FLUOXETINE AND ENERGY EXPENDITURE IN OBESE HUMANS SUBJECTED TO ENERGY RESTRICTION

by

Rachelle Bross

Submitted to the
Faculty of Graduate Studies and Research
in partial fulfilment of the requirements
for the degree of Master of Science

at the

School of Dietetics and Human Nutrition
McGill University
Montreal, Quebec

May 1993

Name RACHELLE BROSS

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

NUTRITION

SUBJECT TERM

0570

SUBJECT CODE

U·M·I

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES

American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES

Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences

Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences

Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY

General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

THESIS SUBMISSION: SHORTENED VERSION OF THESIS TITLE

METABOLIC EFFECTS OF FLUOXETINE

TABLE OF CONTENTS

FLUOXETINE AND ENERGY EXPENDITURE IN OBESE HUMANS SUBJECTED TO ENERGY RESTRICTION

by Rachelle Bross

ABSTRACT

I investigated the effects of continuous administration of fluoxetine, a serotonin reuptake inhibitor, on energy expenditure, body temperature, and thyroid and catecholamine metabolism during weight reduction using a very low calorie diet (VLCD, Optifast, 1757 kJ/day) followed by a balanced deficit diet (BDD, 5016 kJ/day) Fluoxetine (60 mg/day by mouth, n=10) or placebo (n=10) were administered during 3 weeks of inpatient VLCD followed by 8 weeks of outpatient BDD in a double-blind, randomized design A similar amount of weight was lost in both groups during the VLCD, but by the end of the BDD total weight loss in the fluoxetine group was significantly greater (11 0 ± 1 1 kg vs 7.0 ± 1 0 kg, mean ± SEM, p<0 015) Resting metabolic rate (RMR) increased by 4 4 ± 1 8% (p< 0 01) in the fluoxetine group but did not change in the placebo group during the first week of the VLCD, but subsequently decreased significantly in both groups as dieting continued However, RMR remained consistently higher in the fluoxetine group for the duration of the VLCD period No further change in RMR occured in either group during the BDD period The thermic effect of food did not change after VLC dieting plus fluoxetine or placebo treatment Body temperature increased within 2 days of fluoxetine treatment by a mean of 0 3°C, p<0 025 and remained elevated throughout the VLCD but was unchanged in the placebo group VLCD therapy reduced serum levels of T_3, free T_3 Index and 24-hour urinary excretion of dopamine, norepinephrine, metanephrine and normetanephrine equivalently in both groups A thermogenic effect of fluoxetine is demonstrated in humans for the first time The anorectic effect of fluoxetine may be related to its temperature elevating effect.

FLUOXETINE ET DEPENSE ENERGETIQUE CHEZ DES SUJETS HUMAINS OBESES A LA DIETE

par Rachelle Bross

RESUME

Nous avons examiné les effets de l'administration continue de fluoxétine, agent inhibiteur du recaptage de la sérotonine, sur les dépenses énergétique, la température corporelle, les hormones thyroidiennes et le métabolisme des catécholamines pendant une perte de poids induite par un régime à très basses calories (RTBC, Optifast, 1 757 kJ/jour) suivi d'une diète hypocalorique équilibrée (DE, 5 016 kJ/jour) Des comprimés de fluoxétine (60 mg/jour par voie orale, n=10) ou de placebo (n=10) ont été administrés pendant 3 semaines aux patients hospitalisés suivant un RTBC puis pendant 8 semaines dans le cadre d'une DE en clinique externe organisé sous la forme d'un essai randomisé à double insu Pendant le RTBC, la quantité de poids perdu dans chaque groupe a été comparable mais à la fin du DE, c'est dans le groupe à qui avait été administrés des comprimés de fluoxétine que les pertes de poids ont été les plus importantes (moyenne ± SEM 11,0 ± 1,1 contre 7,0 ± 1,0 kg, $p<0.015$) Le métabolisme de repos (MR) a augmenté de 4,4 ± 1,8 % ($p<0.01$), dans le groupe avec fluoxétine mais n'a pas changé dans le groupe avec placebo pendant la première semaine du RTBC, pour décroître de façon marquée dans les deux groupes pendant la diète Toutefois, le MR a toujours été supérieure dans le groupe avec fluoxétine pendant toute la durée du RTBC Le MR n'a pas diminué pendant la DE, quel que soit le groupe L'effet thermique de l'alimentation n'a pas changé après le traitement au fluoxétine ou au placebo La température corporelle a augmenté en moyenne de 0,3°C, $p<0.025$ dans les deux jours qui ont suivi le début du traitement à base de fluoxétine et elle est restée élevée pendant toute la durée du RTBC, sans toutefois changer dans le groupe avec placebo Le RTBC s'est accompagné d'une diminution des concentrations sériques de T_3 et de l'indice de T_3 libre, l'excrétion urinaire sur 24 heures de dopamine, norépinéphrine, métanéphrine, et normétanephrine a été équivalente dans les deux groupes L'effet thermogène de la fluoxétine est ainsi démontré chez l'etre humain pour la première fois L'effet anorexigène de la fluoxétine est peut etre lié à l'effet que cette substance exerce sur la température corporelle

ACKNOWLEDGEMENTS

I would like to thank Dr L.J Hoffer, my Thesis Advisor, and Dr. K. Koski and Dr. E.B Marliss for their guidance and instruction

I thank the staff of the Clinical Investigation Unit (Marie-Josee Bertin and Mary Shingler, Research Nurses, Marla Myers, Research Dietitian) for their high quality of work and patient care.

Financial support for the C.I.U. was provided by the Royal Victoria Research Institute. Financial support for this project was provided by Eli Lilly, Canada, Inc.. Personal financial support from the Fonds pour la Formation de Chercheur et L'Aide à la Recherche is gratefully acknowledged

Sandoz Nutrition Inc provided Optifast 70 as a gift.

Dr. A Tavaroff and Dr R. Gougeon are gratefully acknowledged for their helpful comments

Finally, I thank Marylin Bross and Rebecca Mitchell for their assistance in typing this thesis.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

5-HT	- 5 Hydroxytryptophan
5-HTP	- 5 Hydroxytryptamine
ANOVA	- Analysis of Variance
ATP	- Adenosine Triphosphate
BCM	- Body Cell Mass
BDD	- Balanced Deficit Diet
BIA	- Bioelectrical Impedance
CIU	- Clinical Investigation Unit
CNS	- Central Nervous System
DIT	- Diet Induced Thermogenesis
ECF	- Extracellular Fluid
EE	- Energy Expenditure
F	- Fluoxetine
FFM	- Fat Free Mass
GH	- Growth Hormone
LBM	- Lean Body Mass
NE	- Norepinephrine
OGTT	- Oral Glucose Tolerance Test
P	- Placebo
PSMF	- Protein Sparing Modified Fast
RMR	- Resting Metabolic Rate
RNI	- Recommended Nutrient Intake
RQ	- Respiratory Quotient
SNS	- Sympathetic Nervous System
TBK	- Total Body Potassium
TEE	- Thermic Effect of Exercise
TEF	- Thermic Effect of Food
VLCD	- Very Low Energy Diet

INTRODUCTION

A reduction in the resting metabolic rate (RMR) is a consistent feature of energy restriction (Shetty, 1990) This effect is commonly ascribed to a combination of a reduction in the active tissue mass of the body, and an adaptive decrease in metabolic rate per unit of active tissue (Apfelbaum & Fricker, 1990; Benedict & Miles, 1919; Keys et al. 1950; Grande et al. 1958). The physiological mechanisms governing the reduction in metabolic rate per unit of active tissue are not fully understood, but are thought to include changes in sympathetic nervous system (SNS) activity and catecholamine secretion, thyroid hormone metabolism, and insulin secretion (Shetty, 1990)

The use of drugs to prevent the adaptive reduction in RMR during weight reduction is an attractive concept in obesity treatment, since this would make weight loss faster at any given energy intake. Fenfluramine, a serotonin-agonist drug long used to treat human obesity has recently been shown to increase the RMR of obese humans (Yen & Fuller, 1992), and to potentiate the thermic effect of food (TEF) in both animals (Levitsky et al. 1986b) and humans (Troiano et al 1990; Astrup et al 1992).

Fluoxetine HCl is a serotonin reuptake inhibitor currently in wide use as an antidepressant. In early clinical trials, depressed fluoxetine-treated patients were observed to lose weight. Subsequent trials in obese individuals who were not depressed have confirmed that fluoxetine potentiates weight loss (Yen & Fuller, 1992), this appears largely to be due to decreased food intake, but the mechanisms have not been elucidated in detail. The possibility that fluoxetine might increase weight loss through an effect on energy expenditure has not been previously studied. The present study was therefore designed to determine if fluoxetine has such an effect.

The text which follows includes a review of the basic concepts of energy balance and the determinants of energy expenditure. This is followed by a discussion of the effects of starvation

on energy balance and energy expenditure, an overview of the nutritional and pharmacological influences on energy expenditure, and finally by a rationale for and description of the research plan of the present study.

1. ENERGY BALANCE AND THE DETERMINANTS OF ENERGY EXPENDITURE

1.1. Definitions and Basic Concepts

1.1.1. Calorie (cal) — the amount of energy required to raise the temperature of 1 g of water from 3.5°C to 4.5°C

1.1.2. Kilocalorie (kcal) — the amount of energy required to raise the temperature of 1 kg of water by 1°C

1.1.3. Joule (J) — = 0.239 calories

1.1.4. Thermodynamics

The First Law of Thermodynamics is that energy can neither be created nor destroyed (Park et al. 1992; Brown & Brengelmann, 1965). The human body loses energy continuously to the environment in the form of heat; to sustain life, this energy must be restored The source of this energy is the chemical free energy in the highly structured carbon bonds in food. This energy is quantified by measuring the heat released when a known amount is oxidized in a bomb calorimeter This "heat of combustion" is expressed as kcal per gram. The heat of combustion of glucose is 3.75 kcal/g; of starch is 4.1 kcal/g; of protein is 5 4 kcal/g; of fat is 9 1 kcal/g and of ethanol is 7.1 kcal/g. The commonly accepted values for the usable energy contents of

carbohydrate, protein, fat and ethanol are 4, 4, 9 and 7 kcal/g respectively. They are lower than the total free energy content because of the variability in absorption of the nutrients, the hydration of glucose, the energy remaining in the nitrogenous end products of amino acid oxidation, and the volatility of ethanol which results in some loss from the body. In summary, the human body cannot consume or produce energy; it takes in energy from food, converts it into usable forms to perform life functions, and then passes it on to the environment (Brown & Brengelmann, 1965).

The Second Law of Thermodynamics is that the amount of entropy in a system and its surroundings always increases in a spontaneous reaction (Park et al. 1992; Brown & Brengelmann, 1965). When a nutrient is oxidized, part of its energy is thermodynamically obligated for conversion to heat since the heat energy content of the metabolic end products is greater than the free energy content of the initial nutrient. Man increases the entropy of his surroundings by discharging end products (increased energy) of metabolism of nutrient molecules (low entropy) ingested. The amount of heat lost is not large, with approximately 95% of ingested energy available as free energy. However, the conversion of food energy into usable high energy compounds (such as adenosine triphosphate) is not very efficient. With more than half of potentially available free energy is lost to the environment as heat. This heat loss is essential since it is the basis for unidirectionality of metabolic pathways and their regulators. The fraction of free energy retained in ATP in oxidative pathways is used to maintain life; this includes the maintenance of ionic gradients across membranes, biosynthesis of new molecules, internal work such as blood circulation and external work such as lifting a pen (Newsholme & Leech, 1989).

1.1.5. Energy Balance Energy balance is utilizable energy taken in minus energy expended. When energy in equals energy out, a person is said to be in neutral or zero energy balance. If

energy in is greater than energy out, a person is said to be in positive energy balance and is storing energy When energy in is less than energy out, a person is in negative energy balance and is oxidizing his/her endogenous stores to provide energy (Burszstein et al. 1989)

1.1.6. Energy Storage: When humans ingest carbohydrates, proteins, or fats, they either oxidize them immediately and use the energy released to do work, or transform them into forms that can be stored as potential energy (Burszstein et al 1989) The body stores energy as fat, glycogen and protein. When exogenous energy is not available, as occurs during an overnight fast, the body derives energy from its endogenous fuels (Garrow, 1978). Energy is stored mainly as fat A normal 70 kg man has about 13 kg of fat, which can yield approximately 125,000 kcal (525,000 Kj) on oxidation. These stores can theoretically allow an individual to survive for 50 days, given a daily energy requirement of 2500 kcal Body protein is also lost during a total fast (Danforth, 1985; Brown & Brengelmann, 1965). A normal man has about 1 kg of protein of which 7 kg are intracellular and 4 kg are extracellular. The extracellular or structural proteins in bone and connective tissue are very stable, and are considered to be unavailable for metabolic needs The intracellular proteins, which make up the body's active cell mass, serve many functions but are chiefly enzymes and contractile proteins, there are no storage proteins The estimated 11 kg of protein can potentially yield approximately 45000 kcal (188,000 kJ), or a maximum of 18 days' energy at 2500 kcal/day. However, due to the essential function of protein, loss of protein leads to adverse functional consequences which are conventionally considered to be the cause of death in long term starvation (Hoffer, 1993; Wu & Marliss, 1992) In addition to fat and protein, the body contains between 1000-3000 kcal of carbohydrates stored in the liver and muscles as glycogen. This is available for immediate energy needs, such as exercise, but is of little long-term value by virtue of its limited supply.

1 1 7 Energy Expenditure A large fraction of the energy liberated during substrate oxidation can be retained because reactions in the degradation pathways are linked to the formation of ATP "Energy expenditure" refers to the hydrolysis of ATP, or of other high-energy bonds. Each substrate/fuel has a different heat of combustion and generates a different amount of heat, per litre of oxygen used to burn it. For example, the oxidation of 1 mol (180 g) glucose involves the uptake of 6 mol (134 4 litres) of oxygen (O_2), and the production of 3 mol (134 4 litres) of carbon dioxide (CO_2), 6 mol (108 g) water and the liberation of 665 kcal (Garrow, 1978). The retained energy recovered in ATP (and other high-energy phosphate bonds) is then used for both internal and external work Each fuel also has a characteristic respiratory quotient (RQ), the volume of CO_2 divided by the volume of O_2 consumed for oxidation. The RQ for carbohydrate is 1.0, for fat is 0 7 and for protein 0 8 Despite the large differences in the heat of combustion and RQ for the different fuels, they all produce similar amounts of energy per litre of O_2 used, 5.05 kcal for carbohydrate, 4 46 for protein and 4 74 for fat For a person consuming an adequate mixed fuel diet, and hence with an RQ in the range of 0.75 - 0.85, energy production can be calculated by the following formula (Park et al 1992):

Energy production (kcal/minute) = 4.80 x O_2 (Litre/minute)

Under these conditions oxygen consumption can be used as an indicator of energy expenditure. This equation slightly underestimates energy production when the predominant fuel oxidized is carbohydrate (RQ = 1 0), as may occur during continuous feeding of a high carbohydrate diet and slightly overestimates it when fat is the predominant fuel oxidized (RQ = 0.7), as occurs during consumption of a all protein/carbohydrate free diet or total fasting (Simonson & DeFronzo, 1990) The abbreviated Weir equation (see section 1.3.2.2.) takes into account changes in the RQ and is therefore a more accurate measure of energy expenditure under different nutritional conditions (McClave & Snider, 1992)

1.2. Determinants of Energy Expenditure

Energy expenditure can be divided conceptually into three major components The largest component is the resting metabolic rate (RMR) which accounts for approximately 65-75% of total daily energy expenditure The second largest component is the thermic effect of exercise (TEE) which includes work done on the environment. The TEE of an individual not engaged in heavy labour accounts for 15-20% of total daily energy expenditure, but it can increase two fold with heavy exercise. The third component of energy expenditure is the thermic effect of food (TEF) or diet-induced thermogenesis (DIT) The TEF accounts for 10% of daily energy expenditure, but can vary depending on the amount of energy consumed and on the nutrient composition of the diet (Fugate et al. 1990; Woo et al. 1985; Danforth, 1985)

1.2.1 Resting Metabolic Rate

The RMR is the amount of energy expended by a resting individual in a thermoneutral environment without the effects of meal consumption, physical activity, or other physiological or mental stress (Burszstein et al. 1989; Danforth, 1985; Clark & Hoffer, 1991) The value may be slightly greater than the true basal metabolic rate which, in addition to the above conditions, is measured in the morning upon awakening after 12-18 hours of rest and includes the definition of conditions with respect to circadian rhythms (Garrow, 1978) The energy expended at rest includes the costs of maintaining the biochemical and structural integrity of the body, and the costs of performing internal work, ion pumps, synthesis and degradation of cell constituents, biochemical cycles, and leakage of protons across the mitochondrial membrane (Burszstein et al. 1989).

The RMR is influenced by a number of basic factors, including age, sex and body temperature (Owen, 1988; Burszstein et al. 1989) Much of the effect these factors have on the RMR relates to differences in body composition.

1.2.1.1. Age and RMR

Resting energy expenditure decreases with age. Reported energy requirements vary from 102 kcal (4270 kJ)/kg/day at age 1, to ≈34 kcal (140 kJ)/kg/day between age 30-60 and 20 kcal (85 kJ)/kg/day beyond age 60 (FAO/WHO/UNU, 1985). These variations relate to differences in the composition of the fat free mass and to differences in the total quantity of fat free mass (Weinsier et al. 1992; Holliday, 1971).

1.2.1.2. Sex and RMR

Men have a higher resting energy expenditure than women of the same height and weight (Owen et al 1986; Owen et al. 1987). This sex difference appears to be due to the greater lean tissue mass of males, which appears at age 3, but increases rapidly at puberty, when boys experience a relatively marked increase in skeletal muscles and girls experience a relatively greater increase in adipose tissue (Burszstein et al. 1989). These differences diminish with age, so that by age 70, a man requires only an additional 2.1 kcal (8 kJ)/kg/day as compared to a woman (Burszstein et al. 1989).

1.2.1.3. Body Temperature and RMR

Humans, being homeothermic, regulate their core temperature independently of environmental temperature Early in this century, DuBois (1954) concluded that metabolic rate increases by 13% per degree Celsius (7.2% per degree Fahrenheit) of body temperature when

the increase in temperature is not due to an increased insulation. A change in temperature is not an indicator of energy expenditure, but rather reflects the balance between heat production and dissipation. In the cold, energy expenditure increases to maintain a normal core temperature. When ambient temperature rises to 27°C, energy expenditure is at a minimum in normal subjects. Above this, energy is expended to bring about cooling by sweating or panting, and total energy expenditure increases. When the ambient temperature rises too high, cooling mechanisms become inadequate, body temperature rises and death eventually occurs Different adaptations occur when ambient temperature decreases. Energy expenditure increases to maintain a core temperature of 37°C, but again death will ultimately occur once these adaptive mechanisms are overwhelmed. Between these extremes in ambient temperature is a temperature zone at which energy expenditure is not influenced, termed the zone of thermal neutrality (Elwyn et al. 1981). Other deviations from thermoneutrality alter the RMR. For example, the slight increase in body temperature during the post-ovulatory phase (days 17-26) of the menstrual cycle has been reported to result in about a 5% increase in RMR (Webb, 1986).

Since 1915, when Benedict suggested that the "active body mass" determines the RMR, it has been recognized that the amount of fat free mass in the body (body mass minus total fat mass) is highly predictive of the RMR (Owen, 1988). Fat free mass is thought to most closely reflect the active body mass, but controversy over the proper reference for the active body mass continues up to the present day. Even though RMR and FFM are strongly correlated, the correlation is not linear in normal weight individuals, probably because of the varying compositions of the FFM at different total fat-free masses (Weinsier et al 1992). According to Holliday (Sims & Danforth, 1987), the average RMR of skeletal muscles is 73.7 kJ kg^{-1} d^{-1} (17 6 kcal) and that of visceral organs (brain, heart, kidney and liver) is 1497.2 kJ kg^{-1} d^{-1} (357 7 kcal). During growth the FFM increases, with muscle mass increasing more rapidly than organ mass

(Weinsier et al. 1992; Holliday, 1971). By adulthood, the less metabolically active muscle mass represents about 85% of the combined weight of muscle and organ tissue but accounts for only 25% of the RMR (Weinsier et al 1992). In obesity, where FFM is increased, RMR is proportional to FFM However it is not possible to state the precise contributions of either the increased muscle or increased organ mass to the RMR (Barrows & Snook, 1987).

The RMR of an individual is remarkably constant over periods of 1-2 years); the variations have been reported to range from 1-2% (Astrup et al. 1990b) to 3-6% (Westrate, 1989; Soares & Shetty, 1986) By contrast, in subjects well matched for age, gender, height and weight, interindividual variations are much larger with between subject variability in the order of 16-20% (McClave & Snider, 1992, Astrup et al. 1990b).

1.2.2. Thermic Effect of Exercise

The TEE is the energy expended above the resting level, both during and after physical activity (Woo et al. 1985; Sims & Danforth, 1987). The contribution of exercise to total energy expenditure depends on the intensity of the work performed and the duration of the sum of all activities over the day The variability of this component means that the rate of energy expenditure can be as low as 1.5 times the RMR for clerical work or as high as 15 times the RMR for running In a review of studies pertaining to this issue, Pi-Sunyer and Segal (1992) concluded that there is no effect of exercise on RMR unless the exercise is very prolonged or severe.

It has proven difficult to accurately estimate the true contribution of exercise to energy expenditure This applies not only to free living individuals but also to persons studied in whole body calorimeters In particular, it is difficult to measure the degree of spontaneous small movements (fidgeting) which may be a quantitatively significant contributor to inter-individual differences in energy expenditure (Ravussin et al. 1986). However, recent advances in

technology and particularly the use of doubly labelled water, have increased our understanding of the factors affecting the TEE (Goran et al 1993).

1.2.3. Thermic Effect of Food

The TEF is the increase in energy expenditure immediately following meal ingestion, it includes two components, obligatory and facultative thermogenesis (Woo et al. 1985). Obligatory thermogenesis is the energy expended absorbing, processing and storing nutrients (Danforth, 1985). For example, the cost of converting glucose to glycogen corresponds to about 5% of the glucose energy content; the cost of converting it to lipid accounts for 24% of the glucose energy content (Simonson & DeFronzo, 1990; Flatt, 1992). Digestion, absorption and storage of ingested fat amounts to about 4% of the fat energy being stored. Protein induces the greatest "obligatory" metabolic response, about 25% of the protein energy content The large thermic effect of protein is at least partly due to the high energy cost of protein synthesis, gluconeogensis and ureogenesis (Simonson & DeFronzo, 1990, Flatt, 1992)

Measured TEF is usually greater than predicted by the obligatory component The amount of this difference is referred to as facultative thermogenesis (Danforth, 1985) The determinants of facultative thermogenesis are not fully understood but appear to involve sympathetic nervous system (SNS) activation (Welle et al. 1981). Empirical evidence for this include the reduction of glucose-induced thermogenesis by β -adrenergic blockade using propranolol (Acheson et al. 1984; Acheson et al 1983). Acheson et al (Acheson et al 1983) calculated that the energy cost of storing I g glucose as glycogen is 1 5 kJ/g With concomitant infusion of propranolol to block β -adrenergic receptors, the energy cost decreased by 0 8 kJ/g, a value identical to the theoretical biochemical cost of glycogen synthesis This component is partly due to insulin-mediated activation of the SNS, which in turn exerts its thermogenic effects

via release of norepinephrine (NE) stimulating α_1-adrenoceptors (Astrup et al. 1986) Further evidence of a SNS involvement in the thermic effect of glucose comes from the results of studies by Welle et al (Welle & Campbell, 1983; Welle et al 1981), who reported that plasma NE concentration increases after glucose, but not protein or fat consumption.

Another factor thought to be involved in the mechanism controlling the two components of TEF is insulin. The role of insulin in TEF was established by studies that examined the response to diet and exercise in lean and obese subjects Glucose is generally the nutrient used when examining the role of insulin in thermogenesis Findings over the last few years suggest that insulin resistance and impaired insulin-mediated glucose disposal are associated with a diminished thermic response to glucose. Insulin sensitivity decreases with increasing obesity (Bonadonna et al 1990) Since many studies (Segal et al 1990, Shetty et al. 1981; Bessard et al 1983, Schutz et al 1984a, Segal et al 1984, Schutz et al. 1984b; den Besten et al. 1988; Astrup et al 1990a) report a blunted TEF in obesity it is thought that the impairments in insulin's normal functions are partly responsible for the blunted thermic response to glucose in obesity. Several other studies, however, report no blunting of the TEF in obesity (Owen et al. 1986; Nair et al. 1983, D'Alessio et al 1988, Sharief & MacDonald, 1982; Welle & Campbell, 1983; Felig et al 1983, Swaminathan et al 1985)

Felber et al (1981) showed that the oxidation and disposal of a 100 g oral glucose load was different in obese subjects with and without glucose intolerance Obese subjects without glucose intolerance had a thermic response similar to that of lean subjects; those with impaired glucose tolerance had a significant decrease in glucose oxidation and a marked reduction in glucose storage, both of which would contribute to a blunted TEF. Golay et al (1982) measured oral glucose TEF in lean subjects and in obese subjects with normal glucose tolerance, impaired glucose tolerance, type II diabetes with an increased insulin response, and type II diabetes with

a decreased insulin response. The TEF of glucose was significantly lower in the obese subjects, with the extent of the reduction in thermogenesis proportional to the severity of insulin resistance

More recently, Segal et al (1992) determined the independent effects of obesity and insulin resistance on the thermic effect of both a glucose and mixed nutrient load They observed that the TEF of intravenous glucose during a euglycemic, hyperinsulinemic clamp is blunted in men with low insulin sensitivity, independent of obesity The blunted TEF was related to a significant decrease in glucose storage, the rate of glucose oxidation was not altered These findings are consistent with the greater energy cost of glucose storage than oxidation On the other hand, obesity and insulin resistance are both independently associated with an impaired thermogenic response to a mixed nutrient meal: the TEF of a mixed meal was significantly lower in the groups with low versus high insulin sensitivity, across both levels of obesity (lean and obese), and lower in the obese compared with lean men, at both high or low levels of insulin sensitivity (Segal et al. 1992)

Another example of the controversy of the role of TEF is the impact of physical activity on the TEF Pi-Sunyer and Segal (1992) reviewed the literature and concluded that in lean people, the TEF is greater during exercise than at rest, in obese persons the potentiating effect of exercise on TEF seems to greatly diminish or disappear Also, the TEF after exercise is less in obese than lean subjects. Clearly, the TEF remains controversial as a factor that may contribute to the development or maintenance of obesity.

There is no disagreement on the primary determinants of TEF These are the nutrient composition and energy content of the food consumed (Danforth, 1985; Nair et al 1983) Information about these two determinants comes from many of the recent publications on TEF

All TEF studies agree that protein induces the greatest increase in the metabolic rate (Nair et al 1983, Swaminathan et al 1985) and with the exception of Nair et al (Nair et al 1983) who reported that after protein, fat induces the greatest effect, followed by glucose, the results of other studies concur that after protein, a mixed nutrient meal induces the greatest effect followed by glucose and fat (Swaminathan et al 1985; Lean & James, 1988; Robinson et al. 1990). When expressed as a percentage of the energy content of the test meal the TEF for protein is 25%, for a glucose meal it is 7-10% and for fat the TEF is 6-8% (Simonson & DeFronzo, 1990)

The second determinant of the magnitude of the TEF is the energy content of the test meal. It is well established that the magnitude of the TEF is closely correlated to the energy load provided in the test meal (Segal et al. 1990). Despite this relationship there are significant differences in the methods used to compare the TEF between subjects. In accordance with conclusions of Segal et al (1990), many studies use the same energy load for all subjects, providing anywhere from 300 kcal/1250 kJ to 800 kcal/3340 kJ (den Besten et al. 1988; Welle & Campbell, 1983; Schutz et al 1984b; Felig et al. 1983; Swaminathan et al. 1985; Nair et al. 1983). Other studies use variable energy loads that are adjusted relative to some variable of body weight, composition or basal energy levels (Schutz et al. 1987; Sharief & MacDonald, 1982; Shetty et al 1981, D'Alessio et al. 1988). The results of these studies are difficult to interpret and can be misleading due to the confounding effects of varied meal sizes. Furthermore, as Westrate (1989) points out, when the energy content of a test meal is given on the basis of kg ideal body weight (IBW) or FFM, it is implicitly assumed that the thermic response is correlated to IBW or FFM, and by adjusting the interindividual differences in these variables, comparisons of thermogenic responses will be more valid. Data to support this view have not been reported. In fact Westrate (1989) found that no correlation exists between the TEF, body weight and FFM.

Very little data is available regarding the reproducibility of the TEF Intraindividual variability and interindividual variability have been reported to be 28% and 36% respectively (Westrate, 1989). Recently, Piers et al (British Journal of Nutrition 1992, 67 165-175) reported a somewhat lower intraindividual variability of 18 7%

1.3. Techniques for Measuring Energy Stores and Expenditure

1.3.1. Energy Stores: Body Weight and Body Composition

Body weight measurement is accurate to 0 01% with a simple beam balance (Garrow, 1978). Body weight is a gross measure of the body's mass, but its relationship to the size of the body's energy stores is not simple, nor are changes in energy stores necessarily or fully reflected in weight changes (Garrow, 1978). Because of the limitations associated with body weight, more complex methods are used to describe body composition, and changes in the body's energy stores as a result of energy imbalances. This field of study is especially relevant to obesity research since accurate methods for measuring specific components of body composition are needed to assess the effects of energy restriction

The methods currently in use are based on either a two or four compartment model for body composition. The two-compartment model assumes that the total body mass is composed of two chemically distinct compartments, fat and fat-free mass, whereas the four compartment model divides the body into four chemical groups, water, protein, ash or bone mineral, and fat (Welle et al 1984). Generally speaking, the body is made up of the living cells and the less immediately vital materials such as interstitial fluid, fat and bone (Bray, 1969) Considering the living body as comprising fat mass and fat-free body mass, two terms, the lean body mass (LBM) and the fat-free mass (FFM), are often used synonymously, but are conceptually different (Bray, 1969). The LBM was initially defined as the weight of the body totally devoid of fat except for a small amount of essential lipid FFM is the mass of the body minus the total fat mass

The FFM is a heterogenous compartment containing both metabolically active and inactive components An important aim of body composition research is to determine the size of the metabolically active tissues of the body. Many studies use LBM or FFM to assess the active component to which RMR is correlated, however a large proportion is metabolically inactive, and the losses from the active vs. inactive components do not occur at the same rate in semistarvation The metabolically active component of the FFM is the oxygen-exchanging, potassium-rich, glucose- and/or free fatty acid-oxidizing, work-performing tissue known as the body cell mass (BCM) This includes all the cellular components of the body including muscle, viscera, central nervous system and hematopoietic system as well as cells in cartilage, tendon, bone and adipose tissue (Moore, 1980; Bray, 1969). The BCM is approximately 75% water and comprises 50% of the total FFM of a normal adult (Barrows & Snook, 1987; Foster et al. 1990). The metabolically inactive component of the FFM includes the extracellular fluid (ECF), extracellular solids (bone and connective tissue), and glycogen (and the water associated with it) (Barrows & Snook, 1987; Foster et al. 1990). The ECF is distributed both within (1/4) and without (3/4) the circulation, and in total makes up about 20% of adult body weight. The solid structural component makes up about 15% of body weight.

The other compartment of the human body is the adipose tissue. It is 80% lipid, 15% water, (3% intracellular fluid and 12% ECF) and about 5% protein (Heymsfield & Lichtman, 1992). Since FFM is defined by subtracting body fat from total mass, the water of adipose tissue is included in the FFM Consequently, losses of adipose tissue are associated with an obligatory loss of metabolically inactive FFM.

Obesity evidently alters body composition by increasing total adipose tissue; however, FFM also increases (Heymsfield & Lichtman, 1992). This increased FFM is due both to the FFM component of adipose tissue, and hypertrophy of skeletal muscle and visceral organs (Forbes,

1987). In addition to body composition, fat distribution is also altered in obesity Obesity can be upper-body or abdominal, or it can be lower body or gluteofemoral (the former being common among males and the latter being common among females) (Garrow, 1988) Fat distribution is classified using the ratio of body circumferences measured at the waist and hip, the waist hip ratio. A ratio greater than 0.8 indicates upper-body obesity and a ratio lower than 0 8, indicates lower-body obesity. Interest in the waist:hip ratio has arisen because of the results of population studies suggesting that upper-body obesity is associated with more metabolic complications than lower-body obesity (Björntorp, 1992).

1.3.2. Energy Expenditure

1.3.2.1. Direct Calorimetry

The direct method for measuring metabolic rate involves the measurement of heat losses. Direct calorimetry measures the sum of radiant heat exchange and of convective-, conductive- and evaporative heat transfer. The total heat loss is equal to the rate of energy utilization when body temperature is constant (Westrate, 1989). Direct calorimetry has the advantage of being based solely on conservation of energy and not on any assumptions about the physiology of energy metabolism, but it requires expensive instrumentation. Furthermore, direct calorimetry cannot be used to measure short-term effects of thermogenic stimuli, such as food, on heat exchange due to the large heat storage capacity of the body (Brown & Brengelmann, 1965). As a result, direct calorimetry is infrequently used.

1.3.2.2. Indirect Calorimetry

Indirect calorimetry is the method by which metabolic rate is estimated from measurements of oxygen consumption and carbon dioxide production and through a series of

assumptions and equations (Ferrannini, 1988; McClave & Snider, 1992). The measurement is based on the assumption that all the O_2 consumed (VO_2) is used to oxidize degradable fuels and all the CO_2 produced (VCO_2) is recovered (Burszstein et al. 1989). Calculating energy production in this situation is equivalent to converting the chemical free energy of nutrients into the chemical energy of ATP plus loss of some energy (as heat) during the oxidation process, plus the ultimate conversion of the chemical energy to heat lost to the environment, (plus any external work performed on the environment) so direct and indirect calorimetry should and do, provide identical rates of energy expenditure under steady state conditions (Simonson & DeFronzo, 1990).

The abbreviated Weir equation is frequently used to determine energy expenditure from VO_2 and VCO_2 [energy expenditure = $(3.94 \times VO_2) + (1.11 \times VCO_2)$] (McClave & Snider, 1992). The accuracy of respiratory gas exchange has been reported to be accurate to within 2% (Webb, 1992). Indirect calorimetry is therefore ideal as a method for assessing acute effects of thermogenic stimuli on metabolic rate and for the clinical measurement of RMR.

2. EFFECT OF SEMISTARVATION ON ENERGY BALANCE AND ENERGY EXPENDITURE

2.1. Adaptation and Semistarvation

The concept of adaptation implies the maintenance of an acceptable state through adaptive processes (Waterlow, 1986). There are three types of adaptation important for nutrition: biological/genetic; physiological/metabolic; and behavioral/social (Waterlow, 1986). We are concerned with adaptation of the second kind. Any discussion of any adaptive process must begin by defining "adaptation of what in response to what?" In this case, the discussion will define the adaptation of energy expenditure and body composition to semistarvation.

Semistarvation may be defined as the condition resulting from a prolonged negative energy balance, in which food energy intake is consistently less than total energy expenditure

(Hoffer, 1993) In the presence of negative energy balance, endogenous energy stores are oxidized to make up the energy deficit. This results in weight loss Semistarvation evokes the adaptive processes that minimize the effects of negative energy balance (Shetty, 1990)

2.2. Composition of Weight Loss During Semistarvation

Intracellular protein and glycogen exist in a 25-33% aqueous solution (Van Itallie & Yang, 1977). Normal weight subjects on an energy deficient diet lose weight rapidly during the first several days and much of this initial loss is due to depletion of body glycogen and extracellular and intracellular water (Van Itallie & Yang, 1977). In addition, 15-17 g of water is lost for every 83-85 g of pure fat loss (Garrow, 1978; Goodman & del Pilar Gomez, 1987) Water loss (both intra- and extracellular) accounts for most of the early weight loss in obese subjects consuming energy deficient diets (James et al. 1990).

In the classic Minnesota Experiment, Keys et al (1950) studied the long-term effects of semistarvation on body composition. Thirty-two young men followed a 1570 kcal (6563 Kj) diet (vs. a maintenance diet of 3468 kcal (14496 kJ) for 24 weeks. At the end of the first 12 weeks, water, fat and protein represented 48%, 40% and 12% respectively of the weight lost. By the end of the study, mean total weight loss was 15.9 kg, water and protein accounted for 37% and 9% of the lost mass while fat represented 54%. From these results it was observed that the mass lost corresponded to a mixture of different types of tissues and stored materials which were released in proportions differing according to the duration, intensity and other characteristics of the energy restriction including the nutrient composition of the diet (Keys et al 1950) In other words, the composition and quantity of weight loss changed with respect to how long the volunteers were on the semistarvation diet and what the energy balance was between the energy consumed and energy expended.

Semistarvation challenges the body to reduce energy expenditure as a life saving adaptation This is accomplished by reducing its lean tissue stores and hence RMR enough to reestablish energy balance, but not to such a severe extent that the adverse metabolic consequences of protein deficiency become intolerable. Two processes reestablish protein homeostasis during semistarvation. First, the body increases its efficiency of protein recycling and this reduces the rate of body protein loss. Secondly, as time progresses, the rate of lean tissue loss decreases in part due to the decreasing amount of lean tissue left to be lost. At the same time, the efficiency of retention of dietary protein increases, partly perhaps due to changes in lean tissue mass and to adaptive cellular metabolic changes. As semistarvation proceeds, the lean tissue mass decreases, automatically decreasing endogenous protein loss, while increasing the efficiency of dietary protein retention, until a new equilibrium is established in which the decreased endogenous protein loss is matched by the increased retention of dietary protein, and net body protein loss ceases (Horfer, 1993).

2.3. Decreased Energy Expenditure

Since the beginning of the 20th century, studies have demonstrated a decrease in the RMR as an effect of experimentally induced or therapeutic semistarvation (Shetty, 1990). It has been classically taught that the reduced RMR of semistarvation occurs as a result of changes in body composition and metabolic adaptation (Grande et al. 1958). This new level of energy balance is believed to occur in different phases (Garrow & Webster, 1989; Grande et al. 1958). Acute restriction of energy intake results in an immediate small fall in RMR within a few days during which time little tissue loss has occurred. After several weeks of energy restriction, metabolically active tissue is lost, but this loss does not fully account for the decreased RMR.

In long term starvation, depletion of the body cell mass accounts for most of the reduction in RMR.

After 24 weeks of semistarvation, the Minnesota volunteers had decreased their RMR by 40%. The average decrease per unit of body cell mass (BCM), (estimated from measurements of body density and extracellular space) was 15.5% This implies that the decrease in RMR was not fully accounted for by the loss of active body tissue and that the metabolic rate of the remaining active tissue mass had to have decreased In short-term studies (3 weeks) of semistarvation in non-obese subjects, Grande et al (1958) reported a 21 4% decrease in RMR and a 16.3% decrease in RMR/BCM

Table 1 summarizes the results of studies reporting on the effects of semistarvation on RMR in obese subjects. Energy deficient diets may be classified as low energy (≈ 800 - 1200 kcal/ 3400 = 5016 kJ) or very low energy (< 800 kcal/ 3400 kJ). The low energy diets are the typical balanced deficit diets prescribed to most obese outpatients, while the VLCD's are high-protein, liquid formulas given to patients who are closely supervised and monitored.

In 1969, Bray (1969) studied the effects of 24 days of a 450 kcal/ 1800 kJ diet on the RMR of 14 obese patients. Patients lost an average of 10 3 kg and RMR decreased 15% between the beginning and end of the study. It should be noted that these patients were in a 1000 kcal/ 4180 kJ positive energy balance for 7 days prior to the start of the weight loss diet and this may have had a quantitative effect on the subsequent fall in RMR. Bessard et al (1983) placed 6 obese women on an 11 week protein-supplemented modified fast (PSMF) (exact energy content was not stated). After a 12.1 kg weight loss, RMR fell 14 5% when expressed in absolute terms, and 9.6% when expressed per kg FFM. Welle et al. (1984) reported a 9 4% decrease in RMR in six obese women who followed a 400 kcal/ 1672 kJ diet for 40 days. The fall in the RMR in this study may be somewhat underestimated since the subjects lost weight during the baseline

portion of the diet, implying that some degree of adaptation may have taken place. Ravussin and coworkers (1985) measured RMR before and after a 10-16 week weight loss diet (≈830-1120 kcal/3500-4700 kJ) in 7 obese subjects. Overall RMR decreased 9.0%, but when expressed in terms of FFM, it did not change. In two other studies, investigators (Finer et al. 1986; Barrows & Snook, 1987) reported 25% and 21% falls in RMR respectively. Barrows and Snook (1987) also reported a significant decrease in the RMR:FFM ratio. Hill et al. (Hill et al. 1987) also found a 19.1 and 17 3% reduction in RMR and a 15.3% and 11.6% reduction in the RMR:FFM in both exercising and sedentary obese subjects respectively following five weeks of dieting. den Beston et al (1988) in perhaps the most valid of the studies showing no change in the RMR:FFM, reported a minimal decrease in the RMR and no change in the RMR:FFM despite significant weight loss over an eight week period. Davies et al (1989) compared the metabolic effects of an eight week energy restrictions with 330 kcal/1379 kJ or 780 kcal/3260 kJ per day in two groups of obese inpatients. RMR decreased significantly by 17% in both groups, but RMR:FFM was unchanged. In a four month study by Elliot et al. (1989), RMR decreased 22%. RMR:FFM also decreased significantly by approximately 13%. Garrow and Webster (1989) studied 103 obese women consuming a 800 kcal/3400 kJ diet for three weeks. Mean weight loss was 4.9 kg and RMR fell 8.8%. The adaptive decline in RMR was clearly demonstrated with almost 70% of the total decline in RMR occurring after the first week, when only 1.9 kg of weight was lost. In two other studies, investigators (Hendler & Bonde, 1988; Fricker et al. 1991) placed obese inpatients on very low energy diets for three weeks. Mean weight losses were 8.8 kg (Hendler & Bonde, 1988) and 9.2 kg (Fricker et al. 1991) and the reductions in RMR were 15.3% (Hendler & Bonde, 1988) and 20.0% (Fricker et al. 1991) giving RMR:FFM decreases of 12.5% and 18.0% respectively Finally, in the most recent of these studies, Nelson et al (1992) put 24 obese

women on a 800 kcal/3400 kJ for 3 months and 20 days. Mean weight loss was 12.6 kg, RMR declined significantly by 15.3% and RMR:FFM fell by 7 9%

Combined, the data from the studies presented in Table 1 reveal or demonstrate several interesting points aside from the fact that RMR consistently falls 10-20% during the first few weeks of energy deficiency First, despite similar energy intakes, study durations, and subject profiles, the changes observed in the RMRs were very different This illustrates the reported high degree of interindividual variability in RMR under condition of negative energy balance and weight loss Secondly, the longer the energy restriction, the smaller is the fall in the RMR FFM This supports the classical teachings that the reduction in RMR during energy restriction occurs in phases, with the initial fall reflecting an increase in metabolic efficiency and further decreases in RMR becoming more and more reflective of the loss of FFM until ultimately RMR FFM is not different before and after weight loss Wadden and coworkers (Foster et al 1990) elegantly demonstrated these phases in their study on the long term effects of low and very low energy diets on RMR. The investigators measured RMR and body composition over a 48 week period They compared the effects on RMR of diets with either low (1200 kcal/5021 kJ) or very low energy (420 kcal/1757 kJ). RMR FFM declined by 13 2±3.5% in the low energy diet and 14.2±6.7% in the VLCD from baseline to week 17 of a 48 week study with the rate of the reductions being greatest in the first five weeks of the study With refeeding, the ratios increased and ultimately normalized by week 48

It is apparent that several authors have questioned the concept of metabolic adaptation by reporting no change in RMR FFM after short term semistarvation in obese subjects (Ravussin et al. 1985, den Besten et al 1988; Davies et al 1989) Both Ravussin et al (1985) who reported no change in RMR·FFM and Bessard et al (1983) who did, estimated fat mass by measuring skinfold thickness The discrepancy between their results may in part be explained by the

drawbacks associated with this method, particularly the difficulty in obtaining interpretable measurements in obesity using the calliper method (Fricker et al 1991). Davies et al (1989) also reported no change in RMR FFM despite significant reductions in weight and RMR. However during the first two weeks of energy restriction, RMR fell by 8% and 11% in the 1379 kJ and 3260 kJ groups respectively During the same period total body potassium and therefore FFM did not change in either group (1379 kJ. 3 1±0 5 vs 3.0±0 4 and 3260 kJ: 3.1±0.4 vs 3.1±0.4, where FFM was assumed to have a potassium content of 60 mmol K^+/kg). Despite the ultimate changes in RMR and FFM, the authors failed to recognize the obvious increase in metabolic efficiency per kilogram of metabolizing tissue during the initial phase of the reduction in RMR. Furthermore, the author's use of the total body potassium (TBK) to estimate FFM is questionable since TBK loss has been shown to overestimate the loss of BCM in semistarvation in part, because of potassium loss associated with the glycogen-water pool (Welle et al. 1984). In fact, the N balance results in the study of Davies et al indicate that minimal body N losses occurred during weight reduction. It is therefore unlikely that the decrease of body potassium in their energy restricted obese subjects was due to a loss of the active tissue mass. It appears, therefore, that their conclusion that the RMR.FFM is unchanged in semistarvation is unjustified.

Finally, a problem with most of the studies listed in Table 1 is the failure of the authors to define FFM and account in particular for the metabolically inactive components of the FFM when calculating the RMR:FFM This may reflect a general imprecision in the literature; Garrow, for example, defines the LBM as the sum of all tissues of the body minus the adipose tissue (Garrow, 1978).

In some of the studies discussed thus far, changes in RMR have been evaluated with respect to a loss of total FFM. These studies have not compartmentalized the loss of the lean tissues in terms of what proportion was derived from muscle mass, organ mass or the low energy

metabolizing extracellular tissue In early semistarvation RMR decreases to a greater extent than could be accounted for by the loss of total FFM Since, in early semistarvation, the greatest loss of body protein is from the skeletal muscles which have the lowest metabolic activity of the cells of the BCM (Cohn et al 1981), the latter explanation is unlikely. Furthermore, in the earliest stages of semistarvation, the greatest source of loss from the FFM is from ECF, glycogen-water and adipose tissue water. Therefore, if we accept the conclusions of the authors showing no metabolic adaptation in the metabolically active part of the FFM, RMR per unit FFM should *increase* in early semistarvation. The failure to observe such an increase is evidence that adaptation truly occurs.

Clearly, there is overwhelming evidence for metabolic adaptation in response to semistarvation. These adaptations are seen in the form of weight loss, changes in body composition, reductions in RMR and increased metabolic efficiency of the active tissue mass

2.4. Mechanisms Governing Adaptation of Changes in Energy Expenditure During Semistarvation

Changes in sympathetic nervous system (SNS) activity and catecholamines, in thyroid hormone metabolism and in insulin play key roles in the adaptive response to semistarvation (Shetty, 1990). These changes are not only needed for the use of endogenous fuels during periods of negative energy balance, but are responsible for increased metabolic efficiency of the active cell mass (Shetty, 1990).

2.4.1. SNS and Catecholamines

There is evidence that norepinephrine (NE) released from sympathetic neurons and epinephrine and NE released from the adrenal medulla can influence metabolic processes (Leiter et al. 1984). In humans about 15% of the total energy expenditure has been estimated to be

sympathetically mediated (Landsberg & Young, 1983) Catecholamines have two effects to increase energy expenditure (a) by increasing the rate of cellular metabolism (Himms Hagen, 1976) and (b) by stimulating the conversion of stored fuels into usable energy (Young & Landsberg, 1977b) The increase in cellular metabolism is shown through an elevation in heat production, fuel utilization and O_2 consumption. Because of the stimulatory effects of catecholamines, it is thought that decreases in SNS activity partially explains the fall in RMR observed during semistarvation.

When considering catecholamines and the regulation of energy metabolism, the SNS must be distinguished from the adrenal medulla Increased levels of epinephrine in urine or plasma is good evidence of adrenal medullary stimulation, however, when the adrenal medulla is stimulated, changes in plasma or urinary norepinephrine cannot be assumed to originate from sympathetic nerves, since the adrenal medulla may contribute significantly to the circulating pool of norepinephrine (Landsberg & Young, 1983). SNS activity can be accurately assessed by measurement of NE turnover in individual sympathetically innervated tissue and is a technique not biased by simultaneous changes in adrenal medullary activity (Young & Landsberg, 1981; Landsberg & Young, 1983) This technique is commonly used in animal studies, while studies involving humans, assess SNS activity by plasma and urine levels and to a lesser extent, tracer techniques Measuring SNS activity through plasma or urine concentrations remains controversial It is known that the majority of circulating NE arises from stimulation of the SNS. The fraction of neurotransmitter measurable in the blood (the "spillover concentration") however, is quite small and depends on many complex processes including synthesis, release re-uptake, metabolism and clearance (Fernandez et al 1988) An alternative to measuring NE in plasma is to determine its rate of excretion in urine Animal studies (Kopp et al. 1983) suggest that there is a reasonably good relationship between arterial NE concentration and the rate of urinary NE

excretion, however, it is not clear to what extent this applies in man. Furthermore, it is not clear how changes in catecholamine metabolism may change the proportions of free NE, conjugated NE and the methylated or deaminated metabolites excreted, thus weakening the relationship between SNS activity and urinary NE excretion (MacDonald, 1992) There are limited data reporting on the validity of using urinary catecholamines as a measure of a SNS activity One study reported intraindividual correlations between plasma and urine measurements of 0 7 ($p<0.001$) for epinephrine and NE in experimentally stress-stimulated catecholamine levels in young men (Akerstedt et al 1983). Many researchers, however, rely on urinary catecholamines to demonstrate the effects nutritional factors have on SNS activity (Romoff et al 1979, Kopp et al. 1983, McCargar et al 1988; Troisi et al. 1991, Pasquali et al 1992) It is essential to understand the limitations of the methods used to assess SNS activity when making conclusions about its involvement in metabolic adaptation to energy restriction

Landsberg and Young (1983) established a link between energy intake and SNS activity in a series of rat experiments. Using measurements of NE turnover to estimate sympathetic activity, they demonstrated a reduction in SNS activity during fasting with a controlled sodium intake (Young & Landsberg, 1977a) and stimulation during sucrose overfeeding with controlled sodium intake (Young & Landsberg, 1977c). To avoid the confounding effects of sodium depletion on SNS activity, sodium intake is controlled in studies assessing the effects of altered nutritional status on catecholamines. The SNS is one of the principal pathways involved in the maintenance of blood pressure A reduction in body sodium caused by an inadequate intake will cause blood volume depletion leading to activation of the SNS to maintain blood pressure (MacDonald, 1992). This concept of a reduced catecholaminergic drive during energy restriction has also been demonstrated in human subjects Leiter et al (1984) reviewed studies in which sodium intake was not controlled during energy restriction and found very different results, with

fasting having a stimulatory effect on indexes of adrenergic activity (Romoff et al. 1979). In their studies on the effects of fasting and very low energy diets on sympathetic activity, they observed that both sodium and nutrient intakes interact to determine net catecholamine responses, with the former having a greater impact than the latter. Shetty et al (1979) reported reductions in circulating levels of NE and in an earlier report Kolanowski et al (1975) reported a decrease in daily urinary excretion of NE. More recently Bessard et al (1983) have shown a decrease in urinary NE along with a decrease in RMR:FFM during semistarvation in obese subjects. As in animals, these responses have been shown to relate to the carbohydrate content of the diet (Welle et al 1981; DeHaven et al. 1980). In experimental animals and man, glucose and sucrose stimulate the SNS. In the rat, free access to dilute solutions of sucrose increases NE turnover in heart, liver, pancreas and kidney tissues within one day of administration (Young & Landsberg, 1977c). In human studies, a standard glucose tolerance test increases plasma NE levels in association with increased pulse rate, pulse pressure and systolic blood pressure (Welle et al. 1981) The carbohydrate content of the diet affects SNS activity during energy restriction as well. Obese subjects following a low carbohydrate diet (1370 kcal, 24% carbohydrate) experienced a significant decrease in plasma and urinary NE, while subjects following a high carbohydrate diet (1400 kcal, 59% carbohydrate) had no change in plasma or urine levels (Fagerberg et al. 1984).

With respect to the adrenal medulla, energy restriction of humans has a stimulatory effect causing an increase in urinary epinephrine excretion (Young et al. 1984; Young & Landsberg, 1981). This increase however, has no thermogenic effect, since levels needed to stimulate RMR are considerably higher (Shetty, 1990). Young et al (Young et al. 1984; Young & Landsberg, 1981) have identified specific conditions under which there is a dissociation of sympathetic activity from adrenal medulla secretion. These investigators demonstrated that a brief period of

fasting is associated with sympathetic suppression and adrenal medullary stimulation in normal humans.

Taken together, these results demonstrate that while catecholamines can have a role in metabolic adaptation to semistarvation, they do not prove that the changes found are *causal* with respect to the known changes in metabolic rate (Leiter et al. 1984).

The TEF was also shown to be an important component of the adaptive response to over and underfeeding in small animal studies (Rothwell & Stock, 1981), where it varies proportionately with food intake to minimize weight change. On this basis, the TEF was suggested to be a component of the adaptive response to underfeeding in man as well (James & Trayhurn, 1981).

As was previously discussed, much of the facultative component of the thermic effect of food is thought to be related to the SNS response to glucose. Several investigators have studied the TEF in obese and lean individuals, before and after weight loss, with the aim of establishing whether altered thermogenesis mediated by the SNS is a cause or an effect of the obese state Some (Nelson et al. 1992; den Besten et al. 1988; Astrup et al. 1990a; Bessard et al. 1983, Hendler & Bonde, 1988) have concluded that the TEF does not change after weight loss, whereas others have concluded that it decreases (Schutz et al. 1984b; Apfelbaum et al 1971), another has reported that the TEF actually increases following weight loss (den Besten et al. 1988). Astrup et al (Astrup et al. 1990a) found that severely obese patients (vs lean controls) showed an impaired TEF and a blunted increase in plasma NE concentrations in response to oral glucose. After weight loss, despite normalized plasma glucose and lipid profiles, the increase in plasma NE and the thermic response to glucose were still lower than that of the lean controls. Schutz et al (Schutz et al. 1984b) also found that weight loss in obese subjects was associated with a blunted thermogenic response to a glucose load. However in both these studies, the reduced obese were still obese and had insulin resistance compared to the control group. It has

also been found that acute β -blockade reduced 24 hour energy expenditure (including both RMR and TEF) in the reduced obese but nad no effect on lean controls (Buemann et al. 1992). These findings were taken to suggest that SNS activity is enhanced in the obese. The TEF was lower in the reduced obese compared to the controls, despite empirical evidence of enhanced SNS activity. These findings together with the inconsistent conclusions in the literature suggest that factors in addition to SNS activity are involved in mediating the thermic response to food in the obese.

2.4.2. Thyroid Hormones

Thyroid hormones play an important role in the regulation of the resting metabolic rate (Danforth, 1983). They are also important components of the metabolic adaptations associated with energy restriction (Shetty, 1990). Energy restriction does not affect circulating levels of thyroxine (T_4) since there is no evidence of a change in production rates of T_4 (Shetty, 1990). However, the peripheral conversion of T_4 to the active hormone triiodothyronine (T_3) decreases rapidly (30-40% below normal within 3-4 days), while reverse T_3, which is devoid of activity, increases due to a decrease in its metabolic clearance (Shetty et al. 1979). Gelfand and Hendler (1989) summarized several representative studies showing the magnitude in the fall of T_3 over time in subjects consuming very low calorie diets. From these studies it was also observed that the level of carbohydrate intake has the predominant effect on T_3, rather than the total energy intake. However, both energy and the specific amount of carbohydrates consumed affected the conversion of T_4 to T_3, possibly through their effect on insulin secretion (Azizi, 1978; Danforth & Burger, 1989). The concept of T_3 playing a key role in the adaptive fall in metabolic rate remains controversial. Those supporting the concept emphasize the correspondence between the magnitude of the fall in T_3 and changes in metabolic rate (Phinney et al. 1988); whereas others

report no correlation between changes in T_3 levels and metabolic rate (Barrows & Snook, 1987). Gelfand et al. (1987) showed in obese dieters receiving about 800 kcal/3400 kJ per day, that the fall in both T_3 and RMR are significantly blunted if carbohydrate makes up 80-100% of the energy as compared to a regimen containing no carbohydrate.

In addition to the role T_3 is thought to have in metabolic adaptation, it has also been postulated to play an important role in body protein conservation and in the fall in protein turnover that occurs during semistarvation (Gelfand & Hendler, 1989), given the known effects of thyroid hormone to stimulate whole body and muscle protein breakdown (Gelfand et al. 1987).

As with the SNS and catecholamines, the precise impact of the fall in T_3 with energy restriction is not fully understood, nor is T_3 the only modulating factor (Gelfand & Hendler, 1989). In fact, thyroid and catecholamines interact at the periphery (Shetty, 1990). For example, epinephrine is known to enhance the peripheral conversion of T_4 to T_3; thyroid hormone deficiency is associated with an enhanced SNS activity (NE turnover and plasma appearance rates increase); and T_3 increases the number of tissue NE receptors (Shetty, 1990). These interactions may influence their mutual roles in regulating thermogenesis.

2.4.3. Insulin

Insulin is another pre-eminent hormone that regulates energy balance. In the fed state, insulin regulates the disposition of absorbed nutrients and in the postabsorptive state, with decreases in blood glucose levels, reductions in circulating insulin levels allows for the mobilization of endogenous fuels (Hoffer, 1993).

The thermogenic roles of insulin are demonstrated through its stimulatory effect on Na^+-K^+ pumping across the cell membrane (which alone is estimated to account for about 20% of the RMR) (Durnin, 1967), and through its stimulatory effects on SNS (Acheson et al. 1984).

Insulin is said to mediate cellular thermogenesis by two mechanisms, the former obligatory by increasing glucose uptake, and the latter facultative by increasing catecholamine activity (Shetty, 1990).

Fasting is associated with a significant lowering of circulating insulin levels. This decrease is the primary signal for amino acid mobilization, gluconeogenesis, lipolysis and ketogenesis, all of which are aimed at the mobilization of endogenous fuels as substrates to prevent the development of hypoglycemia (Shetty, 1990). These changes may also be involved in the reduction in RMR during semistarvation.

In assessing the mechanisms which operate to decrease RMR in humans with semistarvation, the importance of the reduction in the activities of SNS and catecholamines, thyroid hormones and insulin cannot be overlooked. How they are each specifically involved in metabolic adaptation clearly remains an area for further study.

3. NUTRITIONAL AND PHARMACOLOGICAL INFLUENCES ON ENERGY EXPENDITURE

The ideal therapy for obesity would maximize fat loss and at the same time maintain protein stores appropriate to the changing size of the person. However, since obesity is a condition characterized by both an increase in fat mass and lean tissue mass (Heymsfield & Lichtman, 1992), weight loss induced by an energy deficit must be composed of both fat and protein (Garrow & Stalley, 1977; Elliot et al. 1989; Barrows & Snook, 1987; Hendler & Bonde, 1988; Fricker et al. 1991). This loss of body protein as well as the reduction in the RMR are part of a successful adaptation to semi-starvation (Hoffer, 1993). One goal of weight reduction therapy is to manipulate these normal responses with the aim of maximizing fat loss. Two approaches can be envisioned to prevent or modify adaptation. The first is to minimize body protein losses and the second approach involves the modification of the changes occurring in

SNS activity and thyroid hormone metabolism. Both modifications should result in smaller reductions in RMR which in turn allows for energy balance to remain negative longer and fat loss to be maximized. The following sections summarize some of these approaches.

3.1. Agents That Influence Fat Free Mass

3.1.1. Exercise

Exercise increases energy expenditure. It can also increase lean tissue mass and hence the RMR, but only when the exercise is geared toward strength training and is combined with an increased energy intake (Smith, 1976).

Exercise is considered a useful component of obesity treatment. However, the specific benefits on energy expenditure and body composition, of combining a weight loss diet with exercise remains uncertain (Heymsfield et al. 1989). Total weight loss, fat and fat free mass loss and change in RMR are among the key variables studied when comparing the effects of diet alone to diet and exercise. Some researchers have reported that diet and exercise produces greater weight loss (Hagen, 1986; Pavlou et al. 1989), greater loss of body fat and preservation of FFM (Hill et al. 1987; Ballor et al. 1988), and a smaller drop in RMR (Lennon et al. 1985; Mole et al. 1989) as compared to diet alone. Other researchers however, have not found such effects with exercise (Heymsfield et al. 1989; Goranzon & Forsum, 1985; Sweeney et al. 1993) Exercise has not been proven as a means to limit lean tissue loss during weight loss programs for the obese. The other benefits of fitness training, including increased aerobic capacity, decreased blood pressure and decreased insulin levels (fasting and after a glucose load) are clearly significant enough to continue recommending exercise as an important component of obesity therapy (Bjorntorp, 1992).

3.1.2. Growth Hormone

Administration of growth hormone (GH) to experimental animals or humans can result in increased mobilization of free fatty acids from adipose tissue, protein accretion and tissue growth (Snyder et al. 1989). The protein sparing actions of GH are thought to be mediated through insulin-like growth factor-I (IGF-I) which is stimulated by GH (Snyder et al. 1989). There is considerable variation in the results of studies assessing the impact of energy restriction on GH. Some show no increase in GH while others show marked increase during fasting (Shetty, 1990). The first study on the metabolic response to GH during fasting in obese subjects reported that fasting caused GH resistance, obliterating the ability of GH to improve negative nitrogen balance (Felig et al. 1971). Danforth (1993) recently reported transiently increased circulating GH concentrations during energy restriction, an effect that is suppressed by the level of carbohydrate in the diet, and stimulated by protein. The increase in GH during energy restriction could have a role in sparing the active tissue mass. This idea stems from the finding that short-term GH administration conserved FFM during energy restriction in obese subjects (Clemmons et al. 1987). Similar results were reported by Snyder et al. (1989) who also noted that an intake of between 12-18 kcal(50-75 kJ)/kg, with >70% of energy as carbohydrate is required for optimal nitrogen-sparing responses to GH. The investigators found that fat loss was significantly greater in the GH-treated group vs placebo, although there were no differences in total weight loss. RMR was not measured in this study. However, similar doses of GH have been shown to increase basal O_2 consumption in obese subjects (Bray, 1969).

3.1.3. Very Low Calorie/Energy Diets (VLCD)

In 1979 an expert panel under the auspices of the Life Sciences Research Office of the Federation of American Societies for Experimental Biology defined "very low calorie diets" as diets

containing less than 800 kcal per day (Life Sciences Research Office, 1979). The current widespread use of these diets stems from the renewed interest, in the 1950's, of total fasting as an alternative treatment for obesity (Gelfand & Hendler, 1989). Unfortunately, total fasting provoked protein malnutrition, which was considered the common cause of fasting related death (Gelfand & Hendler, 1989). In the late 1960's, Bolinger et al (1966) and Apfelbaum et al. (1967) noted that body protein stores could be significantly spared by supplementing a total fast with modest amounts of high quality protein. Today's VLCDs typically consist of fewer than 800 kcal/day and contain complete protein of high biological value, varying low amounts of carbohydrate, and vitamins, minerals and electrolytes (Atkinson, 1990). Gelfand and Hendler (1989) conducted an extensive review of over 100 research papers in order to examine the impact of different nutrients on body protein economy and energy balance during severe energy restriction. N balance was shown to demonstrate a time-dependent pattern whereby daily excretion of N declines over a 2-3 week adaptation period before reaching a stable plateau. Another major conclusion from this review is that when VLCDs of varying composition are compared, the protein intake appears to be the most important determinant of N balance. For example, Hoffer et al (1984) compared a diet containing 1.5 g protein/kg IBW with one containing 0.8 g/kg IBW and 0.7 g carbohydrate/kg IBW. A total energy intake of 550 kcal was the same in both diets. By day 14, all the subjects on the high protein intake achieved N equilibrium; subjects on the lower protein diet were not in N equilibrium and were still in negative balance at the end of the 8 week study. In general, when protein intake was between 30 to 60 g, daily N loss of $\geq$1g/day persisted after three weeks on the diet. Increasing protein to above 60-70 g resulted in almost consistent achievement of N balance by the third week of dieting (some subjects never attain zero N balance). All of the studies provided energy intakes of 200-720 kcal.

These findings support the current guidelines which emphasize the importance of providing generous quantities (≥70 g/day) of high quality protein in VLCDs (Gelfand & Hendler, 1989).

As was discussed previously, metabolic adaptation is a proven response to VLCDs which for the sake of this discussion can be considered synonymous with semi-starvation. Unlike the response to a total fast, the fall in RMR following initiation of a VLCD is not as severe and it seems reasonable to attribute this to its ability to spare lean tissues. The metabolic changes described earlier, including decreased insulin secretion, decreased levels of T_3 (when sodium is provided), and reduced SNS activity, occurred in response to both a severe energy restriction and a limited intake of carbohydrates, typical characteristics of VLCDs. Despite these adaptations, VLCDs continue to be used in selected patients because of their ability to produce rapid and safe weight loss (Atkinson, 1990).

3.2. Agents That Influence Thyroid Hormone Metabolism and the Nervous System

3.2.1. Thyroid Hormones

Results suggesting that the fall in RMR during energy restriction may be related to the concomitant fall in plasma levels of thyroid hormones prompted researchers to evaluate the effectiveness of exogenous T_3 administration in fasting or dieting subjects. The results indicated that T_3 administration prevented both the fall in its plasma levels and the decline in RMR (Welle & Campbell, 1986; Webb, 1986). A problem with these studies however, is that such large amounts of T_3 were administered that T_3 levels actually increased compared to baseline levels. Furthermore, studies revealed that with thyroid hormone therapy, both muscle protein breakdown and body N losses increased (Gardner et al. 1979; Burman et al. 1979) such that weight loss was mainly due to a decrease in FFM.

3.2.2. Nicotine

Nicotine has known appetite-reducing effects and these effects are though to be the cause for the weight gain that occurs after the cessation of smoking (Dallosso & James, 1984). Furthermore, cross-sectional and longitudinal data on energy intake and physical activity indicate that smokers do not differ from non-smokers (Fisher & Gordon, 1985, Stamford et al 1984) Given the findings that smokers weigh less than non-smokers the only remaining determinant of energy balance to account for these differences is metabolic rate. In a recent review (Warwick et al. 1987) of 14 studies that assessed the metabolic effects of smoking, 57% found positive evidence. Audrain et al. (1991) recently reported that nicotine increases RMR by 7.0%. This agrees with results of other investigations reporting similar increases The thermogenic effect of nicotine is thought to be mediated in part through autonomic nervous system activity since nicotine stimulates nicotinic acetylcholine receptors which form part of the autonomic nervous system (of which the SNS is one division) (Vander et al. 1985). Because nicotine has also been shown to stimulate SNS activity (Cryer et al. 1976), the effects of smoking after a meal on the TEF have also been assessed. The results to date are inconclusive (Audrain et al. 1991).

3.2.3. Caffeine

Studies in animals demonstrate that caffeine and other methylxanthines reduce body weight and fat mass by both anorectic and thermogenic effects (Dulloo & Miller, 1984). The stimulatory effect of caffeine on metabolic rate is also well established in man, both at high doses (Acheson et al. 1980) and in the amounts present in a cup of coffee (Dulloo et al 1989) Dulloo et al. (1989) recently reported that administration of 100 mg caffeine increased the RMR of both lean and postobese human volunteers by 3-4% and potentiated the TEF by 25-30% in postobese subjects, but had no significant effect in nonobese subjects. Twenty-four hour energy

expenditure was increased by 5% in both obese and nonobese subjects. They also found that repeated 100 mg doses of caffeine given every two hours for 12 hours immediately increased energy expenditure by 8-11% but had no effect on the subsequent 12 hours. These results support previous findings showing that the thermogenic effects of caffeine are short-lived (Arch et al. 1987). The means by which caffeine increases energy expenditure are not fully understood; however, caffeine has been shown to augment the thermogenic effects of certain sympathetic stimulants (Astrup et al. 1991) which in itself may suggest that caffeine potentiates these effects via adrenergic mechanisms.

3.2.4. Amphetamine

Amphetamine and similar drugs induce weight loss primarily by reducing food intake. A recent study revealed that when administered at a dose of 10 mg/70 kg body weight, twice a day, amphetamine reduced energy intake by 30% (Foltin et al. 1990). Amphetamine seems to exert its effects in the CNS by releasing norepinephrine from central noradrenergic neurons. In man, drug-induced loss of acuity of smell and taste, and increased physical activity are also thought to contribute to weight loss. Several factors make amphetamine less than ideal as a treatment for obesity. Tolerance develops rapidly because the releasable stores of norepinephrine become depleted. Furthermore, amphetamine causes mood enhancement, elation and euphoria and improved physical performance, all of which can lead certain individuals to abuse and addiction. In addition, cessation of this drug appears to be associated with onset of depression.

3.2.5. Ephedrine

Ephedrine is another sympathomimetic drug which stimulates both α and β adrenoceptors; it not only stimulates the release of norepinephrine from sympathetic neurons,

but also has a direct agonist effect on β -adrenergic receptors (Bukowiecki et al 1982). Studies in both lean and obese animals show that chronic ephedrine administration promotes a decrease in body weight and an increase in energy expenditure, without significantly affecting energy intake (Yen et al. 1981). The thermogenic effect of ephedrine has also been demonstrated in humans (Astrup et al. 1985). Recently, Pasquali et al. (1992) demonstrated that in obese subjects following a VLCD, administration of ephedrine partially prevented the fall in RMR significantly improved the N balance, and maintained serum T_3 and urinary catecholamine metabolite levels near pretreatment values. Body weight loss however, showed no difference between ephedrine and placebo therapies possibly because of the short period of drug treatment (2 weeks) Horton and Geissler (1991) confirmed the stimulatory effect of ephedrine on metabolic rate in both lean and obese subjects. They also found that addition of ephedrine to a meal significantly increased the TEF in obese but not lean subjects. The greatest absolute rise in metabolic rate was produced by the combination of meal, ephedrine and aspirin; this enhancement of TEF was also not observed in the lean subjects. The investigators suggested that the aspirin potentiated the effects of ephedrine on sympathetically mediated thermogenesis by preventing the prostaglandin-mediated inhibition of NE release.

3.2.6 L-3,4- Dihydroxyphenylalanine (L-DOPA)

L-DOPA is converted in the neuron to dopamine which acts as a sympathomimetic agent to displace norepinephrine from sympathetic nerve endings (Landsberg & Bruno, 1973). Shetty et al. (1979) administered L-DOPA or placebo to obese subjects at the start of a 24 day VLCD. Venous norepinephrine increased in the L-DOPA group and RMR failed to decrease, as occurred in the placebo group. Serum T_3 levels decreased to the same extent in both groups

The release of stored norepinephrine induced by sympathomimetic drugs occurs widely throughout the body and this norepinephrine can act at a wide variety of adrenergic receptors (α and β). Because of norepinephrine's diverse activities, cardiovascular side effects are common with these drugs and concerns about their safety persist (Cawthorne, 992). Research is currently being done to develop drugs that act on specific receptors, such that a better separation between cardiac and thermogenic effects can be achieved (Jequier et al. 1992).

3.2.7. Serotonin Agonists

Serotonin, like the catecholamines and histamine, is a monoamine neurotransmitter. It is produced in axon terminals from the essential amino acid, tryptophan. Changes in blood levels of tryptophan, resulting from changes in macronutrient intake, or changes in the rate of tryptophan transport from the blood into the extracellular space of the nervous system and into the synaptic terminals, can affect the rate at which serotonin is produced (Vander et al. 1985). Serotonin pathways exist in both the CNS and the peripheral nervous system. "In the periphery, serotonin acts in collaboration with other neuromodulators in a complex network that links sensory receptors generating moment to moment information on the state of the gastrointestinal tract with effectors in musculature, secretory, and absorptive epithelium, blood vasculature; and entero-endocrine cells" (Blundell, 1992). Serotonin releasing neurons occur in virtually all regions of the brain When considering the diffuse serotonergic innervation of the brain, it is easy to understand that the serotonergic system is involved in many cerebral functions, such as control of emotional behaviour, sleep and wakefulness, endocrine function, appetite, body temperature, blood pressure and pain perception (Gothert, 1992).

Serotonin-containing cell bodies can be divided in many distinct nuclei separated into rostral and caudal brainstem groups (Blundell, 1992). The caudal group consists of five main

nuclei and descending projections from this group modulate sensory and motor processing in the spinal cord. The rostral group consists of four main nuclei and they send long axonal pathways projecting into the forebrain to make contact with zones known to be important integrative sites for the control of feeding (eg hypothalamus) (Blundell, 1992).

In addition to the marked diffusion of serotonergic axon terminals in the brain, the heterogeneity of the serotonin (5-hydroxytryptamine, 5-HT) receptors and the differences in distribution of the various 5-HT receptor types and subtypes within the brain makes serotonergic neurotransmission in the brain very complex (Gothert, 1992) The current classification scheme of 5-HT receptors is based on the integrated results of pharmacological, biochemical, electrophysiological and molecular investigations There are currently four receptor types (5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4) and five receptor subtypes (5-HT_1, 5-HT_{1a}, 5-HT_{1b}, 5-HT_{1d} and 5-HT_2 5-HT_2 and 5-HT_{1c}) (Gothert, 1992). The functions of each of these receptors have not been fully described at present.

3.2.7.1. Serotonin and Thermoregulation

Among the varied cerebral functions involving the serotonergic systems, thermoregulation is a function that may be implicated in the control of energy expenditure The autonomic and central control mechanisms regulating body temperature have been well described Based on these results, serotonergic pathways in the hypothalamus were implicated as part of the central mechanisms for heat production (Myers & Waller, 1978) The role of serotonin in thermoregulation is based on anatomical, pharmacological, physiological and metabolic evidence

First, serotonergic nerve endings terminate in the hypothalamic region that has been classically implicated in thermogenesis (Myers & Yaksh, 1969) Destruction of these neurons impairs thermoregulation.

Direct injection of serotonin into the anterior hypothalamus causes a dose-dependent rise in body temperature (Myers & Waller, 1978). Many of the studies involving centrally injected antagonists also support the conclusion that serotonin affects body temperature (Myers & Waller, 1978) Peripherally administered serotonin in mice induces a dose-dependent hypothermia (Sugimoto et al 1992) The hypothermic effects of serotonin are antagonized by peripheral 5-HT_2 antagonists, suggesting that activation of peripheral 5-HT_2 receptors induces hypothermia. This effect may be due to cutaneous vasodilation, which accelerates heat loss from the body (Vanhoutte et al 1981, Myers & Waller, 1978). Temperatures changes have been demonstrated in both animals and humans with a number of serotoninomimetic phenylpiperazines and related agents (M-CPP, MK-212) Body temperature increases temporarily after acute M-CPP administration (Mueller et al 1985; Mueller et al. 1986; Charney et al. 1982; Wozniak et al. 1989; Kahn et al 1992). However, the available antagonist studies are inadequate to identify 5-HT receptor subtypes involved in M-CPP's temperature effects. MK-212 has also been shown to increase temperature in rats (Gudelsky et al. 1986) and in healthy humans but not in schizophrenic subjects (Lee et al. 1992). Through antagonist studies, central 5-HT_2 receptors were implicated in MK-212's hyperthermic response (Murphy et al. 1991). None of these compounds are used clinically and all of the studies involved single dose administration. Azapirones are another group of serotonin agonists which affect temperature. The azapirone, 8-OH-PDAT produces dose-dependent hypothermia in rats and mice (Gudelsky et al. 1986; Wozniak et al 1988; Wozniak et al. 1991), but has never been studied in humans. The hypothermic effects of 8-OH-PDAT may involve the 5-HT_{1a} receptor since this drug is a selective agonist at this site. Fenfluramine, a serotonin agonist used clinically to treat obesity has also been shown to produce temporary hyperthermia after a single administration in rats and chickens (Tagliaferro et al 1982; Sulpizio et al. 1978; Frey, 1975). An increase in temperature among

humans using this drug chronically for obesity therapy has not been described This is not surprising given that the doses used in the animal studies to produce hyperthermia are 10-20x the dose used clinically.

3.2.7.2. Serotonin and Food Intake

The involvement of serotoninergic neurons in the expression of appetite has been recognized for more than 20 years (Blundell, 1992). Most studies investigating this involvement have been done on rats, but studies in the leech and in humans have also been carried out. These investigations include the use of electrical and chemical stimulation of the brain, electrolytic and neurotoxin induced lesions, correlations of energy intake and brain serotonin concentrations after knife cuts, lesions, pharmacologic agonism and antagonism, neurotransmitter precursors and nutrient-transmitter interactions (Blundell, 1992) Diet-induced changes in the concentration of nutrient precursors within the brain can alter the rate of neurotransmitter synthesis. Serotonin is one of the best known neurotransmitters under precursor control. Increased concentration of serotonin metabolites are seen in cerebrospinal fluid and/or plasma after tryptophan ingestion (Anderson, 1988). Research in animals has shown that the relative proportion of protein and carbohydrate in a meal can influence brain amino acid levels and therefore serotonin synthesis. Ingestion of a meal high in carbohydrate preferentially enhances brain tryptophan uptake and serotonin synthesis. In contrast, ingestion of a meal high in protein, results in a decrease in tryptophan uptake into the brain The manner in which dietary-induced changes in serotonin synthesis affects food intake has not been conclusively described to date.

Serotonin does not cross the blood brain barrier, but serotonergic effects can be modulated by drugs that alter its pharmacology in the brain (Wurtman & Wurtman, 1977).

Hyperphagia has been shown to be induced in animals by serotonin receptor antagonists (Silverstone & Schuyler, 1975) or by a serotonin synthesis inhibitor (Breisch et al. 1976). Conversely, hypophagia is observed after increasing serotonin neurotransmission by administration of serotonin itself (directly into the hypothalamus), its precursors (Rezek & Novin, 1975), serotonin receptor agonists (Rezek & Novin, 1975) and by a serotonin releaser (Wurtman & Wurtman, 1977) In addition to the effects of serotonin on feeding behaviour, direct administration of serotonin to rats (subcutaneous injection) resulted in a 20.5 ± 3.3% increase in resting O_2 consumption (Jequier et al. 1992). The increase in VO_2 was though to be mediated by the autonomic nervous system since it was inhibited by an autonomic ganglionic blocker (hexamethonium).

The use of serotonergic agents in the treatment of obesity continues to be the subject of research interest. Much of this interest lies in the fact that unlike amphetamine or other currently available anorectic drugs, serotonergic agonists have not been associated with the same stimulatory and cardiovascular side effects and appears not to be addictive. However the hazards of long term serotonergic therapy for obesity remain uncertain (Munro et al. 1992).

3.2.7.3 Fenfluramine

The only serotonergic drug available for the treatment of human obesity in Canada is d,l-fenfluramine. Fenfluramine causes central serotonergic neurons to release serotonin. Previous work in animals has established that fenfluramine has a broader spectrum of anorectic activity than amphetamine; for example, fenfluramine decreases the motivated search for food (Thurlby et al. 1985), decreases ingestion linked to the palatability of food (Borsini et al. 1985), and reduces stress-induced eating (Antelman, 1979). The principal reason fenfluramine causes weight reduction is because of its centrally mediated anorectic properties (Rowland & Carlton,

1986). Fenfluramine is said to diminish intake by shortening meals rather than by reducing their frequency. The drug is said to work by a meal satiety mechanism rather than by a meal initiation mechanism (Cawthorne, 1992). With the initiation of fenfluramine treatment (and no dietary instruction) an additional weight loss of about 0 25 kg/week will greater than that achieved with placebo (Munro et al. 1992). In obese patients, the weight-lowering effect of short-term (3 months) fenfluramine administration is also well documented. The results of several studies demonstrated that fenfluramine together with a moderate energy restriction significantly reduces hunger and food intake and produces greater weight loss as compared to diet alone (Guy-Grand, 1987).

Long-term clinical studies have shown that continuous administration of fenfluramine to obese patients results in weight loss that is maintained for one year (Douglas et al. 1983). The results of a one year trial involving d-fenfluramine, the International Dexfenfluramine Study (Guy-Grand et al. 1989) recently reported that 15 mg twice daily of d-fenfluramine administration resulted in increased adherence to the weight-lowering program and greater weight loss It was also found that a plateau in weight was achieved after six months (occurring in both fenfluramine and placebo groups) indicating that the drug did not promote further weight loss but helped to avoid the weight regain, which occurred in the placebo group. A recent study by Finer and Finer (1989) supports these findings. Obese subjects lost 14 kg after 8 weeks of a VLCD Patients were then randomized to either drug or placebo treatments associated with a moderate energy restriction for 26 weeks. Fenfluramine-treated patients continued to lose weight (5 8 kg) whereas placebo patients regained 2.6 kg. Finally, it was reported that when drug treatment was stopped after 12 months, there as a 9kg weight regain over the following year (Hudson, 1977).

As interesting as the anorectic properties of this serotoninergic drug are its thermogenic properties. While studying the nature of tolerance to the anorectic effects of amphetamine and

fenfluramine, Levitsky et al. (1981) noticed that when animals continue to receive anorectic drugs, their body weights remained suppressed despite the fact that food intake returns to normal. The lowered body weight persisted as long as drug treatment was maintained and it returns to control levels once treatment stops The investigators also observed that weight regain after drug termination can be achieved without an increase in food intake. These observations suggest that anorectic drugs increase energy expenditure. Artifactual reasons for the above observations such as increased locomotor activity and interference in intestinal absorption of nutrients were ruled out for fenfluramine (Levitsky & Troiano, 1992). A series of studies testing the thermic effect of fenfluramine have been carried out in animals. Levitsky et al. (1986a) administered 20 mg fenfluramine to rats in the postabsorptive state and along with a meal. Energy expenditure was unchanged in the fasted animals, but it increased an additional 10-20% above the increases seen in the meal and placebo group. The authors also found that fenfluramine potentiated the TEF for carbohydrate but not for fat. The same authors then tested whether there was tolerance to fenfluramine's ability to potentiate the TEF by measuring the effect it had on TEF at the beginning and after 15 days of treatment (Levitsky & Troiano, 1992). On day 1 of treatment fenfluramine effectively potentiated the TEF. This effect disappeared by day 15. From this study it was noted that despite the suppression of body weight caused by restricted feeding in the fenfluramine group, metabolic rates postprandially were similar to the control animals who did not lose weight. To test whether fenfluramine prevented the expected fall in energy expenditure TEF was again measured two days after drug treatment was stopped. The TEF of the previously treated animals was significantly suppressed which the authors took to indicate that fenfluramine had indeed prevented the normal fall in metabolism The application of these findings to humans is difficult. The doses used to produce the thermic effects in the animal studies (20 mg or 4 mg/kg) are far larger, on a per kg basis, than doses used in human studies.

To determine whether an acute dose of fenfluramine increases metabolic rate in humans, Troiano et al. (1990) more recently measured the metabolic rate in 16 non-obese young men after either a 750 calorie mixed meal or fasting, with either 60 mg fenfluramine or placebo. The TEF was potentiated by the drug treatment. The results suggested that fenfluramine increased metabolic rate by prolonging the TEF. Fenfluramine had no effect on energy expenditure in the fasting subjects. Similar findings were also reported by Munger et al. (1988) However, most of these studies were carried out in lean subjects whereas fenfluramine is used in obese subjects, and these studies did not specifically test the acute thermogenic effect of the drug. In fact, a recent report (Breum et al. 1990) suggested that fenfluramine did not have any long-term effect on 24 hour energy expenditure in obese subjects. In the latest available study on fenfluramine, Scalfi et al. (1993) submitted seven obese men to four tests in which fenfluramine (30 mg) or placebo were combined with a mixed mean or no meal. In the fasting state RMR increased 6.9% after fenfluramine but not after placebo administration. The TEF of a mixed meal was also significantly higher in the drug group (4.6 ± 1.7% vs 3.6 ± 1.5% - percentage of energy content of the meal). However, this effect did not suggest an additive effect on energy expenditure between fenfluramine alone and food alone (ie. 6.9% + 3.6%)

Based on the studies in animals and humans, the main possible effects of fenfluramine can be summarized as follows, first it reduces food intake by enhancing the serotonin-mediated satiating power of food; second it reduces the motivation for eating under different conditions and finally, it increases the thermic effect of food. However, it is still difficult to relate the acute thermogenic effects of fenfluramine to its weight reducing action. Appropriate longitudinal studies would be necessary to establish that hypothesis.

3.2.7.4. Fluoxetine

Fluoxetine is the first of a new generation of antidepressants that acts by selectively inhibiting serotonin reuptake into presynaptic nerve endings in the brain (Anonymous, 1993). Although weight gain is often reported among depressed patients treated with tricyclic antidepressants, depressed patients treated with fluoxetine commonly lose weight (Cohn & Wilcox, 1985) From the observations that serotonin acts in the CNS to modulate feeding behaviour and that serotonin agonists can alter energy expenditure, fluoxetine, with its enhancement of serotonergic function, has the potential to affect eating behaviour and body weight.

Goudie et al. (1976) were the first to report a decrease in food intake in rats treated with fluoxetine. Wurtman and Wurtman (1977) reported that fluoxetine spared protein intake while reducing total caloric intake in rats. Decreases in food intake and body weight have since been confirmed in several studies in both rats and mice (Rowland et al. 1982; Wong & Fuller, 1987; Yen et al. 1987). Furthermore, like fenfluramine, fluoxetine has been shown to be effective in suppressing feeding behaviour linked to the palatability of food (Leander, 1987), stress induced feeding (Antelman, 1979) and insulin induced feeding (Carruba et al. 1985). Fluoxetine also affects feeding behaviour by reducing meal size but has no effect on meal frequency, which suggests it has a satiating effect (Clifton et al. 1989).

The anorectic properties of fluoxetine have also been demonstrated in humans. A dose response study was done in nondepressed obese adults who were randomized to placebo or fluoxetine (10,20,40 or 60 mg per day) (Levine et al. 1989). After 8 weeks of drug therapy (no diet or exercise instructions were given) mean weight loss was significantly greater in the 60 mg dose group. All of the different drug-dose groups had greater mean weight loss than the placebo group except the 10 mg fluoxetine group. Wise (1992) reviewed six short-term studies of 6-12

weeks which evaluated the anorectic-weight reducing properties of fluoxetine in obese subjects. The dose was either fixed at 60 mg or varied between 20-80 mg Subjects were not given instructions with respect to diet or exercise. The results indicate that fluoxetine-treated patients consistently showed greater weight loss than placebo-treated patients with a mean weight change of about -0.5 kg/week.

In a review of the longer-term studies in obesity, a maximum mean weight loss of about 5 kg has been shown to occur after 12-20 weeks of fluoxetine therapy (Wise, 1992) The pattern of weight loss in fluoxetine treated patients follows the same pattern described earlier for fenfluramine, such that a plateau in body weight occurs after approximately 6 months (Munro et al. 1992). Darga et al. (1991) carried out a 1 year double-blind comparison of 60 mg fluoxetine to placebo in obese patients who also received dietary counselling. Maximum mean weight loss was l2.4 kg, achieved at week 29 for the fluoxetine-diet group Placebo-diet subjects lost 4.5 kg, significantly less weight by that same time. A gradual weight regain occurred from about the 6 month point to the end of the study in subjects given fluoxetine but not in those who received placebo. End of study weight loss was 8.2 ± 1.9 kg in the fluoxetine group and 4.6 ± 1 l kg in the placebo group. An identical study by Marcus et al. (1990) reported somewhat different results. Fluoxetine-treated patients lost 13.9 ± 12.7 kg after l year of drug and diet therapy while those on placebo failed to show any significant weight change. Also, the fluoxetine-treated patients did not regain weight on the medication and continued to lose weight although at a significantly slower rate throughout the year long study.

The therapeutic efficacy of fluoxetine and fenfluramine appears to be about the same as that of the sympathomimetic compounds (Munro et al. 1992). Further research is currently being done to assess the clinical value of fluoxetine as an adjunct to weight loss programs and to elucidate the mechanisms by which serotonin agonists induce weight loss. All serotonin-acting

drugs increase serotonin concentration in serotonergic synapses, thereby making it more available to receptor sites Part of the difference between the different serotonin agonists lies in which receptor subtypes are influenced. Serotonin antagonists specific to the different receptor subtypes are used in conjunction with agonists to determine which subtypes are influenced by which drug and what effects stimulation of that receptor subtype produces. Receptor affinity is one way in which fluoxetine differs from fenfluramine. A study was conducted in which serotonin antagonists were given with fluoxetine or fenfluramine to determine which receptor subtypes are involved with their anorectic properties (Garattini et al. 1992). Surprisingly, fluoxetine induced anorexia was not reduced by any of the antagonists, whereas that induced by fenfluramine was. This suggests that the receptor mechanisms involved in producing anorexia by fluoxetine are different from those of fenfluramine. However, it must be considered that these serotonin antagonists are not equally selective on 5-HT receptors, making a comparison between the effects of fluoxetine and fenfluramine in terms of 5-HT receptor affinity difficult (Garattini et al. 1992). Furthermore, some studies have shown fluoxetine to have no effect on any serotonin receptors (Sommi et al. 1987). Until serotonin antagonists specific to every serotonin receptor subtype are available, mechanistic questions will persist about how fluoxetine and other serotonergic drugs work. Aside from receptor mechanisms, fluoxetine and fenfluramine also differ in their "serotonin-releasing" activities (serotonin reuptake inhibition in the case of fluoxetine). Superfused rat hypocampal synaptosomes, previously loaded with ^{3}H-5-HT, were exposed for three minutes to different concentrations of fluoxetine or fenfluramine. This study showed that fenfluramine-induced release of serotonin was immediate whereas a delay of two minutes was observed with fluoxetine. The dose-response curve was plotted using drug concentrations ranging from 0.03 - 10 μ mol/L. Fenfluramine was saturable, while fluoxetine was not. In addition,

indalpine, an inhibitor of serotonin uptake blocked the effect of fenfluramine, but not of fluoxetine (Garattini et al. 1992)

Fluoxetine's safety has been confirmed in both depressed and obese subjects In terms of adverse side effects, headache, nausea, asthenia, diarrhea, somnolence, insomnia, nervousness, sweating and tremor are the most frequently reported (Cooper, 1988) One aspect of the side effect profile of fluoxetine (nervousness and tremor) could be evidence of a sympathetically mediated stimulation. Lipinski et al (1989) reported that akathisia (nervousness, purposeless movement of feet, legs) induced by fluoxetine can be effectively reduced by propranolol, a ß-adrenergic antagonist. The authors hypothesized that fluoxetine-induced akathisia may be caused by serotonergically mediated inhibition of dopaminergic neurotransmission.

4. SUMMARY AND RATIONALE FOR RESEARCH PLAN

Energy restriction in humans, whether lean or obese, results in a reduction in the RMR In the short term, much of this reduction is adaptive,stemming from the decrease in metabolic activity of the body cell mass; with continuing semistarvation and increased loss of active cell mass, RMR continues to decrease and the latter component assumes a greater and greater role in explaining the decrease in RMR. Metabolic efficiency of the FFM is achieved primarily through reduced sympathetic activity, changes in peripheral thyroid hormone metabolism and lowered insulin secretion. There is also controversial evidence that a decrease in the TEF contributes to the increase in metabolic efficiency that accompanies energy restriction Unfortunately, while these adaptations permit the prolongation of life, they work against one goal of obesity therapy, i.e. to sustain maximal possible rates of fat loss by maintaining a large energy deficit, without excessive loss of lean tissue.

Of the two sides in the energy balance equation, energy intake and energy expenditure, obesity experts have generally focused on modifying an individuals' intakes as a means of achieving safe and effective weight loss, and this remains the preferred approach to achieve long-term weight goals. The development of high-protein, very low energy diets is an example of a dietary modification that spares lean tissues while producing rapid and significant weight loss

Another approach to achieve weight reduction is to increase energy expenditure. The idea of using thermogenic agents is not new. Thyroid hormones may be used for this purpose, but they induce a greater loss of protein than fat. Catecholamine-stimulating appetite suppressants have potent stimulant properties, are prone to abuse, and produce undesirable cardiovascular side effects. On the other hand, serotonin-acting anorectic compounds such as fenfluramine and fluoxetine are less hazardous than the catecholaminergic compounds and are at least as effective in inducing weight loss. A number of pharmacological studies in animals and leans and obese humans have also shown that fenfluramine is thermogenic. Data suggest that acute administration of fenfluramine increases energy expenditure in the fasted state, as well as after the ingestion of food. A direct relationship between fenfluramine's thermogenic effects and its ability to induce weight loss has yet to be described.

In view of the reported thermogenic effect of the serotonin agonist fenfluramine, it was felt justified to study these same effects in fluoxetine. This idea is of interest for two reasons. The first is the issue of whether serotonergic pathways are directly or indirectly involved in thermogenesis. The second is the more practical aspect of weight loss which can be influenced by the adaptive decline in energy expenditure that occurs with the introduction of a VLCD. Any intervention which prevents or blunts this adaptive decline would result in faster and more prolonged weight loss at any given energy intake.

4.1. Hypotheses

1. It was hypothesized that 60 mg fluoxetine administration to obese subjects on a very low energy diet for three weeks would blunt the decrease in RMR that occurs when placebo is administered. In order to test for such an effect of fluoxetine in the most sensitive way, a 1757 kJ/420 kcal/day study diet was chosen for which the fall in RMR is easily and reliably demonstrable. Since metabolic adaptation is mediated via hormonal mechanisms the postulated thermic effect of fluoxetine would potentially be detected through the measurement of urinary catecholamines, serum thyroid hormones and insulin. N balance was measured during the VLCD in order to be able to comment on the composition of weight loss and the resulting body composition changes induced by fluoxetine. It was also desired to know if N retention during the VLCD would be altered by administering this CNS active drug.

2. Further, it was hypothesized that fluoxetine would potentiate the thermic response to a 112.5 g glucose test meal. Because insulin resistance has been reported to reduce the thermic effect of glucose, oral glucose tolerance tests (OGTT) were performed prior to the start of the VLCD in order to be able to account for the difference in TEF among the study subjects due to insulin resistance. The TEF was measured during a weight maintenance baseline period (prior to drug/placebo administration), on the last day of the VLCD and following 8 weeks of a 5016 kJ/1200 kcal balanced deficit diet. This will enable us to address the controversial issue of a blunted TEF following weight loss/energy restriction and how fluoxetine may alter the "blunted" TEF.

3. Finally, it was hypothesized that the RMR would remain higher in fluoxetine- than placebo-treated patients over a subsequent period of 8 weeks with a weight reduction diet of 5012

kJ/1200 kcal using normal foods Because of the slow onset of fluoxetine action (average half life 3-4 days), full pharmacological effect for all subjects was thought to require 18-24 days. To accommodate the possibility of delayed action, the VLCD period was kept to a feasible maximum (21 22 days) and the outpatient period of the study was kept to a maximum of 8 weeks, so that RMR and TEF measurements made during this time could detect the potentially delayed action of fluoxetine

5. EXPERIMENTAL DESIGN (Figure 1)

1 Suitable patients were admitted to the Clinical Investigation Unit (CIU) of the Royal Victoria Hospital, and placed for 4-5 days on a conventional formula diet providing all maintenance needs (inclusion and exclusion criteria are described below). During this time and throughout the subsequent study, all urine was saved in serial 24-hour collections for analysis of N excretion, and the subjects underwent at least 2 measurements of RMR, and one measurement of the thermic effect of a standard glucose meal (TEF).

2. On day 1 of the very low energy diet (VLCD), and following a measurement of the RMR, fluoxetine (F) 60 mg or placebo (P) was administered each morning and a 1757 kJ/420 kcal liquid formula diet was given in three meals. No smoking was allowed. Body weight was recorded daily after voiding and in bed clothes RMR was measured early on the mornings of days 1, 3, 6, 8, 10, 13, 15, 17, 20, 22 (final day of VLCD), in each case prior to administration of that day's fluoxetine dose The TEF was measured on the final day of the VLCD.

3. While continuing fluoxetine or placebo, each subject was "refed" for 3-4 days in hospital with a conventional balance deficit diet (BDD). The subjects were then discharged with dietary counselling to continue this BDD during the outpatient phase

4. Subjects returned for morning follow-up visits which included dietary counselling every two weeks for a total of 8 weeks while continuing on the study medication. Body weight (in standard clothes without shoes) was recorded at each visit. At the 4 week post-discharge visit, RMR was measured. At the 8 week post-discharge visit (the final visit of the study) Both the RMR and the TEF were measured.

6. MATERIALS AND METHODS

6.1. Subjects

Moderately obese women aged 20 to 48 years volunteered to participate in the study Subjects learned of the study through advertisements placed in a local newspaper Potential subjects were required to be in good health with no history of metabolic or cardiac disease, depression and receiving no chronic medication including oral contraceptives An entry criterion was a body mass index (BMI) of 30 kg/m^2 or more Subjects were carefully interviewed and if they appeared likely to benefit medically from weight loss and to be able to adhere to the study protocol, they underwent a complete medical history, physical examination (by Dr L J Hoffer), pregnancy test, haematological tests (complete blood count with differential white cell count and platelets), blood chemistry (sodium, potassium, chloride, CO_2, glucose, urea N, creatinine, triglycerides, cholesterol, uric acid, albumin, total protein, alkaline phosphatase, ALT or AST, thyroid indices and haemoglobin A_1C), urinalysis, electrocardiogram and chest X-ray (postero-anterior and lateral views). Date of the last menstrual period was recorded.

6.2. Research Facility

The subjects were admitted to the Clinical Investigation Unit (CIU) of the Royal Victoria Hospital Nursing procedures included twice daily measurement of heart rate, standing and supine blood pressure and daily determinations of basal temperature while in hospital. Basal temperature was measured with a digital Becton-Dickinson (Toronto, Ontario) basal thermometer (temperature range 35-38^{o}C) Subjects were carefully instructed to take their temperature at the same time each morning, after voiding and before breakfast, by placing the thermometer under the base of the tongue and not to breath through their mouth for 3 minutes. Subjects were weighed each morning at the same time in the same bed clothes, after voiding but before breakfast on a Scale-Tronix^{R} (Ingram & Bell - Meditron, Le Groupe, Inc., Don Mills, Ontario) digital scale (accurate to 0 1 kg) During the baseline period, waist and hip circumferences were measured in order to calculate the waist:hip ratio. Measurements were made on subjects standing up Waist circumference was measured at the narrowest part of the torso, or at the circumference 4 centimetres above the umbilicus, when a natural waist was not evident. Hip circumference was measured in a horizontal plane at the levels of the buttocks (Hardy, 1961). Urine ketones were determined daily using the Chemstrip^{R} 5L (Boehringer Mannheim, Laval, Quebec) to monitor dietary compliance. Subjects were instructed to record days of menstruation in a patient record book. Final preparation of meals were done by either the CIU dietitian or the subjects themselves, under supervision).

6.3. Diets

Upon admission to the hospital, all subjects were given a liquid formula diet calculated to provide maintenance energy requirements. Energy intake was based on the subject's calculated RMR (according to the Harris-Benedict equation) multiplied by 1.5 (Mahalko &

Jonhson, 1980). This intake was calculated to meet the weight maintenance needs of the subjects, which was essential in establishing reproducible baseline data for all the variables measured.This 4-5 day baseline diet consisted of a commercial meal replacement product (Ensure, Ross Laboratories, Montreal, Quebec) and was supplemented with glucose polymer derived from corn starch (Polycose, Ross Laboratories) A standard volume of Ensure was used for all subjects so that protein intake would be constant. When energy requirements were greater than the energy provided in the ensure, additional energy was supplied by Polycose The diet contained 80 grams of protein and fat and varying amounts of carbohydrate (due to the differing amounts of polycose added). The composition of the products used in the baseline diet is given in Table 2.

During the subsequent 21-22 days, all the subjects consumed a liquid formula very low energy diet (Optifast 70, Sandoz Nutrition, Minneapolis, Minn.). The diet provided 1757 kJ/420 kcal per day and at least 100% of the established Canadian Recommended Nutrient Intake for essential vitamins and minerals, with the exception of iodine which provide 94% of the RNI. The composition of the VLCD is given in Table 2. Subjects were instructed to drink at least 2 litres of water daily and to consume no other foods or beverages Subjects were given five prepackaged envelopes of the Optifast powder. The powder in each envelope was combined with 200 ml of water. Two envelopes were consumed at breakfast and at supper, and one envelope was consumed at lunch. Subjects prepared their morning and midday meals in the research kitchen in the presence of the research dietitian.

After completing 3 weeks of the VLCD, subjects were refed on a 5016 kJ/1200 kcal per day balanced deficit diet composed of regular foods. The diet was approximately 15% protein, 55% carbohydrate and 30% fat. It was based on the Good Health Eating Guide which uses a

choice/exchange system (Canadian Diabetes Association, 1980). Subjects were instructed to follow this diet during the entire eight week outpatient phase of the study.

The N content of both the baseline diet and the VLCD (samples of all lots were used) was determined using the Kjeldahl digestion (Munro & Fleck, 1969). All subjects took both the baseline diet (Ensure) and the VLCD (Optifast) from a single lot for the entire study.

6.4. Randomization Procedure

Subjects were assigned at random to either fluoxetine (60 mg) or placebo by a study coordinator at Eli Lilly, Canada, Inc.. This was a randomized double-blind study such that neither the subject, nor any member of the CIU or research team were aware of the randomization list. Fluoxetine and placebo were in capsule form, white in colour and identical in appearance. Bottles were labelled "Fluoxetine: treatment of obesity" and were packaged in a patient kit, identified by a number. Proper randomization was ensured by assigning each kit sequentially, starting with the lowest available number. Each subject had her own kit. Each kit contained three bottles of 35 capsules.

6.5. Collections

Complete 24 hour urine collections were made for all inpatient protocol days. Urine was collected in containers with 15 ml of 12M hydrochloric acid as preservative and was stored at $4^{o}C$ during the 24 hour period. It was then aliquotted and either submitted for analysis or stored at $-20^{o}C$ Collections were analyzed for creatinine, urea N, total N, sodium, potassium, chloride (Wallach, 1981) and urinary catecholamines (epinephrine, norepinephrine, dopamine, metanephrine and normetanephrine)(normal ranges are based on inhospital determinations).

6.6. Blood Drawing

Postabsorbtive venous blood samples were drawn on the first and last day of the baseline period and on days 7,14 and 21 of the VLCD period.

(a) SMAC-16 (Wallach, 1981): Blood was collected in 10 ml red top sterile vacutainers (without anticoagulant) for determination of sodium, potassium, chloride, bicarbonate, urea, creatinine, glucose, uric acid, calcium, phosphorus, total protein, albumin, total bilirubin, alkaline phosphatase, ALT (alanine aminotransferase), AST (aspartate aminotransferase), LDH (lactate dehydrogenase), cholesterol and triglyceride.

(b) Complete Blood Cell Count (Wallach, 1981): Blood was collected in 7 ml lavender top EDTA-K_3 (ethylenediamine tetraacetate) sterile vacutainers for determination of haemoglobin, haematocrit, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils and platelets.

(c) Thyroid Indices: Blood was collected in 10 ml red top sterile vacutainers (without anticoagulant) for determination of T_4 (thyroxine), T_3 uptake, free T_4 index, T_3 and free T_3 index (normal ranges are based on inhospital determinations).

(d) Insulin: Five millilitres of blood was collected in 10 ml test tubes containing 0.5 ml of a Trasylol-Aprotinin - Heparin solution (Trasylol-Aprotinin:10,000 K.I.U./ml, Miles Canada Inc., Etobicoke, Ontario; Heparin:10,000 U.P.S./ml, Organon Teknika, Toronto, Ontario) for determination of insulin. The blood-Trasylol - Heparin mixture was centrifuged at 2800 rpm for 15 minutes, the plasma portion was separated and stored at -12^{o}C.

(e) Fluoxetine and Norefluoxetine: Blood samples were drawn following the collection procedures specified by Eli Lilly Canada, Inc. Blood was collected in 10 ml heparinized vacutainers and was then mixed and centrifuged immediately Heparinized plasma was then transferred to glass tubes (supplied by Eli Lilly Canada, Inc) and immediately frozen at -12^{o}C in

a horizontal position. Frozen specimens were then shipped on dry ice to the Wisconsin Analytical and Research Services for determination of fluoxetine and its metabolite, norefluoxetine. Test results were not disclosed until the study was completed.

(f) Modified Oral Glucose Tolerance Test (OGTT): A modified OGTT was performed on each subject after an overnight fast (10-12 h) during the baseline period. After a fasting blood sample was drawn from an antecubital fossa vein, a 75 g glucose load (Glucodex, Rougier Inc., Quebec) was given. Blood was then drawn two hours later. Blood was collected in 10 ml sterile vacutainers (without anticoagulant) and in 10 ml test tubes containing 0.5 ml of Trasylol - Heparin for determination of glucose and insulin levels respectively.

6.7. Analytical Methods

Urinary creatinine was determined by a modified Jaffe method (Heinegard & Tiderstrom, 1993) on the Beckman Syncron CX4 and CX5 systems (Brea, California). The automated Beckman systems were also used for urea nitrogen determination and for electrolyte determination (Ion Selective Electrode Methodology). These analyses were done by the hospital Biochemistry Laboratory. Urinary catecholamines were determined by weak cation exchange HPLC with electrochemical detection, following sample purification by cation and anion exchange resin chromatography, according to a method published by Biorad (BIORAD, Richmond, California). Urine total N was determined colorimetrically using the Kjeldahl method (Munro & Fleck, 1969) using an automated Technicon Autoanalyzer II (Chauncey, New York).

Haematological tests and blood chemistry (SMAC-16) were determined by the hospital clinical biochemistry laboratory, using standard automated methods (Technicon SMAC II and Technicon H-I System, respectively, Tarrytown, New York). Samples for the thyroid indices were determined in the hospital Endocrinology Laboratory by standard automated radioimmunoassay

techniques (ARIA II Becton Dickinson Immunodiagnostics, Baltimore, Maryland). Plasma samples were analyzed for insulin using a human insulin specific RIA (Linco Research Inc., St. Louis, Missouri).

6.8. Instrumentation

Energy expenditure was measured by continuous indirect calorimetry with the use of a Deltatrac ventilated hood indirect calorimeter (Sensor Medics, Yorba Linda, California). The hood is a 25 litre semiellipsoidal canopy placed over the subject's head and upper body. The ventilated hood method was used because it allows the 3.5 h measurement required to evaluate the thermic effect of food with minimal subject discomfort. Fixed air flow through the hood was 38.01 L/min. The flow was calibrated by combusting 5.00 ml absolute ethanol in a calibration burner unit provided with the instrument (the absolute ethanol used for calibration was carefully protected from ambient moisture using parafilm and an air tight bottle). The total amount of CO_2 produced during the test was used in the following calculation to determine the flow rate:

$$\text{New Flow Rate} = 1.03 \times \frac{3820}{\text{total } CO_2 \text{ production in ml}} \times \text{current flow}$$

The flow rate is relatively high in order to prevent expired CO_2 from accumulating in the hood. The analyzer measured oxygen with a differential paramagnetic sensor and carbon dioxide with an infrared sensor. O_2 consumption and CO_2 production are expressed under standard conditions (STPD): dry gas at $0^{\circ}C$ and 760 mm Hg. Measured VO_2 and VCO_2 consumption takes place under ambient conditions, therefore the Deltatrac corrects the gas volumes to standard conditions. RMR is determined from the measured VO_2 and VCO_2 using the Weir formula (Weir, 1949):

$$RMR = 5.68\ VO_2 + 1.59\ VCO_2 - 2.7\ Nu$$

Where RMR = resting metabolic rate in kcal/24 hours

VO_2 = O_2 consumption in ml/min

VCO_2 = CO_2 production in ml/min

Nu = urinary nitrogen excretion in g/24 hours

Prior to any measurement, gas analyzers were calibrated using a primary standard of 96% O_2, 4% CO_2. The accuracy of O_2 consumption and CO_2 production were then verified by combusting absolute ethanol and verifying the accuracy of the respiratory quotient (RQ = VCO_2/VO_2 = 0.667) and carbon dioxide production rate based on the weight of ethanol consumed. The Deltatrac indirect calorimeter has less than a 2% error in determining the concentrations of O_2 and CO_2 over the full range of concentrations (i.e. 0-100%). And, the stability of the CO_2 analyzer over the full range of concentration has an error of less than 0.2%.

6.9. Resting Metabolic Rate (RMR)

RMR was measured three times during the baseline diet, and every two or three days during the VLCD, thus on days 1, 3, 6, 8, 10, 13, 15, 17, 20 and 22. It was also measured at week 4 and week 8 of the 8 week outpatient phase. Serial measurements on each patient were performed by the same operator at the same time of day (8-9 AM) (Garrow, 1978). The measurements were performed on subjects after a 6-8 hour sleep and after a 10-12 hour fast, in a semi-darkened room with only the subject and tester present. Study medication was not taken prior to the test. If they so wished, subjects could hear restful music played at a low volume to help them relax and prevent them from falling asleep. The room temperature was carefully maintained between 21-24^{o}C by heater/air conditioner. Prior to each test, subjects lay in a semi-recumbent position on a bed for at least 20 minutes. The Deltatrac generates data each minute for VO_2, VCO_2, RQ and energy expenditure. A 15 minute RMR determination was preceded by

5 minutes under the ventilated hood during which time data were collected but not included in the analysis. The steady state, defined as the time during which the coefficient of variability for VO_2 and VCO_2 was less than 5% of the respective mean, was usually achieved in the 15 minute testing duration. If a steady state was not achieved, the RMR was repeated the next day. Studies have reported that repeated RMR measurements on the same subject suggest the existence of a training effect whereby initial measurements of RMR are higher than subsequent measures after the subject becomes adjusted to the testing conditions (McClave & Snider, 1992) To avoid the training effect during the baseline period and as part of the screening procedure, subjects underwent two complete RMR measurements prior to the start of the study. Repeated RMR measurements on all 20 subjects during the baseline diet period were highly reproducible, with a mean coefficient of variability (C.V.) of 2.24 ± 0.27%.

6.10. Thermic Effect of Food

The TEF was assessed on three occasions for each subject, at the end of the baseline diet, the VLCD and the outpatient phase. The TEF protocol was developed based on the results of serial metabolic rate measurements made in 6 normal subjects. Based on the literature, glucose was chosen for the test meal primarily because it induces a relatively strong thermogenic response (7-10%), and this effect is generally completed or on a downward slope after 3 hours. The length of time to consume the test meal, the duration of the TEF measurement, and the scheduling of 10 minute break periods were all varied systematically until the most reproducible results were obtained within an individual.

All began with a measurement of the RMR as described above. Following the RMR, subject sat up on the bed (standing or walking was prohibited) and consumed the test meal over the subsequent 15 minutes. Subjects were given 112.5 g glucose in 450 ml of carbonated water

(Glucodex, 1763 kg/422 kcal). The test meal was divided in three equal parts and consumed in three five minute intervals. Subjects were instructed to drink the test meal slowly so as to avoid discomfort due to nausea which could potentially alter rates of absorption and energy expenditure Subjects were then instructed to resume the same position held during the RMR and the ventilated hood was placed over the subject's head. Metabolic rate was then measured postprandially for 210 minutes, with three ten minute breaks at minute 55, 120 and 165; these breaks allowed the subjects to urinate if desired. Ultimately, 180 discrete measures were made; data for the 30 minutes of break time were generated by interpolating the points from the data immediately before and after As with the RMR all sensory inputs were controlled, i.e., the lights in the room were dimmed, music was played at a low volume and the only other person in the room was the tester (R. Bross). All measurements of RMR or TEF were performed in a closed study room in the McGill Nutrition and Food Science Centre (Crabtree Nutrition Laboratory).

6.11. Calculations

N balance was calculated as the N intake minus the sum of urinary, fecal and miscellaneous losses. N intake was determined to be 12.10 ± 0.10 g and 11.2 ± 0.11 g on the Ensure and Optifast diets respectively by Kjeldhal analysis of the formulas. The values obtained were in agreement with the label claims. Fecal N losses were assumed to be 0.6 g/day based on reported fecal losses in similar subjects following a similar weight loss protocol (Hoffer et al. 1984). Miscellaneous N losses were assumed to be 8 mg/kg/day (FAO/WHO/UNU, 1985). Urinary N was determined from daily 24 hour collections Losses of lean body mass were estimated from changes in N balance, assuming 1 g N is equivalent to 6.25 g of body protein or 31.25 g LBM (Fricker et al. 1991).

The TEF was determined by subtracting the area under the RMR curve (RMR x 210 min) from the area of the TEF curve, where the TEF curve was generated by plotting energy expenditure against time for 210 minutes The difference between the area under the TEF and RMR curves was the postprandial energy expenditure. The TEF was then expressed in two ways (Schutz et al. 1984b):

A. The postprandial energy expenditure (EE) curve averaged over 210 minutes and expressed as a mean value (kcal/min), this was compared to the RMR. This response was expressed as the percentage increase of EE over the pre-meal baseline.

$$\% \text{ increase over RMR} = \frac{\text{postprandial EE (kcal/min)} - \text{RMR (kcal/min)}}{\text{RMR (kcal/min)}} \times 100\%$$

B. The TEF was expressed as a percent of the energy content of the test meal:

$$\text{TEF} = \frac{(\text{postprandial EE (kcal/min)} - \text{RMR (kcal/min)}) \times 210}{1763 \text{ kJ}/422 \text{ kcal}} \times 100\%$$

6.12. Adherence to Protocol

Several measures allowed an estimation of dietary adherence and the reliability of the urine collection. The most important way of determining that the entire baseline or VLCD diets but no other food would be consumed was the selection of responsible, motivated and enlightened subjects, combined with daily interviews with CIU staff (including R. Bross) and study physician. Subjects were observed consuming some of their meals and were questioned about meal schedules, appetite, and the palatability of the formulas. During the inpatient portion of the study, intakes were as controlled as possible within the limits of human experimentation During the outpatient portion of the study, subjects were given dietary counselling every two weeks This included a review of daily dietary records kept by the subjects and 24-hour recalls Furthermore, telephone conversations between the two week visits allowed for a further estimation of

adherence to the outpatient balanced deficit diet. No specific data was collected from these counselling sessions, therefore an exact quantification of the subjects energy and nutrient intakes during the BDD is not possible.

Urine creatinine was examined to confirm the completeness of urine collections. It is used as a measure of completeness because creatinine excretion is directly proportion to muscle mass, and since total muscle mass should not change significantly during the inpatient study, constant amounts of creatinine should be excreted on a daily basis. Urine urea, total N and potassium were monitored to detect increases due to dietary indiscretion or decreases due to failure to take meals. Urine ketones were examined qualitatively to confirm that the carbohydrate intake during the VLCD remained low. Urine ketone concentrations of 0.5 to 10 mmol/L were considered appropriate for the VLCD, given that it contained 30 g carbohydrate. Ketone levels far below this range would be an indication of a higher carbohydrate intake and therefore non-adherence to the diet. Subjects were also weighed at each visit.

Finally, pill counts and plasma levels of fluoxetine and its metabolite, norefluoxetine allowed for an accurate estimation of adherence to the drug protocol.

6.13. Hazards to Subjects and Informed Consent

The nature of the study and its potential hazards were explained to the subjects prior to signing a consent document which had been approved by the Royal Victoria Hospital Human Ethics Committee (see Appendix 1). This committee likewise reviewed and approved the study protocol It was explained to subjects that they were free to withdraw from the study at any time. Subjects were not remunerated for their participation in the study, however a $100 remuneration was given for the final TEF due to the loss of a half day of employment.

6.14. Statistical Analysis

A minimum sample size of 7.8 subjects per treatment group was estimated, based on a power calculation for a one-tailed test and α and β levels of 0.05 (Hall, 1983), given a 3% intraindividual variation of RMR on different days, an intraindividual standard deviation of 3%, and a 5% difference between the response of fluoxetine and placebo subjects (considered physiologically relevant) (Soares & Shetty, 1986). Ten subjects per group were proposed to be ample but not excessive for the purposes of the sample size.

The data for treatment and control groups were compared using repeated measures analysis of variance. Since the study involved making repeated observations on each subject over time, the analysis is a two-factor (treatment and time) analysis of variance with repeated measures on one factor (time). Whenever significant ($p \leq 0.05$) effects were detected for either time or treatment a contrast was done against the baseline variable to determine where significant effects occurred. The repeated measures ANOVA was used to analyze data for RMR, TEF, temperature, weight, pertinent biochemical indices, urinary catecholamines, nitrogen balance and thyroid indices. These variables were analyzed as absolute change from baseline to minimize any interindividual variability at onset. Data for baseline subject characteristics (see table 3), baseline urinary catecholamines, baseline thyroid indices, baseline TEF, plasma glucose and insulin (pre- and post- OGTT) and outpatient nutrient and energy intakes for each group were compared using an unpaired two-tailed t-test. This test was also used to compare the baseline TEF for the glucose intolerant vs. the glucose tolerant subjects. Results are expressed as mean ± SEM.

7. RESULTS

7.1. Subject Characteristics and Diet Composition

Twenty-two subjects entered this study between December 1990 and June 1992. One subject left after two days on the baseline diet because of the difficulties associated with communal living in the CIU The study was discontinued in one other patient after approximately one week on the VLCD because of nonadherence to the study protocol. N balance and rate of weight loss data for subject A D were also not included in the analysis because her low urinary concentrations of urea, sodium, and potassium during the VLCD period, despite consistent creatinine concentrations indicating that she must have consistently underconsumed the prescribed VLCD The subject was later questioned about her adherence to the study diet and insisted that she had indeed consumed all of the VLCD All of A.D.'s other data (other key variables measured) were included in the analysis. Exclusion of her data would result in no change in any of the reported mean values. Furthermore, leaving her data (subject was in the fluoxetine group) in these other analyses would if anything, bias the results against our major conclusions since a more restricted energy intake would work against any thermic effect of fluoxetine by hastening and magnifying metabolic adaptation. Her basal temperature data were also included If they were excluded they would in fact, make the conclusions later reported with regard to temperature even slightly stronger Finally, the temperature data of subject P.R. were not included in the analysis because her mean baseline temperature of 32 8°C was an impossibly low value During the VLCD, P R 's temperatures were similar to her group's mean, indicating that her temperature measurement technique during baseline was not good. If included, this subjects' data makes our conclusions with regard to temperature even stronger. All of P.R 's other data (other key variables measured) were included in the analysis because

there was no reason to doubt their accuracy Thus, results from 20 subjects, l0 subjects per treatment group, were included (with the exceptions described above) in the final analysis

The baseline characteristics of the 20 subjects are shown in Table 3 Subject randomization produced well matched groups with no significant differences between any of the measured variables. Mean baseline energy intakes were not significantly different between the two groups (F: 2505 ± 60 kcal/day, P: 2526 ± 29 kcal/day, p=0 75)

All subjects had further weight to lose at the end of the in-hospital phase, and remained on the balanced deficit diet (BDD) as outpatients Subjects returned for clinical visits and dietary counselling each two weeks for eight weeks. Table 4 lists the prescribed outpatient intakes Prescribed levels of carbohydrate, protein, fat, and energy were not different between the groups

N-analysis of each lot of the baseline diet revealed the N intake was 12.1 ± 0 1g/d and the N intake of the VLCD was 11.2 ± 0.11 g/d. These estimates closely match the N content of the two formula diets (based on a daily intake) which are reported to be 12 8 g N for Ensure and 11.2 g N for Optifast. The cv for the N content among the different lots of formula used was 4 1% for the baseline diet and 2.8% for the VLCD.

7.2. Clinical Course

Apart from one subject, there were no adverse events of medical, or other significance related to either the baseline diet, VLCD or BDD. One subject (L.E.D.), however, had an episode of acute cholecystitis 4 days after concluding the outpatient study. L.E.D had a family history of gallstones/cholecystitis (mother) and had previously presented to the emergency room with nonspecific abdominal pain (01-11-88). Between May 12 and June 10, 1992 L E D experienced three attacks consisting of severe upper abdominal pain, nausea, vomiting and diarrhea An abdominal ultrasound examination revealed several (>3) small stones in the gallbladder and the

diagnosis of cholelithiasis was made The subject underwent a laparoscopic removal of the gallbladder on June 11, 1992 and recovered fully.

The nursing records for the inpatient phase of the study showed that heart rate slowed ($p<0.005$) and blood pressure (postural and recumbent) decreased in both the treatment groups during the VLCD There was no consistent pattern to suggest a difference in response to the VLCD between the two groups. No abnormalities were detected in the ECGs, chest X-rays or hematological tests

Changes in plasma uric acid, AST (aspartate aminotransferase), glucose and insulin are summarized in Table 5 Serum uric acid increased significantly from baseline; 322 ± 22 to 415 ± 35 umol/L in the F-group ($p<0.0001$) and 295 ± 12 to 402 ± 25 umol/L in the P-group ($p<0.0003$) This increase was not different between treatment groups. Finally, there was an initial transient significant increase in serum AST during the first week of the VLCD ($p<0.001$), this increase was not significantly different between treatment groups. Levels of AST were no longer different from baseline during the subsequent two weeks of the VLCD. Both fasting blood glucose and insulin decreased significantly during the VLCD ($p<0.0001$ and $p<0.0003$) and this decrease was similar in both groups. There were no other significant changes in biochemical tests in either treatment group

7.3. Weight loss and N Balance

Changes in body weight are summarized in Table 6 and Figure 2. Average baseline weight in the F-group was 91.5 ± 3.7 kg and 92.8 ± 3.2 kg in the P-group (weight range for n=20· 74.6-112 8 kg) There was no difference in mean weights between groups ($p=0.7$) and no significant changes occurred over the baseline period ($p=0.466$). Weight decreased significantly over the VLCD period ($p<0.0001$) but was not different between groups. Weight loss

at the end of the VLCD was 7.9 ± 0.5 kg and 7.3 ± 0 5 kg in the F- and P-groups respectively. However, the rate of weight loss during the final week of the VLCD was -0 26 kg/day for the F-group and -0.21 kg/day for the P-group (p=0 05) From the last inpatient weight measurement to the final day of the BDD, two months later, there were significant changes in weight over time ($p<0.0048$) and there were significant differences in weight change between groups ($p<0.0053$) There were significant changes in weight from the last day of the VLCD to one month of the BDD (-2.1 ± 0.5 kg, $p<0.0034$) and further significant decreases between one and two months of the BDD ($p<0.0167$) in the F-group such that a total of 3 5 ± 0.9 kg were lost during the BDD period In contrast, the P-groups's mean weight did not change significantly from the last inpatient day to either one month (0.5 ± 0.5 kg) or two months of (-0 2 ± 0 8 kg) of BDD By the final day of the BDD, body weight had decreased 11 0 ± 1.1 kg to 80 6 ± 3 5 kg in the F group and 7 0 ± 1.0 kg to 85.9 kg in the P-group. Ultimately, the F-group lost significantly more weight during the entire protocol than the P-group ($p<0.015$), with weight decreasing 12.0 ± 1.2% and 7 4 ± 1.4% respectively. The cv of final weight loss among the F- and P-subjects was much higher than during the VLCD alone, 32.5% and 46.4% respectively.

N balance results are summarized in Table 7 and Figure 3. Subjects in the F-group were in N-equilibrium during the baseline diet, P-subjects, however, were in slightly positive N balance (0.8 ± 0.3, $p<0.03$). There was no significant difference in N balance at baseline between the two groups ($p=0.48$). The balance fell to -3.7 ± 0.4 g/day and -4.6 ± 0.5 g/day during the first week of the VLCD in the F- and P-groups respectively. By the end of the second week, N balance became less negative in both groups and remained in slight negative balance during the third week of the VLCD. Throughout the VLCD, N-balance was significantly lower than baseline in both treatment groups ($p<0.01$) There were no significant difference between the F-group

and P-group in N balance at any time during the inpatient study. Cumulative N losses during the VLCD were 61 1 ± 4 8 g and 71.5 ± 6.6 g in the F- and P-groups respectively.

Using the factor of 31 25 to convert N to lean wet tissue, the total mean loss of lean tissue at the end of the VLCD was 1 9 ± 0 2 kg and 2.2 ± 0.2 kg for the F- and P-groups respectively. These losses account for 26.1 ± 2.0% of the total weight lost in the F-group and 32.3 ± 3.7% in the P-group

7.4. Resting Metabolic Rate

Changes in RMR are summarized in Table 8 and Figure 4. Initial average resting metabolic rate was 1 12 ± 0 03 kcal/min in the F-subjects (n=10) and 1.09 ± 0.04 kcal/min in the P-subjects (n=10), (RMR range for n=20: 0.94 - 1.28 kcal/min). Baseline RMR was not significantly different between the two groups (p=0.44) Interindividual variability in baseline RMR was typically high, with cv's of 7.9% and 10.3% for the F-group and P-group respectively. RMR did not change significantly during the baseline period (p=0.49) and intraindividual variability was minimal (cv = 2 2 ± 0.3%) Measured baseline RMR and predicted RMR using the Harris-Benedict (1919) and Owen et al (1986) equations are shown in Table 9. The RMR (n=20) by ventilated hood was 6.1% lower than predicted by the Harris-Benedict equation (p<0 05) and 8 9% higher than predicted by the Owen equation (p<0.05).

There was a significant change in RMR over the VLCD period (p<0.0001). Final inpatient values of RMR (kcal/min) were 1.05 ± 0 03 in the F-group and 0.98 ± 0.03 in the P-group. This corresponds to a percentage change of -6.7 ± 1.2% and -9.7 ± 1.5% respectively from baseline. The effect of fluoxetine vs. placebo on the change in RMR over the entire 3 week period was borderline significant (p<0.09). However, analysis of data grouped weekly during the VLCD produced different results. During week one of VLCD, there was a significant effect of treatment

on RMR ($p<0.04$). For the F-subjects, RMR first increased significantly ($p<0.002$) and then returned to baseline levels by day 6. For the P-subjects RMR decreased significantly ($p<0.035$) This significant treatment effect disappeared during the second week of the VLCD ($p=0.37$) RMR was below baseline by the end of the second week in both groups ($p<0.003$), but the RMR's of the F-subjects fell below baseline after 9.8 ± 0.88 days, whereas RMR of the P-subjects fell below baseline after 5.6 ± 0.62 days. This difference is statistically significant ($p<0.001$) By the third week of the VLCD, the treatment effect again approached significance ($p<0.07$) This was mainly due to a general increase in RMR on day 17 in the F-subjects resulting in a significant difference between treatment groups at that time ($p<0.024$) RMRs continued to be significantly lower than baseline in both F- and P-groups ($p<0.0001$) by the last day of the VLCD. There were no further significant changes in RMR from the last inpatient measurement to measurements made after one and two months of the BDD. RMR remained significantly lower than baseline during the BDD in both the F-subjects ($p<0.008$) and P-subjects ($p<0.017$) However, there was a treatment effect for the change in RMR from the last inpatient measurement ($p<0.025$) This difference derives from the nonsignificant decreasing trend in RMR in the F-group and the nonsignificant increasing trend in the P-group By the final day of the BDD, RMR had fallen 0.091 ± 0.027 kcal/min to 1.03 ± 0.027 kcal/min and 0.066 ± 0.023 kcal/min to 1.024 ± 0.029 kcal/min in the F- and P-groups respectively This change in RMR was not significantly different between groups. This represented a percentage change from baseline of $-7.9 \pm 2.5\%$ in the F-group and $-5.7 \pm 2.0\%$ in the P-group.

7.5. Thermic Effect of Food

Mean TEF values of both groups at baseline, and after the VLCD and BDD are presented in Table 10 and Figure 5. There were no significant differences in TEF at baseline, after the

VLCD and after the BDD. Furthermore, both groups had similar TEF values before and after weight loss. Neither fluoxetine, nor weight loss were detected as having an effect on TEF.

7.6. Basal Temperature

Changes in basal temperature are summarized in Table 11 and Figure 6. Initial average basal temperature in the F-group was 36.5 ± 0.2°C (n=9) and 36.5 ± 0.1°C in the P-group (basal temperature range for n=19: 35.4-37.3°C). There was no difference between groups ($p=0.92$) and basal temperature did not change significantly over the baseline period ($p=0.67$). There was not a significant effect of time for basal temperature from baseline over the length of the VLCD in either treatment group ($p=0.37$). However, there was a significant treatment effect, whereby F-subjects had significantly higher basal temperatures during the VLCD than the P-subjects ($p<0.025$), with a mean increase of 0.28 ± 0.1°C versus a mean decrease of -0.12 ± 1.2°C in the P-subjects. Temperature increased almost concurrent to the start of the active study medication, with changes approaching significance on day 2 and 3 of the VLCD period ($p<0.06$ and $p<0.07$). The maximum increase in temperature reached by the F-subjects was 0.4 ± 0.16°C on days 10, 12 and 18

The timing of the post-ovulatory phase of the menstrual cycle was recorded in the treatment groups in order to rule out the possibility that temperature changes observed in the F-group were due to a disproportionate amount of subjects being in that phase during the VLCD. The results show that 4 subjects in the F- and P-groups were in the post-ovulatory phase during week one of the VLCD, 2 and 3 subjects in the F- and P-groups respectively during the second week, and 3 subjects in each treatment group during the third week of the VLCD. One subject, G.G., did not menstruate during the inpatient portion of the study and information about her last

menstrual cycle was not obtained. Therefore, the results are based on 9 subjects in the F-group and 10 subjects in the P-group.

7.7. Thyroid Hormones

Changes in thyroid variables are summarized in Table 12 and Figure 7. Baseline concentrations of T_4, free T_4 index, T_3, free T_3 index and T_3 uptake were similar in both treatment groups and there were no significant variations during the baseline period. The results show no difference in the responses of subjects in the two treatment groups. In contrast, there was a significant effect of time on the change in serum T_4 from baseline ($p<0.03$) with levels increasing after one week of VLCD ($p<0.054$) and subsequently decreasing, such that ultimately T_4 levels were not different from baseline. Serum T_3 levels decreased significantly over time ($p<0.0007$) in both groups. This decrease was significant for all three weeks of the VLCD T_3 uptake increased significantly over time during the VLCD ($p<0.0023$). Free T_4 index did not change significantly during the VLCD from baseline levels in the F-group. However, during the second week of the VLCD, levels were significantly higher than baseline in the P-group ($p<0.008$). Free T_3 index decreased significantly in both groups with the onset of the VLCD ($p<0.0001$) but did not change within the VLCD period. Despite the changes in the thyroid indices, all measurements remained within normal limits.

7.8. Catecholamines

Changes in urinary catecholamines are summarized in Table 13 and Figure 8 Baseline excretion of dopamine, norepinephrine (NE), metanephrine and normetanephrine were not significantly different between the F- and P-groups. However, excretion of epinephrine were significantly different ($p<0.05$), although in all cases, levels were within normal limits. Statistical

analysis of the catecholamine data were based on the absolute changes from baseline for each variable, therefore the difference in baseline epinephrine levels is eliminated (with respect to the statistical analysis). The results for dopamine, norepinephrine, metanephrine and normetanephrine showed no difference in the responses of F-subjects or P-subjects. In all cases, levels decreased significantly during the VLCD. In the cases of dopamine, norepineprhine and normetanephrine, levels decreased significantly by the first week of the VLCD ($p<0.0001$ for all). In contrast, the decrease in metanephrine reached significance during the second week of the VLCD and remained reduced throughout the third week. Epinephrine did not change significantly with time during the VLCD but there was a significant effect of treatment ($p<0.011$). There was no change in epinephrine levels from baseline during the first two weeks of the VLCD in the P-group. F-subjects, on the other hand, had significantly higher urinary epinephrine levels during the VLCD than baseline period with a maximal increase of 28 ± 7 nmol/d ($p<0.0044$) during the second week of the VLCD. Epinephrine increased significantly in the F-group within the first week of the start of the active study medication and remained significantly elevated through the second week of the VLCD It was no longer significantly elevated by the third week of the VLCD. The data were analyzed based on 9 subjects per group because two urine samples were lost (one per group) during the VLCD.

7.9. Oral Glucose Tolerance Test

The normal ranges (based on lean individuals) used for fasting serum glucose and insulin were 3.9-6.1 mmol/L and 35-145 pmol/L (Wallach, 1981) Normal ranges (based on lean individuals) used for a blood sample taken two hours after a 75 g glucose load were glucose concentration below 7.8 mmol/L and insulin levels below 1078 pmol/L (National Diabetes Data Group, 1979). According these criteria described above, eight subjects were categorized as

glucose intolerant/insulin resistant and eight subjects were categorized as having a normal glucose tolerance/insulin sensitive (this categorization was done on subjects who underwent the modified OGTT prior to treatment randomization) Of the 8 subjects characterized as intolerant, 4 were subsequently randomized to the F-group and 4 to the P-group Since subjects with glucose intolerance were equally distributed among the treatment groups, there is no possibility of a confounding effect due to this factor. Mean fasting serum glucose and insulin for the resistant and sensitive groups were 5.4 ± 0.9 mmol/l, 184 1 ±62 0 pmol/l and 4 8 ± 0 6 mmol/l, 160.5 ± 44.8 pmol/l respectively. Two hours after a 75 g glucose load serum glucose was 9.6 ± 1.7 mmol/l ($p<0.047$) and 6.7 ±0.8 mmol/l ($p=0.08$) and insulin levels were 1335.7 ± 728 3 pmol/l ($p=0.137$) and 290.7 ± 109.9 pmol/l ($p=0.291$) in the resistant and sensitive groups respectively. From the data obtained through the modified OGTT, prerandomization baseline TEF values were separated and compared using an unpaired t-test based on whether subjects were normal or glucose intolerant/insulin resistant Normal subjects had a TEF of 8 81 ± 0 97% vs 7.79 ± 1.05% in the glucose intolerant/insulin resistant subjects when expressed as a percent increase above the RMR. We were unable to detect any difference in the TEF between these two groups ($p=0.5$). Also a significant correlation between degree of obesity (%IBW) and fasting serum insulin was not detected ($r=0.215$, $p=0.363$).

8. DISCUSSION

The main question addressed by this study was whether administration of fluoxetine would modify energy expenditure in obese subjects during weight loss. It was hypothesized that fluoxetine would blunt the decrease in RMR in obese subjects on a VLCD, increase the thermic effect of a glucose test meal, and maintain the RMR at a higher level during a BDD when compared to placebo. Instead, the present results indicate that fluoxetine given at the same time

as a VLCD increased the RMR. This effect was only temporarily, for the RMR subsequently decreased in the way typical of adaptation to energy restriction. Fluoxetine administration did not increase the thermic response of a glucose test meal during weight reduction and it did not result in the RMR remaining higher during the BDD. Importantly, fluoxetine increased basal temperature, a condition which was sustained throughout the VLCD.

Several factors are known to play important roles in regulating energy expenditure. The primary known mechanisms which operate to control the facultative component of the RMR in humans involve the activities of the SNS and catecholamines and thyroid hormones. In addition to changes in body temperature and body composition, which themselves change RMR, these other factors could also partly explain the findings in this study. Therefore, urinary catecholamines, and several thyroid hormones were measured. The results of these measurements do not indicate a clear difference between the F- and P-groups that would account for the differences in RMR and body temperature that were observed.

8.1. Resting Metabolic Rate

Within three days of concurrent fluoxetine and the VLCD, RMR increased significantly from baseline levels but then gradually decreased as dieting continued. The RMR of the F-subjects remained above their baseline throughout the first 12 days of the VLCD. VLCD dieting alone was associated with a steady reduction in RMR. Figure 4 illustrates that after the first week of the VLCD, the decreasing trend of RMR in F-subjects follows a similar pattern as in the P-subjects, except that the RMR of F-subjects remained consistently higher. RMR has been reported to decrease 8-20% (Table 1) after approximately three weeks of moderately severe energy restriction (Welle et al 1984; Garrow & Webster, 1989; Hendler & Bonde, 1988; Fricker et al. 1991). This suggests that fluoxetine has an immediate effect on RMR, but this effect does not block its

adaptive decrease during energy restriction, an effect mediated in part by decreased T_3 and SNS activity. Our results for the P-subjects (-9.7%) agree with other reports for RMR reduction during a VLCD. The 6.7% decrease observed in the F-subjects is somewhat lower

Fenfluramine has recently been shown to increase the RMR 6 9% in weight stable obese subjects after acute administration (Scalfi et al. 1993). No other study to our knowledge has demonstrated a similar effect in this drug or any other serotonin agonist in obese subjects in negative energy balance. Nor has this been demonstrated after chronic administration in the present study; RMR was measured 24 hours after the last dose of the drug. In this study, we report an increase in RMR in obese subjects actively losing weight on a VLCD, with fluoxetine administration.

The most significant finding in this study which supports the contention of a thermogenic effect of fluoxetine is the significant increase in basal temperature among the F-subjects To our knowledge no other study has described this effect before. The almost immediate increase in temperature could account for the increase in RMR Since every 1°C increase in body temperature increases RMR by 13% (DuBois, 1954), the mean temperature increase of 0 3°C on day 3 of the VLCD is consistent with the observed 4.4% increase in RMR.

Figures 4 and 6 demonstrate the parallel increase in RMR and temperature that occurred during the first week of the VLCD. During the subsequent 2 weeks of the VLCD, however, temperature remained elevated but the RMR decreased. That RMR decreased despite the temperature elevation, suggests that heat loss from the body decreases in parallel to the decrease in RMR thus allowing temperature to be maintained. This decrease in heat dissipation is not unique to the fluoxetine group, for temperature was also maintained in the placebo group despite the significant decrease in RMR.

Central serotonin systems affect thermoregulation in several species (Myers & Waller, 1978). It is therefore possible that fluoxetine stimulates centrally mediated mechanisms controlling temperature in humans. The present data raise the possibility that the thermogenic effects of fluoxetine are not mediated directly through thyroid hormones or SNS activation, but are due to a direct effect on the temperature regulating centre of the brain, in effect resetting the "thermostat", which secondarily increases thermogenesis to maintain the new higher body temperature Additionally, it appears this effect was insufficient to prevent the decrease in RMR induced by the normal adaptation to semistarvation. Hence, the effect of fluoxetine to increase energy expenditure to maintain body temperature is not sustained during the adaptation to semistarvation. The increased body temperature under these circumstances is presumably maintained by voluntarily increasing body insulation (Figure 9).

Fluoxetine is not the only serotonergic drug reported to increase body temperature in humans. As was discussed earlier, fenfluramine increases temperature in rats and chickens (Tagliaferro et al 1982; Sulpizio et al 1978, Frey, 1975). However, these temperature effects occur after acute administration of fenfluramine in doses much higher than used clinically, and the dose of fluoxetine used in this study (8, 13, or 20 mg/kg body weight). A temperature elevating effect of fenfluramine has not been reported in the clinical trials. The phenylpiperazine compound, M-chlorophenylpiperazine (M-CPP) (a metabolite of the antidepressant drug trazodone) has also been shown to increase body temperature in weight stable healthy volunteers in single-dose studies (Mueller et al. 1985; Mueller et al. 1986; Charney et al. 1982). Temperature elevations were in the order of 0.5^{o}C following acute administration of 0.5 - 0.75 mg/kg M-CPP Research on the properties of this compound has focused on its potential use as a pharmacological probe of serotonergic responsivity in human. This agent is not used

clinically, and it is unknown whether its effects on temperature would persist with chronic use, nor which receptors M-CPP directly influences in humans.

The temperature elevating effect of 60 mg fluoxetine given chronically is a novel finding. This increase in body temperature may not only explain its thermogenic properties but, could also relate to some of its anorectic properties. Body temperature has been described as a nonchemical correlate of food ingestion. The increase in metabolic rate induced by eating tends to raise body temperature slightly and this constitutes a signal inhibitory to eating It is possible that the increase in basal temperature caused by fluoxetine lowers the "threshold" temperature at which an inhibitory signal is transmitted following food ingestion, thereby resulting in earlier feelings of satiety and less total energy intake within a meal. This speculation fits in well with the satiety effects of fluoxetine (Clifton et al. 1989).

Ovulation produces increases in body temperature. This factor can however be ruled out as one of the causes of the increase in basal temperature among the F-subjects because the post-ovulatory period was similarly distributed throughout the VLCD between the two treatment groups.

The temperature data obtained in this study also provides surprising evidence that the onset of action of fluoxetine is much earlier than expected. From the 4 day half-life described for fluoxetine, full effect was only expected to be seen after about 4 half-lives or 16 days, and while this may be true of its antidepressant or anorectic properties, it does not seem to be the case for its effects on temperature. The only other reference to an immediate effect with fluoxetine administration is from the clinical observations made by a psychiatrist The doctor noted that after switching one subject from a tricyclic antidepressant to fluoxetine "she became almost hypomanic for a time and then settled down somewhere just above euthymia" (Kramer,

1990). Thus, our results provide new information about the effects of fluoxetine on basal temperature and on the onset of action of the drug with respect to its thermogenic properties.

With respect to the hormonal/neurotransmitter factors, the catecholamine data obtained in this study are consistent with the well-known link between energy intake and SNS activity (Young & Landsberg, 1977a; Shetty et al. 1979; Kolanowski et al. 1975; Davies et al. 1989; Bessard et al 1983). In our study urinary norepinephrine decreased by 34% in the F-subjects and 49.6% in the P-group during the VLCD. Urinary dopamine, metanephrine and normetanephrine excretion also decreased in both groups. Taken together with the significant decreases in norepinephrine excretion, these results are consistent with the conclusion that SNS activity decreases in response to a VLCD Contrary to our results with norepinephrine, however, urinary epinephrine excretion increased significantly in the fluoxetine group, whereas no change in its excretion occurred in the placebo group. Our placebo group responded exactly as would be expected from previous results (Davies et al 1989; Pasquali et al. 1992, Bessard et al. 1983). The increase in urinary epinephrine excretion in the F-group suggests an increase in adrenal medullary epinephrine secretion (Landsberg & Young, 1983) An increase of this magnitude has previously been reported in normal male volunteers subjected to short-term total fasts (Cryer, 1980) It is not clear from the literature however, whether the 27% increase during the first week of the VLCD is enough to actually increase the RMR. In fact, there was no correlation between the change in epinephrine excretion and the change in RMR ($r=0.203$, $p=0.405$). Furthermore, although by the second week of the VLCD, epinephrine excretion had risen maximally by 58%, the fall in RMR was no different in the F-group than in the P-group. These findings suggest that fluoxetine-induced epinephrine secretion is not the primary determinant of the elevated RMR.

Sodium intake on the VLCD was 40 mmol/day (40 meq), close to the cutoff point of 50 mmol/day, at which according to Romoff et al (1979) sodium intake will stimulate norepinephrine

and epinephrine production despite a low energy and carbohydrate intake Our results do not agree with these suggestions possibly because the sodium intake was high enough not to interfere with the effects of a restricted nutrient intake. The reductions in urinary excretion we observed in norepinephrine agree with other published results where the sodium intake was >40 mmol/d (Davies et al. 1989; Pasquali et al 1992).

The second factor playing a key role in the regulation of energy expenditure are the thyroid hormones. As was expected, T_4 levels remained relatively unchanged while T_3 levels declined significantly during the VLCD (Davies et al 1989, Young & Landsberg, 1977b, Welle et al. 1984; Hill et al 1987; Krotkiewski et al. 1981). Serum T_3 decreased by 20% in both F- and P-groups within the first week of the VLCD By the end of the VLCD, T_3 had fallen 40% and 29% in the F- and P-groups respectively. The decrease in T_3 concentration in the present study confirms what several other groups reported (Shetty, 1990, Gelfand & Hendler, 1989, Azizi, 1978, Danforth & Burger, 1989), none of which, however, provide clear understanding of the role of reduced T_3 concentrations in mediating changes in metabolic rate during semistarvation In fact, there was no correlation between the change in T_3 and change in RMR in our study ($r = -0.35$, $p=0.246$). For example, T_3 levels decreased 20% in the first week of the VLCD, but there was no decrease in RMR in the P-group. Furthermore, the similar decrease in T_3 in the F group with the concurrent increase in RMR strongly suggests that the thermic effect of fluoxetine is not mediated via thyroid hormone metabolism. The significance of the transient 7% increase ($p=0.05$) in the T_4 in both groups is difficult to determine and although not a common result, is not a unique observation (Krotkiewski et al. 1981; Azizi, 1978)

The third factor thought to play a role in regulating energy expenditure is insulin The decrease in serum insulin levels in both the F- and P-groups supports what other investigators have reported (Clifton et al. 1989; Durnin, 1967, Krotkiewski et al 1981) Within the first week

of the VLCD, insulin levels fell by one third in both the F- and P-groups and remained decreased throughout the VLCD Given that the thermic effect of insulin is partially mediated by SNS activation, our results provide some support for this relationship.

In summary, current knowledge indicates that the decreased energy expenditure that occurs during semistarvation involves changes in SNS activity, thyroid hormones and insulin Measurements of plasma hormones and urinary catecholamines give an incomplete view of hormonal status and SNS activity without study of hormone kinetics and receptor binding. Nevertheless, our findings are consistent with the hypothesis that thyroid, insulin and catecholamine hormones/neurotransmitter have a key regulatory role Fluoxetine does not appear to increase energy expenditure by stimulating SNS activity or T_3 Fluoxetine seems to interact with catecholamine metabolism, but its main effect on RMR appears to be through body temperature. However, the exact mechanism by which fluoxetine induces its transient thermogenic effect during a VLCD is not completely answered by our present results

The total weight loss over the 3-week VLCD period was similar for both treatment groups and comparable to previous reports with this type of diet (Krotkiewski et al 1981, Davies et al. 1989; Cohn et al. 1981; Fricker et al. 1991; Welle et al 1984). It is noted, however, that weight loss of the F-group during the final week of the VLCD was significantly greater than for the placebo group ($P = 0.05$). It can be assumed by this stage of a VLCD that nonadipose extracellular fluid and glycogen losses are trivial Under these conditions, it is possible to calculate the relative contributions of lean tissue loss (from N balance data) and adipose tissue loss (from energy balance) For example, weight loss in the P group can be predicted assuming that 1 g negative N balance is equivalent to 31 g of lean tissue loss and 100 kcal negative energy balance is equivalent to a loss of 10.6 g of pure fat, and 12.2 g of adipose tissue (assuming 9 45 kcal/g fat, and adipose tissue is 85% pure fat) (Hoffer et al 1985) If is assumed that total energy

expenditure in the P-group was 1.5 X RMR during the final of dieting, their predicted total weight loss is 0 202 kg/day, a value that is within 4% of the observed weight loss (p=0.7). The difference in weight loss between the treatment groups implies there was a 400 kcal/day higher daily energy expenditure in the F-group

Indeed, although not statistically significant, there was a 100 kcal/day higher RMR in the F-group during this 7 day period The statistical power of this analysis is 0.52, given a between-subject variability of 0 05 kcal/min and a total of 10 subjects per treatment group Therefore, it is possible and plausible that the 6 5% difference in RMR was significant during the last week of the VLCD

However, even if it is assumed that RMR was indeed 6.5% greater in the F-group during this period, this is insufficient to fully account for the difference in observed weight losses. For example, a similar calculation to that done for the P-group predicts a weight loss of 0.222 kcal/day by the F-group, 15% less than observed ($p < 0.0001$), and leaves 38 g/day of greater weight loss in the F-group unaccounted for. This suggests that some components of energy expenditure other than the RMR, was greater in the F-group than in the P-group. Of the different factors that contribute to the non-resting component of energy expenditure, fidgeting is one that has been identified as a possible explanation for differences in 24-hour energy expenditure (Ravussin et al. 1986). Anotner possible source of the difference in energy expenditure may be that F-subjects had to generate more heat than P-subjects to maintain their elevated body temperature. This is possible, since except for the brief period in the RMR test room, where ambient temperature was carefully controlled, the living quarters of the CIU were not kept within the thermoneutral range.

During the outpatient phase of the study, F-subjects lost considerably more weight than the P-subjects, who actually gained a small, statistically insignificant amount of weight between

the end of the VLCD and the end of the BDD. In contrast to the VLCD weight loss, variability in total weight loss from baseline to the end of the BDD is considerable, suggesting that adherence to the BDD was variables The greater weight loss among F-subjects seems to confirm the known anorectic effect of fluoxetine However, no data were collected to enable us to conclude that F-subjects adhered better to the BDD than P-subjects

Fluoxetine did not produce greater body N losses than placebo It has been calculated that the excess weight in obesity is 70-78% fat and 22-30% lean tissue (Forbes, 1987) The calculated losses of lean and fat mass in both F- and P-groups are consistent with this composition of weight loss Lack of a difference in N-balance between the two groups indicates that fluoxetine does not potentiate energy expenditure by sparing lean tissue during weight loss, nor, did the increased RMR induced by fluoxetine result in protein wasting Compared to the incidence of side effects reported for F-treated patients (Wise, 1992), fewer were reported in this study where fluoxetine treatment was combined with a VLCD Since N-balance data were not obtained during the BDD, we cannot draw conclusions about the longer term effects of fluoxetine on N-balance; however the continued weight loss combined with the stable RMR in F subjects during the BDD provides some evidence that fluoxetine does not increase N excretion in the long-term. The increased rate of weight loss induced by fluoxetine during the final week of the VLCD is evidence of fluoxetine's thermogenic properties The most likely explanation for the greater total weight loss during the BDD was a decreased food intake among the F-subjects, was probably due to fluoxetine's anorectic properties.

8.2. Thermic Effect of Food

The second major question addressed in this work was whether fluoxetine administration would potentiate the thermic effect of a glucose test meal. Contrary to our hypothesis, chronic

fluoxetine administration (60 mg/day) does not potentiate the thermic effect of glucose in obese women following a VLCD or BDD The baseline TEF data obtained in this study indicate that 112 5 g of glucose increases the RMR by 8 14 ± 0.81% in the F-subjects and 8 66 ± 1.11% in the P-subjects, when expressed as a percent of the energy content of the test meal the TEF is 4.49 ± 0 40% and 4 52 ± 0 49% respectively In obese subjects with normal glucose tolerance the thermic response to a 75-100 g oral glucose load is 6 ± -2% (% over energy load) (Simonson & DeFronzo, 1990, Nair et al 1983) Among subjects with impaired glucose tolerance and/or insulin resistance the TEF is reported to be significantly lower (Golay et al. 1982, Felber et al. 1981) When expressed as a percent increase over RMR, the TEF of glucose in obese subjects is reported by two groups to be 12.2% (Welle & Campbell, 1983) and 8.5% (Schutz et al. 1984b). The present baseline results compare with the lower end of the reported ranges. Part of the discrepancy between the present data and the higher TEF results may be because of differences in the TEF test protocol Welle et al (Welle & Campbell, 1983) took blood samples every 30 minutes during the 3 hour test which may possibly interrupt the rested state required by these measurements Furthermore, they reported a 5% increase over baseline RMR with a noncaloric test meal over 3 hours. This 5% possibly represents nonspecific stimulation of their testing procedures and if subtracted from their TEF results (12.2-5 = 7.2%) puts their results in line with mine. Finally, our subjects were given breaks, which most subjects used as an opportunity to urinate. Following the consumption of 400-500 ml of fluid in the fasted state, it is not unreasonable to consider that the urgency to void after approximately one hour can increase energy expenditure, such urination breaks were not mentioned as part of other TEF test protocols (Nair et al 1983, Schutz et al 1984b)

The TEF did not change in either the F- or P-group after the VLCD and after the BDD. This lack of change in the TEF following weight loss is in agreement with some (Nelson et al.

1992; den Besten et al 1988; Clugston & Garlick, 1982, Bessard et al 1983, Hendler & Bonde, 1988) but is at variance with other studies reporting a reduced TEF after weight loss (Schutz et al. 1984b; Apfelbaum et al. 1971) Thus chronic fluoxetine administration in combination with a 3 week VLCD and a 2 month BDD did not potentiate the TEF in our obese subjects Given the large interindividual variability of 37 7% for the TEF, the number of subjects that would have been needed to show a clinically significant change is more than double than that present in this study. We cannot, therefore, rule out the possibility that the finding of no change in the TEF following treatment is false. It is difficult to compare our results to those of the fenfluramine studies in which TEF increased, because of the major difference in protocol The studies reporting increased TEFs with fenfluramine involved weight stable lean subjects (Troiano et al 1990; Munger et al. 1988) or weight stable obese subjects (Scalfi et al 1993) and in all cases involved the acute effects of fenfluramine (i e drug was administered at the same time as the test meal), whereas in the present study the most recent dose of fluoxetine was 24 hours before No conclusion can be made about the effects of fluoxetine under conditions similar to those described for fenfluramine; however, the longer peak action of fluoxetine than fenfluramine leads me to predict that it would not potentiate the TEF if administered acutely with food

The present study also addressed the issue of a reduced thermic response to glucose among patients with glucose intolerance and/or insulin resistance Data obtained during the baseline period indicate that 8 subjects had abnormally high fasting serum glucose levels and abnormally high insulin levels two hours after a 75 g glucose load The thermic effect of glucose was not significantly reduced in these subjects compared (by an unpaired two-tailed t test) to the 12 normal obese subjects and neither was the TEF significantly correlated with the degree of insulin resistance. It is also interesting to note, that despite improved glucose and insulin levels following weight loss, the TEF did not increase among those subjects with high baseline levels.

These results are in contrast to previously reported results (Felber et al 1981; Golay et al. 1982; Segal et al 1992) The difference may just be a factor of the sample size and the sample population studied Furthermore, the fasting insulin levels of our subjects who had abnormally high insulin levels after the 75 g glucose load are much lower than the insulin resistant obese subjects described by Segal et al (1992) (184 05 ± 20 5 pmol/L vs 293 17 ± 30 86 pmol/L). In fact, our subjects classified as glucose intolerant/insulin resistant had fasting levels almost identical to those subjects described by Segal et al as being insulin sensitive (162.01 ± 30.86 pmol/L) Due to the nonspecificity of most conventional immunoassays to differentiate between insulin and proinsulin, it is not possible to make a direct comparison between the total insulin levels in our subjects and those of other researchers without knowing the nature of the assay used (Reaven et al 1993) Thus, while our results do not support the relationship between insulin resistance and blunted glucose-induced TEF, they do not refute the possibility that the TEF could be reduced in the presence of more severe insulin resistance

8.3. Resting Metabolic Rate and the Balanced Deficit Diet

Finally, our study assessed the ability of fluoxetine administration to maintain the RMR at a higher level compared to baseline levels during the BDD. The RMR results obtained during the BDD do not fully address this question because while the F-subjects continued to lose weight, the P-subjects did not. The RMRs are in fact lower in the F-group than in the P-group As would be expected, the RMR began to increase in the P-subjects during the 2 months where they were weight stable, suggesting that the adaptive mechanisms which slowed their metabolic rate were reversing (Cohn et al 1981) The F-subjects continued to lose significant amounts of weight during the BDD and yet their RMR did not decrease significantly. It is difficult to draw conclusions from these findings, nevertheless, it would be anticipated that the RMR would

continue to decrease as long as energy balance was negative and subjects are losing weight that partially includes active lean tissue The fact that RMR failed to decrease could even suggests fluoxetine was inducing a positive effect on energy expenditure The continued weight loss during the BDD may relate in part to the thermogenic properties of fluoxetine and to its anorectic properties which supports its known effects on appetite (Levine et al 1989, Wise, 1992, Darga et al. 1991, Marcus et al 1990) Basal temperature, thyroid hormones and urinary catecholamines were not measured during the outpatient period, which limits the abilities to comment further about the possible mechanisms of the thermic effect of fluoxetine during this phase of the study. Nevertheless, the outpatient RMR and body weight results suggest that fluoxetine may have contributed to the lack of a fall in RMR and accelerated weight loss by increasing energy expenditure.

9. CONCLUSIONS

It is concluded that administration of 60 mg per day of fluoxetine to obese female subjects during a very low energy diet results in a transient increase in resting metabolic rate, as well as a sustained elevation in basal temperature, but no potentiating effect on the thermic effect of glucose either following a very low energy diet or following a balanced deficit diet

The increased RMR, taken together with the increase in basal temperature, suggest that brain serotonergic pathways may affect energy expenditure by directly affecting temperature regulation From these findings it is not certain that the weight loss observed after administration of fluoxetine to humans is entirely due to its anorectic properties (Levine et al 1989, Wise, 1992, Darga et al 1991; Marcus et al. 1990); we can however rule out the involvement of a potentiated TEF Further investigation is needed to define the role(s) of serotonin neurons in hypothalamic

regulation of temperature and to define further the mechanism(s) by which serotonergic drugs affect body weight through anorexia or increased energy expenditure.

The increase in temperature and RMR observed during the first week of the VLCD leads us to speculate that if fluoxetine were combined with a more moderate energy deficit, a more sustained increase in RMR could be achieved This in turn could produce relatively greater weight loss than by diet alone The greater effect of metabolic adaptation to reduce RMR induced by the VLCD versus the smaller effect of fluoxetine to increase RMR secondary to the increase in temperature, could explain why RMR did not remain elevated throughout the VLCD and BDD There may exist a specific cut off point in negative energy balance beyond which the thermogenic effect of fluoxetine becomes ineffective

Therapeutic application of fluoxetine is already justified by its abilities to produce weight loss with a relatively low frequency of adverse side effects Our findings do not support the use of fluoxetine as an adjunct to VLCDs because no additional weight was lost and based on the lack of significant difference in N balance, the composition of the weight loss was also apparently not affected However, the sustained increase in body temperature combined with the short-lived increase in RMR and the sustained RMR during the BDD suggest that fluoxetine combined with a BDD could be justified Again, research for this situation is required before any recommendations can be made

The present results indicate, for the first time, that fluoxetine, like amphetamine and nicotine both, reduces energy intake and increases energy expenditure and body temperature. In this regard, further studies are also necessary to test the theory that body temperature regulation may be involved in the control of appetite and that some anorectic agents may work by altering temperature regulation This consideration can also be used to support the theory that certain subsets of the obese population are hypometabolic. Abnormal metabolic regulation

could mean that in order to achieve the threshold temperature at which the signal to stop eating is transmitted, more food energy than normal must be consumed Thus, a defect in thermoregulation may be linked to abnormal appetite control

Overall, the observations made in this study may be important in the treatment and etiology of obesity, particularly if a reduced energy expenditure capacity is thought to be involved.

REFERENCES

Acheson, K , Jequier, E & Wahren J (1983) Influence of beta-adrenergic blockade on glucose induced thermogenesis in man J Clin Invest 72 981-986

Acheson, K J , Zahorska-Markiewicz, B , Pittet, P , Anantharaman, K & Jéquier, E (1980) Caffeine and coffee their influence on metabolic rate and substrate utilization in normal weight and obese individuals Am J Clin Nutr 33 989-997

Acheson, K J , Ravussin, E , Wahren, J & Jequier, E (1984) Thermic effect of glucose in man. obligatory and facultative thermogenesis J Clin Invest 74 1572-1580

Akerstedt, T , Gillberg, M , Hjemdahl, P , Sigurdson, K., Gustavsson, I , Daleskog M;, & Pollare, T (1983) Comparison of urinary and plasma catecholamine responses to mental stress. Acta Physiol.Scand 117. 19-26

Anderson, G.H (1988) Metabolic regulation of food intake. In: Modern Nutrition In Health and Disease (3rd Ed (Shils, M E & Young, V R , eds.) Lea & Febiger, Philadelphia.

Anonymous (1993) Fluoxetine hydrochloride, Clinical investigation brochure, Eli Lilly and Company, Indianapolis

Antelman, S M (1979) The importance of stress in assessing the effects of anorectic drugs. Current Medical Research Opinion 6 (suppl 1). 73-82.

Apfelbaum, M., Bost-Sarron, J , Brigant, L. & Dupin, H. (1967) La composition du poids perdu de la diète hydrique Gastroenterologia 108: 121-134

Apfelbaum, M , Bostsarron, J & Lacatis, D. (1971) Effect of caloric restriction and excessive caloric intake on energy expenditure. Am J.Clin Nutr 24: 1405-1409

Apfelbaum, M & Fricker, J (1990) Metabolic effects and complications of VLCD In: Progress of obesity research 1990 (OOMURA, Y , ed) pp 481-488. John Libbey & Co. Ltd.,

Arch, J R S , Piercy, V & et al., (1987) Thermogenic and lipolytic drugs for the treatment of obesity. Old ideas and new possibilities Recent advances in obesity research 5: 300.

Astrup, A., Lundsgaard, C., Madsen, J. & Christensen, N.J. (1985) Enhanced thermogenic responsiveness during chronic ephedrine treatment in man. Am J Clin Nutr. 42: 83-94.

Astrup, A., Bulow, J., Christensen, N.J., Madsen, J. & Quaade, F. (1986) Facultative thermogenesis induced by carbohydrate: a skeletal muscle component mediated by epinephrine. Am.J.Physiol. 250: E226-E229.

Astrup, A., Andersen, T., Christensen, N.J., Bulow, J., Madsen, J., Breum, L. & Quaade, F. (1990a) Impaired glucose-induced thermogenesis and arterial norepinephrine response persist after weight reduction in obese humans. Am J Clin Nutr. 51: 331-337.

Astrup, A., Thorbek, G., Lind, J. & Isaksson, B. (1990b) Prediction of 24-h energy expenditure and its components from physical characteristics and body composition in normal-weight humans. Am.J.Clin Nutr. 52: 777-783.

Astrup, A., Toubro, S., Cannon, S., Hein, P. & Madsen, J. (1991) Thermogenic synergism between ephedrine and caffeine in healthy volunteers: a double-blind, placebo-controlled study. Metabolism 40: 323-329.

Astrup, A., Toubro, S., Christensen, N.J. & Quaade, F. (1992) Pharmacology of thermogenic drugs. Am.J.Clin.Nutr. 55: 246S-248S.

Atkinson, R.L. (1990) Usefulness and limits of VLCD in the treatment of obesity. In. Progress in obesity research 1990. (OOMURA, Y., ed.) pp. 473-480. John Libbey and Company.

Audrain, J.E., Klesges, R.C., DePue, K. & Klesges, L.M. (1991) The individual and combined effects of cigarette smoking and food on resting energy expenditure. Int J Obes. 15: 813-821.

Azizi, F. (1978) Effect of dietary composition on fasting-induced changes in serum thyroid hormones and thryotropin. Metabolism 27: 935-942.

Ballor, D.L., Katch, V.L., Becque, M.D. & Marks, C.R. (1988) Resistance weight training during caloric restriction enhances lean body weight maintenance. Am.J.Clin Nutr. 47: 19-25.

Barrows, K. & Snook, J.T. (1987) Effect of a high-protein, very-low-calorie diet on resting metabolism, thyroid hormones, and energy expentiture of obese middle-aged women. Am.J.Clin.Nutr. 45: 391-398.

Benedict, F G & Miles, N (1919) In Human Vitality and Efficiency Under Prolonged Restricted Diet. (Carnegie Institute Of Washington No 280, Washington, D C

Bessard, T , Schutz, Y & Jéquier, E (1983) Energy expenditure and postprandial thermogenesis in obese women before and after weight loss Am J Clin Nutr 38: 680-693.

Bjorntorp, P (1992) Physical exercise in the treatment of obesity. In: Obesity. (Björntorp, P. & Brodoff, B N , eds) pp 708-711 J B Lippincott, Philadelphia

Bjorntorp, P (1992) Regional obesity In: Obesity (Bjorntorp, P. & Brodoff, B. N., eds.) pp. 579-586 J P Lippincott Company, Philadelphia.

Blundell, J E (1992) Serotonin and the biology of feeding. Am.J.Clin.Nutr. 55: 155S-159S.

Bolinger, R E , Luker, B P , Brown, R.W , Guevara, L. & Steinberg, R. (1966) Metabolic balance of obese subjects during fasting. Arch Intern Med. 118: 3-8.

Bonadonna, R C , Groop, L , Kraemer, N., Ferrannini, E , Del Prato, S. & DeFronzo, R.A. (1990) Obesity and insulin resistance in humans: a dose-response study Metabolism 39: 452-459.

Borsini, F , Bendotti, C & Samanin, R (1985) Salbutamol, d-amphetamine and d-fenfluramine reduce sucrose intake in freely fed rats by acting on different neurocnemical mechanisms. Int.J.Obes 9 277-283

Bray, G A. (1969) Effect of caloric restriction on energy expenditure in obese patients. Lancet Aug.23: 397-398

Breisch, S T , Zemlan, F P & Hoebel, B.G (1976) Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. Science 192: 382-385.

Breum, L , Astrup, A , Andersen, T., Lammert, O., Nielsen, E., Garby, L. & Quaade, F. (1990) The effect of long-term dexfenfluramine treatment on 24-hour energy expenditure in man. A double-blind placebo controlled study Int J.Obes 14. 613-621.

Brown, A C & Brengelmann, G. (1965) Energy metabolism. in: Physiology and biophysics. (Ruch, & Patton, , eds) pp. 1030-1049.

Buemann, B., Astrup, A , Madsen, J & Christensen, N J (1992) A 24 h energy expenditure study on reduced-obese and nonobese women effect of beta-blockade Am J Clin Nutr 56 662 670

Bukowiecki, L , Jahjah, L & Follea, N (1982) Ephedrine, a potential slimming drug, directly stimulates thermogenesis in brown adipocytes via beta-adrenoreceptors Int J Obes 6 343 350

Burman, K D , Wartofsky, L , Dinterman, R E , Kesler, P & Wannemacher, R W J (1979) The effect of T3 and reverse T3 administration on muscle protein catabolism during fasting as measured by 3-methylhistidine excretion Metabolism 28 805-813

Burszstein, S , Elwyn, D H , Askanazi, M D & Kinney, J M (1989) Energy metabolism, indirect calorimetry, and nutrition, pp 1-166 Williams & Wilkins, Baltimore

Canadian Diabetes Association, (1980) Good Health Eating Guide,

Carruba, M.O , Ricciardi, S , Spano, P & Mantegazza, P (1985) Dopaminergic and serotoninergic anorectics differentially antagonize insulin- and 2-DG-induced hyperphagia Life Sci 36 1739-1749

Cawthorne, M A (1992) Thermogenic drugs In: Obesity (Bjorntorp, P & Brodoff, B N , eds) pp 762-777. J B. Lippincott Company, Philadelphia

Charney, D.S., Heninger, G R , Reinhard, J.F., Steinberg, D.E & Hafstead, K M (1982) The effects of intravenous l-tryptophan on prolactin and growth hormone and mood in healthy subjects Psychopharmacology 77 217-222.

Clark, H.D. & Hoffer, L.J. (1991) Reappraisal of the resting metabolic rate of normal young men Am.J.Clin.Nutr. 53 21-26

Clemmons, D.R., Snyder, D K., Williams, R. & Underwood, L E (1987) Growth hormone administration conserves lean body mass during dietary restriction in obese subjects J.Clin.Endocrinol.Metab. 64: 878-883.

Clifton, P.G., Barnfield, A M. & Philcox, L (1989) A behavioural profile of fluoxetine-induced anorexia. Psychopharmacology (Berl) 97: 89-95.

Clugston, G A & Garlick, P J (1982) The response of protein and energy metabolism to food intake in lean and obese man Human Nutr.. Clin.Nutr. 36C. 57-70.

Cohn, J.B. & Wilcox, C. (1985) A comparison of fluoxetine, imipramine, and placebo in patients with major depressive disorder J Clin.Psychiatry 46: 26-31.

Cohn, S H., Gartenhaus, W., Sawitsky, A., Rai, K., Zanzi, I., Vaswani, A., Ellis, , Yasumura, S., Cortes, E. & Vartsky, D (1981) Compartmental body composition of cancer patients by measurement of total body nitrogen, potassium, and water. Metabolism 30: 222-229.

Cooper, G L (1988) The safety of fluoxetine - an update. Br.J.Psychiatry 153 (suppl.3): 77-86.

Cryer, P.E., Haymond, M W , Santiago, J V. & Shah, S D. (1976) Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N.Engl J.Med 295 573-577.

Cryer, P.E. (1980) Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. N Engl.J.Med. 303: 436-444.

D'Alessio, D.A., Kavie, E.C., Mozzoli, M.A., Smalley, K.J., Polansky, M., Kendrick, Z.V., Owen, L.R., Bushman, M.C., Boden, G. & Owen, O.E. (1988) Thermic effect of food in lean and obese men. J.Clin.Invest. 81: 1781-1789.

Dallosso, H.M. & James, W.P. (1984) The role of smoking in the regulation of energy balance. Int.J.Obes. 8: 365-375.

Danforth, E.,Jr. (1993) Hormonal adaptations to energy balance and imbalance and the regulation of energy expenditure In: Hormones, thermogenesis and obesity (Lardy, H. & Stratham, F., eds.) Elsevier Science Publishing Company, Amsterdam.

Danforth, E.,Jr. & Burger, A.G. (1989) The impact of nutrition on thyroid hormone physiology and action. Ann Rev Nutr. 9: 201-227.

Danforth, E.J. (1983) The role of thyroid hormones and insulin in the regulation of energy metabolism. Am J.Clin.Nutr. 38: 1006-1017.

Danforth, E J. (1985) Diet and obesity Am J Clin Nutr 41 1132-1145

Darga, L L., Carroll Michals, L , Botsford, S J & Lucas, C P (1991) Fluoxetine's effect on weight loss in obese subjects Am J Clin Nutr. 54 321-325

Davies H.J , Baird, I M , Fowler, J , Mills, I H , Baillie, J E , Rattan, S & Howard, A N (1989) Metabolic response to low- and very-low-calorie diets Am J Clin Nutr 49 745-751

DeHaven, J., Sherwin, R , Hendler, R & Felig, P (1980) Nitrogen and sodium balance and sympathetic-nervous-system activity in obese subjects treated with a low-calorie protein or mixed diet. N Engl J.Med 302. 477-482

den Besten, C., Vansant, G , Weststrate, J A & Deurenberg, P (1988) Resting metabolic rate and diet-induced thermogenesis in abdominal and gluteal-femoral obese women before and after weight reduction Am J Clin Nutr. 47 840-847

Douglas, J G., Gough, J , Preston, P G., Frazer, I , Haslett, C , Chalmers, S R & Munro JF , (1983) Long-term efficacy of fenfluramine in treatment of obesity Lancet 19, 1 384-386

DuBois, E.F (1954) Energy metabolism. Ann.Rev.Physiol. 16 125-134

Dulloo, A.G., Geissler, C.A , Horton, T , Collins, A & Miller, D S. (1989) Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers Am J.Clin Nutr 49 44-50

Dulloo, A.G & Miller, D.S (1984) Thermogenic drugs for the treatment of obesity sympathetic stimulants in animal models Br J Nutr 52 179-196

Durnin, J.V.G.A. (1967) Energy, work and leisure, Heinemann Educational Books, London
Elliot, D.L , Goldberg, L , Kuehl, K S. & Bennett, W M (1989) Sustained depression of the resting metabolic rate after massive weight loss Am.J.Clin Nutr 49 93-96

Elwyn, D.H., Kinney, J.M & Askanazi, J (1981) Energy expenditure in surgical patients Surg.Clin North Am 61: 545-556

Fagerberg, B , Andersson, O., Nilsson, U., Hedner, T., Isaksson, B & Björntorp, P (1984) Weight-reducing diets: role of carbohydrates on sympathetic nervous activity and hypotensive response Int.J.Obesity 8 237-243

Felber, J.P. Meyer, H.U., Curchod, B., Iselin, H.U., Rousselle, J., Maeder, E., Pahud P;, & Jequier, E (1981) Glucose storage and oxidation in different degrees of human obesity measured by continuous indirect calorimetry Diabetologia 20: 39-44.

Felig, P., Marliss, E.B & Cahill, G.F. (1971) Metabolic response to growth hormone during prolonged starvation J.Clin.Invest. 50: 411-421.

Felig, P , Cunningham, J , Levitt, M., Hendler, R. & Nadel, E. (1983) Energy expenditure in obesity in fasting and postprandial state. Am.J.Physiol 244: E45-E51.

Fernandez, P.G , Snedden, W., Vasdev, S., Bolli, P. & Rabin, E.Z. (1988) Can plasma catecholamine levels be a useful index of sympathetic nervous system activity? Can.J.Cardiol. 4: 169-176.

Ferrannini, E. (1988) The theoretical bases of indirect calorimetry: a review. Metabolism 37: 287-301.

Finer, N., Swan, P.C. & Mitchell, F.T. (1986) Metabolic rate after massive weight loss in human obesity. XIREPLACE:1,0,121 70: 395-398.

Finer, N., Finer, S. & Naoumova, R.P. (1989) Prolonged use of a very low calorie diet (Cambridge diet) in massively obese patients attending an obesity clinic: safety, efficacy and additional benefit from dexfenfluramine. Int.J.Obes. 13 Suppl 2: 91-93.

Fisher, M. & Gordon, T. (1985) The relation of drinking and smoking habits to diet: the Lipid Research Clinics Prevalence Study. Am.J.Clin.Nutr. 41. 623-630.

Flatt, J P (1992) The biochemistry of energy expenditure. In: Energy metabolism:Tissue determinants and cellular corollaries. (Kinney, J. M. & Tucker, H. N., eds.) pp. 123-125. Raven Press Ltd , New York.

Foltin, R.W., Kelly, T.H. & Fischman, M.W. (1990) The effects of d-amphetamine on food intake of humans living in a residential laboratory. Appetite. 15: 33-45.

Food and Agriculture Organization/World Health Organization/United Nations University Expert Consultation (1985) Energy and Protein Requirements Technical Report Series No 724, World Health Organization, Geneva

Forbes, G B. (1987) Human Body Composition, pp 1-350. Springer-Verlag, New York

Foster, G.D., Wadden, T.A., Feurer, I.D , Jennings, A S , Stunkard, A.J , Crosby, L O , Ship, J & Mullen, J.L (1990) Controlled trial of the metabolic effects of a very-low-calorie diet short-and long-term effects Am.J Clin Nutr. 51 167-172.

Frey, H H. (1975) Hyperthermia induced by amphetamine, p-chloroamphetamine and fenfluramine in the rat. Pharmacology 13. 163-176

Fricker, J., Rozen, R., Melchior, J -C & Apfelbaum, M (1991) Energy-metabolism adaptation in obese adults on a very-low-calorie diet Am J.Clin.Nutr 53· 826-830

Fugate, L , Kaye, G., Perrin, D , Riley, R & Sewell, S (1990) Very Low Calorie Diets in the Management of Obesity, Ross Laboratories, Columbus, Ohio

Garattini, S , Bizzi, A., Codegoni, A,M , Caccia, S & Mennini, T (1992) Progress report on the anorexia induced by drugs believed to mimic some of the effects of serotonin on the central nervous system. Am.J Clin.Nutr. 55· 160S-166S

Gardner, D.F., Kaplan, M M , Stanley, C.A & Utiger, R.D (1979) Effect of tri-iodothyronine replacement on the metabolic and pituitary responses to starvation N Engl J Med 300 579-584

Garrow, J.S. (1978) Energy balance and obesity in man, 2nd Ed Elsevier Science Publishing Company, New York.

Garrow, J.S. (1988) Obesity and Related Diseases, Churchill Livingstone, London

Garrow, J S & Stalley, S. (1977) Cognitive thresholds and human body weight Proc Nutr.Soc 18A (abs.)

Garrow, J.S & Webster, J D. (1989) Effects on weight and metabolic rate of obese women of a 3.4 MJ (800 kcal) diet Lancet 1: 1429-1431.

Gelfand, R.A , Hutchinson Williams, K.A., Bonde, A.A., Castellino, P & Sherwin, R.S. (1987) Catabolic effects of thyroid hormone excess: the contribution of adrenergic activity to hypermetabolism and protein breakdown. Metabolism 36: 562-569.

Gelfand, R A & Herndler, R (1989) Effect of nutrient composition on the metabolic response to very low calorie diets learning more and more about less and less. Diabetes Metab.Rev. 5: 17-30.

Golay, A , Schutz, Y , Meyer, H.U., Thiebaud, D., Curchod, B., Maeder, E., Felber, & Jequier, E. (1982) Glucose-induced thermogenesis in nondiabetic and diabetic obese subjects. Diabetes 31: 1023-1028

Goodman, M.M. & del Pilar Gomez, M. (1987) Decreased myofibrillar proteolysis after refeeding requires dietary protein or amino acids. Am.J.Physiol. 253: E52-E58.

Goran, M.I., Beer, W.H., Wolfe, R.R., Poehlman, E.T. & Young, V.R. (1993) Variation in total energy expenditure in young healthy free-living men. Metabolism 42. 487-496.

Goranzon, H. & Forsum, E (1985) Effect of reduced energy intake versus increased physical activity on the outcome of nitrogen balance experiments in man. Am.J.Clin.Nutr. 41: 919-928.

Gothert, M (1992) 5-Hydroxytryptamine receptors. Drug Research 42: 238-246.

Goudie, A J , Thornton, E.W. & Wheeler, T.J. (1976) Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia. Evidence for serotoninergic inhibition of feeding. J.Pharm.Pharmacol. 28: 318-320.

Grande, F , Anderson, J.T. & Keys, A. (1958) Changes of basal metabolic rate in man in semistarvation and refeeding. J.Appl.Physiol. 12: 230-238.

Gudelsky, G.A., Koenig, J.I. & Meltzer, H Y. (1986) Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. Neuropharmacology 25: 1307-1313.

Guy-Grand, B (1987) Therapeutic use of dexfenfluramine in obesity. In: Body weight control: The physiology, clinical treatment and prevention of obesity. (Bender, A. E. & Brookes, L. J., eds.) pp. 280-285. Churchill Livingstone, Edinburgh.

Guy-Grand, B , Crepaldi, G , Lefebvre, P , Apfelbaum, M , Gries, A & Turner, P (1989) International trial of long-term dexfenfluramine in obesity Lancet Nov 11 1142-1145

Hagen, R.D. (1986) The effects of aerobic conditioning and/or calorie restriction in overweight men and women Med Sci Sports Exerc 18 87-94

Hall, J.C. (1983) A method for the rapid assessment of sample size in dietary studies Am.J.Clin.Nutr. 37· 473-477

Hardy, J.D. (1961) Physiology of temperture regulation. Physiol Rev 41 521

Harris, J.A. & Benedict, F.G. (1919) Individuals and measurements considered. In: A biometric study of basal metabolism in man (pp 25-70 Carnegie Institution of Washington, Washington

Heinegard, D. & Tiderstrom, G (1993) Determination of serum creatinine by a direct colorimetric method Clin.Chim.Acta 43 305-310.

Hendler, R & Bonde, A.A.,III (1988) Very-low-calorie diets with high and low protein content· impact on triiodothyronine, energy expenditure, and nitrogen balance. Am.J.Clin Nutr. 48 1239-1247.

Heymsfield, S.B., Casper, K , Hearn, J. & Guy, D (1989) Rate of weight loss during underfeeding relation to level of physical activity. Metabolism 38 215-223.

Heymsfield, S.B. & Lichtman, S. (1992) Assessment of body composition·An overview In Obesity. (Bjorntorp, P & Brodoff, B N , eds.) pp 37-54 J B. Lippincott Company, Philadelphia

Hill, J.O., Sparling, P.B., Shields, T W & Heller, P A. (1987) Effects of exercise and food restriction on body composition and metabolic rate in obese women Am J Clin.Nutr 46· 622-630

Himms Hagen, J (1976) Cellular thermogenesis Annu Rev Physiol. 38 315-351

Hoffer, L.J., Bistrian, B.R , Young, V R , Blackburn, G L & Matthews, D E (1984) Metabolic effects of very low calorie weight reduction diets J Clin Invest 73 750-758

Hoffer, L J., Bistrian, B.R , Blackburn, G L. (1985) Composition of weight loss resulting from very low calorie protein only and mixed diets In Management of obesity by severe caloric restriction (Blackburn, G.L & Bray, G A., eds.) PSG Publishing Co , Inc , Littleton, MA

Hoffer, L J (1993) Starvation In. Modern nutrition in health and disease (8th Ed (Goodhart, R. S & Shils, M E , eds) Lea & Febiger, Philadelphia

Holliday, M.A (1971) Metabolic rate and organ size during growth from infancy to maturity and during late gestation and early infancy Pediatrics 474 169-179.

Horton, T.J & Geissler, C.A (1991) Aspirin potentiates the effect of ephedrine on the thermogenic response to a meal in obese but not lean women. Int.J.Obes. 15: 359-366.

Hudson, K.D (1977) The anorectic and hypotensive effect of fenfluramine in obesity. J R Coll Gen.Pract 27: 497-501.

James, W P & Trayhurn, P (1981) Thermogenesis and obesity Br.Med.Bull. 37: 43-48.

James, W P T., McNeill, G & Ralph, A (1990) Metabolism and nutritional adaptation to altered intakes of energy substrates Am J Clin Nutr. 51 264-269.

Jequier, E , Munger, R & Felber, J P (1992) Thermogenic effects of various beta-adrenoceptor agonists in humans their potential usefulness in the treatment of obesity. Am.J.Clin.Nutr. 55: 249S-251S

Kahn, R S , Knott, P., Gabriel, S , DuMont, K , Mastroianni, L & Davidson, M. (1992) Effect of m-chlorophenylpiperazine on plasm homovanillic acid concentrations in healthy subjects. Biol Psych iatry 31 1055-1061

Keys, A , Brozek, J , Henschel, A , Mickelsen, O & Taylor, H L (1950) The Biology of Human Starvation, The University of Minnesota Press, Minneapolis

Kolanowski, J , Jeanjean, M & Lambert, A E (1975) Hormonal adaptation to short term total fast in the obese In Jequier E,ed Regulation du bilan d'energie chez l'homme Geneve, Editions Medecine et Hygiene 112-116

Kopp, U , Bradley, T. & Hjemdahl, P (1983) Renal venous outflow and urinary excretion of norepinephrine, epinephrine, and dopamine during graded renal nerve stimulation Am.J.Physiol. 244 E52-E60

Kramer, P D. (1990) The new you. The Psychiatric Times Medicine & Behavior March: 45-46.

Krotkiewski, M., Toss, L., Björntorp, P & Holm, G (1981) The effect of a very-low-calorie diet with and without chronic exercise on thyroid and sex hormones, plasma proteins, oxygen uptake, insulin and c peptide concentrations in obese women Int J Obesity 5 287-293

Landsberg, L. & Bruno, S J (1973) 3,4-Dihydroxyphenylalanine (dopa), dopamine and norepinephrine storage in the rat heart after L-dopa--further evidence for norepinephrine release Biochem Pharmacol. 22. 417-425

Landsberg, L. & Young, J.B. (1983) The role of the sympathetic nervous system and catecholamines in the regulation of energy metabolism. Am.J.Clin Nutr 38. 1018-1024

Lean, M.E & James, W.P. (1988) Metabolic effects of isoenergetic nutrient exchange over 24 hours in relation to obesity in women Int J Obes. 12. 15-27

Leander, J D. (1987) Fluoxetine suppresses palatability-induced ingestion. Psychopharmacology (Berl) 91: 285-287.

Lee, H.S , Bastini, B , Friedman, L., Ramirez, L. & Meltzer, H Y (1992) Effect of the serotonin agonist, MK-212, on body temperature in schizophrenia Biol Psychiatry 31 460-470

Leiter, L.A., Grose, M., Yale, J -F. & Marliss, E B (1984) Catecholamine responses to hypocaloric diets and fasting in obese human subjects Am J Physiol 247 E190-E197

Lennon, D., Nagle, F , Stratman, F., Shrago, E. & Dennis, S (1985) Diet and exercise training effects on resting metabolic rate. Int.J Obes 9. 39-47.

Levine, L.R., Enas, G.G , Thompson, W L , Byyny, R.L , Dauer, A D , Kirby, R W , Kreindler T G., Levy, B., Lucas, C P & McIlwain, H.H (1989) Use of fluoxetine, a selective serotonin-uptake inhibitor, in the treatment of obesity a dose-response study (with a commentary by Michael Weintraub) Int. J Obes 13. 635-645

Levitsky, D A., Strupp, B J & Lupoli, J (1981) Tolerance to anorectic drugs pharmacological or artifactual. Pharmacol Biochem Behav 14 661-667

Levitsky, D.A., Schuster, J.A., STALLONE, D & Strupp, B.J (1986a) Modulation of the thermic effect of food by fenfluramine Int.J Obesity 10 169-173

Levitsky, D A , Schuster, J A & Strupp, B.J (1986b) Modulation of the thermogenic effect of food by fenfluramine Int J Obesity 10. 169-173

Levitsky, D A & Troiano, R (1992) Metabolic consequences of fenfluramine for the control of body weight Am J Clin Nutr 55: 167S-172S

Life Sciences Research Office, (1979) Research needs in management of obesity by severe caloric restriction, Washington, D C..

Lipinski, J F , Mallya, G., Zimmerman, P. & Pope, H G (1989) Fluoxetine-induced akathisia: clinical and theoretical implications J Clin.Psychiatry 50 9.

MacDonald, I A (1992) Sympathetic nervous system and malnutrition. In. Energy metabolism Tissue determinants and cellular corollaries (Kinney, J. M. & Tucker, H. N., eds.) pp. 247-265 Raven Press Ltd , New York.

Mahalko, J R & Jonhson, L K (1980) Accuracy of predictions of long term energy needs. J Am Dietet Ass 77 557-561.

Marcus, M D , Wing, R R., Ewing, L , Kern, E., McDermott, M. & Gooding, W. (1990) A double blind, placebo-controlled trial of fluoxetine plus behavior modification in the treatment of obese binge-eaters and non-binge-eaters Am.J.Psychiatry 147 876-881

McCargar, L J , Clandinin, M T , Fawcett, D M & Johnston, J L (1983) Short-term changes in energy intake and serum insulin, neutral amino acids, and urinary catecholamine excretion in women. Am J Clin Nutr 47 932-941.

McClave, S.A & Snider, H L (1992) Use of indirect calorimetry in clinical nutrition Nutrition in Clinical Practice 7 207-221

Mole, P.A , Stern, J.S., Schultz, C L., Bernauer, E.M. & Holcomb, B.J. (1989) Exercise reverses depressed metabolic rate produced by severe caloric restriction. Med Sci.Sports Exerc. 21: 29-33

Moore, F.D (1980) Energy and the maintenance of the body cell mass. J P.E.N. 4: 228-260.

Mueller, E.A., Murphy, D.L. & Sunderland, T. (1985) Neuroendocrine effects of M-Chlorophenylpiperazine, a serotonin agonist, in humans J.Clin.Endocrinol Metab 61. 1179-1184.

Mueller, E.A., Murphy, D.L & Sunderland, T. (1986) Further studies of the putative serotonin agonist, m-chlorophenylpiperazine evidence for a serotonin mediated mechanism of action in humans. Psychopharmacology 89 388-391

Munger, R., Lavielle, R., Arnaud, O., Schutz, Y. & Jequier, E (1988) Enhanced diet induced thermogenesis in humans after administration of dexfenfluramine(abstract) In. 1st European congress on obesity. (pp. 283. E.A.S.O., Stockholm.

Munro, H.N. & Fleck, A. (1969) Analysis of tissues and body fluids for nitrogenous constituents In: Mammalian Protein Metabolism, Volume 3 (Munro, H N , ed) pp. 424-525 Academic Press, New York.

Munro, J.F., Scott, C. & Hodge, J. (1992) Appraisal of the clinical value of serotoninergic drugs. Am.J.Clin.Nutr. 55: 189S-192S.

Murphy, D.L., Lesch, K.P., Aulakh, C.S. & Pigott, T.A. (1991) Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans Pharmacological Reviews 43. 527-552

Myers, R. & Waller, M.B. (1978) Thermoregulation and Serotonin In. Serotonin in Health and Disease Volume II: Physiological Regulation and Pharmacological Action (Essman, W B , ed.) pp. 1-67. Spectrum Publications Inc., New York.

Myers, R.D. & Yaksh, T.L. (1969) Control of body temperature in the unanaesthetized monkey by cholinergic and aminergic systems in the hypothalamus J Physiol 202 483-500

Nair, K S., Halliday, D & Garrow J S (1983) Thermic response to isoenergetic protein, carbohydrate or fat meals in lean and obese subjects Clinical Science 65. 307-312

National Diabetes Data Group, (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28: 1039-1057

Newsholme, E.A & Leech, A.R. (1989) Biochemistry for the medical sciences, John Wiley & Sons,

Owen, O.E , Kavle, E., Owen, R.S., Polansky, M., Caprio, S., Mozzoli, M.A., Kendrick, Z.V., Bushman, M C. & Boden, G. (1986) A reappraisal of caloric requirements in healthy women. Am.J Clin Nutr. 44: 1-19.

Owen, O E , Holup, J.L., D'Alessio, D.A., Craig, E.S., Polansky, L.R., Smalley, K.J., Kavle, E.C., Bushman, M.C., Owen, L.R., Mozzoli, M.A., Kendrick, Z.V. & Boden, G.H. (1987) A reappraisal of the caloric requirements of men Am.J.Clin.Nutr. 46: 875-885.

Owen, O.E. (1988) Resting metabolic requirements of men and women. Mayo.Clin.Proc. 63: 503-510

Park, H.S., Kelly, J.M. & Milligan, L.P. (1992) Energetics and cell membranes. In: Energy metabolism: Tissue determinants and cellular corrollaries. (Kinney, J. M. & Tucker, H. N., eds.) pp. 411 Raven Press Ltd , New York.

Pasquali, R., Casimirri, F., Melchionda, N., Grossi, G., Bortoluzzi, L., Morselli Labate AM;, , Stefanini, C. & Raitano, A. (1992) Effects of chronic administration of ephedrine during very-low-calorie diets on energy expenditure, protein metabolism and hormone levels in obese subjects Clin.Sci 82 85-92

Pavlou, K N., Krey, S & Steffee, W P. (1989) Exercise as an adjunct to weight loss and maintenance in moderately obese subjects. Am.J.Clin.Nutr. 49: 1115-1123.

Phinney, S.D , LaGrange, B.M., O'Connell, M. & Danforth, E.J. (1988) Effects of aerobic exercise on energy expenditure and nitrogen balance during very low calorie dieting. Metabolism 37: 758-765.

Pi Sunyer, F X & Segal, K.R. (1992) Relationship of diet and exercise. In: Energy metabolism Tissue determinants and cellular corollaries. (Kinney, J. M. & Tucker, H. N., eds.) pp. 187-210. Raven Press Ltd., New York.

Ravussin, E., Burnand, B., Schutz, Y. & Jéquier, E. (1985) Energy expenditure before and during energy restriction in obese patients. Am.J.Clin.Nutr. 41: 753-759.

Ravussin, E., Lillioja, S., Anderson, T E., Christin, L. & Bogardus, C. (1986) Determinants of 24-hour energy expenditure in man: methods and results using a respiratory chamber J.Clin.Invest. 78: 1568-1578.

Reaven, G.M., Chen, Y.D.I., Hollenbeck, C B., Sheu, W.H H., Ostrega, D & Polonsky, K S (1993) Plasma insulin, C-peptide, and proinsulin concentrations in obese and nonobese individuals with varying degrees of glucose tolerance. J Clin.Endocrinol Metab 76. 44-48

Rezek, M. & Novin, D. (1975) The effects of serotonin on feeding in the rabbit. Psychopharmacologia. 43. 255-258.

Robinson, S M., Jaccard, C., Persaud, C., Jackson, A A., Jequier, E & Schutz, Y. (1990) Protein turnover and thermogenesis in response to high-protein and high-carbohydrate feeding in men. Am.J.Clin.Nutr. 52 72-80.

Romoff, M.S., Keusch, G , Campese, V.M., Wang, M.S., Friedler, R.M , Weidmann, P. & Massry SG , (1979) Effect of sodium intake on plasma catecholamines in normal subjects J.Clin.Endocrinol Metab. 48: 26-31.

Rothwell, N.J. & Stock, M.J. (1981) Regulation of energy balance. Annu Rev Nutr 1. 235-256.

Rowland, N., Antelman, S.M. & Kocan, D. (1982) Differences among 'serotonergic' anorectics in a cross-tolerance paradigm: do they all act on serotonin systems? Eur.J Pharmacol 81. 57-66

Rowland, N.E. & Carlton, J. (1986) Neurobiology of an anorectic drug. fenfluramine Prog.Neurobiol. 27: 13-62.

Scalfi, L., D'Arrigo, E., Carandente, V., Coltorti, A. & Contaldo, F. (1993) The accute effect of dexfenfluramine on resting metabolic rate and postprandial thermogenesis in obese subjects: a double-blind placebo-controlled study. Int.J.Obesity 17: 91-96

Schutz, Y., Bessard, T. & Jequier, E. (1984a) Diet-induced thermogenesis measured over a whole day in obese and nonobese women. Am J.Clin.Nutr. 40: 542-552

Schutz, Y., Golay, A., Felber, J.P. & Jequier, E. (1984b) Decreased glucose-induced thermogenesis after weight loss in obese subjects. a predisposing factor for relapse of obesity? Am.J.Clin.Nutr 39: 380-387.

Schutz, Y , Bessard, T & Jequier, E (1987) Exercise and postprandial thermogenesis in obese women before and after weight loss Am J Clin Nutr 45 1424-1432

Segal, K R , Presta, E & Gutin, B (1984) Thermic effect of food during graded exercise in normal weight and obese men Am J Clin.Nutr 40 995-1000

Segal, K R , Edano, A , Blando, L & Pi Sunyer, F X (1990) Comparison of thermic effects of constant and relative caloric loads in lean and obese men Am J.Clin Nutr 51 14-21

Segal, K R , Albu, J , Chun, A , Edano, A , Legaspi, B & Pi Sunyer, F X (1992) Independent effects of obesity and insulin resistance on postprandial thermogenesis in men J Clin Invest 89· 824-833

Shariet, N N & MacDonald, I (1982) Differences in dietary-induced thermogenesis with various carbohydrates in normal and overweight men Am.J Clin Nutr 35. 267-272.

Shetty, P S , Jung, R T & James, W P.T (1979) Effect of catecholamine replacement with levodopa on the metabolic response to semistarvation Lancet Jan 13 7''-79.

Shetty, P S , Jung, R T , James, W P , Barrand, M.A & Callingham, B A (1981) Postprandial thermogenesis in obesity Clin Sci 60 519-525

Shetty, P S (1990) Physiological mechanisms in the adaptive response of metabolic rates to energy restriction Nutr Res Rev 3 49-74

Silverstone, T & Schuyler, D (1975) The effect of cyproheptadine on hunger, calorie intake and body weight in man Psychopharmacologia 40: 335-340

Simonson, D.C & DeFronzo, R.A. (1990) Indirect calorimetry. methodological and interpretative problems [see comments]. Am.J.Physiol. 258· E399-E412

Sims, E A & Danforth, E J (1987) Expenditure and storage of energy in man J Clin.Invest 79· 1019-1025

Smith, N J (1976) Gaining and losing weight in athletics JAMA 235 149-151

Snyder, D.K., Clemmons, D.R & Underwood, L E (1989) Dietary carbohydrate content determines responsiveness to growth hormone in energy-restricted humans J Clin Endocrinol Metab 69 745-752.

Soares, M.J & Shetty, P.S (1986) Intra-individual variations in resting metabolic rates of human subjects. Human Nutr.: Clin.Nutr. 40c: 365-369.

Soares, N.J. & Shetty, P.S. (1986) Intra-individual variation in resting metabolic rates of human subjects. Human Nutr.: Clin.Nutr. 40C: 365-370

Sommi, R.W , Crismon, M.L. & Bowden, C.L. (1987) Fluoxetine a serotonin-specific, second-generation antidepressant. Pharmacotherapy 7: 1-15.

Stamford, B.A., Matter, S , Fell, R D., Sady, S , Cresanta, M K & Papanek, P. (1984) Cigarette smoking, physical activity, and alcohol consumption: relationship to blood lipids and lipoproteins in premenopausal females Metabolism 33: 585-590

Sugimoto, Y., Yamada, J & Horisaka, K (1992) Activation of peripheral serotonin2 receptors induces hypothermia in mice. Life Sci 48: 419-423

Sulpizio, A., Fowler, P.J. & Macko, E. (1978) Antagonism of fenfluramine-induced hyperthermia a measure of central serotonin inhibition Life Sci. 22: 1439-1446.

Swaminathan, R., King, R.F., Holmfield, J., Siwek, R.A., Baker, M. & Wales, J K (1985) Thermic effect of feeding carbohydrate, fat, protein and mixed meal in lean and obese subjects Am.J.Clin.Nutr. 42: 177-181.

Sweeney, M.E , Hill, J O., Heller, P.A., Baney, R. & DiGirolamo, M (1993) Severe vs moderate energy restriction with and without exercise in the treatment of obesity: efficiency of weight loss Am.J.Clin.Nutr. 57: 127-134.

Tagliaferro, A.R., Roberts, J.S. & Davis, J R. (1982) Effects of fenfluramine on food intake, body temperature and energy metabolism in the adult rat Fed.Proc 41: 942

Thurlby, P.L., Garattini, S. & Samanin, R. (1985) Effects of serotonin antagonists on the performance of a simple food acquisition task in rats treated with fenfluramine isomers. Pharmacol.Res.Commun. 17: 1129-1139.

Troiano, R.P., Levitsky, D A & Kalkwarf, H.J (1990) Effects of dl-fenfluramine on thermic effect of food in humans. Int J Obesity 14 647-655.

Troisi, R J., Weiss, S T , Parker, D R., Sparrow, D., Young, J.B. & Landsberg, L. (1991) Relation of obesity and diet to sympathetic nervous system activity. Hypertension 17: 669-677.

Van Itallie, T.B & Yang, M -U (1977) Current concepts in nutrition: diet and weight loss. N.Engl J Med 297: 1158-1161.

Vander, A J , Sherman, J.H & Luciano, D.S. (1985) Human physiology, 4th Ed. pp. 172-228. McGraw-Hill Book Company, New York.

Vanhoutte, P M., Verbeuren, T J. & Webb, R.C. (1981) Local modulation of adrenergic neuroeffector interaction in the blood vessel well Physiol.Rev. 61: 151-247.

Wallach, J. (1981) Interpretation of Diagnostic Tests. A Handbook Synopsis of Laboratory Medicine, 3rd Ed Little, Brown and Company, Boston.

Warwick, P M , Chapple, R S & Thomson, E.S. (1987) The effect of smoking two cigarettes on resting metabolic rate with and without food Int.J.Obes. 11: 229-237.

Waterlow, J C. (1986) Metabolic adaptation to low intakes of energy and protein. Ann.Rev.Nutr. 6: 495-526.

Webb, P. (1986) 24-hour energy expenditure and the menstrual cycle. Am.J.Clin.Nutr. 44: 614-619.

Webb, P. (1992) Calorimetry in the study of obesity. In: Obesity. (Björntorp, P. & Brodoff, B. N., eds.) pp 91-99 J.P. Lippincott Company, Philadelphia.

Weinsier, R L , Schutz, Y & Bracco, D (1992) Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans Am J Clin Nutr 55 790-794

Weir, J.B. (1949) New methods for calculating metabolic rate with special reference to protein metabolism J.Physiol. 109 1-9.

Welle, S., Lilavivat, U. & Campbell, R.G. (1981) Thermic effect of feeding in man· increased plasma norepinephrine levels following glucose but not protein or fat consumption Metabolism 30: 953-958.

Welle, S.L., Lilivivat, U. & Campbell, R.G. (1981) Thermic effect of feeding in man increased plasma norepinephrine levels following glucose but not protein or fat consumption Metabolism 30: 953-959.

Welle, S.L., Amatruda, J.M., Forbes, G.B. & Lockwood, D.H. (1984) Resting metabolic rates of obese women after rapid weight loss. J.Clin.Endocrinol.Metab. 59: 41-44.

Welle, S.L. & Campbell, R G. (1983) Normal thermic effect of glucose in obese women. Am.J.Clin.Nutr. 37: 87-92.

Welle, S.L. & Campbell, R.G. (1986) Decrease in resting metabolic rate during rapid weight loss is reversed by low dose thyroid hormone treatment. Metabolism 35: 289-291.

Westrate, J.A. (1989) Resting metabolic rate and diet induced thermogenesis: studies on humans on individual differences and on the impact of nutritional and non-nutritional factors, Unilever Research Laboratory,

Wise, S.D. (1992) Clinical studies with fluoxetine in obesity. Am.J.Clin.Nutr. 55· 181S-184S

Wong, D.T. & Fuller, R.W. (1987) Serotonergic mechanisms in feeding. Int.J Obes 11 Suppl 3: 125-133.

Woo, R., Daniels Kush, R. & Horton, E.S. (1985) Regulation of energy balance. Annu.Rev.Nutr 5: 411-433.

Wozniak, K.M., Aulakh, C.S., Hill, J.L. & Murphy, D.L. (1988) The effect of 8-OH-DPAT on temperature in the rat and its modification by chronic antidepressant treatments Pharmacology Biochemistry & Behavior 30: 451-456.

Wozniak, K.M., Aulakh, C.S., Hill, J.L. & Murphy, D.L. (1989) Hyperthermia induced by m-CPP in the rat and its modification by antidepressant treatments. Psychopharmacology 97: 269-274.

Wozniak, K.M., Durcan, M.J. & Linnoila, M. (1991) Is NAN-190 an effective agonist of the hypothermia and hyperglycemia induced by the 5-HT1a receptor agonist, 8-OH-DPAT? Eur.J.Pharmacol. 195: 253-256

Wu, G. & Marliss, E.B. (1992) Interorgan metabolic coordination during fasting and underfeeding: an adaptation for mobilizing fat while sparing protein in humans. In: The Biology of Feast and Famine, Volume 10. (Anderson, G. H. & Kennedy, S. H., eds.) pp. 219-244. Academic Press, Orlando.

Wurtman, J.J. & Wurtman, R.J. (1977) Fenfluramine and fluoxetine spare protein consumption while suppressing caloric intake by rats. Science 198: 1178-1180.

Yen, T.T., McKee, M.M & Bemis, K.G. (1981) Ephedrine reduces weight of viable yellow obese mice (Avy/a). Life Sci. 28: 119-128.

Yen, T.T., Wong, D.T. & Bemis, K.G. (1987) Reduction of food consumption and body weight of normal and obese mice by chronic treatment with fluoxetine: a serotonin reuptake inhibitor. Drug.Devel.Res 10: 37-47.

Yen, T.T. & Fuller, R.W. (1992) Fluoxetine for weight loss. Am.J.Clin.Nutr. 55: 177S-180S.

Young, J.B., Rosa, R.M. & Landsberg, L. (1984) Dissociation of sympathetic nervous system and adrenal medullary responses. Am.J.Physiol. 247: E35-E40.

Young, J.B. & Landsberg, L. (1977a) Suppression of sympathetic nervous system during fasting. Science 196: 1473-1475.

Young, J B. & Landsberg, L. (1977b) Catecholamines and intermediary metabolism. Clin.Endocrinol.Metab. 6: 599-631.

Young, J.B. & Landsberg, L. (1977c) Stimulation of the sympathetic nervous system during sucrose feeding. Nature 269: 615-617.

Young, J.B. & Landsberg, L. (1981) Effect of concommitant fasting and cold exposure on sympatoadrenal activity in rats. Am.J.Physiol. 240: E314-E319.

TABLE 1. DATA ON THE EFFECTS OF SEMISTARVATION ON RMR AND ON THE EVOLUTION OF THE RELATIONSHIP OF RMR TO FAT FREE MASS (RMR:FFM) IN OBESE ADULTS

REFERENCE[a]	SEMISTARVATION DIET		Mean Weight Loss (kg)	% Decrease in RMR	RMR.FFM Evolution
	KJ/kcal per day	Mean Duration (days)			
1	1800/450	24	10.3	15	-
2	PSMF	77	12.1	14.5	↓9.6%
3	1672/400	40	10.8	9.4	-
4	3500-4700/830-1120	91	12.6	9.0	no change
5	1600/380	170	34.5	25.0	-
6	2050/490	127	18.6	21.0	↓6.7%
7	3400/800	35	8.0 (sedentary) 8.2 (exercising)	17.3 19.1	↓11.6% ↓15.3%
8	4180/1000	56	10.P (Abdominal/obese) 9.6 (gluteal-femoral obese)	9.1 2.6*	no change no change
9	1379/330 3260/780	56 56	15.7 11.8	17.0 17.0	no change
10	1254/300	113	28.3	22 0	≈↓13 0%
11	3400/800	21	4.9	8 8	-
12	1839/440	21	8.8	15.3	↓12.5%
13	VLCD (energy not specified)	21	9.2	20 0	↓18.0%
14	3400 800	≈110	12 6	15 3	↓7 9%

* not significant [a] see Table 1 continued

Table 1 Continued...

Refences:

1: Bray, 1969

2: Bessard et al., 1983

3: Welle et al., 1984

4. Ravussin et al., 1985

5 Finer et al., 1986

6 Barrows & Snook, 1987

7. Hill et al., 1987

8: den Beston et al., 1988

9. Davies et al., 1989

10: Elliot et al., 1989

11: Garrow & Webster, 1989

12: Hendler & Bonde, 1988

13: Fricker et al., 1991

14. Nelson et al., 1992

TABLE 2. NUTRIENT COMPOSITION OF BASELINE DIET, VLCD AND GLUCOSE POLYMER

NUTRIENT COMPOSITION	ENSURE PER 235 ml	OPTIFAST PER DAY	GLUCOSE POLYMER PER 100 g
ENERGY (KJ)	1045	1755 6	1590
ENERGY (Kcal)	250	420	380
PROTEIN (g)	8.74	70	
FAT (g)	8.84	2	
CARBOHYDRATE (g)	34.08	30	94 0
VITAMIN A (I.U)	620 40	5000	
VITAMIN D (I U)	49.35	400	
VITAMIN E (I.U.)	5.7	3.375	
VITAMIN K (mg)	0.009	0.1	
VITAMIN C (mg)	37.27	90	
FOLIC ACID (mg)	0.05	0.4	
THIAMINE (mg)	0.38	2.25	
RIBOFLAVIN (mg)	0.42	2.6	
PYRIDOXINE (mg)	0.49	3 0	
VITAMIN B_{12} (mg)	0.00148	0.006	
NIACIN (mg)	4.94	20	
BIOTIN (mg)	0.04	0.36	
PANTOTHENIC ACID (mg)	1.25	10	
SODIUM (g)	0.17	0.92	0.11
SODIUM (mmol)	7.39	40	4 8
POTASSIUM (g)	0.28	1955	0.01
CHLORIDE (g)	0.26	-	0.223
CALCIUM (mg)	120.0	1000	30
PHOSPHOROS (mg)	120.0	1000	5
MAGNESIUM (mg)	50.0	400	
IODINE (mg)	0.01998	0.15	
MANGANESE (mg)	0.49	4.0	
COPPER (mg)	0.26	2 0	
ZINC (mg)	3.76	15	
IRON (mg)	2 23	18	
CHLORINE (g)	0 14	0 1	

TABLE 3. BASELINE CHARACTERISTICS OF SUBJECTS

Variable	Fluoxetine (n=10)	Placebo (n=10)
Age (years)	32.0 ± 3.2	33.0 ± 3.9
Weight (kg)	91.5 ± 3.7	92.8 ± 3.2
Height (m)	1.64 ± 0.02	1.65 ± 0.01
BMI[a] (kg/m^2)	34.0 ± 1.3	34.1 ± 1.3
% IBW[b]	136.0 ± 5.3	136.7 ± 5.1
Waist:Hip	0.86 ± 0.02	0.83 ± 0.01
RMR (kcal/min)	1.121 ± 0.026	1.089 ± 0.036
Temperature (°C)	36.5 ± 0.1	36.5 ± 0.1

All data are mean ± SEM
[a] Body Mass Index
[b] Ideal Body Weight (based on BMI = 25 kg/m^2)

TABLE 4. NUTRIENT COMPOSITION OF BALANCED DEFICIT DIET

Variable	Fluoxetine (n=10)	Placebo (n=10)
Carbohydrate (g/day)	152 ± 4	151 ± 7
Protein (g/day)	75 ± 1	71 ± 2
Fat (g/day)	37 ± 1	35 ± 2
Energy Intake (kcal/day)	1239 ± 15	1199 ± 16
(kJ/day)	5179 ± 63	5012 ± 67

ALL DATA ARE MEAN ± SEM

TABLE 5. PLASMA ASPARTATE AMINOTRANSFERASE (AST), PLASMA URIC ACID, PLASMA GLUCOSE AND PLASMA INSULIN CONCENTRATION BEFORE AND AFTER A VLCD AND FLUOXETINE (n=10) OR PLACEBO (n=10) TREATMENT

	GROUP	BASELINE	VLCD7	VLCD14	VLCD21
AST	F	23.8±2.3	36.6±4.3*	26.1±3.6	28.5±3.7
(6-35 U/L)∞	P	23.7±2.7	27.0±3.1	25.0±2.7	23.3±2.8
URIC ACID	F	322±22	469±45**	484±42**	415±35**
(147-353 umol/L)∞	P	295±12	419±19**	430±31**	402±25**
GLUCOSE	F	5.1±0.2	4.5±0.2**	4.3±0.2**	4 4±0.3**
(3.9-5.8 mmol/L)∞	P	5.1±0.2	4.2±0.2**	4.2±0.2**	4.2±0 2**
INSULIN	F	167±26	111±13**	114±12*	119±21
(35-145 pmol/L)∞	P	150±12	104±17**	106±13**	109±12**

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM

* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)
∞ normal range

TABLE 6. BODY WEIGHT DURING VLCD AND BDD AND FLUOXETINE (N=10) OR PLACEBO (N=10) TREATMENT

	GROUP	BASELINE	VLCD7	VLCD14	VLCD22	BDD 1 MO	BDD 2MO
WEIGHT (KG)	F	91.5±3.7	87.9±3.8**	85.8±3.8**	80.8±3.1**	81.9±3.6**	80.6±3.5**
	P	92.8±3.2	89.5±3.1**	87.5±3.0**	83.9±4.3**	86.5±2.9**	85.9±2.8**
WEIGHT CHANGE	F	-	-3.6±0.2**	-5.7±0.3**	-7.9±0.5**	-9.6±0.8**a	-11.0±1.1**a
	P	-	-3.3±0.1**	-5.3±0.3**	-7.3±0.5**	-6.3±0.6**	-7.0±1.0**

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM

* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)
a significantly different from placebo group ($p<0.05$)

TABLE 7. NITROGEN BALANCE DURING THE VLCD AND FLUOXETINE (N=9) AND PLACEBO (N=10) TREATMENT

STUDY DAY	FLUOXETINE	PLACEBO
BASELINE	0.4±0.5	0.8±0.3
1	-1.5±0.4*	-1.6±0.5*
2	-3.7±0.4**	-4.3±0.4**
3	-4.5±0.6**	-5.2±0.4**
4	-4.6±0.4**	-5.7±0.3**
5	-4.0±0.5**	-5.5±0.4**
6	-4.0±0.4**	-5.0±0.4**
7	-3.9±0.6**	-5.2±0.4**
8	-4.0±0.4**	-4.7±0.4**
9	-3.9±0.4**	-4.6±0.4**
10	-3.6±0.6**	-4.5±0.6**
11	-3.4±0.5**	-4.5±0.4**
12	-3.5±0.5**	-4.3±0.5**
13	-3.1±0.6**	-3.6±0.6**
14	-2.8±0.6**	-4.0±0.5**
15	-3.0±0.6**	-3.7±0.4**
16	-2.9±0.6**	-3.6±0.4**
17	-2.7±0.6**	-3.4±0.5**
18	-3.1±0.6**	-3.3±0.4**
19	-2.7±0.7**	-3.2±0.4**
20	-2.8±0.6**	-3.3±0.4**
21	-2.7±0.7**	-3.5±0.3**
22	-1.1±0.6**	-2.1±0.4**

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM

* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)

TABLE 8. CHANGE IN RMR (KCAL/MIN) DURING VLCD AND BDD AND FLUOXETINE (N=10) OR PLACEBO (N=10) TREATMENT

STUDY DAY	FLUOXETINE RMR (KCAL/MIN)	FLUOXETINE CHANGE IN RMR	FLUOXETINE %CHANGE RMR	PLACEBO RMR (KCAL/MIN)	PLACEBO CHANGE IN RMR	PLACEBO % CHANGE RMR
BASELINE	1 121±0 026	-	-	1.089±0 036	-	-
VLCD WK 1						
DAY 3	1 171±0 029**	0 049±0 012**a	4 4±1 1	1 092±0 040	0 003±0 014	0 3±1 4
DAY 6	1.121±0 029	-0 001±0 015	-0 1±1.3	1 044±0 042	-0.045±0 018*	-4 3±1 8
DAY 8	1 119±0.029	-0 002±0 017	-0 1±1 5	1 042±0 038	-0 047±0 022	-4 3±2 1
VLCD WK 2						
DAY 10	1 089±0 034	-0 032±0 019	-2 9±1 8	1 025±0 038*	-0.064±0 019**	-5 9±1 9
DAY 13	1 062±0 030**	-0 060±0 013**	-5 4±1.3	1 024±0 033	-0.065±0 023*	-5 8±2 1
DAY 15	1 065±0 032*	-0 056±0 014**	-5 1±1.4	1 008±0 032*	-0 081±0 020**	-7 3±1 6
VLCD WK 3						
DAY 17	1 076±0 030a	-0 045±0 013**a	-4 1±1 1	0 974±0 025**	-0 116±0 026**	-10 2±2 0
DAY 20	1 030±0 031**	-0.092±0 014**	-8 3±1 3	0 977±0 034**	-0 112±0 019**	-10 2±1 6
DAY 22	1 048±0 031**	-0.074±0 013**	-6 7±1.2	0.983±0 033**	-0 106±0 017**	-9 7±1 5
BDD						
MONTH 1	1.021±0 029**	-0 100±0.017**	-8.9±1.5	1 031±0 036**	-0 058±0 016**	-5 4±1 6
MONTH 2	1 030±0 027**	-0 091±0 027**	-7 9±2 5	1 024±0 029*	-0 066±0 023*	-5 7±2 0

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM
* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)
a significantly different from placebo group ($p<0.05$)

TABLE 9. MEASURED AND PREDICTED RESTING METABOLIC RATE (KCAL/DAY) AT BASELINE

	Resting Metabolic Rate (kcal/day)		
Subject #	Deltatrac[a]	Harris Benedict[b] (1919)	Owen et al[c] (1986)
1	1567	1650	1400
2	1819	1830	1514
3	1542	1460	1342
4	1428	1650	1390
5	1615	1660	1407
6	1743	1690	1516
7	1700	1710	1461
8	1665	1830	1537
9	1498	1850	1606
10	1530	1530	1371
11	1783	1730	1496
12	1560	1730	1450
13	1353	1580	1334
14	1419	1650	1395
15	1839	1770	1573
16	1553	1620	1420
17	1707	1750	1563
18	1502	1780	1484
19	1559	1730	1479
20	1407	1630	1454

Statistical differences were determined using Repeated Measures ANOVA

Methods used to determine RMR with different superscripts are significantly different ($p<0.05$)

TABLE 10. TEF AT BASELINE, AFTER THE VLCD AND AFTER THE BDD IN FLUOXETINE (N=10) AND PLACEBO (N=10) GROUPS

	THERMIC EFFECT OF GLUCOSE		
	BASELINE	POST-VLCD	POST-BDD
FLUOXETINE			
% INCREASE OVER RMR	8 14±0 81	7 65±0 78	9 36±0 92
% OF ENERGY INGESTED	4 49±0 40	3 95±0 39	4 76±0 44
PLACEBO			
% INCREASE OVER RMR	8 66±1 11	8 79±1 10	9 64±0 85
% OF ENERGY INGESTED	4 52±0 49	4 17±0 47	4 85±0 39

All data are mean ± SEM

TEF did not change significantly from baseline and is not significantly different between groups

TABLE 11. BASAL TEMPERATURE (°C) DURING THE VLCD AND FLUOXETINE (N=9) AND PLACEBO (N=10) TREATMENT

STUDY DAY	FLUOXETINE TEMPERATURE	FLUOXETINE CHANGE	PLACEBO TEMPERATURE	PLACEBO CHANGE
BASELINE	36.5±0.1	-	36.5±0.1	-
1	36.6±0.1	0.1±0.1	36.4±0.1	-0.1±0.1
2	36.7±0.1*	0.2±0.1[a]	36.5±0.1	-0.1±0.1
3	36.7±0.1[a]	0.2±0.1	36.5±0.1	-0.1±0.1
4	36.8±0.1*[a]	0.3±0.1[a]	36.4±0.1	-0.2±0.1
5	36.8±0.1[a]	0.3±0.1	36.5±0.1	0.0±0.1
6	36.8±0.1[a]	0.3±0.1	36.5±0.1	0.0±0.1
7	36.9±0.1*[a]	0.3±0.1[a]	36.5±0.1	-0.1±0.1
8	36.9±0.1*[a]	0.3±0.1[a]	36.4±0.1	-0.1±0.1
9	36.8±0.1[a]	0.3±0.1[a]	36.4±0.1	-0.1±0.1
10	36.9±0.1*[a]	0.4±0.1	36.5±0.1	0.0±0.2
11	36.8±0.1[a]	0.3±0.1[a]	36.3±0.1	-0.3±0.1
12	36.9±0.0*[a]	0.4±0.1[a]	36.4±0.1	-0.2±0.1
13	36.8±0.1[a]	0.3±0.2[a]	36.4±0.1	-0.2±0.1
14	36.8±0.1[a]	0.3±0.1[a]	36.4±0.1	-0.2±0.1
15	36.8±0.0[a]	0.3±0.1[a]	36.4±0.1	-0.1±0.1
16	36.8±0.1[a]	0.3±0.1[a]	36.3±0.1	-0.2±0.2
17	36.8±0.1[a]	0.3±0.2	36.5±0.1	-0.1±0.1
18	36.9±0.1[a]	0.4±0.2	36.5±0.1	0.0±0.1
19	36.8±0.1[a]	0.3±0.2	36.5±0.1	-0.1±0.2
20	36.8±0.1[a]	0.2±0.2[a]	36.3±0.1	-0.2±0.1
21	36.9±0.1[a]	0.3±0.1[a]	36.3±0.2	-0.2±0.2
22	36.6±0.1	0.1±0.2	36.5±0.1	-0.1±0.1

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM

* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)
[a] significantly different from placebo group ($p<0.05$)

TABLE 12. THYROID HORMONES BEFORE AND AFTER A VLCD AND FLUOXETINE (n=10) OR PLACEBO (n=10) TREATMENT

	GROUP	BASELINE	VLCD7	VLCD14	VLCD21
T_4 (64-144 nmol/l)$^{\infty}$	F	111±4	119±5*	111±3	112±4
	P	109±6	116±7*	118±7	106±7
FREE T_4 INDEX (63-125)$^{\infty}$	F	106±4	110±4	111±3	115±6
	P	96±5	104±4	117±5*	101±5
T_3 (1.4-2.6 nmol/l)$^{\infty}$	F	2.0±0.1	1.6±0.1**	1.4±0.1**	1.2±0.1**
	P	2.1±0.1	1.7±0.1**	1.5±0.1**	1.5±0.1**
FREE T_3 Index (1.3-2 3)$^{\infty}$	F	1.9±0.1	1.4±0.1**	1.4±0.1**	1.3±0.1**
	P	1.9±0.1	1.6±0.1*	1.5±0.1**	1.4±0.1**
T_3 UPTAKE (0 22-0.32)$^{\infty}$	F	0.29±0.01	0.28±0.01	0 30±0.01*	0.31±0.01*
	P	0.27±0.01	0.28±0.01	0 29±0 01*	0.29±0.01*

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM

* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)
$^{\infty}$ normal range

TABLE 13. URINARY CATECHOLAMINES DURING VLCD AND FLUOXETINE (N=9) OR PLACEBO (N=9) TREATMENT

STUDY DAY	GROUP	DOPAMINE (<2630 nmol/d)$^{\infty}$	NOREPINEPHRINE (<591 nmol/d)$^{\infty}$	EPINEPHRINE (<136 nmol/d)$^{\infty}$	METANEPHRINE (281-1841 nmol/d)$^{\infty}$	NORMETANEPHRINE (502-2531 nmol/d)$^{\infty}$
BASELINE	F	2856+196	294+31	50+6	618+63	1718+185
	P	2972±304	265±39	34±5	485±51	1574±233
VLCD5	F	2429±146**	246±31**	66±10**a	623±44	1288±163**
	P	2845±229**	220±29**	40±7	425±91	1187±145**
VLCD6	F	2395±149**	239±28**	62±7**	580±63	1155±107**
	P	2819±236**	218±27**	39±4	428±50	1181±115**
VLCD12	F	2165±193*	194±26**	79±12**a	556±59	894±79**
	P	2542±258*	166±28**	37±6	477±66	922±94**
VLCD13	F	2163±157*	200±24**	73±16	562±83	842±93**
	P	2413±254*	158±24**	40±7	387±64*	848±77**
VLCD19	F	1997±115*	170±21**	70±11	437±50**	743±67**
	P	2412±226*	132±20**	43±8	406±45	708±70**
VLCD20	F	2092±101*	194±20**	67±7	512±43	799±74**
	P	2314±219*	134±20**	38±7	412±52	694±72**

Statistical differences were determined using Repeated Measures ANOVA
All data are mean ± SEM
* significantly different from baseline value ($p<0.05$)
** significantly different from baseline value ($p<0.01$)
a significantly different from placebo group ($p<0.05$)
$^{\infty}$ normal range

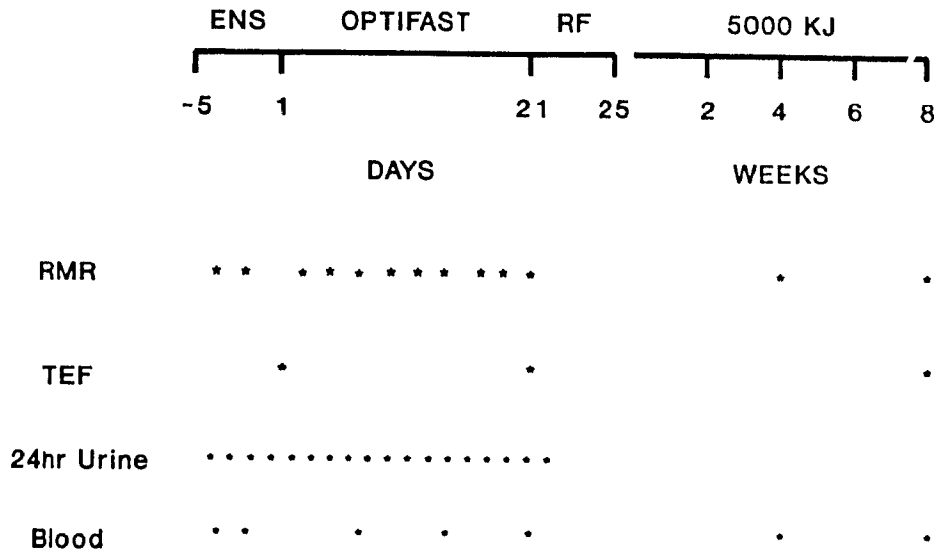

FIGURE 1. EXPERIMENTAL DESIGN.

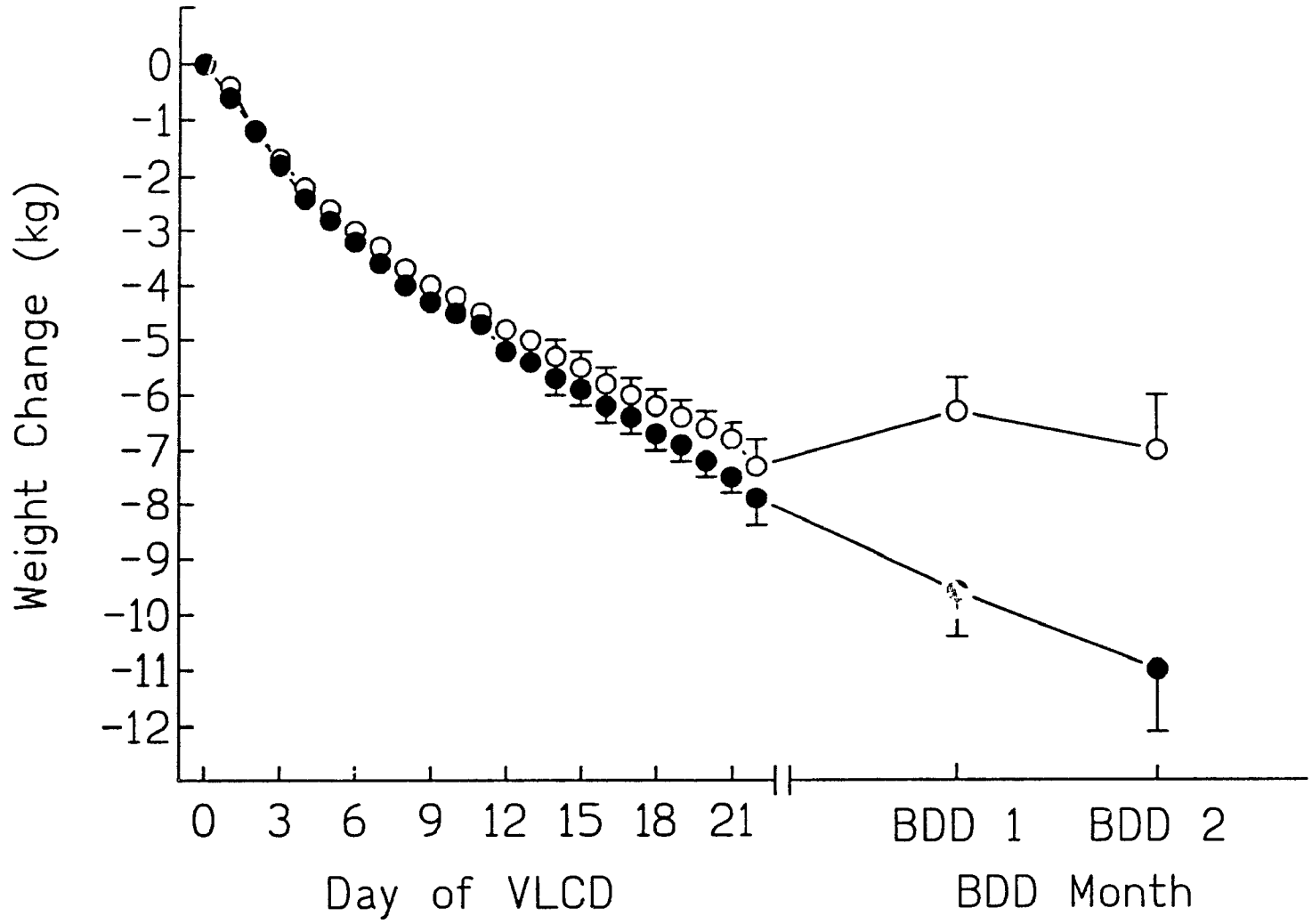

FIGURE 2. CHANGE IN WEIGHT FROM BASELINE DURING THE VLCD AND BDD. OPEN CIRCLES: PLACEBO GROUP, CLOSED CIRCLES: FLUOXETINE GROUP.

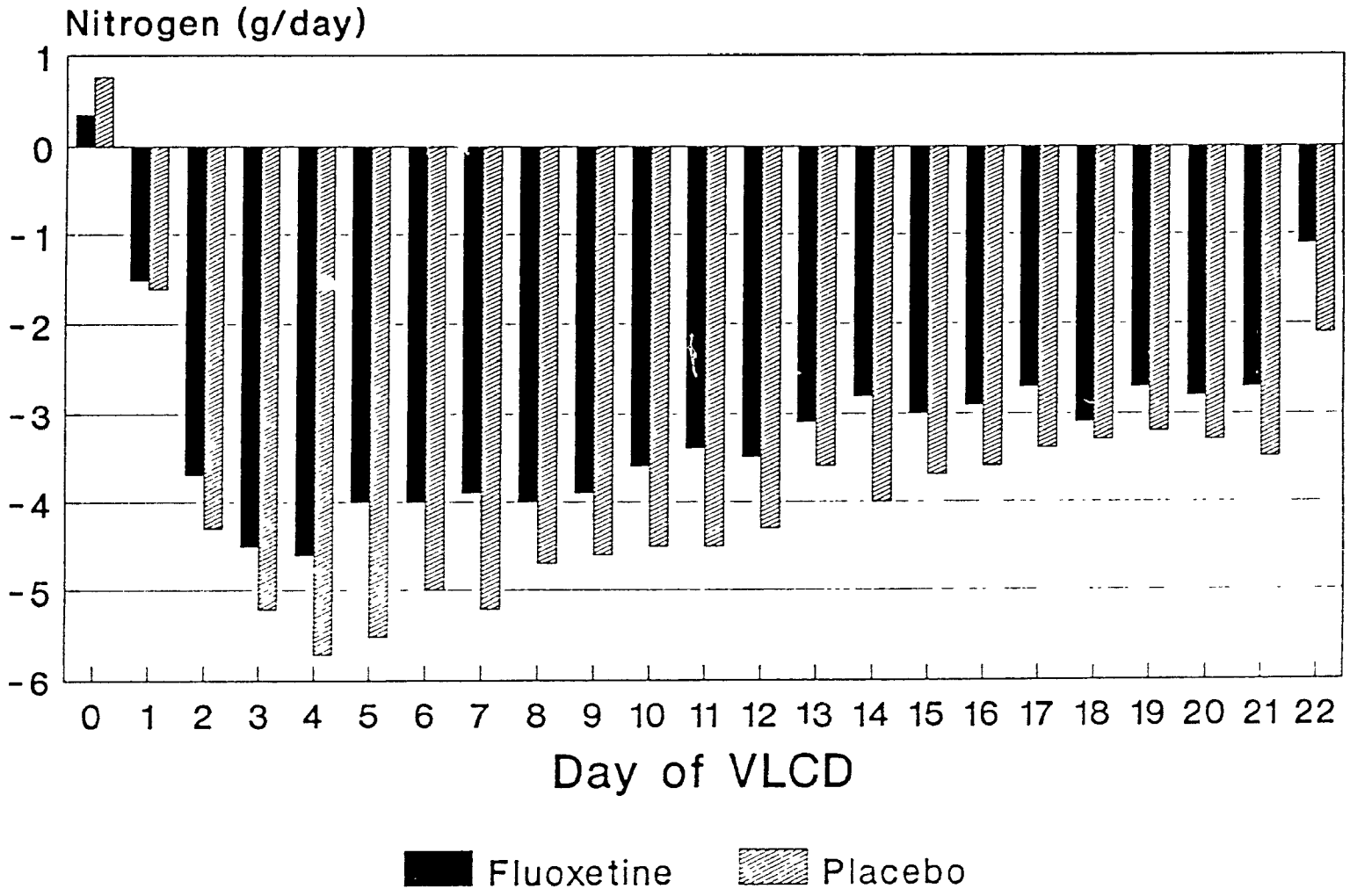

FIGURE 3. NITROGEN BALANCE DURING THE VLCD IN FLUOXETINE (N=9) AND PLACEBO (N=10) GROUPS.

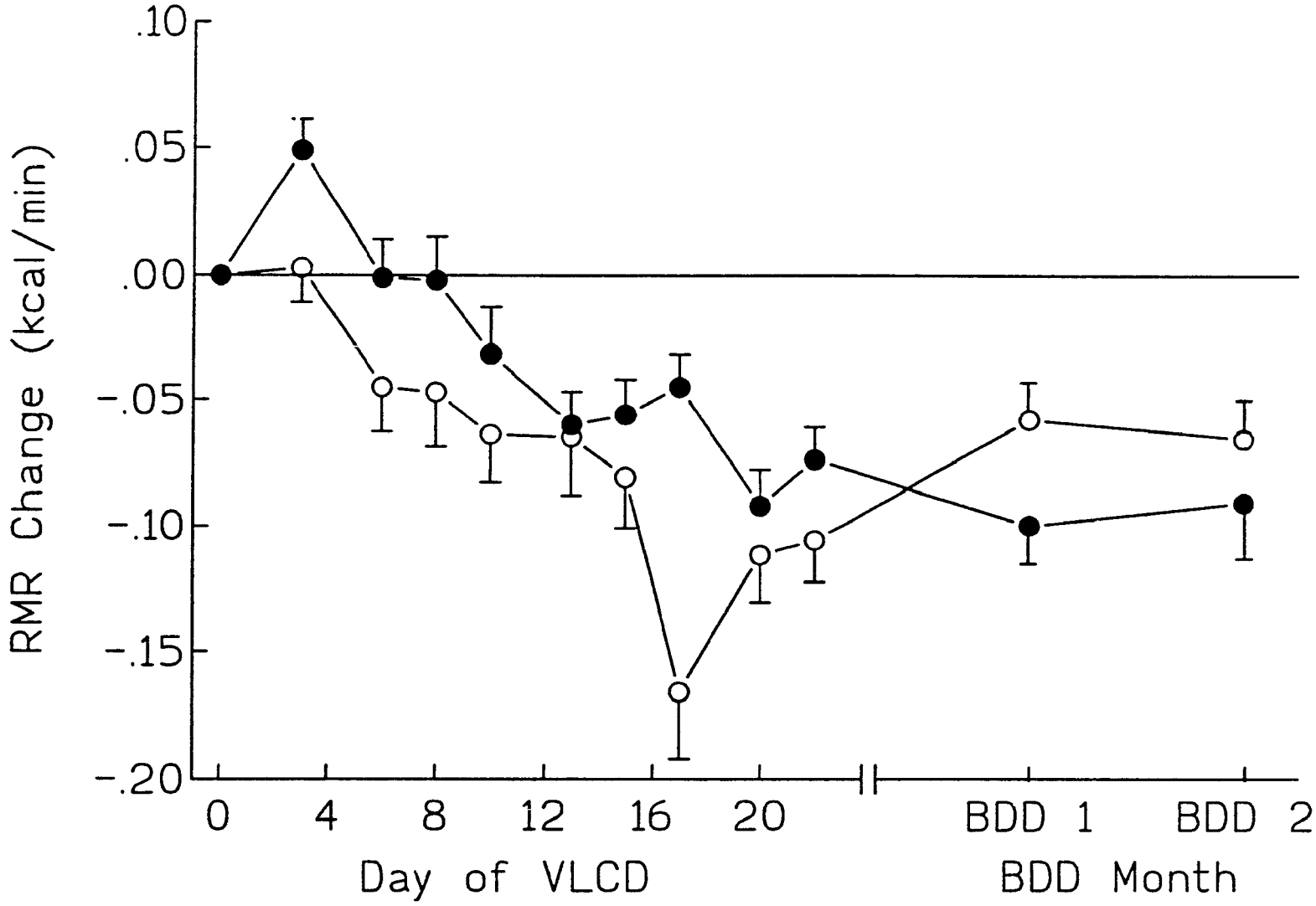

FIGURE 4. CHANGE IN RESTING METABOLIC RATE (KCAL/MIN) FROM BASELINE DURING THE VLCD AND BDD. OPEN CIRCLES: PLACEBO GROUP, SHADED CIRCLES: FLUOXETINE GROUP.

TEF AT BASELINE, AFTER THE VLCD AND AFTER THE BDD IN FLUOXETINE AND PLACEBO GROUPS

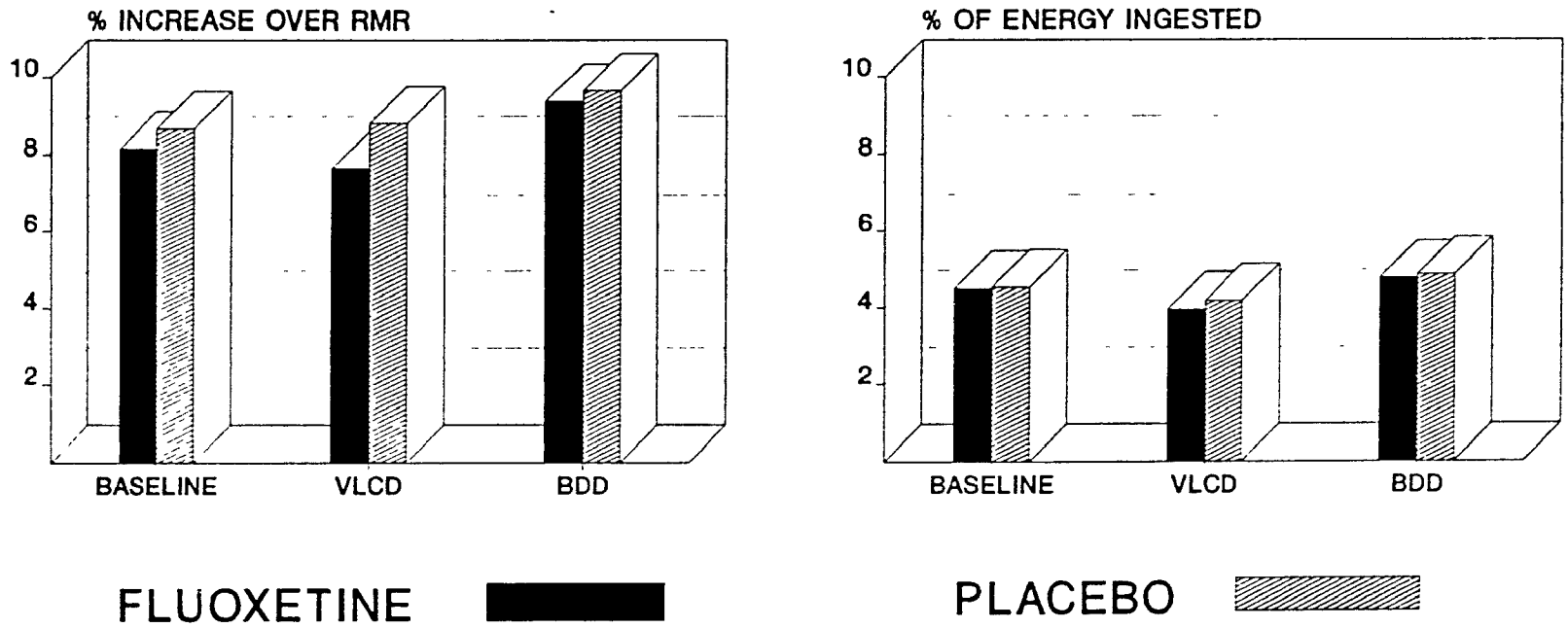

FIGURE 5. TEF AT BASELINE, AFTER THE VLCD AND AFTER THE BDD IN FLUOXETINE (N=10) AND PLACEBO (N=10) TREATMENT.

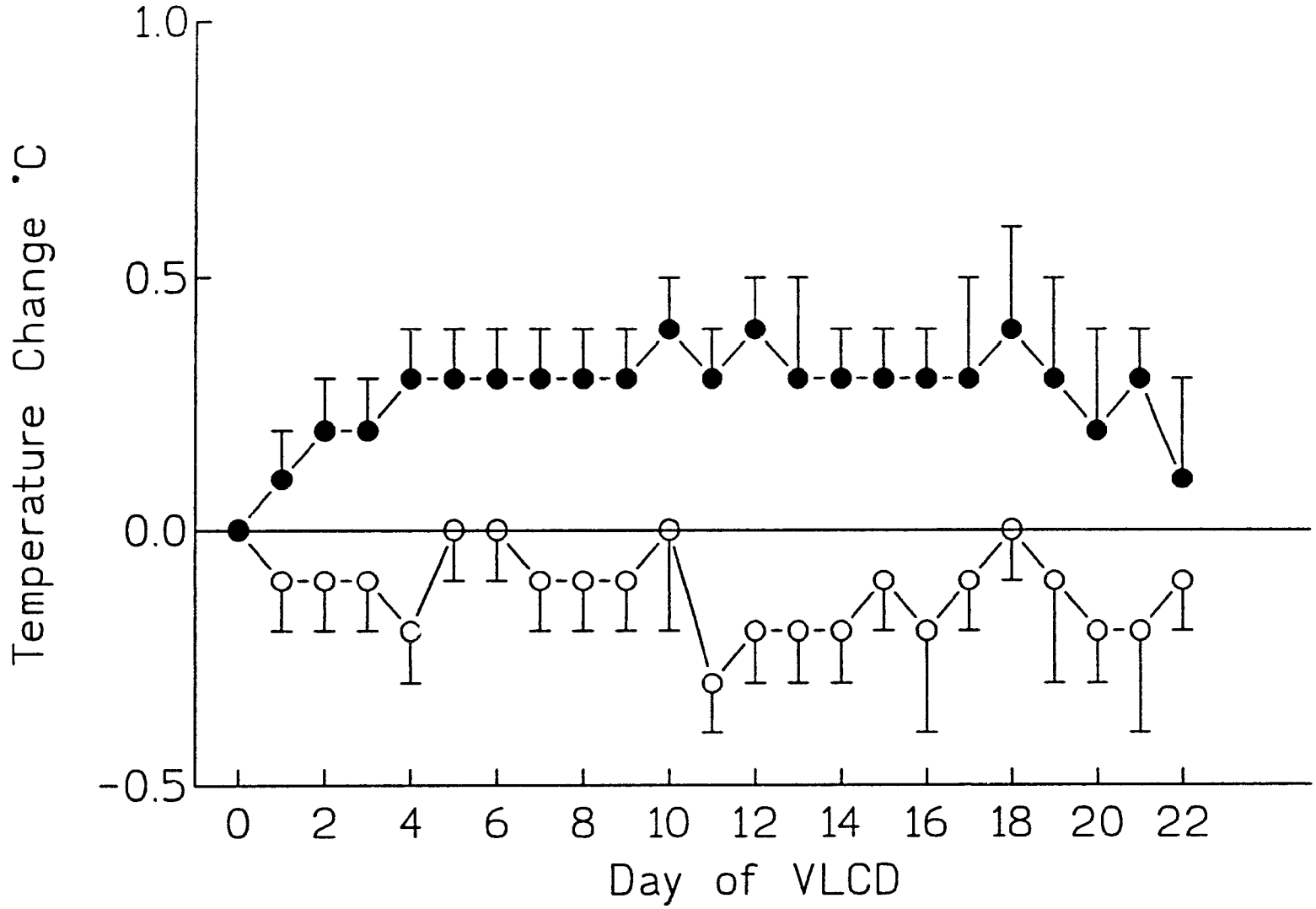

FIGURE 6. CHANGE IN BASAL TEMPERATURE FROM BASELINE DURING THE VLCD. OPEN CIRCLES: PLACEBO GROUP, SHADED CIRCLES: FLUOXETINE GROUP.

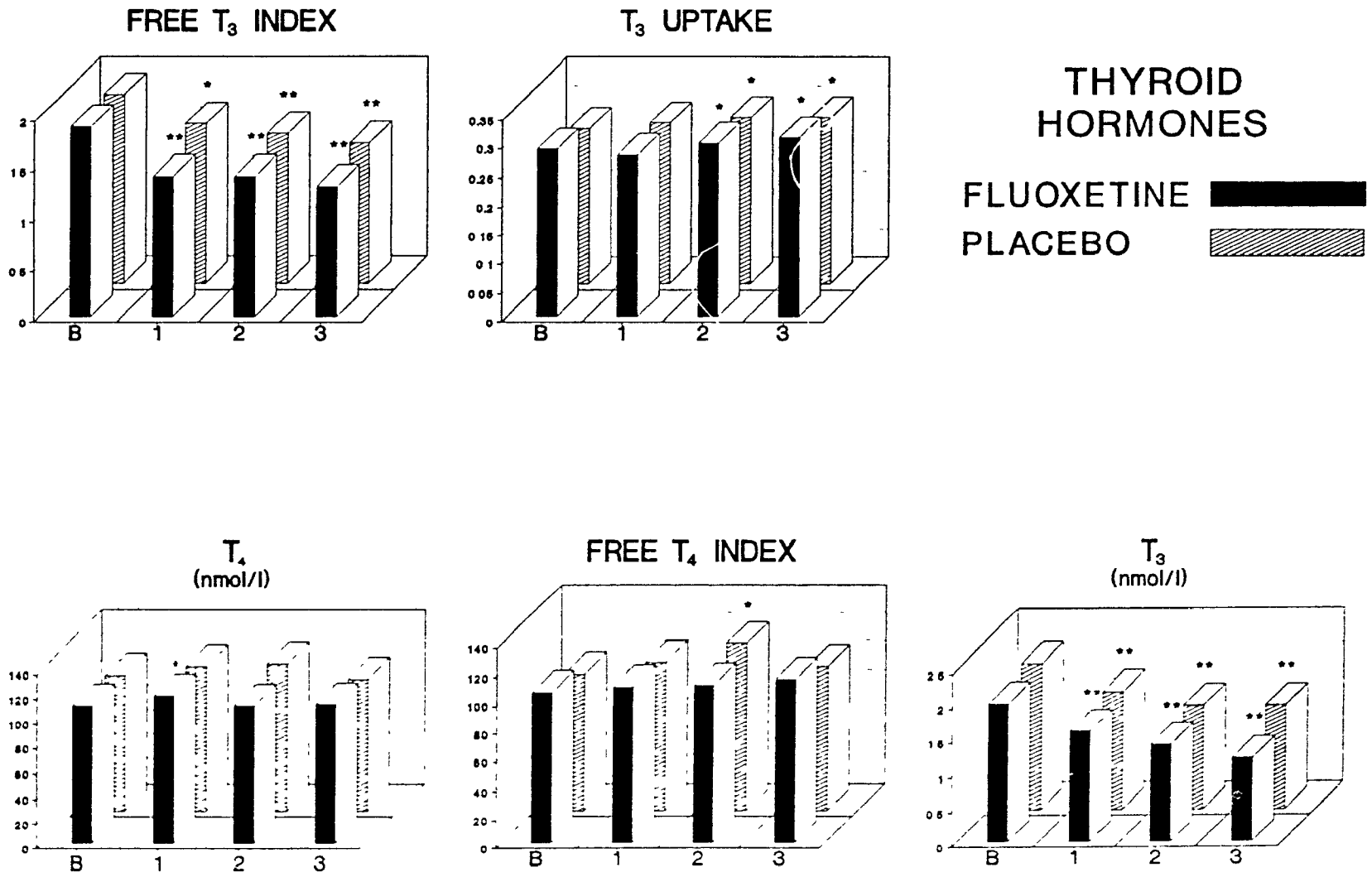

FIGURE 7. THYROID HORMONES BEFORE AND AFTER A VLCD AND FLUOXETINE (N=10) OR PLACEBO (N=10) TREATMENT.

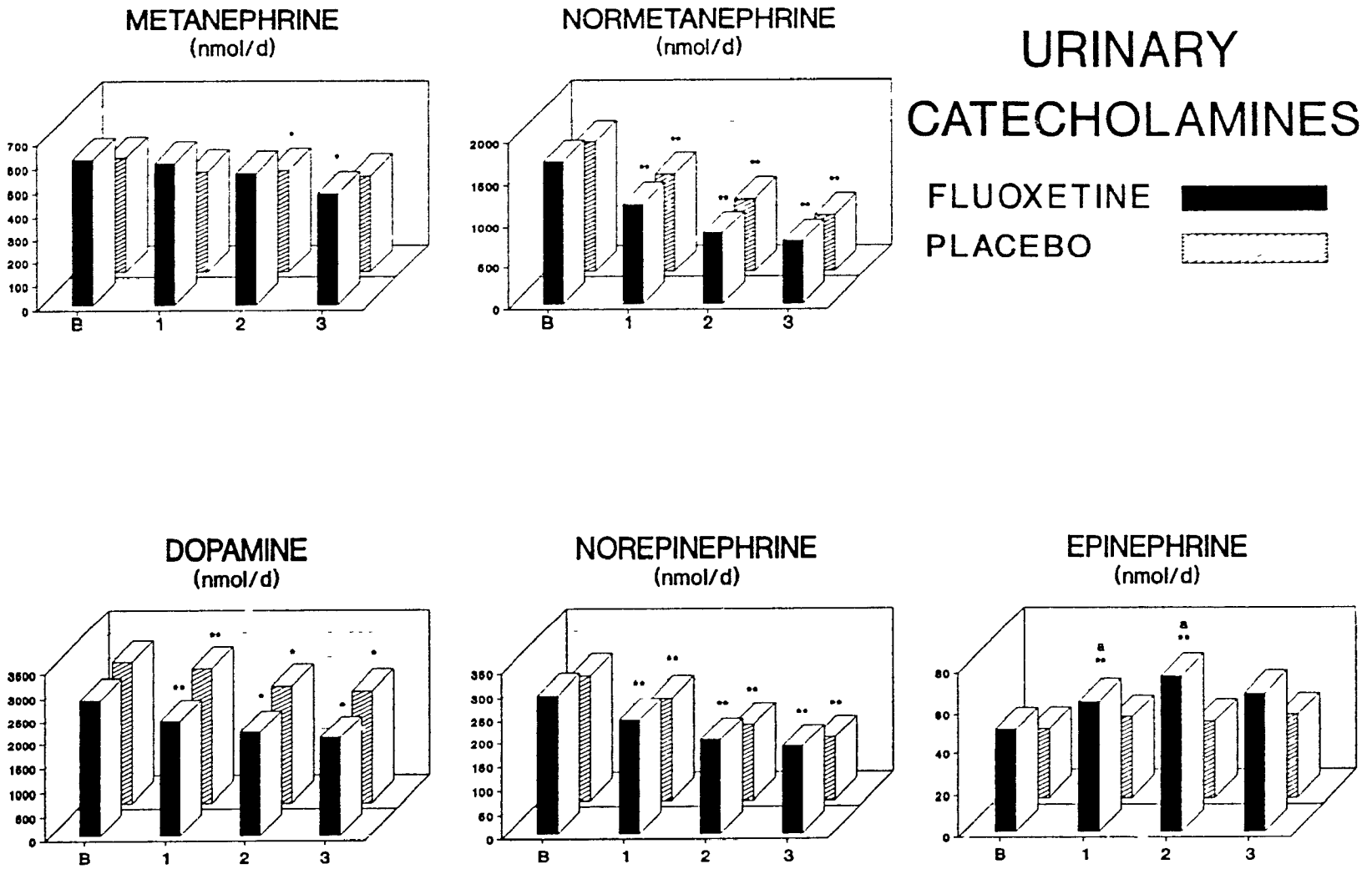

FIGURE 8. CATECHOLAMINES BEFORE AND AFTER A VLCD AND FLUOXETINE (N=10) OR PLACEBO (N=10) TREATMENT.

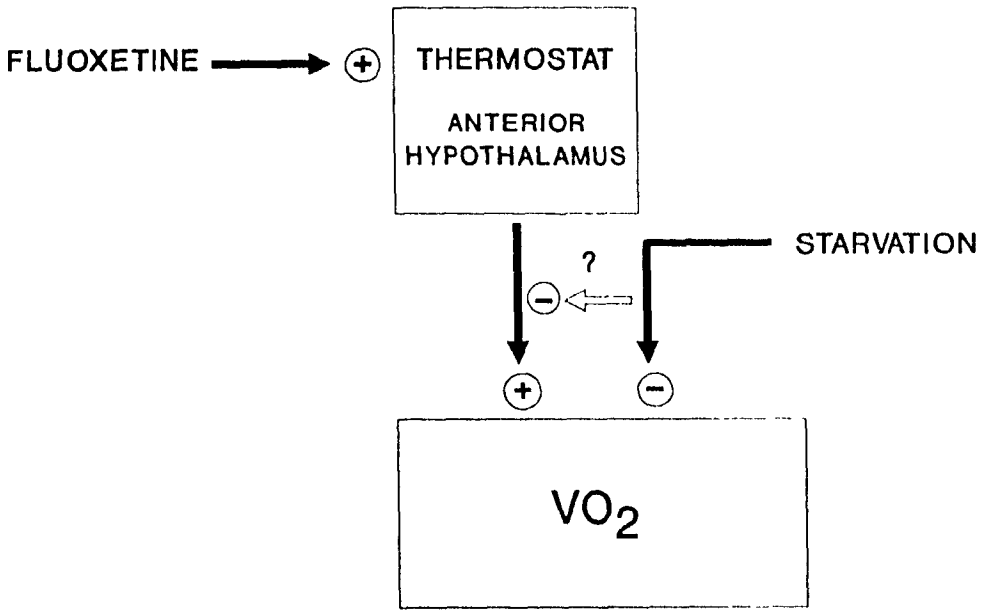

FIGURE 9. FLUOXETINE AND BASAL TEMPERATURE: POSSIBLE MECHANISM

APPENDIX 1

Informed Consent Document

Patient Name: _____ Date of Birth: ___ /___ / ___

Introduction: I agree to participate in a research study of a new investigational drug, fluoxetine, sponsored by Eli Lilly. MY PART IN THIS STUDY WILL LAST ABOUT 12 weeks (about 4 weeks during which I remain in hospital and 8 weeks outside of the hospital). About 20 other people will also be in this study.

The purpose of the study is to test if fluoxetine treatment will affect the resting metabolic rate (RMR) and the thermic effect of a standard test meal while I am in hospital and on a liquid formula very low energy diet providing about 500 Calories per day, as well as to test the effect of fluoxetine on metabolism while I am out of the hospital and eating a conventional weight reduction diet. These measurements indicate the rate at which the body is burning calories. The RMR involves lying comfortably on a bed under a clear canopy hood for 15-30 minutes. The thermic effect of a meal requires lying comfortably on a bed before and for the 4 hours following consumption of a stndard test meal.

Procedure: In this study, I will take one of two special drugs. One is fluoxetine, (the investigational drug) and a second is a placebo (sugar pill) that has no action. During the study I will receive either placebo or fluoxetine. Neither I nor the study physician will know which of these substances I am taking. This "double-blind" procedure is necessary to ensure unbiased observations and assessment of the efficacy of this new treatment by myself and the study physician.

Before entering the study, I will be interviewed by the study physician, who will take my history (including my consumption of alcohol and my previous smoking habits) and complete a general physical examination. At the beginning of the study I will have blood and urine tests done. There may be some pain or bruising associated with the venipuncture (blood drawing). These tests will be done to ensure that I meet the medical requirements for participating in the study. If I meet these requirements and decide to participate, I will be seen by the study physician and/or her/his research assistant daily while I am in hospital and twice monthly outside of the hospital. The purpose of these evaluations will be to determine whether the treatment is working and to be sure it is not causing me any harm. At every visit after leaving the hospital, I will be seeing a dietician and doctor. I will be weighed, and have my pulse and blood pressure taken. In hospital, I will follow a conventional diet for a "baseline" period of 4-5 days. I will then commence the study medication (or placebo) and the very low energy diet. On day 1, 5, 10, 13, 17 & 21, RMR will be measured in hospital

and once/month outside of the hospital. Thermic effect of a meal will be measured while I am on a normal diet, after 21 days of the low energy diet and on one outpatient follow-up visit. I realize that these tests are being done for the purpose of monitoring my physical condition prior to and after the study. There will be no charge to me for these tests and the clinic visits.

My reactions to the medication I am taking during the study will be carefully monitored. It is extremely important that the study physician or the research assistant be aware of all other drugs which I take, in addition to the study drug, while I am participating in the study. This even includes over-the-counter medications (non-prescription drugs). It is important that I discuss all other medications I have taken during the study with the study physician at each of the interviews. Also, drinking of caffeine-containing or alcoholic beverages and smoking must be avoided.

It is also important that I discuss with the study physician or research assistant all unpleasant or unusual symptoms which I experience, as well as any positive effects which I may have from the medication.

Risks: I understand there may be risks for me in being in this study. Fluoxetine has been given to over 6,000 clinical trial patients; 1.3 million patients in total. In some of these people, certain side effects have been observed. The most common side effects include nausea, nervousness, inability to sleep, headache, shaking, anxiety, sleepiness, dry mouth, increased sweating, diarrhea and dizziness. Allergic reactions to the medication, including hives, rash, joint pain and swelling can occur in some people. If I develop a rash or hives, I will tell Dr. Hoffer immediately.

Convulsions have been reported very rarely. In one of these cases, the patient swallowed 35 times more medicine than should have been taken. One elderly female patient developed an inflammatory condition of the lungs called pulmonary alveolitis. The relationship to fluoxetine is uncertain.

Because sleepiness could be a side effect with fluoxetine, I should not drive or operate complicated machinery such as a car or truck if I feel sleepy.

If I become pregnant during this study, there may be risks to me and my fetus or child that are not known. Women who are capable of becoming pregnant and who wish to participate in this study must be using a medically accepted form of birth control. If I am of child-bearing potential, I will tell the study physician or research assistant which method of birth control I am currently using.

I understand I am to tell Dr. Hoffer immediately if I have any unusual health experiences, injury or bad effect.

Drugs and procedures in this study may involve risks to me that are not known.

Very low energy diets result in a rapid weight loss. They are considered safe when medically supervised and used by properly selected patients. These diets could be dangerous for a fetus or unborn child. It is essential that women not be pregnant while following such a diet. These diets may be associated with side effects which include feelings of chilliness, constipation, temporary loss of menstruation or temporary increased rate of hair loss, although these side effects are most common after periods longer than the 3 weeks involved in this study. There may be an increased risk of a gall bladder attack (cholecystitis).

Benefits: I may benefit from this study in the following ways: I may see an increase in RMR which may help me to lose weight. Even if I am taking the placebo, I will benefit from weight loss during three weeks of monitored dieting with a very low energy diet in hospital, and outpatient follow-up on a conventional weight reduction diet after I leave the hospital. I will receive the benefits of evaluation of symptoms and general health discussions with the study physician, the health information from the laboratory tests, and finally, the chance to contribute to a scientific investigation which may be of benefit to patients in the future and to Eli Lilly.

Confidentiality: Records from the study which identify me will be confidential except they may be given to and inspected by the sponsor of the study (Eli Lilly), the Health Protection Branch and the Food and Drug Administration, and will not otherwise be released except as required by law. The sponsor will receive a signed copy of this consent agreement.

Compensation: If I follow the directions of the study physicians in charge of this study, and I am physically injured because of any substance or procedure properly given me under the plan for this study, Eli Lilly agrees to pay all medical expenses necessary to treat such injury which are not covered by my own insurance.

Payments: I will not have to pay for any of the medicines, medical examinations or laboratory tests that are required in my part of this study.

Voluntary Participation: I volunteer to participate in this study. I may quit at any time. If I decide not to participate, or I quit, I will not be penalized and will not give up any benefits which I had before entering this study. If I decide not to participate, or I quit, I will notify the study physician.

Stopping the Study: I understand that the study physician or Eli Lilly may stop my being in the study at any time without my consent. My participation may be discontinued if the study physician judges that it is in my best interest; if I fail to comply with study procedures; if the project is withdrawn by the sponsor; if the sponsor deems it appropriate; or for other reasons.

I understand that upon completion or termination of the study I may not take a class of drugs call MAO inhibitors for a period of 5 weeks afterward.

Information: I can get more information or answers to my questions about the study, my participating in the study, and my rights from Dr. Hoffer, who can be telephoned at (514) 843-1665. If the study physician learns of important new information that might affect my desire to remain in this study, the study physician will tell me.

Qualifications: The study physician has told me that I cannot be in this study if I am pregnant or breast feeding a child. I also cannot be in this study if I abuse alcohol or drugs; regularly use psychotropic drugs, including lithium; am being treated with certain high blood pressure, antidepressant or thyroid medications; have glaucoma, a history of urinary retention, a history of seizures, or have had a serious illness that is not under control. I am between the ages of 18 and 45 years.

Understanding: The study physician has explained this study and this consent form to me. The study physician has answered all my questions to my satisfaction. I understand what will happen if I agree to be part of this study.

VOLUNTEERS'S NAME _____

VOLUNTEER'S SIGNATURE _____

DATE: _____ TIME: _____

PHYSICIAN'S SIGNATURE: _____

WITNESS'S SIGNATURE: _____

DISPOSITION OF THIS DOCUMENT

1. Give one copy of the document to the patient.
2. Keep one copy of the document in the investigator's records.
3. Forward one copy of the document to Eli Lilly attached to the Informed Consent page of the case report form along with Visit 1.