans by contact with faeces of triatomine bugs, known as 'kissing bugs' or reduviid bugs. In the past decade, Chagas disease has been increasingly detected in the United States of America, Canada and Europe. This has been attributed to population mobility between Latin America and the rest of the world [125]. About 6 million to 7 million people are estimated to be infected worldwide, mostly in Latin America where Chagas disease is endemic. The highest prevalence rates are found in Bolivia, followed by Argentina. Brazil and Mexico both have prevalence rates of 1%; however these countries have about one third of all *T. cruzi* infections because of their large populations.

Recently, it was estimated that there are about three hundred thousand (300,000) people with chronic *T. cruzi* infection in the United States of America, mostly immigrants from endemic countries. Based on recent census data, the overall prevalence in the at-risk popoulation was estimated to be 1.3%. Other modes of transmissions such as blood transfussion, organ transplantation, congenital transmission and laboratory accidents involving transmission of *T. cruzi* have been reported in the United States of America. To date, five cases of transfusion-related transmission of *T. cruzi* in the United States (two in Canada) and three cases of organ transplantation–related transmission have been reported.

The cost of treatment for Chagas disease remains substantial. A 2013 study noted that the global cost of Chagas disease is estimated to be at $7.19 billion per year and $188.80 billion per lifetime with more than 10% of these costs emanating from the USA and Canada, where Chagas disease has not been traditionally endemic. The authors also noted that substantial proportion of the cost emerges from lost productivity from cardiovascular disease-induced early mortality [126].

1.3.2 Biology of *Trypanosoma cruzi*

At least three morphogenetic forms of *T. cruzi* have been recognized in its life cycle: trypomastigotes, amastigotes and epimastiotes. Trypomastigotes are the extracellular non-dividing forms found in the mammalian hosts; the metacyclic trypomastigotes is the form of *T cruzi* that is capable of infecting humans. Amastigotes are also found in the mammalian hosts and these are the intracellular dividing form of *T. cruzi*. The epimastigote form divides in the midgut of reduviid bugs.

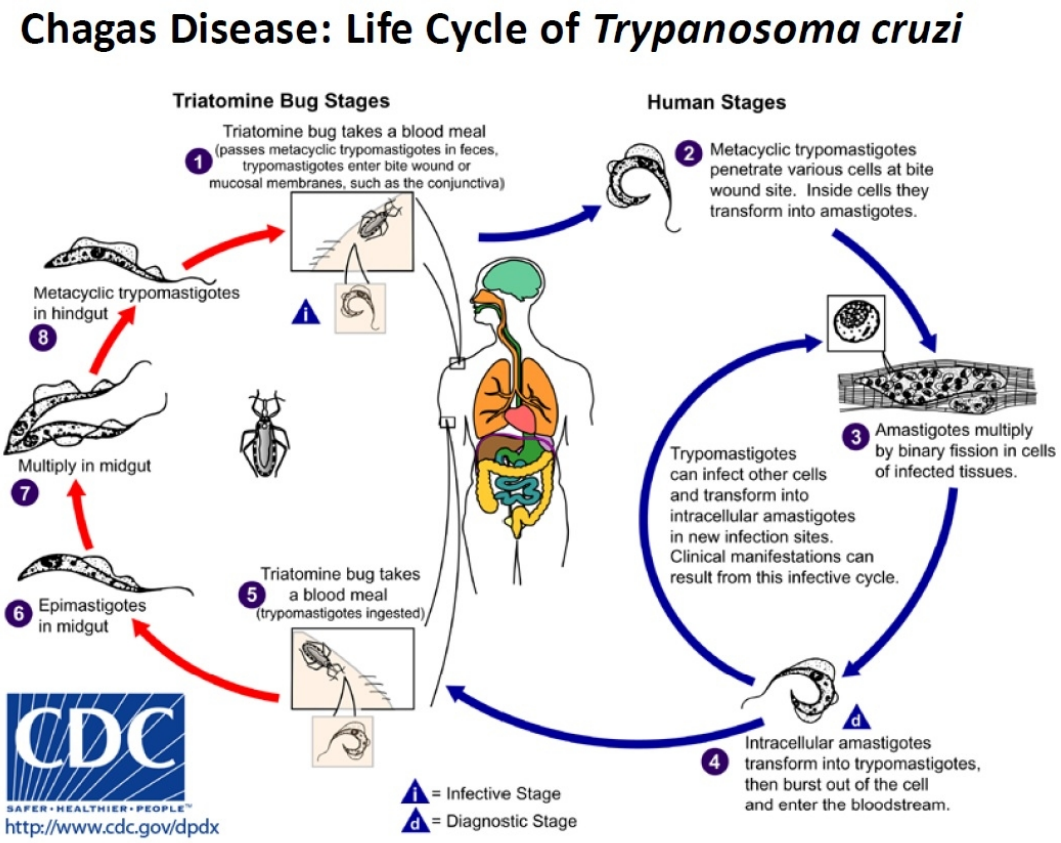

Figure 4: Lifecycle of Trypanosoma cruzi; Adapted from CDC.gov

Differentiation of epimastigotes to metacyclic trypomastigotes occurs in the insect's hindgut. During a blood meal, trypomastigotes are deposited via defecation; this can result in transmission when it comes in contact with vulnerable surfaces like the bug bite site, conjunctivae, and nasal

mucosa. Trypomastigotes are resistant to complement-mediated lysis; the mechanism of resistance has not been fully elucidated. Host cell entry by *T. cruzi* is a complex and active process involving a variety of factors such as fibronectin, LDL receptor, phospholipase A2, trans sialidase, and 60 kDa penetrin. Once in the cell, trypomastigotes transform into amastigotes, capable of intracellular division. Trypomastigotes and amastiotes synthesize TC-TOX, an acid-stable hemolytic protein capable of lysing the membrane of the parasitophorous vacuole [127]. The parasite is then liberated where it multiplies freely by binary fission in the cytosol. Host cells rupture and release amastigotes and trypomastigotes into the blood stream. These can go on to infect other cells and organ systems.

1.3.3 Other modes of transmission

Other modes of transmission such as transmission via transfusion of whole blood or derivative products obtained from chronically ill donor infected with *T. cruzi* [128] and through transplantation of organs with presumably containing intracellular amastigotes have been documented [129]. Several studies have documented cases of congenital transmission in endemic countries [130-132]; transmission of parasite is transplacental and the likelihood of transmission increases with the level of circulating parasites in infected mothers [133, 134]. Two other mechanisms of transmission worth mentioning are oral transmission resulting from the ingestion of food and drink that is infected with parasites and transmission through laboratory accidents.

1.3.4 Clinical Manifestation of Chagas disease

The acute phase of Chagas disease begins 1-2 weeks after vector-borne *T. cruzi* exposure and lasts 4-8 weeks. It is characterized by trypomastigote parasitemia and detectable by microscopy. Most patients with acute chagas have only mild and non-specific symptoms or are asymptomatic. In some patients there is local inflammation and swelling at the site of inoculation, known as a chagoma. Inoculation via the conjunctiva leads to unilateral periorbital edema known as Romana's sign. Other manifestations may include fever, lymphadenopathy, hepatosplenomegaly, nausea, vomiting, diarrhea and rash. Severe acute disease occurs in less than 1% of patients and

carries a substantial risk of mortality. Manifestations of severe acute disease may include acute myocarditis with foci of myocytolytic necrosis, pericardial effusion and meningoencephalitis [135, 136]. The presence of arrhythmias, heart blocks, and heart failure in the acute phase of chagas disease is an indicator of poor prognosis.

Dilated cardiomyopathy is an important manifestation of chronic Chagas disease that occurs several years after the onset of acute infection. Other clinical manifestations of chronic Chagas include thromboembolic events, myocardial infarction, apical aneurysm, complications of central and peripheral nervous system, mega esophagus, and megacolon.

1.3.5 Immunology of Chagas disease

Evidence has shown the importance of both humoral and cell-mediated components of the immune system in Chagas disease. In two separate experiments, *Tarleton et al.* [137] demonstrated high parasitemia and early death in mice depleted of CD8 cells and in beta-2 microglobulin-deficient mice. The role of cytokines, such as interleukins 1, 2, 5, 6, 10, tumor necrosis factor alpha (TNF-alpha) and gamma interferon (INF-y) are well documented in the pathogenesis of *T. cruzi* infection.

In acute Chagas disease, there is a robust inflammatory response in infected tissues. This inflammation is necessary to clear the infection [125]. It is believed that innate immune cells, such as natural killer (NK) cells, neutrophils, and macrophages play an important role in this process. NK cells are important sources of interferon gamma and tumor necrosis factor alpha, which are critical for the activation of macrophages to eliminate the parasite [137].

1.3.6 Chagas disease and adipose tissue

Adipocytes, once considered a static storage compartment for triglycerides, are also considered to be active endocrine cells playing a critical role in various metabolic and immune responses [125]. Adipocytes are involved in systemic lipid homeostasis and release adipocyte-specific hormonal

factors (such as adiponectin, leptin, resistin, visfatin) and mediators of inflammation collectively known as adipokines.

Adipose tissues, containing adipocytes among other cells, is a target for many infections including *T. Cruzi* infection. *T. cruzi* has high affinity for low density lipoprotein and high density lipoprotein [138]. Parasitism of adipose tissues by *T. cruzi* may create a state of low-grade chronic inflammation. *Nagajyothi et al* demonstrated that *T. cruzi* utilizes LDL receptor to invade host cells [139].

Experimental studies have demonstrated a significant reduction in plasma adiponectin in mice infected with *T. cruzi* [139, 140]. Pro-inflammatory markers such as TNF –alpha, IL-1 beta, INF-y and toll-like receptors (TLRs) were markedly elevated in adipose tissues from mice infected with *T. cruzi* [140].

1.3.7 Treatment of Chagas disease

Nifurtimox and benznidazole are the only FDA approved drugs with proven efficacy against Chagas disease [136]. When used in the acute setting, these drugs reduce severity of symptoms and shortens clinical course of the disease. They have no role in the therapy of *T. cruzi* chronic infections. The disadvantages to these drugs are the side effects and the long duration of treatment.

Nifurtimox (*Lampi, Bayer 2502*) is a nitrofuran that interferes with *T. cruzi* carbohydrate metabolism by inhibiting pyruvic acid synthesis. The drug is administered orally in three to four divided doses for ninety days. Dose regimen varies by age; for children younger than 10 years, 10-15mg/kg/day; for children 11-16 years, 12.5-15mg/kg/day. Above 17 years of age, the dose is 8-10 mg/kg/day. Side effects include tremors, irritability, insomnia, psychosis, neuropathy and hemolytic anemia [125].

Benznidazole (*Rochagon, Roche 7-1051*), a nitroimidazole derivative, is considered a more potent trypanocidal agent than nifurtimox. It is known to induce the formation of free radicals and electrophilic metabolites leading to potentially lethal double-stranded DNA breaks in *T. cruzi* DNA [141]. The drug is administered orally in two divided doses daily for sixty days. For

individuals younger than 12 years, the dose is 10 mg/kg/day; for those 12 years or older, the dose is 5-7mg/kg/day. Side effects include hypersensitivity reactions, such as exfoliative dermatitis, bone marrow suppression and peripheral neuropathy.

2.0 CHAPTER 2 -- EXPERIMENTAL PART

2.1 Aims of the study

Obesity and the metabolic syndrome have a negative reputation as a major detriment to health and longevity. The metabolic syndrome, as presently construed, produces immune activation and metabolic alterations that promote complications of obesity and diseases of later life, such as myocardial infarction, stroke, diabetes, Alzheimer's disease and cancer. Using an evolutionary approach, we hypothesized that for millions of years, the channelling of host resources into immune defenses starting early in life, ameliorating the effects of infectious diseases, such as Chagas disease. Throughout human history, the so-called syndrome has benefited individuals by enhancing host defenses against infectious diseases. In addition to support during acute self-limited infections (e.g. community acquired pneumonia), we raise the possibility that the major benefits of the processes associated with the metabolic syndrome accrue in controlling the widespread potentially ravaging infections that the body cannot self-cure, including American trypanosomiasis, the subject of the thesis.

The specific aims are:

1. To assess the effect of obesity and the metabolic syndrome (induced by a high fat diet) on the course of *T. cruzi* infection.

2. To investigate the relationship between high fat feeding and parasitemia and parasite burden.

3. To assess the effect of metformin treatment [which is known to ameliorate the metabolic syndrome] on the course of *T. cruzi* infection

2.2 Materials and methods

2.2.1 Study design

As stated earlier, there are several animal models of the metabolic syndrome. However, no animal model can be expected to mimic all the features of the metabolic syndrome as seen in humans. Other common models like the streptozotocin model, and the genetic models including ob/ob and db/db mouse model, can be useful in evaluating specific molecular mechanisms in development of obesity in mice and the metabolic syndrome in humans is not a monogenic disorder.

We opted for a dietary model as it mimics, albeit imperfectly, the features of the metabolic syndrome in humans: obesity, dyslipidemia and insulin resistance. CD-1 inbred mice were fed a high fat diet to induce obesity and the metabolic syndrome. CD-1 inbred mice are robust, inexpensive, and come without any genetic manipulation. CD-1 mice were subsequently infected with *Trypanosoma cruzi*, a protozoa causing Chagas disease. *Trypanosoma cruzi* infection is widely studied by a world-renowned research group at the Albert Einstein College of Medicine, in New York. Our study was conducted in close collaboration with the group at Albert Einstein College of Medicine and the Feinstein Institute for Medical Research.

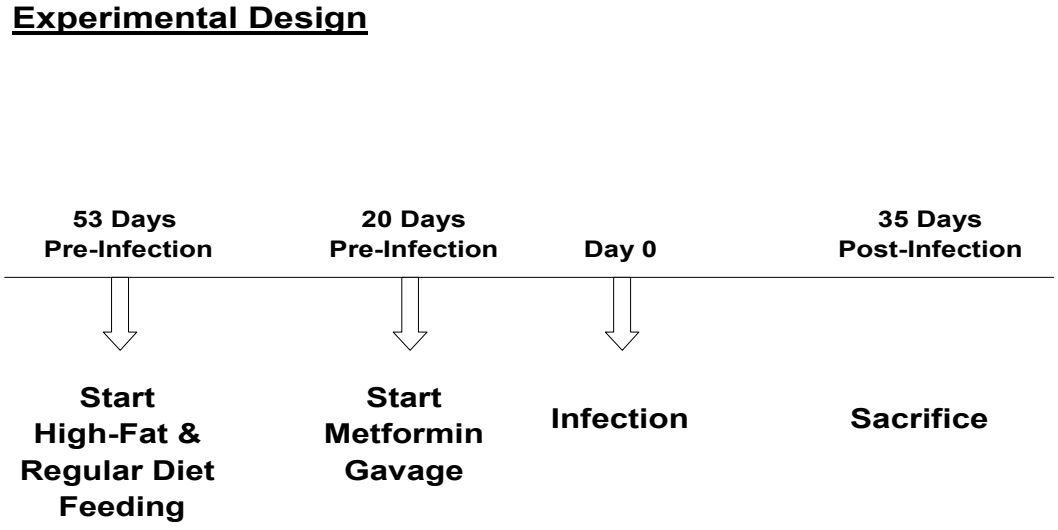

Table 7: Basic experimental design

2.2.2 Ethics statement

All animal experiments followed standard care principles and procedures for the use of experimental animals (Guide for the Care and Use of Laboratory Animals, National Research Council) as described by the National Institutes of Health and the Office of Laboratory Animal Welfare. All experiments were performed on an animal use protocol (20130101) approved by the Institutional Animal Care and Use Committees of the Albert Einstein College of Medicine.

2.2.3 Experimental animals

Five-week-old CD-1-inbred mice (n=220), weighing on average 28 g (Charles River Laboratories, Wilmington, MA, United States), were maintained on a 12-h light–dark cycle in a temperature and humidity controlled room. Animals were housed in groups of five per cage with free access to water and food. Body weights were recorded every 2 weeks, and cages were changed three times per week.

Mice were randomly divided into a high fat diet (HFD) group (n=100) or a regular diet (RD) group (n=120). Mice fed a regular diet included 20 uninfected mice, 20 uninfected mice treated with metformin, 40 infected mice and 40 infected mice treated with metformin. Mice fed a high fat diet included 20 uninfected mice, 20 uninfected mice treated with metformin, 30 infected mice and 30 infected mice treated with metformin. The high fat diet consisted of (by kilocalories) 60% fat with added cholesterol, 20% protein and 20% carbohydrate; the regular diet consisted of 10% fat, 20% protein and 70% carbohydrate (Research Diets Inc., New Brunswick, NJ). Diets were matched for sucrose. All mice were fed the assigned diets for the duration of the experiment.

	Regular Diet	High Fat Diet
Uninfected	**20**	**20**
Uninfected + Metformin	**20**	**20**
Infected	**40**	**30**
Infected + Metformin	**40**	**30**

Table 8: Experimental groups based on diet. Mice were grouped in based on whether they were fed a regular diet vs a high fat diet, infected vs uninfected and whether they were treated with daily metformin

2.2.4 Metformin gavage

At 10 weeks of age (20 days before infection), a subset of mice were started on metformin (Research Grade, Sigma-Aldrich, St. Louis, MO) as indicated earlier. The metformin was administered daily by gavage (after weighing and blood draws) by using a bulb tipped gastric gavage needle attached to a syringe. During this procedure, mice were maintained in an upright (vertical) position as the gavage needle passes along the side of the mouth into the esophagus and toward the stomach. This continued through day 71 post-infection. No placebo and no sham gavages were utilized.

2.2.5 Infection of mice

After weighings, metformin administration and blood drawings, on the day of infection (designated as postinfection day 0), subsets of mice in both high fat diet and regular diet groups were infected intraperitoneally with 5×10^4 trypomastigotes Brazil strain of *T. cruzi*, maintained by passage in C3H/Hej mice (Jackson Laboratories, Bar Harbor, ME), as described by Tanowitz et al. [142]. Trypomastigotes of Brazil strains were harvested from the supernatants of infected fibroblast. Experimental mice were then categorized into eight subgroups.

2.2.6 Parasitemia

Parasitemia was determined at 10, 15, 20, 24, 27, 30, 33, 35 and 38 days post infection (dpi). Twenty microliters of tail blood obtained by venesection of the tail was diluted and incubated in 18 µl of 0.89% ammonium chloride in phosphate-buffered saline (PBS), and the resulting solution was transferred to a Neubauer haemocytometer, and parasites were counted by microscopy. Data are expressed as the mean of parasites per millilitre of peripheral blood [143, 144].

2.2.7 Fasting glucose measurements

Mice were fasted for 4 h in the morning, after which blood was drawn from ten animals per group on the day of infection, post infection day 15 and 30 (16 animals per group at day minus 20, before the first dose of metformin) for the measurement of blood glucose. Glucose was measured on whole blood with AlphaTRAK 2 blood glucose test strips and monitoring system (Abbott) [145]. Results are expressed as a mean (+/- standard error of the mean).

2.2.8 Oral glucose tolerance test

Oral glucose tolerance test was carried out on day 28 post-infection after 6-h fasting. Five mice per group were used for each determination. The mice were weighed, and their baseline glucose was measured using an AlphaTRAK® blood glucose monitoring system. Mice were gavaged orally with a glucose solution of 2-mg glucose/g body weight, and blood glucose was measured after 15, 30 and 60 min [145]. Data are represented as mean glucose plotted against time at 0, 15, 30 and 60 min.

2.2.9 Body composition analysis

On day 70 post-infection, we used magnetic resonance spectroscopy, employing the Echo Medical System EchoMRI-100, to determine body composition, expressed as fat mass and lean mass, in live mice unrestrained without anaesthesia.

Prior to scanning, EchoMRI was calibrated, and the weights of individual mice were determined. In this experiment, two mice were used for each infected group, and three mice per uninfected (control) group. Mice closest to the median weight of that group were selected for screening.

2.2.10 Sacrifice of mice

On day 35 post-infection, mice (n=54) were sacrificed by cervical dislocation. Liver, heart and white adipose tissues were harvested; liver and heart were weighed. All harvested tissues were stored at -80 °C and/or fixed in formalin for future studies.

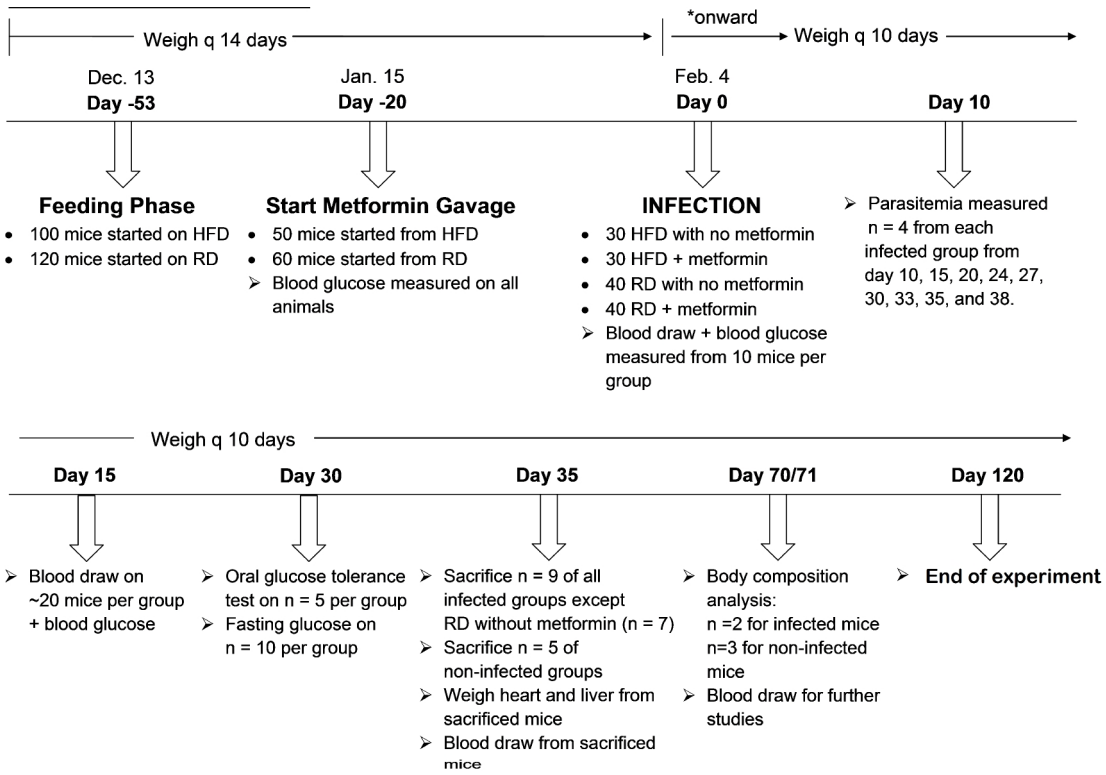

Table 9: Experimental timeline

2.2.11 Immunoblot analysis of heart tissue

Heart tissues harvested from mice sacrificed 35 days post-infection were sliced, homogenized in an ice-cold lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich) and centrifuged

at 10 000 rpm at 4 °C for 15 min. The protein was estimated in the supernatant using a Bradford assay according to the standard protocol and separated by using sodium dodecyl sulphate polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose membranes (Whatman-GE Healthcare Life Sciences). Membranes were blocked with 5% non-fat milk and 3% bovine serum albumin in phosphate-buffered saline with 0.1% Tween for 60 min and incubated with the appropriate primary antibodies at 4 °C overnight. After appropriate washing steps, membranes were incubated with horseradish peroxidise conjugated secondary antibodies for 60–90 min. An enhanced chemiluminescence detection system (Thermo Scientific) was used for developing the immunoblots.

Photographic films were used. Scanned immunoblot images were used for densitometric quantification using IMAGEJ software [138]. Band intensity was normalized to guanine nucleotide dissociation inhibitor band intensity in respective columns. Results are expressed as mean plus or minus the standard error of the mean.

2.2.11.1 Antibodies

Primary antibodies used for the immunoblotting experiments were anti-AKT dilution – 1 : 5000 (Cell Signaling Technology, Danvers, MA), anti-phospho-AKT (Ser473) dilution – 1 :5000 (Cell Signaling Technology), anti-guanosine diphosphate dissociation inhibitor (GDI) dilution – 1 : 3000 (Invitrogen), anti-interferon (IFN)-gamma dilution – 1 : 2000 (MAB485, R&D Systems) and anti-tumour necrosis factor (TNF)-alpha dilution – 1 : 2000 (MAB410, R&D Systems). Secondary antibodies were anti-rabbit Immunoglobulin G (IgG), Horse Radish Peroxidase (HRP)-linked antibody, anti-mouse IgG and HRP-linked antibody (Cell Signaling Technology).

2.2.12 Quantitative Determination of Parasite Load in Tissue by PCR

Heart tissue, liver tissue, and white adipose tissue (WAT) were harvested and stored at -80 °C on dpi 35. DNA was isolated from these tissues and purified using Trizol Reagent (Life Technologies) according to standard protocol.

A standard curve in the range of 50 picogram (pg) to 50 nanogram (ng) for the quantification of *T. cruzi* DNA by real time PCR was developed using the T. cruzi 195-bp repeat DNA-specific primers TCZ-F (5'-GCTCTTGCCCACAAGGGTGC-3') and TCZ-R (5'-CCAAGCAGCGGATAGTTCAGG-3') and genomic DNA purified from T. cruzi epimastigotes [140]. Quantitative PCR was performed using samples containing 50ng of genomic DNA, 0.5 uM TCZ-F and TCZ-R primers (that amplify a 182-bp product), LightCycler FastStart Master SYBER Green 1 mix containing MgCl2 (Roche Applied Science), and PCR grade water (Roche Applied Science) to a final total volume of 20 ul.

To normalize the amount of tissue analyzed in each PCR reaction (i.e. correcting intra-sample variations of the initial sample amount, DNA recovery and sample loading), a parallel reaction was done for each sample using 50ng of genomic DNA and murinespecific 18S primers.

These reaction mixes were loaded into Roche LightCycler Capillaries, capped, centrifuged for 5 minutes at 1800 rpm, and placed in the iQ5 LightCycler (Bio-Rad). In the denaturation phase [140], the capillary was heated to 95 °C at a 20 °C/s ramp and held for 10 min. During the 45 cycles of the amplification phase, there were three steps: 95 °C for 2 s, 57 °C for 5 s, and 72 °C for 10 s, all at a 20 °C/s ramp. During extension, the fluorescence intensity was acquired as single colour detection (SYBER Green 1). The third phase was a hold at 95 °C at a 20 °C/s ramp for 0 s, 65 °C at a 20 °C/s ramp for 15 s, and finally 95 °C at a 0.1 °C/s ramp for 0 s. During the melting phase, the acquisition setting was set at "continuous." The final phase was the cooling phase, which lasted 30 s at 40 °C at a 20 °C/s ramp.

Data were acquired and analyzed with LightCycler version 3.0 software. Each run contained 2 negative controls (no DNA added to the reaction mix), and each DNA sample was quantified in duplicate. Duplicate values for each DNA sample were averaged and the number of parasites per cell was calculated by dividing the number of parasites (copies of *T. cruzi* DNA obtained by real time PCR) by number of cells (copies of host DNA obtained by real time PCR).

2.2.13 Cytokine, Insulin and Leptin measurements in serum

On post infection day 70, blood was collected from the periorbital plexus in unanaesthetized mice after four hours fasting with a 100 µL microhaematocrit tube inserted in the lateral canthus of the orbit. With gentle pressure and side to side rotation, blood was collected in microcentrifuge tubes and and stored on crushed ice for no longer than 20-30 minutes before being centrifuged at 8000 rpm at 4°C for 10 minutes. Plasma samples were aliquoted and stored at -80°C for further analyses. Cytokines (IL 6, TNF alfa and Interferon gamma) were determined by multiplex analysis using Luminex xPONENT for MagPix version 4.2(Luminex, Tx) to quantify data and Millipore analyst version 3.5.5(Millipore Corporation, MA) to analyze data.

Insulin and Leptin assays were performed in the Hormone Assay Core of the Einstein Diabetes Research and Training Center using Mouse Insulin ELISA (ALPCO - catalog # 80-INSMS-E01) and Mouse Leptin ELISA (EMD Millipore -catalog # EZML-82K) respectively. Samples were ran in duplicates.

2.2.14 Histopathologic analysis of heart tissue

On day 35 post infection, mouse organs were harvested and fixed with phosphate-buffered formalin. Liver and heart samples from each experimental group were processed for paraffin embedding and optimum cooling temperature (OCT) embedding. Five micrometer-thick sections of paraffin embedded tissues were cut with a microtome, then stained with H&E to examine the extent of inflammation, fibrosis, and parasitemia [144].

Scoring was performed for the atrium, right and left ventricles and base in the following manner: In areas where no inflammation or fibrosis were observed, the score of 0 was applied; in the event of minimal inflammation or fibrosis, a score of 1 was applied; in like manner, a score of 2, 3 and 4 was given to a slide showing mild, moderate and extensive inflammation and fibrosis, respectively. For each heart examined, composite scores for inflammation and fibrosis were then derived. Although four distinct areas of the heart were examined, regional differences were not statistically significant. The maximal score for inflammation or fibrosis was 16. These scores were ranked, and statistical significance among groups was determined with use of

nonparametric Wilcoxon analysis. Mean scores for each group were calculated as well.

2.3 Cell culture experiment to determine the effect of metformin

2.3.1 Mammalian cell culture

Human foreskin fibroblasts (ATCC CRL 1475) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1% penicillin– streptomycin at 37 °C in 5% CO2. The fibroblasts were plated in 24-well plates (Falcon™ Tissue Culture Plates) and Nunc™ four-well dishes (Sigma-Aldrich) at a density of 2.5×10^5 per millilitre 24 h prior to the start of the experiment to achieve 80–90% confluence.

Eight hours prior to infection, half of the wells in each plate were pre-treated/incubated with selected concentrations of metformin (0, 5, 10, and 50 μg/mL) (Sigma-Aldrich).

2.3.2 Infection of Human Foreskin Fibroblasts

Tissue culture-derived trypomastigotes were generated by weekly passage in confluent monolayers of fibroblast cells in DMEM containing 10% foetal bovine serum. Trypomastigotes, harvested from cell culture supernatants, were washed two times and re-suspended in DMEM. Confluent monolayers of fibroblasts were infected with parasite at a density of 5×10^5 and incubated with 0–50 μg/mL of metformin at 37 °C in 5% CO_2. Infection was allowed to proceed for 24h. The number of live parasites/trypomastigotes and infected host cells in each well was determined with a hemocytometer at 24h post-infection. Plates were washed two times with 10% phosphate-buffered saline at 37 °C, fixed with 4% paraformaldehyde and stained with May–Grünwald–Giemsa according to a standard protocol [146].

Next, two concentrations of metformin (0, and 10 μg/mL) were used to pre-treat wells in 24-well plates (Falcon™ Tissue Culture Plates) plated with fibroblast, as described above. Confluent monolayers of fibroblasts were infected with parasite at a density of 5×10^5 and incubated with 0–10 μg/mL of metformin at 37 °C in 5% CO_2. Infection was allowed to proceed for 24, 48 and 72

hours. The number of live parasites/trypomastigotes and infected host cells in each well was determined with a hemocytometer at 24, 48 and 72 h post-infection. Plates were washed, fixed and stained with May–Grünwald–Giemsa as described above.

2.4 Statistical analysis

The results are shown as means, +/- the standard error of the mean. Statistical analysis was performed using Student's t-test or analysis of variance (ANOVA and Statistical Analysis Software) as appropriate. Because of the uneven distribution of the data, the non-parametric version of t-test (the Mann– Whitney U-test) and non-parametric version of analysis of variance (the Kruskal–Wallis test) were used where appropriate. Results were considered statistically significant with a p-value < 0.05.

For histopathology analyses, statistical significance among groups was determined with use of nonparametric Wilcoxon analysis. Mean scores for each group were calculated as well.

2.5 Results

2.5.1 Mortality of CD-1 mice during acute *T. cruzi* infection

The impact of diet on the course of acute *T. cruzi* infection was examined on five-week-old CD-1-inbred mice (n = 220), who were placed either on a high fat or regular diet. All mice were fed the assigned diets for the duration of the experiment.

As expected, when infected with the Brazil strain of *T. cruzi*, CD-1 mice maintained on a regular diet (RD) displayed a high mortality rate (Figures 5A and 6). The first death in this group occurred on day 26 post-infection (Figures 5A and 6). Deaths were recorded until day 42 post-infection, by which time mortality had reached 55%. CD-1 mice fed a high fat diet (HFD), which was begun 53 days prior to infection and continued throughout the study, displayed a markedly attenuated mortality (Figures 5B and 6). Overall, the mice on the high fat diet had a death rate of 20% as compared with the mice on the regular diet (55%).

The co-administration of metformin resulted in a marked drop in mortality in both diet groups (Figures 5C and D and 6). In regular diet mice, there was a reduction in mortality from 55% to 25%. In high fat diet mice, the metformin reduced mortality from 20% to 3% (Figures 5B and D and 6).

The goal of the high fat diet was to induce the metabolic syndrome and thereby to test the hypothesis that the activation of the immune system with the metabolic syndrome would improve the outcomes of *T. cruzi* infection. We anticipated that the addition of metformin would produce modestly reduced weight, a modest diminution in blood glucose and a diminution in some features of immune activation. How these and other perturbations would affect survival was difficult for us to predict, given the multiple countervailing effects of fat feeding and of metformin. The marked further improvement in survival observed here with metformin may not occur under other experimental conditions. (If metformin is uniformly effective in *T. cruzi* infections, we would need to search for a heretofore unknown parasiticidal effect of metformin. In our hands, no such effect was detected in cell culture –see later in the text.)

A similar study done by our neighbors Nagajyothi et al. showed that mice fed a HFD 30 days prior to infection displayed 92% survival compared to 40% survival for RD-fed mice due to acute infection. These data demonstrates that HFD has a protective effect on the mortality seen during acute *T. cruzi* infection in this murine model [147].

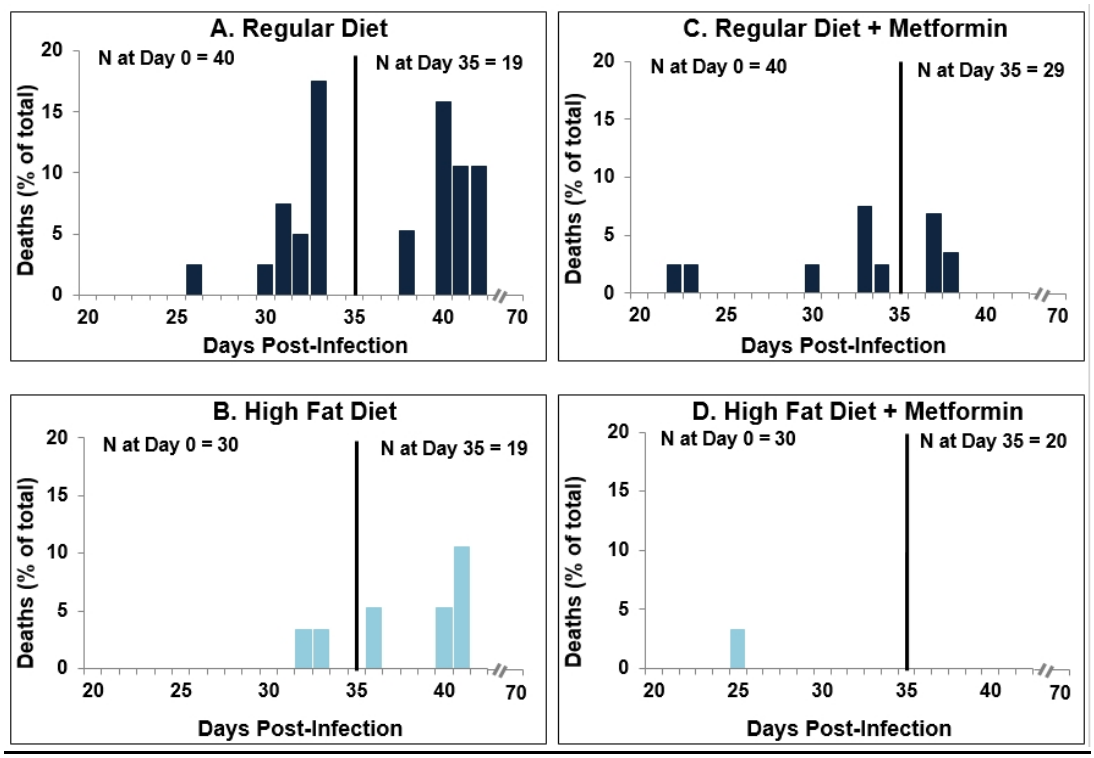

Figure 5: The impact of diet on the course of acute *T. cruzi* infection.

Figure 5A & 5B: CD-1 mice were fed a chow diet until they were 5 weeks old. At that time, the mice were placed on either a high fat diet or a matched regular diet for the remainder of the experiment. 53 days later (designated day 0), some of the mice were infected with *T. cruzi*. Deaths were recorded on each day from day 0 to day 70. Note that on day 35 (marked by the vertical line), some animals were sacrificed for more in-depth study. The height of each bar represents the deaths that occurred on that day, expressed as a percentage of the total animals in that group on day 0 (or of all animals in that group on day 35 post-sacrifice). No deaths occurred in the uninfected mice.

Figure 5C & 5D: Starting 20 days prior to infection, each mouse was given metformin, once daily (50 mg/kg by gavage).

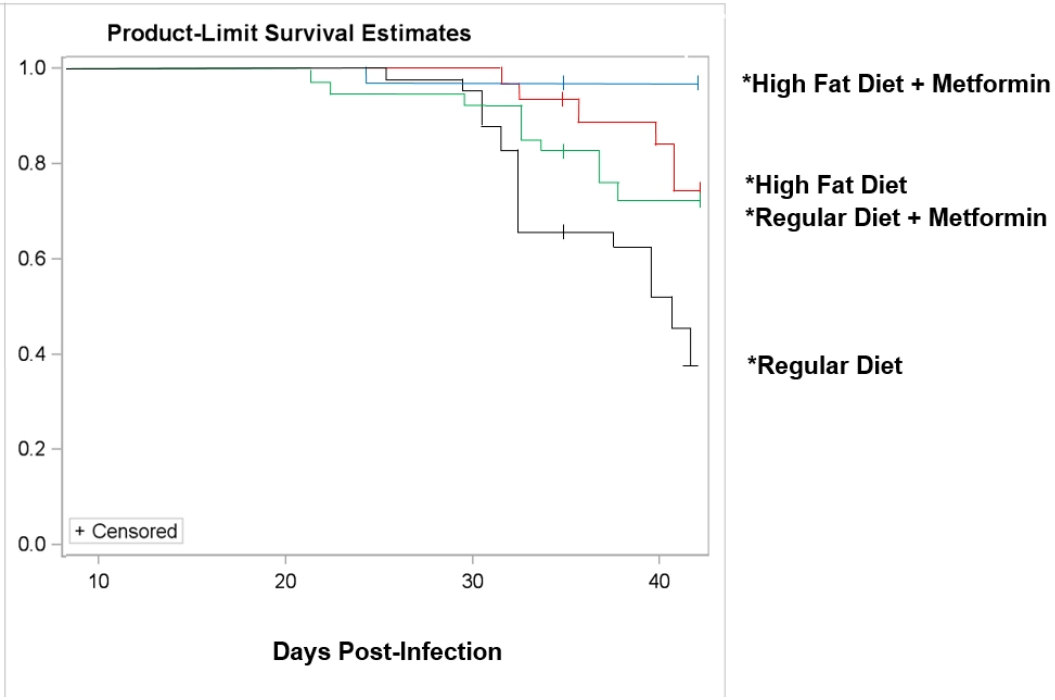

Figure 6: Kaplan-Meier survival plot: The same data that were presented in figure 5A-D are re-expressed here in the form of a Kaplan-Meier survival curve. The different Kaplan-Meier curves for each group were found to be statistically significant (using the log-rank test) for diet (p=0.0003) and metformin treatment (p=0.002). Survival probability at day 41 was lower among the animals without metformin treatment (56.8%), compared with those on metformin (82.5%), and among mice on a regular diet (58.3%) compared with the mice fed a high fat diet (85.3%).

2.5.2 Body weight of CD-1 mice

Five-week-old CD-1-inbred mice (n = 220), weighing on average 28 g were randomly divided into a high fat diet (HFD) (n = 100) or a regular diet (RD) (n = 120). Mice fed a regular diet included 20 uninfected mice, 20 uninfected mice treated with metformin, 40 infected mice and 40 infected mice treated with metformin. Mice fed a high fat diet included 20 uninfected mice, 20 uninfected mice treated with metformin, 30 infected mice and 30 infected mice treated with metformin. Body weights were recorded once every 2 weeks.

As expected, pre-infection mice fed the high fat diet (HFD) revealed a much greater weight gain than those on regular diet (RD) (Figure 7A and B). Post-infection, the regular diet mice displayed an attenuation of weight gain and then a modest weight loss (Figure 7B) in comparison with the

uninfected mice (Figure 7A). High fat diet mice on day 5 post-infection began a steep weight loss so that their weights approached those of the infected regular diet mice (Figure 7B).

This is consistent with the effect of *T.cruzi* infection on adipocytes [148, 149]. Pre-infection, mice treated with metformin had an initial fall in weight and persistently lower weights than their respective non-metformin-treated mice, both with the high fat diet and regular diet (Figure 7A and B). After infection, both the high fat diet (HFD) and regular diet (RD) mice treated with metformin showed a gradual modest weight loss, resulting in somewhat lower weights throughout the course of the experiment in comparison with their respective non-metformin-treated mice (Figure 7B).

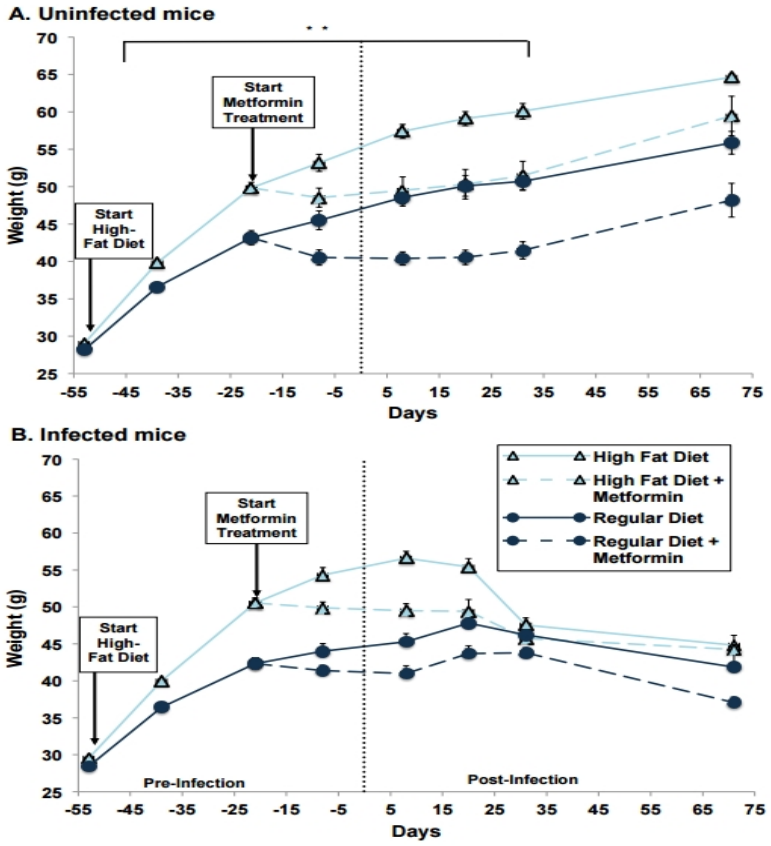

Figure 7: Body Weights of CD-1 mice

Figure 7A & 7B: Diets were started 53 days prior to infection. Metformin was started 20 days prior to infection. Both were continued throughout the remainder of the experiment. Each point represents the mean weight (+ SEM) of all animals in a given group.

7A- Uninfected Mice: Body weights between regular diet-fed mice and high fat diet-fed mice differed significantly at day -53 ($p=0.0052$), day -39 ($p=0.0009$), and day -20 ($p=0.0011$). Following metformin treatment, body weight between RD, RD + Metformin, HFD, and HFD + Metformin groups continued to differ significantly at days -8 ($p=0.0061$), day 8 ($p=0.0353$), day 20 ($p=0.0353$), and day 30 ($p=0.0323$), but not on day 70 ($p=0.0638$).

7B- Infected mice: Body weights between regular diet-fed mice and high fat diet-fed mice differed significantly at day -53 ($p<0.0001$), day -39 ($p<0.0001$), and day -20 ($p<0.0001$). Following metformin treatment, body weight between RD, RD + Metformin, HFD, and HFD + Metformin groups continued to differ significantly at days -8 ($p<0.0001$), day 8 ($p<0.0001$), day 20 ($p<0.0001$), day 30 ($p=0.0293$), and day 70 ($p=0.0347$).

2.5.3 Body composition analysis of CD-1 mice

On day 70 post-infection, we used magnetic resonance spectroscopy, employing the Echo Medical System EchoMRI-100, to determine body composition, expressed as fat mass and lean mass, in live mice unrestrained without anesthesia.

The infected regular diet mice weighed 25% less than their uninfected counterparts, consistent with earlier studies. Almost all of this weight loss was due to a reduction in fat mass (Figure 8). Lean mass was almost entirely preserved. Infected animals on the high fat diet weighed 32.2% less than their uninfected counterparts, again because of a reduction in fat mass (Figure 8).

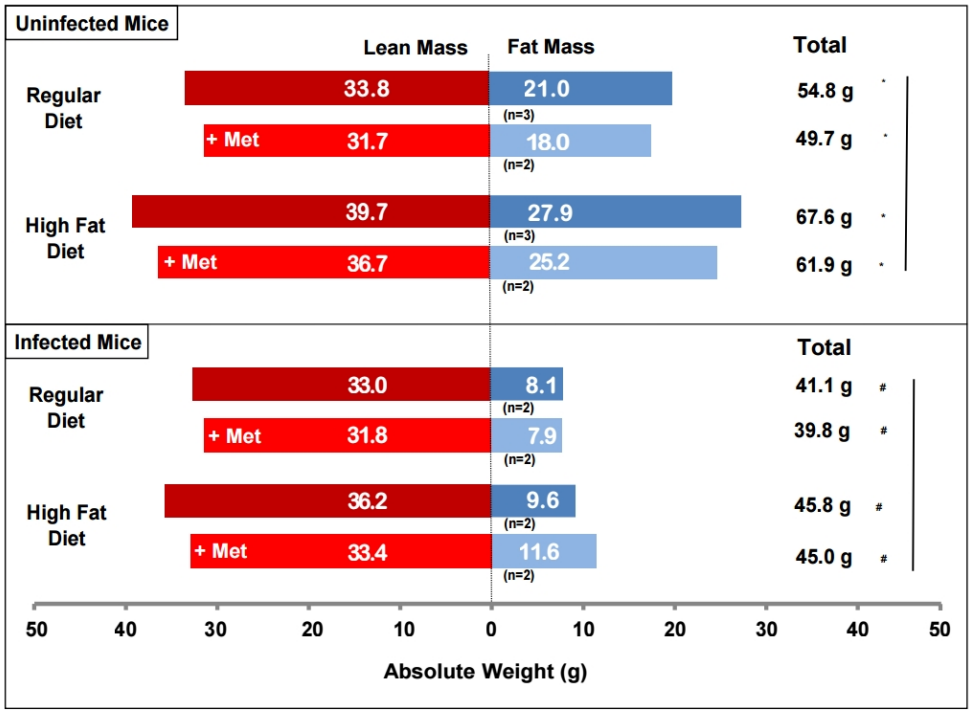

Figure 8: Body Composition analysis of CD-1 mice. Body composition, measured by magnetic resonance spectroscopy, was done on live mice (without anesthesia) to measure fat mass and lean (fat-free) mass. The measurements were conducted 70 days post-infection. Mice were selected for body composition studies by choosing those that were at or closest to median weight in each experimental group. Among uninfected mice, there was a statistically significant difference in lean mass (p=0.0038) and total weight (p=0.0038) among the 4 diet/treatment groups. No such difference was detected in fat mass (p=0.1208), % fat mass (p=0.6217), or % lean mass (p=0.5230). Among infected mice, there was a significant difference in total weight among the groups (p=0.0095), but not in fat mass (p=0.4762), lean mass (p=0.5619), % fat mass (p=0.3429), or % lean mass (p=0.6857).

2.5.4 Fasting blood glucose measurements

Blood glucose, following a 4-hour fast, was measured 20 days pre-infection and on days 0, 15 and 30 post-infection (Figure 9). As expected, high fat diet (HFD) mice had significantly higher 4-h fasting blood glucose levels compared with regular diet (RD) mice in both uninfected and infected groups (Figure 9A and B). In addition, treatment with metformin resulted in a decrease in blood glucose levels in high fat diet mice, regardless of infection status (Figure 9A and B). This effect was not detected in regular diet mice. In high fat diet mice, infection resulted in a progressive and substantial fall in blood glucose levels from around 220 mg/dL at day 0 to 110

mg/dL by day 30 post-infection (Figure 9B). For regular diet mice, the fall in blood glucose post-infection was more modest and gradual. By day 30 post-infection, the blood glucose levels in both groups were nearly identical, despite the slightly greater weight, which can be attributed to fat mass, in the high fat diet mice (Figure 9B).

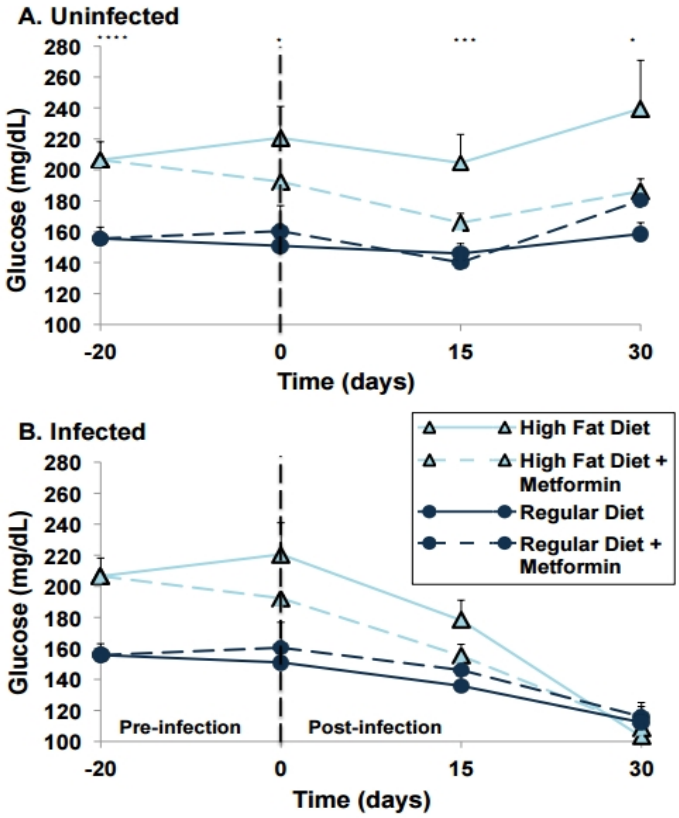

Figure 9: Fasting Blood Glucose Measurement In CD-1 Mice

Mice were fasted for 4 hours in the morning, after which blood was drawn from 10 animals per group (16 animals per group at day -20, before the first dose of metformin) for measurement of blood glucose. Results are expressed as a mean (+ SEM).

9A- Uninfected: Fasting glucose levels differed significantly among uninfected mice groups at days -20 ($p<0.0001$), day 0 ($p=0.021$), day 15 ($p=0.0004$) and day 30 ($p=0.019$). Pairwise comparisons were performed for day 15 and day 30 using the Bonferroni method of correction for multiple comparisons. For day 15, the following comparisons remained statistically significant: RD+Metformin vs. HFD ($p=0.0007$), RD+Metformin vs HFD+Metformin ($p=0.0036$), and between RD vs HFD ($p=0.0028$). For day 30, HFD vs RD+Metformin remained statistically significant ($p=0.0073$).

9B- Infected: Fasting glucose levels differed among infected mice groups at day -20 (p=0.0021) and at day 0 (p=0.0003), but not on day 15 (p=0.0554) or on day 30 (p=0.75).

2.5.5 Oral glucose tolerance test

At 28 days post-infection, oral glucose tolerance was determined after a 6-h fast. To our surprise, there was no difference in glucose tolerance in the uninfected group between regular diet and high fat diet mice. In infected animals, high fat diet mice displayed enhanced glucose tolerance when compared with regular diet mice (Figure 9). Consistent with the 4-h fasting glucose levels on day 30 post-infection (Figure 9), the fasting glucose was highest in the high fat diet-fed animals in both infected and uninfected animals (Figure 10). The uninfected regular diet mice had fasting glucose levels about half those of the high fat fed (p=0.009). Regular diet + metformin mice had fasting glucose levels that were (to our surprise) about 50% higher than their counterparts without metformin (Figure 10). At 1 h after glucose administration, all uninfected mice showed marked elevations of blood glucose to similar levels, seemingly uninfluenced by the fasting level. Infected mice (28 days after infection) on regular diet had fasting blood glucose levels around 100 mg/dL, irrespective of metformin administration (Figure 10). With high fat diet, fasting blood glucose was slightly higher (p=0.028). With high fat diet, metformin had little effect. Following oral glucose administration (infected mice) with regular diet, results were similar, with and without metformin. The high fat diet-fed mice, to our surprise, had post glucose levels that were below their normal diet counterparts. Again, unexpectedly, the metformin-treated animals were more hyperglycemic (p=0.226) (Figure 10).

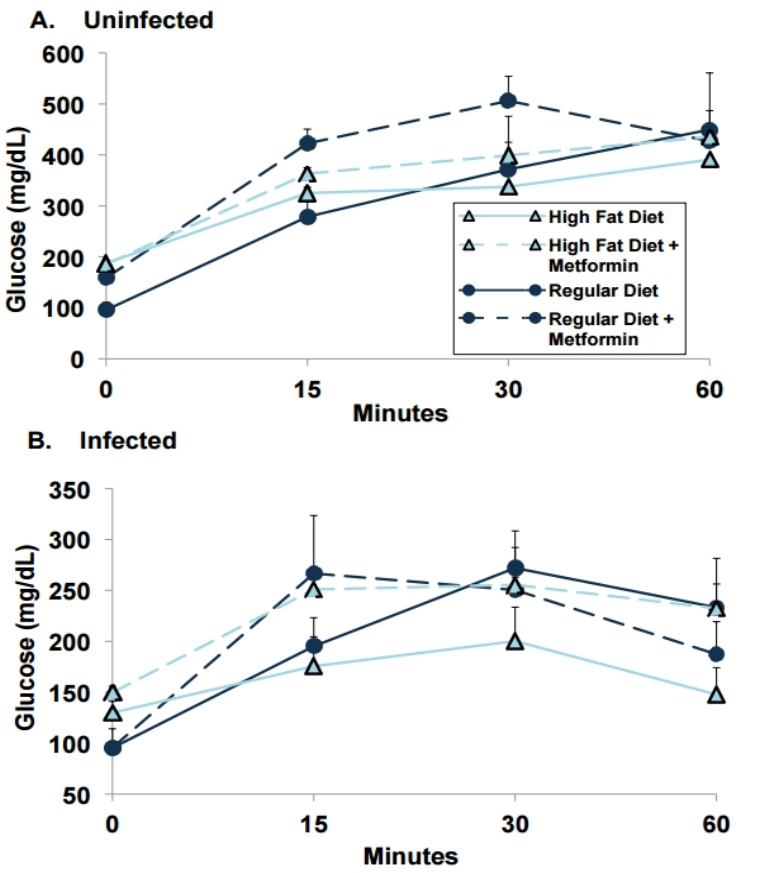

Figure 10: Oral Glucose Tolerance Test (OGTT) In CD-1 Mice

OGTT was done on post infection day 28 after 6 hours fasting. Five mice per group were used for this determination. Data is represented as mean glucose plotted against time at 0, 15, 30 and 60 minutes for regular diet fed mice, regular diet fed mice treated with metformin, high fat diet fed mice, and high fat diet fed mice treated with metformin.

2.5.6 Immunoblot analysis of tissue

2.5.6.1 Heart tissue AKT and Phospho AKT

The heart, a classical target for insulin, is a major target of *T. cruzi*. AKT is a major step downstream of the receptors for insulin and receptors for insulin-like growth factor 1 (IGF 1) receptors, typically associated with the metabolic (rather than growth promoting) effects of these hormones. Previous studies in adipocytes [150] showed twofold or greater increases in total AKT and phospho-AKT 4 days postinfection of normal fed mice. In our study at 35 days post-infection, heart tissue displayed a significant increase in pAKT, irrespective of diet or metformin

treatment (Figure 11). Regular diet mice had higher levels of pAKT than high fat diet mice at 35 days post-infection. The effect of metformin on pAKT was not significant. Measurements of total AKT showed much less difference between infected and uninfected mice. Interestingly, metformin-treated infected mice demonstrated a significant reduction of total AKT in both diet groups (Figure 11). Inflammation is typically linked to insulin resistance. However, it is important to note that with inflammation, insulin sensitivity varies widely from normal to impaired from tissue to tissue and from pathway to pathway in the same tissue.

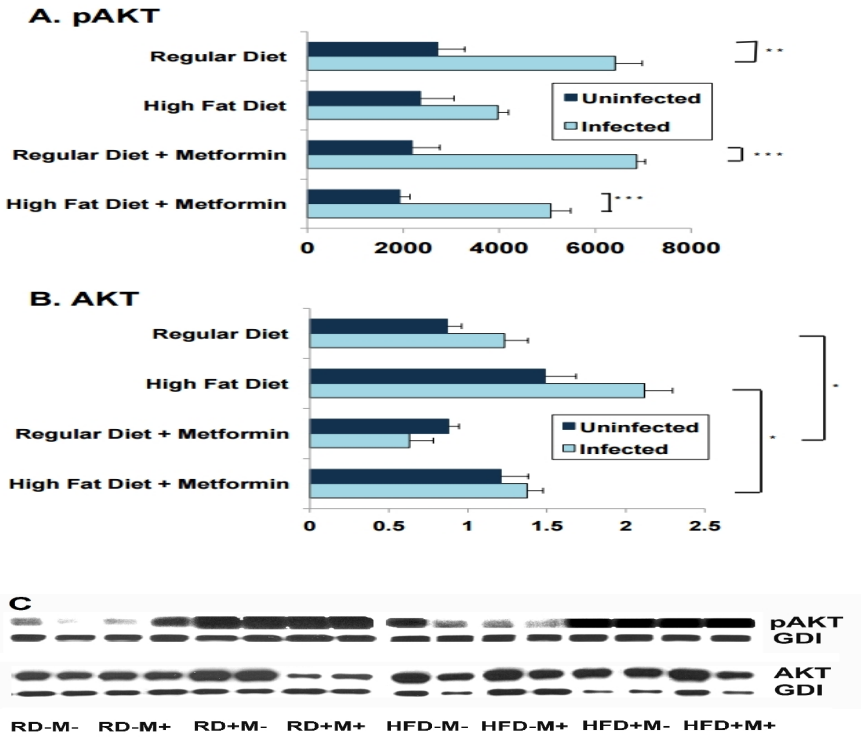

Figure 11: pAKT/AKT 35 dpi levels in heart tissue

AKT/p-AKT expression of heart tissue at 35dpi. Under RD and HFD conditions, infection modulates the phosphorylation of AKT. Metformin significantly decreases AKT expression in RD+M+ and HFD+M+ when compared to infection alone. While a HFD increases AKT expression in both HFD+M- and HFD+M+ when compared to RD mice of the same groups $p<0.05$.

A significant difference was determined using Student's t-test. $p<.05$ *, $p<0.01$ **, $p<0.005$ ***.

Western blot of RD fed mice (RD-M-), RD treated with Metformin (RD-M+), RD infected (RD+M-), RD treated with Metformin and Infected (RD+M+), HFD fed mice (HFD-M-), HFD treated with Metformin (HFD-M+), HFD infected (HFD+M-), and HFD treated with Metformin and Infected (HFD+M+).

2.5.6.2 Interferon-gamma

The IFN-gamma levels in hearts obtained from mice sacrificed on day 30 post-infection were several-fold (at least three times) higher in infected mice compared with uninfected mice. Metformin treatment substantially lowered IFN-gamma in the uninfected mice but had no effect in tissue of infected mice (Figure 12A).

2.5.6.3 Tumor necrosis factor-alpha

The TNF-alpha levels in hearts on day 30 post-infection were elevated to levels 1.5 to 5 times greater in infected animals than in uninfected animals. High-fat diet in both infected and uninfected mice displayed a significant increase. Metformin did not alter this observation (Figure12B).

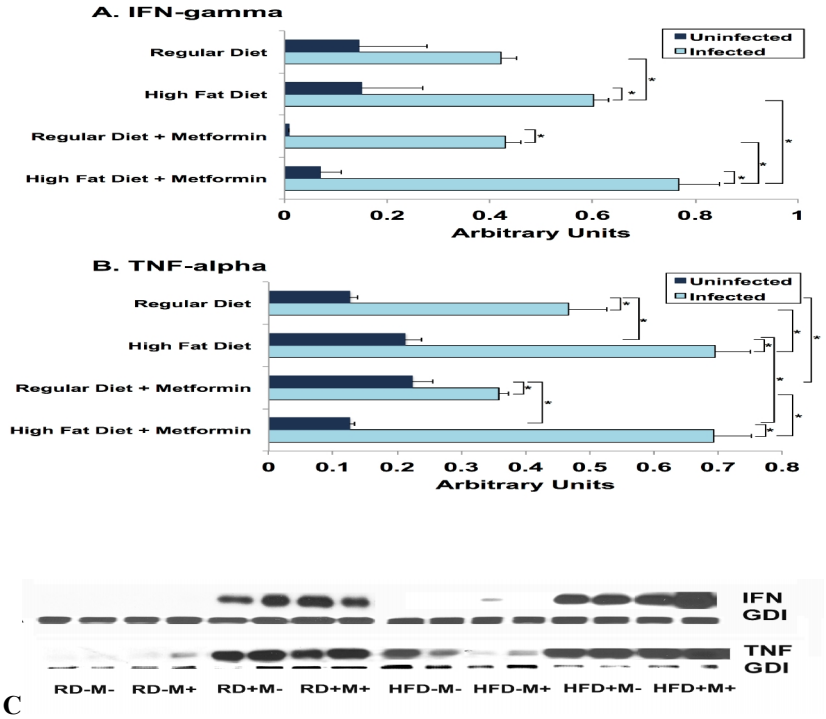

Figure 12: IFN-gamma and TNF-alpha levels in heart tissue on 35dpi

Western blot of RD fed mice (RD-M-), RD treated with Metformin (RD-M+), RD infected (RD+M-), RD treated with Metformin and Infected (RD+M+), HFD fed mice (HFD-M-), HFD treated with Metformin (HFD-M+), HFD infected (HFD+M-), and HFD treated with Metformin and Infected (HFD+M+). A significant difference was determined using Student's t-test. $p<.05$ *, $p<.01$ **, $p<.005$ ***.

A) INF-ϒ expression of heart tissue at 35dpi. INF- ϒ expression is independent of Metformin treatment; however, it is dependent on diet as shown by the significant difference of INF- ϒ expression between RD+M+ and HFD+M+ and between RD+M- and HFD+M- $p<.05$.

B) TNF-α expression of heart tissue at 35dpi. In uninfected HFD fed mice, Metformin reduced TNF-α expression.

C) Immunoblots of INF-ϒ and TNF-α with GDI as control

2.5.7 Insulin and Leptin in serum by day 70 post-infection

2.5.7.1 Insulin

In uninfected animals, plasma levels of insulin (4-h fasting) on day 70 post-infection were very similar for regular diet and high fat diet mice. Metformin in regular diet mice produced a marked lowering of plasma insulin but had no discernible effect in high fat diet mice (Figure 13A).

In the infected mice 70 days post-infection, plasma insulin levels were markedly diminished to levels 50% or less than their uninfected counterparts (Figure 13A). The decreased insulin levels in the infected groups irrespective of the diets fed or drug treatment are likely due to low blood glucose levels often associated with *T. cruzi* infection [140]. In addition, leptin levels fell mainly because of the loss in fat cells in infected mice [140]. One possibility is that the very low leptin levels in *T. cruzi*-infected mice mimic the leptin levels found in severe starvation. We suggest the possibility that with starvation, the low leptin levels we observe cue the fall in plasma insulin to protect against hypoglycemia and prevent shutting down ketone production.

2.5.7.2 Leptin

At 70 days post-infection, plasma leptin concentrations in the uninfected mice followed the expected pattern (Figure 13B). The high fat diet mice, which were heavier (Figure 7) and fatter (Figure 8), also had higher leptin levels than their regular diet counterparts (Figure 13B). The metformin-treated mice weighed less (Figure 7A and B) and were leaner (Figure 8) than their non-drug counterparts, but leptin levels reflected this difference only for regular diet mice (Figure 13B).

With the infected mice, plasma leptin levels were markedly reduced below levels observed in their uninfected counterparts, probably reflecting the dramatic shrinkage of fat stores (Figure 8). Leptin levels in infected mice fed with a high fat diet were about twice as high as in the infected regular diet group (Figure 13B).

These differences in plasma leptin were greater than the differences in weights and body composition in the subgroups of infected animals.

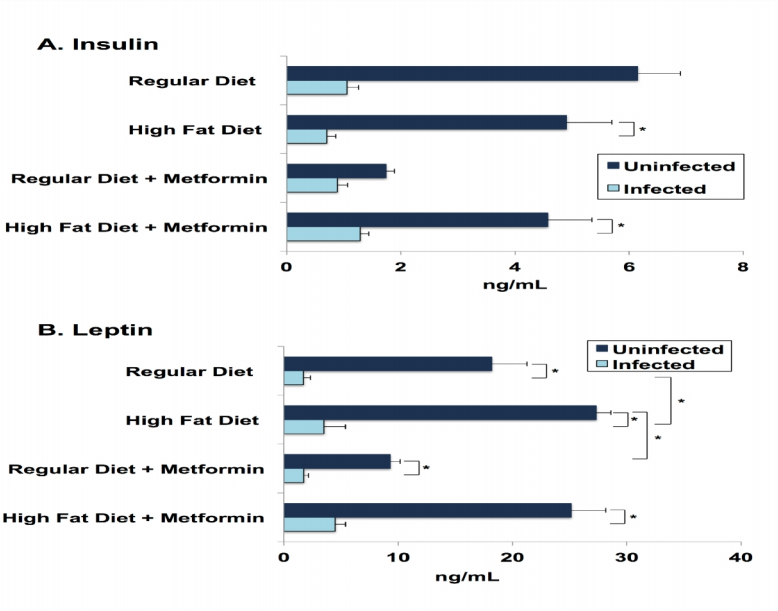

Figure 13. Insulin and Leptin in serum by day 70 post-infection

2.5.8 Serum cytokine levels by day 70 post-infection

Previous studies have shown that the acute phase of *T. cruzi* infection is associated with elevated plasma levels of interleukin 6 (IL-6), TNF-alpha and IFN-gamma, compared with uninfected mice [151, 152].

Typically, IL-6 production increases during obesity. In our study, CD-1 mice fed with a high fat diet irrespective of their infection status, have elevated serum IL-6, and as expected, infected mice on a high fat diet have increased level of IL-6, compared with uninfected mice fed with a

high fat diet (Figure 12A). There was no significant difference in the regular diet mice irrespective of their infection status. Metformin did not have a significant effect on IL-6 in regular diet mice (Figure 14A).

No significant differences in serum TNF-alpha of uninfected mice in both diet groups were observed at day 70 post-infection. Infected mice fed with regular diet have elevated serum TNF-alpha. No difference is observed in metformin-treated mice fed a high fat diet or a regular diet irrespective of their infection status (Figure 14B).

Interferon-gamma production is shown to correlate with effective control of *T. cruzi* parasitism [153, 154]. It enhances macrophage killing of *T. cruzi* in vitro and enhances resistance to *T. cruzi*. Infected mice fed a high fat diet and treated with metformin displayed a significant increase in IFN-gamma especially when compared with metformin-treated uninfected mice fed a high fat diet (Figure 14C).

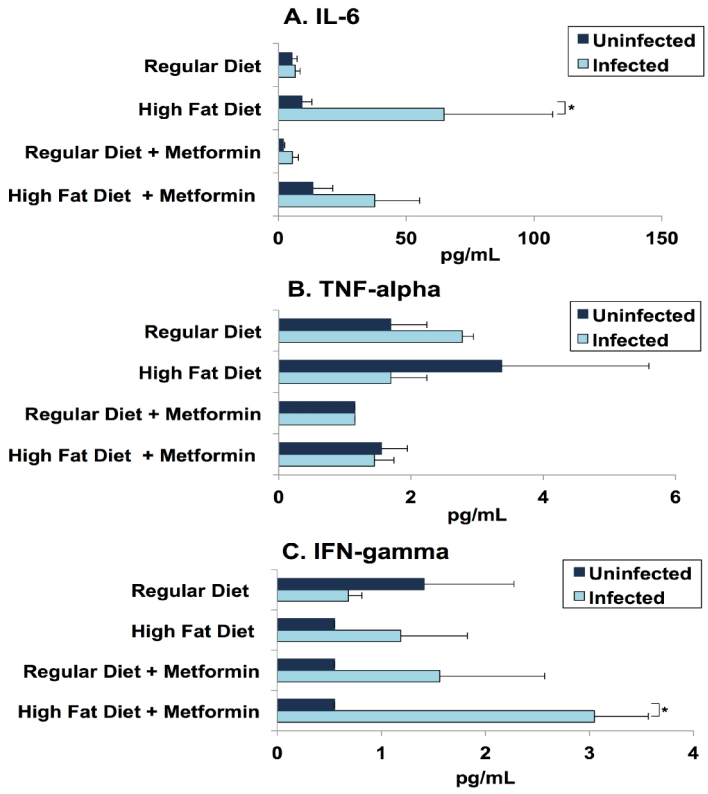

Figure 14. Serum cytokine levels by day 70 post-infection.

On day 70 post-infection, blood was collected and processed as described earlier. Minimum levels of detection in picograms per milliliter are 1.1 for interleukin 6 (IL-6), 1.1 for interferon (IFN)-gamma and 2.3 for tumour necrosis factor (TNF)-alpha. Each bar represents the mean (+/-standard error of the mean) concentration in picograms per milliliter. The number of pools (n=5) is listed for each group. Serum IL-6 (A), TNF-alpha (B) and IFN-gamma (C) levels were determined using Luminex XPONENT for Magpix.

2.5.9 Histopathologic analysis of heart tissue

On day 35 post infection, mouse were sacrificed; heart samples from each experimental group were processed for paraffin embedding and optimum cooling temperature (OCT) embedding. Five micrometer-thick sections of paraffin embedded tissues were cut with a microtome, then stained with H&E to examine the extent of inflammation, fibrosis, and parasitemia [144]. Scoring was performed for the atrium, right and left ventricles and base as described earlier. Although four distinct areas of the heart were examined, regional differences were not statistically significant.

There were no significant histological differences in the hearts obtained from HFD and RD fed uninfected mice (Figure 15A). Mice infected with *T. cruzi*, irrespective of the type of diet displayed inflammatory reactions and fibrosis (Figure 15B).

Histological scoring of hearts ranged from 0 to 4+ in each of the categories of degenerating cardiac fibers, inflammation, fibrosis and presence of adipocytes. No statistically significant difference was noted between infected HFD and infected RD fed mice (Figure 15C).

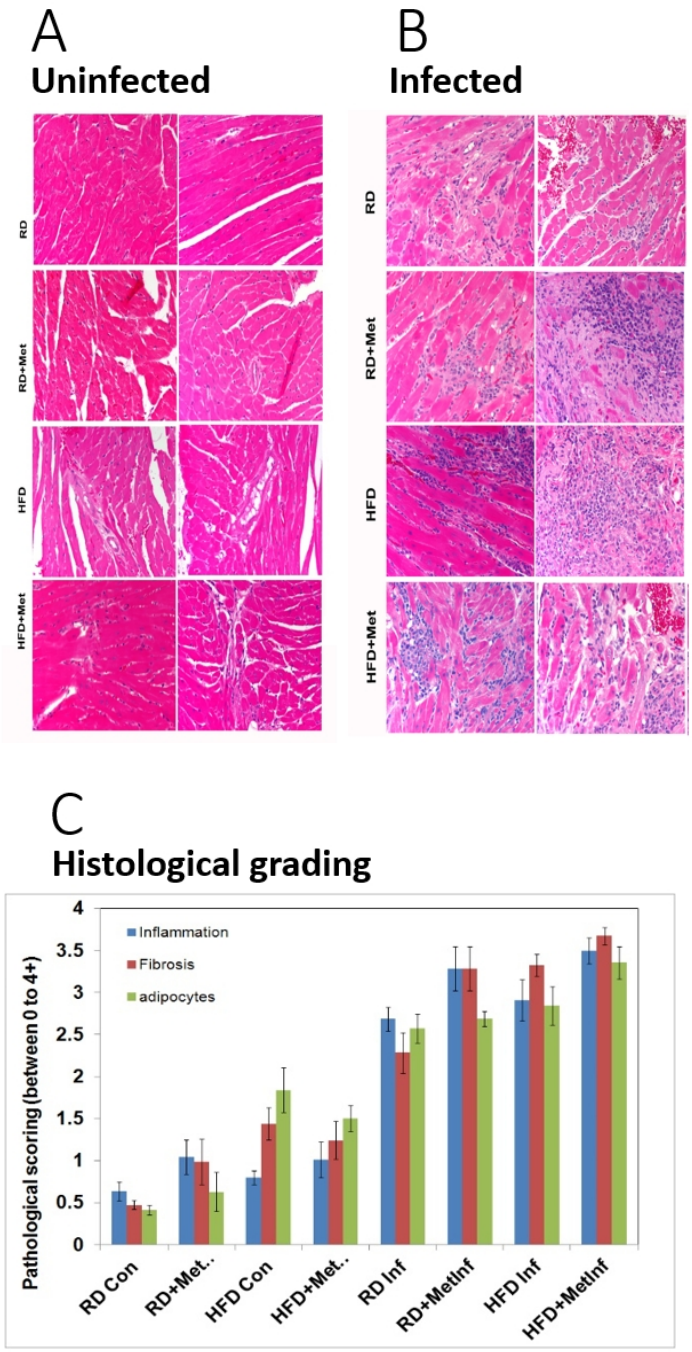

Figure 15: Histology of heart tissue: A-Uninfected mice, B- Infected mice, C- Histological grading

RD: Regular diet, HFD: High fat diet, RD+Met: Regular diet + metformin, HFD + Met: High fat diet + metformin, RD con: Regular diet control, RD inf: Regular diet infected, RD+Met inf: Infected regular diet +metformin, HFD con: High fat diet control, HFD inf: High fat diet infected, HFD+Met inf: Infected high fat diet +metformin.

2.5.10 Parasitemia

Parasitemia was evaluated using samples from tail blood by counting in a Neubauer hemocytometer. In regular diet mice, parasite levels in blood began to increase 15 days post-infection, reached a peak at day 25 and then fell slowly to undetectable levels by day 33 (Figure 16). In high fat diet mice, parasitemia reached its peak later (at day 30 post-infection) and cleared the blood rapidly, becoming undetectable by day 38 post-infection. A nonparametric comparison Kruskal–Wallis test demonstrated statistical significance ($p<0.01$) amongst all four groups on days 27, 30, 33 and 35 post-infection. The high fat diet group had a greater parasitemia, demonstrated by the larger area under the curve (Figure 16).

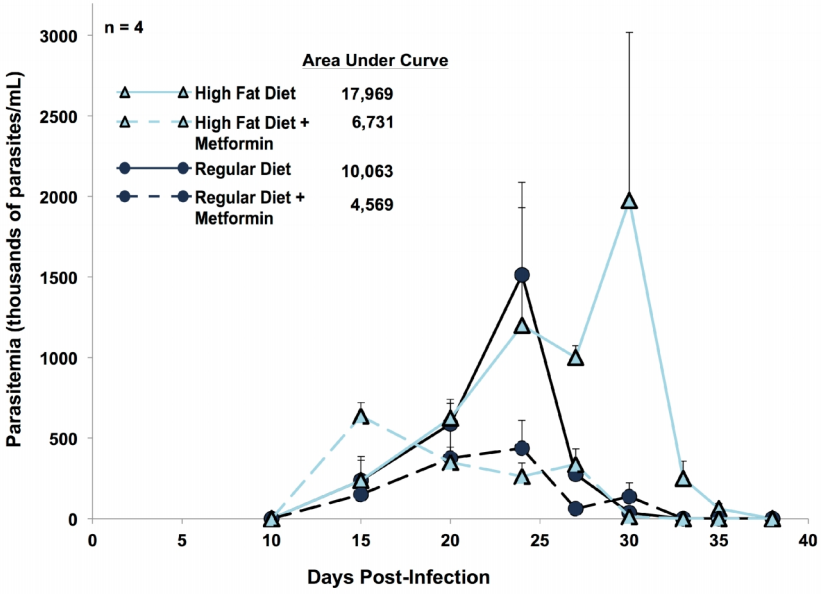

Figure 16: Parasitemia:

Blood was drawn from 4 mice per group at intervals from post-infection day 10 through day 38 for measurements of parasites. Each point represents the mean (+ SEM). The numbers in the key represent the relative areas under each curve. A non-parametric version of ANOVA, the Kruskal-Wallis test, demonstrated that there was a statistically significant difference in parasitemia levels amongst all groups on day 27 ($p=0.0001$), day 30 ($p=0.0035$), day 33 ($p=0.0286$), and day 35 ($p=0.0286$) post-infection.

2.5.11 Quantitative Determination of Parasite Load in Tissue by PCR

On dpi 35, heart tissue, liver tissue, and omental fat (white adipose tissue) were harvested and stored at -80 °C. Quantitative determination of parasite load in these tissues was done by PCR as described earlier.

In general, high parasite load is seen in heart tissue and omental fat when compared to liver tissue (Figure 17). A higher parasite load is seen in the myocardium and white adipose tissue (omental fat) of RD fed mice (Figure 17A and 17B). The difference is statistically significant in mice fed a RD versus HFD as seen in omental fat and liver tissue (Figure 17A and 17C).

Note: parasite load by PCR in heart tissue was determined using pooled samples

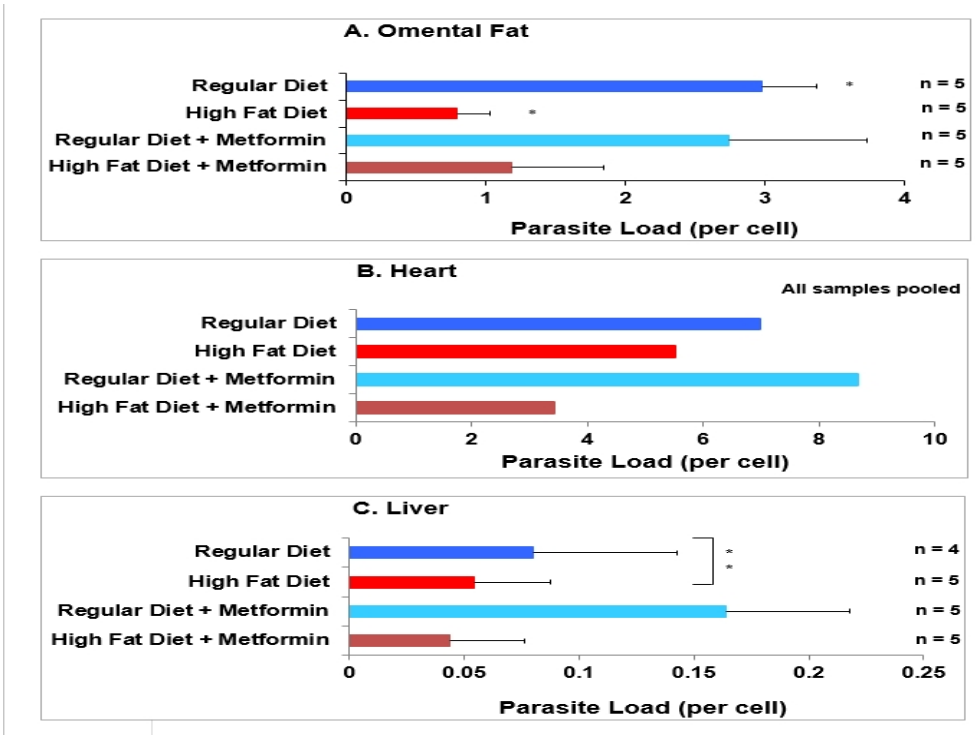

Figure 17: Quantitative determination of parasite load in WAT, heart and liver tissues by PCR.

A-Omental fat; B-Heart tissue (using pooled samples) and C- Liver tissue; *denotes $p<0.01$ and ** $p<0.001$

2.5.12 Effects of metformin on *Trypanosoma cruzi*

To study the effects of metformin on *T. cruzi*, Human Foreskin Fibroblasts (HFF) were cultured in 12 well plates. Eight hours prior to infection with trypomastigotes, half of the wells in each plate were pre-treated/incubated with selected concentrations of metformin (0–50 µg/mL). Infection was allowed to proceed for 24, 48 and 72 h. The number of live parasites/trypomastigotes and infected host cells in each well was determined with a haemocytometer at 24, 48 and 72 h post-infection.

In both diet groups, parasite levels in blood (expressed as areas under the curve) were about 40% as high with metformin as in its absence (as shown in figure 16). Metformin in vitro had no effect on parasites growing on human fibroblasts when cells were exposed to metformin (up to 50 µg/L) for up to 72 h (Figure 18).

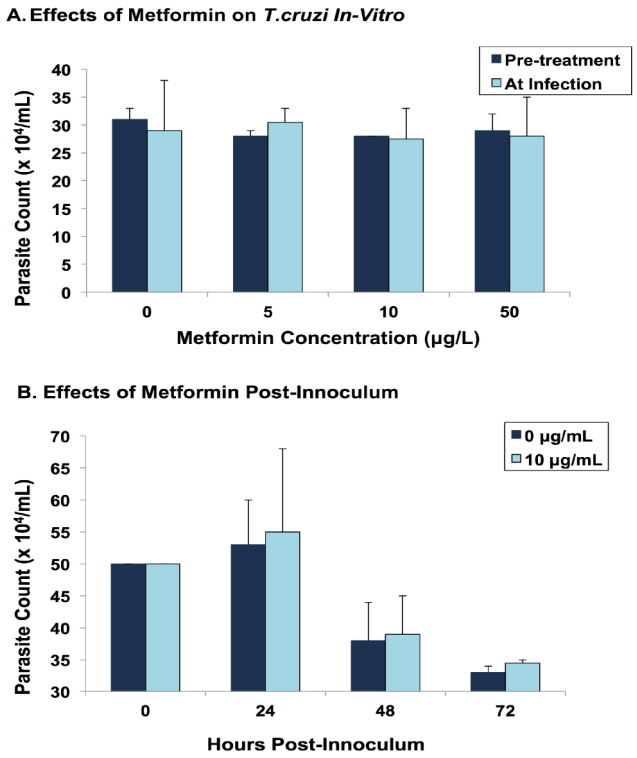

Figure 18: In Vitro Effects of Metformin

3.0 CHAPTER 3: DISCUSSION AND CONCLUSION

3.1 Discussion

Obesity is very widespread and detrimental to health. Obesity brings with it many changes including heightened immune function, and a higher prevalence of many disorders, including major cardiovascular diseases, diabetes, cancer and Alzheimer disease. Obesity is also associated with shortened lifespan. The detrimental effects of obesity are linked to the "metabolic syndrome", a broad range of changes in metabolic processes and immune function. The increasing prevalence of the metabolic syndrome parallels the rise in the prevalence of obesity and IDF estimates that one-quarter of the world's adult population has the metabolic syndrome.

As a first approximation, we agree with this formulation. However, this formulation has some weaknesses. Firstly, crude mortality rates increase with increasing BMI but as the BMI approaches the normal range, mortality rates reverse so that individuals with reduced BMI have elevated mortality - yielding the notorious poorly explained J-shaped curve. Secondly, a multiplicity of medical and surgical conditions have been reported where short term and medium term outcomes are better for overweight patients. These conditions are placed under the heading of "obesity paradox". Recalling Dr. Faust, the medieval German legend, who chose short term benefits and sacrificed long term best outcomes, we suggest that the Faust Phenomenon may apply better than Obesity Paradox. Adiposity brings short time benefits but at cost of long term deficits. Thirdly the medical community has introduced a binary system for the metabolic syndrome ---- yes, patient has it or no, the patient does not have it, despite the fact that all of the changes that are considered components of the metabolic syndrome are continuous variables.

Using an evolutionary approach, we hypothesized that for millions of years, the channeling of host resources into immune defenses starts early in life, combating potentially lethal infectious conditions, like pneumonia and sepsis as well as chronic infections, such as tuberculosis and Chagas disease.

The present study constitutes an experiment to test our overarching hypothesis about the close ties that exist between nutrition and host defenses. We hypothesize that the metabolic syndrome represents one part of a rich program that we term the immuno-metabolic coalition that protects the host against infectious invaders. The magnitude of the defense effort is linked to the host's internal measurements regarding the availability of calories. With low levels of calorie stores, the host defenses are likely reduced. This phenomenon is often associated with the well-described immune deficiency of undernutrition; indeed, death from starvation is often due to infection. As calorie stores increase, host defenses likewise increase. With excess calories, mostly deposited as fat in omentum and viscera, greater activation of the immune system occurs and brings with it a complex of findings, including those grouped under the rubric of the metabolic syndrome.

Over the short term, especially in the young, immune activation is beneficial. Only when prolonged over the years especially in older adults, do the detrimental effects dominate. The persistence of high levels of inflammation and other features of the syndrome (hypertension, insulin resistance, disorders of lipids and so on) over years produce a high prevalence of cardiovascular complications, hyperglycemia, cancer and other disorder characteristics of the metabolic syndrome and premature ageing. Paradoxically, in the long run, the emergence of hyperglycemia or severe vascular disease may undermine the heightened defenses of the metabolic syndrome.

In the present study, we showed that 8 weeks of overfeeding, in the form of a high fat diet, diminished the lethality of *T.cruzi* in mice from 55% to 20%. This result is consistent with the recently published article from our group; high fat diet mice starting on the day of infection reduced mortality from 60% to 15% [147]. High fat diet started 30 days before infection dropped mortality from 60% to 8% [147]. The benefits of overfeeding were clear, despite the moderate hyperglycemia that accompanied the high fat diet. In our mice, metformin, a medication widely used to control hyperglycemia (and weight gain) in patients with diabetes, further reduced mortality.

We chose CD-1 mice because the course of *T. cruzi* infection in them is well described [145] and because CD-1 mice mimic the metabolic syndrome when fed a high fat diet [155]. Overfed animals with obesity have elevated levels of circulating leptin. Leptin is a robust broadly acting

promoter of immune activation, e.g. as with metabolic syndrome. One note of caution is that typical high fat diets, as used in the experiments here, are exceptionally rich in saturated fats, which strongly activate Toll-like receptor 4 (TLR4) receptors [156], probably to a much greater degree than might be observed with more mixed and balanced diets of overweight humans. We avoided congenitally obese rodents, for example, ob/ob, Zucker, db/db and knockouts in whom leptin or the leptin receptor system was markedly diminished because leptin action may play a key role in nutrition-related immune activation [157, 158]. Also, it has been demonstrated that the disruption in leptin signalling during acute T. cruzi infection is associated with increase mortality [159]. We also tried to minimize pathologic hyperglycemia (as may have happened here), fearing that the abnormal glycemia might undermine body defences [160-168].

At the start of the experiment, mice were fed a pre-selected diet for 53 days prior to infection. Their respective diets were continued throughout the course of the experiment. On the day of infection, mice on the high fat diet had an estimated average weight of 55 g, which was about 25% more than the mice fed with a regular diet. After infection, weights of the high fat diet mice fell precipitously but always remained higher than the weights of infected regular diet mice. We failed to track individual animals and therefore do not know whether those destined to die differed in weight from those that survived. We have amended protocol for future studies to correct that weakness. On the day of infection, high fat diet mice (after a 4-h fast) on average had higher blood glucose levels (220 mg/dL) compared with regular diet mice (150 mg/dL), consistent with our conclusion that by the time of infection, high fat diet mice had well-developed metabolic syndrome, including modest hyperglycemia. Over the 30 days following infection, glucose levels in blood of infected mice fell continuously so that blood glucose in high fat diet and regular diet mice converged. In many bacterial infections, there is a stress-induced increase in blood glucose, which was absent here. In *T. cruzi* infection in mice, hypoglycemia is a well-described observation [140, 169]. There are several possible explanations for this observation. It is possible that the parasite metabolizes glucose. In addition, Nagajyothi et al. [145] have demonstrated that there is a marked impairment in hepatic gluconeogenesis in the setting of murine Chagas disease, which may help explain the reduction in blood glucose observed here. In our study, in high fat diet mice, with and without infection, metformin-treated mice showed modest reductions in blood glucose but these failed to reach statistical significance.

Other studies have shown that mortality with other infectious diseases, such as pneumonia, appear to be related to adiposity. In a retrospective study [170] with patients in one veteran's hospital, the 30-day mortality with community-acquired bacterial pneumonia was four times higher in underweight patients (BMI < 18.5) than in those with obesity (BMI > 30). Those patients designated as normal weight and overweight showed intermediate values suggesting that these differences were not overly dependent on an accumulation of susceptibility factors in the underweight. The authors thus suggest that obesity may exert a protective effect against 30-day mortality from community-acquired bacterial pneumonia.

Our hypothesis was that the metabolic syndrome, brought on by the high fat diet, produces a concatenation of immunological, inflammatory and metabolic changes that act in congruence to improve the host's defenses against infectious diseases such as *T. cruzi* infection [28, 171]. In particular, the increase in adipogenesis with the high fat diet presumably contributes to the increased activation of immune system [172]. That the high fat diet mice had less than half the death rate of regular diet mice supports this hypothesis. Previously, we demonstrated that infection alone is associated with increased lipolysis and a reduction in fat mass; both processes are reduced by feeding a high fat diet [147]. In mice infected with *T. cruzi* and fed a high fat diet, there is an increase in parasite load in the white adipose tissue and a reduction in parasite load in the heart. Thus, we believe that adipose tissue may potentially act as a 'sponge' to take up the parasites, reducing parasite load in the heart. A weakness in our interpretation derives from the fact that the trypomastigotes use the low-density lipoprotein (LDL) receptor for entry into its major host tissue target, the adipocyte [139]. The metabolic syndrome typically includes elevation in circulating LDL levels [173]; this elevation typically downregulates the cell surface level of LDL receptors [174]. It is possible that a reduction in LDL receptors contributed to the easing of the infection.

Metformin, very widely used in the treatment of type 2 diabetes, typically reduces blood glucose levels and the manifestations of the metabolic syndrome. Metformin's best-studied target molecule is adenosine mono phosphate (AMP)-activated protein kinase, but other targets have emerged [175, 176]. We treated select mice with metformin to reduce the severity of the metabolic syndrome. The dramatic reduction in mortality in metformin-treated mice surprised us (Figure 5). That mice treated with metformin had reduced levels of parasites in blood raised the

possibility of a direct toxic effect of the drug on the parasites (Figure 16). That metformin had no effect on the viability of trypanosome in vitro (Figure 18) suggests that metformin itself is unlikely toxic to the parasites but does not exclude the possibility that in vivo metabolites of metformin might have deleterious effects on the parasite. Evidence suggests that metformin alters the host organism's production and release of specific cytokines involved in inflammatory responses. Note that metformin activates AMP-activated protein kinase in macrophages [177] and that this activation leads to an inhibition of release of high mobility group box 1 (HMGB1), a master cytokine; in addition to being an inflammatory cytokine on its own, HMGB1 is also a stimulator of other mediators of inflammation. It acts downstream on an array of other inflammatory cytokines to orchestrate an organized inflammatory response [178]. Tsoyi et al. [179] showed that metformin attenuated the pro-inflammatory response in a mouse model of lethal endotoxemia and improved the survival of these mice through inhibition of HMGB1 protein release. Zhang et al. [180] demonstrated that metformin protects against cardiomyocyte injuries by inhibiting the expression of HMGB1 protein and the expression of receptors for advanced glycation end products (RAGE), a signal transduction ligand for HMGB1. High glucose levels increase the expression of RAGE and HMGB1 by increasing the production of reactive oxygen species (ROS) [181]. Metformin significantly inhibits the production of ROS [182], which suggests that metformin may inhibit the expression of HMGB1 by limiting the production of ROS. Andrews et al. [183] explained that obese patients treated with metformin had lower levels of high sensitivity C-reactive protein (hsCRP), a marker of inflammation, and decreased expression of TNF-alpha, a pro-inflammatory cytokine. This suggests that metformin may alter the mix of inflammatory cytokines to reach a level that is favorable yet balanced in regard to (i) mounting an immune response against the *T. cruzi* parasite, whilst (ii) limiting cell, tissue and organ damage that results from self-perpetuated inflammation.

Our emerging view is that the metabolic syndrome represents only the late tail-end of the metabolic continuum. Excess deposits of fat, especially abdominal fat, pre-dispose to shortened life span [184, 185]. Reconciliation of our present finding suggests that adiposity determines statistical life-span i.e. overall "lease on life", but when the leaseholder is faced with a life threatening condition, adiposity helps rescue the individual in the short term (e.g. community acquired pneumonia or sepsis) or medium-term (e.g. tuberculosis or trypanosomiasis). Adiposity

is good in the near term, bad in the long term, a Faustian arrangement. Blaser et al. have gone further, suggesting that the metabolic syndrome may be a mechanism for culling the herd, accelerating the demise of the post-reproductive individuals who are in physical decline but still eating a full measure or more of food from the common. Possibly this program has evolved to be adaptive for the group, rescuing the young and accelerating death of the overweight post-reproductive set.

We have suggested that infections in general are associated with better survival in overweight patients, but also recognize that outcomes in some individual infections may be worse with obesity [186, 187]. These infections include H1N1 influenza A [188-191], and hepatitis B and C [192-195]. Overall, the influence of obesity on individual infectious diseases needs further study.

3.2 Conclusions

The last decades of the 20th century and the first part of the 21st has been characterized by an epidemic of obesity and an increase in the recognition of the negative health consequences of obesity, including the metabolic syndrome. In our study we reviewed epidemiologic studies where obesity brought with it better outcomes for a clutch of major infectious diseases including acute illnesses such as pneumonia, and sepsis and protracted illnesses such as TB, and Chagas disease. To show that the relationship was cause and effect related, we experimentally reproduced the benefit of obesity in our studies of mice with Chagas diseases; short term overfeeding of mice with a high fat diet enhanced survival.

Our finding that a high fat diet producing the metabolic syndrome markedly reduced mortality amongst mice infected with *T. cruzi* is consistent with our broadly based hypothesis that immune-metabolic interactions and the associated metabolic syndrome appear to protect against the consequences of many serious conditions such as sepsis, pneumonia and Chagas disease.

Introducing an evolutionary lens, we raise the possibility that extra nutrition with immune activation were highly beneficial in protecting the populace, especially the young, from the ravages of infectious illnesses that typically decimated young humans. Only within the last century in wealthy countries has clean water, immunizations, antibiotics and other public health advances turned the tide. Now with rampant obesity, longer life-spans, and infection control, the benefits of the added weight have diminished while the deficits have increased.

3.2.1 Future Paths

In the long run, obesity over decades is detrimental to health and longevity. We have catalogued infections and non-infectious conditions where in the short run and medium run, obesity was associated with a better outcome. (Dubbed the "obesity paradox", there are over 800 Pub Med citations linked to such conditions). Can we derive future therapeutic pathways from these findings? We raise the possibility that short term administration of modest doses of adipocyte-associated hormones to thin patients with select conditions may reproduce safely the advantage noted in some of the conditions linked to the obesity paradox. To support this suggestion, we turn to patients with lipodystrophy. They are completely lacking adipose tissue and as expected have an absence of fat cell–derived hormone leptin. They also have hypertriglyceridemia and severe insulin resistance, usually accompanied by diabetes mellitus. Replacement therapy with leptin alone resolves these abnormalities [196]. That replacement of only one fat cell hormone in fat cell deficient patients was enough to restore patient to metabolic health provides us with a potential model --- In select conditions, can safe doses of one or a few adipocyte-derived messenger molecules to thin patients enhance outcomes?

In the previous paragraph, we proposed unbundling adiposity – selecting one or a very small fraction of hormones associated with obesity to manipulate therapeutically. As noted in Table 5, obesity is associated with elevated levels of many secretory products and diminished levels of a small number, e.g. adiponectin.

We favor exploring (initially in animal models) each condition where outcomes in the obese differ from the thin to determine the value of adding or blocking one or a few fat cell–associated hormone-like agents.

In implementing these experimental approaches, several cautions should be front and center

1) Abnormalities brought on by serious hyperglycemia may mask benefits

2) Obesity brings with it many abnormalities, of which the hormonal ones are but a part.

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