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**Prediction of cellular ATP generation from foods
in the adult human - application to
developing specialist weight-loss foods**

**A thesis presented in partial fulfilment
of the requirements for the degree of**

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in
Nutritional Science**

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Abstract

For the accurate prediction of the potential 'available energy' of a food at the cellular level (i.e. ATP generation from food) it is necessary to be able to predict both the quantity and location of uptake (upper-tract or colon) for each energy-yielding nutrient. The objective was to develop a valid model ('Combined Model') for predicting the (potential) ATP available to the body from absorbed nutrients across the total digestive tract. The model was intended for the adult human under conditions where energy intake \leq energy expenditure and all absorbed nutrients are catabolised. The development of the model involved two parts: (i) the experimental development of a dual *in vivo* – *in vitro* digestibility assay ('dual digestibility assay') to predict human upper-tract nutrient digestibility, as modelled by the rat upper digestive tract, and colonic digestibility, as predicted by fermenting rat ileal digesta in an *in vitro* digestion system containing human faecal bacteria; and (ii) the development of a series of mathematical equations to predict the net ATP yielded during the post-absorptive catabolism of each absorbed nutrient at the cellular level.

A strong correlation ($r=0.953$, $P=0.047$) was found between total tract organic matter digestibility (OMD), as predicted with the newly developed dual *in vivo* – *in vitro* digestibility assay and with that determined in a metabolic study with humans for four mixed diets ranging considerably in nutrient content. There were no statistically significant ($P>0.05$) differences for mean OMD between the predicted and determined values for any of the diets.

The Combined Model (dual *in vivo* – *in vitro* digestibility assay + stoichiometric predictive equations) was applied to three meal replacement formulations and was successfully able to differentiate between the diets in terms of both energy digestibility and predicted ATP yields. When the energy content of each diet was compared to that of a baseline food (dextrin), some metabolisable energy (ME) models gave considerably different ratios compared to that predicted by the Combined Model. By way of example, for Diet C a ratio of 0.96 (Atwater and FDA models) was found

versus 0.75 (Combined Model). Thus, the model has practical application for predicting dietary available energy content, particularly in the research and development of specialised weight-loss foods, where it may be more accurate than some current ME models. Uniquely, the Combined Model is able to define a food in terms of ATP content (mol ATP / g food) using recent estimates of cellular P/O ratios and therefore, directly relates dietary energy intake to the quantity and form (ATP) of energy ultimately delivered at the cellular level.

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Abbreviations

ΔG	Free Energy
AA	Amino Acid
AA_d	Amino Acids Present In The Diet
AA_i	Amino Acids Present In The Ileal Digesta
ADH	Alcohol Dehydrogenase
ADP	Adenosine Diphosphate
Ala	Alanine
AMG	Amyloglucosidase
AMP	Adenosine Monophosphate
ANOVA	Analysis Of Variance
AOAC	Association Of Analytical Chemists
Arg	Arginine
Asn	Asparagine
Asp	Aspartic Acid
ATP	Adenosine Triphosphate
ATP_a	Available ATP (ATP Yield)
ATP_{AA}	ATP Yield From Amino Acids
ATP_d	ATP Cost Of Digestion
ATP_{FA}	ATP Yield From Fatty Acids
ATP_{net}	Net ATP Yield
ATP_{SCFA}	ATP Yield From Short Chain Fatty Acids
ATP_{ST+SU}	ATP Yield From Starch And Sugars
ATP_t	ATP Cost Of Absorption / Transportation
BMR	Basal Metabolic Rate
BW	Body Weight
CHO	Carbohydrate
CoA	Coenzyme A
CV	Coefficient Of Variation
Cys	Cysteine
dE	Digestibility Of Energy
DE	Digestible Energy
DF	Dietary Fibre

dHE	Increment Of Heat Energy
DIT	Dietary Induced Thermogenesis
DM	Dry Matter
DMD	Dry Matter Digestibility
ER	Endoplasmic Reticulum
EtOH	Ethanol
FA	Fatty Acid
FA_d	Fatty Acids Present In The Diet
FADH₂	Flavin Adenine Dinucleotide H ₂
FA_i	Fatty Acids Present In The Ileal Digesta
FAO	Food And Agriculture Organization
FDA	Food And Drug Administration
FE	Faecal Energy
FFA	Free Fatty Acid
FID	Flame Ionisation Detector
GaE	Gaseous Energy
GC	Gas Chromatography
GE	Gross Energy
GI	Gastrointestinal
GL	Glucose
Gln	Glutamine
Glu	Glutamic Acid
Gly	Glycine
GP	Glycerol Phosphate
GTP	Guanosine Triphosphate
GY	Glycerol
HE	Heat Energy
HF	High Fibre
His	Histidine
IE	Intake Energy
Ile	Isoleucine
Leu	Leucine
LF	Low Fibre
LPL	Lipoprotein Lipase

Lys	Lysine
ME	Metabolisable Energy
MEOS	Microsomal Ethanol Oxidising System
Met	Methionine
MF	Mixed Fibre
NADH	Nicotinamide Adenine Dinucleotide H
NADPH	Nicotinamide Adenine Dinucleotide Phosphate H
N_d	Nitrogen Present In The Diet
NDF	Neutral Detergent Fibre
NE	Net Energy
NEAT	Non-Exercise Activity Thermogenesis
NEFA	Non-Esterfied Fatty Acids
N_i	Nitrogen Present In The Ileal Digesta
NR	Not Reported
NSP	Non-Starch Polysaccharide
OFN	Oxygen-Free Nitrogen
OM	Organic Matter
OMD	Organic Matter Digestibility
OM_{D+F}	Organic Matter That Is Digested And Fermented By The Body
OM_i	Organic Matter Present In The Ileal Digesta
OM_{uf}	Unfermented Organic Matter At The End Of Incubation
P	P-Value (Probability)
PE	Pectin
PEG	Polyethylene Glycol
Phe	Phenylalanine
Pro	Proline
PSP	Phenolsulphonphthalein
PVTC	Post-Valve T-Caecum
r	Correlation Coefficient
RE	Retained Energy
RMR	Resting Metabolic Rate
RS	Resistant Starch
SAPU	Small Animal Production Unit
SCFA	Short Chain Fatty Acid

SE	Standard Error
SE	Surface Energy
SEM	Standard Error Of The Mean
Ser	Serine
SI	Le Système International D'unités
ST	Starch
ST_d	Starch Present In The Diet
ST_i	Starch Present In The Ileal Digesta
SU	Sugars
SU_d	Sugars Present In The Diet
SU_i	Sugars Present In The Ileal Digesta
TAG	Triacylglycerol
TAG_d	Triacylglycerol Present In The Diet
TAG_i	Triacylglycerol Present In The Ileal Digesta
TCA	Tricarboxylic Acid
TEE	Total Energy Expenditure
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
UC	Unavailable Carbohydrate
UCP	Uncoupling Protein
UE	Urinary Energy
UV	Ultraviolet
Val	Valine
VFA	Volatile Fatty Acid
VLDL	Very Low-Density Lipoprotein
WB	Wheat Bran
WHO	World Health Organization

Preface

After ingestion, the energy-providing nutrients in food (carbohydrate, fats, protein, and for some individuals, ethanol) undergo a series of catabolic reactions in the human digestive tract, and then (primarily) in hepatocytes to release energy from their chemical bonds. This energy then becomes available to the body, primarily in the form of ATP (the universal currency of chemical energy in the body) and is subsequently converted into other forms of energy such as mechanical energy, thermic energy and so on. However, not all of the energy present in ingested food is ultimately converted to ATP due to the energy requirements involved with the digestion, absorption and intermediary metabolism of food, which vary with the type of food and the nutrients ingested. Some energy is also lost through the heat of fermentation of undigested dietary material entering the large intestine. Furthermore, nutrients vary in their degree of digestibility and absorption (i.e. uptake from the gut) and the efficiency by which they yield energy that is ultimately useful to the body (net ATP gains), with the energy made available to the body via short chain fatty acids from nutrients fermented in the hindgut being less than that obtained from direct nutrient uptake in the upper-tract. For the accurate prediction of the potential 'available energy' (ATP) at the cellular level it is therefore important to be able to predict both the quantity and location of uptake (upper-tract or colon) for each nutrient. The use of metabolisable energy (ME) systems (e.g. Atwater system), as commonly used for food labelling purposes, may not be the most appropriate or accurate means of predicting the useful energy at the cellular level because amongst other weaknesses, ME systems do not account for the unique features of each diet, such as differences in digestibility or inter-nutrient interactions that may affect nutrient assimilation. A valid alternative means needs to be found to model and predict the available energy content of a food for the research and development of foods required to deliver a specific quantity of energy to the body at the cellular level, such as those specifically designed for weight-loss. The need for such foods is growing in importance due to the rapid increase in overweight and obese persons in recent years.