FLINDERS UNIVERSITY OF SOUTH AUSTRALIA

THE ROLE OF SIRTUIN 1 DURING HIGH-FAT FEEDING

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DECLARATION

I hereby certify that this thesis entitles “The Role of Sirtuin 1 During High-Fat Feeding” does not contain material which has been accepted for the award of any degree or diploma; and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text of this thesis.

Kamelya Aliakbari

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADP	Adenosine diphosphate ribose
Akt	Protein kinase B
BAC	Bacterial artificial chromosome
BAX	Apoptosis regulator gene
BMI	Body mass index
bp	Base pair
cDNA	Complementary DNA
cGMP	Cyclic guanosine monophosphate
Ct	Cycle threshold
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DTT	Dithiothreitol
Duox1	Dual oxidase 1
Duox2	Dual oxidase 2
E	Efficiency
eNOS	Endothelial nitric oxide synthase
FOXO	Forkhead box O3
GADD45	Growth arrest and DNA-damage-inducible protein
GOI	Gene of interest
HDL	High density lipoprotein
HFD	High fat diet
H2O2	Hydrogen peroxide
IDT	Integrated DNA Technology
iNOS	Inducible nitric oxide synthase
kDa	The unified atomic mass unit
MnSOD	Extracellular superoxide dismutase
NAD^+	Nicotinamide adenine dinucleotide (Oxidised)
NADH	Nicotinamide adenine dinucleotide (Reduced)
NADPH	Nicotinamide adenine dinucleotide phosphate
NC	Normal chow
NCF1	Neutrophil cytosol factor 1
NO	Nitric oxide
NOSs	Nitric oxide synthase family of enzymes
Nox1	NADPH oxidase 1
Nox2	NADPH oxidase 2
Nox3	NADPH oxidase 3
Nox4	NADPH oxidase 4
Nox5	NADPH oxidase 5

O_2	Molecular oxygen
$O_2•^-$	Superoxide
OH•	Hydroxyl radicals
$ONOO^-$	Proxynitrate
PAGE	Polyacrylamide gel electrophoresis
PARP-1	Poly adenosine diphosphate ribose polymerase 1
PBS	Phosphate buffered saline
PCNA	Proliferating cell nuclear antigen
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα	Peroxisome proliferator-activated receptor alpha
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PUMA	p53 upregulated modulator of apoptosis
PVDF	Polyvinylidene fluoride
p21	Cyclin-dependent kinase inhibitor 1
p53	Tumor suppressor p53
REF	Reference gene
RIN	RNA integrity number
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
SEM	Standard standard error of the mean
SREBP1	Sterol regulatory element-binding transcription factor 1
SIRT1	Sirtuin 1
SIRT1-KI	Sirtuin 1 knockin mice
SOD	Superoxide dismutase
SOD1	Cytosolic superoxide dismutase
SOD2	Extracellular superoxide dismutase
SOD3	Mitochondrial superoxide dismutase
TBE	Tris-borate-EDTA
TNF-α	Tumor necrosis factor alpha
UCP2	Uncoupling protein 2
WAT	White adipose tissue
WHO	World Health Organisation
WT	Wildtype

1. ABSTRACT

Background: More than half of the Australian adult population is overweight or obese. High-fat feeding is the main culprit for these staggering statistics. Oxidative stress, inflammation and decreased nitric oxide bioavailability in obese patients increase their likelihood of developing stroke or dementia.

Purpose: Sirtuin 1 (SIRT1) is a protein deacetylase with known antioxidant properties and ability to enhance nitric oxide bioavailability. Studies from our laboratory and others have shown that high-fat feeding leads to SIRT1 depletion within the vasculature. In the present study, we tested the hypothesis that SIRT1 overexpression during high-fat feeding would attenuate the phenotypes of vascular ageing, including inflammation and oxidative stress.

Methods: Wildtype (WT) and SIRT1 overexpressing mice (SIRT1-KI) were fed either a normal diet or high-fat diet for two months. At the end of the study, whole blood, plasma and vascular samples were obtained and stored for analysis.

Results: WT mice on a high-fat diet displayed decreased SIRT1 protein expression and increased nitrotyrosine expression, as measured in the carotid artery, which were both prevented in SIRT1-KI mice.

Conclusions: Our results highlight the potential benefits of targeting SIRT1 as a therapeutic strategy in reducing the clinical complications associated with vascular impairment during high-fat feeding.