Abstract

Cardiac Fatty acids β-oxidation (FAO) markedly increases during maturation and become the major source of energy for the adult heart. However, excessive rates of FAO can compromise cardiac function in obesity, diabetes, and heart failure. Lysine acetylation has recently been identified as a potentially important pathway involved in the control of energy metabolism. In mitochondria, this post-translational acetylation is catalyzed by general control of amino acid synthesis 5-like 1 (GCN5L1), while SIRT3 is a major deacetylase. Despite the fact that a number of FAO enzymes can be acetylated, whether this post-translational modification activates or inhibits FAO enzymes is a matter of debate. We therefore examined the role of lysine acetylation on cardiac FAO during maturation (1-day, 7-day, and 21-day old rabbit), and in hearts from high fat (HF) diet-induced obese mice, and abdominal aortic constriction (AAC)-induced HF obese mice. Cardiac FAO rates were significantly increased during maturation. Activities of the FAO enzymes, long chain acyl CoA dehydrogenase (LCAD) and β-hydroxyacyl CoA dehydrogenase (β-HAD) were increased in hearts from 7-, and 21-day vs 1-day old rabbits, and were associated with LCAD and β-HAD hyperacetylation. Increased overall myocardial protein acetylation during maturation was associated with increased mitochondrial acetyltransferase GCN5L1 expression, while expression of the mitochondrial deacetylase, SIRT3, did not change. Increased expression of the nuclear deacetylase SIRT1 and

a decrease in cardiac SIRT6 expression during maturation was accompanied by decreased acetylation of peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC-1α) and increased ATP production. Acetylation of the glycolytic enzymes hexokinase (HK) and phosphoglycerate mutase (PGM) were increased; scenarios consistent with the decrease in glycolytic activity seen in 7-, and 21-day vs. 1-day old hearts. High FAO rates in isolated working hearts from diet-induced obese mice was found to be positively correlated with an increase in activity of the FAO enzymes, LCAD and β-HAD. An increase in LCAD and β-HAD acetylation was also observed, which was associated with a decrease in SIRT3 expression and no change in the expression of GCN5L1. To further examine the role of acetylation in regulating FAO, we used SIRT3 KO mice to investigate the role of lysine acetylation on cardiac FAO. Although cardiac work was similar, cardiac FAO rates were increased in SIRT3 KO versus WT mice (422±29 vs 291±17 nmol.g dry $wt^{-1}.min^{-1}$, respectively, $p<0.05$). This was accompanied by a decrease in glucose oxidation rates in SIRT3 KO versus WT mice (1262±121 vs 1983±174 nmol.g dry $wt^{-1}.min^{-1}$, respectively, $p<0.05$). Cardiac lysine acetylation was increased in SIRT3 KO mice compared to WT mice, which included an increased acetylation and activity of LCAD and β-HAD. Obesity and heart failure were induced in mice by feeding a HF diet (60% kcal from fat) for 4 weeks and producing an AAC. At 4 wk post-AAC, mice were either switched to a low-

fat (LF) diet (4% kcal from fat; HF AAC LF) or maintained on a HF Diet (HF AAC HF) for a further 10 wk period. After 18 weeks, HF AAC HF mice weighed significantly higher than HF AAC LF mice. Cardiac hypertrophy evident in HF AAC HF mice was associated with SIRT1 mediated inhibition of FoxO1 while activation of FoxO1 and AMPK blunted this effect in HF AAC LF mice. In isolated working hearts, insulin stimulated glucose oxidation rates were also significantly increased in HF AAC LF hearts, compared to HF AAC HF hearts. Activity of the fatty acid β-oxidation enzyme, LCAD was significantly increased in hearts from HF AAC HF vs HF AAC LF mice, and was associated with LCAD hyperacetylation. Heart failure observed in vivo by echocardiography in HF AAC HF was significantly improved in HF AAC LF mice. These results suggest a key regulatory role of acetylation in controlling cardiac energy metabolism. However, we conclude that lysine acetylation increases, rather than decreases, FAO in the heart.

Dedication

I dedicate this thesis to my parents, Yousef and Heyam, to my beautiful wife, Maryam, and our little angel, Rital. This thesis is also dedicated to my sisters, Asma, Tamador, Isra and brothers, Mohammed and Asem.

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List of Abbreviations

FAO	Fatty Acid Oxidation
CoA	Coenzyme A
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
HFD	High Fat Diet
AAC	Abdominal Aortic Constriction
LCAD	Long Chain Acyl CoA Dehydrogenase
β-HAD	Beta-Hydroxy Acyl CoA Dehydrogenase
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1 alpha
GLUT4	Glucose Transporter Type 4
HK	Hexokinase
PGM	Phosphoglycerate Mutase
LDH	Lactate Dehydrogenase
HIF1α	Hypoxia Inducible Factor 1 Alpha
MPC	Mitochondrial Pyruvate Carrier
PDH	Pyruvate Dehydrogenase
PDK	Pyruvate Dehydrogenase Kinase
PDHP	Pyruvate Dehydrogenase Phosphatase
IR	Insulin Receptor
IRS	Insulin Receptor Substrate
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PIP3	Phosphatidylinositol 3-phosphate
PH	Pleckstrin Homology
PDK1	Phosphoinositide dependent protein kinase 1
Akt (PKB)	Protein Kinase B
PPARs	Peroxisome Proliferator Activated Receptors
FACS	Fatty Acyl CoA Synthetase
CPT1	Carnitine Palmitoyltransferase 1

3-KAT	**3-ketoacyl CoA Thiolase**
PFK1	**Phosphofructokinase 1**
FFA	**Free Fatty Acids**
FABP	**Fatty Acids Binding Protein**
FATP	**Fatty Acids Transporter Protein**
ACS	**Acyl CoA Synthase**
CrAT	**Carnitine Acetyltransferase**
VLDL	**Very Density Lipoproteins**
TAG	**Triacylglycerol**
LPL	**Lipoprotein Lipase**
ATGL	**Adipose Triglyceride Lipase**
HSL	**Hormone Sensitive Lipase**
MGL	**Monoglyceride Lipase**
MAG	**Monoacylglycerol**
HSLAS	**Health Sciences Lab Animal Services**
OGTT	**Oral Glucose Tolerance Test**
AUC	**Area Under the Curve**
LV	**Left Ventricle**
AMPK	**Adenosine Monophosphate Activated Protein Kinase**
MCD	**Malonyl CoA Decarboxylase**
$MCD^{-/-}$	**Malonyl CoA Decarboxylase Knockout**
GSK3β	**Glycogen Synthase 3 Beta**
ERRα	**Estrogen Related Receptor Alpha**
PKC	**Protein Kinase C**
P70S6K	**Ribosomal Protein S6 Kinase**
mTOR	**Mammalian Target of Rapamycin**
FoxO	**Forkhead box O**
AICAR	**Aminoimidazole-4-Carboxamide Ribonucleotide**
P38MAPK	**P38 Mitogen Activated Protein Kinase**
TCA	**Tricarboxylic Acid**
ETC	**Electron Transport Chain**

FAD	**Flavin Adenine Dinucleotide**
GCN5	**General Control of Amino Acids 5**
GCN5L1	**General Control of Amino Acids 5 Like Protein 1**
SIRT	**Silent mating type Information Regulation 2 homolog**
HDAC	**Histone Deacetylase**
HAT	**Histone Acetyl Transferase**
NAD	**Nicotinamide Adenine Dinucleotide**
TSA	**Trichostatin A**
NAM	**Nicotinamide**
DIO	**Diet Induced Obesity**
TAC	**Transverse Aortic Constriction**
AAC	**Abdominal Aortic Constriction**
HF-AAC-HF	**High Fat- Abdominal Aortic Constriction-High Fat**
HF-AAC-LF	**High Fat- Abdominal Aortic Constriction-Low Fat**
BMI	**Body Mass Index**
TDI	**Tissue Doppler Imaging**
E/A	**Early to Late ventricular filling**
E'	**Mitral annulus early diastolic velocity**
A'	**Late diastolic mitral annulus velocity**
HW	**Heart Weight**
TL	**Tibia Length**