Insulin sensitivity and nutrient utilisation in skeletal muscle

by

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the Degree of Doctor of Philosophy

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Summary

Obesity is a condition in which fat accumulation in adipose tissue is in excess to an extent that health may be impaired. Insulin resistance is integral to the pathophysiology of obesity-related metabolic complications. Central adiposity and skeletal muscle mass and function determine insulin sensitivity and metabolic risk. A high visceral fat-to-skeletal muscle mass-ratio contributes to an unfavourable metabolic profile.

Epidemiological and experimental studies suggest that high dietary saturated fat intake is deleterious while polyunsaturated fatty acids (PUFAs), in particular n-3 PUFAs of marine origin, may be advantageous to metabolic health.

The aim was to determine the effect of subcutaneous (SC) and visceral (IAB) fat, and long-chain saturated, n-3 and n-6 PUFAs, and the interactions between them, on insulin sensitivity and the pathways regulating energy metabolism in skeletal muscle. Thereby an adipose tissue-conditioned media-skeletal muscle myotube co-culture system was developed.

Adipose tissue-conditioned medium (CM) was generated from SC and IAB fat biopsy of obese humans. Viability of the tissue explants was confirmed by the measurement of lactate dehydrogenase activity in the CM and nuclear DNA fragmentation of tissue explants. The concentrations of cytokines (leptin, adiponectin, interleukin (IL)-1β, IL-

6, IL-8, tumor necrosis factor-α, resistin and plasminogen activator inhibitor-1) and long-chain fatty acids were determined in CM.

CM from IAB but not SC fat reduced insulin-stimulated glucose uptake. The effect of IAB fat was predominantly mediated by IL-6 via the activation of a nuclear factor kappa B/mammalian target of rapamycin complex 1 (NFκB/mTORC1)-dependent pathway.

Palmitic acid (PA; 16:0) reduced insulin-stimulated glucose uptake, an effect mediated by intramuscular accumulation of ceramide and the activation of NFκB and mTORC1. The effects of fatty acids were similar in the presence of CM from either fat depot, where the effect of PA was partially reversed by docosahexaenoic acid (DHA; 22:6n-3) and completely by linoleic acid (LA; 18:2n-6).

The effect of each fatty acid in the presence or absence of CM from each fat depot on mRNA expression of key genes regulating muscle energy metabolism was determined. Protein phosphorylation of adenosine monophosphate-activated protein kinase (AMPK)-α and acetyl-coenzyme A carboxylase (ACC)-β were also determined. PA increased SCD1 mRNA. DHA and LA increased AMPKα2 mRNA and AMPKα and ACCβ protein phosphorylation.

Microarray analysis was used to determine the global gene expression changes in PA- and DHA-treated L6 myotubes. DHA down-regulated lipogenic genes and up-regulated genes which were involved in β-oxidation and mitochondrial function.

When compared to PA, DHA down-regulated genes which were involved in lipid synthesis, endoplasmic reticulum metabolism and mitogen-activated protein kinase activity.

Taken together, pro-inflammatory cytokines from IAB fat and PA induced insulin resistance in skeletal muscle and both were at least partly mediated by a NFκB/mTORC1-dependent pathway. In contrast, DHA and LA may improve insulin sensitivity by diverting fatty acids towards oxidation and subsequently reducing substrate availability for the formation of lipid metabolites including ceramide. A reduction in PA intake and substitution (rather than addition) of DHA and LA, together with a reduction in overall energy intake and increase in physical activity, is optimal for metabolic health.

Declaration

Name: Yan Yan Lam Degree Program: Doctor of Philosophy

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Publications

Manuscripts submitted for publication:

1. Lam, Y.Y., Janovská, A., McAinch, A.J., Belobrajdic, D.P., Hatzinikolas, G., Game, P., Wittert, G.A. Adipokines from visceral fat inhibit insulin-stimulated glucose uptake in skeletal muscle cells: the roles of the nuclear factor kappa B and mammalian target of rapamycin pathways.

2. Lam, Y.Y., Janovská, A., McAinch, A.J., Hatzinikolas, G., Cavuoto, P., Game, P., Wittert, G.A. Insulin-stimulated glucose uptake and pathways regulating energy metabolism in skeletal muscle cells: the effects of subcutaneous and visceral fat, and long-chain saturated, n-3 and n-6 polyunsaturated fatty acids.

Manuscripts in preparation:

1. Lam, Y.Y., Buchanan, G., Heilbronn L.K., Hatzinikolas, G., Wittert, G.A. The gene expression profile of skeletal muscle cells in response to long-chain saturated and n-3 polyunsaturated fatty acids: implications for nutrition, health and disease prevention.

2. Lam, Y.Y., Wittert, G.A. Review: The effects of free fatty acids on insulin sensitivity and energy metabolism in skeletal muscle.

3. Lam, Y.Y., Wittert, G.A. Review: *In vitro* models to study the relationship between central obesity, inflammation and insulin resistance in skeletal muscle.

Abbreviations

AA	Arachidonic acid
ACC	Acetyl-coenzyme A carboxylase
AGPAT	1-acylglycerol-3-phosphate acyltransferase
AICAR	5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
aPKC	Atypical protein kinase C
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
BH	Benjamini & Hochberg
BMI	Body mass index
BP	Biological process
BSA	Bovine serum albumin
CACT	Carnitine-acylcarnitine translocase
CBP	cAMP-responsive element-binding protein-binding protein
CC	Cellular component
CM	Adipose tissue-conditioned medium
cm	Centimetre
CO_2	Carbon dioxide
CoA	Coenzyme A
CPT	Carnitine palmitoyltransferase
C_T	Threshold cycle
Ctrl	Control
DAG	Diacylglycerol
DEPC	Diethyl pyrocarbonate
DGAT	Diacylglyceroltransferase
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
dSAT	Deep subcutaneous adipose tissue
dsDNA	Double-stranded DNA
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid disodium salt dihydrate
EGTA	Ethylene glycol-bis (2-aminoethylether) *–N,N,N',N'*-tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
FABPpm	Plasma membrane fatty acid-binding protein
FAME	Fatty acid methyl ester
FAS	Fatty acid synthase
FAT/CD36	Fatty acid translocase
FATP	Fatty acid transport protein
FBS	Fetal bovine serum
g	Gram
G-6-P	Glucose-6-phosphate
GLUT	Glucose transporter
GO	Gene Ontology
GPAT	Glycerol-3-phosphate acyltransferase
h	Hour
HCl	Hydrochloric acid
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
HS	Horse serum
IAB	Visceral
IκB	Inhibitor protein inhibitor kappa B
IKK	Inhibitor of kappa B kinase
IL	Interleukin
IR	Insulin receptor
IRS	Insulin receptor substrate

JAK	Janus kinase
JNK	c-Jun NH2-terminal kinase
kb	Kilobase
kg	Kilogram
L	Litre
LA	Linoleic acid
LDH	Lactate dehydrogenase
LPL	Lipoprotein lipase
m	Metre
M	Molar
MAPK	Mitogen-activated protein kinase
MCP	Monocyte chemoattractant protein
MEM	Minimal Essential Medium
MEK	Mitogen-activated protein kinase kinase
MF	Molecular function
µg	Microgram
mg	Milligram
min	Minute
µl	Microlitre
ml	Millilitre
µM	Micromolar
mM	Millimolar
mm	Millimetre
µm	Micrometre
mmol	Millimol
mRNA	Messenger ribonucleic acid
mTORC1	Mammalian target of rapamycin complex 1
mU	Milliunit
MUFA	Monounsaturated fatty acid
NaF	Sodium fluoride
NaOH	Sodium hydroxide
NaPPi	Sodium pyrophosphate tetrabasic decahydrate

NFκB	Nuclear factor kappa B
nPKC	Novel protein kinase C
ng	Nanogram
nM	Nanomolar
nm	Nanometre
PA	Palmitic acid
PAI	Plasminogen activator inhibitor
PAP	Phosphatidate phosphohydrolase
PBS	Phosphate buffered saline
PCA	Principal Components Analysis
PDC	Pyruvate dehydrogenase complex
PDK	Pyruvate dehydrogenase kinase
PDP	Pyruvate dehydrogenase phosphatase
PDPK	3-phosphoinositide-dependent protein kinase
PDTC	Pyrrolidinedithiocarbamate
PFK	Phosphofructokinase
PGC	Peroxisome proliferator-activated receptor-γ coactivator
PIP2	Phosphatidylinositol(4,5)-bisphosphate
PIP3	Phosphatidylinositol(3,4,5)-trisphosphate
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PMSF	Phenylmethylsulfonyl fluoride
PP	Protein phosphatase
PPAR	Peroxisome-proliferator-activated receptor
PTP	Protein-tyrosine phosphatase
PUFA	Polyunsaturated fatty acid
RIN	RNA Integrity Number
RMA	Robust Multi-array Average
RNA	Ribonucleic acid
RNAse	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute

RT-PCR	‘Real Time’ Polymerase Chain-Reaction
SAPK	Stress-activated protein kinase
SBTI	Soybean trypsin inhibitor
SC	Subcutaneous
SCD	Stearoy-coenzyme A desaturase
sec	Second
SEM	Standard error of mean
SFA	Saturated fatty acid
SOCS	Suppressor of cytokine signalling
SPT	Serine palmitoyltransferase
SREBP	Sterol regulatory element binding protein
sSAT	Superficial subcutaneous adipose tissue
STAT	Signal transducers and activators of transcription
TBE	Tris-borate-EDTA
TCA	Tricarboxylic acid
TNF	Tumor necrosis factor
TSC	Tuberous sclerosis complex
U	Unit
UCP	Uncoupling protein
UV	Ultraviolet
pg	Picogram
V	Volt
v	Volume
w	Weight
w.w.	Wet weight
WHR	Waist-to-hip ratio
yr	Year

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