FACTORS INFLUENCING THE THERMIC EFFECT OF FOOD

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My African man boasts,
The extra fat on his belly,
Is a status symbol!
But the Dutch man says,
it kills his athleticism.

My African beauty queen believes,
The adipose rump,
Is a magnetic nectar,
And a golden treasure!
But the Dutch blonde admits,
It makes her clumsy!

My erudite cardiologist warns,
The extra butter on the toast,
Though tasty,
Cholesterolises and kills the heart!
But my oncologist professes,
Low cholesterol promotes cancer!

All of these people,
Might be right after all,
So claims my polymathic friend.
Indeed,
The extra CALORIE in the fat,
Is a paradox!!.

CONTENTS

Acknowledgements			vi
Declaration			vii
Summary			viii
List of Tables			xi
List of Figures			xiii
Abbreviations			xv
CHAPTER 1		GENERAL INTRODUCTION	1
CHAPTER 2		LITERATURE REVIEW	7
	2.1	Introduction	8
	2.2	Energy Intake	8
	2.3	Energy Expenditure	10
	2.4	Causes of the Thermic Effect of Food	15
	2.5	Factors Influencing the Thermic Effect of Food	25
		Meal Composition	25
		Energy Content of a Meal	30
		Meal Frequency	31
		Exercise	33
		Body Composition	35

	2.6	Measurement of Energy Expenditure	39
		Direct Calorimetry	39
		Indirect Calorimetry	40
		Non-Calorimetric Methods	43
CHAPTER 3		SUBJECTS AND GENERAL METHODOLOGY	
	3.1	Subjects	47
	3.2	Methods	56
		Determination of Metabolic Rate	56
		Determination of Basal Metabolic Rate	62
		Determination of Post-prandial Metabolic Rate	63
		Test Meals	64
CHAPTER 4		EFFECT OF MEAL COMPOSITION AND ENERGY CONTENT ON THE THERMIC EFFECT OF FOOD	
	4.1	Introduction	67
	4.2	Subjects and Methods	68
	4.3	Results	71
	4.4	Discussion	75
CHAPTER 5		EFFECT OF MEAL FREQUENCY ON THE THERMIC EFFECT OF FOOD	
	5.1	Introduction	80
	5.2	Subjects and Methods	81
	5.3	Results	84
	5.4	Discussion	88

CHAPTER 6	EFFECT OF EXERCISE ON THE THERMIC EFFECT OF FOOD		
	6.1	Introduction	93
	6.2	Subjects and Methods	94
	6.3	Results	99
	6.4	Discussion	103
CHAPTER 7	EFFECT OF BODY COMPOSITION ON THE THERMIC EFFECT OF FOOD		
	7.1	Introduction	109
	7.2	Subjects and Methods	109
	7.3	Results	113
	7.4	Discussion	121
CHAPTER 8	GENERAL DISCUSSION		129
REFERENCES			136

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DECLARATION

I Joyce L.D. Kinabo do hereby confirm that the work presented in this thesis was carried out by me and has not been submitted before to any other University. The following publications are based on the work described in this thesis.

1. Kinabo, J. and Durnin, J.V.G.A. (1990). Thermic effect of food in humans: Effect of meal composition and energy content. British Journal of Nutrition (In press).

2. Kinabo, J. and Durnin, J.V.G.A. (1990). The effect of meal frequency on thermic effect of food. European Journal of Clinical Nutrition (In press).

3. Kinabo, J. and Durnin, J.V.G.A. Thermic effect of food in obese and non-obese subjects. (In preparation, to be submitted to the European Journal of Clinical Nutrition).

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Signature..... Date.29.5.90

SUMMARY

The studies described in this thesis were designed to determine the potential factors that may influence the magnitude of the thermic effect of food (TEF), and thus, its role in energy balance in humans. The factors studied were meal composition, energy content of a meal, meal frequency, exercise and body composition.

In all studies, female (women) subjects were used, and their basal metabolic rate (BMR) and post-prandial metabolic rate (PP-MR) were measured by open circuit indirect calorimetry using the Douglas bag technique. Two different meal compositions were used: (1) high carbohydrate-low fat (HCLF) with 0.70, 0.19 and 0.11 of the energy content from carbohydrate, fat and protein, respectively, and (2) low carbohydrate-high fat (LCHF) with 0.24, 0.65 and 0.11 of the energy content from carbohydrate, fat and protein, respectively. The energy content of the meals for each composition were 2520 kJ (600 kcal) and 5040 kJ (1200 kcal). Thus, there were four different meal combinations. Both the HCLF and LCHF meals were used in all the experiments, except in those on exercise in which only the HCLF meal was used. Meals comprised ordinary food items such as cornflakes, bread, cheese, eggs and orange juice and were administered in the form they are normally consumed.

The effect of meal composition and energy content of a meal on TEF was studied in 16 non-obese subjects. On four separate occasions, after BMR measurements, each subject consumed one of the four food combinations. Post-prandial metabolic rate (PP-MR) was measured for 5 h following complete ingestion of the test meal while the subjects were in the resting state (lying down). It was found that while energy content of the meals had a significant (P=0.001) effect on TEF, meal composition did not significantly (P=0.49) influence TEF. TEF was found to be 9 and 7 percent of the ingested meal for the 2520 kJ (600 kcal) and 5040 kJ (1200 kcal) meals, respectively. In all the subjects, and for all meals, PP-MR had not returned to the pre-meal level 5 h after the meal.

The effect of meal frequency on TEF was examined in 18 non-obese subjects. Eight subjects consumed the HCLF meal and 10 subjects consumed the LCHF meal. On two separate occasions, after BMR measurements, each subject consumed the appropriate diet either as one large meal containing 5040 kJ (1200 kcal) or as two smaller meals (180 min apart), each containing 2520 kJ (600 kcal). Post-prandial metabolic rate (PP-MR) was then measured for a total of 6 h following complete ingestion of the test meals. TEF values calculated for 6 h after consuming the HCLF meal did not show significant (P=0.94) differences between the two feeding regimens. Similarly, no significant (P=0.64) differences in TEF were found for the LCHF meal between the two feeding regimens. Additionally, the two meal compositions did not show a significant (P=0.57) effect on TEF. However, in the feeding regimen of two meals there was superimposition of the TEF of the first meal into that of the second meal. And the TEF calculated for the second meals were found to be significantly higher than those of the first meals (HCLF, P=0.012; LCHF, P=0.001). TEF expressed as a percent of energy intake was 7 percent for all feeding regimens as well as meal compositions.

To determine the effect of exercise on TEF, the energy expenditure of walking on a treadmill at 4.8 km/h at 0 percent slope before and after a meal (HCLF, 2520 kJ (600 kcal)) was determined in non-obese subjects (n=28) and compared to that of subjects who did not perform any exercise (chapter 4). No significant differences were found in energy expenditure between walking in the fasting state and walking in the fed state (P=0.79). The PP-MR measured under resting conditions 20 min after ingestion of a meal was found to be 24 percent above the pre-meal level. However, the energy expenditure of walking in the fed state increased by only 1 percent above the pre-meal energy expenditure of walking. The results seem to suggest that performance of exercise after meal resulted into a lower TEF. As for the PP-MR measured at 45, 95 and 150 min after exercise, this did not differ significantly from that measured at corresponding times in subjects who did not perform exercise (chapter 4) (P=0.40, 0.70, 0.84, respectively). Exercise significantly lowered the TEF, and it did not potentiate the TEF when this was measured for several hours after cessation of exercise. In addition there was no

significant difference between BMR and resting metabolic rate measured 30 min after subjects had exercised for 15 min while in the fasting state, which seem to suggest that there was no prolonged effect of exercise on metabolic rate.

The effect of body composition on TEF was evaluated by determining post-prandial metabolic rate (PP-MR) of obese subjects (n=21) after consumption of the four test meals. The values obtained were then compared with those measured in non-obese subjects (chapter 4). Energy content of the meals was found to significantly influence TEF (P=0.005) in obese subjects as was found for non-obese subjects. Meal composition did not show significant influence on TEF (P=0.09) in these subjects. However, at both levels of energy intake (2520 and 5040 kJ) the mean TEF values for obese subjects were 20 percent lower (p=0.001) than those for non-obese subjects.The results have also shown that there was no correlation between TEF and any of the physical characteristics of the subjects. However, it is not clear from these results whether the low TEF observed in these studies was due to obesity or that the low TEF caused obesity.

In conclusion, these studies have shown that the magnitude of TEF was significantly influenced by the energy content of a meal and body composition, but not by meal composition and meal frequency. Performance of exercise after ingestion of a meal caused a reduction in TEF. These results seem to suggest that meal composition and meal frequency are not important determinants of TEF, and that exercise does not potentiate the TEF.

LIST OF TABLES

Table 1.	Studies which have shown that meal composition has no significant effect on the thermic effect of food	27
Table 2.	Studies which have shown that meal composition has a significant effect on the thermic effect of food	28
Table 3.	Studies which have shown lower thermic effect of food in obese than lean subjects	36
Table 4.	Studies which have shown no differences in thermic effect of food between obese and lean subjects	37
Table 5.	Constituents of the test meals	65
Table 6.	Physical characteristics of the subjects	69
Table 7.	Basal metabolic rate (BMR) and thermic effect of food in 16 non-obese subjects after ingestion of a high carbohydrate-low fat (HCLF) and low carbohydrate high fat (LCHF) meals	72
Table 8.	Physical characteristics of the subjects	82
Table 9	Energy expenditure in non-obese subjects after ingestion of high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) meals under two different feeding regimens	85
Table 10.	Thermic effect of food in non-obese subjects after ingestion of a high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) meals under two feeding regimens	89
Table 11.	Physical characteristics of the subjects	95
Table 12.	Basal metabolic rate (BMR) and energy expenditure of walking in the fasting state in non-obese female subjects	100

Table 13.	Energy expenditure of walking in the fasting and fed state for subjects who followed protocol 1	101
Table 14.	Energy expenditure of walking in the fasting and fed state in subjects who followed protocol 2	104
Table 15.	Physical characteristics of obese subjects	110
Table 16.	Physical characteristics of obese and non-obese subjects	111
Table 17.	Basal metabolic rate (BMR) in obese subjects	114
Table 18.	Basal metabolic rate (BMR) in obese and non-obese subjects	115
Table 19.	Thermic effect of food in obese subjects after ingestion of high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) meals	118
Table 20.	Thermic effect of food in obese and non-obese subjects after ingestion of a high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) meals	120

LIST OF FIGURES

Fig. 1.	A theoretical model of various factors known to influence basal metabolic rate in humans	11
Fig. 2.	A theoretical model for the thermic effect of food	16
Fig. 3.	Metabolic pathways during the absorptive state	17
Fig. 4.	Metabolic pathways during the post-absorptive state	18
Fig. 5.	Substrate cycles known to influence metabolic rate	20
Fig. 6.	Equipment for the determination of body density by underwater weighing showing subject resting with her head out of water prior to initiation of weighing procedure	53
Fig. 7.	Underwater weighing showing subject completely immersed in water	55
Fig. 8.	The Douglas bag system showing collection of expired air from the subject	59
Fig. 9.	Increase in metabolic rate above basal metabolic rate (BMR) in non-obese subjects after ingestion of four different test meals	73
Fig. 10.	Thermic effect of food in non-obese subjects after ingestion of four different test meals	74
Fig. 11.	Increase in metabolic rate above basal metabolic rate in non-obese subjects after ingestion of high carbohydrate-low fat (HCLF) and low carbohydrate- high fat (LCHF) meals administered either as a single large meal or two small meals	86
Fig. 12.	Thermic effect of food in non-obese subjects after ingestion of a high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) meals administered either as a single large meal or as two	

	small meals	87
Fig. 13.	Energy expenditure of walking in the fasting and in the fed state in subjects who followed protocol 1	102
Fig. 14.	Energy expenditure of walking in the fasting and fed state in subjects who followed protocol 2	105
Fig. 15.	Increase in metabolic rate above basal metabolic rate in obese subjects after ingestion of four different test meals	117
Fig. 16.	Thermic effect of food in obese subjects after ingestion of four different test meals	119
Fig. 17.	Increase in metabolic rate above basal metabolic rate in obese and non-obese subjects after ingestion of high carbohydrate-low fat meals	122
Fig. 18.	Increase in metabolic rate above basal metabolic rate in obese and non-obese subjects after ingestion of low carbohydrate-high fat meals	123
Fig. 19.	Thermic effect in obese and non-obese subjects after ingestion of four different test meals	124

ABBREVIATIONS

BMR	Basal metabolic rate
HCLF	High carbohydrate-low fat
LCHF	Low carbohydrate-high fat
RMR	Resting metabolic rate
MR	Metabolic rate
PP-MR	Post-prandial metabolic rate
FFM	Fat free mass
TEF	Thermic effect of food
WHR	Waist:Hip ratio
STPD	Standard temperature and pressure, dry
AHR	Abdominal:Hip ratio
PEP	Phosphoenopyruvate
PYR	Pyruvate
G	Glucose
G-6-P	Glucose-6-phosphate
Fru-6-P	Fructose-6-Phosphate
Fru-1-6P2	Fructose-1-6-Diphosphate
ATP	Adenosine Triphosphate
UWW	Underwater weighing

CHAPTER 1

GENERAL INTRODUCTION

Two major components are involved in the regulation of energy balance. These are energy intake and energy expenditure. The difference between these two components constitutes what is called energy balance. It is through a long term interaction and adjustments of these two major components which determine the composition of the energy stores of the body and thus, body weight.

Obesity and energy deficiency, therefore, are both manifestations of chronic body energy imbalance. Obesity may arise either due to excess energy intake or defective energy expenditure or both. Energy deficiency arises primarily due to food or energy deficiency and in most occasions it occurs in association with deficiencies of other nutrients like protein (e.g in protein-energy malnutrition), iron and vitamins.

In many parts of the world, obesity (Sims, 1986; Simopolous, 1987) and energy deficiency (Narasinga, 1985) underly some of the most serious health problems. Obesity is considered to be the underlying cause for heart diseases, hypertension, diabetes mellitus, digestive diseases and cancer (Bray, 1987). Estimates of the prevalence of obesity range from 10 percent to 50 percent of the adult population (Bray, 1987; Duernberg and Hautvast, 1989) in industrialised countries. Reports on the prevalence of obesity in developing countries (Adadevoh, 1974; Richards and deCessares, 1974) are scanty. This is because attention has been focused mainly on undernutrition and energy deficiency.

Energy deficiency, on the other hand, lowers immunity and therefore predisposes the population to infection and morbidity. It has a far reaching economical implication in that it lowers the work capacity of the population. It is estimated that energy deficiency affects about 23 percent of the adult population in developing countries (Naiken and Perisee, 1985).

Energy deficiency is more common in developing countries. Many people living in these areas experience seasonal food shortages, partly due to inadequate storage facilities. And often food shortage coincides with peak labour demand for agricultural activities.

It is probable that people have acquired adaptability mechanisms to cope with the seasonal perturbations in energy intake. Various possible adaptive mechanisms such as reduction in basal metabolic rate (BMR), in body size, in daily physical activity (Ferro-Luzzi, 1986; James, 1987; Durnin and Drummond, 1988) and a reduction of the energy cost of muscular work (Saibene, 1990) have been suggested. However, the exact adaptative mechanisms are yet to be fully elucidated (Shetty and James, 1984; Waterlow, 1986; Minghelli, in press).

The importance of obesity and energy deficiency has been well recognised by various organisations such as the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO). Although obesity has been investigated extensively, neither the aetiology nor the regulatory mechanisms of obesity are clearly understood (Jequier, 1987; Wurtman and Wurtman, 1987).

The identification of possible defects and adaptability capabilities in the regulation of energy balance continues to be of interest to Nutritionist and other related fields. An understanding of the mechanisms involved in the regulation of energy balance would provide vital information needed for setting up human energy requirements.

Human studies have shown that the relationship between energy intake and energy expenditure is a highly complex one and at the moment is not clearly defined. The wide inter- individual as well as intra-individual variations in energy intake and energy expenditure which cannot be explained by the variations in body weight and composition suggest that there could be other yet unidentified factors which regulate energy balance. Application of multidisciplinary approach to the study of energy balance borrowing concepts from endocrinology, neurology, genetics, molecular biology, physiology and nutrition should provide new insights into how energy intake and energy expenditure interact to influence energy metabolism and body composition.

1.1 COMPONENTS OF ENERGY BALANCE

Energy intake

Energy intake represents the amount of energy which may theoretically be expected to be released on the complete metabolism (total metabolisable energy) of the food ingested by an individual in a day. Currently, little is known as to how much food should an individual consume (Durnin, *et. al.*, 1973; FAO/WHO/UNU 1985). This may partly be attributed to methodological problems associated with energy intake and energy expenditure measurements in free living populations.

Energy expenditure

Total energy expenditure can be divided into three major components, namely, basal metabolic rate (BMR), physical activities and thermic effect of food (TEF). The three components are not entirely discrete but are useful divisions when attempting to investigate factors that might regulate or control them (Sims and Danforth, 1987). Of the three components, BMR and TEF contribute up to 80 percent of the total daily energy expenditure for people living in developed countries. However, for people living in developing countries, the contribution of each of these components is yet to be defined.

1.2 THERMIC EFFECT OF FOOD

TEF is defined as the increase in metabolic rate above BMR after ingestion of a meal. This increase in metabolic rate after meal has been associated with the energy cost of digestion, absorption and storage of food in the body. There is increasing evidence to suggest that TEF may also have a regulatory component which is mediated through the sympathetic nervous system (Landsberg and Young, 1983), and some hormones such as insulin due to its role in nutrient storage (Ravussin, *et.al.*, 1985) and thyroid (Ingbar and Braverman, 1986) . The role played by genetic factors in determining TEF is also of current interest (Borgadus *et al.*, 1985).

On average TEF accounts for 10 percent (Jequier, 1984) of daily energy expenditure, but this can vary from one individual to another. Thus, its quantitative role in the regulation of energy balance is still unclear. Both the mechanisms that underly its production and the factors that influence its magnitude are poorly understood.

Knowledge of the effect of the factors that influence TEF is important in understanding the role of TEF in the overall energy balance regulation under different nutritional conditions, such as in obesity and energy deficiency. Currently, little is known about the possible role of the adaptive mechanisms regulating TEF under chronic energy deficiency (Waterlow, 1985).

Various factors such as nutritional status (Brooke and Ashworth, 1972; Garrow, 1986), energy content of the ingested meal (Clough and Durnin, 1970; Belko *et. al.*, 1986), meal composition (Garrow and Hawes, 1972; Schwartz *et. al.*, 1985) , number of meals per day (meal frequency) (Belko and Barbieri, 1987), and exercise (Miller and Mumford, 1967; Segal *et. al.*, 1983) have been shown to influence the magnitude of TEF, hence energy balance. However, results from different studies differ widely. This area of nutrition is still one of controversy.

1.3 STUDY OBJECTIVES

The present studies were carried out to examine the influence on TEF of meal composition, energy content of the ingested meal, meal frequency, body composition and exercise in humans and possibly assess the role of TEF in the overall energy balance.

Of the five factors investigated, energy content and body composition significantly influenced the magnitude of TEF. Comparison of the TEF in obese and non-obese subjects showed that the TEF in obese subjects was 20 percent lower than that in non-obese subjects. There was no significant effect of meal composition and meal frequency on TEF. However, exercise resulted in a lower TEF.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The main components involved in energy balance regulation are energy intake and energy expenditure. Since the components of energy balance are not entirely discrete, it is appropriate that all different aspects of energy intake and energy expenditure are considered in human energy balance studies. In this chapter, various components of energy balance are reviewed with particular emphasis on TEF.

2.2 ENERGY INTAKE

Energy intake is the total metabolisable energy of the food ingested by an individual. Energy intake is regulated by a complex system involving both external as well as internal voluntary and involuntary controls (Kissillef and VanItallie, 1982; Forbes, 1988). However, it remains unclear which control systems from a variety of neural and humoral mechanisms are dominant in controlling energy intake (Nicholl *et al.*, 1985). External factors (non-physiological) which have been suggested to control food intake include: palatability, social set up (culture) and economic (cost and availability of food). It is the interaction between the internal and external signals which controls food intake. The actual availability of the ingested energy is also affected by various factors such as gastro-intestinal diseases as well as the bioavailability of the nutrients from the food matrix. Regulation of energy intake is complex, poorly understood, and of growing concern in a world with problems of starvation and obesity morbidity (Nicholl *et al.*, 1985).

Despite a number of studies on this subject, it is still not clearly understood what determines how much energy should an individual consume (Durnin *et al.*, 1973; Daly *et al.*, 1985). This may partly be due to the problems associated with the food intake studies. The main problems encountered in energy intake studies include: first, the accurate determination of the subjects customary energy intake ; second, the conversion

of this information to energy and nutrient intake, third the length of time food intake should be measured, and, fourth the wide inter-individual as well as intra- individual variations in energy intake (Hegsted, 1974).

Various methods have been used to estimate energy intake in man. These include dietary recall (Burke, 1947) where the subject is asked to recall as accurately as possible food consumed within 24 h prior to the interview. The quality of food has to be estimated. Although the method is simple to execute, it does not provide a reliable estimate of the individual's intake or a description of customary intake. Furthermore, it requires a cooperative and literate informant, a trained interviewer and standard format (Reed and Burke, 1954). However, it can provide an estimate of group average intake (Graham, 1982).

A second method is the weighed inventory (Widdowson, 1936). In this method the participating subject is asked to weigh each item of food before it is consumed and any left overs are weighed as well. This is to give an indication of the amount of food actually consumed by the subject. From a record of the items consumed, the actual nutrient intake is calculated using food composition tables. It has several advantages over the recall method in that it does not require the presence of the researcher, it can be fairly accurate and reproducible. However, it is tedious and requires a high degree of motivation on the part of the subject. In some cases this has been shown to interfere with the normal eating pattern of the participating subject, consequently, leading to underreporting and underestimation of the actual intake. The weighing and recording of the food items may not be easy for illiterate individuals ; thus selection of subjects may introduce significant bias. Food tables are also widely used to estimate energy intake. But with introduction of pre-cooked meals and given the fact that items of diet and amounts change, it means food tables have to be updated more often. This may not be a simple task since chemical analysis of the food is a complicated, time consuming and costly exercise. However, introduction of nutrition labelling should help to alleviate the problem.

2.3 ENERGY EXPENDITURE

While energy intake is considered to be the metabolisable energy of the food ingested, energy expenditure can be divided into three major components, basal metabolic rate (BMR), thermogenesis (cold or food induced) and energy expenditure due to physical activities.

Basal metabolic rate

BMR accounts for about 60-75 percent of the total daily energy expenditure of man. It is defined as the energy expended by the body for maintenance of normal body functions and homeostasis under complete rest without the influence of a meal or muscular exertion. BMR is measured in the morning, 12-14 h after the last meal or vigorous physical activity. Before the measurement the individual must be allowed to lie quietly in a thermoneutral environment for at least one half hour (Benedict, 1915; du Bois, 1927; Boothby and Sandiford, 1929; Boothby *et. al.*, 1936). In practice BMR measured in this way is considered to be approximately equal to energy expenditure of subjects during sleep (FAO/WHO/UNU, 1985).

Determinants of BMR (Fig. 1) include constitutional factors such as age, sex and genes and variable factors such as body weight and composition, preceding diet, drugs and stress. Constitutional factors determine the mass of metabolically active tissues such as liver, kidney and brain and also the composition of different tissues of the body. There is evidence to suggest that BMR may be regulated genetically. Measurements of BMR in lean and obese diabetic and non-diabetic Pima Indian volunteers revealed an inheritable component in BMR (Daniels *et al.*, 1982; Borgadus *et al.*, 1985). Moreover, retrospective studies of adopted twins in Denmark (Stunkartd *et. al.*, 1986) have suggested a strong genetic component in BMR. The implication of these results is not clear but the evidence supports a genetic factor in the regulation of BMR.

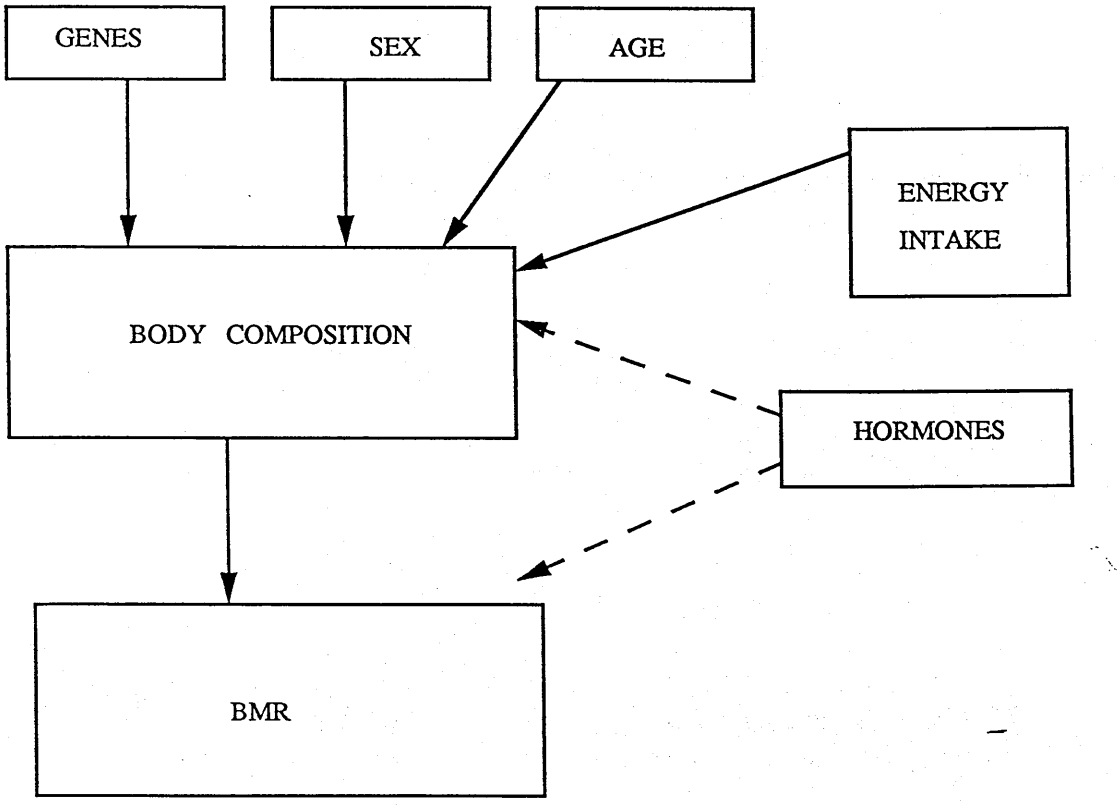


Fig. 1. A theoretical model showing various factors known to influence basal metabolic rate (BMR).

BMR is higher in children (when expressed per kg of body weight) and declines in old age mainly due to loss of fat free mass. Moreover, BMR also shows variation with sex. Females have lower BMR compared to males and this is attributed to differences in body composition. Females tend to have a higher percent of body fat content and thus less fat free mass compared to males. Environmental factor, in particular diet determines the size of fat free mass and fat mass of the body (Owen *et al.* 1987).

It has been suggested that the variability which is observed when comparing individuals of different age, sex and weight disappears when values are expressed per unit of fat free mass (Webb, 1981; Jequeir, 1987). However, even when this is done there is still up to 30 percent difference between individuals despite same body weight, age, sex and habits (Jequier, 1987). The source of the variability is not easily identified (Woo, *et.al.*, 1985). However, this could suggest the existence of yet unidentified factors controlling BMR.

The expression of BMR values per unit of fat free mass may provide a simple way of comparing individuals but it does not take into account the basic differences in energy demanding processes at cellular level. Since most of the biochemical processes at cellular level are regulated and controlled by hormones, it is pertinent to assume that the differences in BMR which can not be explained by differences in fat free mass may be attributed to differences in the levels of and sensitivity to hormones.

Hormones such as thyroid hormones (Ingbar and Braverman, 1986; Jequier, 1987), catecholamines (Danforth, 1983) are known to influence BMR. For instance, thyroid hormones are known to control the respiratory processes in the mitochondria by regulating the amount of oxidative enzymes and cofactors, thus oxygen consumption (Ingbar and Braverman, 1986; Franklyn and Sheppard, 1989). Since BMR is usually measured by indirect calorimetry which essentialy is a measure of oxygen consumption , any factor which regulate or affect oxygen consumption would in turn influence BMR. It is interesting to note that until recently, the thyroid hormone status of patients was assessed by measuring oxygen consumption at rest (Lamb *et al.*, 1984). It has been shown (Jequier, 1987) that thyroid hormones are responsible for approximately 30

percent of BMR, since in completely athyreotic humans the BMR falls to about 70 percent of control values. Thyroid hormones have also been shown to modify the action of other hormones especially that of catecholamines and to regulate the sodium pump (Na+ dependent ATPases) all of which could be related to BMR (Danforth, 1983). However, the exact mechanisms by which thyroid hormones regulate oxygen consumption hence metabolism are yet to be fully elucidated.

Environmental factors such as level of energy intake, drugs and stress are known to influence BMR. Energy underfeeding may reduce BMR by one third (Keys *et al.* 1950; Jung *et al.*, 1980; Shetty and James, 1984). The initial fall of BMR due to underfeeding may occur without greater losses in fat free mass. This suggests that the effect is probably dominated by a fall in metabolic activity at cellular level and not so much by the decline in the fat free mass (Jung *et al.*, 1980). Futhermore, it has been shown that during underfeeding there is a fall in circulating triiodothyronine (Ingbar and Braverman, 1986) and catecholamines (Jung *et al.*, 1980) and this may partly account for the initial fall of BMR. On the other hand, energy overfeeding cause an increase in BMR (Schutz, *et al.*, 1985) especially if overfeeding occurs on the day just prior to BMR measurement. However, this could be related to the prolonged effect of food on metabolic rate, since it has been shown that the effect of a large meal on the day prior to BMR measurement could be detected on the following day (Schutz *et al.*, 1985). The long term effect of overfeeding on BMR is due to increased fat free mass associated with overfeeding.

It is most probable that the changes occurring during energy overfeeding and underfeeding are modulated by endocrine and sympathetic tone, but the biochemical basis is unclear (Woo *et al.*, 1985; Garrow, 1987).

Physical activity

Another component of total daily energy expenditure is the energy expended due to the performance of physical activities (work). Of all the components of energy

expenditure, energy expenditure due to physical activity is the most variable and therefore can easily be altered.

Energy expenditure due to physical activity may account for about 10-20 percent of the total daily energy expenditure (Horton *et al.*, 1983; Woo *et al.*, 1985), and its role in energy balance depends on both the intensity and duration of doing an activity. Although physical activity is obviously energetic and can increase oxygen consumption by about 100-300 percent of basal level, the effect of activity on daily energy expenditure of most animals is quite small, sometimes accounting for about 5 percent of energy expenditure . In modern affluent societies, energy sparing devices have drastically reduced the intensity as well as the duration of performing physical activities. This has considerably diminished the magnitude of physical activity in energy expenditure (Bray, 1987). It has been shown (Durnin, 1973) that about 75 percent of the average person's day is spent in relatively sedentary activities such as sitting, standing and short distance walking. Of this time, about 15-37 percent of the total daily energy on physical activity is spent on sitting activities and is by far the dominant type of activity. Standing is of little importance for most people in developed countries. Walking is a common activity but short duration of walking makes it less significant in daily energy expenditure.

It is generally appreciated that alteration in nutritional status, which may have major effect on the BMR, seem to have no significant effect on the efficiency of performing physical activity (Spurr, 1988). However, it is not clear whether nutritional status has any influence on work capacity. Furthermore, little is known about the influence of other variables such as endocrine status, and cardiovascular work on work capacity, and also the effect of parasitic infestation on the performance of physical activity. Currently few data are available, thus, more research is needed to elucidate the role of these variables in work capacity particularly in deprived societies (Durnin and Drummond, 1988; Spurr, 1988).

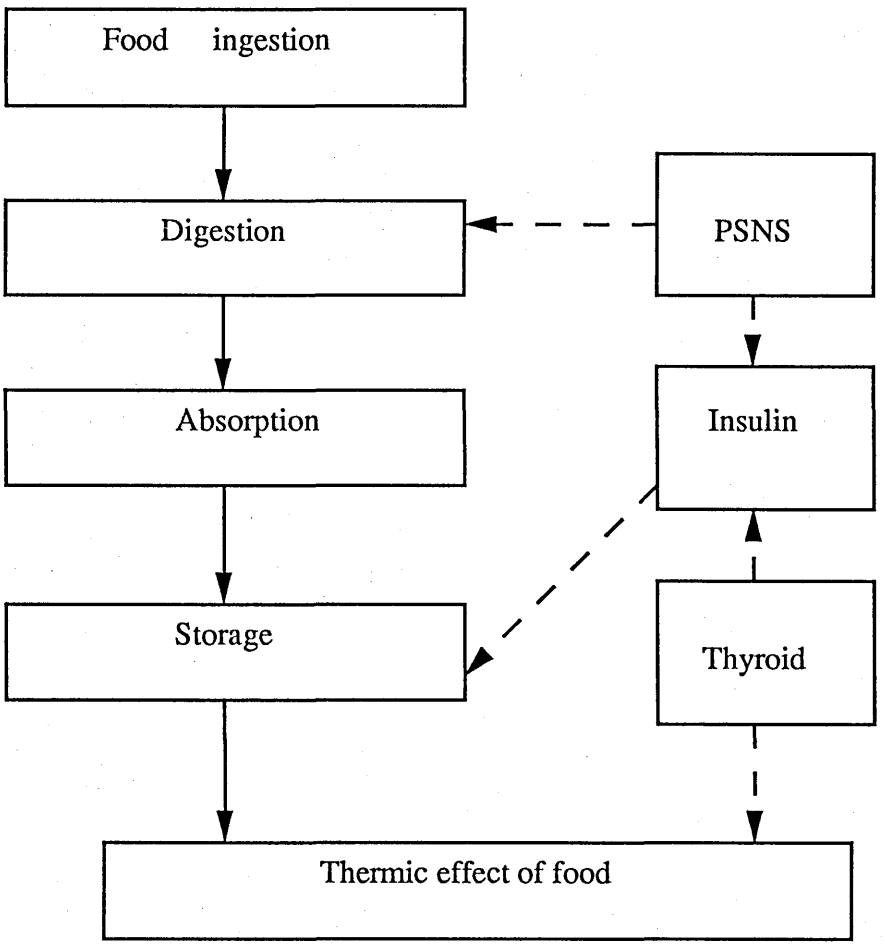
Thermic effect of food

Another component of total daily energy expenditure is TEF. The textbooks by Benedict and Carpenter (1918) and that of Lusk (1928) give a detailed account of most of the classical work on TEF, then known as specific dynamic action (SDA). The present review is intended to present the current knowledge on TEF with particular emphasis on the factors which influence TEF. TEF is accounted for largely by the energy cost of digestion, absorption and storage of the ingested nutrients in the body and also a small component related to the regulatory mechanisms of digestion, absorption and storage of food (Fig.2). Recent research indicates that a complex network of nutrient and hormonal factors interact to cause and regulate TEF in humans (Woo *et al.*, 1985)

Despite the amount of work on TEF, the mechanisms underlying the nature of action of TEF are not fully understood. However, several mechanisms such as substrate cycling (Newsholme, 1972), activation of the sympathetic nervous system (Landsberg and Young, 1984), release and conversion of thyroid hormones (Thyroxine to Triiodothyronine) (Ingbar and Braverman, 1986) and insulin (Ravussin *et. al.*,, 1983), have been suggested.

2.4 CAUSES OF THERMIC EFFECT OF FOOD

Presence of food in the gastro-intestinal tract starts a series of events leading to the production of digestive enzymes which hydrolise the food into smaller molecules. During the absorptive state (period immediately after meal) products of digestion pass from the digestive tract into the liver, muscles and adipose tissue (Fig. 3). The major source of energy to the body during this period is glucose. However, during the post-absorptive state (Fig. 4) the opposite happens. Nutrients flow from the storage organs such as the liver and adipose tissue into circulation to be used by other tissues as source of energy (Fig. 4). Because the brain, renal medulla and erythrocytes utilise mostly glucose



PSNS=Parasympathetic nervous system

Fig. 2. A theoretical model of the thermic effect of food. Solid lines indicate obligatory components and broken lines indicate regulatory components

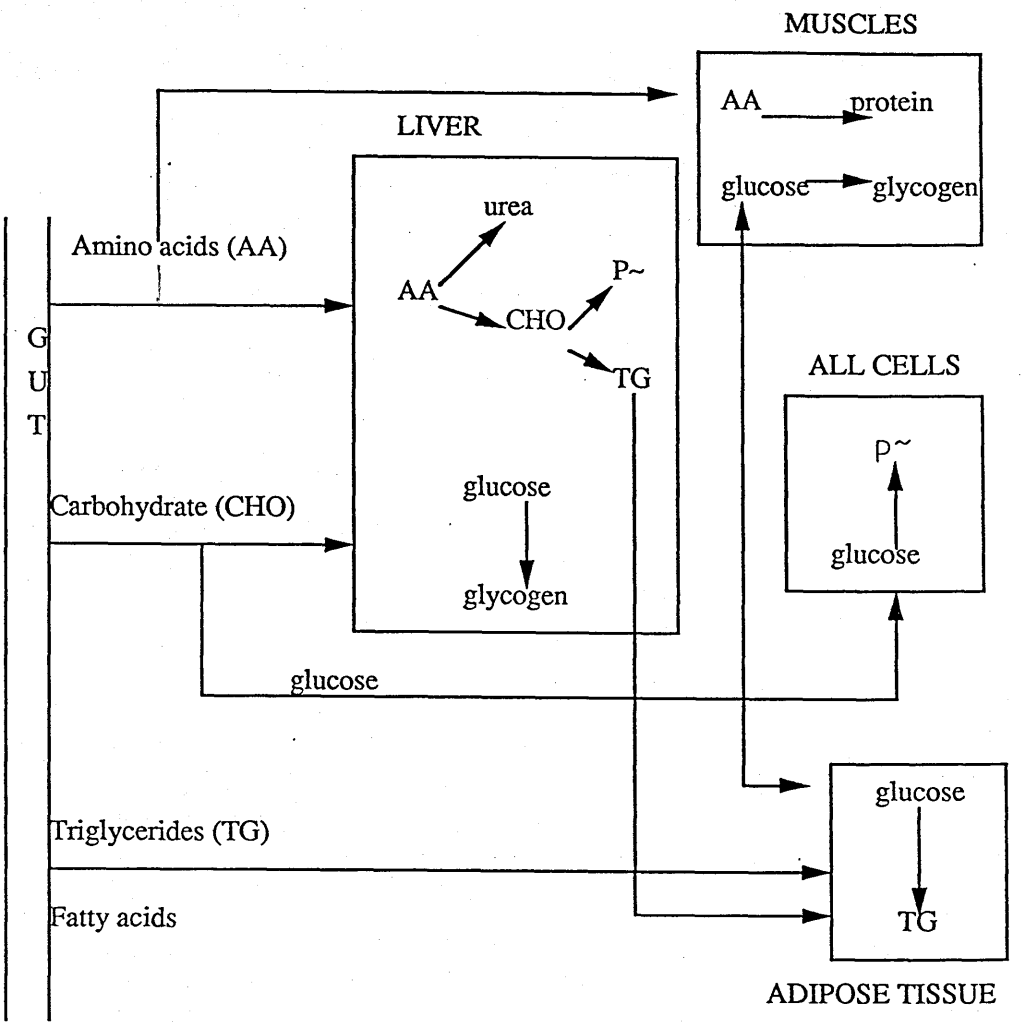
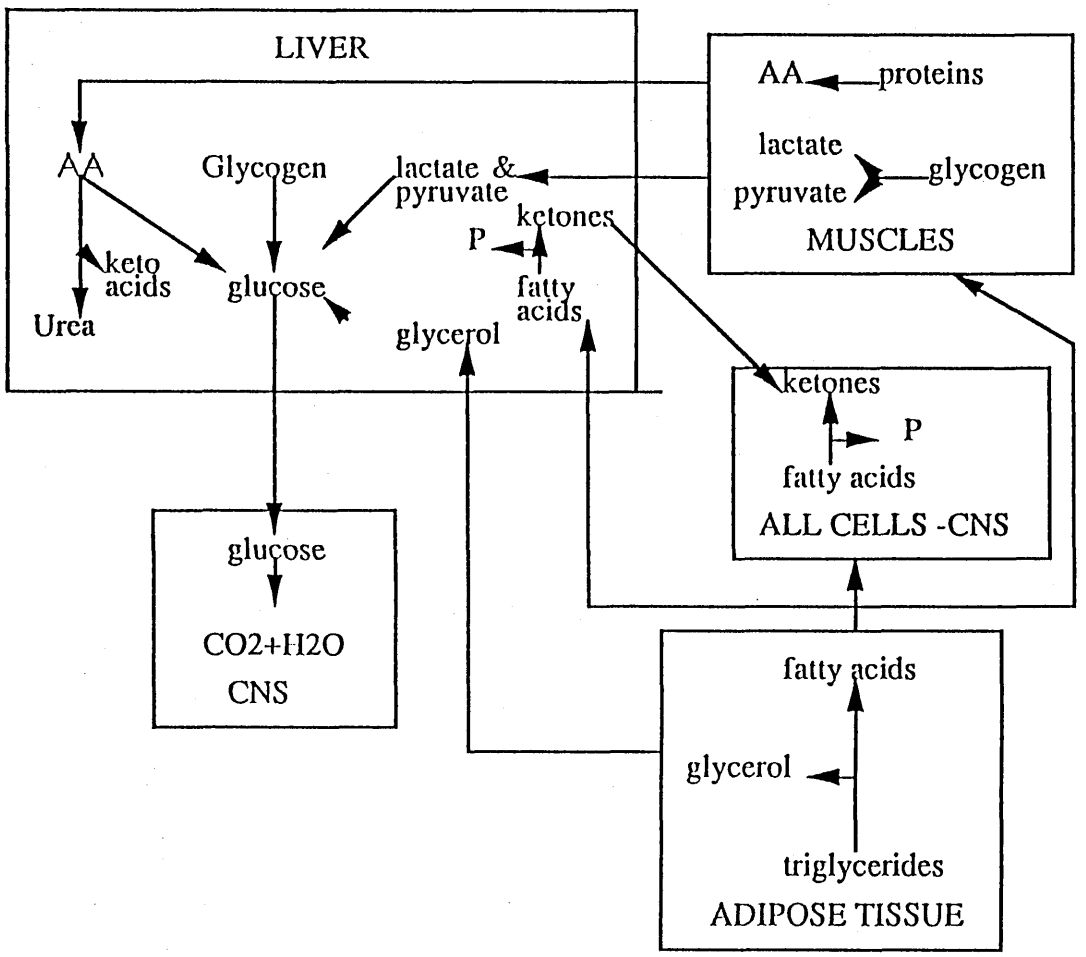


Fig 3. Metabolic pathways of absorption, showing the direction of flow of nutrients during the absorptive state.



AA= Amino acids; CNS= Central nervous system

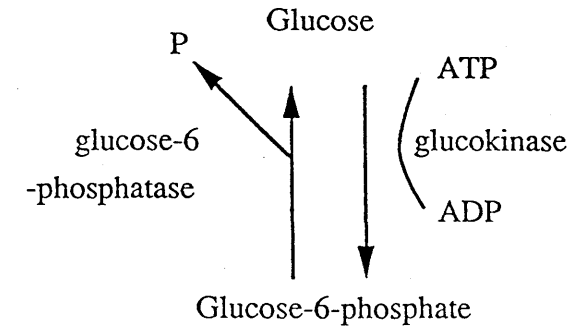
Fig. 4. Metabolic pathways showing the direction of flow of nutrients during the post-absorptive state

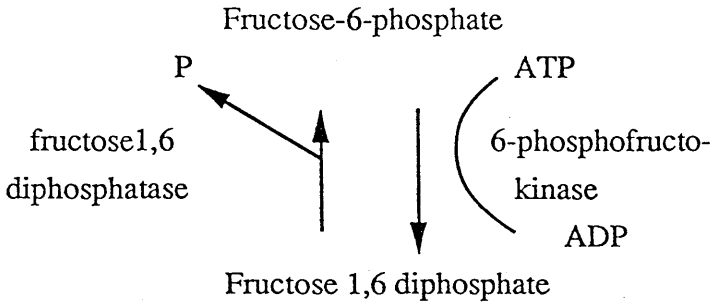
for energy, the blood glucose is maintained constant by glycogenolysis and gluconeogenesis. The energy supply to most other cells is therefore switched from glucose to fatty acids.

These various adjustments are regulated and controlled by hormones such as insulin, glucagon and catecholamines. These three hormones are directly involved in the control of organic metabolism. However, other hormones such as thyroid hormones are involved in a permissive way.

Substrate cycling

During the absorptive state the concentration of products of digestion in circulation increases. This leads to an increase in the rate of substrate cycling in the major metabolic pathways (glycolysis and Krebs cycles) especially along the glucose to glucose-6-phosphate (G to G-6-P), fructose-6-phosphate (Fru-6-P) to fructose 1, 6, diphosphate (Fru 1, 6, P2), phosphoenol pyruvate (PEP) to pyruvate (PYR) (Fig. 5) , amino acid to protein and fatty acids to triglycerides in both the liver and muscles (Newsholme , 1980). According to Newsholme and Carbtree, (1978), the increased rate of substrate cycling results in a greater demand for energy, hence oxygen consumption. It is now thought that the cycle between PEP and PYR may be the major site for acute hormone action on the entire glycolysis pathway, since pyruvate kinase is the only enzyme known to be involved in the interconversion of PEP and PYR. Current knowledge also suggests that hormonal regulation at Fru-6-P and Fru 1, 6,P2 is mediated by changes in the level of Fru 2,6,P2. When Fru 2,6,P2 levels are high the activity of 6-phosphofructokinase (Fig. 5) is high, and therefore, the activity of Fru,1,6 phosphatase is inhibited. However, when Fru 2,6, P2 levels are low, Fru 1,6,phosphatase activity is enhanced, and that of 6-phosphofructokinase is inhibited and gluconeogenic flux predominates. The level of Fru 2,6 P2 has been shown (Claus *et al.*, 1984) to be under acute hormonal control. Glucagon is known to lower the level of Fru-2,6,P2, and hence raise gluconeogenic flux. The action of insulin, however, is to prevent the level of Fru-





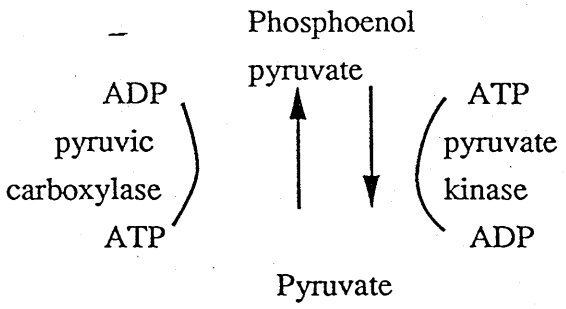


Fig. 5. Substrate cycles

2,6,P2 from falling and because of this glucose storage is enhanced, which leads to increased oxygen consumption (Claus *et al.*, 1984; Pilkis *et al.*, 1986). It is possible that differences in enzyme and hormone levels between individuals, (for example in insulin resistance or diabetic patients) may partly be responsible for the differences in TEF between subjects.

Although the mechanisms sound plausible, the estimation of the energy cost of these cycles has never been demonstrated. Furthermore, there is little experimental evidence to suggest involvement of this mechanism in TEF (Blaxter, 1989). However this is probably the first biochemical explanation for TEF (Newsholme, 1978) and therefore needs further investigation.

The availability of products of digestion or substrates play a major role in increasing the rate of these cycles, it is also possible that changes in the concentration of hormones and enzymes in blood and the extent of excitation of the nervous system associated with feeding may determine the rate of the cycles (Pilkis *et al.*, 1986).

Sympathetic nervous system

The actions of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS) on different visceral organs are complex and varied. In general, activity in the SNS tend to decrease gastric and intestinal motility and tone, to inhibit secretion and to cause constriction of the sphincters. Consequently leading to reduced digestive function. However, it increases the rate of glycogenolysis, gluconeogenesis and lipolysis in the liver.

On the other hand, parasympathetic nervous system activity enhances digestive function by increasing gastric and intestinal motility and tone, stimulating secretion and relaxing sphincters.

A variety of techniques have been used to investigate the role of sympathoadrenal system in the regulation of TEF. Studies based on either chemical or surgical removal of the sympathetic nerves and adrenal medulla or on the administration of adrenergic

blocking agents, have provided much of the basic information about the contribution of the sympathoadrenal system on TEF (Landsberg and Young, 1983). Assessment of sympathoadrenal activity has usually been based on the measurements of catecholamines in urine and plasma. Plasma and urinary noradrenalin levels provide an index for SNS activity.

Catecholamines are involved in mobilisation of glycogen stores, stimulate glucose synthesis from non-carbohydrate precursors and stimulate the release of free fatty acids from triglyceride stores in the adipose tissue. However, it has been shown (Landsberg and Young, 1983) that during starvation and food deprivation sympathoadrenal activity is reduced (Shetty and James 1984). This could be a control mechanism by the body to preserve energy. Refeeding restores catecholamines turnover toward normal level.

The role of sympathetic nervous system in TEF is still controversial. While some studies have shown that noradrenalin increases after meal (Le Blanc *et al.*, 1984) and after glucose infusion (Robertson and Porte, 1974; Welle *et al.*, 1981), others could not establish any significant involvement of the sympathetic nervous system on TEF (Zwillich *et al.*, 1981; Seaton *et al.*, 1984; Gallen *et al.*, 1990). Beta-adrenergic blockade of the sympathetic nervous system with propanolol showed partial inhibition (Acheson *et al.*,1983; DeFronzo *et al.*, 1984) and was without effect (Vernet, 1987) on TEF.The study by Schwartz *et al.*, (1988) suggested that alpha-receptors may also be involved in TEF, since blockade of alpha-receptors with clonidine reduced TEF.

These results are surprising considering the known beta-receptor-mediated effects of noradrenalin.

There are reports to suggest that the sympathetic nervous system is involved in the regulatory mechanisms associated with underfeeding (Shetty and James, 1984) and overfeeding (Landsberg and Young, 1984). However, evidence for the participation of sympathetic nervous system in these situations at present is limited.

The parasympathetic nervous system has also been shown (Natch *et al.*, 1987; Deriaz *et. al.*,, 1989) to participate in TEF. Blockade of the parasympathetic nervous system by atropine caused a 60 percent reduction in TEF , suggesting that

parasympathetic nervous system is involved in TEF. This would be expected because of the role the parasympathetic nervous system plays in the digestive processes.However, blockade of the parasympathetic nervous system with atropine did not influence the TE of glucose/insulin administered intravenously (Deriaz, *et. al.*,, 1989). These results seem to suggest that the presence of food in the stomach is important for the parasympathetic nervous system mediated responses.

Role of Hormones in TEF

Various hormones such as thyroid (Hesse, *et al.*, 1981; Danforth and Burger, 1984; Ingbar and Braverman, 1986; O'riodarn *et. al.*, 1988) and Insulin (Ravussin, *et al.*, 1983) have been shown to play a role in TEF.

Thyroid hormones

During starvation including overnight fast, there is a decrease in plasma triiodothyronine and an increase in reverse triiodothyronine but no change in plasma thyroxine (Portnay *et al.*, 1975; Chopra *et al.*, 1975; Jung *et al.*, 1978; Ingbar and Braverman, 1986; Hendler, 1988). Caloric restriction has been shown to cause a 53 percent reduction in serum triiodothyronine with simultaneous increase in reverse triiodothyronine in obese subjects (Spaulding *et al.*, 1976). Furthermore, marginal energy intake significantly influenced the peripheral conversion of thyroxine to triiodothyronine (Garrel *et al.*, 1984). Studies (Hesse *et al.*, 1981; Dauncey *et al.*, 1983) have shown that feeding causes an increase in the secretion of triiodothyronine and thyroxine from the thyroid gland and also increases peripheral conversion of thyroxine to triiodothyronine. At the same time plasma reverse triiodothyronine concentration decreases. The conversion of thyroxine to a more thermogenically triiodothyronine is thought to be one of the mechanisms that causes an increase in oxygen consumption after meal (Hesse *et al.*, 1981). Dauncey *et al.*, (1983) showed that the level of plasma triiodothyronine increases immediately after ingestion of a meal and reaches a peak at

approximately 60 min after completion of a meal. The level of triiodothyronine remains elevated for more than 3 h. The magnitude to which thyroid hormone increases after meal has been shown to be influenced by the energy content of the ingested meal but not by meal composition (Ingbar and Braverman, 1986). Danforth *et al.*, (1979) observed that energy overfeeding had no effect on the concentration of thyroxine in circulation but increased that of triiodothyronine irrespective of the composition of the diet ingested.

Insulin

Another hormone considered to play an important role in TEF is insulin. Insulin is essential for glucose metabolism. It controls the passage of glucose and other nutrients (amino acids and fatty acids) into the cells, it also inhibits gluconeogenesis and enhances lipogenesis. It has been shown that plasma insulin levels rise rapidly within 30 min of glucose infusion (Ravussin *et al.*, 1985) and after ingestion of a mixed meal (Hendler *et al.*, 1988), and continues to rise progressively for 3 h. The rise in plasma insulin levels increase the rate of glucose uptake and disposition by the cells, hence oxygen consumption.

Studies (Ravussin *et al.*, 1985; Borgadus *et al.*, 1985) have shown that TEF is lower in diabetic patients and some obese subjects (insulin resistance obesity). This observation suggests that insulin is involved in TEF. The conversion of glucose to glucose-6-phosphate is an energy demanding process, which is catalysed by an enzyme glucose-6-kinase. Because of insulin deficiency in diabetic and insulin resistance in some obesity, the action of glucose-6-kinase is restricted; therefore passage of glucose in to the cells is limited. This is probably why TEF is low in diabetic and insulin resistance obese (Lusk, 1928).

These observations suggest that insulin and thyroid hormones work in concert to control nutritionally induced alterations in energy expenditure thus TEF. But the entire mechanism is yet to be fully elucidated.

2.5 FACTORS INFLUENCING THE THERMIC EFFECT OF FOOD

Various factors have been shown to influence TEF. These include meal composition, energy content of the ingested meal, meal frequency, exercise and body composition.

Effect of meal composition

The effect of meal composition on metabolic rate was first demonstrated by Rubner (1902) (cited by Lusk, 1928). It was observed that protein had a greater effect on metabolic rate than carbohydrate and fat (Dubois, 1927; Lusk, 1928). Protein increased metabolic rate by 20-30 percent, carbohydrate by 6 percent and fat by 4 percent. These studies led to the formulation of the term SDA. The term was used to describe the increase in metabolic rate elicited by ingestion of protein foods.

According to Flatt (1978), assessment of the amount of ATP utilised in the processing of food in the body was taken to represent SDA. Processing of protein required 6 moles of ATPs, equivalent to about 25 percent of the ingested energy, and that of carbohydrate needed about 0.5-8.7 moles of ATPs, that is 2-7 percent of the energy content of glucose, and fat required required 2.3-4.6 moles of ATPs representing 2-4 percent of the energy ingested. The SDA of carbohydrate and fat depends on whether the absorbed nutrient is directly oxidised or converted to storage form. For example, it is 2 percent if it is directly oxidised, 7 percent if it is converted to glycogen and 25 percent if converted to fat. Similarly, the SDA of fat is 2 percent if it is directly oxidised, and 4 percent if it is stored.

The SDA of protein is the same whether amino acids are converted in to protein or directly oxidised, because the cost of gluconeogenesis and ureogenesis is similar to that involved in protein synthesis (Flatt, 1978).

Increased fat intake coupled with low SDA was thought to be a recipe for obesity. However, subsequent studies on the effect of meal composition on metabolic rate have

produced conflicting results, and the exact effect of meal composition on metabolic rate has not been established.

Although meal composition has been reported to have a significant effect on TEF (Glickman *et al.*, 1948; Welle *et al.*, 1981; Dauncey and Bingham, 1983; Schwartz *et al.*, 1985), several other studies have shown that TEF is independent of meal composition (Durnin and Ferro-Luzzi, 1960; Miller and Mumford, 1967; Pittet *et al.*, 1974; Rosenberg and Durnin, 1978; Hurni *et al.*, 1982; Belko and Barbieri, 1987). The reasons for these differences are not clear. However, several physiological and methodological differences (Table 1 and 2) may partly account for the widely different results from different laboratories. Lack of uniformity with regard to type of food, duration of measurement, sample size and technique for measuring energy expenditure may all account for the observed variations between studies in different laboratories.

The type of foods used have ranged from pure nutrients such as glucose and amino acids (Garrow and Hawes, 1972; Pittet *et al.*, 1974) to mixed normal meals (Rosenberg and Durnin, 1978; Dauncey and Bingham, 1983). In most studies, these experimental diets were administered either as liquid meals (Pittet *et al.*, 1974; Schwartz et al., 1985; Poehlman, 1988) or solid meals (Rosenberg and Durnin, 1978; Dauncey and Bingham, 1983). It is surprising that even in the studies where the same type of food was used, significant differences between studies were observed (Table 1 and 2). This could probably be due to differences in the techniques used to measure energy expenditure.

The Techniques for measuring energy expenditure have ranged from short term (3-5 h) indirect calorimetry (Pittet et al 1974; Rosenberg and Durnin, 1978) to long term (25 h) direct calorimetry (Dauncey and Binghan, 1983; Acheson *et al.*, 1984). This has sometimes made comparison between studies difficult, particularly with regard to those studies (Dauncey and Bingham, 1983; Acheson *et al.*, 1984) in which exercise bouts were introduced during the experiment.

Although it is appropriate to design diets to meet the energy requirement of the subject, it is often not necessary if the experiment is only of a short duration. In some of

Table 1. Studies which have shown that meal composition has no significant effect on TEF

Composition			Energy	
P	CHO	Fat	content	
%	%	%	kcal	References
-	100	-	650	Garrow and Hawes, 1972
100	-	-	650	Garrow and Hawes, 1972
-	100	-	650	Garrow and Hawes. 1972
-	100	-	200	Pittet *et al.* 1974
50	50	-	400	Pittet *et al.* 1974
100	-	-	200	Pittet *et al.* 1974
15	42.5	42.5	600	Rosernberg and Durnin 1978
11	14	75	2793	Schutz *et al.* 1984
11	80	9	2693	Schutz *et al.* 1984
12	60	28	2842	Schutz *et al.* 1984
17	81	2	2000	Hurni, 1982
22	57	21	2000	Hurni, 1982
17	17	66	1500	Morgan and York, 1983
15	45	40	1516	Belko *et al.*, 1986
30	32	38	1462	Belko *et al.*, 1986
45	16	38	1408	Belko *et al.*, 1986
19	64	17	766	Henry and Emery,1986
17	66	17	500	Robinson and York,1988

P=Protein; CHO= Carbohydrate.

Table 2. Studies which have shown that meal composition has a significant effect on TEF

Composition			Energy	
P	CHO	Fat	content	
%	%	%	kcal	References
7	54	39	NP	Glickman *et al.* 1948
37	46	17	NP	Glickman *et al.* 1948
37	15	48	2470	Dauncey and Bingham,1983
3	49	48	2469	Dauncey and Bingham,1983
-	100	-	300	Nair *et al.* 1983
100	-	-	300	Nair *et al.* 1983
-	-	100	300	Nair *et al.* 1983
15	85	-	800	Schwartz *et al.* 1985
15	-	85	800	Schwartz *et al.* 1985
-	100	-	400	Swaminathan, 1985
-	-	100	400	Swaminathan, 1985
100	-	-	400	Swaminathan,1985
12	54	34	400	Swaminathan, 1985
15	45	40		Ravussin *et al.* 1985
17	42	42	2390	Zed and James, 1986
1	5	94	3420	Zed and James, 1986

P= Protein; CHO= Carbohydrate; NP= Not provided.

the experiments (Sharief and MacDonald, 1982; Poehlman, 1988) the administered food was tailored to the requirement of the subjects. Since energy content is known to significantly influence TEF, calculation of meal size according to the energy requirements will have a significant influence on the final interpretation of the results. The results may be related to the energy content rather than the composition of the ingested meal. In general, people do not eat food on the basis of predetermined energy requirements. It is probable that differences in body composition observed between individuals who consume different diets (high carbohydrate or high fat) could be related to the total energy intake associated with a particular diet and not necessarily on a low TEF.

High carbohydrate (starch) meals which are common in developing countries are known to have low energy density and to be bulky, thus effective energy intake is low compared to high fat diets which are known to have high energy density. Another property of fat meals is that they improve the palatability of the meals and encourage people to eat more. The prevalence of obesity in Western societies is more related to high energy intake through high fat diets and not to low TEF of fat diets as previously conceived (Lusk, 1928).

Food intake surveys all over the world (Widdowson, 1947; McKillop, 1990 Personal communication) have shown that, protein intake contributes about 10-15 percent of total energy intake. This amount is considered to be adequate for mantaining nitrogen balance in adults (FAO/WHO/UNU, 1985). Incorporation of about 45 percent (Glickman *et al.*, 1948; Dauncey and Bingham, 1983) and 100 percent of protein (Pittet *et al.*, 1974) in the test meals represents an unrealistically high intake of protein.

To accurately determine the effect of meal composition on TEF, more research is needed using common foods.

Effect of energy content on TEF

TEF is the energy expended in digestion, absorption and storage of nutrients in the body. It is pertinent to assume that the magnitude of TEF will increase as the amount of food to be processed increases. However, this is not the case since different studies have provided widely different observations. While some investigations have shown that TEF is directly related to the energy content of the ingested meal (Wanchholder and Franz, 1944; Stock, 1966; Swindells, 1972; Morgan *et al.*, 1982; Hill *et al.*, 1984; Karst *et al.*, 1984; Belko *et al.*, 1986), others have failed to establish any relation between TEF and energy content of a meal (Glickman *et al.*, 1948; Bradfield and Jordan , 1973; Bray *et al.*, 1974). It should however be noted that, in most of these studies, the number of subjects have ranged from two (Stock, 1966) to 10 (Swindells, 1972) and it can be expected that with such small numbers of subjects, small variations between subjects may significantly influence the final results and hence the interpretation.

Hill *et al.* (1984) demonstrated that by progressively increasing the energy content of a meal (500-1500 kcal), TEF was found to increase systematically, but not in a linear fashion. Their results also showed that, as the energy content of the meal increased, the magnitude and duration of TEF also increased, but unfortunately, the duration of measurement was not extended for long enough time to allow for metabolic rate after each meal to return to the basal level. Since the portion of TEF measured after each meal did not represent total TEF, the nonlinear relationship observed may therefore be not surprising. It is probable that the relationship could have been linear if total TEF was measured. Studies that have measured total TEF have not been reported.

Although TEF has been shown to vary with energy content of a meal, the overall energy balance, however, will depend on the relationship between energy intake and energy expenditure. If energy expenditure is less than energy intake, whatever level of TEF is attained due to meal size , the overall energy balance will be positive, suggestive of weight gain. Positive energy balance is inevitable whenever high TEF due to large meal is not coupled with increased energy expenditure through physical activity.

Apart from acute effects of energy content of a meal on metabolic rate, energy content of a meal has a long term effect on BMR. Overfeeding studies (Dalloso *et al.*, 1982; Schultz *et al.*, 1985) have shown that excess energy intake (overfeeding) affects metabolic rate for a prolonged period of up to 24 h.

Studies to determine the optimum combination level of energy intake and physical activity are needed. Such studies are valuable in providing data for weight control programmes.

Effect of meal frequency on TEF

Meal frequency has a considerable effect on the metabolism and body composition of experimental animals (Fabry and Tapperman, 1974; Adams *et al.*, 1981). However, the evidence in humans is unclear. The effect of meal frequency in humans has been examined extensively in relation to weight reduction and nutrient utilisation.

Meal frequency and weight changes

It is unclear whether meal frequency has a significant influence on body weight changes. While some studies have reported that eating more than four meals per day (nibbling) causes weight loss (Gwinup *et al.*, 1963; Seaton and Duncan, 1964; Young *et al.*, 1971 ; Dalloso *et al.*, 1982), and eating less than three meals per day (gorging) causes weight gain (Fabry *et al.*, 1964; Fabry, 1967), others have failed to establish any significant relationship between meal frequency and body weight changes (Bortz, *et al.*, 1966; Swindells *et al.*, 1968; Garrow *et al.*, 1981). The hypothesis that meal frequency has a significant influence on weight changes seem to suggest that individuals who eat less than three meals per day stimulate more lipogenesis than those who eat more than four meals per day. This is suprising because the tendency to gain weight depends on the relationship between energy intake and energy expenditure. If energy intake exceeds energy expenditure there will be a positive energy balance, which in physical terms it will appear as weight gain. It is unfortunate that in most of these studies metabolic rate was

not measured. This was neccessary so as to establish the level of energy intake for each participant. It is probable that some individuals had high levels of energy expenditure and that the amount of food which was administered was inadequate for them to maintain energy balance. Since the levels of activity in these studies were not controlled, this could have contributed to descripancies between different studies.

Plasma lipid metabolism

The study by Fabry *et al.* (1964) on 1359 men (age 60-64) showed that men who consumed less than three meals per day had higher levels of cholesterol and phospholipids than those who consumed more than five meals per day. Other studies have also reported similar observations (Gwinup et al., 1963; Bortz *et al.*, 1966; Irwin and Feeley, 1967; Young *et al.*, 1971, 1972; Peters *et al.*, 1979). These results suggested that the high cholesterol level associated with less frequent meal pattern was a predisposing factor to the development of cardiovascular diseases particularly ischaemic heart disease. However, other studies (Okey, *et al.*, 1960; Finkelstein and Fryer 1979) have shown that meal frequency had no significant influence on serum lipid levels and thus, ischaemic heart disease.

Glucose tolerance

It has been shown (Fabry *et al.*, 1964; Young *et al.*, 1972) that glucose intolerance was common among individuals who consumed three or less meals per day than amoung those who consumed more than five meals per day. Peters *et al.* (1979) found that blood glucose level was significantly lower after a more frequent feeding pattern (more than four meals per day) than after a less frequent pattern. Moreover, studies have shown (Fabry *et al.*, 1964; Young *et al.*, 1972; Peters *et al.*, 1979) that individuals who consume small number of meals per day tend to ingest more energy per meal than those who eat more often. Since plasma insulin response is directly related to the amount of energy to be processed, it is pertinent to assume that individuals who consume less than three meals per day will have more insulin released per meal than those who eat small amounts of

food more often. This could imply that less frequent meal pattern may increase the chances for the onset of diabetes especially for those predisposed to the disease.

Energy expenditure

Studies on the effect of meal frequency on energy expenditure are few. However, meal frequency has been shown to have no significant effect on BMR (Garrow *et al.*, 1981), 10 h oxygen consumption (Belko and Barbieri, 1987), 24 h energy expenditure (Dalloso *et al.*, 1982) and 21 days energy expenditure (Swindells, 1968). These results suggest that TEF is not influenced by meal frequency, and therefore, do not support the hypothesis that eating large number of meals stimulate more thermogenesis and may aid in weight reduction programmes.

Effect of exercise on TEF

Although the effect of exercise on TEF has been extensively studied, its influence on TEF is far from clear. While several investigations have shown that TEF is potentiated by exercise (Miller *et al.*, 1967; Bray, 1977; Miller and Wise, 1975; Whipp *et al.*, 1976; Zahorska, 1980; Stock, 1980; Hill *et al.*, 1984; Young *et al.*, 1987), others have observed no significant influence of exercise on TEF (Dalloso *et al.*, 1982; Welle, 1984; Belko, *et al.*, 1986; Garby *et.al.*, 1987). Moreover, it has been reported that exercise has a prolonged effect on metabolic rate (Passmore and Johnstone, 1960; Bielisnki *et al.*, 1985; Shah *et al.*, 1988) but this has not been confirmed in other studies (Hammernsen *et al.*, 1984; Pacy *et al.*, 1985; Akabas *et al.*, 1985; Bingham *et al.*, 1989).

It has been suggested that the type of exercise employed may partly account for the different results that have been reported from various studies. The types of exercise that have been used include step test (Miller *et al.*, 1967; Stock, 1980), cycling (bicycle ergometer) (Bray *et al.*, 1974; Zahorska, 1980; Welle, 1980; Dalloso, 1980) and walking or running on a treadmill (Swindells, 1972; Belko, *et al.*, 1986). For all these different types of exercises, choice of subjects is important because it has been shown (Passmore

and Durnin, 1955) that considerable differences do exist depending on the experience of the subject in performing a specific exercise. It is thus probable that the variation in results between laboratories may be due to subject variability in exercise performance.

That exercise potentiates TEF is surprising because during exercise, various changes such as blood flow and gastric secretion and emptying are markedly affected. Blood flow to the splanchnic bed accounts for about 25 percent of total blood distributed to the body, and that to the muscles is about 20 percent. Under resting conditions, gastric mucosal blood flow increases markedly after ingestion of a meal (Davenport, 1977). Intestinal blood flow also increases after food intake and remains elevated by about 30 percent above normal for several hours. The mechanism is unclear, but it is probably partly mediated by hormones and it is possible that the elevated intestinal metabolism associated with secretion, absorption and motility may also increase blood flow via accumulation of vasodilator metabolites (Davenport, 1977). During exercise, oxygen demand to the exercising muscles increases. These changes demand intergrated response of the cardiovascular system to effect redistribution of blood. Blood flow to the exercising muscles increases by about 200-300 percent during moderate exercise. This increase is due to arteriolar dilatation in the exercising muscles. At the same time, vasoconstriction occurs in the splanchnic and renal vascular beds. The decline in blood flow that occurs which in turn determines stomach activity, is usually proportional to the severity of the exercise (Hellenbrandt *et al.*, 1934)).

Gastric emptying is prolonged by all types of exercise. The more severe the exercise, the greater the delay in emptying and in completing the digestive cycle (Hellenbrandt *et al.*, 1934).

The function of the gastrointestinal secretion and absorption is achieved through adequate blood flow in the gastrointestinal tract. If blood to the gastrointestinal tract is curtailed as in exercise, these processes are markedly reduced (Vander *et al.*, 1987), thus reducing digestion and absorption processes. This has also been observed during period of recovery from exercise (Hellenbrandt and Tapper, 1934).

Effect of body composition on TEF

Obesity is a manifestation of chronic energy imbalance. It arises when energy intake is greater than energy expenditure, which leads to positive energy balance. However, it is not clear whether this imbalance results from an excessive energy intake (gluttony and sloth) and/or defective energy expenditure (Garrow, 1981; Jequier and Schutz, 1985). It is likely that both mechanisms play a role. While hyperphagia is regarded as the primary cause of obesity in some individuals, a defective energy expenditure mechanism may contribute to weight gain in other individuals. Garrow, (1981) and Felig *et al.*, (1983) have shown that the level of energy intake of some obese individuals is not different from that of lean individuals. It is now generally recognized that obesity is probably a heterogeneous disorder. Broadly, it has been considered a disorder of energy balance but there is increasing evidence that development of obesity in humans is of complex and multifactorial aetiology, involving genetic and environmental components that affect regulatory and metabolic events.

Because of the increased fat free mass which accompanies the large adipose tissue in obesity, obese subjects generally have a more elevated BMR and energy cost of physical activity, particularly in those activities involving movement of the whole body such as walking and climbing stairs (Jequier, 1987) compared to lean subjects. The TEF, however, has been found in some studies (Table 3.) to be lower in obese than in lean subjects. But some other studies (Table 4.) have observed no significant differences in TEF between obese and lean subjects. The reasons for the conflicting results are unclear. Furthermore, obesity is considered to be a heterogeneous disorder, and therefore the type of subjects and the number of subjects studied could have contributed to the discrepancy in results between different studies. For instance, in most of these studies (Table 3 and 4) sample sizes have ranged from four subjects to ten subjects.

Several mechanisms have been suggested as possible causes for low TEF in obese individuals. These include, insulin resistance (Ravussin *et al.*, 1983) and blunted thermogenic response to noradrenalin (Ravussin *et al.*, 1985; Bazelman *et al.*, 1985).

Table 3. Studies which have shown that TEF is lower in obese than in non-obese subjects

Meal composition			Energy			
P	CHO	Fat	content	Duration	Sample	
%	%	%	kcal	h	size	Reference
-	100	-	200	2.5	11	Pittet *et al.*, 1976
78	16	3	800	5	4	Kaplan, 1976
-	100	-	400	3	55	Golay, 1982
14	46	40	900	4	-	Segal, 1983
15	85	-	800	5	6	Schwartz, 1983
17	54	29	1200	5	6	Bessard, 1983
-	100	-	400	3	-	Jequier, 1984
100	-	-	400	1	-	Noack, 1985
-	-	100	400	2.5	6	Swaminathan, 1985
12	55	33	400	2.5	6	Swaminathan, 1985
100	-	-	200	10	17	Steiniger *et al*,1987
24	38	38	*	5	11	Molnar *et al.*, 1985
15	45	40	*	14	20	Schutz *et al.*, 1984

P=Protein; CHO=Carbohydrate

* Calculated according to body weight.

Table 4. Studies which have shown no differences in TEF between obese and lean subjects

Meal composition			Energy			
P	CHO	Fat	content	Duration		
%	%	%	Kcal	h	n	Reference
-	-	-	200	-	-	Bradfield, 1973
-	-	-	100	3	-	Zahorska, 1980
22	47	31	575	2		Shetty, 1981
100	-	-	300	2.5	10	Nair, 1982
-	100	-	300	2.5	10	Nair, 1982
-	-	100	300	2.5	10	Nair, 1982
-	100	-	1200	3	11	Sharief, 1982
-	100	-	350	3	11	Sharief, 1982
-	100	-	400	3	13	Welle, 1983
14	46	40	900	4	10	Segal, 1983
15	45	40	800	3	10	Felig, 1983
not specified			800	-	10	Blaza, 1983
17	58	25	1200	6	-	Scalfi, 1985
100	-	-	400	2.5	22	Swaminathan, 1985
-	100	-	400	2.5	22	Swaminathan, 1985
Ensure			800	3.5	10	Kush *et al.*, 1986

P=Protein; CHO=Carbohydrate

Insulin resistance

Ravussin *et al.* (1983) have shown that the defect in the thermic effect of infused glucose in obese subjects was due to decreased tissue sensitivity to insulin. Consequently, resulting in lower rate of glucose uptake by the cells. Because glucose storage as muscle glycogen is an energy requiring process, impairment of glucose storage due to insulin resistance in obese subjects may help to explain at least in part why some obese subjects have low TEF. However, since the action of insulin in glucose storage is indirect (through activation of enzymes), it is possible that insulin resistance observed in some obese subjects may partly be due to reduced enzyme production. This hypothesis is supported by an observation that exogenous insulin does not suppress hepatic glucose output in some obese subjects (Caballero, 1987). At present, the role of enzyme activity in TEF and obesity is not known.

Response to noradrenalin

When noradrenalin was infused into lean and obese subjects, obese and post-obese subjects had a lower thermogenic response to noradrenalin than lean subjects (Jung *et al.*, 1979). However, Ravussin *et al.* (1985) showed that beta- adrenergic blockade with propanolol gave similar effect in obese as well as in lean subjects, which may seem to suggest that the sympathetic nervous system responsiveness is not blunted in obesity. Moreover, total body clearence of noradrenalin did not show any significant differences between obese and lean Pima indians (Kush *et al.*, 1986).

When noradrenalin responsiveness was studied (Bazelmans *et al.,* 1985) in relation to overfeeding and underfeeding in obese subjects, it was observed that noradrenalin turnover did not change in response to overfeeding and underfeeding. Failure to observe any significant changes in the levels of noradrenalin during overfeeding and underfeeding suggest that the sympathetic nervous system is probably blunted in obese subjects, since in normal subjects both underfeeding and overfeeding

produce marked changes in the levels of noradrenalin. The role of sympathetic nervous system in regulating TEF needs to be elucidated.

2.6 MEASUREMENT OF ENERGY EXPENDITURE

Measurements of human energy expenditure is applied in many areas of clinical and research investigation such obesity, undernutrition, exercise, and a number of disease states such as trauma, infection and cancer.

There are many methods available for measuring energy expenditure in humans under resting and active conditions. Each has its advantages and disadvantages with regard to accuracy, complexity, versatility, availability and cost (Garrow, 1978; Jequier, 1981; Segal, 1987). Both direct and indirect calorimetry have been applied to assess metabolic rate and short- and long term energy balance.

Direct calorimetry

The work of Lavoisier and Laplace (1783) and also that of Atwater and Benedict (1903) is accredited for the development of direct calorimetry as we know today. Lavoisier and Laplace measured the heat dissipated by the animal enclosed in a adiabatic double walled chamber containing ice. The amount of heat dissipated by the animal was assessed by measuring the amount of melted ice. The work of Atwater and Benedict was based on the isothermal principle. The heat dissipated by the subject raised the temperature of water flowing through the calorimeter. However, in both cases the amount of heat dissipated was calculated as a product of temperature difference between the inflow and outflow and the rate of flow of the cooling medium.

The development of more sensitive heat sensing devices and more precise techniques has allowed accurate measurement of the heat losses by man (Webb, 1985). Currently, three types of calorimeters are used to assess heat loss in man. These are,

isothermal calorimeter as developed by Atwater and Benedict (1903), the gradient layer (Benzinger and Kitzinger, 1949) and water cooled garment (Webb *et al.*, 1972).

The method of direct calorimetry measures the amount of heat dissipated by the body in the form of radiation, convection, conduction and evaporation. The heat released in this way is regarded as representing the total heat released by metabolism in the body. Direct calorimetry has the advantage over the indirect method in that it is more accurate and it can be used to measure rates of heat exchange for a long period of time (up to 24 h) without causing much discomfort to the participating individuals. However, because of the delayed response between heat production and heat loss, due to the body's capacity to store heat energy, direct calorimetry is inappropriate for short term measurements of heat exchange, for example in the assessment of TEF and effect of exercise (Jequier, 1987). Although the method is considered accurate, it cannot be used to measure energy expenditure in a large sample of free living individuals . Moreover, the equipment is complex and expensive to construct.

Indirect calorimetry

In indirect calorimetry, heat production or metabolic rate is determined from measurements of oxygen consumption and carbon dioxide production. When the amount of excreted nitrogen is also known information on the type and rate of fuel oxidation within the body can be calculated (Frayn, 1983; Jequier and Felber, 1988). However, a steady state of carbon dioxide production and respiratory exchange must be reached and the subjects should have normal acid-base balance. When these conditions are achieved indirect calorimetry can have an accuracy within 2-5 percent for energy expenditure (Garrow, 1978; Jequier and Felber, 1988; Blaxter, 1989).

Indirect calorimetry or respiratory calorimetry is based on the principle that during oxidation of organic molecules in the body, oxygen is consumed in amounts related to the energy or heat liberated. For each litre of oxygen consumed there is a known amount of heat which is being liberated by the body . Nevertheless, the amount

of heat liberated per litre of oxygen consumed is not constant, it varies depending on the proportions of carbohydrate, fat and protein being oxidised. Measurement of oxygen consumption, therefore, provides an estimation of the amount of heat liberated by the body, and thus, metabolic rate.

Unlike the direct calorimetry, indirect calorimetry has a short response time. This is attributed to the fact that the body has a low capacity to store oxygen and anaerobic production of ATP is limited. Because of its flexibility, versatility and short response time, indirect calorimetry is widely used in the assessment of short term effects of exercise and food on metabolic rate.

In indirect calorimetry, two techniques are used. These include closed-circuit indirect calorimetry and open- circuit indirect calorimetry

Closed-circuit indirect calorimetry

In this technique the subject is made to breath through a closed system containing pure oxygen. The expired air is passed through soda lime where carbon dioxide is removed and the expired oxygen is passed back to the respirometer. The difference in volume of oxygen before and after a known period of time is a measure of oxygen consumption. By using appropriate conversion factors metabolic rate (kJ/min) of the subject can be estimated.

Open-circuit indirect calorimetry

Subjects inspire atmospheric air and the expired air is collected in a bag and then analysed for oxygen and carbon dioxide content using specially designed gas analysers. Development of the computerised systems for measuring oxygen consumption has made it possible to have an 'on line' gas analysis for example in the ventilated hood system or the respiration chamber. There is a wide variety of apparatus being used in the study of oxygen consumption. They range from respiration chambers (Jequier, 1987) to respirometers (Douglas, 1911) (Chapter 3).

The respiration chamber as described by Jequier and Schutz (1983) is an air tight room which forms an open circuit ventilated indirect calorimetry. Outside air is continously drawn in to the chamber and the flow rate of air at the outlet is measured using a pneumotachograph with a differential manometer. A fraction of the extracted air is continously analysed for oxygen and carbon dioxide concentrations. In respiration chambers subjects have room to sleep, eat and do exercise; therefore it is possible to measure energy expenditure over a long period of several hours to few days without causing discomfort to the subject. The respiration chamber is considered to be the most accurate open circuit indirect calorimetry method. However, the disadvantage with this method is the artificial conditions of living in a closed environment. Its influence on metabolic rate of the individual is yet to be established.

On the other hand, the respirometers are used to collect expired air via tight fitting face mask or mouth piece. These may be uncomfortable but they have been shown to be tolerated by a wide range of subjects (Segal, 1987). When used properly they give results comparable to those obtained using either direct or respiratory chamber methods. Another method is that which use the ventilated hood system first described by Benedict (1930). The principle of the ventilated hood system is that a stream of air is forced in to a transparent hood placed over the head of the subject and made air tight at the neck. The rate of metabolism can be determined by measuring the amount of air flowing through the hood and by measuring oxygen and carbon dioxide concentration in the inflow and

outflow . The ventilated hood system is comfortable and it can be used to measure energy expenditure in patients without causing much discomfort to the subject (Segal, 1987).

The choice of a particular system, however, would depend on it complexity, availability, cost, the accuracy to be achieved and application. For instance, the Douglas bag cannot be used to measure energy expenditure in patients or young children but it is an appropriate piece of apparatus when used in normal health individuals (Segal, 1987).

Non-calorimetric methods

There are a variety of non-calorimetric methods for estimating energy expenditure in man. These include techniques based on physiological measurements (such as heart rate and pulmonary ventilation), human observation and recording methods (time and motion studies and activity diaries), and kinematic recordings such as radar and mechanical activity meters. The errors inherent in these methods are too great to permit accurate measurement of energy expenditure.

The prediction of energy expenditure from heart rate depends largely on individually determined regression lines. The relation between the two variables is not linear within the usual range of measurements (80 and 110 beats per min), this is primarily due to the variations in stroke volume. It has been shown (Warnold and Lerner, 1977) that during vigorous exercise there is a high correlation between heart rate and metabolic rate, but in resting conditions the heart may vary between 60 and 80 beats per min without a significant change in metabolic rate. Thus it is necessary to make two calibration lines for each subject. The study by Dauncey and James, (1979) showed that when heart rate method was compared with the respiration chamber, the average error of the heart rate method was 3 (SD 10) percent at a light level of activity and 3 (SD 7) percent at moderate levels of activity. These large standard deviations suggest that there are wide variations between individuals, and because of this prediction of energy expenditure from heart rate is uncertain.

Activity diary and kinematic recording methods (minute by minute account of activities) are based on the principle that if the energy cost of various activities which make up the day of the subject are known, then by recording the amount of time spent in each activity, the number of calories for each activity and total energy expenditure per day can computed. It has been observed (Edholm *et al.*, 1955) that wide inter- as well as intra-individual variations in energy expenditure exist and the error could be up to 20 percent. This may have a significant consequence especially when estimating energy requirements for different individuals. Since prediction of energy expenditure using the activity diary method is based on values determined in the laboratory while the subject is performing the task (Edholm *et al.*, 1955; Passmore and Durnin, 1955), it is probable that these values may not represent the values for energy expenditure in free living conditions. However, they are useful methods to obtain an index of physical activity.

Currently, a simple and non intrusive method has been used to measure energy expenditure in free living individuals. The double isotopically labelled water ($^{2}H_2$ ^{18}O) has shown some prospects (Schoeller and Webb, 1984; Schoeller *et al.*, 1986). Although this method does permit individuals to continue with their normal day-to-day activities, it is expensive and requires access to a specialised mass spectrometer for analysis of the samples. The method is based on the observation made by Lifson (1949) that the oxygen of the respiratory carbon dioxide mixes freely with the oxygen of body water. Therefore by measuring carbon dioxide production, energy expenditure can be estimated. However, the technique is based on a number of inherent assumptions:

1. The body water volume is constant.
2. Rates of water flux and carbon dioxide production are constant.
3. Isotopes label only water and carbon dioxide in the body and that they leave the body as water and carbon dioxide.
4. The isotopic enrichment in water and carbon dioxide leaving the body are the same as in the body water.
5. Water or carbon dioxide do not enter the animal across the skin or lung surfaces.

Validation studies have shown (Klein *et al.*, 1984; Schoeller and Webb, 1984; Tatner and Bryant, 1989) that the mean discrepancy between the traditional methods is generally less than 10 percent.

The longer experiment period of about 5 to 14 days and relatively easy way of sample collection have encouraged wide application of the technique. However, more research is required to establish the validity of the various assumption stated above (Woo *et al.*, 1985; Jequier *et al.* 1987), particularly those related to the effects of fractionation and compartmentation of the isotopes .

CHAPTER 3

SUBJECTS AND GENERAL METHODOLOGY

3.1 SUBJECTS

Eighty three female subjects participated in these studies. Subjects were recruited by posters and through informal talks. Forty three of the subjects were either students or workers at Glasgow university. Forty others were recruited from the general public around the Glasgow area.

The details of the study were explained to them before being asked to give their consent to participate. Only those who were healthy (personal declaration), non-smoking and taking no medication were recruited.

The physical characteristics of the subjects were assessed by anthropometry and body fat content by skinfold measurements and underwater weighing.

Weight

The weight of the subjects was recorded using a Weylux electronic digital weighing scale (Weylux model 824/890, UK.), while the subjects were in a fasting state and after emptying the bladder. The weighing was carried out while the subject was wearing a pre-weighed light indoor clothing. Weight was recorded to the nearest 0.1 kg . The difference between the recorded weight and the weight of the clothing was taken as representing the weight of the subject. Weight was recorded on each test day and the average of the measurements recorded on different days was taken as representing the weight of the subject at the time of the study.

Height

Height was measured using a wall stadiometer (Holtain Ltd. Grymych, Dyfed,UK). The subject (without shoes) stood with heels together on a horizontal plane (floor) near the stadiometer. The back of the subject was straight against the vertical bar of the stadiometer and the frankfurt plane of the head in the horizontal position. The head-

bar was then brought down gently on to the head and the reading was recorded to the nearest centimetre.

Body circumferences

Body circumferences were measured using a flexible steel tape (Harpenden Anthropometry tape (2m) Holtain Ltd. Grymch, Dyfed, UK) while the subject was standing. The tape was placed firmly around the position of measurement and the reading was recorded to the nearest centimetre. Circumference measurements were made at six positions as follows:

Breast: This was taken at the widest circumference around the breast area.

Upper arm circumference: This was measured midway between the inferior border of the acromion process and the tip of olecranon process while the arm was hanging slightly away from the subject's side.

Waist: Minimum circumference at the level of the waist narrowing as seen from the front.

Buttock: Maximum circumference over the buttocks.

Thigh: at the level of the gluteal fold.

Calf: This was measured at the maximum circumference of the calf muscle while the subject was in a sitting position with the leg bent 90^0 at the knee.

Measurement of body fat content

The composition of the human body has physiological, medical and nutritional importance. The distinction between the relatively inert components (fat) of the body and those that are metabolically active (fat free mass) is useful in the interpretation of energy metabolism investigation and for making inferences as to the nutritional (energy) status of the subjects.

The current knowledge of the composition of the human body is based on the studies performed in the fifties and early sixties on human cadavers (Widdowson *et al.*, 1951; Forbes *et al.* 1956;). In these studies the density of human cadavers was determined and the body was chemically analysed for fat content. Formulas were developed to convert density values to percent fat. These formulas formed the basis of all the indirect methods used to determine body composition.

Indirect methods

Most of the indirect methods are based on the assumption that the body is divided in to two distinct components, fat mass and fat free mass. Body fat is considered to be anhydrous, to contain no potassium and to have a constant density of about 0.90 x 10^3 kg/m^3. The fat free mass is considered to have a constant density of 1.10 x 10^3 kg/m^3 (Keys and Brozek, 1953) and a water content of 73 percent.

Various indirect techniques are used to determine body composition. These include, measurement of total body water (Halliday and Miller, 1977), total body potassium (Burch and Spiers, 1953), skinfolds (Durnin and Womersley, 1974), density by underwater weighing (Behnke *et al.,* 1942; Siri, 1961; Durnin and Rahaman, 1967). Other methods include computerised tomography, neutron activation analysis and total body calcium. In the present study two techniques were used to assess body fat content. These were skinfold measurements and underwater weighing and these are described in detail.

Skinfolds measurement

It has been suggested that about 50 percent of the store of body fat in humans is located just beneath the skin (subcutaneous fat). However, the exact proportions of internal and subcutaneous fat to total body fat content are not known. This may partly be attributed to the difficulty associated with the assessment of body fat content in humans.Nevertheless, measurement of skinfold thickness at various sites of the body has been shown to give a good indication of the amount of subcutaneous fat and total body fat

(Edwards, 1950; Tanner, 1965). Various sites ranging from single skinfold over the triceps (Seltzer and Mayer, 1965) to 15 sites (Strakova and Markova, 1971) have been used . Probably the most established is that of Durnin and Womersley (1974) using 4 sites: biceps, triceps, subscapular and suprailiac. This is because subcutaneous fat layer varies from place to place and the distribution is not the same in different individuals or in the same individual at different ages. Equations for predicting body fat or body density from a combination of measurement of skinfolds at different sites have been developed (Brozek and Keys, 1951; Keys and Brozek, 1953; Durnin and Womersley 1974). Durnin and Womersley (1974) have developed regression equations according to age and sex which to some extent compensate for age and sex related differences in fat distribution in the body.

In comparison with other techniques, skinfold measurement is the easiest to perform and the cheapest. And it does not need a great deal of practice to learn to measure skinfold accurately even in more inexperienced observers differences of only 3 percent can be observed.(Durnin, *et. al.*, 1971) Although there may be statistically significant differences in the total skinfolds between observers, the amount of the difference has been shown to have no significant effect on the prediction of total body fat content in the subject (Durnin *et al.*, 1971). Also estimation of body fat by skinfold measurements has been shown (Garrow, 1978) to underestimate the amount of fat in obese subjects. This is probably attributed to the higher proportion of internal fat in obese subjects than in lean subjects. Skinfold measurement can be used successfully in screening a large group of subjects to identify those who may be grossly lean and also in identifying individuals who need immediate energy supplementation especially in famine situations.

In the studies presented in this thesis skinfold thicknesses of the subjects were measured using a Harpenden skinfold caliper (Holtain Ltd. Grymych, Dyfed, UK) at four sites namely, biceps, triceps, subscapular and suprailiac regions. Measurements were taken on the right hand side of the body of the subject while standing in a relaxed fashion.

Biceps: A vertical skinfold on the front of the arm directly above the centre of the cubital fossa at the level of the mid-point of the muscle belly while the arm was hanging vertically..

Triceps: A mark was made at the back of the arm, half way between the inferior border of the acromion process and the tip of the olecranon process and directly in line with the elbow and acromion process.using a metallic tape (Harpenden Anthropometric tape (2m) Holtain Ltd, Grymych, Dyfed, UK). A vertical skinfold was measured at this mark.

Subscapular: Below the tip of the inferior angle of the right scapular, at 45^0 to the vertical.

Suprailiac: A vertical skinfold above the anterior superior iliac spine in the mid-axillary line of the abdomen.

At the site where the measurement was to be made, the skin was pinched firmly between the thumb and the forefinger and pulled up slightly away from the underlying muscle tissue. The caliper was then placed at the site, the finger and thumb removed, and quickly the measurement was recorded to the nearest millimetre. Measurements were taken in triplicate by the same observer and the mean of the three measurements represented the skinfold thickness of a particular site. Body fat content was calculated using regression equations developed by Durnin and Womersley (1974).

Determination of body density by underwater weighing

This technique employs the Archimedes principle which basically states that when an object is immersed in water it displaces the amount of water equal to its volume. It is also based on the fact that fat floats in water because its density is less than the density of water. Determination of body density requires the measurement of body volume. This is achieved by weighing the subject on an accurate scale in air and then weighing the subject while totally submerged in water. Since the body submerged in water is pushed up by a force equal to the volume of the water it displaces, the volume of water displaced by the subject is a measure of body volume and is obtained by subtracting weight of the subject in water from that recorded in air as shown below:-

$$\text{Volume of the body} = \frac{\text{weight in air - weight in water}}{\text{density of water}}$$

In this study body density of the subjects was determined by the method described by Durnin and Rahaman (1967) and Durnin and Womersley (1974)..

The equipment for measuring body density by underwater weighing included:

a) A tank (1.38m x 1.19m x 1.19m) with a capacity to hold up to 1900 litres of water.

b) A chair (0.76m x 0.51m and height of 0.86m), connected by hanging nylon strings to an electronic load cell (Western Load Cell Co. Ltd. Scotland) which was in turn connected to a digital display unit.

c) A 4 litre anaesthetic bag, mouth piece and nose clip.

d) Pure oxygen (99.2 percent).

Underwater weighing: After weighing the subject in air, the subject was asked to wear a swimming suit and then to sit down on a chair placed in a tank containing warm (36.5 ^{O}C) water. Subjects were asked to hold on to the sides of the chair with their hands and rest their feet on the cross bar just below the chair.(Fig. 6).The whole procedure was explained to the subject while she was in a sitting position. After being satisfied that the subject was confident and relaxed, a nose clip was fitted and the following procedure was used :

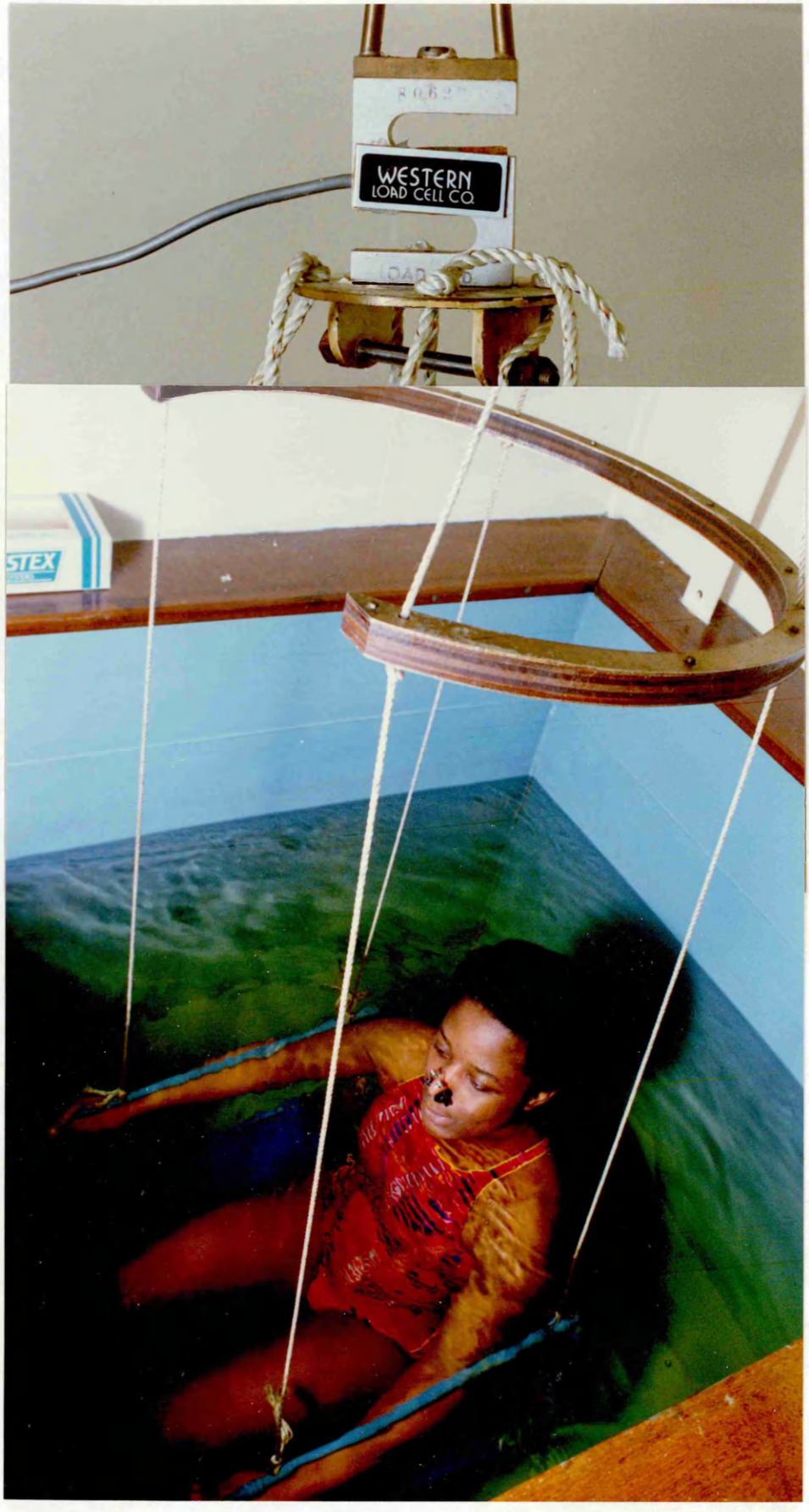


Fig. 6. Equipment for the determination of body density by underwater weighing. With the subject sitting on a weighing chair , her head just above the level of the water.

1. The subject was asked to take full expiration.

2. While holding breath and mouth firmly closed, the subject gently bent forward and lowered her head until it was completely immersed in water (Fig. 7). This position was maintained, keeping the chair as steady as possible to allow the reading on the digital display unit to stabilise (about 10-15 seconds) and immediately underwater weight was recorded.

Determination of residual lung volume: Residual lung volume is the volume of air left in the lungs after a maximum expiration. When using the underwater weighing technique to estimate body fat it is obligatory that the value of underwater weight has to be corrected for the air remaining in the lungs at the moment of measurement. Residual lung volume can be determined by various methods. Those commonly used include the helium dilution (Siri, 1956) or the nitrogen wash-out (Durnin and Rahaman, 1967; Durnin and Womersley, 1974) techniques. In this study residual lung volume was determined by the nitrogen wash-out technique.

Because it is usually not possible to measure residual lung air while the subject is still immersed in water, to minimise this error subjects were asked to make a maximum expiration just before immersion and to hold breath while their weight was being recorded. Immediately after the underwater weight has been recorded as described above a signal was given and the subject surfaced. A mouth piece attached to one limb of a three way tap was then placed in between the lips. Another end of the tap was connected to an anaesthetic rubber bag containing pure oxygen. The subject held the breath until the lips were tightly over the mouth piece. The tap was then opened and the subject breathed in and out of the bag three times thereby allowing the nitrogen in the lungs to mix with the oxygen in the bag. At the end of the third expiration the tap was closed. The expired air in the bag was then analysed for the oxygen and carbon dioxide as described later. The amount of nitrogen in the bag was used to compute residual lung volume. Residual lung volume and body density were calculated using a computer programme based on the method of Durnin and Rahaman (1967) and that of Durnin and Womersley (1974).

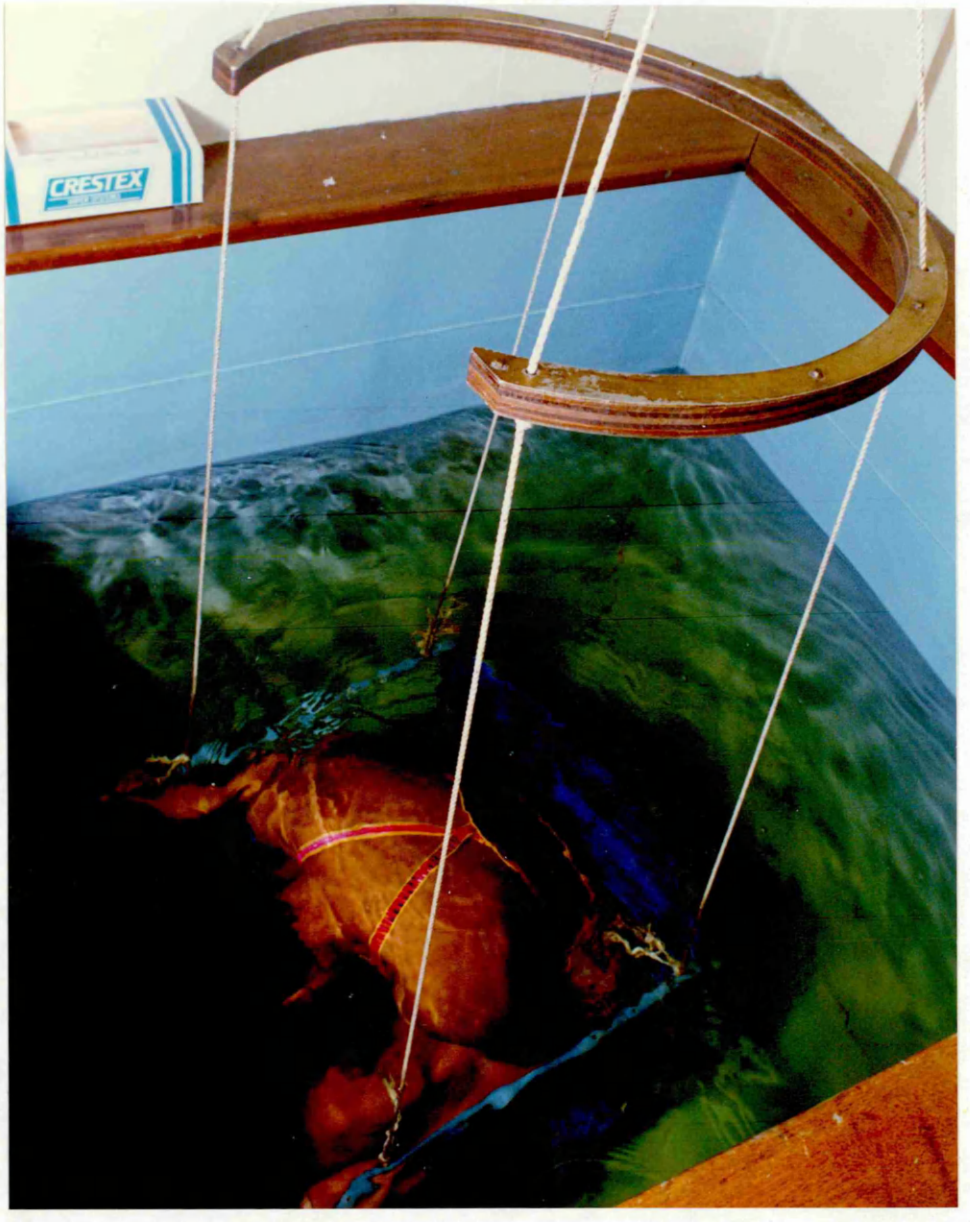


Fig. 7. Underwater weighing, showing the subject's head completely submerged under water.

$$\text{Body density} = \frac{\text{Weight in air}}{\text{Body volume - Residual volume}}$$

Body fat content was estimated using Siri's (1956) equation :-

Percent fat= (4.95/D-4.50)100,

or

kg fat= 4.95/D-4.50W

where D= Body density, and W = weight of the subject in air. Fat free mass was calculated as a difference between weight of subject in air and weight of fat.

For each subject two measurements were made. But occasionally if the difference between the two measurements was not within 3 percent , a third measurement was made.

Estimation of body fat by underwater weighing is considered to be more accurate than by skinfold measurement. This is because it takes into account both the subcutaneous and internal fat deposits. However, it has some disadvantage, it requires greater cooperation by the participating subject and it is complicated by the determination of residual lung volume.

3.2 METHODS

Determination of metabolic rate

In all the studies presented in this thesis, metabolic rate of the subjects before and after ingestion of a meal was determined by open circuit indirect calorimetry using the Douglas bag technique (1911). In this technique subjects breath in atmospheric air but the expired air is collected in a bag for subsequent gaseous analysis and metabolic rate calculations. It has the advantage over the other methods in that it is portable, flexible and also the subject can make the necessary adjustments without external help. However, in the studies described in this thesis all manipulations on the equipment were carried out by the observer.

The equipment for the Douglas bag technique included:-

1) Douglas bag (Cranlea & Co. Birmingham, UK), which is a large non-diffusable plastic bag supplied as of 100 or 200 litres capacity.

2) Flexible corrugated plastic tubing (length 122 cm, ID 2.86 cm) (Cranlea & Co. Birmingham, UK)

3) Rudolph low resistance two way valve No. 1400 (Kansas city MO. U.S.A.).

4) Three way aluminium tap.

5) Mouthpiece, noseclip and stopwatch.

6) A paramagnetic oxygen analyser (Servomex type 570 SYBRON, Servomex Ltd., Crowborough, Sussex, England)

7) Infrared carbon dioxide analyser (PK Morgan Ltd., Chatham, Kent, England)

8) Gas meter (Parkinson-Cowan Ltd., London, England) calibrated at 50-150 l/min.

9) A mixture of 4.05 percent CO_2 : 16.30 percent O_2

10) A mixture of 6.06 percent CO_2 : 15.62 percent O_2

11) Oxygen-free nitrogen.

The standard gas mixtures were supplied by British Oxygen Co. Ltd., Great Westhouse, Brentford, England.

Calibration of the gas analysers: Oxygen and carbon dioxide analysers were calibarted on each test day prior to the start of the experiment. They were first set at zero by introducing oxygen free nitrogen and then calibrated using standard gas mixtures containing either 4.05 percent CO_2 : 16.30 percent O_2 or 6.06 percent CO_2 : 15.62 percent O_2 (tested by Schollender). The span of the oxygen analyser was set at 20.93 percent using fresh dried atmospheric air. Oxygen free nitrogen was introduced again to reset the analysers at zero.

Collection of expired air

The subject was fitted with a nose clip and breathed through a mouthpiece connected to a two way Rudolph low resistance valve. The subject inspired atmospheric air and the expired air was directed through flexible plastic tubing to the Douglas bag (100 litres capacity) (Fig.8). Interposed between the bag and one end of the plastic tubing was a three way aluminium tap. This was used to allow the subject to breath either into the bag or to the atmosphere. During gas collection, the subject was allowed 3-5 min to be in respiratory equilibrium under the experimental condition before actual gas collection. This was also intended to make the subject relax and get used to breathing through the apparatus. After the initial equilibration time, the three way alluminium tap was then turned so that the expired air was collected in the bag for specified time. The tap was then closed , bag disconnected from the breathing system and taken away for gaseous analysis.

Analysis of expired air

A sample of expired air was introduced into the analysers through a side tube attached to the Douglas bag. One min was allowed for the reading on the analysers to stabilise. The displayed readings were recorded and were taken as representing the carbon dioxide and oxygen content of the expired air. The side tube was shut off and the volume of expired air was measured using the gas meter. The temperature of expired air was recorded using a thermister attached to the gas meter. The volume of expired air (pulmonary ventilation) was then corrected for the amount of water vapour at standard temperature and presure dry (STPD), using a correction factor from a standard nomogram (Consolazio et al., 1963), or calculated as described below.

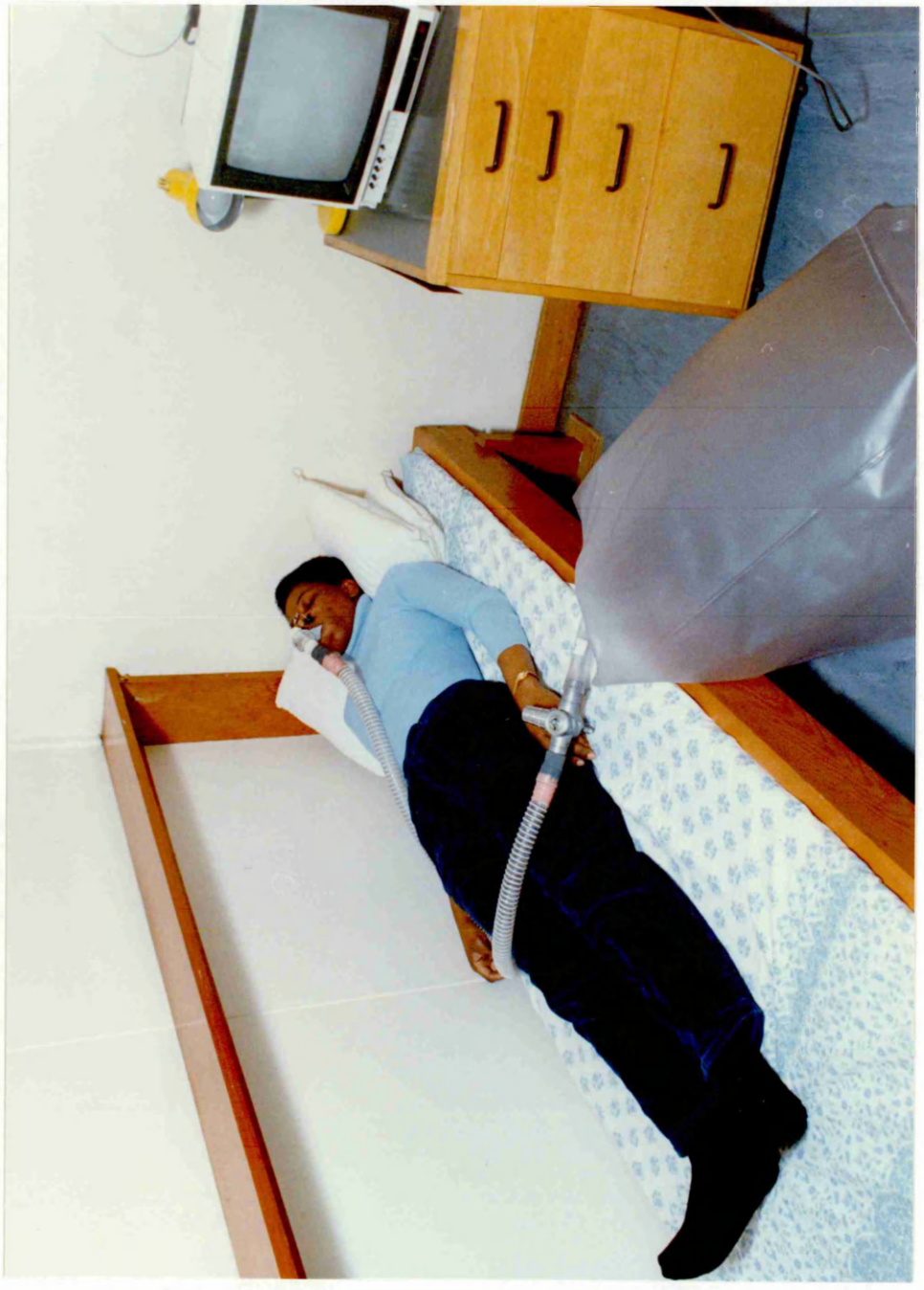


Fig. 8. The Douglas bag system indicating the different components which are used during gas collection.

Calculation of metabolic rate

In this study metabolic rate was calculated using Weir's (1949) method.Previously, indirect calorimetry required a detailed and elaborate procedure to determine the proportions of the different nutrients oxidised in the body by measuring both gaseous exchange as well as nitrogen excreted in the urine. The calculations were often so cumbersome that the effect of protein was ignored. The equation developed by Weir (1949) has taken into account the effect of protein in calculating metabolic rate from respiratory exchange alone without necessarily having to measure urinary nitrogen.

This equation is based on the assumption that a fixed percentage (11-14 percent) of the total calories expended by the body arise from protein metabolism. For this reason, in the studies described in this thesis, it was considered unnecessary to determine urinary nitrogen. It has been shown that changes in total calories from protein do not significantly alter metabolic rate (Weir, 1949).

In the present studies, oxygen consumption and carbon dioxide production were calculated from measured values of pulmonary ventilation, carbon dioxide, oxygen and nitrogen content of the expired air. It is from these set of data that the metabolic rate or energy expenditure of the subject was subsequently computed.

Oxygen consumption rate (l/min) was obtained by multiplying ventilation rate (l/min STPD) by 'true' oxygen as provided in the O_2, CO_2 and RQ nomogram by Dill (Consolazio et al., 1963). However, exact values of 'true oxygen' were calculated as described below.

Metabolic rate (kcal/min) was calculated by multiplying ventilation rate (l/min STPD) by the calorific value per litre of expired air. The calorific value of expired air when protein is assumed to contribute 11-14 percent is equivalent to one twentieth of the difference between oxygen in the inspired air and that in the expired air. According to Weir, (1949) and Consolazio et al., (1963) the calorific value of expired air is independent of CO_2 in the expired air, and thus of the respiratory quotient.

The following data were obtained:-

Barometric pressure.....P mmHg

Volume of expired air.....Ve l/min

Temperature of expired air.....Te oC

Oxygen content of expired air.....O_2e %

Carbon dioxide content of expired air.....CO_2e %

Nitrogen content of expired air.....$N_2e=(100-O_2e+CO_2e)$ %

Oxygen content of inspired air (O_2i).....20.93 %

Carbon dioxide content of inspired air (CO_2i).....0.03 %

Nitrogen content of inspired air (N_2i).....79.04 %

Volume of expired air (Body temperature and pressure saturated with water vapour BTPS) was corrected for moisture at standard temperature and pressure, dry (STPD 0^0C, 760 mmHg, dry) using a correction factor:-

$$\text{Correction factor (F)} = \frac{273+37}{273+{}^0C} \times \frac{P-p}{P-47}$$

Where P= Atmospheric pressure; p= saturated vapour pressure; ^0C= Temperature of the expired air.

$$\text{Oxygen consumption} = \frac{(20.93 \times N_2e)}{(79.04)} - O_2e \times \frac{Ve \text{ (l/min STPD)}}{100}$$

Calculation of metabolic rate:

$$\text{Metabolic rate} = \frac{(20.93 - O_2e)}{20} \text{ (kcal/l)} \times Ve \text{ (l/min)}$$

Where $\frac{20.93-O_2e}{20}$ is the calorific value of the expired air.

Determination of basal metabolic rate

Instructions for subjects: For the determination of BMR, subjects were instructed to refrain from any strenous activities 24 h before the test day. They were also instructed to abstain from food and drinks especially caffeine containing beverages as well as alcohol by 20.00 h on the night preceding each test day, not to eat anything the following morning, and also to keep a record of the foods eaten and activities carried out on the day preceding each test day.

On the day of the experiment, subjects reported to the laboratory at 08.00 h after an overnight fast. The subject was weighed, and the stage of her menstrual cycle recorded and then allowed to rest lying down in bed in the supine position for 30 min in a room maintained at 18^0-$20\ ^0$C depending on the subject's comfortability. After 30 min of rest the subject was fitted with a nose clip and mouth piece, and breathed through a low resistance two way valve for at least 3 min. After the subject had become accustomed to breathing through the apparatus, two 10 min samples of expired air were collected in Douglas bags, analysed and metabolic rate calculated as described in section 3.3. If the difference between the two bags was not less than 3 percent, a third sample of expired air was collected, analysed and metabolic rate calculated. The average metabolic rate calculated from these three measurements was taken as representing the BMR of the subject. BMR was determined on each test day prior to the ingestion of a test meal.

The initial measurement was called BMR because it was determined according to the conditions required for BMR determinations as described by Benedict (1915), du Bois (1927) and Boothby *et al.* (1936).

What exactly constitutes the conditions under which 'basal' metabolic rate is measured has become in the recent past a source of confusion, and the impression seems to have arisen that 'basal' metabolic rate implies that the subject has been admitted to a hospital or to a metabolic unit or chamber the night before and BMR is measured before getting out of bed in the morning. Whatever the rationale of this view, this is not how almost all the fundamental work on BMR was carried out (Benedict, 1915; du Bois,

1927; Boothby et al., 1936). An extract from the book by du Bois (1927), describes the requirements: The subject " must be instructed to take no food in the evening after 8 o'clock and nothing in the morning except a cup of caffeine free coffee without milk or sugar. He must be brought to the labolatory without fatigue, he must lie quitely for at least one half hour before the test. The atmosphere of the room should be quiet and confident". These are precisely the conditions under which BMR was measured in the studies described in this thesis.

Determination of post-prandial metabolic rate

After BMR determinations were completed, subjects consumed the test meal while in a sitting position. The meal had to be consumed within 10 min if it contained 2520 kJ (600 kcal) or within 20 min if it contained 5040 kJ (1200 kcal). After consuming a meal, the subject was asked to lie in a supine position and remain in that position for the duration of the experiment.

Post-prandial metabolic rate (PP-MR) measurements were started 10 min after complete ingestion of the test meal. Samples of expired air were collected intermittently for the period of the experiment. During each measurement, two samples of expired air were collected, analysed and metabolic rate calculated as described above.

To avoid discomfort from prolonged use of the mouth piece and noseclip, these were removed in between measurements (rest period). However, they were again fitted to the subject during the last 3 min of the rest period to ensure that the subject was supine, quiet, relaxed and breathing comfortably through the valve before gas collection. Subjects were allowed to read, listen to music or watch television between measurements, but were requested to remain in a supine position most of the time, and to be immobile during the actual measurement.

Test meals

Four different test meals were used. Their energy content and nutrient composition were computed using the food composition Tables of McCance and Widdowson (Paul & Southgate, 1978). The constituents of the test meals are presented in Table 5. Meals were of high carbohydrate- low fat (HCLF) of which 0.70, 0.19 and 0.11 of the enegry content was obtained from carbohydrate, fat and protein, respectively, and low carbohydrate-high fat (LCHF) with 0.24, 0.65 and 0.11 of the energy content from carbohydrate, fat and protein, respectively. The energy content of the test meals were 2520 kJ (600 kcal) and 5040 kJ (1200 kcal).

In all studies, meals were presented in the form they are normally consumed. The composition of the test meals was chosen to represent the different diets eaten in different societies. Protein was maintained constant at 0.11 of the energy content of the test meals. This was considered to be within the normal range of intake (WHO/FAO/UNU, 1985). Moreover, dietary surveys in various parts of the world have shown that the majority of people take 10-15 percent of their calories from protein (Widdowson, 1947; McKillop, (1990); personal communication). Except for few tribes such as the Eskimos and the Masai which their diet is predominatly high in protein.

The energy content of the test meals represented the average level of intake for breakfast (2520 kJ; 600 kcal) and for dinner (5040 kJ; 1200 kcal) for most people in Scotland.

Table 5. Constituents of the test meals

Meals							
A		B		C		D	
Item	(g)	Item	(g)	Item	(g)	Item	(g)
C/Flakes	80	Egg	60	C/Flakes	50	Bread	90
Milk	150	M'nnaise	20	Sugar	20	Butter	30
O/Juice	75	Butter	20	Milk	250	C/Cheese	60
Bread	40	Bread	80	Bread	90	Tomato	100
Butter	6			Butter	10	D/Cream	80
Jam	15			M'malade	50	Sugar	20
				Yoghurt	100	S'berrie	100
				O/Juice	440		

C/Flakes= Cornflakes; O/Juice= Orange juice; M'nnaise= Mayonnaise; M'malade= Marmalade; C/Cheese= Cheddar cheese; D/Cream= Double cream; Bread= Brown soft rolls; S'berries= Strawberries.

Meals A and C : high carbohydrate-low fat (HCLF) containing 2520 kJ (600 kcal) and 5040 kJ (1200 kcal), respectively.

Meals B and D; low carbohydrate-high fat (LCHF) containing 2520 kJ (600 kcal) and 5040 kJ (1200 kcal), respectively.

CHAPTER 4

EFFECT OF MEAL COMPOSITION AND ENERGY CONTENT ON THE THERMIC EFFECT OF FOOD

4.1 INTRODUCTION

Although meal composition has been reported to influence TEF (Garrow and Hawes, 1972; Welle *et al.*, 1981; Dauncey and Bingham, 1983; Schwartz *et al.*, 1985), several studies have shown that TEF is not influenced by meal composition (Miller *et al.*, 1967; Pittet *et al.*, 1974; Rosenberg and Durnin, 1978; Hurni *et al.*, 1982; Belko *et al.*, 1986). While several investigators have shown that TEF is directly related to the energy content of a meal (Hill *et al.*, 1984; Karst *et al.*, 1984; Belko *et al.*, 1986), others have failed to establish any relationship between TEF and energy content of a meal (Glickman *et al.*1948; Bradfield and Jourdan, 1973; Bray *et al.*, 1974). The reasons for these different results are not clear. However, methodological differences as well as the type and form of meals used may partly account for the results between laboratories.

Since early experiments on TEF protein was regarded as more thermogenic than carbohydrate and fat (Lusk, 1928), therefore its proportion in the diet has been shown to have a significant effect on TEF (Dauncey and Bingham, 1983; Zed and James, 1986). However, other workers have observed no significant difference in TEF between meals containing different proportions of protein (Bradfield and Jourdan, 1973; Belko *et al.*, 1986). Moreover, it has been demonstrated that the increase in energy expenditure after ingestion of a meal does not appear to result from an increase in the protein synthesis (Garlick, 1986) or urea formation (Garrow and Hawes, 1972).

In the present study, the effect on TEF of two factors , namely: (1) meal composition: high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF), and energy content: 2520 kJ (600 kcal) and 5040 kJ (1200 kcal) of the ingested meal was examined in non-obese subjects. The two composition were chosen to represent the two extreme diets eaten in different societies. The main aim of this study was to establish whether consuming isoenergic meals of different composition would have a different effect on TEF and thus energy balance (Lusk, 1928), and also to examine the effect of the level of energy intake on TEF.

4.2 SUBJECTS AND METHODS

Subjects

Sixteen healthy, adult, non-obese female subjects participated in the study. None was taking any medication during the period of the study. Their physical characteristics are presented in Table 6. There were no significant differences between subjects in terms of age, weight, height, body fat and fat free mass.

Subjects were instructed to abstain from food and drinks (especially caffeine containing beverages as well as alcohol) by 20 h on the night preceding each test day and not to eat anything the following morning. They were also asked to keep a record of food eaten and activities carried out on the day immediately preceding the test day and to refrain from strenuous activities at least 24 h prior to the test day. All subjects were familiar with the protocol by the time of the experiment.

Test meals

Four meals were used. Their composition and constituents are described in chapter 3 and presented in Table 5.

Determination of basal metabolic rate

The BMR of the subjects was determined as described in chapter 3.

Determination of post-prandial metabolic rate

After BMR determinations were completed, the subjects consumed one of the test meals, which had to be consumed within 10 min if it contained 2520 kJ (600 kcal) or within 20 min if the meal contained 5040 kJ (1200 kcal). Each subject consumed four

Table 6. Physical characteristics of subjects

	Mean	SD	Range
Age (years)	22	5.9	18-40
Height (m)	1.64	0.05	1.56-1.74
Weight (kg)*	55.8	3.8	50.1-61.6
Body fat (%)†	24	2.6	20-28
FFM (kg)	42.9	3.0	39.0-47.8
BMI (kg/m2)	20.8	0.2	18.6-23.4
BMR (kJ/kgFFM per d)	124	7.5	110.0-140.0

b.wt.,body weight; BMI, body mass index; BMR, basal metabolic rate; FFM, fat free mass.

* Average of weights recorded on four different days.

† Estimated from four skinfold thicknesses (biceps, triceps, subscapular and supra iliac) by the method of Durnin & Womersley (1974).

different test meals each on separate occassion. The order of presentation of the test meals was randomized separately for each subject.

PP-MR measurements were started 10 min after complete ingestion of the test meal. Samples of expired air were collected intermittently for a period of 5 h. The first three measurements were taken at 10 min intervals, and the next five measurements at 20 min intervals.

Expired air was collected, analysed and metabolic rate calculated as described in chapter 3. The average values obtained from the two bags were taken as representing the PP-metabolic rate at each time a measurement was made. For each subject, the increase in metabolic rate above BMR (PP-MR -(BMR)) was plotted against time. TEF was calculated as the area under the metabolic rate versus time curve using the trapezoidal method.

General conditions such as subject comfort were all observed as described in chapter 3.

Statistical analysis

The results were assessed by 2 x 2 factorial analysis of variance (ANOVA) using the minitab statistical package (Ryan, *et.al.*, 1985). The BMR values and the overall increase PP-MR values were compared using the Student's paired t-test. Values are presented as Mean ± Standard error , unless otherwise stated.

4.3 RESULTS

TEF

The mean BMR and the 5 h TEF values of the four test meals are presented in Table 7. The mean BMR values were similar in all test meal situations with an average of 3.63 ± 0.07 kJ/min (0.86 ± 0.02 kcal/min) or 123 ± 0.45 kJ/kg fat free mass/day. The mean coefficient of variation (CV) in BMR within individual subjects was 5 percent (range 1-10 percent) indicating good reproducibility. There was no indication to suggest that BMR values varied with the stage of the menstrual cycle. The mean coefficient of variation in weight within individual subjects was 0.8 percent (range 0.3-1.7 percent).

All subjects showed a significant increase in metabolic rate after all test meals (Fig. 9). The overall mean increase in metabolic rate after ingestion of 2520 kJ (600 kcal) meals were 0.79 kJ/min (0.19 kcal/min) (95 percent confidence interval 0.71, 0.85) for the HCLF meal and 0.79 kJ/min (0.19 kcal/min) (95 percent confidence interval 0.71, 0.86) for the LCHF meal. The corresponding values for the 5040 kJ (1200 kcal) meals were 1.26 kJ/min (0.30 kcal/min) (95 percent confidence interval 1.17, 1.34) and 1.21 kJ/min (0.29 kcal/min) (95 percent confidence interval 1.17, 1.31). This corresponded to an increase of 21 and 33 percent above the BMR for the 2520 kJ (600 kcal) and 5040 kJ (1200 kcal) meals, respectively.

The 5 h TEF values (Table 7) were 228 ± 11.8 kJ (54 ± 2.8 kcal) and 228 ± 9.6 kJ (54 ± 2.3 kcal) for the 2520 kJ (600 kcal) HCLF and LCHF meals, respectively. Those for the 5040 kJ (1200 kcal) meals were 356 ± 20.4 kJ (85 ± 4.9 kcal) and 340 ± 15.8 kJ (81 ± 3.8 kcal) for the HCLF and LCHF , respectively (Fig. 10).

There was no significant effect of meal composition on TEF (P=0.49). However, the energy content of the test meals had a significant effect on TEF (P=0.001), with meals of higher energy content having 5 h TEF values significantly greater (55 percent) than those for the low energy content. Meal composition and energy content did not show significant interaction in influencing TEF (P=0.50).

Table 7. Basal metabolic rate (BMR) and the thermic effect of food (TEF) of female subjects (n=16) after ingesting four different test meals

	MEALS							
	A		B		C		D	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
BMR(kJ/min)	3.70	0.3	3.65	0.3	3.69	0.3	3.47	0.3
(Kcal/min)	0.88	0.1	0.87	0.1	0.88	0.1	0.83	0.1
TEF(kJ/5h)	228	12	228	14	356	20	340	16
(Kcal/5h)	54	3	54	2	85	5	81	4
TEF % EI*	9.0	0.5	9.0	0.6	7.0	0.4	7.0	0.3

* EI= Energy intake

A= High carbohydrate-low fat (HCLF) 2520 kJ (600 Kcal) meal

B= Low carbohydrate-high fat (LCHF) 2520 kJ (600 Kcal) meal

C= High carbohydrate-low fat (HCLF) 5040 kJ (1200 Kcal) meal

D= Low carbohydrate-high fat (LCHF) 5040 kJ (1200 Kcal) meal.

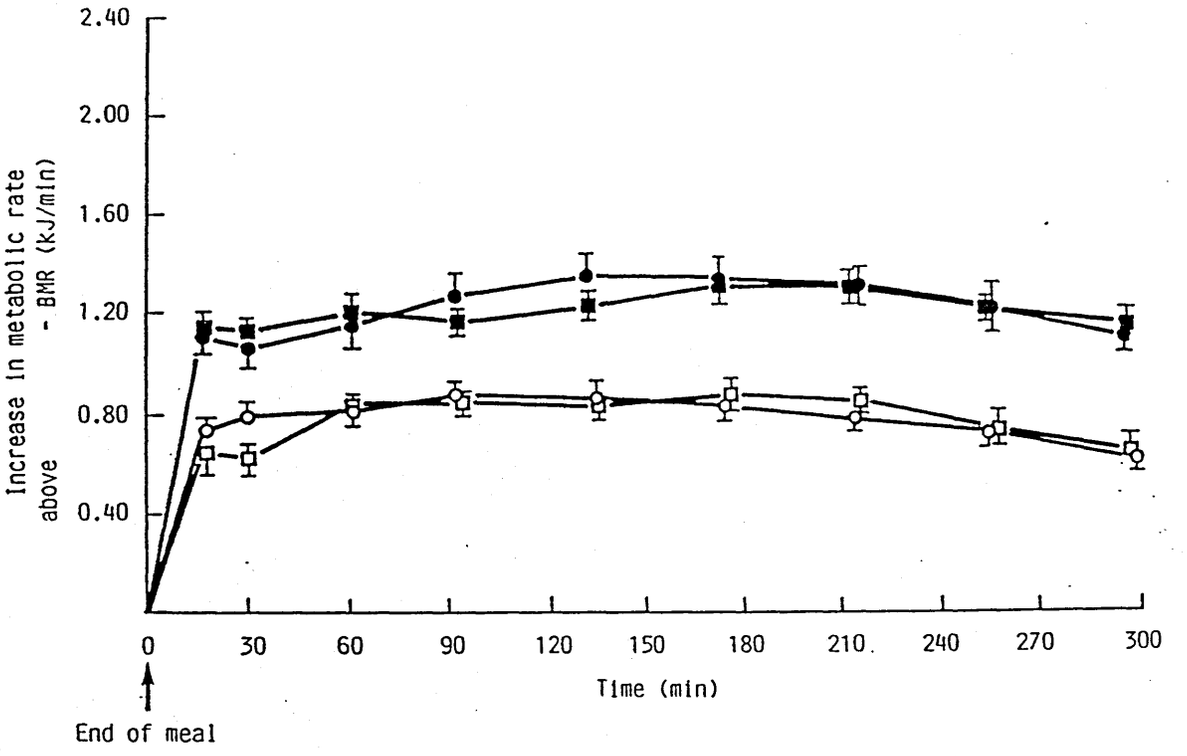


Fig. 9. Increase in metabolic rate above basal metabolic rate (BMR) in kJ/min (Mean ± SE) in non-obese subjects (n=16) after ingestion of four different test meals: high carbohydrate-low fat (2520 kJ (600 kcal)) [O], low carbohydrate-high fat (2520 kJ (600 kcal)) [[]], high carbohydrate-low fat (5040 kJ (1200 kcal)) [●], low carbohydrate-high fat (5040 kJ (1200 kcal)) [■].

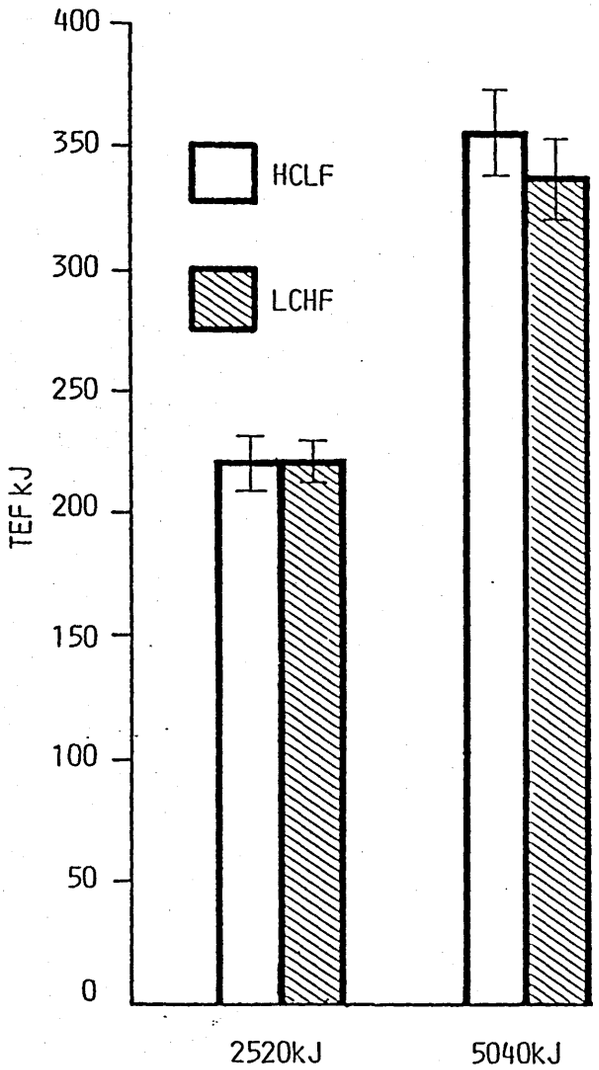


Fig. 10. Thermic effect of food (TEF) in kJ (Mean ± SE) in non-obese subjects (n=16) after ingestion of high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) meals at two levels of energy intake 2520 kJ (600 kcal) and 5040 kJ (1200 kcal).

The 5 h TEF values expressed as percentage of energy content of the ingested meals were 9 percent for the 2520 kJ (600 kcal) and 7 percent for the 5040 kJ (1200 kcal) meals. Since total TEF was not measured, TEF obtained here underestimate to some degree (especially after the 5040 kJ meals) the total percentage of the energy content of the meal actually expended for TEF.

Inter-subject variability

There was significant inter-subject variability in TEF values (P=0.001), the coefficient of variation (CV) was found to be 18.6-24.6 percent.

4.4 DISCUSSION

Effect of meal composition on TEF

This study did not establish any relationship between meal composition and TEF. There were no significant differences in the 5 h TEF values between HCLF and LCHF meals for equal energy content. These results agree with those of other studies (Pittet *et al.*, 1974; Rosenberg and Durnin, 1978; Belko *et al.*, 1986). However, other workers have reported significant differences in TEF values between meals of different composition (Dauncey and Bingham, 1983; Schwartz *et al.*, 1985; Zed and James, 1986). The reasons for the differences are not clear. It is probable that methodological differences as well as the form and type of meal used may partly account for such differences.

In most studies (Table 1 and 2.), either 100 percent pure single nutrients (Gomez *et al.*, 1972; Garrow and Hawes, 1972; Pittet *et al.*, 1974; Nair *et al.*, 1983; Swaminathan, 1985), pure mixed nutrients (Garrow and Hawes, 1972; Pittet *et al.*, 1974), single nutrient meals (Garrow and Hawes, 1972; Welle *et al.*, 1981) or mixed meals (Swindells, 1972; Rosenberg and Durnin, 1978; Dauncey and Bingham, 1983;

Schwartz *et al.*, 1985; Belko *et al.*, 1986) were used to examine TEF in man. These are not the kind of food people would normally eat. Of these, only a few used solid meals (Swindells, 1972; Rosenberg and Durnin, 1978; Dauncey and Bingham, 1983). In the present study, meals were presented in the form in which are normally eaten (chapter 3). The effect of meal composition on TEF has been examined previously at only one level of energy intake (Table 1 and 2.). In the present study, the effect of meal composition was examined at two levels of energy intake (2520 kJ (600 kcal) and 5040 kJ (1200 kcal). At both levels, meal composition did not have any significant effect on TEF. This is surprising, as it had been expected that at high levels of energy intake the HCLF meal would stimulate more thermogenesis than the LCHF meal since more energy is required for the storage of carbohydrate than fat (Flatt , 1978; Garrow, 1978; Acheson *et al.*, 1984). This observation may suggest that perhaps de novo lipogenesis is not an important pathway in carbohydrate metabolism. Alternatively this could mean that the energy required for carbohydrate storage is probably not as high as suggested by Flatt, 1978.

Effect of energy content on TEF

In this study, meals of high energy content (5040 kJ (1200 kcal) had TEF values significantly greater than those of low energy content (2520 kJ (600 kcal). The results are in accord with those reported from other studies (Hill *et al.*, 1984; Karst *et al.*, 1984; Belko *et al.*, 1986; Steiniger *et al.*, 1987). However, other studies have reported no significant differences in TEF values between meals of different energy content (Swindells, 1972; Bradfield and Jourdan, 1973; Bray *et al.*, 1974). The results obtained in the present study suggest that the magnitude of TEF is related to the energy content of the ingested meal. But the exact relationship could not be established because the experiment was terminated before metabolic rate returned to a basal level. The study by Hill *et al.*, (1984), suggested a quadratic relationship between TEF and the energy content of the meal. They demonstrated that by progressively increasing the energy of the meal, TEF increased but not in a linear fashion. Unfortunately, the duration of the

measurement was not extended for long enough to allow for the metabolic rate to return to basal. It is possible that TEF has a linear relationship to energy content of the meal.

When the 5 h TEF values were expressed as percentage of the energy content of the ingested meals, they were found to be 9 and 7 percent. The estimated TEF is 10 percent (Ravussin *et al.*, 1985; FAO/WHO/UNU, 1985). Since total TEF was not measured, the values obtained here underestimated the actual amount of energy expended for TEF and it is possible that the values may be greater than the estimated 10 percent. However, the values obtained here are consistent with those reported by other workers. Miller *et al.*, (1967) reported TEF values which were 7-10 percent of the energy contentof 1550-13440 kJ meals. Similarly Belko *et al.*, (1986) observed that the TEF values were 5-7 percent of the energy content of 6000-8300 kJ.

The PP-MR values obtained in the present study were 21 and 33 percent above the BMR for the 2520 kJ (600 kcal) and 5040 kJ (1200 kcal) meals, respectively. These results are similar to those reported by other workers using meals of same energy content (Miller *et al.*, 1967; Swindells, 1972; Rosenberg and Durnin, 1978; Dalloso *et al.*, 1982), and taken together, they seem to suggest that TEF measured under resting conditions may attain a magnitude equivalent to one quarter of the BMR. This cannot be considered insignificant in energy balance as has been suggested (Davidson and Passmore, 1986; Swindells, 1972; Garrow, 1978). The results of Schwartz *et al.* (1985) are in accord with the results obtained in the present study.

Inter-subjects variability

There was marked inter-subject variability in the 5 h TEF values coefficient of variation 18.6-24.6 percent. This was surprising because there were no significant differences between subjects in terms of weight, fat free mass and fat content. The reasons for the variation is unclear. However, it may be speculated that this variation could be due to inherent differences with regard to efficiency in digestion, absorption and

disposition of nutrients in the body which in turn may reflect inherent differences in either hormone and/or enzyme levels.

Duration of TEF

It is evident that, TEF measured under resting conditions in this study constituted a significant increase in metabolic rate. However, it is possible that, the overall quantitative importance of TEF could have been underestimated, especially that of the 5040 kJ (1200 kcal) meals because none of the subjects studied had their metabolic rate values returned to baseline 5 h after meal. Other studies have also made similar observations (Schwartz *et al.*, 1985; Belko and Barbieri, 1987). One overfeeding study (Schutz *et al.*, 1985) showed that TEF was so prolonged that it lasted not only for the whole day but also throughout the night until the following morning, when the effect was still apparent.

From these results, and considering the average feeding pattern (three meals per day), there is strong indication that the TEF of one meal merges into the TEF of the next meal.

The reasons for this prolonged effect is unknown. However, it pertinent to assume that the presence of food in the body stimulates various biosynthetic and oxidation processes. Although these processes take place all the time, their rate increases (self accelerating) immediately after a meal because of the high influx of nutrients to the cells. These processes will proceed at a diminishing rate until all the nutrients have been processed and stored. According to Flatt (1978), even after the nutrients have been stored there is a certain probability of being re-utilised in the biosynthetic and oxidation processes. Nutrient intake therefore, depending on the amount to be processed, will increase energy expenditure far beyond the post-prandial phase. If this theory is true, then it will help to explain at least in part the response observed here (especially with the high energy meals) and in other studies as well.

CHAPTER 5

EFFECT OF MEAL FREQUENCY ON THE THERMIC EFFECT OF FOOD

5.1 INTRODUCTION

Feeding frequency is often cited as one of the variables which can influence energy balance in man. Infrequent meal pattern ('gorging': less than three meals per day) has been shown in some studies to induce adaptive changes in the intestinal absorption of glucose and fat (Fabry, 1969), to enhance lipogenesis (Fabry *et al.*, 1964; Terpestra *et al.*, 1978), to increase body weight (Dalloso *et al.*, 1982) and increase blood serum cholesterol level (Gwinup *et al.*, 1963; Irwin and Feeley, 1967; Young *et al.*, 1972). Some of these factors are regarded as risk factors for coronary heart diseases (Fabry and Tapperman, 1970; Adams and Morgans, 1981).

In contrast to these observations, other investigations have failed to establish any significant relationship between meal frequency and weight and body composition changes or nitrogen metabolism (Swindells *et al.*, 1968; Finkelstein and Fryer, 1971; Young *et al.*, 1971; Garrow *et al.*, 1981). For example, Bortz *et al.* (1966) found no difference in weight loss in obese women fed diets of the same energy content in either one, three or nine meals per day.

The view that an infrequent meal pattern is more fattening than a more frequent (more than four meals per day) meal pattern, seems to suggest that the thermic effect of food (TEF) of a more frequent meal pattern is higher than that of an infrequent meal pattern. However, Belko and Barbieri (1987) found no difference in the TEF between four meals and two meals measured over a period of 10 h. Furthermore, meal frequency has been shown to have no influence on the 24 h (Dalloso *et al.*, 1982) and 21 days (Swindells *et al.*, 1968) energy expenditure.

Studies of the relationship between meal frequency and TEF are few. The aim of the present investigation was to determine the effect on TEF of meal composition and frequency of ingestion in non-obese subjects.

5.2 SUBJECTS AND METHODS

Subjects

Eighteen women subjects, divided in to two groups for experimental purposes participated in this study. Their physical characteristics are shown in Table 8. There were no significant differences between the two groups of subjects in terms of age (P=0.26), weight (P=0.53), height (P=0.56), body fat and fat free mass (P=0.55). None of the subjects was under medication during the time of the study. Subjects were instructed to curtail vigorous activities on the day preceding each test day. They were also instructed to avoid food as well as drinks by 20.00 h on the night before the test day and not to eat anything the following morning.

Meals

Two meal compositions were used, these were HCLF (C) and LCHF (D) containing 5040 kJ (1200 kcal). The composition and constituents of the meals are described in chapter 3 and presented in Table 5.

Experimental design

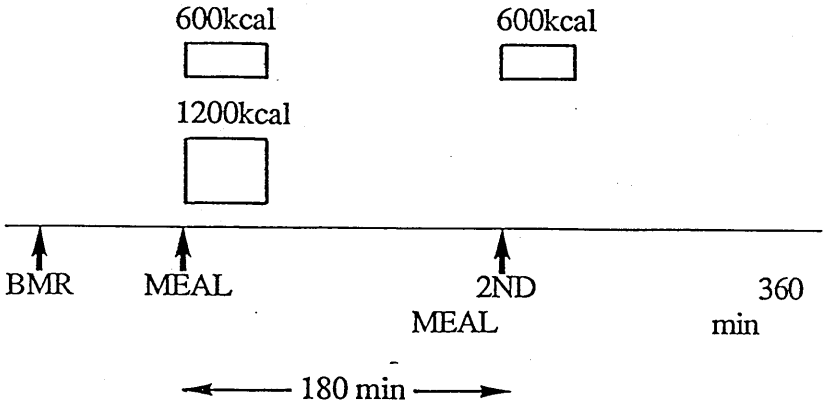


Table 8. Physical characteristics of the subjects

	Age (years)	Body Weight (kg)	Height (m)	BMI (kg/m2)	Body Fat* (%)	FFM (kg)	BMR (kJ/d)
Group A (n=8)							
Mean	24	55.2	1.63	21	23	42.4	5295
SD	6.0	4.2	0.08	1.4	1.7	3.1	557
Range	19-34	50-63	1.53-1.76	18-22	21-26	38.7-46.9	4776-6495
Group B (n=10)							
Mean	20	57.2	1.65	21	23	43.6	5429
SD	7.5	7.7	0.05	2.2	5.0	4.6	561
Range	18-29	40-68	1.59-1.73	16-23	15-30	34.2-48.5	4818-6368

* Estimated from skinfold measurements by the method of Durnin & Womersley (1974).

Group A (n=8) consumed a HCLF meal and group B (n=10) consumed a LCHF meal. On two separate occasions, each subject consumed the appropriate diet (HCLF or LCHF) either as one meal of energy value of 5040 kJ (1200 kcal) or as two smaller meals each containing about 2520 kJ (600 kcal). The second meal was given after a duration of 180 min. The order of eating the meal or meals was randomised and a period of at least 1 week was allowed between treatments.

Determination of basal metabolic rate

Each subject arrived at the laboratory at 8.00 h after an overnight fast (at least 12 h). After emptying the bladder, bodyweight was measured (to the nearest 0.1 kg) and the BMR of the subjects was determined as described in chapter 3.

Determination of post-prandial metabolic rate

After BMR determination, each subject consumed the appropriate diet either as one large meal containing 5040 kJ (1200 kcal) or two small meals each containing 2520 kJ (600 kcal). The second meal was given after an interval of 180 min and each was consumed within 10 min while the one large meal was consumed within 20 min. Collection of expired air started 10 min after complete ingestion of the meal and continued intermittently for 6 h. During the first 90 min, samples were collected with intervals of 10 min between measurements and thereafter with interval of 20 min. Expired air was collected and analysed as described in section in chapter 3.

Metabolic rate was calculated for each bag as described in chapter 3. TEF was calculated as the area under the increase in metabolic rate versus time curve using the trapezoidal method.

Comfort of the subjects was ensured as described in chapter 3.

Statistical analysis

Differences between the feeding regimens and between the two groups were assessed for significance using Student's paired and unpaired t-test, respectively, by the Minitab statistical package (Ryan *et. al.*, 1985). Values are presented as Mean ± Standard error, unless otherwise stated.

5.3 RESULTS

Energy expenditure

Mean BMR and 6 h PP-MR values are presented in Table 9. The mean BMR values of the subjects who consumed a HCLF (group A) meal was 3.68 ± 0.10 kJ/min (0.88 ± 0.02) kcal/min) and that of the subjects who consumed a LCHF meal (group B) was 3.77 ± 0.08 kJ/min. These values represent the average of the two BMR values of each individual subject measured on two separate occasions. There were no significant differences (P=0.63) in the mean BMR between the two groups of subjects. The mean coefficient of variation (CV) in BMR within individual subjects was 3.6 percent (range 1-8.5 percent).

There was a significant increase in PP-MR after ingestion of a meal in all subjects and in all meal situations (Fig. 11 and Table 9). The overall 6 h PP-MR values in subjects who consumed the HCLF meal were 4.81± 0.08 kJ/min (1.15 ± 0.02 kcal/min) for the one large meal and 4.69 ± 0.08 kJ/min (1.12 ± 0.02 kcal/min) for the combined two small meals. The values for the LCHF meal were 4.83 ± 0.04 kJ/min (1.15 ± 0.01 kcal /min) for the one large meal and 4.70 ± 0.08 kJ/min (1.12 ± 0.02 kcal/min) for the combined two small meals. There were no significant differences in the overall 6 h PP-MR between the two feeding regimens (HCLF, P=0.56; LCHF, P=0.20).

The mean 6 h TEF values (Table 9 and Fig. 12) for the HCLF meal were 377.0 ± 30 kJ (90.0 ± 7.2 kcal) and 381.0 ± 26.5 kJ (91.0 ± 6.3 kcal) for the one large meal

Table 9. Energy expenditure in subjects who consumed high carbohydrate-low fat (HCLF, Group A) and low carbohydrate-high fat (LCHF, Group B) meals under two different feeding regimens

	HCLF Group A (n=8)				LCHF Group B (n=10)			
	One meal (5040 kJ)		Two meals combined (2520 kJ each)		One meal (5040 kJ)		Two meals combined (2520 kJ each)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
BMR (kJ/min)	3.78	0.17	3.58	0.13	3.84	0.17	3.69	0.08
PP-MR(kJ/min)	4.81	0.08	4.69	0.08	4.83	0.04	4.70	0.08
TEF (kJ)	377.0	30.0	381.0	26.5	356.0	23.0	340	15.9
TEF % EI	7.5	0.6	7.6	0.5	7.1	0.5	6.8	0.3

BMR = Basal metabolic rate; PP-MR = Post-prandial metabolic rate (Mean values over 6h); TEF = Thermic effect of food; EI = Energy intake

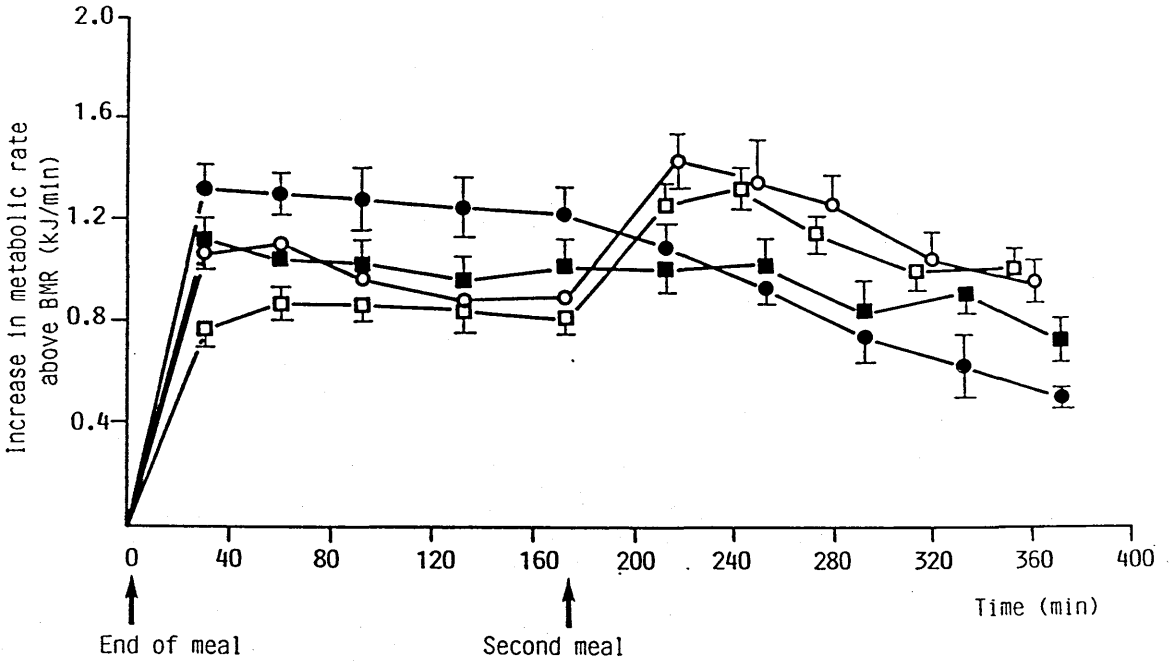


Fig. 11. Increase in metabolic rate above basal metabolic rate (BMR) in kJ/min (Mean ± SE) in non-obese subjects after ingestion of a high carbohydrate-low fat (n=8) meal administered either as a single large meal (5040 kJ (1200 kcal)) [●] or as two smaller meals (2520 kJ (600 kcal)) each [O] and a low carbohydrate-high fat (n=10) meal either as a large single meal (5040 kJ (1200 kcal)) [■] or as two smaller meals (2520 kJ (600 kcal)) each [□].

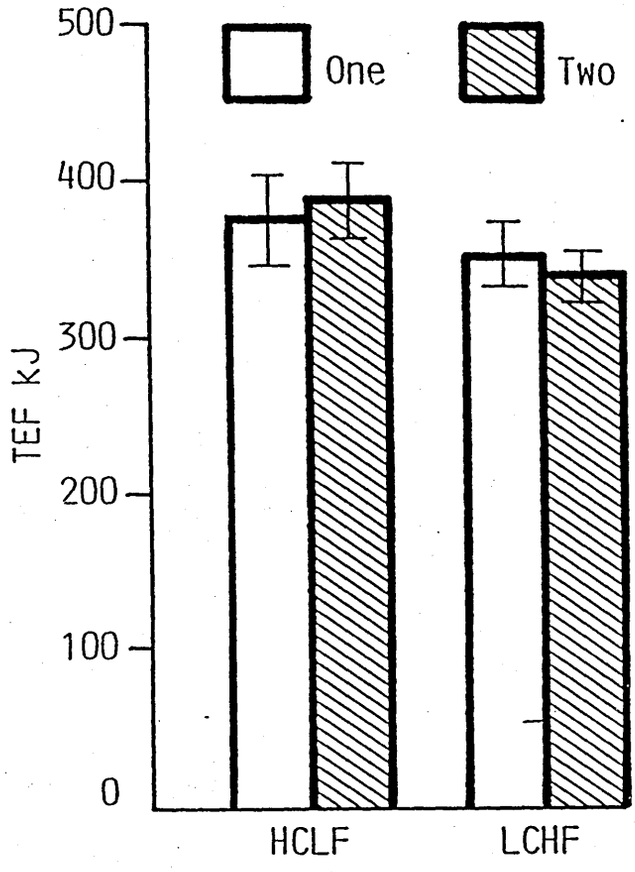


Fig. 12. Thermic effect of food (TEF) in kJ (Mean ± SE) in non-obese subjects after ingestion of high carbohydrate-low fat (HCLF; n=8) and low carbohydrate-high fat (LCHF; n=10) meals administered either as a single large meal or two small meals.

and two combined small meals, respectively, and that of the LCHF were 356.0 ± 23.0 kJ (85.0 ± 5.5 kcal) and 340.0 ± 15.9 kJ (81.0 ± 3.8 kcal) for the one large meal and the combined two small meals, respectively. For both meal compositions there were no significant differences in the 6 h TEF values after consuming one large meal or two small meals (HCLF, P=0.94; LCHF, P=0.64). Moreover, no significant differences (P=0.57) in 6 h TEF values were found between the two meal compositions. Nevertheless, in the two meals feeding regimen, there was a significant increase in TEF after the second meal was consumed (HCLF, P=0.012; LCHF, P=0.001) (Table 10).

The 6 h TEF values expressed as percent of the ingested energy were 7.5 for the HCLF one large meal and 7.6 percent for the combined two small meals. The values for the LCHF meal were 7.1 for the one large meal and 6.8 for the combined two small meals. No significant differences in TEF expressed as a percent of energy intake were observed between the two feeding regimens as well as between the two meal compositions (P=0.45).

Inter-subject variability

There was significant (P=0.001) inter-subject variability in TEF. Coefficient of variation (CV) was found to be 16-23 percent.

5.4 DISCUSSION

The results of the present investigation have shown that the frequency of eating meals has no significant influence on TEF of mixed meals. There was no significant differences in the 6 h TEF values between one large meal (5040 kJ; 1200 kcal) and combined two small meals (2520 kJ; 600 kcal) . This agrees with the results of some other studies (Swindells *et al.*, 1968; Dalloso *et al.*, 1982; Belko and Barbieri, 1987). Furthermore, the lack of significant differences in TEF values between subjects who consumed a HCLF meal and those who consumed a LCHF meal indicates that TEF is not affected by meal

Table 10. Thermic effect of food (TEF) in subjects who received a high carbohydrate-low fat (HCLF, group A) and low carbohydrate-high fat (LCHF, group B) meals under the two meals feeding regimen (first and second meal)

	HCLF Group A (n=8) meals				LCHF Group B (n=10) meals			
	first		second		first		second	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TEF (kJ)	157.0	9.2	224.0	20.0	133.0	7.0	207.0	9.6
TEF % EI	6.2	0.4	8.9	0.8	5.3	0.3	8.3	0.3

TEF = Thermic effect of food; EI = energy intake

composition (carbohydrate and fat content) (chapter 4). Other investigations have also shown similar results (Garrow and Hawes, 1972; Pittet *et al.*, 1974; Rosenberg and Durnin, 1978; Belko *et al.*, 1986).

In the present study, the TEF calculated for the feeding regimen of two meals showed that the TEF of the second meal resulted in significantly higher values than that of the first meals in both groups of subjects (Table 10). This observation suggests that there was super-imposition of the TEF of the first meal in to that of the second meal. Consequently, the TEF of the second meal was significantly higher than that of the first meal, but the total TEF did not differ significantly from that of a single meal regimen. This observation also suggests that under an average feeding pattern of three meals per day, the TEF of one meal merges in to the TEF of the next meal, but the overall TEF per day is related to the total energy ingested.

In spite of the 6 h measurement period in the present study, PP-MR was still 13-27 percent above pre-meal level at the end of 6 h suggesting that total TEF was underestimated in both feeding regimens. However, total and accurate measurement of TEF is difficult to achieve. To illustrate this point, if the total duration of TEF is extrapolated graphically, the effect of a 5040 kJ (1200 kcal) meal would take about 10 h to disappear. Because it is generally not practicable to collect air from subjects for about 10 h (using the Douglas bag), it is evident that this problem of underestimation remains an inherent limitation of the methodology. Nevertheless, the proportion of TEF which was not measured in this study represented a small fraction (13-27 percent) of total TEF. It is not suggested that this proportion is insignificant, but it can be estimated from graphical extrapolation without affecting the final interpretation of the results.

The hypothesis that meal frequency has a significant influence on weight changes seem to suggest that individuals who eat less than three meals per day stimulate more lipogenesis than those who eat more than four meals per day. However, lack of effectiveness in achieving weight reduction with simple alteration of meal pattern has been reported by other investigations. Their reports indicated that frequency of feeding did not

affect skinfold thickness (Thomas and Call, 1973), body weight or body composition (Nunes and Canham, 1963; Swindells *et al.*, 1968; Young *et al.*, 1971).

The results of the present study do not support the view that eating large number of meals is more thermogenic and therefore less fattening than eating small numbers of large meals. The study has also shown that the metabolic response due to meal frequency is not influenced by the composition of the meal. From these results, it is evident that meal frequency and meal composition are not important factors related to TEF, and that TEF is related to the total energy ingested.

The implication of these results is that, the success of weight loss or gain depends on energy balance through out the day and not on meal frequency. Weight is lost by ingesting less energy than expended and not by the manipulation of the frequency or composition of energy intake.

CHAPTER 6

EFFECT OF EXERCISE ON THE THERMIC EFFECT OF FOOD

6.1 INTRODUCTION

It is unclear whether exercise potentiates TEF. There have been reports to support the hypothesis that exercise potentiates TEF (Miller and Mumford, 1967; Miller and Wise, 1975; Bray *et al.*, 1974; Whipp *et al.*, 1976; Zahorska, 1980; Hill *et al.*, 1984; Maehlum *et al.*, 1986; Young *et al.*, 1986). However, other studies (Clough and Durnin, 1968; Swindells, 1972; Dalloso and James, 1984; Welle, 1984; Pacy, *et al.*, 1985; Belko *et al.*, 1986; Schutz *et al.*, 1987) have failed to establish any relationship between exercise and TEF. Exercise has also been shown (Passmore and Johnson, 1960; Bielisnki *et al.*, 1985; Maehlum *et al.*, 1986; Shah *et al.*, 1988) to have a prolonged effect on RMR, but this has not been confirmed in other investigations (Harmansen *et al.*, 1984; Pacy *et al.*, 1985; Akabas, 1985; Bingham, *et al.*, 1989).

The physiological basis of the prolonged effect of exercise on resting metabolic rate is not well understood (Schutz *et al.*, 1987). However, it is thought to be associated with the repayment of oxygen debt generally referred to as excess post exercise oxygen consumption (EPOC). During strenuous exercise, the delivery of oxygen to the exercising muscles fails to meet the immediate oxygen demands, and the muscle begins to utilise anaerobic metabolism for the supply of energy. After exercise, oxygen consumption may remain elevated for periods of few minutes to several hours depending on the intensity of exercise performed (Newsholme, 1978; Maehlum *et al.*, 1986). During this recovery period, oxygen is consumed in excess of that required to support the metabolism of the resting muscles. The EPOC has been considered to involve some form of reversal of the anaerobic processes that occur at the beginning of exercise (Newsholme, 1978).

It has been proposed that, the EPOC is probably used to regenerate the depleted high-energy phosphate stores, to replenish the oxygen stores in the blood and muscle (Newsholme, 1978; Lamb, 1984) and to oxidise the lactic acid produced in the hypoxic muscle. However, the existence, duration and role of the EPOC in energy regulation is still controversial.

The studies described in this chapter were carried out to investigate the effect of exercise on TEF, to examine whether exercise potentates TEF, and also to establish whether exercise has an influence on post exercise oxygen consumption in the fasting and fed state. The exercise involved walking on a treadmill at 4.8 km/h (3.0 mph) at zero percent gradient. Walking was preferred to other types of exercises such as cycling and running in order to minimise differences in mechanical efficiency between individuals in carrying out the exercise. It is known that, on average, every individual is likely to posses similar experience in walking (Passmore and Durnin, 1955; Waterlow, 1986), but for other types of exercises, there are considerable differences between individuals depending on experience.

6.2 SUBJECTS AND METHODS

Subjects

A total of 28 healthy subjects participated in this study. Their physical characteristics are shown in Table 11. None of the subjects studied was under any medication during the period of study. Their body fat content was assessed by both skinfold thickness measurements and underwater weighing. The skinfold measurement underestimated body fat by 7.5 percent compared to the underwater weighing.

Meal

A high carbohydrate-low fat (HCLF: A) meal containing 2520 kJ (600 kcal) was used. The composition of the meal was calculated as described in chapter 3. The meal contained 0.70, 0.19 and 0.11 of the energy from carbohydrate, fat and protein, respectively.

Table 11. Physical characteristics of the subjects.

	Mean	SD	Range
Age (Years)	22.6	4.4	17-34
Weight (kg)	59.2	7.6	38.7-71.9
Height (m)	1.66	0.07	1.52-1.82
Body fat (%)			
skinfolds*	23	4.7	13-32
UWW†	25	5.1	16-34
FFM (kg)			
skinfolds	45.0	4.8	33-56
UWW†	44.8	4.4	39-57
BMI(kg/m2)	21	2.4	16-25

BMI= Body mass index; FFM= Fat free mass; UWW= Underwater weighing.

* Estimated from four (biceps, triceps, subscapular and suprailiac) skinfold thicknesses by the method of Durnin and Womersley (1974).

† Estimated by underwater weighing using Siri's (1956) equation.

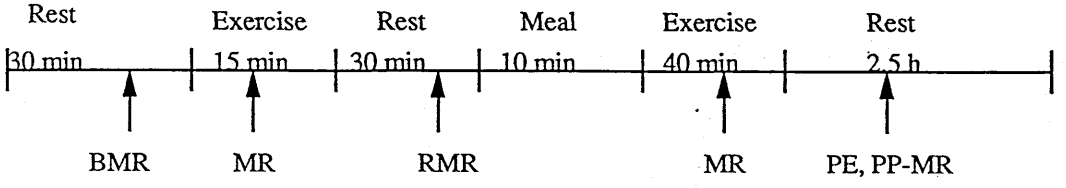
Determination of Basal Metabolic Rate

The BMR values of all the subjects were determined as described in chapter 3.

Determination of the energy expenditure of walking before and after a meal

The effect of walking on TEF was examined using two different protocols as follows:

Protocol 1. The main objective of this study was to examine whether exercise potentiates TEF during exercise and for several hours after the cessation of exercise. It has been suggested (Welle, 1984) that when exercise is performed immediately after meal, the glycogen depletion in the exercising muscle would cause an increase in the activity of the gastro-intestinal tract and the liver to replace the depleted glycogen. Thus energy expenditure after meal and after exercise will be higher. It has been reported that performance of exercise immediately after ingestion of a meal result in a higher energy expenditure because of the added effect of TEF (Miller and Mumford 1967).



BMR= Basal metabolic rate; RMR= Resting metabolic rate; PE,PP-MR= post exercise, post prandial metabolic rate; MR = Metabolic rate.

Eleven subjects participated in this protocol. Immediately after BMR determinations were completed, the subject was asked to walk on a motor-driven treadmill (Quinton Instruments Company, Seattle, Washington) for a period of 15 min. The treadmill speed was set at 4.8 km/h and zero percent slope. Subjects were allowed few minutes at the beginning of walking in order to get used to walking on the treadmill. After the subject had become accustomed to the feeling of walking on the treadmill, a further 5 min was allowed for the subject to at least attain a steady state. At the same time, they were asked to fit the nose clip and

mouth piece ready for gas collection. After ensuring that the subject was confident and comfortable, two 5 min samples of expired air were collected in Douglas bags. The expired air was analysed for CO_2 and O_2 content, and the metabolic rate was calculated as described in chapter 3. The average metabolic rate value calculated from the two bags was considered to represent the energy expenditure of walking (4.8 km/h; zero percent gradient) of the subjects in the fasting state (post absorptive conditions).

The subject was then allowed to rest lying down in the supine position in a room maintained at 18-20^{0}C for 30 min. This was to allow the metabolic rate of the subjects to return to basal level before ingesting a meal. In view of the prolonged effect of exercise on metabolic rate (Shah et al., 1988) it was considered necessary to reassess metabolic rate after exercise so as to establish a new baseline to be used in the subsequent analysis of the data. At the end of the 30 min resting period, one 10 min sample of expired air was collected, analysed and metabolic rate determined. The subject consumed the test meal within 10 min while in a sitting position, and immediately thereafter, walked on the treadmill at 4.8 km/h at zero percent gradient for 40 min. During this period, a total of four 5 min samples of expired air were collected at intervals of 5 min, analysed and metabolic rate determined as described above. The average of the four values was taken as representing the energy expenditure of walking of the subjects in the fed state.

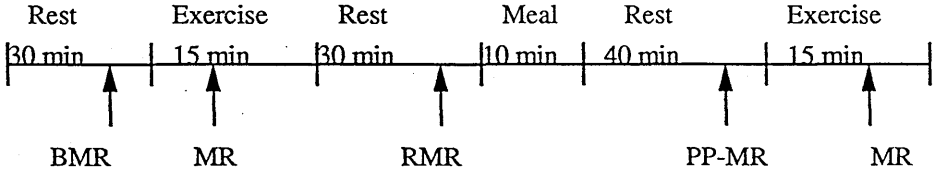
The subject was then asked to rest in the supine position for another 2.5 h. During this time, three measurements of metabolic rate were made (as described in chapter 3) at 45, 95 and 150 min after cessation of exercise. The main aim was to examine the effect of exercise on post exercise and post-prandial metabolic rate.

Protocol 2. In this protocol exercise was performed after PP-MR had attained peak increase after ingestion of a 2520 kJ (600 kcal) meal. The main aim was first to determine PP-metabolic rate after meal and then examine whether the measured PP-metabolic rate will increase or decrease during exercise. An increase in PP-MR would suggest exercise potentiates TEF.

Seventeen subjects participated in this protocol. Their BMR, energy expenditure of walking in the fasting state (post absorptive) and pre-meal RMR measured 30 min after walking were determined as described in protocol 1 above.

After BMR, energy expenditure of walking in the fasting state and pre-meal RMR determinations were completed, each subject consumed the test meal (while in a sitting position) within a period of 10 min. The subject was asked to lie down quietly, and 20 min after complete ingestion of a meal the resting PP-MR was determined using two 10 min samples of expired air.

Forty min after completion of the meal, the subject was asked to walk on the treadmill (4.8 km/h, zero percent gradient) for 15 min. During this period, two 5 min samples of expired air were collected and metabolic rate determined. The average of the two values represented the energy expenditure of the subjects in the fed state.



BMR=Basal metabolic rate; RMR=Resting metabolic rate; PP-MR=Post prandial metabolic rate; MR= Metabolic rate.

Post-Prandial Metabolic Rate

To investigate whether performing exercise before taking a meal might potentiate TEF, the post-prandial RMR values for all the subjects in each of the two protocols in this study were compared statistically using Students' t-test with those for the subjects who did not perform any exercise as described in chapter 4.

Statistical analysis

Differences between walking in the fasting and fed states and also between subjects who performed the exercise and those who did not perform any exercise were assesed for

significance using the Student's paired and unpaired t-test. Values are presented as Mean ± Standard error, unless otherwise stated.

6.3 RESULTS

The BMR, energy expenditure of walking in the fasting state and pre-meal RMR are presented in Table 12. The mean BMR was 3.94 ± 0.08 kJ/min (0.94 ± 0.02 kcal/min). The pre-meal RMR measured 30 min after subjects had finished walking was 4.15 ± 0.08 kJ/min (0.99 ± 0.02 kcal/min). There was no significant difference (P=0.07) between BMR and pre-meal RMR despite the 15 min period of walking on a treadmill at 4.8 km/h and zero percent slope (Fig.13 and 14). The mean value for the energy expenditure of walking in the fasting state was 16.48 ± 0.46 kJ/min (3.93 ± 0.11 kcal/min).

Protocol 1

The mean values for energy expenditure of walking in the fasting and the fed state are presented on Table 13 and Fig. 13. These were 15.37 ± 0.75 kJ/min (3.67 ± 0.18 kcal/min) and 15.66 ± 0.75 kJ/min (3.74 ± 0.18 kcal/min), respectively. These values were not significantly different (P=0.78). The energy expenditure of walking in the fed state increased by only 2 percent above the pre-meal (fasting state) energy expenditure of walking. The mean values for the post-exercise and PP-MR at 45, 95 and 150 min were, respectively, 4.69 ± 0.21 kJ/min (1.12 ± 0.05 kcal/min), 4.65 ± 0.21 kJ/min (1.11 ± 0.05 kcal/min) and 4.60 ± 0.21 kJ/min (1.10 ± 0.05 kcal/min). These values represented a 21, 20 and 19 percent increase in metabolic rate above BMR values (P= 0.40, 0.70, 0.84, respectively).

Table 12. Basal metabolic rate (BMR), energy expenditure of walking in the fasting state and pre-meal resting metabolic rate (RMR) in 28 lean female subjects.

	BMR (kJ/min)	Energy expenditure of walking (kJ/min)	Pre-meal RMR (kJ/min)
Mean	3.94	16.48	4.15
SE	0.08	0.46	0.08

Table 13. Energy expenditure of walking in the fasting and in the fed state in subjects (n=11) who followed protocol 1.

	BMR	Energy expenditure of walking		Post-meal post exercise RMR		
		fasting state	fed state	45 min	95 min	150 min
	kJ/min	kJ/min	kJ/min	kJ/min	kJ/min	kJ/min
Mean	3.88	15.37	15.66	4.69	4.66	4.60
SE	0.20	0.75	0.75	0.21	0.21	0.21

BMR= Basal metabolic rate; RMR= Resting metabolic rate.

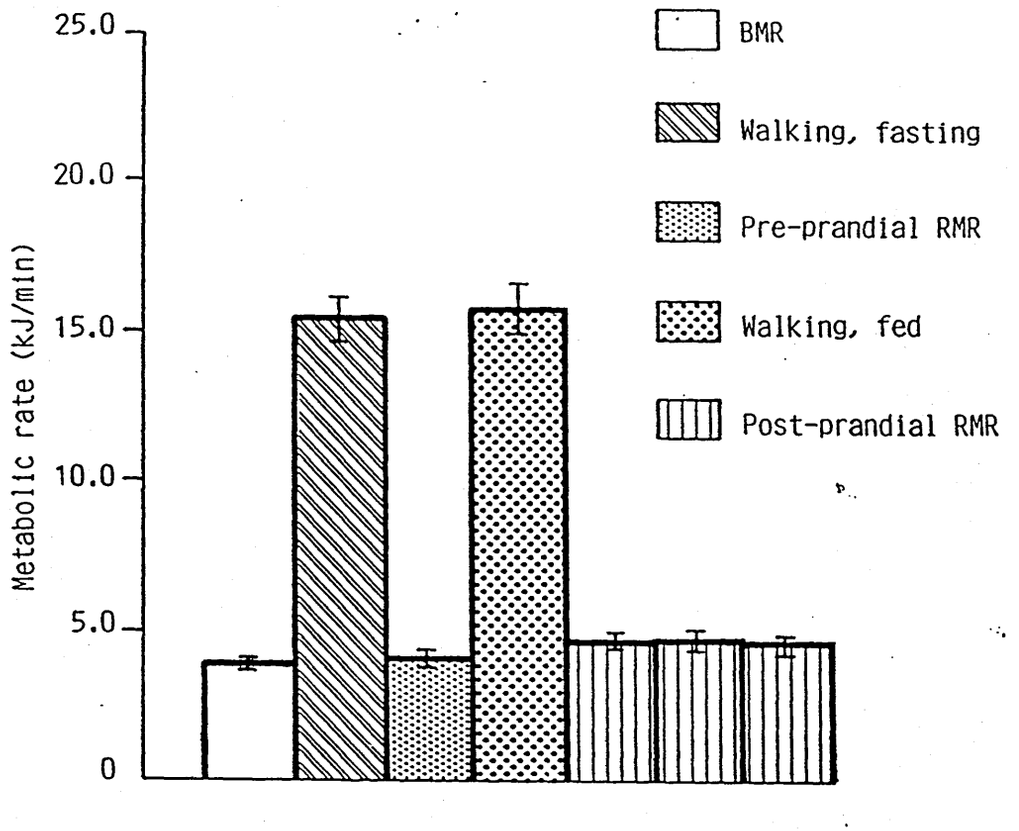


Fig. 13. Energy expenditure of walking in the fasting and fed state and resting metabolic rate (RMR) in kJ/min (Mean ± SE) in subjects who followed protocol 1 (n=11).

Protocol 2

The mean values for the energy cost of walking in the fasting and fed states are presented in Table 14, and Fig. 14. These were 17.24 ± 0.50 kJ/min (4.12 ± 0.12 kcal/min) for the energy expenditure of walking in the fasting sate and 17.41 ± 0.46 kJ/min (4.16 ± 0.11 kcal/min) for the energy expenditure of walking in the fed state. No significant differences were found between these values (P=0.79). The energy expenditure of walking in the fed state showed a 1 percent increase above that measured while the subjects were in the fasting state. The PP-MR measured 20 min after ingestion of a meal and before the second phase of walking on the treadmill was 4.94 ± 0.08 kJ/min (1.18 ± 0.02 kcal/min), about 24 percent increase in metabolic rate above the BMR (P=0.18).

PP-MR in exercising subjects and in those who did not perform any exercise

No significant differences were found when the PP-MR values measured at 45, 95 and 150 min after exercise for the subjects in protocol 1 were compared with those measured at the corresponding times for the subjects who did not perform any exercise (chapter 4). The P values were, respectively, 0.40, 0.70 and 0.84. Similarly, for protocol 2, the mean PP-MR measured 20 min following ingestion of a meal and before the second phase of walking on the treadmill did not differ significantly (p=0.18) from that obtained for the subjects who did not perform any exercise as described in chapter 4.

6.4 DISCUSSION

These results suggest that exercise does not potentiate TEF, since no significant differences were found in energy expenditure between walking in the fasting state and walking in the fed state. Further evidence is provided by the lack of significant difference between BMR and pre-prandial RMR measured 30 min after exercise. Moreover, the post-prandial RMR values measured after 15 min (protocol 2) and 40 min (protocol 1) of walking on the treadmill at 4.8

Table 14. Energy expenditure of walking in the fasting and in the fed state in subjects (n=17) who followed protocol 2.

	BMR kJ/min	energy expenditure of walking fasting kJ/min	post prandial RMR* kJ/min	energy expenditure of walking fed kJ/min
Mean	3.98	17.24	4.94	17.41
SE	0.20	0.50	0.07	0.47

BMR= Basal metabolic rate; RMR= resting meatbolic rate.

* Measured 40 min after ingestion of a meal and before the second bout of exercise.

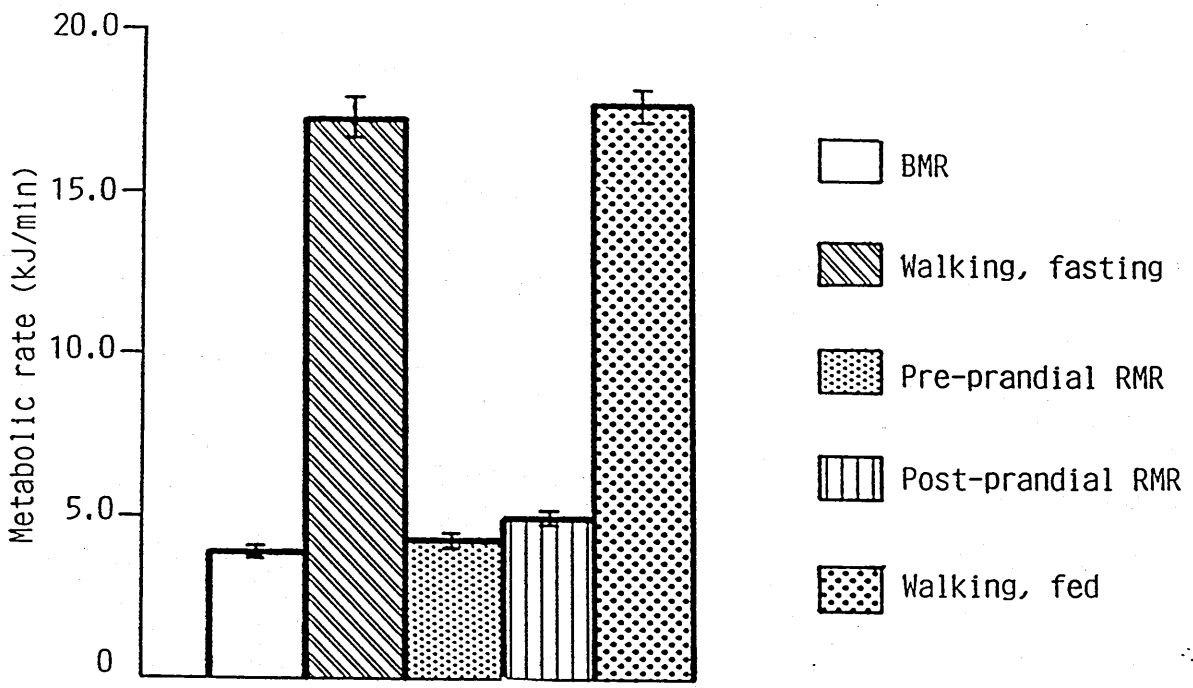


Fig. 14. Energy expenditure of walking in the fasting and fed state and resting metabolic rate (RMR) in kJ/min (Mean ± SE) in subjects who followed protocol 2 (n=17).

km/h at zero percent slope were not significantly different from those measured at corresponding times in subjects who did not perform any exercise (Chapter 4).

According to the hypothesis of potentiation of TEF by exercise, one would have expected to observe a significantly higher energy expenditure of walking in the fed state compared to the fasting state. But this was not observed in the present study, suggesting that the effect of meal on metabolic rate was temporarily curtailed and that of exercise maintained. It is therefore evident that performance of exercise resulted in a lower TEF. This finding is consistent with the results from other studies (Dalloso and James, 1984; Welle, 1984; Belko *et al.*, 1986; Schutz *et al.*, 1987). Schutz *et al.* (1987) have shown that exercise did not potentiate TEF in elderly men. Nevertheless, other workers have reported that exercise significantly potentiated the TEF (Miller and Mumford, 1967; Miller and Wise, 1975; Young *et al.*, 1986). The reasons for these differences are obscure. It has been reported that the type of exercise employed may partly account for the different results between laboratories.

Failure to observe any increase in TEF could be attributed to various physiological processes that occur during exercise. The functions of the gastro-intestinal tract, primarily secretion and absorption are achieved through adequate blood flow . Under resting conditions gastric blood flow increases markedly when acid secretion is increased particularly after ingestion of a meal (Hellebrandt and Hoopes, 1934; Davenport, 1977). Intestinal blood flow also increases after meal and remains elevated by about 30 percent above pre-meal levels for several hours (Hellebrandt *et al.*, 1934) . If the gastro-intestinal tract blood flow is inadequate, these functions are also markedly reduced. In the resting state, the gut and liver receive about 25 percent (Davenport, 1977; Vander *et al.*, 1987) of the total blood supply to the body. This is considered to be the highest compared to that supplied to other organs and tissues of the body. During exercise, even mild walking, blood supply to the gut is reduced while that to the muscles is increased. The increase in blood flow to the muscle is due to elevated cardic output and to arteriolar dilatation in the exercising muscles. Simultaneously, vasoconstriction occurs in the splanchnic and renal vascular beds and their blood flow tends to decline in proportion to the severity of exercise. Because of reduced blood flow which occurs in the gastro-intestinal tract during exercise, gastro-intestinal functions such as secretion, digestion and absorption are also

reduced. It is therefore reasonable to speculate that exercise might not significantly potentiate TEF.

Furthermore, during exercise, release of insulin has been shown to be significantly reduced (Harris, 1966;). Because most of the energy required by exercising muscles is provided through the oxidation of glycogen and fatty acids stored in the muscles, the contribution of the liver and gastrointestinal tract to total carbohydrate utilisation during exercise is small (Bergstrom *et al.*, 1967). The claim that potentiation of TEF by exercise could be due to the increased demand for glycogen and glucose by the exercising muscle and therefore increased liver activity (Welle, 1984) could not be supported by the study of Bergstrom and Hultman (1967) and Wahren *et al.*, (1971) and also by the results obtained in the present study. Since PP-MR measured for 2.5 h after the cessation of exercise in the present study was not significantly different from that measured in subjects who did not perform exercise, this may suggest that there was no greater demand for glycogen. The study by Bergstrom and Hultman, (1967) has shown that depending on the type of diet and the severity of exercise, glycogen levels in the muscles may take 2-3 days to increase back to the pre-exercise levels. However, Piehl (1974) observed that there was a marked increase in the storage of glycogen in the muscle during the first 5 h after exercise, depending on the severity of exercise.

It has been shown (Hellebrandt and Hoopes, 1934; Ramsbottom and Hunt, 1974) that all types of exercise delay the completion of the digestive cycle and the emptying time especially when exercise is done immediately after a meal, and that the extent of the delay of these processes vary with the severity of exercise. Additionally, these functions have been shown to be suppressed even during recovery from exercise (Hellebrandt and Tepper, 1934). These points may help to explain why there were no significant differences between PP-MR with exercise and PP-MR without exercise.

In conclusion, the results of the present study have shown that there was a significant reduction of TEF due to exercise, and that , exercise did not potentiate TEF when this was measured for several hours after cessation of exercise.

CHAPTER 7

EFFECT OF BODY COMPOSITION ON THE THERMIC EFFECT OF FOOD

7.1 INTRODUCTION

Low TEF in obese individuals has generally been suggested as an important contributory factor to the development of obesity (Jequier and Schutz, 1985 ;Jequier, 1987). However, evidence to support such views is conflicting. While some studies (Table 3) have shown that TEF is lower in obese subjects than in lean subjects, other investigations have found no significant differences in TEF between obese and lean subjects (Table 4.).

The purpose of the experiments described here was to examine the effect of body composition on TEF using meals of different composition (HCLF and LCHF) and energy content (2520 and 5040 kJ). Additionally, it was of interest to compare the data obtained here with those obtained in non-obese subjects in chapter 4 in order to determine whether body composition might influence TEF.

7.2 SUBJECTS AND METHODS

Subjects

A total of twenty one obese female subjects participated in the study. None of the subjects was on a slimming programme, was diabetic or hypertensive (personal history). Their physical characteristics were determined as described in chapter 3 and are presented in Table 15. There were no significant differences in terms of age ($p=0.25$), weight ($p=0.20$), height ($p=0.46$), body fat content ($p=0.55$) and FFM ($p=0.26$) between subjects who ingested a HCLF (group A) and those who ingested a LCHF meal (group B). However, body fat content measured by skinfold thickness was 20 percent lower than that measured by underwater weighing.

To make comparison of TEF between obese subjects and non-obese subjects, the data obtained for non-obese subjects in chapter 4 were used here. There were significant differences in some of the physical characteristics (Table 16) between the obese subjects used here and the non-obese subjects described in chapter 4. The mean body weight of obese subjects was 43

Table 15. Physical characteristics of obese subjects.

	Age (Years)	Body Weight (kg)	Body Height (m)	Fat (%)*	FFM (kg)	BMI (kg/m2)
Group A (n=10)						
Mean	37.9	78.3	1.62	39.0	47.2	30.0
SD	13.6	11.4	0.07	7.27	5.7	3.5
Range	17-48	62.4-93.3	1.50-1.72	35-44	37.7- 55.6	26- 36
Group B (n=11)						
Mean	31.8	84.6	1.64	40.9	49.7	31.8
SD	7.9	10.3	0.05	4.6	3.9	4.6
Range	20-47	71.3-100.6	1.54-1.73	37-50	42.8-55.2	25-38

FFM= Fat free mass; BMI= body mass index.

* Estimated by underwater weighing using Siri's (1956) equation.

Table 16. Physical characteristics of obese and non-obese** subjects

	Age (years)	Body Weight (kg)	Height (m)	BMI kg/m2)	Body Fat (%)	FFM (kg)	W:H ratio	A:H ratio
Obese (n=21)								
Mean	34	79.9	1.63	31	42	48.3	0.83	0.99
SD	11.0	11.9	0.04	4.1	4.1	5.5	0.09	0.09
Range	17-48	61-101	1.5-1.7	25-38	34-50	38-56	0.8-1.0	0.8-1.2
Non-obese** (n=16)								
Mean	22	55.8	1.64	21	24	42.6	0.72	0.87
SD	6.0	3.6	0.04	0.16	2.4	3.2	0.04	0.04
Range	18-40	50-62	1.5-1.4	19-24	20-28	39.0-48	0.6-0.8	0.8-1.0

BMI= Body mass index; FFM= fat free mass; W:H= Waist:hip ratio; A:H= Abdominal hip ratio.

** from chapter 4

percent greater than that of non-obese subjects (p=0.001), body fat was 75 percent (p=0.001) and FFM was 13 percent higher in obese (p=0.001) than in non-obese subjects. However, there were no significant differences in height between non-obese and obese subjects (P=0.68).

Meals

Four different meals were used. The composition of the meals was calculated as described in chapter 3 and are presented in Table 5. The test meals used here were similar to those used in the other experiments (chapter 4).

Experimental design

To examine whether the composition of the test meals might have a significant influence in TEF of obese subjects, twenty one obese subjects were divided in to two groups for experimental purposes. Subjects in group A (n=10) consumed high carbohydrate -low fat meals (HCLF: A and C) and those in group B (n=11) consumed low carbohydrate-high fat meals (LCHF: B and D) . Each obese subject consumed two different test meals, each meal on a separate occasion.

Methods

Both BMR and PP-metabolic rate were determined as described in chapter 3 and chapter 4.

Statistical analysis

The effect of body composition, meal composition and energy content on TEF were analysed by 3 x 3 factorial analysis of variance using the Genstat Statistical package (Alvey *et.al.*, 1983). Unpaired Students' t-test was used to assess differences in physical

characteristics between the obese subjects as measured here and those of non-obese subjects described in chapter 4. The results are presented as Mean ± Standard error, unless otherwise stated.

7.3 RESULTS

Basal Metabolic Rate

The mean BMR values (Table 17) were 4.23 ± 0.16 kJ/min (1.01 ± 0.04 kcal/min) and 4.77 ± 0.13) kJ/min (1.14 ± 0.03 kcal/min) for subjects who consumed a HCLF (group A) and a LCHF (group B), respectively. These values were equivalent to 6086 ± 234 kJ/day (1454 ± 56.0 kcal/day) and 6873 ± 184 kJ/day (1642 ± 44.0 kcal/day) for group A and B, respectively. The difference was significant (p=0.016). When BMR values were expressed per kg FFM they were found to be 130 ± 4.6 kJ/kg FFM/day (31 ± 1.1 kcal/kgFFM/day) and 137 ± 3.76 kJ/kgFFM/day (32.7 ± 0.90 kcal/kgFFm/day) , the difference was not significant.

The mean BMR values presented in Table 18 include the mean BMR values for non-obese subjects presented in chapter 4. The mean BMR values of obese subjects were significantly (p=0.001) higher (25 percent) than those of non-obese subjects. When BMR values were expressed per kg of body weight, they were found to be 25 percent lower in obese subjects than in non-obese subjects (p=0.001). However, BMR values expressed per kg of FFM were 11 percent higher in obese subjects (p=0.001) than in non-obese subjects (p=0.001), this will be discussed later.

Table 17. Basal metabolic rate (BMR) in obese subjects

	Group A (n=10)		Group B (n=11)	
	Mean	SE	Mean	SE
BMR (kJ/min)	4.23	0.16	4.77	0.13
(kcal/min)	(1.01)	(0.04)	(1.14)	(0.03)
BMR (kJ/day)	6086.0	234.0	6873.0	184.0
(kcal/day)	(1454.0)	(56.0)	(1642.0)	(44.0)
BMR (kJ/kgFFM/day)	130.0	4.6	137.0	3.8
(kcal/kgFFM/day)	(31.0)	(1.1)	(32.8)	(0.9)

FFM= Fat free mass.

Table 18. Basal metabolic rate (BMR) in obese and non-obese** subjects

	Obese (n=21)		Lean**(n=16)	
	Mean	SE	Mean	SE
BMR (kJ/min)	4.55	0.13	3.63	0.07
(kcal/min)	(1.09)	(0.03)	(0.88)	(0.02)
BMR (kJ/day)	6560	172	5233	103
(kcal/day)	(1567)	(41)	(1250)	(25)
BMR				
(kJ/kgbwt/day)	80	1.9	92	1.5
(kcal/kgbwt/day)	(19)	(0.5)	(22)	(0.4)
BMR				
(kJ/kgFFM/day)	134	2.9	121	1.9
(kcal/kgFFM/day)	(32)	(0.7)	(29)	(0.5)

kgbwt= kg body weight; kgFFM= kg fat free mass;

** From chapter 4.

Thermic effect of food in obese subjects

There was a significant increase in metabolic rate immediately after ingestion of a meal in all subjects. The overall increase in metabolic rate above BMR (Fig. 15) averaged 20 and 12 percent for the HCLF and LCHF 2520 kJ (600 kcal) meals, respectively and that for the 5040 kJ (1200 kcal) meals were, respectively, 24 and 20 percent. The overall increase in metabolic rate above BMR was slightly higher for the HCLF than for the LCHF meals containing 2520 kJ, but the difference did not attain statistical significance (P=0.10).

The mean 5 h TEF values are presented in Table 19 and Fig. 16. The mean 5 h TEF values of subjects who consumed a HCLF meals were 201.0 ± 15.0 kJ (48 ± 3.6 kcal) and 288.0 ± 16 kJ (68.8 ± 3.8 kcal) for the 2520 kJ (600 kcal) and 5040 kJ (1200 kcal) meals, respectively. The corresponding values for subjects who consumed a LCHF meals were 159 ± 20 kJ (38.0 ± 4.8 kcal) and 272 ± 36 kJ (65.0 ± 8.6 kcal). Meal composition did not have any significant effect on TEF (p=0.09). However, the energy content of the ingested meals had a significant effect on TEF (p=0.005). Meals containing 5040 kJ (1200 kcal) had a higher TEF compared to meals containing 2520 kJ (600 kcal) in both groups of subjects. There was no significant (p=0.247) interaction between meal composition and energy content of the ingested meals in influencing TEF.

Thermic effect of food in obese and non-obese subjects

Results obtained in this study were then compared to those obtained in non-obese subjects (chapter 4). All subjects (obese and non-obese) showed significant increase in metabolic rate after ingestion of a meal (Fig 17 and 18). However, the magnitude of the increase was found to be smaller in obese than in non-obese subjects.The mean 5 h TEF values are presented in Table 20 and Fig. 19. The mean 5 h TEF values in obese subjects after ingestion of a LCHF (2520 kJ) and both the HCLF and LCHF meals at 5040 kJ were significantly lower by about 20 percent than those obtained in non-obese subjects (p=0.001).

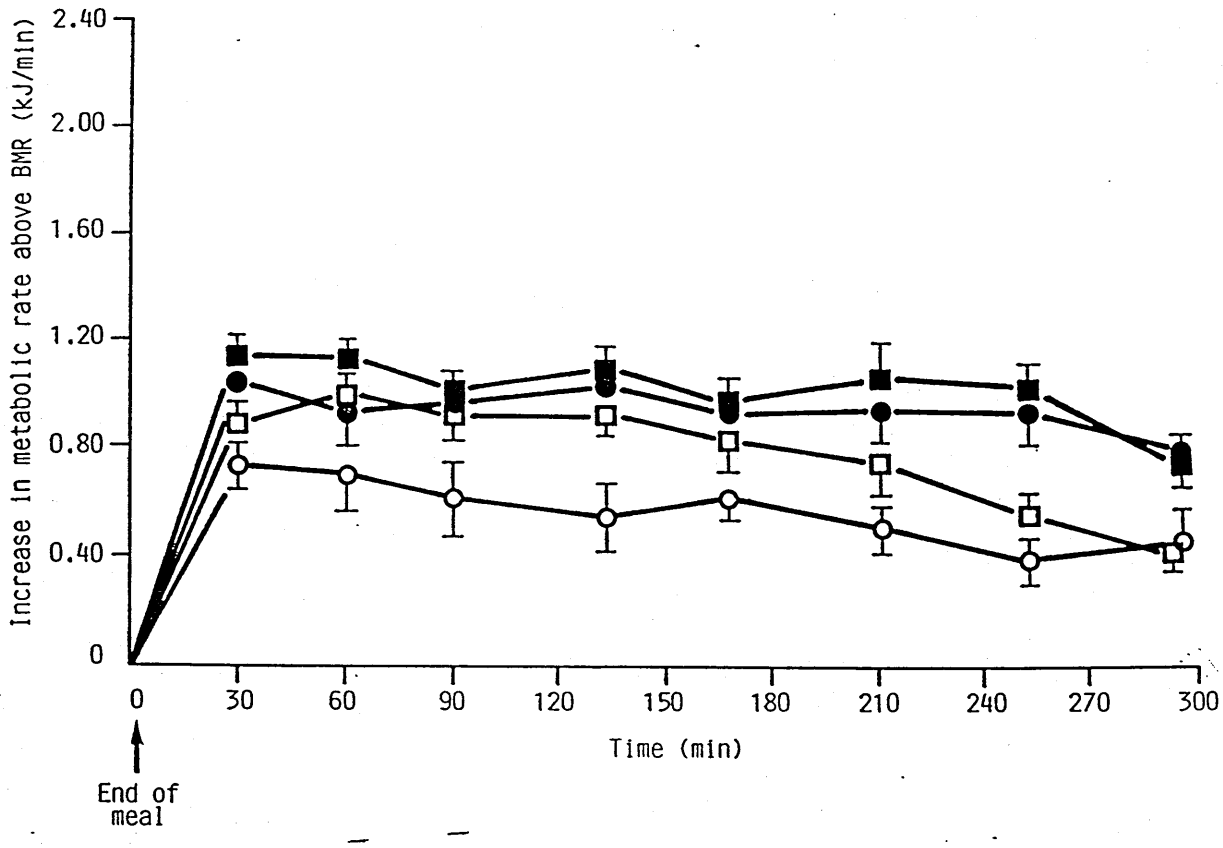


Fig. 15. Increase in metabolic rate (MR) above basal metabolic rate (BMR) in kJ/min (Mean ± SE) in obese subjects after ingestion of four different test meals: high carbohydrate-low fat (HCLF; n=10) at two levels of energy intake 2520 kJ (600 kcal) (□) and 5040 kJ (1200 kcal) (■) and low carbohydrate-high fat (LCHF; n=11) at two levels of energy intake 2520 kJ (600 kcal) (O) and 5040 kJ (1200 kcal) (●).

Table 19. Thermic effect of food (TEF) in obese subjects.

	Group A (n=10)				Group B (n=11)			
	2520 kJ		5040 kJ		2520 kJ		5040 kJ	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TEF (kJ)	201.0	15.0	288.0	16.0	159.0	20.0	272.0	36.0
(kcal)	(48.0	3.5	68.9	3.8	38.5	4.8	64.7	8.6)
TEF % EI	8.0	0.6	6.0	0.3	6.0	0.7	5.0	0.3

TEF= Thermic effect of food; EI= Energy intake.

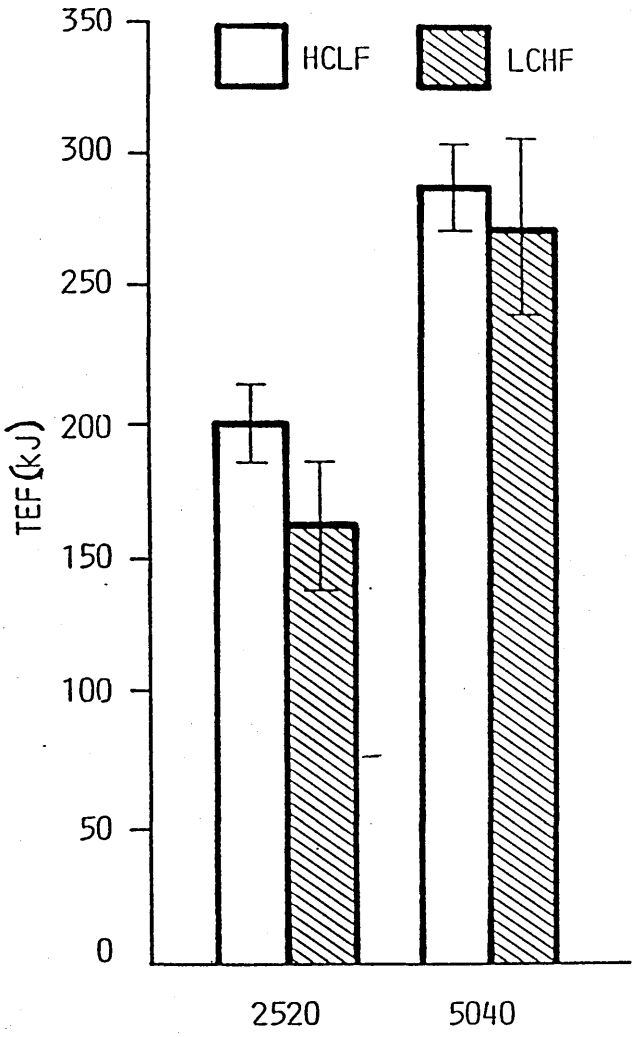


Fig. 16. Thermic effect of food (TEF) in kJ (Mean ± SE) in obese subjects after ingestion of high carbohydrate-low fat (HCLF; n=10) and low carbohydrate-high fat (LCHF; n=11) at two levels of energy intake 2520 kJ (600 kcal) and 5040 kJ (1200 kcal).

Table 20. Thermic effect of food (TEF) in obese (n=21) and non-obese** (n=16) subjects after ingestion of a high carbohydrate -low fat (HCLF) meal containing 2520 kJ (A) (600 kcal) and 5040 kJ (C) (1200 kcal) and a low carbohydrate-high fat (LCHF) containing 2520 kJ (B) (600 kcal) and 5040 kJ (D) (1200 kcal).

	Obese (n=21)		Lean(n=16)	
	Mean	SE	Mean	SE
TEF (kJ)				
meals				
A	201.0	15.0	228.0	12.0
B	159.0	20.0	228.0	10.0
C	288.0	16.0	356.0	21.0
D	272.0	36.0	340.0	16.0
TEF % EI				
meals				
A	8.0	0.6	9.0	0.5
B	6.0	0.7	9.0	0.6
C	6.0	0.3	7.0	0.4
D	5.0	0.3	7.0	0.3

EI= Energy intake

** From chapter 4.

However, that of the 2520 kJ (600 kcal) HCLF was found to be comparable to that measured in non-obese subjects (p=0.58). The reason for this could not established.

The 5 h TEF values expressed as percent of energy intake of 2520 kJ (600 kcal) were 6-8 percent in the obese subjects and 9 percent in the non-obese subjects. For the 5040 kJ (1200 kcal) these were 5-6 percent in the obese subjects and 7 percent in the non-obese subjects. In both non-obese and obese subjects meal composition did not have any significant (p=0.209) effect on TEF. Similarly energy content had a significant effect on TEF in both non-obese and obese subjects (p=0.206). Lack of significant interaction between meal composition and energy content in influencing TEF was observed in both non-obese and obese subjects (p=0.66). In addition, the correlation coefficients between TEF and age, body weight, fat free mass and fat mass did not suggest any meaningful association. This could be due to small sample size particularly that of obese subjects.

About 24 percent of the obese subjects had TEF values which were comparable to those measured in non-obese subjects.

7.4 DISCUSSION

Basal Metabolic Rate

The BMR of obese subjects expressed as absolute values was found to be higher than that of non-obese subjects. This could be due to their large FFM associated with increased body fat content compared to that of non-obese subjects (Jequier, 1987). Several other studies have reported similar results (Table 3 and 4).

In this study, BMR of obese subjects expressed per kg FFM was also higher than that of non-obese subjects. This was surprising because it is known that such differences are not usually seen when BMR is expressed per kg FFM (Webb, 1981). The high BMR values per kg FFM in obese subjects are due to their higher FFM compared to non-obese subjects. This should be expected, because during the development of obesity both body

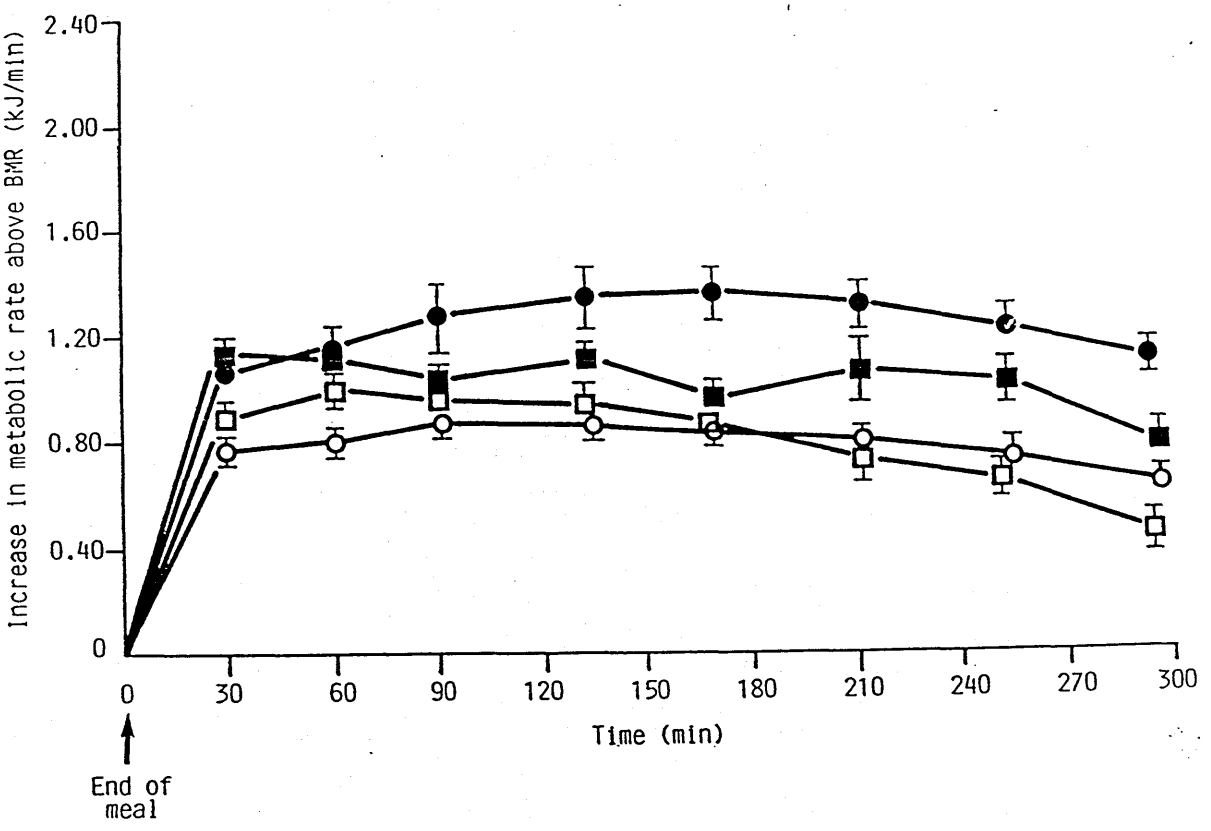


Fig. 17. Increase in metabolic rate (MR) above basal metabolic rate (BMR) in kJ/min (Mean ± SE) after ingestion of high carbohydrate-low fat (HCLF) meals at two levels of energy intake 2520 kJ (600 kcal) (open symbols) and 5040 kJ (1200 kcal) (closed symbols) in obese (n=10) ([]) and non-obese (n=16) (O) subjects.

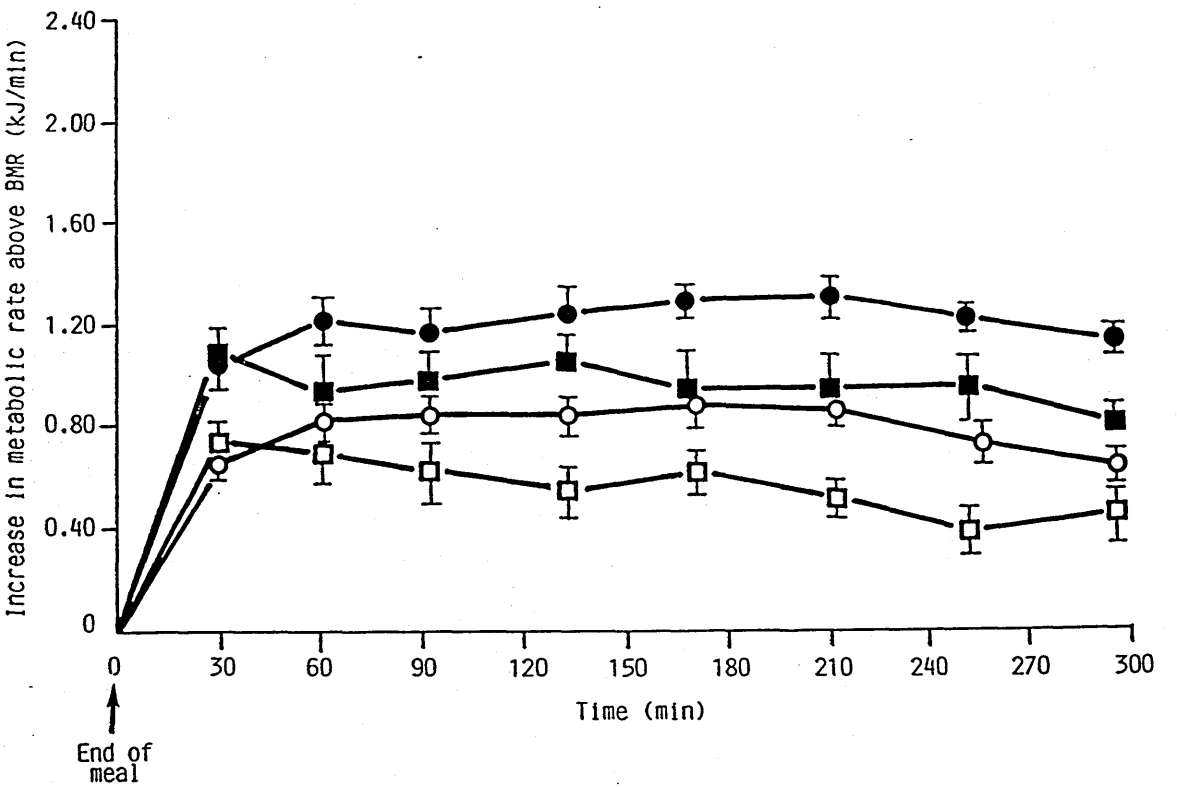


Fig. 18. Increase in metabolic rate (MR) above basal metabolic rate (BMR) in kJ/min (Mean ± SE) after ingestion of low carbohydrate-high fat meals (LCHF) at two levels of energy intake 2520 kJ (600 kcal) (open symbols) and 5040 kJ (1200 kcal) (closed symbols) in obese (n=11) ([]) and non-obese (n=16) (O) subjects.

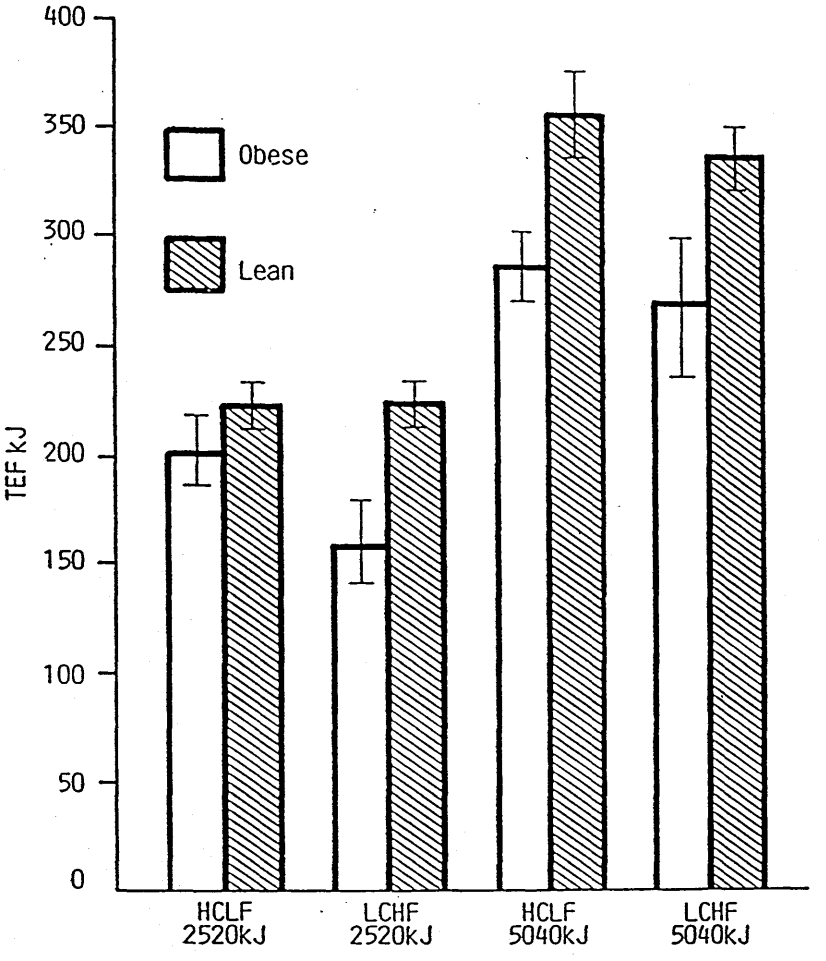


Fig. 19. Thermic effect of food (TEF) in kJ (Mean ± SE) in obese (n=10 HCLF; n=11 LCHF) and non-obese (n=16) subjects after ingestion of four different test meals: high carbohydrate-low fat (HCLF) and low carbohydrate-high fat (LCHF) at two levels of energy intake 2520 kJ (600 kcal) and 5040 kJ (1200 kcal).

fat and FFM increases (James *et al.*, 1978; Ravussin *et al.*, 1982) . It is most likely that the high BMR values in the obese could be attributed to the increased FFM accompanying the increased adipose tissue (Jequier, 1987). However, when BMR was expressed per kg body weight, it was found to be lower in obese than in non-obese subjects. It has been reported (FAO/WHO/UNU, 1985) that BMR per unit body weight tends to vary with weight in that BMR per kg is higher in short and light individuals, and lower in tall and heavy individuals. This is probably related to the fat/FFM ratio per kg body weight. The fat/FFM in obese subjects is higher than in non-obese subjects and this may partly account for the low BMR expressed per kg body weight in obese subjects.

Thermic effect of food

Effect of meal composition on TEF

The results of the present study have shown that meal composition did not have any significant influence on TEF. The 5 h TEF values for subjects who consumed HCLF meals were not statistically different from those for subjects who consumed LCHF meals. These results are consistent with those reported in other studies (Pittet *et al.*, 1976; Segal 1987; Steiniger *et al.*, 1987) and also those reported in this thesis chapter 4, but not with those reported in some other studies (Swaminathan *et al.*, 1985; Schwartz *et al.*, 1985; Zed and James, 1986). In these studies, it was found that TEF of high fat meals were significantly lower compared to either high carbohydrate meals (Schwartz *et al.*, 1985) or high protein meals (Swaminathan *et al.*, 1985; Zed and James, 1987). Furthermore, the TEF of high fat meals have been shown (Schutz *et al.*, 1984; Zed and James, 1986) to be inversely correlated to fat mass. They also established that the TEF of a fat meal is much lower in individuals with familial obesity. In the present study, however, TEF in obese subjects did not show any significant correlation with body fat content, despite admission by some of the subjects that they have been obese all their life. Den Besten *et al.* (1988) also made a similar observation. In this study they observed that the TEF in subjects with abdominal obesity was slightly higher but not significant compared to subjects with

gluteal-femoral type of obesity. However, the TEF of the HCLF (2520 kJ) in the present study was found to be higher than that of the LCHF meal at the same energy level the reason for this could not be established, since that of the 5040 kJ did not show any significant differences between the HCLF and the LCHF meals.

Effect of energy content on TEF

The energy content of the ingested meal had a significant effect on 5 h TEF. In both meal compositions the 5 h TEF values of small meals (2520 kJ; 600 kcal) were lower than those of the large meals (5040 kJ; 1200 kcal). These results are in accord with the results reported by Steiniger *et al.* (1987) and those which were obtained in non-obese subjects (chapter 4). These results seem to suggest that TEF is influenced by the energy content of the ingested meal. Since TEF is a measure of the energy cost of processing the ingested food, it seems likely that the magnitude of TEF will tend to vary depending on the amount of food to be processed.

When TEF was expressed as a percent of the ingested energy, it showed that obese subjects expended only a small proportion of the ingested energy in digestion , absorption and assimilation processes in the body. Considering the amount of energy ingested, this observation suggests that there was a large proportion of the ingested energy was available for storage. This would imply that some obese subjects are capable of utilising energy more efficiently compared to non-obese subjects. TEF therefore, could be considered as an indicator for characterising and identifying individuals with high energy efficiency.

Subject variability

There was wide inter-subject variability in the 5 h-TEF values. However, the variation was more marked in subjects who consumed LCHF meals (Coefficient of variation 39-42 percent) than in subject who consumed HCLF meals (Coefficient of variation 17-19 percent). The reasons for the difference between groups are unclear, since there were no significant differences in the physical characteristics of the subjects between

the two groups. Moreover, there were no significant correlations between TEF and any of the physical characteristics of the subjects, suggesting that these parameters are not correlated with TEF. However, the wide inter-individual variability could be due to inherent differences in digestion and storage process.

TEF in obese and non-obese subjects

To test the hypothesis whether TEF is lower in obese than in non-obese subjects, results obtained in this study were compared with those obtained in non-obese subjects in the study described in chapter 4. It was found that in all meal compositions and at both levels of energy intake the 5 h TEF values for obese subjects were significantly lower than those for non-obese subjects. This was also true when TEF was expressed as percent of energy intake. These results seem to suggest that obese subjects spend less energy in digestion, absorption and assimilation processes compared to non-obese individuals. What this implies is that obese subjects are more efficient than non-obese subjects in converting ingested energy in to stored energy.

Several factors may account for the low TEF in obese subjects. Those considered to play a significant role include insulin resistance (Golay, 1982; Borgadus *et al.*, 1985) and low thermogenic response to noradrenalin (Jung *et al.*, 1979; O'dea, 1982; Schutz *et al.*, 1984). Moreover, genetic factors have also been implicated (Ravussin *et al.*, 1985), and this could also account for the wide inter-individual variations in TEF seen here, particularly for the obese subjects. It may be speculated that the low TEF in obese subjects could also be an adaptive mechanism to conserve energy to meet the high demand for energy created by the increased body size. Since the level of energy expenditure is high in obese (BMR and physical activity), it is pertinent to assume that their energy requirements would also be high. And to meet the new level of energy requirements perhaps on the same level of energy intake obese subjects would need to adjust their energy expenditure, and one possible way of doing that would be to reduce energy expenditure due to TEF.

Duration of TEF

In both obese and non-obese subjects the 5 h TEF values measured were underestimates of TEF. This is because the PP-MR at the end of 5 h for the 2520 kJ (600 kcal) meals was still 10 percent and 18 percent above the pre-meal level for obese and non-obese subjects, respectively, and the corresponding values for the 5040 kJ (1200 kcal) meals were 18 percent and 30 percent (Fig. 17 and 18). These results suggest that TEF in obese subjects is of smaller magnitude and shorter duration compared to that of non-obese subjects. There has been reports (Swaminathan *et al.*, 1985; Zed and James, 1986) to suggest that the low TEF in obese subjects could be due to delayed thermogenic response after a meal. However, the results of the present study do not support this hypothesis. This is also supported by the observations made by Jequier and Schutz (1983) and Schutz *et al.* (1984) . In these studies they measured TEF in both obese and non-obese subjects using a respiration chamber for 15 h (Jequier and Schutz, 1983) and 24 h (Schutz et al., 1984) and found that the TEF was significantly and consistently lower in obese subjects than in non-obese subjects, and that there was no delayed response in PP-MR.

In conclusion, the results of the present study have demonstrated that TEF is influenced by body composition and energy content of the ingested meal but not by meal composition.

CHAPTER 8

GENERAL DISCUSSION

The studies described in this thesis were carried out to examine the factors which may influence the magnitude of TEF in humans. Knowledge of the factors which influence TEF is important in understanding the role of TEF in the overall energy balance. Moreover, such knowledge is valuable in estimating human energy requirements and for designing feeding regimens for individuals on parenteral nutrition.

The factors examined in the present studies included, meal composition, meal frequency, energy content of the meal, exercise and body composition.

It is evident from these studies that TEF constituted a considerable proportion of energy expenditure. However, it was shown that the magnitude of the TEF was not significantly influenced by the composition of the ingested energy. In addition, there were no interactions between meal composition and energy content of a meal, meal frequency or body composition in influencing the TEF. Lack of significant influence of meal composition on TEF suggest that meal composition is not an important factor in influencing the magnitude of TEF.

According to Flatt (1978), the energy cost of digestion and storage of nutrients has been shown to vary depending on the type of nutrient to be stored. It was found to be higher with protein then carbohydrate and least with fat. However, this conclusion was based on theoretical calculations based on the chemical structure of the nutrient. Studies (Table 1 and 2) using real foods have produced conflicting results. The results of the present studies do not support the hypothesis that the TEF of a high fat meal is significantly lower compared to that of a high carbohydrate meal, as once believed to be (Lusk, 1928).

It is possible that differences in body composition between individuals who consume different diets may be attributed to the total energy intake associated with a particular diet and not on the low TEF of that diet. Because most carbohydrate foods are bulky and have low energy density, effective energy intake from high carbohydrate meals tend to be lower compared to that from high fat foods. Moreover, high fat foods are more palatable and less bulky and therefore there is a tendency for people to eat more. Thus, it is quite easy to attain a positive energy balance on a high fat meal than it is with a high carbohydrate meal.

Although some studies (Dauncey and Bingham, 1983; Zed and James, 1986) have shown that the TEF is higher with protein meals compared to carbohydrate or fat meals, others

(Garrow and Hawes, 1972; Pittet *et al.*, 1974; Belko *et al.*, 1986) have shown that TEF is not influenced by the protein content of the meal. In the present studies the protein content of the test meals was maintained constant and within the recommended level of protein intake (FAO/WHO/UNU, 1985).

Another factor examined in the present studies was the effect of meal frequency on TEF. The main objective of this study was to find out whether ingestion of isoenergetic meals either as one large meal or two smaller meals would influence the TEF, hence affect energy balance.

The results have shown that there were no significant differences in TEF between the two feeding regimens, suggesting that the TEF is not influenced by meal frequency. Furthermore, failure to observe any significant differences between meals of different composition (HCLF and LCHF) in this study indicates that TEF is not affected by meal composition. The feeding regimen of two meals showed that there was superimposition of the TEF of one meal into that of the second meal. This observation may suggest that under an average feeding pattern of three meals per day the TEF of one meal merges into that of next meal. However, the total TEF did not differ from that of a single meal regimen. Lack of significant differences between the two feeding regimens implies that TEF is related to the energy content of the ingested meal. The results obtained in the present study are in accord with those reported in other studies (Swindells, 1968; Garrow *et al.*, 1981; Dalloso *et al.*, 1982; Belko and Barbieri, 1987). These results taken together, seem to suggest that meal frequency is not an important determinant of TEF. These findings do not support the view that a more frequent meal pattern stimulates more thermogenesis hence less fattening than a less frequent meal pattern. The observation by Hipsley (1964) in New Guinea that there were no signs of obesity in a population where people are practically on a feeding regimen of two meals per day and sometimes one meal per day, is evidence that positive energy balance is not related to the patterning of energy intake. The overall relationship between energy intake and total energy expenditure is what determines energy balance.

It was also shown, in the present studies that the TEF was significantly influenced by the energy content of a meal. In addition, lack of significant differences in TEF between the

two feeding regimens as observed in the meal frequency study, further suggested that the magnitude of TEF depends on the energy content of the ingested meal. This was because total energy ingested in both feeding regimens was same (5040 kJ; 1200 kcal) . From these results it is evident that TEF is related to the energy content of the ingested meal, but the exact relationship is not clear. The study by Hill *et al.* (1984) suggested a quadratic relationship between TEF and the energy content of the meal. In this study they observed that by progressively increasing the energy content of the meal from 2000-6000 kJ , the TEF increased, but not in a linear fashion. Unfortunately, the duration of the measurement was not extended for long enough time to allow for the metabolic rate to return to a basal level. Since TEF measured after each meal did not represent total TEF, the non-linear relationship observed may not be surprising. It is probable that the relationship could have been linear if total TEF was measured. The TEF measured in the present studies was also underestimated, since none of the subjects studied had their metabolic rate returned to a basal level 5 to 6 h after completion of the meal.

Since TEF is largely due to the energy cost of digestion and storage of the ingested nutrients in the body, it is most likely that the energy cost of these processes, and thus TEF would vary depending on the amount of food (energy) to be processed. Although TEF has been shown to vary with the energy content of the meal, the overall energy balance, however, will depend on the relationship between energy intake and total energy expenditure. Positive energy balance is inevitable whenever high TEF due to large meal is not coupled with increased energy expenditure.

In the study to examine the effect of body composition on TEF, it was shown that TEF values in obese subjects were lower compared with those measured in non-obese subjects. These results suggest that body composition is an important determinant of TEF. The observation that TEF is lower in obese subjects support the hypothesis that obese subjects have a thermogenic defect which favour weight gain (Bessard et al., 1983; Jequier, 1987). But it is not clear from these results whether the defect is a consequence of obesity or a cause of obesity. Other studies have reported that post obese subjects have lower BMR and TEF compared to non-obese subjects (Bessard *et al.*, 1983; Geissler *et al.*, 1987; Shah *et al.*, 1988) this may

explain at least in part that the low TEF in obese subjects is a cause of obesity not a consequence of obesity.

About 24 percent of the obese subjects in the present study had TEF values comparable to those of non-obese subjects. It is probable that not all obese individuals have low TEF. There are reports (Jequier, 1984; Simopolous, 1987) to suggest that obesity is a heterogeneous condition, elicited by various aetiologies. It is possible that among the obese individuals, there are those who utilise energy more efficiently and therefore gain weight without overeating and others who gain weight by overeating but with normal energy expenditure. Measurement of TEF can be considered as a suitable indicator for characterising people with high efficiency of energy utilisation.

The reasons for the low TEF in obese subjects are not clear. Insulin resistance (Ravussin *et al.* 1985) and blunted noradrenalin response (Jung *et al.*, 1980) have been suggested as possible mechanisms responsible for the low TEF in obese. A defect in the synthesis of some enzymes (Pilkis *et al.*, 1986; Goodridge, 1986) particularly those catalysing processes of energy metabolism could also be a possibility.

Another factor investigated was exercise. The main objective of this study was to find out whether exercise potentiates TEF. It was found that exercise did not potentiate TEF. Actually, performance of exercise after meal cuased a reduction in TEF.

The suggestion that TEF is influenced by exercise is surprising, because the two are controlled by different and opposing mechanisms. Performance of exercise immediately after meal interferes with blood distribution which in turn slows down digestion processes. This may explain why there were no differences in the energy expenditure of walking before and after ingestion of a meal. Indeed, exercise caused a reduction in TEF. Furthermore, PP-MR measured for 2.5 h after meal and after the cessation of exercise was not different from that measured in subjects who did not perform any exercise. The results do not support the hypothesis that exercise has a prolonged effect on RMR and that it can potentiate the TEF. It is unlikely therefore, that this could be recommended as a therapy in slimming programmes (Bingham *et al.*, 1989).

In the present studies, the cause of the within- subject variability in the BMR could not be established. Although the stage of the menstrual cycle has been suggested (Solomon *et. al.*, 1982) to influence the BMR, in the present studies the stage of the menstrual cycle and familiarisation to the protocol did not seem to have any significant influence on the BMR of the subjects. The wide inter-subject variability in BMR between obese and non-obese subjects was attributed to the differences in body composition (Owen *et al.*, 1987). However, when BMR was expressed per kilogram of fat free mass, that of obese subjects was higher than that of non-obese subjects. This is surprising because it has been suggested (Owen *et al.*, 1987) that differences in BMR between individuals of different age, body composition are not easily discernible when BMR is expressed per kilogram of fat free mass. The high BMR in obese subjects as observed in this study suggest that obese subjects have got a larger fat free mass compared to non-obese subjects (Jequier, 1987).

An evident feature in these studies has been the wide inter-subject variability in TEF. The cause of the variability is not clear. However, this may be due to inherent differences in digestion and disposition of nutrients in the body and also due to differences in the level of hormones such as thyroid and insulin which have been shown to play a role in TEF. This may have important implications in energy balance and may give an indication as to which subjects are likely to become obese, given the favourable conditions.

It is evident from these studies that, the TEF measured under resting conditions constituted a sizeable increase in metabolic rate. There is a possibility, however, that the overall quantitative importance could have been underestimated because none of the subjects measured had their metabolic rate returned to a baseline 5 to 6 h after ingestion of a meal. The reasons for this prolonged effect are unknown. The presence of food in the gastro-intestinal tract provokes a series of events and reactions leading to the storage of nutrients in the body. It is pertinent to assume that the rate of these reactions will proceed at a diminishing rate until all the nutrients have been processed. This observation seems to suggest that under an average feeding pattern of three meals per day and considering the prolonged effect of food on metabolic rate as observed in the present studies, it is probable that the TEF remain elevated for a considerable period of time probably until late at night. Unfortunately, studies (Dauncey *et al.*, 1982; Schutz

et al., 1985) which have measured 24 h energy expenditure and TEF included in their protocol exercise bouts some of which had to be performed at 22.00 h.

Lack of uniformity in the techniques, protocols and sample size used in TEF studies may have contributed to the wide variation in results between different laboratories (Table 1, 2, 3 and 4). The quantitative role and importance of TEF in energy balance could be enhanced if similar methods were used and terms standardised. In addition, recent research indicate that a complex network of nutrient and hormonal factors interact to cause and regulate TEF in humans, thus multidsciplinary approach to the study of TEF should provide new insight in understanding the role of TEF in energy balance.

In conclusion, under the present experimental conditions the present studies have shown that ingestion of food cause a significant increase in metabolic rate which may attain a magnitude of about 9 percent of the ingested energy. The studies have also shown that the PP-MR may remain elevated for a longer period than once believed to be. Of the five factors investigated, the energy content of a meal and body composition had a significant influence of both the magnitude and duration of PP-MR, hence TEF. It is unlikely that meal composition and meal frequency could have any significant influence on both the magnitude and duration of TEF. However, it is appreciated that meal composition and meal frequency can have a significant influence on the overall energy balance if they influence the amount of energy intake. For instance, high fat meals due to their palatability and high energy density may have a considerable influence on energy intake. Exercise perfomed immediately after a meal resulted in a lower TEF and it did not seem to potentiate the TEF when this was measured for several hours after cessation of exercise. Although TEF varies with the energy content of a meal, a positive energy balance is inevitable if energy intake is higher than energy expenditure despite high TEF values due to large meals.

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