ENDOCANNABINOIDS AND SKELETAL MUSCLE GLUCOSE UPTAKE

A Thesis Submitted by

Paul Cavuoto

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THESIS SUMMARY

Obesity is a risk factor for type 2 diabetes mellitus and cardiovascular disease. Obesity, in particular when the fat is predominantly visceral, is associated with insulin resistance and a reduced ability to increase the rate of fat oxidation in response to an increase in dietary fat intake. Skeletal muscle is a primary site for insulin-stimulated glucose uptake. Insulin responsiveness in skeletal muscle is regulated by a number of factors including growth hormone, cortisol, sex steroids, cytokines secreted by inflammatory cells and adipocytes, fatty acids, and fatty acid derivatives such as the endocannabinoids.

The most abundant endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are synthesised from arachidonic acid. They have autocrine or paracrine mechanisms of action which are rapidly terminated by cellular uptake and subsequent metabolism by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) which degrades AEA and 2-AG, respectively. AEA and 2-AG are ligands for the cannabinoid receptor type 1 (CB_1) and the cannabinoid receptor type 2 (CB_2); both are 7 transmembrane domain G-protein coupled receptors. AEA and 2-AG also bind to the transient receptor potential channel-vanilloid sub-family member 1 (TRPV1). TRPV1 is a putative six-transmembrane domain protein with a pore region between segments five and six

and cytoplasmic N and C termini. TRPV1 was initially discovered as a receptor for capsaicin, the main pungent component of hot chilli. Activation of TRPV1 leads to an increase in intracellular calcium either by entry through the plasma membrane or through calcium release of intracellular stores.

Endocannabinoids and their receptors form part of an endogenous system that regulates a number of homeostatic functions, including food intake (appetite and motivation to eat via effects in the hypothalamus and nucleus accumbens shell), the regulation of fat mass and intermediary metabolism. An overactivity of the endocannabinoid system in obesity may serve to maintain fat mass and may also underlie some of the associated metabolic consequences. Several studies have shown that inhibition of CB_1 in obese animal models improved the metabolic profile and reversed the deleterious effects of obesity on metabolism. The majority of this data was based on the effects of endocannabinoids on adipose tissue and liver. The studies that form the basis of this thesis examined the effect of endocannabinoids on glucose uptake and metabolism in skeletal muscle.

It was initially shown that CB_1 inhibition improves basal glucose uptake in primary cultures obtained from obese, but not lean humans. This is consistent with the notion of an "overactive endocannabinoid system" apparent even in the *ex-vivo* system of primary culture (**Chapter 3**). These data could not however all be

explained by the presence of a single type of endocannabinoid receptor in skeletal muscle. In a series of studies messenger RNA for CB_1, CB_2, TRPV1 and the enzyme FAAH was shown to be present in human and rat skeletal muscle biopsies, primary cultures of human skeletal muscle and a rat skeletal muscle cell line (L6) (**Chapter 4**).

Subsequent experiments to determine the effect of endocannabinoids on basal and insulin-stimulated glucose uptake and receptors mediating these effects were performed in L6 cells (**Chapter 5**). Chronic (24 h), but not acute (30 min) exposure to AEA and 2-AG increased insulin-stimulated glucose uptake and the effect of 2-AG was greater than that of AEA. 2-AG was used in subsequent studies. 2-AG-mediated glucose uptake was ameliorated by inhibition of CB1 (SR141716), CB_2 (SR144528) or TRPV1 (SB366791) with no additional effect when more than one receptor was blocked concurrently. These studies are the first to demonstrate the presence of TRPV1 in skeletal muscle and that it has a role in glucose regulation.

To investigate a role for TRPV1 on glucose metabolism *in vivo*, targeted mutant mice with a deletion of the TRPV1 gene were utilised. The studies described in **Chapter 6** measured glucose tolerance in $TRPV1^{-/-}$ mice in comparison to wild-type mice in response to a standard or high fat diet (HFD) via intraperitoneal glucose tolerance testing. At baseline the $TRPV1^{-/-}$ mice were able to clear a glucose load

more efficiently than their wild-type counterparts. After 18 weeks of high fat feeding, body weight of the wild-type mice increased significantly and glucose tolerance was impaired. In contrast, the TRPV1$^{-/-}$ mice were resistant to diet induced obesity, but their glucose tolerance was similar to that of the wild-type mice. The reason for the discrepancy between adiposity and glucose tolerance is unknown, however, *in vitro* studies describing an effect of endocannabinoids to increase insulin-stimulated glucose uptake via TRPV1 suggests a role for this receptor in the regulation of glucose utilisation. The novel observations relating to TRPV1 offer a new perspective on endocannabinoid mediated effects on peripheral metabolism with potential therapeutic implications. Further studies are required to determine the relationship between the effects of endocannabinoids on peripheral metabolism and the emerging role of TRPV1 in diabetes and obesity.

THESIS DECLARATION

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PUBLICATIONS ARISING FROM THIS THESIS

PUBLISHED MANUSCRIPTS

Cavuoto P, McAinch AJ, Hatzinikolas G, Janovská A, Game P, Wittert GA. *The expression of receptors for endocannabinoids in human and rodent skeletal muscle.* Biochemical and Biophysical Research Communications, 2007, **364**(1): 105-110.

Cavuoto P, Wittert GA. *The role of the endocannabinoid system in the regulation of energy expenditure.* Best Practice & Research: Clinical Endocrinology & Metabolism, 2009, **23**(1): 79-86.

MANUSCRIPTS IN PREPARATION

Cavuoto P, McAinch AJ, Janovská A, Hatzinikolas G, Lam YY, Cameron-Smith D, Wittert GA. *SR141716, a selective CB1 inverse agonist, increases basal glucose uptake in human skeletal muscle myotubes derived from obese patients.*

Cavuoto P, Wittert GA, Janovska A, Lam YY, Hatzinikolas G, Blackshaw LA. *TRPV1 Mediates Discordant Effects on the Regulation of Fat Mass and Glucose Metabolism*.

OTHER MANUSCRIPTS IN PREPARATION

Lam YY, Janovská A, McAinch AJ, Hatzinikolas G, **Cavuoto P**, Game P, Wittert GA. *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*.

PUBLISHED ABSTRACTS

Cavuoto P, McAinch AJ, Hatzinikolas G, Wittert GA. *Effects of cannabinoid receptors on skeletal muscle oxidative pathways.* Obesity Reviews, 2006, **7**(supp.2): 130.

Wittert GA, **Cavuoto P**, Hatzinikolas G, Blackshaw LA. *TRPV1 Mediates Discordant Effects on the Regulation of Fat Mass and Glucose Metabolism.* Diabetes, 2010, **59**(supp.1A): LB31.

CONFERENCE PRESENTATIONS

ORAL PRESENTATIONS

Cavuoto P, McAinch AJ, Janovská A, Hatzinikolas G, Lam YY, Cameron-Smith D, Wittert GA. *The effect of the selective CB1 inverse agonist SR141716 on human skeletal muscle myotubes*. The 2009 Post Graduate Research Expo (Finalist), Faculty of Health Sciences, University of Adelaide, Adelaide, Australia, September 1, 2009.

Cavuoto P, McAinch AJ, Janovská A, Hatzinikolas G, Lam YY, Cameron-Smith D, Wittert GA. *The effect of the first selective cb1 blocker rimonabant on human skeletal muscle myotube gene expression*. Centre of Clinical Research Excellence (CCRE) seminar, Discipline of Medicine, University of Adelaide, Adelaide, Australia, November 14, 2007.

Cavuoto P, McAinch AJ, Janovská A, Hatzinikolas G, Lam YY, Cameron-Smith D, Wittert GA. *The effect of the first selective CB1 blocker rimonabant on human skeletal muscle myotube gene expression* (Hot Topics). Australasian Society for the Study of Obesity 16th Annual Scientific Meeting, Canberra, Australia, August 31-September 2, 2007.

POSTER PRESENTATIONS

Wittert GA, **Cavuoto P**, Hatzinikolas G, Blackshaw LA. *TRPV1 mediates discordant effects on the regulation of fat mass and glucose metabolism.* The 70th Scientific Sessions, American Diabetes Association (ADA), Orlando, USA, 25-29 June 2010.

Cavuoto P, McAinch AJ, Janovská A, Hatzinikolas G, Lam YY, Cameron-Smith D, Wittert GA. *The effect of the selective CB1 inverse agonist SR141716 on human skeletal muscle myotubes.* The 2009 Australian Diabetes Society and Australian Diabetes Educators Association (ADS-ADEA) Annual Scientific Meeting, Adelaide, Australia, 26-28 August 2009.

Cavuoto P, Janovská A, Lam YY, Wittert GA. *Endocannabinoids and the regulation of insulin-stimulated glucose uptake in rodent skeletal muscle.* The 2008 Postgraduate Research Expo, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia, 23 July 2008.

Lam YY, McAinch AJ, Janovská A, Hatzinikolas G, **Cavuoto P**, Wittert GA. *An adipose tissue-myotube co-culture system to study nutrient utilisation in skeletal muscle cells.* The 2007 Postgraduate Research Expo, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia, 23 October 2007.

Cavuoto P, McAinch AJ, Janovská A, Hatzinikolas G, Lam YY, Cameron-Smith D, Wittert GA. *The effect of the selective CB1 blocker SR141716 on human skeletal muscle myotubes.* The 2007 Postgraduate Research Expo, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia, 23 October 2007.

Lam YY, McAinch AJ, Janovská A, Hatzinikolas G, **Cavuoto P**, Wittert GA. *An adipose tissue-myotube co-culture system to study nutrient utilisation in skeletal muscle cells.* Australasian Society for the Study of Obesity 16th Annual Scientific Meeting, Canberra, Australia, August 31-September 2, 2007.

McAinch AJ, **Cavuoto P**, Hatzinikolas G, Cameron-Smith D, Wittert GA. *The effect of the selective CB1 antagonist sr141716 on human skeletal muscle myotube gene*

expression. The 4th Asia-Oceania Conference on Obesity, Seoul, Korea, 9-11 February 2007.

LIST OF ABBREVIATIONS

Δ^9-THC	delta-9-tetrahydrocannabinol
2-AG	2-arachidonoyolglycerol
ACC	acetyl coenzyme A carboxylase
ACEA	arachidonyl-2′-chloroethylamide hydrate
ADTP	Australasian Digital Thesis Program
AEA	anandamide
AMPK	AMP-activated protein kinase
AUC	area under the curve
BAT	brown adipose tissue
BDNF	brain derived neurotrophic factor
BMI	body mass index
BP	base-pairs
CaMKK	calmodulin-dependent protein kinase kinase
cAMP	cyclic AMP
CB_1	cannabinoid receptor type 1
CB_2	cannabinoid receptor type 2
CGRP	calcitonin-gene-related peptide
CoA	Coenzyme A
CPT-1	carnitine palmitoyltransferase-1
C_T	critical threshold

DAG	diacylglycerol
DOG	deoxy-D-glucose
DRG	dorsal root ganglia
ECM	extra cellular matrix
ECs	endocannabinoids
EDL	extensor digitorum longus
eNOS	endothelial nitric oxide synthase
FAAH	fatty acid amide hydrolase
FA	fatty acid
FAF	fatty acid free
FBS	fetal bovine serum
FFAs	free fatty acids
G-6-P	glucose-6-phosphate
GC-MS	gas chromatography coupled to mass spectrometry
HEK	human embryonic kidney
HFD	high fat diet
HPLC	high pressure liquid chromatography
HS	horse serum
IR	insulin receptor
K^+_A	A-type potassium
K_{ir}	inwardly rectifying potassium
MAGL	monoacylglycerol lipase

MAPK	mitogen-activated protein kinase
MBH	mediobasal hypothalamus
MEF2C	myocyte enhancer factor 2C
NGF	nerve growth factor
NHS	National Health Survey
NRFs	nuclear respiratory factors
PDC	pyruvate dehydrogenase complex
PDKs	pyruvate dehydrogenase kinases
PDK4	pyruvate dehydrogenase kinase 4
PEA	*N*-palmitoylethanolamine
PGC-1α	peroxisome proliferator-activated receptor γ coactivator 1α
PhD	Doctor of Philosophy
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKB	protein kinase B (also known as Akt)
PKC	protein kinase C
PPARγ	peroxisome proliferator-activated receptor γ
PVDF	polyvinylidene difluoride
RT-PCR	reverse transcription polymerase chain reaction
RTX	resiniferatoxin
SF1	steroidogenic factor 1
SOL	soleus

SP	substance P
SREBP-1c	sterol-regulatory element-binding protein-1c
T2DM	type 2 diabetes mellitus
TBS/T	tris-buffered saline/0.1% Tween 20
TG	triglycerides
TRPV1	transient receptor potential channel-vanilloid sub-family member 1
VMH	ventro medial hypothalamus
WHO	World Health Organisation