DISSERTATION ETH *No. 14003*

METABOLIC EFFECTS OF MEALS AND SUBSEQUENT FOOD INTAKE

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of

DOCTOR OF NATURAL SCIENCES

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ZURICH, FEBRUARY 2001

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Never assume anything!

(E. Tan)

This thesis is dedicated to my parents

Food is a very good anorexic agent, which induces a depression of hunger and inhibits eating. However one major side effect of this anorexic commodity is that it can lead to weight gain. ...

(Blundell JE, Burley VJ: Prog Obes Res (1990) Ch. 72: 453-457)

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TABLE OF CONTENTS

TABLE OF CONTENTS **5**

ABSTRACT **8**

ZUSAMMENFASSUNG **10**

INTRODUCTION **13**

EATING BEHAVIOR AND POTENTIAL INFLUENCES 13
Physiological Mechanisms of Hunger and Satiety *14*
Preabsorptive mechanisms 14
Postabsorptive mechanisms 15
Central integration 16

SCOPE 16
Study I: Fiber *17*
Study II: Fat *17*
Study III: Lactate *18*

METHODS 18
The universal eating monitor in the human studies (Studies I and II) 18
The infusion cages in the rat study 20

DIETARY FIBER IN BREAKFAST **23**

INTRODUCTION 23

METHODS 24
Subjects *24*
Test breakfasts *24*
Design and Procedure *26*
Experiment 1 26
Experiment 2 27
Questionnaires *27*
Universal Eating Monitor (UEM) *28*
Blood analyses *28*
Statistics *28*

RESULTS 29
Lunch intake *29*
Microstructure of lunch *29*
Questionnaires *31*
Plasma metabolites and hormones *32*
Experiment 1 32
Experiment 2 39

DISCUSSION 40

DIETARY FAT LEVEL AND EFFECTS OF A HIGH FAT MEAL **45**

INTRODUCTION 45

METHODS 46
General design *46*
Subjects *46*
Procedure Phase I *46*
Procedure Phase II *47*
Data transformation and statistics *50*

RESULTS 50
Subject characteristics *50*
Respiratory measurements *50*
Blood parameters *51*
Plasma glucose, insulin, and lactate 51
Plasma triglycerides (TG), free fatty acids (FFA), and β-hydroxy-butyrate (BHB) 52
Lunch intake and microstructure of eating *54*
Lunch intake and microstructure of eating *55*
Questionnaire ratings *55*

DISCUSSION 56

LACTATE AS SATIETY SIGNAL IN RATS **61**

INTRODUCTION 61

METHODS 62
Animals *62*
Surgery *62*
Test procedure *63*
Experiments *63*
Blood sampling *64*
Data collection and analysis *64*

RESULTS 65
Feeding responses to hepatic portal vein infusions *65*
Comparison between hepatic portal vein and vena cava infusions *65*
Plasma glucose and lactate in response to hepatic portal vein infusion *66*

DISCUSSION 70
Perspectives *74*

GENERAL DISCUSSION **77**

PERSPECTIVES **80**

LITERATURE **83**

CURRICULUM VITAE **91**

ACKNOWLEDGEMENTS **93**

Seite Leer /
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ABSTRACT

The control of eating behavior of humans in their natural environment is very complex. Eating behavior is affected by many nutritional, physiological, psychological, sociological and cultural factors. In the work presented in this thesis, I intended to address the physiological role of some so-called pre- and postabsorptive satiety signals. The main emphasis in this context was on short-term prandial or postprandial metabolic mechanisms, which may affect the size and duration of a meal and/or the duration of the subsequent intermeal interval.

In the first of three series of studies, the effects of four equienergetic breakfasts with varying fiber and macronutrient composition on hunger and satiety ratings, on subsequent lunch intake, and on postprandial carbohydrate and fat metabolism were investigated in normal weight male subjects. In Experiment 1, lunch was offered at a predetermined time, whereas in Experiment 2 the subjects were free to choose when to eat lunch. Consumption of either a commercially available high fiber cereal (HFC, 10% fiber), a medium fiber cereal (MFC, 7% fiber), a low fiber cereal (LFC, 3% fiber), or a standard continental breakfast (close to 0% fiber) on nonconsecutive days did not differentially affect hunger and satiety ratings, the size or microstructure of the subsequent lunch, and the breakfast to lunch intermeal interval (in Experiment 2). Plasma concentrations of glucose, lactate, and insulin increased more after the LFC breakfast than after the other breakfast varieties. A reactive postprandial hypoglycemia occurred after the LFC breakfast, shortly before lunch. The plasma concentrations of fat metabolites (triglycerides, free fatty acids, β-hydroxy-butyrate) and of glucagon were not differentially affected by the breakfast varieties. The results are consistent with the assumption that energy content of a meal is the major determinant of subsequent energy intake in man, and that fiber content and macronutrient composition have but a modulating effect.

A special experimental setup was chosen in the second study in order to investigate whether a high fat meal has different short-term effects on food intake and metabolism in man, dependent on habitual dietary fat consumption. After determining dietary fat intake in 40 subjects based on a seven day recording period, the effect of a high fat breakfast (52% of metabolizable energy from fat) on postprandial fat and carbohydrate metabolism as well as on subsequent lunch intake was investigated in 28 lean, male subjects with habitual dietary fat intake between 21 and 44% (of daily energy intake; assigned to either a low, medium or high fat group). Correlational analysis and comparisons between a low fat group (LF, fat intake $\leq$ 35%, n = 10) and a high fat group (HF, fat intake $\geq$ 40%, n = 11) with similar BMI (LF: 22.7, HF: 22.4) demonstrated that the fat level of the habitual diet did not affect the baseline values and the postprandial changes in the respiratory quotient and in the plasma levels of glucose, insulin, lactate, free fatty acids, β-hydroxy-butyrate, and triglycerides. Only the area under the curve for insulin was higher and the lactate/insulin ratio was lower in the HF group than in the LF group. Moreover, hunger and satiety ratings and lunch intake (amount, duration, microstructure of eating) after the high fat breakfast were similar in all subjects. Thus, the habitual level of dietary fat did not alter the acute affects of a fat-rich breakfast on whole body and hepatic fatty acid oxidation and eating behavior at lunch under the present test conditions. Yet, long-term high fat intake appears to have

subtle effects on postprandial metabolism, which are consistent with early signs of a developing insulin resistance.

The observed prandial and postprandial changes in the plasma level of lactate in these first two studies as well as reports of a marked food intake suppressive effect of parenteral lactate administrations in animals prompted us to study the prandial and postprandial effects of lactate on food intake more closely. We infused lactate in the hepatic portal vein (0.5, 1.0, 1.5 mmol lactate/meal) or into the vena cava (1.0, 1.5 mmol lactate/meal) of ad lib fed rats during their first spontaneous nocturnal meal in order to investigate the acute effects of lactate on spontaneous feeding. Infusions (5min, 0.1ml/min) were remotely controlled, and a computerized feeding system recorded meal patterns. In separate crossover tests, meal size decreased independent of the infusion route after 1.0 and 1.5 mmol, but not after 0.5 mmol lactate. The subsequent intermeal interval (IMI) tended to decrease only after the vena cava infusion of 1.0 mmol lactate. The size of the second nocturnal meal increased after the 1.0 mmol lactate infusion. Hepatic portal infusion of 1.5 mmol lactate increased the satiety ratio (subsequent IMI [min] / meal size [g]) by 175 %, which was higher than the insignificant 43 % increase after vena cava infusion. Hepatic portal infusion of 1.5 mmol lactate also increased systemic plasma lactate but not glucose concentration at 1 min after the end of infusion. The results are consistent with the idea that meal-induced increases in circulating lactate play a role in the control of meal size (satiation). Moreover, the results suggest that lactate also contributes to postprandial satiety and that the liver is involved in this effect. The exact mechanisms of lactate's inhibitory effects on feeding and the site(s) where lactate acts to terminate meals remain to be identified.

Some of the above findings support the theory of a metabolic control of food intake, but not all data confirm the results reported in the literature, in particular as far as the possible effects of dietary fiber on eating are concerned. This may be due to methodological differences. With the experimental designs chosen, however, new and interesting findings were obtained that do deserve closer attention and should be further investigated.

ZUSAMMENFASSUNG

In einer alltäglichen, natürlichen Umgebung ist das Essverhalten beim Menschen äusserst komplex gesteuert, und die Nahrungsaufnahme wird durch viele physiologische, psychologische, soziologische und kulturelle Faktoren beeinflusst. In der vorliegenden Arbeit wollte ich die physiologische Rolle einiger prä- und postabsorptiver Sättigungssignale untersuchen. Meine Hauptanliegen waren speziell die kurzfristigen prandialen und postprandialen Mechanismen, welche die Grösse und Dauer einer Mahlzeit und/oder die Dauer des Intervals bis zur nachfolgenden Mahlzeit beeinflussen können.

Die erste von drei Studien befasste sich mit den Auswirkungen von vier isoenergetischen Frühstücken mit unterschiedlichem Nahrungsfaser- und Makronährstoffgehalt auf Hunger und Sättigung, auf das nachfolgende Mittagessen sowie auf den postprandialen Kohlenhydrat- und Fettstoffwechsel. Diese Studie wurde an normalgewichtigen, männlichen Probanden in zwei Experimenten durchgeführt, in denen das Mittagessen entweder zu einem vorbestimmten Zeitpunkt angeboten wurde (Experiment 1), oder in dem die Probanden selbst den Zeitpunkt des Mittagessens bestimmen konnten (Experiment 2). Der Verzehr eines handelsüblichen Müslis mit hohem (10%), mittlerem (7%) und niedrigem (3%) Fasergehalt (HFC, MFC und LFC), oder ein Standard-Frühstück (annähernd 0% Fasern) hatten keinen unterschiedlichen Einfluss auf Hunger und Sättigung, Grösse und Mikrostruktur des folgenden Mittagessens und auf die Zeitspanne zwischen Frühstück und Mittagessen (in Experiment 2). Die Plasmakonzentrationen von Glukose, Laktat und Insulin stiegen nach dem LFC-Frühstück stärker an als nach den anderen Frühstücksvarianten. Eine reaktive, postprandiale Hypoglykämie trat nach dem LFC-Frühstück kurz vor dem Mittagessen auf. Die Plasmakonzentrationen von Metaboliten des Fettstoffwechsels (Triglyzeride, freie Fettsäuren, β-Hydroxy-Butyrat) und von Glukagon wurden durch die Frühstücksvarianten nicht unterschiedlich beeinflusst. Die Resultate stehen im Einklang mit der Annahme, dass der Energiegehalt einer Mahlzeit die wichtigste Determinante für die nachfolgende Energieaufnahme ist. Der Faser- und Makronährstoffgehalt scheinen diesbezüglich höchstens einen modulierenden Effekt zu besitzen.

Ein spezieller experimenteller Aufbau wurde in der zweiten Studie gewählt, um zu untersuchen, ob eine Mahlzeit mit hohem Fettgehalt die nachfolgende Nahrungsaufnahme und den Stoffwechsel in Abhängigkeit vom gewohnten Fettkonsum unterschiedlich beeinflusst. Nachdem bei 40 Probanden der gewohnte Fettkonsum anhand einer siebentägigen Aufzeichnung der Diät bestimmt war, wurde bei 28 dieser schlanken, männlichen Probanden mit einem Fettkonsum zwischen 21 und 44% (der täglichen Energieaufnahme; eingeteilt entweder in eine Niedrigfett-, Mittelfett- oder Hochfett-Gruppe) die Auswirkung eines fettreichen Frühstücks (52% der metabolisierbaren Energie aus Fett) auf den postprandialen Fett- und Kohlenhydratstoffwechsel sowie auf das nachfolgende Mittagessen untersucht. Eine Korrelationsanalyse und ein Vergleich zwischen der Niedrigfett-Gruppe (LF, Fettaufnahme $\leq$35%, n = 10) und der Hochfett-Gruppe (HF, Fettaufnahme $\geq$ 40%, n = 11), welche einen ähnlichen BMI (LF: 22.57, HF: 22.4) aufwiesen, zeigte, dass der Fettgehalt der gewohnten Diät keine Auswirkungen auf die Basalwerte und die postprandialen Veränderungen des respiratorischen Quotienten und der Plasmaspiegel

von Glukose, Insulin, Laktat, der freien Fettsäuren, β-Hydroxy-Butyrat und Triglyzeriden. Bei der HF-Gruppe war lediglich die Fläche unter der Kurve des Verlaufs der Insulinkonzentration grösser und der Laktat/Insulin-Quotient war etwas niedriger als bei der LF-Gruppe. Ausserdem waren Hunger, Sättigung und die Aufnahme des Mittagessens (Grösse, Dauer, Mikrostruktur) nach dem fettreichen Frühstück bei allen Probanden ähnlich. Demzufolge hatte der gewohnte Fettkonsum (zumindest in den untersuchten Grenzen) keinen Einfluss auf die akuten Auswirkungen des fettreichen Frühstücks auf die Fettsäurenoxidation und das Essverhalten beim Mittagessen. Langfristiger hoher Fettkonsum scheint jedoch subtile Effekte auf den postprandialen Stoffwechsel zu haben, welche sich mit den frühen Anzeichen bei der Entwicklung von Insulinresistenz vereinbaren lässt.

Die beobachteten prandialen und postprandialen Veränderungen der Plasmaspiegel von Laktat in diesen beiden ersten Studien sowie Berichte über einen verzehrsreduzierenden Effekt von Laktat nach parenteraler Verabreichung bei Versuchstieren unter bestimmten Bedingungen veranlassten uns dazu, die prandialen und postprandialen Effekte von Laktat auf die Nahrungsaufnahme näher zu untersuchen. Wir infundierten Laktat in die Pfortader (0.5, 1.0, 1.5 mmol Laktat/Mahlzeit) oder in die Vena cava (1.0, 1.5 mmol Laktat/Mahlzeit) von ad-libitum-gefütterten Ratten während der ersten spontanen Mahlzeit in der Dunkelphase, um die akuten Effekte von Laktat auf die spontane Futteraufnahme zu untersuchen. Die Infusionen (5 Minuten, 0.1 ml/min) erfolgten ferngesteuert und das Mahlzeitenmuster wurde über einen Computer kontinuierlich aufgezeichnet. In separaten Crossover-Tests war die Mahlzeitengrösse unabhängig vom Infusionsweg nach 1.0 und 1.5 mmol, nicht aber nach 0.5 mmol Laktat verringert. Das nachfolgende Mahlzeiteninterval (IMI) war nach der Infusion von 1.0 mmol Laktat in die Vena cava tendenziell verkürzt. Die Grösse der zweiten Mahlzeit der Dunkelphase war nach Infusion von 1.0 mmol Laktat erhöht. Infusionen von 1.5 mmol Laktat in die Portalvene erhöhten den Sättigungs-Quotienten (nachfolgendes IMI [min] / Mahlzeitengrösse [g]) um 175%, was deutlich höher war als die nach Infusion in die Vena cava beobachtete, nicht signifikante Erhöhung von 43%. Infusionen von 1.5 mmol Laktat in die Portalvene erhöhten ebenfalls die systemische Plasmakonzentration von Laktat, nicht aber die Glukose-Konzentration 1 Minute nach Beendigung der Infusion. Die Resultate unterstützen die Annahme, dass eine mahlzeiteninduzierter Anstieg der Plasmakonzentration von Laktat zur Beendigung der Mahlzeit beiträgt. Zudem deuten die Resultate darauf hin, dass Laktat an der Aufrechterhaltung der Sättigung nach einer Mahlzeit beiträgt und dass die Leber daran beteiligt ist. Der genaue Mechanismus des Hemmeffekts von Laktat auf die Futteraufnahme sowie der Ort, an dem Laktat diesen Effekt ausübt, müssen jedoch noch identifiziert werden.

Einige der oben beschriebenen Befunde unterstützen die Hypothese, dass die Nahrungsaufnahme einer metabolischen Steuerung unterliegt. Nicht alle Resultate stehen jedoch im Einklang mit Befunden aus der Literatur, insbesondere was den möglichen Einfluss der Nahrungsfasern auf das Essverhalten betrifft. Dies dürfte mit methodischen Unterschieden zusammenhängen. Generell konnten mit den gewählten Versuchsansätzen jedoch einige neue und interessante Resultate gewonnen werden, welche nähere Aufmerksamkeit verdienen und intensiver untersucht werden sollten.

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INTRODUCTION

EATING BEHAVIOR AND POTENTIAL INFLUENCES

Food intake is one of the most basic behaviors and requirements of life. Its well-regulated control is a question of survival. However, as much as research has investigated and analyzed the elements of food intake behavior, we are far from completely understanding the underlying control mechanisms.

In adult humans body weight is kept fairly stable, although there is an abundance of appetizing foods available varying in nutritional value and energy content (De Castro 1993). Monitoring the energy and nutrient contents of the ingested foods and adjusting ingestion to the total energy expended by the processes that maintain the bodily functions achieve body weight constancy.

Animals and humans display a distinct circadian meal pattern that is characterized by phases of food intake (meals) and phases when no food is ingested (intermeal interval). Meal taking is regulated by external and internal factors resulting in hunger and appetite as well as satiety sensations, which either promote or inhibit eating, and thus determine the size of a meal and the duration of the subsequent intermeal interval. Consequently, research addresses the basic questions concerning the external and internal factors responsible for the initiation and termination of spontaneous meals.

In particular in humans the meal pattern is influenced by a myriad of external factors; i.e. social surroundings, working conditions, food availability, conditioned time factors, etc. strongly influence eating (e.g. de Castro 1997, de Castro *et al.* 1992). In addition, the timing of meals for humans in industrialized countries is usually rather fixed due to social constraints, which often work against the control food intake by physiological demand (Bellisle, LeMagnen 1981). Previous studies in humans have shown a strong correlation between the duration of the premeal interval and meal size, suggesting that meal size in humans is influenced by the time elapsed since the preceding meal (De Castro 1985, De Castro 1987, Bellisle *et al.* 1999). Cognitive restriction and environmental as well as social constraints in turn determine the frequency of meals (Bellisle 1979, de Castro 1997). In part as a consequence of that, humans adjust food intake to changes in energy requirements by modulating meal size and not frequency (Campbell *et al.* 1977).

On the other hand, in animals, such as free running rats with ad-libitum access to food, a correlation between meal size and the postmeal interval has been found (Davies 1977), which means that the size of a meal determines how long the animal waits until it consumes the next meal. This suggests that internal physiological processes mainly determine when a meal is consumed (De Castro 1985). Interestingly, under time isolation, a correlation between meal size and the duration of the postmeal interval was also evident in humans, just like in animals (Bernstein *et al.* 1981).

Many behavioral and psychological factors have to be taken into account when designing human studies of eating behavior. External influences should be kept to a minimum, if the internal physiological factors of eating control are of foremost interest.

Depending on the experimental design, external factors could either be kept constant throughout the study or mostly be eliminated, e.g. by isolation of the subjects from external time cues, social contacts, and other potentially interfering factors. For instance, in time isolation studies, besides the shift to a longer circadian cycle, the intermeal intervals were lengthened without a proportional increase of meal sizes (Green *et al.* 1987). Furthermore, in pleasant social context, humans tend to over-consume food (de Castro 1997). Therefore, when investigating the physiological control mechanisms of food intake, time cues and social contacts should be eliminated under laboratory conditions.

In rat studies, eliminating the external factors is undoubtedly an easier task, because unlike in humans the rat's surroundings and housing conditions can be controlled for weeks. Yet, besides the room temperature, humidity and light cycle, the behavior of the investigator can have great effects on meal patterns. When the experimenter enters the rat room, the activity of the animals usually increases, and, according to own experience, this is also often accompanied by an initiation of eating. All disruptive factors should therefore be eliminated, particularly in short-term studies of food intake in single meals. This requires handling the rats to allow them to adjust to the experimental routine, keeping the presence of the experimenter in the animal room to a minimum and bound to a strict schedule, treating all animals in the same manner, to mention only a few factors. By disturbing the animals in the least possible way, the treatment effects on single meal size can be reliably measured and ascribed to physiological processes, rather than external factors.

Physiological Mechanisms of Hunger and Satiety

Preabsorptive mechanisms

Physiological satiety signals derive either from preabsorptive or postabsorptive sites. Preabsorptive signals develop in the gastrointestinal tract, starting with oral sensory specific satiety evoked by single food items (Rolls *et al.* 1986), and continue to origin from all the way through the intestinal tract until the nutrients' digestion products are absorbed into the blood stream. These preabsorptive and mostly prandial satiety signals are major contributors to meal termination, i.e. satiation (Blundell, Burley 1987, De Graaf *et al.* 1999).

Hedonic characteristics mostly provide a way of classifying foods before large amounts of it are consumed. This allows for judging the tasted food as edible. High palatability initially stimulates eating. In addition, sensory stimuli of food ensure through sensory specific satiety that a variety of different foods are ingested (Rolls *et al.* 1986). Sensory specific satiety applies particularly to the consumed food, which is liked less than a food with different sensory characteristics (Rolls *et al.* 1986). Palatability can shape eating behavior in interaction with physiological mechanisms (Bellisle, LeMagnen 1981). A high systemic glucose utilization may for instance influence hedonic responses in the way that glucose is liked less than if glucose utilization is low.

When a food bolus reaches the stomach, more preabsorptive satiety signals are generated. Hunger and satiety have long been associated with gastrointestinal motility (see Wisen, Hellström 1995). Especially an inhibition of gastric emptying provides a strong satiety signal. As food is periodically ingested and stored in the stomach, gastric emptying supplies the intestine with a nearly constant flux of nutrients, in part

predigested and liquefied. The storage capacity of the stomach helps to protect the systemic circulation from an overflow of nutrients (Wisen, Hellström 1995). Gastric emptying therefore participates in the link between meal ingestion and the metabolic utilization of nutrients at the cellular level (Carbonnel *et al.* 1994). The physiological slowing of gastric emptying seems to be linked to increased feelings of satiety (Carbonnel *et al.* 1994). Gastric emptying is mostly controlled from the duodenum through a negative feedback system (Vidon *et al.* 1989). The rate of gastric emptying is thus limited by the rate of duodenal clearance (Hunt 1983).

Satiety signals are modulated by the nutrient composition as well as the energy content of the ingested food. The satiating efficiency of the ingested foods is of particular interest for studies of nutrition and appetite (Burley *et al.* 1993). Fiber seems to have an inhibitory effect on gastric emptying and may thus influence meal termination (Eastwood 1992), delay the postprandial return of hunger (Raben *et al.* 1994b; Benini *et al.* 1995), and increase postprandial satiety (Burley *et al.* 1993). An increase in fiber content is usually accompanied by a reduced energy density and an increased volume of the food, which tends to decrease energy intake (Leitzmann, Hixt 1986). Finally, some evidence suggests that fibers slow down the rate of nutrient absorption in the intestine, resulting in a delayed appearance of nutrients in the blood plasma (Eastwood 1992). This, in turn, may influence metabolic satiety signals (Burley *et al.* 1993).

It has been proposed that the premeal stomach contents are associated with subjective hunger in humans, and this in turn is related to the size of the meal (De Castro, Elmore 1988). Thus, the amount of food present in the stomach at meal onset may determine meal size (De Castro, Kreitzman 1985, De Castro 1999). Furthermore, especially the amount of protein and fat present in the stomach appears to have an influence on prandial regulatory mechanisms, an effect that may be related to the satiety value of these nutrients (De Castro, Kreitzman 1985).

In single meal studies it has been shown that the microstructure of eating, the course of the rate of food intake within a meal, is very sensitive to manipulations of feeding conditions (Bellisle, Le Magnen 1981), and to palatability and type of food eaten (Bobroff, Kissileff 1986). There is a distinction between decelerated and non-decelerated eaters. Decelerated eaters are usually characterized by a decrease in the eating rate over the course of the meal, whereas non-decelerated eaters show hardly any change in eating rate for the duration of the meal (Westerterp-Plantenga *et al.* 1990). It was proposed that normal-weight and obese subjects differ in eating style, i.e. that normal-weights show a more decelerated and the obese a more non-decelerated eating rate (Meyer, Pudel 1972). It should be mentioned, however, that some studies could not find such a relationship between obesity and eating rate (Kaplan 1980; Westerterp-Plantenga *et al.* 1990). Other factors, such as age and restrained eating, may also change the rate of eating within the course of a meal (Westerterp-Plantenga *et al.* 1990). The microstructure of a meal may therefore be sensitive to several individual characteristics in subjects. Despite these limitations, the microstructure of eating is considered to be a useful parameter for investigations into the physiological control of basic and manipulated meals (Westerterp-Plantenga *et al.* 1990, Westerterp-Plantenga *et al.* 1992).

Postabsorptive mechanisms

Postabsorptive satiety mechanisms are activated as soon as the digested nutrients are absorbed into the blood stream, and they sustain postprandial satiety for some time after

a meal; i.e. postabsorptive satiety signals influence mainly the duration of the intermeal interval. Furthermore, in humans where the intermeal interval is often fixed by sociocultural constraints, postprandial satiety mechanisms may also influence the size of the next meal because humans mainly regulate food intake by changing meal size, not the frequency of meals (Campbell *et al.* 1977).

It is generally believed that the depletion of a certain nutrient, concurrent with a low fuel utilization, contributes to the initiation of the next meal by weakening the satiety signals in the postabsorptive state. In the attempt to replete the nutrient, food intake is stimulated until repletion reaches a certain level, which in turn suppresses hunger and terminates the meal (De Castro 1993). Over the years, many theories have tried to explain in detail how this is regulated. All these theories of the metabolic control of food intake have the common assumption that eating is inversely related to fuel utilization, with a high rate of fuel oxidation leading to satiety.

After absorption, most nutrients enter the hepatic portal vein and first pass through the liver. This, together with other findings, forms the basis for the presumed role of the liver in food intake regulation. The concept of a metabolic control of eating based on fuel utilization requires, however, that nutrient availability and substrate oxidation are monitored by sensors which are connected to central structures that integrate these signals into the overall food intake regulation (Langhans 1996). The liver is proposed to be a major site for such metabolic sensors (Langhans 1996). The effects of the satiety signals generated by hepatic glucosensors and other hepatic metabolic sensors appear to depend on intact afferent nerves, which feed the hepatic metabolic signals into the central circuitry that controls eating (Langhans 1996). The liver therefore seems to represent the link between dietary sources of energy, the extrahepatic tissues, which are the main users of energy, and the central regulation of ingestion.

Central integration

It would exceed the scope of this thesis to go into details concerning central mechanisms of food intake control. Several brain areas from the hindbrain to the hypothalamus, and various neurochemicals are involved in the complex integration and of the peripheral signals and in shaping a coordinated behavioral response. In any case, the peripheral satiety signals inform central structures of the metabolic status, i.e. of ingested and absorbed nutrients, nutrient utilization, storage depots, etc. Central structures integrate the afferent signals and create a coordinated response. The central integration of the satiety signals is followed by the resulting efferent signals to evoke specific food intake behavior.

SCOPE

The emphasis of this thesis was on the investigation of short-term satiating effects of single meals and the metabolic processing of the fuels derived from such meals on subsequent meal ingestion.

The thesis comprises two human studies which were designed to investigate the effects of single meals on prandial and postprandial metabolic (blood metabolites and hormones), behavioral (microstructure of meal intake), and psychological (subjective feelings of hunger and satiety) variables, as well as of one animal study, which

concentrated on a single metabolite and its immediate effects on eating patterns (meal size and duration, intermeal interval).

In the two experiments of Study I, the effects of different types of breakfast on subsequent food intake in young male subjects were investigated. Study II looked into the effects of a high fat meal on fatty acid oxidation and subsequent food intake in males adapted to high or low dietary fat intake. In Study III, lactate, which is proposed to provide a metabolic satiety signal, was intravenously administered in a rat model to define its role in spontaneous meal consumption.

Study I: Fiber

Nutritionists recommend an increase in fiber consumption due to their positive effects on digestion, gastrointestinal motility, prevention of colon cancer, as well as the positive influences on postabsorptive mechanisms. Previous studies have concentrated mainly on investigating the effects of meals, which were composed in the laboratory containing artificially high or low amounts of fiber. The findings are interesting, but the acceptability of such high fiber foods (at times exceeding 20% of fiber) is usually quite low (Golden *et al.* 1995). In addition, most studies so far used a mixture of foods produced in the laboratory. Therefore, the intention of our study was to use fiber products that are commercially available and well established on the Swiss market and are widely accepted by the population. These were then prepared according to the manufacturers suggestions.

In Study I we used three breakfast cereals with varying fiber (3%, 7%, 10%) and macronutrient content, as well as a standard continental breakfast (0% fiber), to investigate the effects of these breakfasts on postprandial carbohydrate and fat metabolism and subsequent food intake with a fixed or a freely chosen intermeal interval.

Study II: Fat

According to nutritionists, dietary fat intake is too high in industrialized countries. Long-term high fat intake is believed to contribute to obesity, which in turn is held responsible for many diseases such as high blood pressure, diabetes, and joint and back problems. Therefore, it is usually recommended to keep fat intake at below or around 30% (Sutter-Leuzinger, Sieber 1998). In fat intake studies so far mostly obese subjects with a supposedly high fat intake were compared to lean subjects with a more moderate fat consumption. However, there are also lean humans eating high fat diets, and this raises the question why they don't overeat and become overweight. In addition to differences in physical activity, which of course markedly affect energy balance, one of the reasons may be an enhanced fatty acid oxidation, which enables them to utilize most of the fat energy instead of storing it in adipose tissue.

In Study II we therefore investigated whether lean subjects adapted to a high fat intake of $\geq 40\%$ of total daily energy intake have changes in postprandial fat metabolism, in particular hepatic fatty acid oxidation, compared to subjects eating lower fat diets ($\leq 35\%$). The second question was whether such changes, in case they occurred, might influence subsequent food intake.

Study III: Lactate

In human Studies I and II, the profile of the plasma metabolites and hormones helped to assess the postabsorptive carbohydrate and fat metabolism under these conditions. It was observed that, among other meal-induced metabolic changes, plasma lactate increased during and after a meal. Since parenteral administration of lactate was shown to decrease food intake (Langhans *et al.* 1985a; Langhans *et al.* 1985b; Racotta *et al.* 1977), lactate may provide a metabolic satiety signal. The feeding suppressive effect of lactate seems to depend on intact hepatic branch vagal afferents (Langhans *et al.* 1985b; Nagase *et al.* 1996), suggesting that the satiety effect of lactate originates in the liver. After absorption from the gut, glucose is taken up into the blood and partly transformed to the 3-carbon metabolite lactate by the mucosa cells. Glucose and lactate, both appear in the hepatic portal vein. In Study III we attempted to simulate and exaggerate the prandial lactate appearance in the hepatic portal vein by remotely controlled infusions in the rat, in order to investigate the role of lactate as a prandial satiety signal.

By implanting a catheter into the hepatic portal vein, we were able to infuse lactate during spontaneous meals to determine whether an experimentally increased lactate level during a meal reduces meal size and possibly influences the postmeal interval. In addition, the effects of hepatic portal vein versus vena cava lactate infusions on eating were compared, in an attempt to address the role of the liver in the effect of lactate on food intake.

METHODS

All methods employed are described in detail in the respective method sections of the three studies. Therefore, just brief descriptions of two particular pieces of equipment, which deserve closer attention, are presented below.

The Universal Eating Monitor (UEM) in the human studies (Studies I and II)

One of the instruments used for generating eating behavior data in this thesis was the ***Universal Eating Monitor*** (UEM) developed by H.R. Kissileff in 1980 (Kissileff *et al.* 1980). The UEM is an apparatus especially suited to study eating behavior during the course of a single meal because it allows continuous online recordings by means of measuring weight changes of the plate placed upon it.

The subjects consume their meals alone (to minimize social effects on the amount of food consumed (e.g. de Castro 1997)) while sitting at the experimental table, which has a non-visible built-in scale underneath a false plate that fits into an opening of the table. The food plate with a homogenous meal is placed on the plate. The homogenous meal prevents subjects from picking on single food items, and allows assessing the actual amount consumed and its macronutrient composition. The scale underneath measures the weight of the food plate every 2 seconds and transmits the data online via an interface to a computer (Olivetti M 300) in an adjacent room. The rate, size, and duration, i.e. the microstructure of the meal, can be determined with a custom-designed program (VZM®, Krügel, Munich, Germany). The subjects are not aware of the scale. Additionally, a video surveillance camera (Phillips, VSS 3440) allows continuous observation and recording of the proceedings in the eating room.

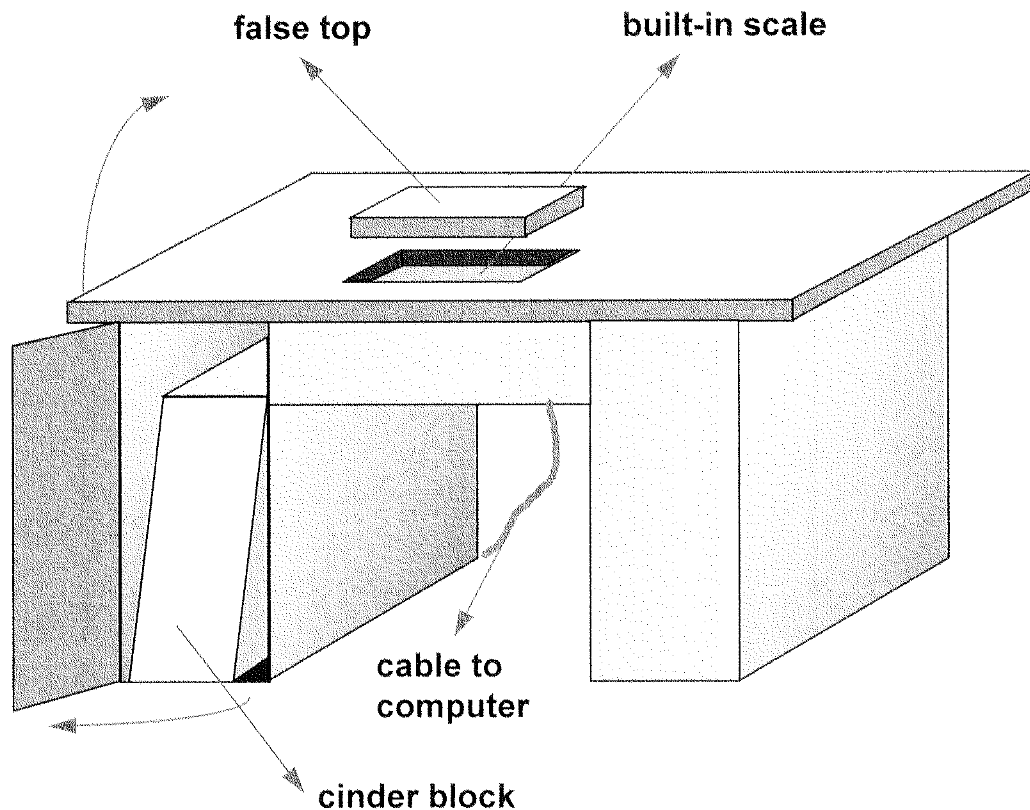

Figure 1: *The universal eating monitor (UEM) built according to Kissileff (Kissileff 1980).*

The ***Cumulative Intake Curve*** (CIC) is determined by graphing the cumulative amount of food consumed versus time. The curve can be best described by a quadratic equation ($y = a + bx + cx^2$) whose coefficients are determined by a non-linear regression analysis (Kissileff *et al.* 1982).

After fitting the whole curve to a quadratic equation, coefficient 'b' describes the initial phase of the meal and represents the rate of intake. Coefficient 'c' describes the decelerating phase of the meal when the rate of intake decreases until the meal comes to an end. The deceleration towards the end of the meal reflects the onset of internal satiating mechanisms (Kissileff *et al.* 1982; Stellar, Shrager 1985). For further details, see method sections of Studies I and II below.

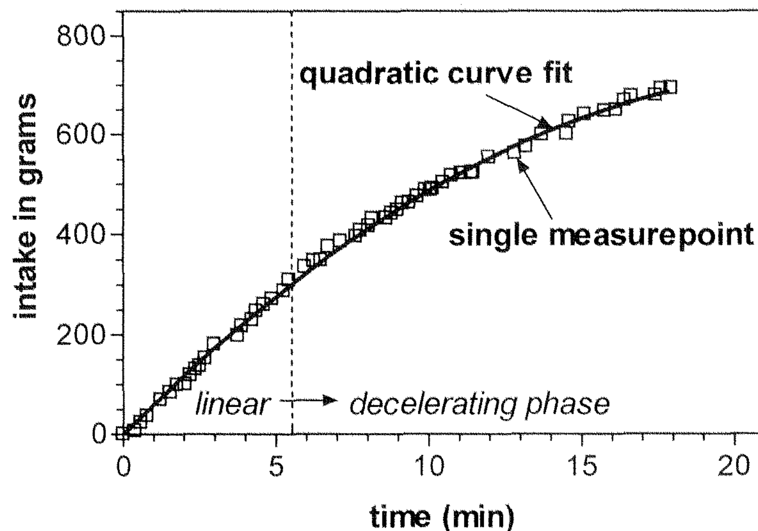

Figure 2: *The cumulative intake curve. Graph of the cumulative amount of food intake during a meal versus the duration of the meal measured by the universal eating monitor (UEM, Kissileff 1980).*

The infusion cages in the rat study (Study III)

Sixteen single housed rat cages for remotely controlled infusions were installed (see Figure 3). The custom design of the cages allows for continuous recording of spontaneous food intake and remotely controlled infusions in relation to spontaneous meals in ad lib fed rats. Therefore, experiments under comparatively ecological situations are possible, and any disturbance by the presence of the experimenter or by the manipulation can be ruled out.

The cages are made of Plexiglas. Through the hinged front door the experimenter can handle the rat. The rat stands on a stainless steel grading floor with a sawdust drawer underneath to catch the droppings. The rat has ad lib access to water through a water bottle mounted on the back wall of the cage. A bridge, about 5 cm off the floor, allows ad lib access to food. Food is placed into the spill resistant food cup, which rests on a scale. The scale is interfaced with a computer (Olivetti M 300) in an adjacent room. A video surveillance camera (Phillips, VSS 3440) allows continuous observation of the rats.

Through a custom designed program (VZM®, Krügel, Munich, Germany) the computer records the weight of the scale at intervals of 30 seconds. According to the preset meal parameters (minimum meal size, minimum meal duration, minimum intermeal interval), the computer output delivers the timing, size, and duration of the individual meals, and the duration of the intermeal intervals.

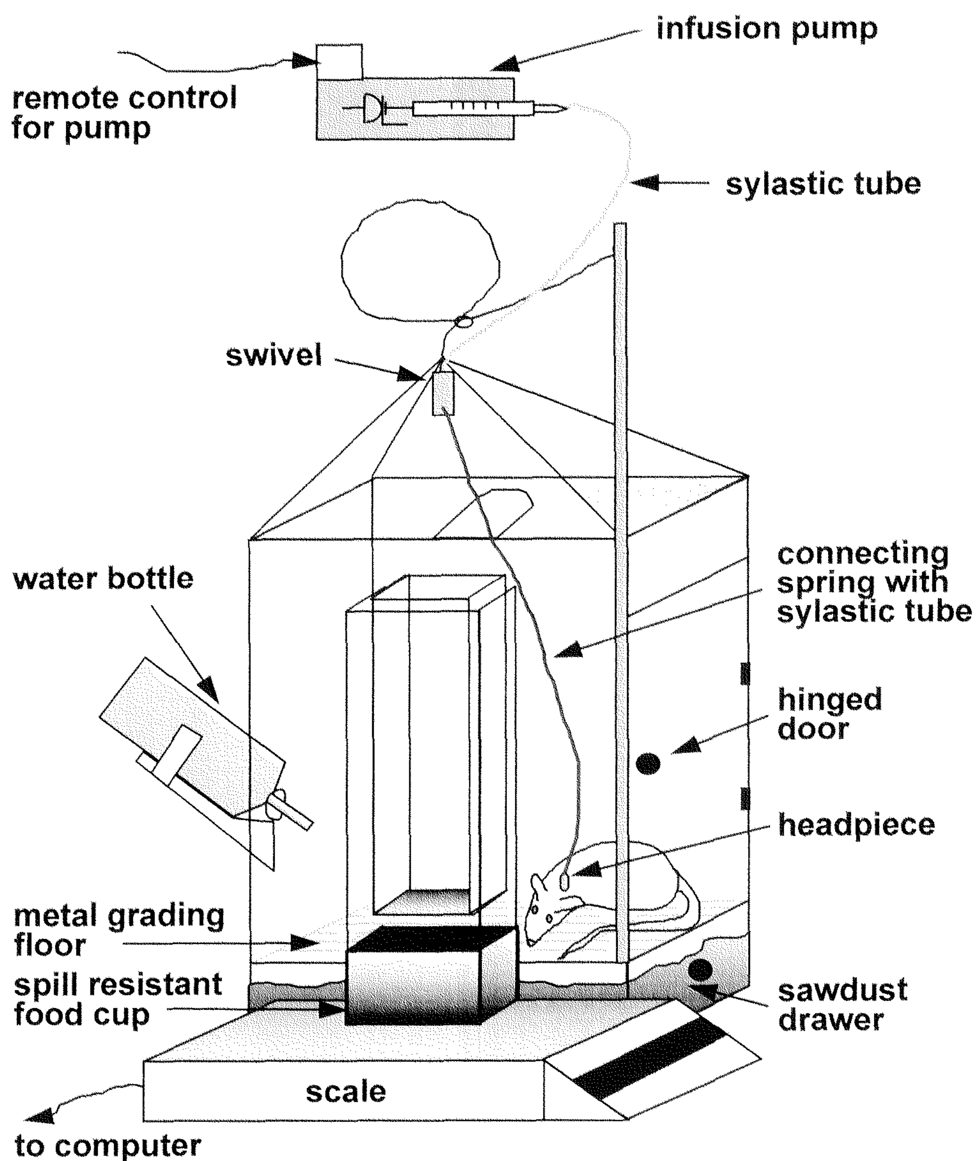

Figure 3: *Plexiglas cage for remotely controlled metabolite and hormone infusions.*

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DIETARY FIBER IN BREAKFAST

BASED ON:

Silberbauer C., Frey-Rindova P., Langhans W.: Breakfast with different fiber and macronutrient content do not differentially affect timing, size or microstructure of the subsequent lunch.
Zeitschrift für Ernährungswissenschaft / European Journal of Nutrition (1996) 35: 356-368

INTRODUCTION

Dietary fibers have repeatedly been reported to reduce hunger or increase satiety ratings (e.g. DiLorenzo *et al.* 1988; Leathwood *et al.* 1988; Rigaud *et al.* 1987), in particular in people on low calorie diets (Astrup *et al.* 1990), and to affect both satiation (the process that terminates eating) and satiety (the subsequent state that prevents eating) (see Blundell, Burley 1987). In one study the ingestion of a high fiber breakfast decreased subsequent lunch intake (Levine *et al.* 1989). In another study, however, moderate differences in the fiber content of a test breakfast did not influence hunger ratings or subsequent energy intake at lunch (Burley *et al.* 1987). These discrepancies are presumably due to procedural differences and the use of different forms of fiber. Thus, the generality of an effect of dietary fiber on energy intake is open to discussion. The possible mechanisms of such an effect are also unclear. Gastrointestinal factors such as a retardation of gastric emptying by dietary fiber (DiLorenzo *et al.* 1988; Leathwood *et al.* 1988; Levine *et al.* 1989), or delayed absorption of glucose from the small intestinal lumen (Jenkins *et al.* 1978; Torsdottir *et al.* 1989; van Amelsvoort, Weststrate 1992; Westerterp-Plantenga *et al.* 1990), as well as metabolic factors related to differences in postprandial glycemia or insulinemia may be involved (Delargy *et al.* 1993; Leathwood *et al.* 1988; Raben *et al.* 1994b; Torsdottir *et al.* 1989; van Amelsvoort *et al.* 1992).

There are also disparate findings with respect to the influence of macronutrient content on subsequent energy intake. When compared with a high carbohydrate supplemented meal, an equienergetic high fat supplementation diminished the satiating efficiency of the meal as evidenced by differences in energy intake in a test meal 90 min later (Blundell *et al.* 1993). In another study, the macronutrient composition of a liquid breakfast had no effect on the subsequent lunch or on total energy intake for the rest of the day (de Graaf *et al.* 1992). Moreover, covert manipulations of macronutrient or energy contents of meals apparently lead to subsequent caloric but not macronutrient compensation (Foltin *et al.* 1992).

The present study investigated the effects of equienergetic, real breakfasts with varying fiber and macronutrient contents on subsequent energy intake and on postprandial carbohydrate and fat metabolism. The effects of four breakfast varieties were tested. A short-term influence of the breakfasts on hunger and satiety might be reflected by changes in the size or timing of the subsequent lunch. Therefore, in a first experiment

lunch was offered at a predetermined time after breakfast, whereas in a second experiment subjects were isolated from time cues and allowed to decide individually when to have lunch. To assess the metabolic consequences of the test breakfasts, plasma carbohydrate and fat metabolites, insulin and glucagon were measured before and at various times after the test breakfasts, as well as before and after the subsequent target lunch (only in the first experiment). In addition to the amount eaten at lunch, the microstructure of eating was recorded using the universal eating monitor (UEM; described in Kissileff *et al.* 1980). Information about subjective hunger ratings and postprandial feelings were obtained by questionnaires.

METHODS

Subjects

Eighteen male normal weight subjects, age 21 to 32 y, participated in the study (9 in each experiment). Two subjects were later excluded because one was unable to participate in all four test trials of Experiment 1, and because blood could not be obtained from one subject in Experiment 2. The subjects weighed 60.2 to 81.2 kg, and their height was 168 to 190 cm. All subjects usually ate breakfast between 0630 h and 0930 h, lunch between 1130 h and 1400 h, and dinner between 1800 h and 2130 h, before going to bed between 2230 to 0130 h. Only a few subjects consumed snacks between meals. Subjects estimated drinking 10 to 25 dl of fluids a day. Regular physical activity was reported by nine subjects (between 4 and 10 h/week). Four subjects were smokers with an average of 2, 15, 17, and 20 cigarettes a day. The study was approved by the ethics committee of the University Hospital Zurich, and all subjects gave their written consent after the experimental procedure had been explained to them.

Test breakfasts

The four breakfast varieties were: [1] A commercially available high fiber Swiss breakfast cereal with raisins (HFC) (Bio-Huus-Müesli®, Bio-Familia AG, Sachseln), [2] a medium fiber breakfast cereal (MFC) with dried strawberry chunks (Frutta Crunch®, Bio-Familia AG, Sachseln), [3] a low fiber high sucrose cereal (LFC) consisting of sweetened puffed rice (Smacks®, Kellogg Company, USA), and [4] a standard continental breakfast consisting of two white rolls, butter, strawberry jam, and apricot yogurt (Table 1). The HFC cereal consisted of oat flakes, corn flakes, sultanas, barley flakes, sunflower seeds, honey-wheat bits, almonds, hazelnuts, apple pieces, and wheat flakes. The MFC cereal was made of whole oat flakes, wheat flakes, unrefined sugar, peanut oil, coconut, hazelnuts, honey, freeze-dried strawberries, raspberries, wheat germ, glucose, pectin, and salt. The LFC cereal was produced from wheat, sugar, glucose syrup, honey, plant fats, lecithin, vitamins, and iron.

TABLE 1: ENERGY AND MACRONUTRIENT COMPOSITION OF MEALS (CALCULATED)

BREAKFAST		Energy	Carbohydrates	Fat	Fiber	Protein
High fiber cereal	125 g	2038 kJ	86.3 g *[1]	8.8 g	12.5 g	13.8 g
250 ml milk	3.7% fat	700 kJ	12.0 g	9.2 g	--	8.0 g
20 ml cream	35% fat	28 kJ	6.4 g	0.7 g	--	4.2 g
	Total	***2766 kJ***	***104.7 g***	***18.7 g***	***12.5***	***26.0 g***
Medium fiber cereal	125 g	2236 kJ	75.0 g *[2]	20.6 g	8.75 g	12.5 g
220 ml milk	2.8% fat	528 kJ	11.0 g	6.2 g	--	7.0 g
	Total	***2764 kJ***	***86.0 g***	***26.8 g***	***8.75 g***	***19.5 g***
Low fiber cereal	125 g	2033 kJ	105.0 g *[3]	1.9 g	3.75 g	10.4 g
250 ml milk	3.7% fat	700 kJ	12.0 g	9.3 g	--	8.0 g
30 ml cream	25% fat	31 kJ	10.5 g	0.7 g	--	7.2 g
	Total	***2764 kJ***	***127.5 g***	***11.9 g***	***3.75*** g	***25.6*** g
Standard	breakfast					
2 white bread buns	90 g	1206 kJ	46.8 g	7.2 g	--	8.0 g
butter	20 g	634 kJ	0.1 g	16.6 g		0.1 g
jam	50 g	560 kJ	33.0 g	--		0.3 g
yogurt	180 g, 0.1% fat	369 kJ	14.4 g	0.2 g		7.2 g
	Total	***2769 kJ***	***94.3 g***	***24.0 g***	--	***15.6 g***
LUNCH (Total amount offered)						
Risotto						
tomato risotto	250 g	3750 kJ	185.0 g	5.0 g	--	22.5 g
butter	20 g	634 kJ	0.1 g	16.6 g		0.1 g
Parmesan cheese	25 g	400 kJ	0.0 g	7.0 g		8.0 g
minced pork	250 g	1825 kJ	0.0 g	25.0 g		50.0 g
	Total	***6609 kJ***	***185.1 g***	***53.6 g***	--	***80.6 g***

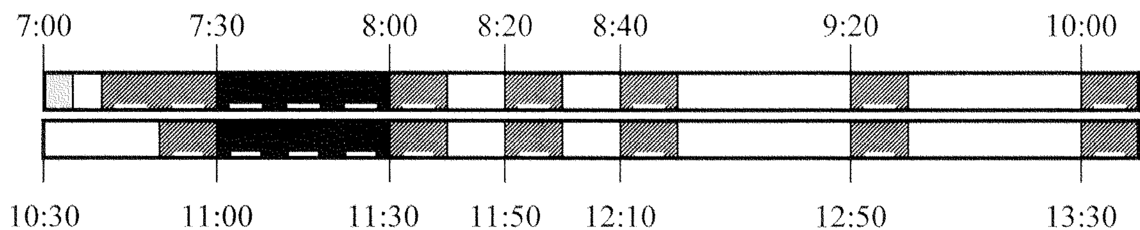
Declarations according to the manufacturers
*(*1 sugar content not available, *2 22.0 g sugars, *3 61.0 g sugars)*

Design and Procedure

Experiment 1

A fully repeated-measures design was employed. Independent variables were the composition of the test breakfasts (see above) and the timing of blood sampling (12 time points). Dependent variables were plasma metabolite and hormone levels, parameters of meal microstructure, and questionnaire data. The experiment was performed on 12 test days over a period of 20 working days. Each subject participated on 4 days with a minimum intermittent period of 1 day. Smoking, strenuous exercise, and alcohol consumption were prohibited after 1700 h the day before tests. Between 1800 h and 2100 h, subjects consumed a pre-packaged dinner consisting of fresh meat tortellini (250 g), canned tomato sauce (190 g), grated Parmesan cheese (50 g), fresh carrot salad (160 g), and canned fruit cocktail (227 g including syrup). The dinner provided 5029 kJ (161.6 g carbohydrates, 46.7 g fat and 44.2 g protein). The dessert fruit cocktail could be eaten until 2300 h. Subjects were instructed to drink 330 ml mineral water at dinner. Additional bottles of mineral water were provided for individual fluid demands.

EXPERIMENT 1



EXPERIMENT 2

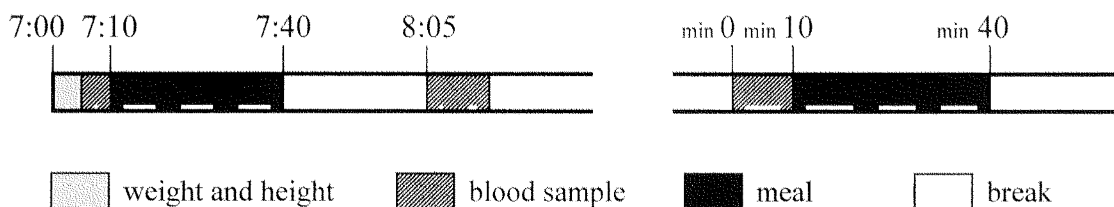


Figure. 1: *Schedule of experimental proceedings for the first subject on each test day. Experiment 1: Second and third subject with delay of 30 min and 60 min, respectively. Experiment 2: Second, third and fourth subject with delay of 25 min, 50 min, and 75 min, respectively.*

Three subjects were tested daily, beginning at 0700, 0730 and 0800 h. Upon arrival at the laboratory on the morning of the test day, each subject had fasted for at least 8 to 9 hours. Figure 1 (top) shows the daily proceedings for each subject. After weight and height measurements, a 20-gauge Vialon catheter (Becton, Dickinson, Switzerland) was placed into the antecubital vein and a fasting blood sample was drawn for baseline measurements. Eleven more blood samples were taken at various times, starting immediately after breakfast (see Fig. 1). Breakfast lasted 30 minutes, including completion of the two questionnaires given before and after the meal. Subjects were allowed to drink bottled mineral water with the meals and throughout the test period at their discretion. Between breakfast and lunch, subjects engaged in sedentary activities such as reading and watching TV.

A hot lunch was served 3.5 hours after the onset of breakfast. The lunch consisted of risotto (tomato flavored rice stew; Knorr-Nährmittel AG, Thayngen) with bite-size pieces of meat. Subjects were instructed to eat as much of the oversized serving (6609 kJ, carbohydrates 185.1 g, fat 53.6 g, protein 80.6 g) and to drink as much of the provided mineral water as they wanted. Lunch lasted 30 minutes, including answering the questionnaires before and after the meal. Subjects remained in the laboratory after lunch, engaging only in sedentary activities, until all blood samples were drawn.

Experiment 2

Basically the same design and procedure as in Experiment 1 were employed, with the following alterations (see Figure 1, bottom): [1] only three blood samples (baseline, after breakfast, just prior to lunch) were drawn by venapuncture. [2] Four subjects were tested daily, beginning at 0700, 0725, 0750, and 0815 h. [3] The subjects themselves could determine when they wanted to consume the target lunch. Watches were taken from the subjects upon arrival at the laboratory, and other potential time cues were not available. After breakfast subjects engaged in sedentary activities, e.g. listening to music, in separate rooms. As soon as the subjects felt hungry enough to consume a warm meal, they came to the blood sampling room. After the third and last blood sample was drawn and the questionnaire was filled out, the same hot target lunch as in Experiment 1 was served.

Questionnaires

Subjects filled out a questionnaire before and after breakfast and lunch in each experiment. The questions addressed the subjects' degree of hunger and thirst and their perception of the meal. Some questions (not reported here) were posed to obscure the major purpose of the study. Answers were given through visual analogue scales ranging from 0 - 107 mm (e.g. from "not at all" (0 mm) to "extremely" (107 mm)). Other questions were answered by marking the appropriate expression on a five-point scale. Example: "no desire to eat" (1 point), "strong desire to eat immediately" (5 points). The questionnaire before each meal focused on the subjects' current hunger and thirst state, and the questionnaire after each meal inquired about the satisfaction of postprandial sensations.

Universal Eating Monitor (UEM)

The amount eaten at lunch and the microstructure of the meal (i.e. the cumulative food intake curve) was recorded with a scale integrated in the table underneath the plate (Kissileff *et al.* 1980). Subjects were not aware of the scale but were informed that they were supervised through a video camera during the meal. The weight of the plate was constantly monitored by the built-in scale, and recorded on-line by a computer in an adjacent room. By graphing the cumulative weight of the food eaten against time, a curve results that can be best described by a quadratic equation ($y = a + bx + cx^2$) (Kissileff *et al.* 1982). A non-linear regression analysis determines the coefficients of the *cumulative intake curve*, where 'b' describes the initial rate of eating, and 'c' reflects the deceleration of eating and is, therefore, considered to be an expression of internal mechanisms of satiation (Kissileff *et al.* 1982; Stellar *et al.* 1985).

Blood analyses

Each blood sample (the first 2 ml discarded) was drawn into a 2.7 ml sodium-fluoride tube and a 4ml EDTA-tube of which 1 ml was transferred into a glass tube containing 50 ml Aprotinin (500 KIU, Boehringer, Mannheim) for later analysis of glucagon. All tubes were centrifuged at 3800 rpm (≅ 1900 G) for 10 min at 4°C, and the plasma was removed and stored at -20°C for analysis of metabolites and hormones. Insulin and glucagon were determined by commercially available radioimmunoassays (Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden; Glucagon Double Antibody, Diagnostic Products Corporation, Los Angeles, CA). Glucose, lactate, triglycerides (TG), free fatty acids (FFA), and β-hydroxy-butyrate (BHB) were determined by standard colorimetric and enzymatic methods adapted for the Cobas Mira auto-analyzer (Hoffman LaRoche, Switzerland) (Langhans *et al.* 1992b; Surina *et al.* 1993).

Statistics

One way repeated measure analyses of variance (ANOVA) were used to detect differences between breakfast varieties in energy intake at lunch, cumulative intake curve coefficients, questionnaire ratings, plasma levels of metabolites and hormones, and time between breakfast and lunch in Experiment 2. Changes in plasma metabolite and hormone levels with time were also analyzed by ANOVA. When appropriate, additional comparisons of means were done with Student Newman Keuls post-hoc tests. Pearson product moment correlations were used to correlate energy intake, plasma levels of metabolites and ratings. To compare overall glucose, lactate and insulin responses to the test breakfasts and to the target lunch, the integrated areas under the curves' main peaks around breakfast and lunch (= net response; see Results for exact time points) were calculated for each subject in Experiment 1. P values < 0.05 were considered significant. *SigmaStat* software version 1.0 (Jandel Corporation, San Rafael, CA) was used for statistical calculations.

RESULTS

Lunch intake

The amount of food consumed during the fixed time lunch (Experiment 1) or the self-timed lunch (Experiment 2) did not differ significantly after the HFC, MFC, LFC or standard breakfasts (Fig. 2A, 2B) (Experiment 1: $F(3,7) = 2.77$, $P > 0.07$), Experiment 2: $F(3,7) = 1.01$, $P > 0.40$). Lunch duration did also not differ significantly after the 4 breakfast varieties in both experiments (Experiment 1: $F(3,7) = 0.16$, $P > 0.92$, Experiment 2: $F(3,7) = 1.84$, $P > 0.18$). In Experiment 2, the breakfast-lunch intermeal interval ranged from 172 to 345 minutes and was not influenced by the breakfast variety ($F(3,7) = 0.94$, $P > 0.43$) (Fig. 2B). Water intake during lunch was around 300ml for all breakfast varieties in both experiments and was also not significantly different (Experiment 1: $F(3,7) = 0.54$, $P > 0.66$; Experiment 2: $F(3,7) = 1.27$, $P > 0.31$).

EXPERIMENT 1

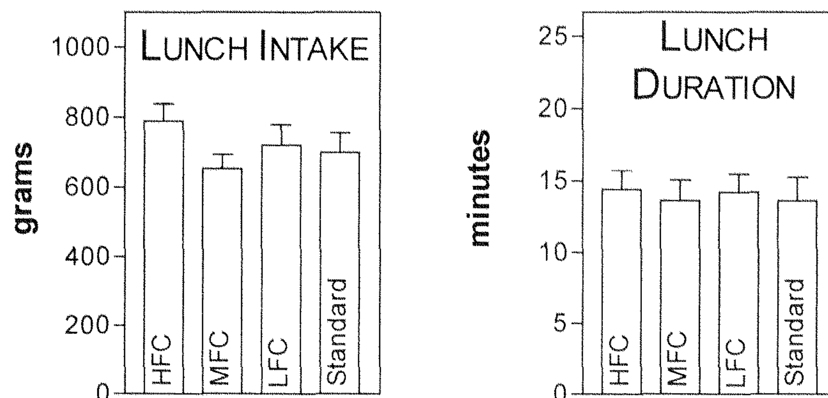


Figure 2A: *Experiment 1: Size and duration of the fixed time lunch after the HFC, MFC, LFC, and standard breakfast. Bars represent means ± SEM of 8 subjects.*

Microstructure of lunch

As indicated by the mean cumulative food intake curves for the breakfast varieties (Figs. 3A and 3B), the microstructure of eating during the fixed time lunch (Fig. 3A) and the self-timed lunch (Fig. 3B) was also not affected significantly by the four breakfast varieties. The plots presented in Figures 3A and 3B were obtained by performing a non-linear regression analysis on the microstructure data recorded by the UEM, fitting a quadratic equation to the cumulative intake curve (Kissileff *et al.* 1982; Rigaud *et al.* 1987), plotting intake as a function of time, and extending the average curves for the mean meal duration.

EXPERIMENT 2

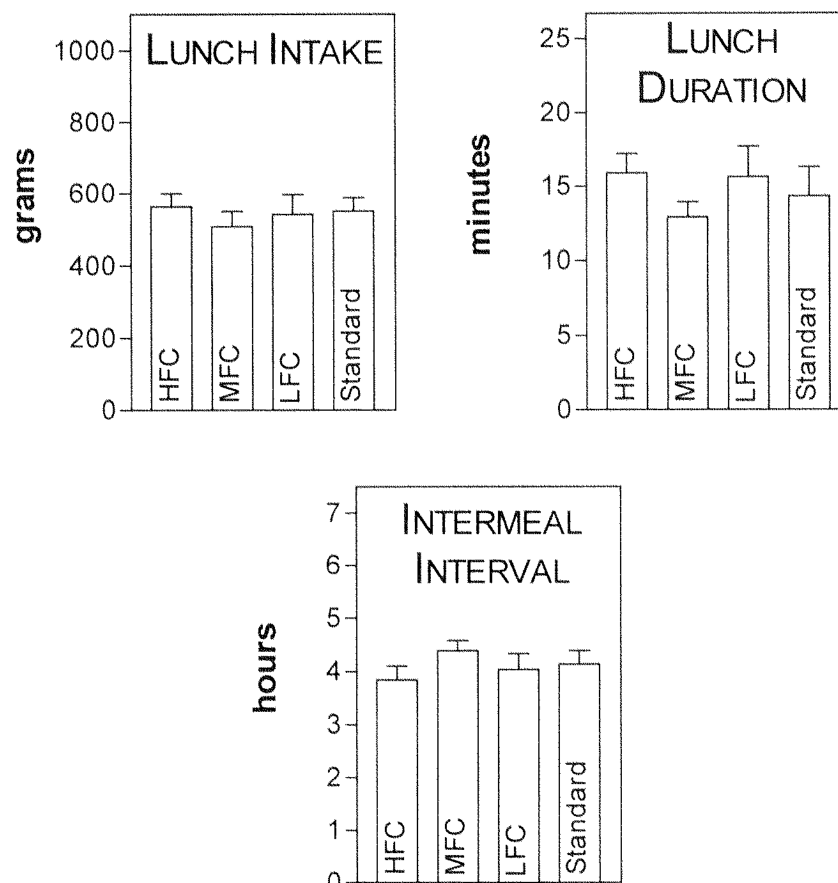


Figure 2B: *Experiment 2: Size and duration of the self-timed lunch and duration of the intermeal interval (breakfast to lunch) after the HFC, MFC, LFC, and standard breakfast. Bars represent means ± SEM of 8 subjects.*

There was no significant difference in the rate (b) or deceleration (c) coefficients between HFC, MFC, LFC, and the standard breakfast in either experiment. In both experiments lunch duration was negatively correlated with the rate of intake (Experiment 1: $r = -0.55$, $P < 0.01$; Experiment 2: $r = -0.83$, $P < 0.01$). In Experiment 2 the duration of the intermeal interval was positively correlated with the intake rate (b: $r = 0.41$, $P < 0.03$), and negatively correlated with the deceleration of intake (c: $r = -0.43$, $P < 0.02$), but it was not significantly correlated with the amount eaten at lunch ($r = -0.09$, $P > 0.65$). Furthermore, there was a positive correlation of the deceleration of the cumulative intake curve with the amount eaten ($r = 0.59$, $P < 0.01$) and with lunch duration ($r = 0.71$, $P < 0.01$).

Although the results of the two experiments in this study cannot be directly compared (different subjects, different days), it is interesting to note some overall differences between the microstructure of lunch in both situations: [1] There was less variability in the cumulative intake curves between the breakfast varieties in Experiment 1 than in Experiment 2. [2] Despite the somewhat longer intermeal interval in the self-timing

situation, subjects in Experiment 2 consumed generally less food during lunch than subjects in Experiment 1 ($F(1,15) = 20.8$, $P < 0.01$). As this was not associated with a reduced lunch duration ($F(1,15) = 0.19$, $P > 0.67$), the overall rate of intake (expressed by the coefficient b of the cumulative intake curve) was lower in Experiment 2 ($b = 0.80 \pm 0.05$, mean ± SEM) than in Experiment 1 ($b = 1.11 \pm 0.05$) ($F(1,15) = 17.6$, $P < 0.01$).

EXPERIMENT 1

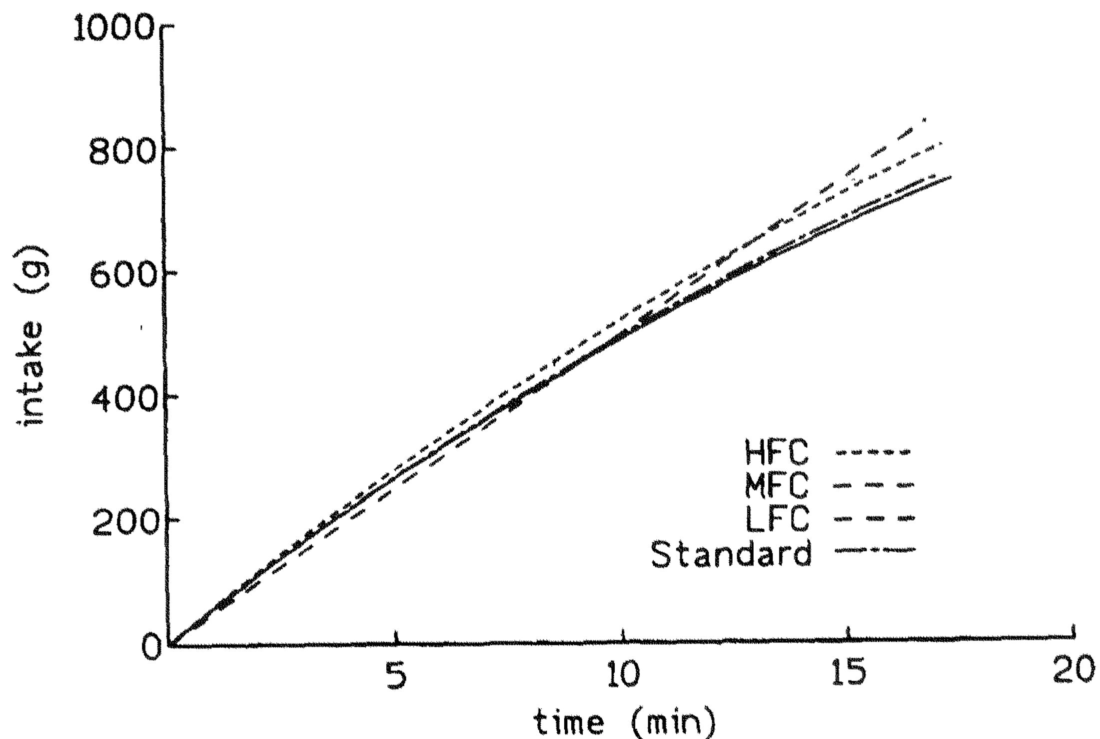


Figure 3A: *Experiment 1: Fitted cumulative intake curves at the fixed time lunch plotted with the mean coefficients for each breakfast variety.*

Questionnaires

The subjects liked all test breakfasts similarly in both experiments (Tables 2 and 3). Hunger ratings before and after the meals as well as the prandial declines and post-breakfast increases in hunger ratings (Δ^1, Δ^2 in Tables 2 and 3) were also not affected significantly by the different test breakfasts. The HFC and standard breakfasts (Experiment 1; $F(3,7) = 3.82$, $P < 0.05$) and the MFC breakfast (Experiment 2; $F(3,7) = 4.12$, $P < 0.02$) were estimated to have a longer lasting satiety effect than the LFC breakfast. After lunch, postprandial fullness ($r = 0.40$, $P < 0.05$), but not remaining hunger sensations ($r = 0.11$, $P > 0.5$), was positively correlated with the amount eaten at lunch.

EXPERIMENT 2

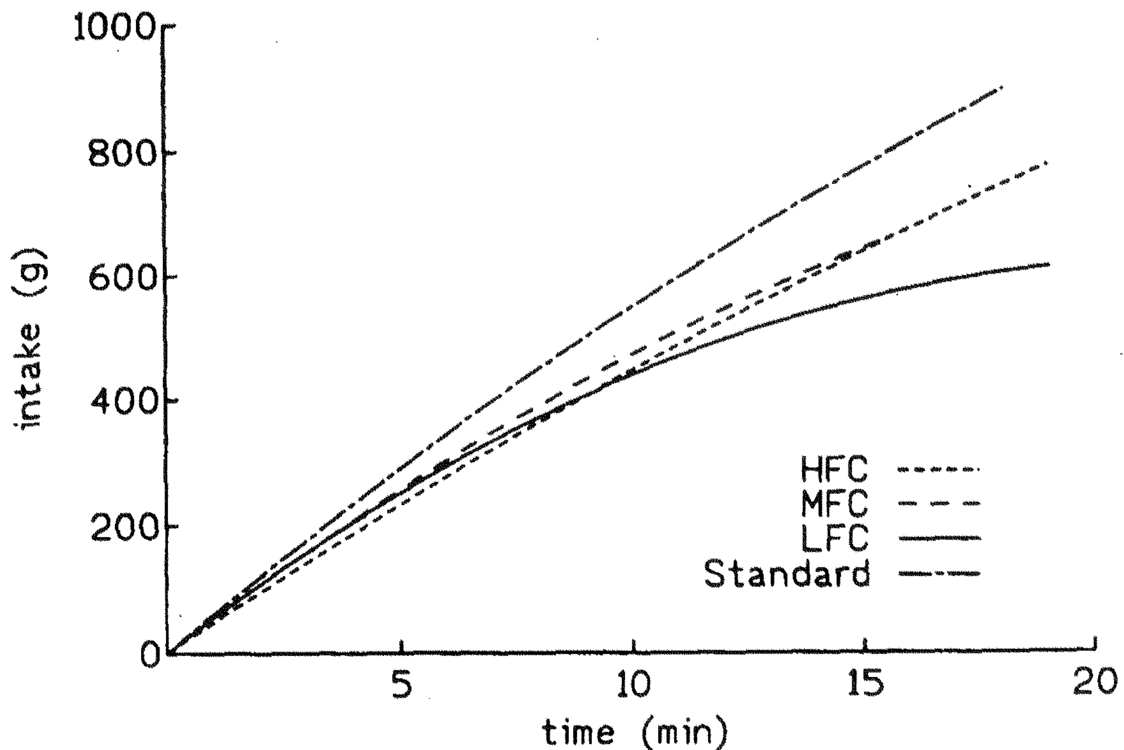


Figure 3B: *Experiment 2: Fitted cumulative intake curves at the self-timed lunch plotted with the mean coefficients for each breakfast variety.*

Plasma metabolites and hormones

Experiment 1

The plasma glucose concentration changed significantly with time ($F(11,31) = 19.2$, $P < 0.001$) and varied between breakfasts (Fig. 4). With the LFC breakfast, it was higher than with the other breakfasts at 60 min and lower at 210 min. The overall glucose response to the breakfasts (area under the curve between 0 and 80 min) was also greater after the LFC than after the other breakfasts ($P < 0.05$ in post-hoc test after significant ANOVA, $F(3,7) = 4.01$). The breakfasts did not affect the plasma glucose level or overall glucose response after lunch (area under the curve between 210 and 290 min; $F(3,7) = 0.45$, $P > 0.7$), nor did the glucose response after breakfast differ from the response after lunch ($F(1,31) = 0.49$, $P > 0.5$). Only with the LFC breakfast, plasma glucose level was lower before lunch (210 min) than at baseline (0 min)($F(1,7) = 32.9$, $P < 0.001$). After the HFC and MFC breakfasts, the plasma glucose level was significantly lower than after the subsequent lunch (40 min vs. 250 min) (HFC: $F(1,7) = 11.9$, $P < 0.01$; MFC: $F(1,7) = 10.4$, $P < 0.01$). Finally, there was no significant correlation between the glucose level before lunch and hunger ratings at the corresponding time ($r = 0.07$, $P > 0.16$).

TABLE 2: QUESTIONNAIRE RATINGS CONCERNING HUNGER AND SATIETY SENSATIONS BEFORE AND AFTER MEALS (EXPERIMENT 1)

BREAKFAST		Questions	HFC	MFC	LFC	Standard	P-value
Before breakfast		How strong is your desire to eat? (Answers 1-5)	3.1 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	3.3 ± 0.5	NS
		How full do you feel?	20.5 ± 7.0	14.4 ± 4.9	18.6 ± 8.5	18.4 ± 7.8	NS
	A	How hungry are you right now?	58.3 ± 5.8	63.1 ± 6.3	55.0 ± 6.0	58.9 ± 6.5	NS
After breakfast		How did the meal taste?	62.1 ± 7.2	74.2 ± 7.5	60.3 ± 9.4	85.3 ± 5.9	NS
		How full do you feel after this meal?	69.8 ± 8.3	53.5 ± 10.4	70.6 ± 9.5	63.3 ± 8.3	NS
	B	How hungry are you after this meal?	27.0 ± 7.9	43.9 ± 10.4	30.4 ± 10.7	27.4 ± 10.3	NS
	Δ^1	Prandial decrease of hunger (= A - B)	31.3 ± 8.8	19.3 ± 12.2	24.6 ± 12.7	31.5 ± 12.6	NS
		How thirsty are you after this meal?	17.4 ± 5.1	13.6 ± 4.3	27.3 ± 10.1	12.0 ± 3.7	NS
		How long do you think you will be satiated after this meal?	61.5 ± 9.3	46.6 ± 10.2	25.0 ± 6.0	55.3 ± 10.2	0.025 [1]
LUNCH							
Before lunch		How strong is your desire to eat? (Answers 1-5)	3.0 ± 0.2	3.3 ± 0.3	3.5 ± 0.3	3.3 ± 0.4	NS
		How full do you feel?	28.1 ± 8.5	24.3 ± 10.4	12.6 ± 4.5	21.1 ± 6.6	NS
	C	How hungry are you right now?	59.9 ± 8.2	56.8 ± 10.2	65.5 ± 6.2	61.4 ± 6.6	NS
	Δ^2	Postprandial increase in hunger (= C - B)	32.9 ± 10.8	12.9 ± 14.9	35.1 ± 13.1	34.0 ± 12.5	NS
After lunch		How did the meal taste?	63.9 ± 4.9	60.4 ± 6.0	67.3 ± 7.1	65.0 ± 5.3	NS
		How full do you feel after this meal?	93.0 ± 3.4	88.1 ± 3.8	86.1 ± 5.7	92.8 ± 3.1	NS
		How hungry are you after this meal?	6.5 ± 2.4	8.4 ± 3.4	10.3 ± 4.3	4.8 ± 2.1	NS
		How thirsty are you after this meal?	27.9 ± 8.0	15.5 ± 5.1	37.9 ± 11.6	18.3 ± 6.57	NS
		How long do you think you will be satiated after this meal?	65.5 ± 10.7	67.3 ± 8.4	59.3 ± 11.9	64.0 ± 6.9	NS

Values are means ± SEM of 8 subjects; 0 = weakest and 107 (in mm of visual analogue scale or 5 respectively) = strongest sensation of the feeling. HFC = High fiber cereal, MFC = Medium fiber cereal, LFC = Low fiber cereal.

[1] Student-Newman-Keuls test: $F(3,21) = 3.82$; HFC different from LFC, LFC different from standard breakfast.

TABLE 3: QUESTIONNAIRE RATINGS CONCERNING HUNGER AND SATIETY SENSATIONS BEFORE AND AFTER MEALS (EXPERIMENT 2)

BREAKFAST		Questions	HFC	MFC	LFC	Standard	P-value
Before breakfast		How strong is your desire to eat? (Answers 1-5)	3.3 ± 0.3	3.0 ± 0.3	3.3 ± 0.2	3.3 ± 0.3	NS
		How full do you feel?	14.0 ± 2.7	22.7 ± 4.6	17.3 ± 4.4	17.4 ± 4.3	NS
	A	How hungry are you right now?	74.1 ± 4.8	64.9 ± 5.8	68.6 ± 3.0	70.9 ± 6.6	NS
After breakfast		How did the meal taste?	61.1 ± 9.66	71.9 ± 7.7	48.9 ± 10.3	64.6 ± 9.26	NS
		How full do you feel after this meal?	81.4 ± 4.4	82.9 ± 3.4	79.3 ± 7.5	75.9 ± 3.7	NS
	B	How hungry are you after this meal?	34.6 ± 12.1	14.6 ± 3.4	21.4 ± 6.1	23.3 ± 5.8	NS
	Δ[1]	Prandial decrease of hunger (= A - B)	39.6 ± 13.7	50.3 ± 6.5	47.3 ± 6.6	47.6 ± 7.8	NS
		How thirsty are you after this meal?	48.4 ± 11.9	62.9 ± 8.3	52.6 ± 11.3	42.5 ± 12.6	NS
		How long do you think you will be satiated after this meal?	51.6 ± 9.5	66.0 ± 6.7	39.1 ± 5.54	33.4 ± 6.0	0.02 [1]
LUNCH							
Before lunch		How strong is your desire to eat? (Answers 1-5)	3.3 ± 0.2	3.1 ± 0.1	3.5 ± 0.3	3.6 ± 0.3	NS
		How full do you feel?	13.0 ± 3.4	19.8 ± 3.3	14.6 ± 4.2	13.8 ± 4.2	NS
	C	How hungry are you right now?	76.3 ± 4.8	72.5 ± 4.8	78.1 ± 5.6	76.9 ± 5.5	NS
	Δ[2]	Postprandial increase in hunger (= C - B)	41.7± 12.0	57.9 ± 6.1	56.8 ± 8.6	53.6 ± 6.7	NS
After lunch		How did the meal taste?	69.5 ± 6.7	74.0 ± 5.3	79.5 ± 5.9	73.5 ± 5.9	NS
		How full do you feel after this meal?	78.1 ± 10.0	81.3 ± 5.6	89.1 ± 3.7	89.4 ± 3.6	NS
		How hungry are you after this meal?	6.6 ± 1.3	11.3 ± 2.7	6.3 ± 1.6	7.9 ± 1.8	NS
		How thirsty are you after this meal?	28.0 ± 11.6	42.1 ± 11.0	33.3 ± 13.1	24.9 ± 10.2	NS
		How long do you think you will be satiated after this meal?	71.7 ± 11.5	80.0 ± 5.9	64.5 ± 11.5	77.5 ± 6.9	NS

Values are means ± SEM of 8 subjects; 0 = weakest and 107 (in mm of visual analogue scale or 5 respectively) = strongest sensation of the feeling. HFC = High fiber cereal, MFC = Medium fiber cereal, LFC = Low fiber cereal.

[1] Student-Newman-Keuls test: $F(3,20) = 4.12$; MFC different from LFC and standard breakfast.

The plasma lactate concentration (Fig. 4) also changed with time ($F(11,31) = 14.1$, $P < 0.001$) and differed between breakfasts, i.e. it was higher after the LFC breakfast than after the other breakfasts at 80 and 120 min (Fig. 4). The overall lactate response (area under the curve between 40 and 160 min) to the LFC breakfast was also greater than the response to the other breakfasts ($P < 0.05$ in post-hoc test after significant ANOVA, $F(3,7) = 6.33$). The lactate response was generally greater after breakfast than after lunch ($F(1,31) = 23.6$, $P < 0.001$). After the MFC, LFC, and standard breakfasts, plasma lactate concentration was higher than after lunch ($Ps < 0.05$ for 80 vs. 290 min).

The plasma insulin concentration also changed with time ($F(11,31) = 36.0$; $P < 0.001$) (Fig. 5) and significant treatment differences were observed after breakfast (60 min):

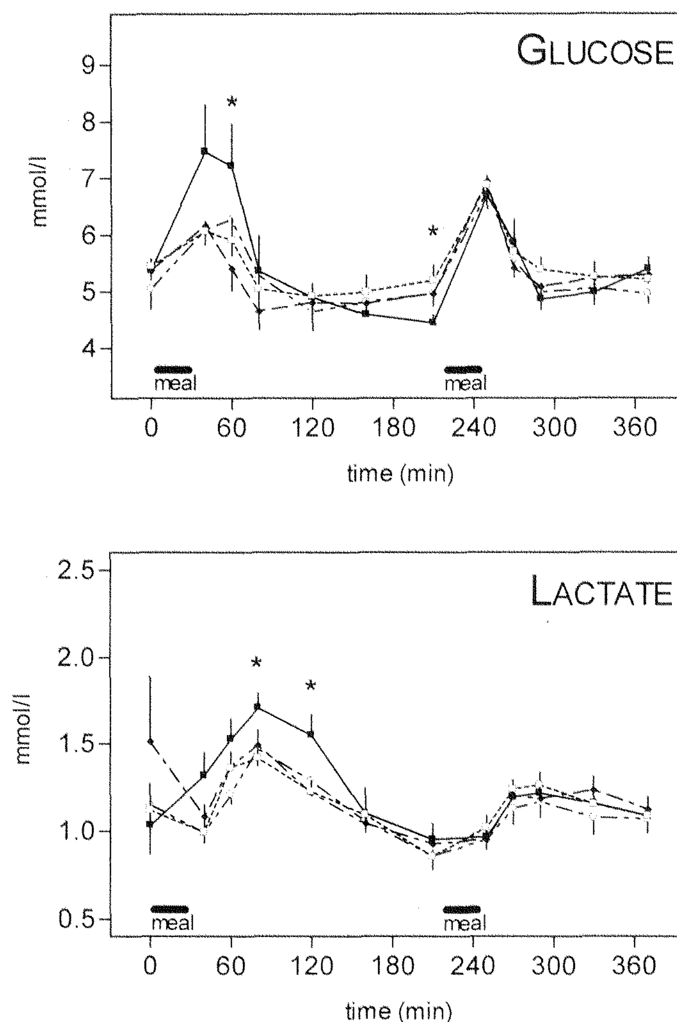


Figure 4: *Effect of HFC, MFC, LFC, and standard breakfast on plasma glucose (top) and plasma lactate (bottom) concentrations. Values are means ± SEM of 8 subjects. * Indicates significant treatment differences, $P < 0.05$ in post-hoc tests after significant ANOVA (glucose: 60 min, $F(3,7)=3.27$, 210 min, $F(3,7)=5.59$; lactate: 80 min $F(3,7) = 3.63$, 120 min $F(3,7) = 6.29$).*

The LFC breakfast increased plasma insulin concentration more and triggered a greater overall insulin response (area under the curve between 0 and 160 min) than the HFC and MFC breakfasts, ($P < 0.05$ in post-hoc test after significant ANOVA, $F(3,7) = 6.10$).

The overall insulin response to all breakfasts was not significantly different from the response to lunch ($F(1,31) = 0.23$; $P > 0.62$). Plasma insulin generally dropped to about baseline level before lunch. In general, postprandial hyper-insulinemia was also similar following breakfast and lunch, but with the HFC ($F(1,7) = 4.77$, $P < 0.05$) and the standard breakfast ($F(1,7) = 5.08$, $P < 0.05$), plasma insulin was higher after lunch than after breakfast (250 vs. 40 min). Insulin levels just before lunch (210 min) were negatively correlated with the reported satiety state at that time ($r = -0.47$, $P < 0.01$) and positively correlated with the amount eaten at lunch ($r = 0.43$, $P < 0.05$).

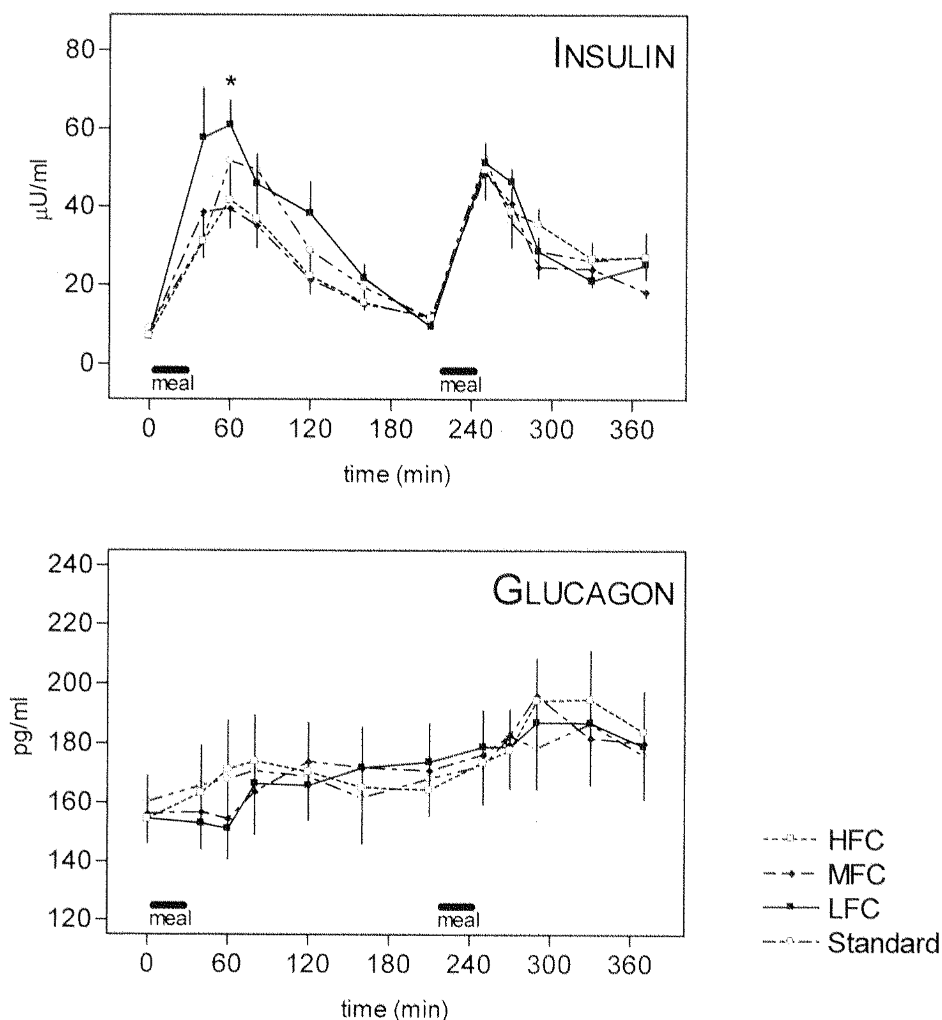


Figure 5: *Effect of HFC, MFC, LFC, and standard breakfast on plasma insulin (top) and plasma glucagon (bottom) concentrations. Values are means ± SEM of 8 subjects. * Indicates significant treatment differences, $P < 0.05$ in post-hoc tests after significant ANOVA ($F(3,7) = 3.53$)*

Plasma glucagon levels gradually increased with time (F(11,31) = 19.2, P < 0.001) (Fig. 5), but there was no significant difference between breakfast varieties at any time. The insulin/glucagon ratio (Fig. 6) was also calculated for all individual plasma samples and was significantly higher at 40 and 120 min after the LFC breakfast than after the other breakfast varieties.

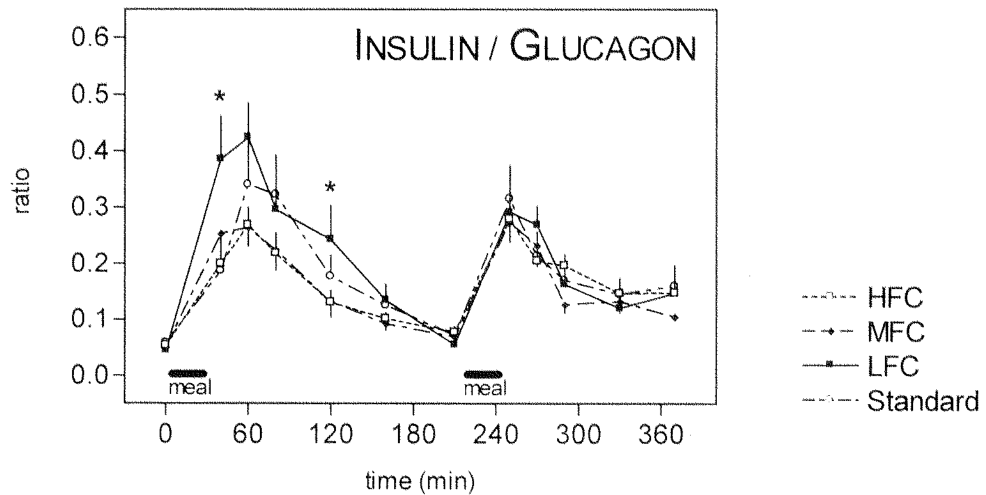


Figure 6: *Effect of HFC, MFC, LFC, and standard breakfast on insulin/glucagon ratio. Values are ratio means ± SEM of 8 subjects. * Indicates significant treatment differences, P < 0.05 in post-hoc tests after significant ANOVA (40 min F(3,7) = 4.22, 120 min F(3,7) = 3.66).*

Plasma β-hydroxy-butyrate (BHB) decreased with time (F(11,31) = 13,5, P < 0.001) (Fig. 7). Basal plasma concentrations of BHB varied considerably between subjects. This was mainly due to one subject whose BHB value was always particularly high in the first four plasma samples. No significant treatment differences in plasma BHB level were observed between breakfast and lunch. After lunch, plasma BHB level was lower at 270 min with the LFC breakfast than with the MFC breakfast. At 290 and 330 min plasma BHB was lower with the standard breakfast than with the HFC or MFC breakfast.

The plasma free fatty acid (FFA) concentration changed with time (F(11,31) = 25.1, P < 0.001) (Fig. 7). More specifically, it decreased during breakfast and increased after lunch. Significant differences between test breakfasts were found at 160 min, when the plasma FFA level was higher after the HFC than after the LFC or the standard breakfasts, and higher after the MFC than after the LFC breakfast. Plasma FFA level was generally higher after lunch than after breakfast (290 vs. 80 min, P < 0.05 for MFC, LFC, and standard breakfast). With the standard breakfast the FFA level was significantly higher after the subsequent lunch (330 min) than with the other breakfasts (Fig. 7).

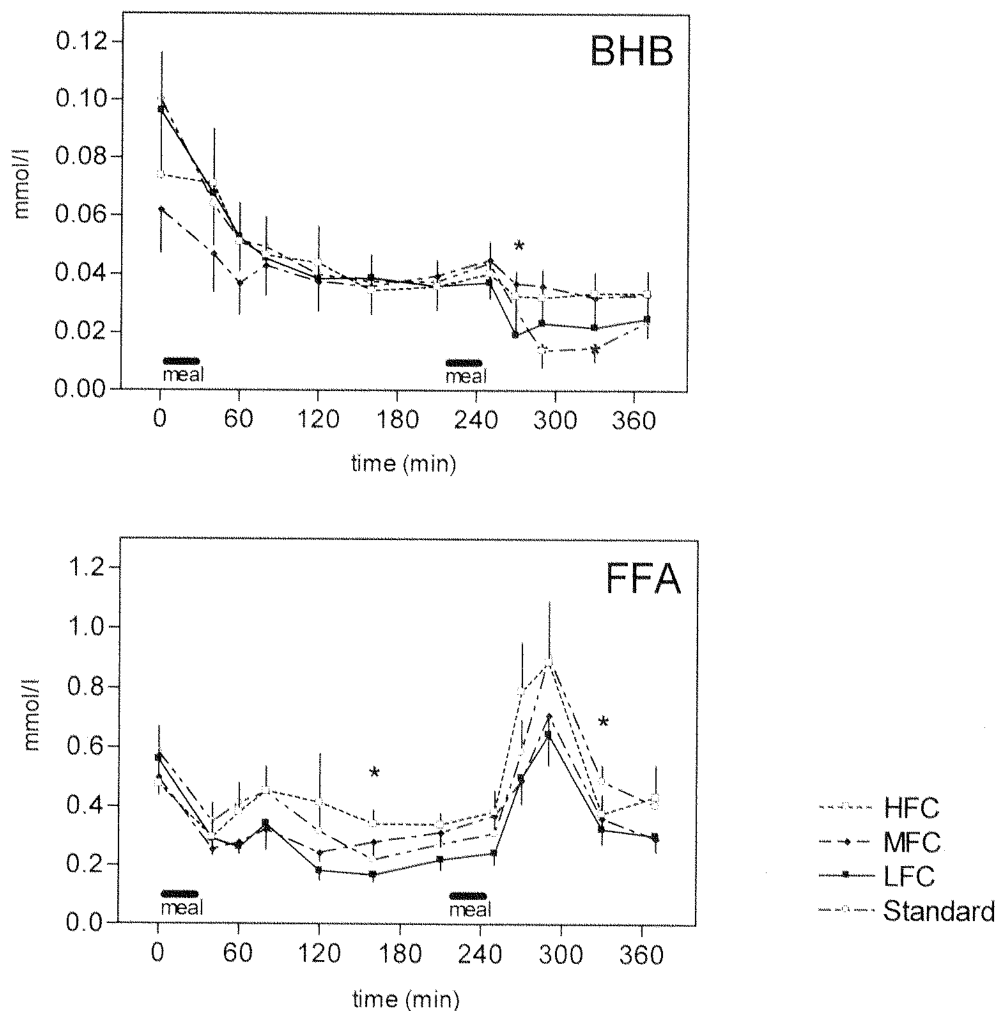


Figure 7: *Effect of HFC, MFC, LFC, and standard breakfast on plasma β-hydroxybutyrate (BHB, top) and free fatty acids (FFA, bottom) concentrations. Values are means ± SEM of 8 subjects. * Indicates significant treatment differences in post-hoc tests after significant ANOVA (BHB: 270 min $F(3,7) = 3.17$, 290 min $F(3,7) = 6.42$, 330 min $F(3,7) = 5.15$; FFA: 160 min $F(3,7) = 7.24$, 330 min $F(3,7) = 4.78$).*

The plasma triglyceride level ($F(11,31) = 45.7$, $P < 0.01$) gradually increased, and total plasma protein level ($F(11,31) = 4.44$, $P < 0.01$) (Fig. 8) decreased with time, but there was no significant difference between breakfast varieties for these parameters at any time.

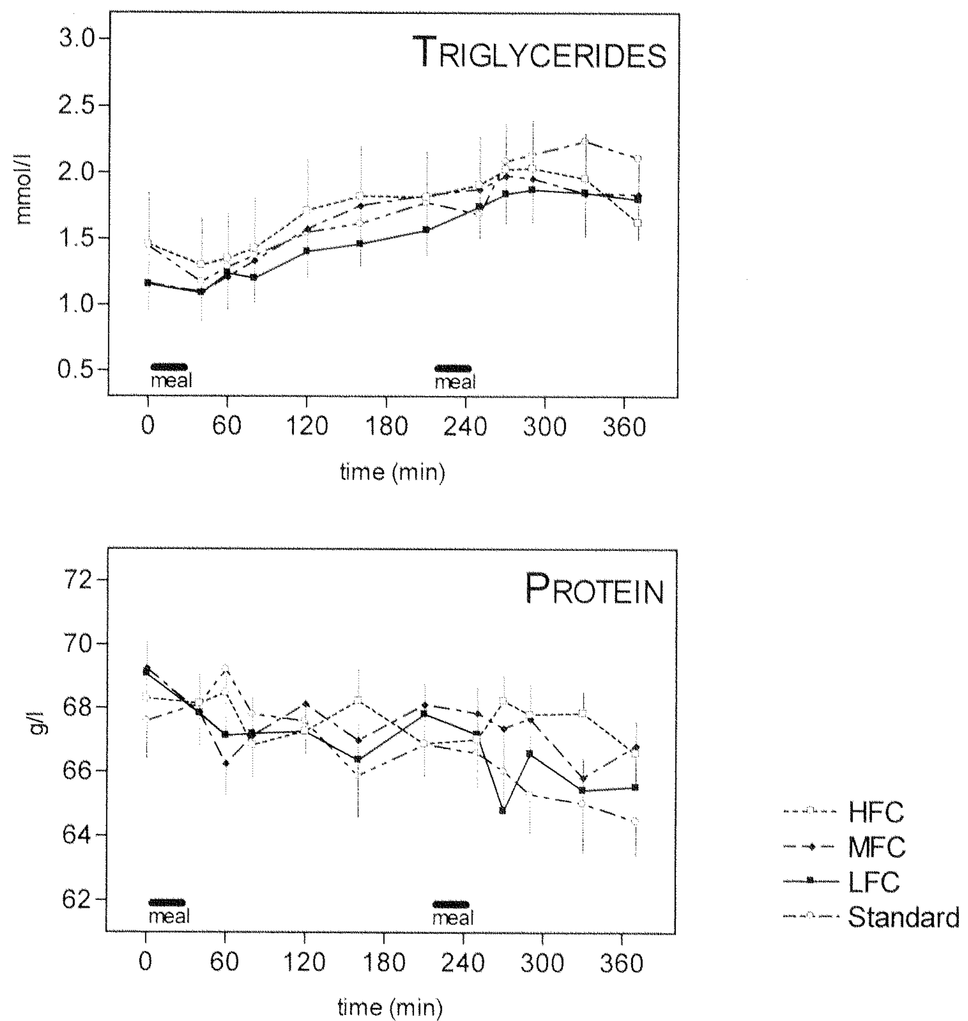


Figure 8: *Effect of HFC, MFC, LFC, and standard breakfast on plasma triglyceride (top) and plasma protein (bottom) concentrations. Values are means ± SEM of 8 subjects.*

Experiment 2

As far as can be judged from the limited number of samples, the postprandial metabolic responses to the 4 breakfast varieties were similar to the responses in Experiment 1 and are therefore not presented. Again plasma glucose level just prior to lunch was lowest after the LFC breakfast ($F(3,7) = 7.68$, $P < 0.002$). None of the other metabolite and hormone levels at that time differed between the breakfast varieties. Hunger ratings prior to lunch and glucose level were again not correlated, but there was a significant positive correlation between the glucose level just before lunch and the duration of the intermeal interval ($r = 0.37$, $P < 0.04$).

DISCUSSION

In the present study, equienergetic, "real" breakfasts with varying fiber content and nutrient composition did not differentially affect hunger ratings, energy intake and microstructure of eating during the subsequent lunch, served at a predetermined or at a self-selected time. This is in line with and extends several previously reported failures of dietary fiber (Blundell *et al.* 1993; Burley *et al.* 1987; de Graaf *et al.* 1992) or macronutrient content (de Graaf *et al.* 1992; Foltin *et al.* 1992) to affect energy intake. Yet, reduced hunger ratings or energy intake in response to dietary fiber ingestion have also been reported (Astrup *et al.* 1990; di Lorenzo *et al.* 1988; Levine *et al.* 1989; Raben *et al.* 1994b; Rigaud *et al.* 1987). These discrepancies are presumably due to procedural differences. In some studies in which dietary fiber did reduce hunger ratings or subsequent energy intake, higher amounts of fiber were used (Astrup *et al.* 1990; di Lorenzo *et al.* 1988; Levine *et al.* 1989), the fiber was given over longer periods of time (Astrup *et al.* 1990; Rigaud *et al.* 1987), or the high and low fiber test meals were not equienergetic (Levine *et al.* 1989). Considering that the HFC (10% fiber) and MFC (7% fiber) breakfasts contained a fairly high amount of fiber, the present results also suggest that fiber content of a test meal must be above 10% to influence subsequent energy intake under normal ecological conditions.

Unlike some other studies testing the effect of dietary fiber on energy intake, the macronutrient content of the breakfasts used in this experiment varied because the main goal was to compare "real" breakfasts, i.e. commercially available cereals prepared close to the suggestions of the manufacturer. Therefore, the breakfasts provided the same amount of energy but had a different macronutrient content. It is unlikely that an effect of dietary fiber on lunch intake was obscured by the varying macronutrient content because the target lunch was served about 3 hours after completion of breakfast, at a time when an immediate effect of breakfast macronutrient composition on energy intake had presumably ceased (Blundell *et al.* 1993). Furthermore, nutrient composition of liquid breakfasts used elsewhere had no effect on the energy intake of a lunch consumed 3.5 hours later (de Graaf *et al.* 1992). On the other hand, fiber supplementation of breakfast has been reported to affect lunch intake 3.5 hours later (Levine *et al.* 1989).

The fiber content and the nutrient composition of the breakfasts used in this study were apparently not able to change the timing of the subsequent lunch or the amount eaten. As meal size and the timing of meals seem to be in part controlled by different factors (Langhans *et al.* 1992), the present results indicate that fiber content and nutrient composition of breakfast do not influence the maintenance of satiety or the mechanism(s) that terminate food intake at lunch.

The shape of the cumulative intake curve is considered to reflect learning processes and internal mechanisms regulating food intake (Kissileff *et al.* 1982; Westerterp-Plantenga *et al.* 1990). The mean cumulative intake curves of each test day in this study were remarkably similar to the curves described by Kissileff *et al.* (Kissileff *et al.* 1982). We did not see a pronounced deceleration of intake towards meal end in any of the experiments, as described for normal weight subjects by Westerterp-Plantenga *et al.* (Westerterp-Plantenga *et al.* 1990). However, non-decelerated cumulative intake curves

are not unusual for normal weight, young males (Kaplan 1980; Westerterp-Plantenga *et al.* 1990). Although a direct comparison of the curves of both experiments is inappropriate, the smaller variability of the cumulative intake curves, the higher rate of intake and the greater lunch intake, despite the somewhat shorter intermeal interval in Experiment 1 compared to Experiment 2, seem to suggest that the normal physiological controls of eating were somehow weakened by the fixed timing of the lunch in Experiment 1. Further studies are necessary to clarify this interesting point, in particular as most short-term human eating studies employ fixed time target meals.

Although the breakfast varieties did not significantly affect post-breakfast hunger ratings and lunch intake, the subjects believed that the HFC and standard breakfast (Experiment 1) or the MFC breakfast (Experiment 2) would suppress hunger for a longer time than the LFC breakfast. This seems to reflect a judgment of the satiating efficiency of foods based on the perceived nutritional value: The sweet LFC may have been considered less nutritional than the HFC or MFC, two products that are advertised and conceived as healthy and very nutritional.

There were significant postprandial metabolic differences between treatments, particularly for glucose, insulin, and lactate. In earlier studies, a delayed postprandial increase in plasma glucose after the consumption of complex carbohydrates has been reported (Delargy *et al.* 1993; Leathwood *et al.* 1988; Torsdottir *et al.* 1989), presumably due to slower glucose absorption from the intestines into the blood. The higher fiber content of the HFC and MFC may have resulted in the slower uptake of glucose into the blood stream, resulting in the attenuated increase in blood glucose. Glucose and insulin, except on LFC breakfast days, increased faster after lunch than after breakfast. Whether or not this reflects a delaying effect of the breakfast fiber content (in particular of the HFC and MFC breakfasts) on glucose absorption cannot be judged from the present data. On the other hand, the overall glucose, insulin, and lactate responses to breakfast were more pronounced after the LFC than after the other test breakfasts. The higher glycemic response to the LFC might be a result of the higher sugar and lower fiber content of the breakfast. The fiber content of the breakfasts may have delayed glucose absorption somewhat, however, as carbohydrate and fiber content of the breakfasts varied, it cannot be judged from the present results to what extent the fiber content alone influenced the glycemic response.

A reactive postprandial hypo-glycemia was detected after the LFC breakfast in both experiments, i.e. shortly before lunch plasma glucose concentration was lower on days with the LFC breakfast than on the other experimental days. As indicated by the high plasma lactate concentration after the LFC breakfast, the reactive hypo-glycemia reflects an enhanced glucose utilization, which can be attributed to the high insulin response. Considering that a premeal decline in blood glucose may provide a metabolic pattern that contributes to meal initiation (Campfield *et al.* 1990; Louis-Sylvestre *et al.* 1980), which has recently been described also in man (Campfield *et al.* 1995), it is interesting to note that the preprandial glucose level was correlated with the duration of the self-controlled intermeal interval in Experiment 2. This is compatible with the idea that blood glucose level is somehow involved in the maintenance of satiety. On the other hand, we found no significant correlation between the blood glucose concentration and the hunger ratings before lunch.

The rise in the plasma FFA level after lunch is presumably due to hydrolysis of ingested triglycerides by lipoprotein lipase. This rise appeared particularly pronounced on days

when the HFC or the standard breakfast were served, but there is no clear explanation for this finding since no other blood parameter showed treatment differences. The increase in plasma FFA after lunch was not accompanied by an increase in plasma BHB, indicating that hepatic fatty acid oxidation was not enhanced. The reasons for this dissociation are unknown. Whether the higher plasma FFA concentration after the HFC breakfast reflects an inhibitory effect of dietary fiber on fatty acid oxidation (Raben *et al.* 1994b), or whether it coincides with enhanced fatty acid oxidation, as might be possible considering the usual positive correlation between plasma FFA concentration and fatty acid oxidation and the low insulin/glucagon ratio for HFC, cannot be decided from the present results.

In summary, the present study demonstrates that "real" breakfasts with different fiber content and nutrient composition have some distinct effects on postprandial metabolic variables, but do not differentially affect the timing, the size, or the microstructure of the subsequent lunch. They also do not alter subsequent hunger and satiety ratings. The results are consistent with the assumption that energy content of a meal is the major determinant of subsequent food intake and that fiber content and nutrient composition have only a modulating effect on subsequent energy intake.

DIETARY FAT LEVEL AND EFFECTS OF A HIGH FAT MEAL

BASED ON:

Silberbauer C.J., Jacober B., Langhans W.: Dietary fat level and short-term effects of a high fat meal on food intake and metabolism.
Annals of Nutrition & Metabolism (1998) 42: 75-89

INTRODUCTION

Fat oxidation is enhanced during fasting, when carbohydrates are in short supply. Yet, baseline fatty acid oxidation in humans increases also with increases in the dietary fat level (e.g. Rumpler *et al.* 1991; Verboeket-van de Venne *et al.* 1994), and a failure to adequately enhance fatty acid oxidation in response to an increase in fat ingestion may be a predisposing factor for obesity (Schutz *et al.* 1989; Astrup *et al.* 1994). To a certain degree, fatty acid oxidation is acutely enhanced after a high fat meal in humans and rats, even when carbohydrates are available (Surina *et al.* 1993; Griffiths *et al.* 1994; Surina-Baumgartner *et al.* 1996). This is interesting because several findings suggest a role of fatty acid oxidation in the maintenance of satiety. Thus, inhibition of fatty acid oxidation is associated with enhanced eating, in particular in rats consuming a fat-rich diet (Scharrer *et al.* 1986; Friedman *et al.* 1990), and this effect is primarily due to a shortening of the intermeal interval (Langhans *et al.* 1987). However, these experiments were done in rats, and it has not yet been thoroughly investigated whether fatty acid oxidation affects food intake in humans.

The increase in fatty acid oxidation with high fat intake is presumably due to an increased activity of the mitochondrial enzymes for fatty acid oxidation (Bremer *et al.* 1984). The fat content of the habitual diet may therefore also determine the acute fatty acid oxidation response to a fat-rich meal. In addition, if fatty acid oxidation contributes to postprandial satiety in humans, the satiating effect of a high fat meal may be different in people accustomed to high or low fat intake. These assumptions were tested in the present study.

Plasma metabolite and hormone levels as well as respiratory measurements were used to assess the acute effects of a high fat breakfast on postprandial fat and carbohydrate metabolism in subjects accustomed to diets with differing levels of fat. Lunch intake was quantified using the universal eating monitor (UEM; Kissileff *et al.* 1980). Additional questionnaire ratings addressed the subjective levels of hunger and satiety.

METHODS

General design

Phase I of the two phase study was conducted to determine the average daily fat intake in an homogenous group of subjects, and to identify subjects with fairly stable but differing fat intake for phase II, in which the effects of a high fat breakfast on metabolic parameters and lunch consumption were assessed. The study protocol was approved by the ethics committee of the University Hospital Zurich.

Subjects

Forty, non-smoking, male subjects, age 29 ± 4 y (mean ± SEM, range: 24 to 37 y), body weight 72.3 ± 1.6 kg (range: 58.6 to 115.5), participated in phase I of the study. All subjects signed their consent after the purpose and procedure of the study was explained to them. The subjects had an average BMI of 22.4 ± 0.3 (18.3 to 26.5) and their average body fat content was 10.5% ± 0.6% (5.5% to 15.5%), as estimated by a bio impedance analyzer (Akern-Rjil Systems, BIA 101/5, Bio Impedance Analyzer). This body fat content is very low, but still within the range (4.6 to 19.0, mean: 12.8) measured in male students of similar BMI using the same method of determination (T. Talluri, Akern SRL, personal communication). In a previous study of ours, the bio impedance analyzer and skin fold measurements yielded similar BMI values (unpublished data). The bio impedance analyzer also gave estimates for lean body mass (LBM) and daily metabolic rate. All 40 subjects exercised at least once a week (average total exercise time per week: 5.3 h ± 0.7 h (0.5 h to 12.9 h)).

Procedure Phase I

All subjects recorded their daily dietary intake by means of a pocket computer for a full seven-day period, including a weekend. After each main meal subjects recorded the kind and amount of food they had just consumed by choosing the corresponding food item(s) from the 400 items listed in the pocket computer. The computer recorded the time, the food items that were chosen and the amount of those food items consumed. Subjects were also given a digital scale for measuring the weight of the foods they would consume during the meals. They were instructed to weigh the food whenever possible, and to otherwise estimate the amount through either a selection of provided photographs of variously sized meals, or by indicating the amounts through household measurements such as tablespoon, pieces, etc. From the list of 400 food items, subjects chose an average of 68 ± 19 items (12 to 98 items) in the seven day recording period.

The data from the pocket computer were analyzed to determine the average daily fat consumption (EBIS, Version 1.1, Stuttgart 1993). Ten of the forty subjects were excluded from further participation in phase II of the study because of inaccurate or incomplete dietary reports (weighing < 50% of the food items eaten), unstable day to day fat consumption (within subject SD of 7 days > 10%), changes of body weight of more than 1kg, excessive physical activity (>mean + 3SD) or excessive alcohol

consumption (> mean + 3SD). The remaining 30 subjects were asked to participate in phase II of the study. For further comparison, the 30 subjects were divided into three groups according to their percentage energy intake from fat. A low fat (LF, ≤ 35% of total energy intake, n = 11, because two subjects had 35% energy intake from fat) and a high fat group (HF, ≥ 40%, n = 12 because three subjects had 40% energy intake from fat) were determined. Yet, data are reported for only 10 LF and 11 HF subjects (see Table 1). The data from two subjects with a BMI > 27 (one LF and one HF) were omitted. The usual fat consumption of the remaining 28 subjects (21-44% of daily energy intake) was later correlated with metabolic and eating parameters.

Procedure Phase II

The experiment was performed on 13 non-consecutive days in a period of 25 consecutive days with each subject participating once. The day before the test trial, strenuous exercise and alcohol consumption after 1700 h were prohibited. On the evening before the test trial subjects consumed a prepackaged dinner consisting of vegetable tortellini (250 g), tomato sauce (190 g), Parmesan cheese (50 g), carrot salad (130 g), and a canned fruit cocktail (227 g) for dessert. The dinner provided 4771 kJ (147.7 g carbohydrates, 41.1 g fat, 42.2 g protein). Subjects were instructed to consume dinner between 1800 h and 2100 h, and the dessert until 2300 h. A minimum of one 330 ml bottle of mineral water had to be consumed at dinner, before going to bed, and before coming to the institute the next morning. Additional bottles of mineral water were provided for individual fluid demands.

Three subjects were tested daily, beginning at 0700 h, 0725 h, and 0750 h. Upon arrival at the laboratory, subjects had fasted for at least 8 to 9 hours. Figure 1 shows the daily proceedings for each subject. After arriving at the institute, a baseline respiratory measurement was taken followed by three more measurements between breakfast and lunch (35 min, 100 min, 250 min; see Figure 1). The four respiratory measurements were taken while the subject was sitting in a comfortable chair. The respiratory quotient (RQ) was determined in sessions lasting 15 minutes using the OXYCON SIGMA metabolic cart (B.V. Mijnhardt, Netherlands) with a ventilated hood system.

After the baseline respiratory measurement, a 20-gauge Vialon catheter (Becton Dickenson, Switzerland) was placed into the antecubital vein, and a baseline blood sample was drawn. Six more blood samples were drawn between breakfast and lunch. Each blood sample (13 ml, the first 2 ml discarded) was drawn into a 2ml EDTA-fluoride tube (Monovette), a 4ml Li-Heparin tube (Monovette), and a 4ml K_2EDTA (Monovette) tube. All tubes were centrifuged at 3000 rpm (≅ 1500 G) for 15 min at 4°C, the plasma was removed and stored at -20°C for later analysis of metabolites and insulin. Insulin was determined using a commercially available radioimmunoassay (Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). Glucose, lactate, triglycerides (TG), free fatty acids (FFA), and β-hydroxy-butyrate (BHB) were determined by standard colorimetric and enzymatic methods adapted for the Cobas Mira auto-analyzer (Hoffman LaRoche, Switzerland) (Surina *et al* 1993; Langhans *et al.* 1992b).

TABLE 1: SUBJECT CHARACTERISTICS

	Age (y)	Weight (kg)	Height (cm)	BMI (kg/m^2)	Body fat (%)
LF (n = 10)	29.8 ± 1.3	73.6 ± 2.5	179.7 ± 2.8	22.7 ± 0.4	10.7 ± 0.5
HF (n = 11)	29.5 ± 1.2	72.5 ± 2.7	179.7 ± 1.7	22.4 ± 0.6	10.7 ± 0.9
P-value	--	--	--	--	--

	Fat consumption (%)	Fat consumption (g)	Weekly exercise (kJ)	Daily energy intake (kJ)
LF (n = 10)	30.9 ± 1.5	100 ± 8.1	9499± 1837	12805 ± 1036
HF (n = 11)	41.3 ± 0.4	130 ± 7.0	7108 ± 1655	11992 ± 727
P-value	< 0.0001	< 0.02	--	--

Values are means ± SEM; LF = low fat group (n=10), HF = high fat group (n=11)

After the baseline blood sample, a high fat (52% of total energy from fat) breakfast was served (Table 2). A dietitian designed the breakfast. It provided 5204 kJ (111.0 g carbohydrates, 71.0 g fat, 36.2 g protein), and consisted of a wheat/rye bread roll (150 g), butter (20 g), cheese (22 g), scrambled eggs (2 midsize eggs) with cream (30 g) and butter (20 g), raspberries (220 g) with sugar (11 g) and whipped cream (30 g), and mineral water (ad lib). After the meal, further blood samples and respiratory measurements were taken until lunch was served (see Fig. 1). Subjects remained in the laboratory until after lunch. They engaged in sedentary activities such as reading, watching videos, and talking.

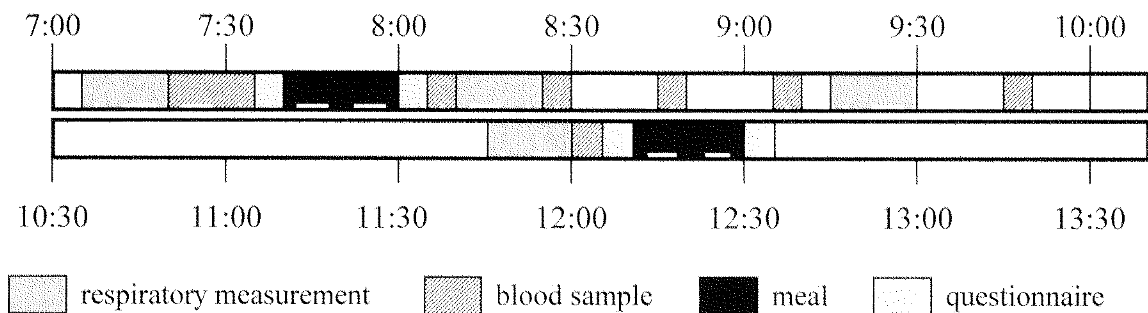


Figure 1: *Schedule of experimental proceedings for the first subject on each test day. Second and third subject with delay of 25 and 50 min, respectively.*

A hot lunch (Table 2) was served 4,5 hours after the onset of breakfast (see Figure 1). It consisted of an oversized serving of a mushroom flavored rice stew (risotto) (27% of total energy from fat), with a bite sized meat substitute (250 g Cornatur®), butter (20 g), and Parmesan cheese (50 g). The lunch provided 6259 kJ (193 g carbohydrates, 46 g fat, 79 g protein). Subjects consumed lunch from the UEM (Kissileff *et al.* 1980). The amount eaten at lunch and the microstructure of the meal was recorded with a scale integrated in the table underneath the plate. Subjects were not aware of the scale but were informed that they were surveillanced through a video camera during the meal. The weight of the plate was constantly monitored by the built-in scale, and recorded on-line by a computer in an adjacent room. By graphing the cumulative weight of the food eaten against time, a curve results that can be best described by a quadratic equation ($y = a + bx + cx^2$) (Kissileff *et al.* 1982).

A non-linear regression analysis determines the coefficients of the cumulative intake curve, where 'b' describes the initial rate of eating, and 'c' reflects the deceleration of eating and is considered to be an expression of internal mechanisms of satiation (Kissileff *et al.* 1982; Stellar *et al.* 1985). A slope change of more than 10% towards the end of the meal would be enough to characterize a subject as being a decelerated eater, indicating undisturbed satiation mechanisms in healthy, normal weight males (Westerterp-Plantenga *et al.* 1990).

TABLE 2: COMPOSITON OF MEALS (CALCULATED)

BREAKFAST		*Energy*	*Carbohydrates*	*Fat*	*Protein*
wheat/rye bread	150 g	1560 kJ	75.0 g	1.5 g	13.5 g
butter	20 g	634 kJ	0.1 g	16.6 g	0.1 g
cheese	22 g	195 kJ	0.0 g	2.6 g	5.7 g
raspberries	220 g	462 kJ	22.0 g	1.1 g	2.2 g
whipped cream	30 g	426 kJ	1.0 g	10.5 g	0.6 g
powdered sugar	11 g	187 kJ	11.0 g	0.0 g	0.0 g
2 eggs	106 g	680 kJ	0.8 g	11.6 g	13.4 g
cream	30 g	426 kJ	1.0 g	10.5 g	0.6 g
butter	20 g	634 kJ	0.1 g	16.6 g	0.1 g
	Total	***5204 kJ***	***111.0 g***	***71.0 g***	***36.2 g***
LUNCH (Total amount offered)					
mushroom risotto	250 g	3875 kJ	190.0 g	8.75 g	22.5 g
butter	20 g	634 kJ	0.1 g	16.6 g	0.1 g
Parmesan cheese	50 g	800 kJ	0.0 g	14.0 g	16.0 g
minced Cornatur® (meat substitute)	250 g	950 kJ	2.5 g	6.25 g	40.0 g
	Total	***6259 kJ***	***192.6 g***	***45.6 g***	***78.6 g***

Declarations according to the manufacturers

Subjects filled out a short questionnaire before and after breakfast and lunch. The questions addressed the subjects' degree of hunger and thirst and their perception of the meal. Some questions (not reported here) were posed to obscure the major purpose of the study. Answers were given through visual analogue scales ranging from 0 - 100 mm (e.g. from "not at all" (0 mm) to "extremely" (100 mm)). The questionnaire before each meal focused on the subjects' current hunger and thirst state, and the questionnaire after each meal inquired about the satisfaction of postprandial sensations.

Data transformation and statistics

Pearson product moment correlation coefficients were calculated for all 28 subjects between their usual fat consumption determined in Phase I of the study and the blood plasma parameters, respiratory measurements, questionnaire ratings and microstructure of lunch intake. Student's t-test was used to test lunch intake differences between the LF and HF groups (n = 10 and 11, respectively) for significance. Metabolic responses to the test breakfast and questionnaire ratings were statistically analyzed by a two-way repeated measures analysis of variance with time and group as factors. In case of overall significant differences, further comparisons were done by the Student-Newman-Keuls post-hoc test. In addition, the areas under the curve were calculated for plasma metabolite and insulin levels of the LF and HF groups and compared by Student's t-test. P-values < 0.05 were considered significant.

RESULTS

Subject characteristics

The BMI of the 28 subjects which completed phase II of the study was positively correlated with their %-body fat content ($r\ [28] = 0.60$, $P < 0.001$), and their LBM correlated highly with the estimated metabolic rate ($r\ [28] = 0.98$, $P < 0.001$). The daily energy intake of the 28 subjects correlated with LBM ($r\ [28] = 0.68$, $P < 0.001$), estimated metabolic rate ($r\ [28] = 0.64$, $P < 0.01$), and total energy expenditure for physical activity ($r\ [28] = 0.63$, $P < 0.01$). Energy expenditure for physical activity, expressed in kJ per week, was estimated (McArdle *et al.* 1996) from the duration and type of physical activity, by taking into account the intensity and the subjects' body weight. There was no significant difference between the weekly energy expenditure for physical activity of the LF and HF groups, which was 74 ± 13 % (SEM) and 59 ± 16 % of daily energy intake for the LF and HF groups, respectively (Table 1). This high level of physical activity presumably contributed to the low body fat content.

Respiratory measurements

Significant time ($F = 3.18$, $P < 0.05$) but not group ($F = 0.03$, $P = 0.87$) effects without a significant time x group interaction ($F = 1.61$, $P = 0.20$) were observed for the respiratory quotient (RQ) (Figure 2). Baseline RQ was also not correlated with the usual fat consumption ($r\ [28] = -0.18$, $P = 0.34$). RQ slightly increased during the meal for both groups and returned to about baseline values thereafter (40 min vs. 250 min,

P < 0.05). There was no correlation between the RQ before lunch and hunger before lunch (r [28] = -0.05, P = 0.81) or lunch size (r [28] = -0.01, P = 0.96).

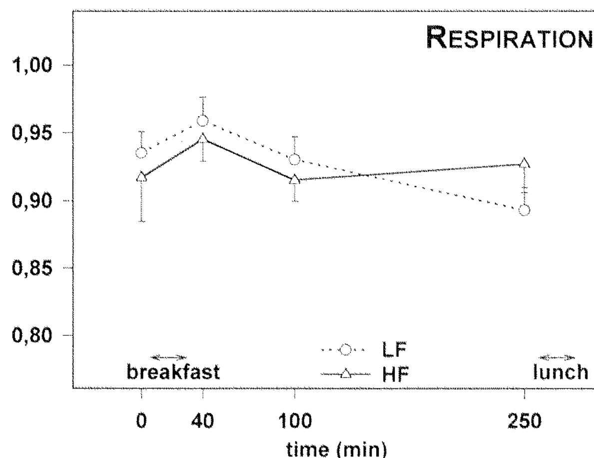


Figure 2: Effect of high fat breakfast on RQ in the LF and HF groups. Values are means ± SEM of 10 (LF) and 11 (HF) subjects, respectively.

Blood parameters

Plasma glucose, insulin, and lactate

The plasma glucose, insulin, and lactate levels (Figure 3) changed over time (glucose: F(6,114) = 35.2, P < 0.0001; insulin: F(6,114) = 23.91, P < 0.0001; lactate: F(6,114) = 12.81, P < 0.0001), but there were no group differences (glucose: F(1,19) = 1.37, P = 0.26; insulin: F(1,19) = 2.69, P = 0.12; lactate: F(1,19) = 0.64; P = 0.70) and no group x time interactions (glucose: F(6,114) = 0.86, P = 0.53; insulin: F(6,114) = 0.94, P = 0.47; lactate: F(6,114) = 0.64, P = 0.70) for any of the three. Plasma glucose increased during the breakfast (0 min vs. 30 min, P < 0.05) in particular in the HF group, decreased thereafter to a well below baseline nadir at 70 min after the onset of breakfast (0 and 30 min vs. 70 min, P < 0.05), and increased again (70 min vs. 265 min, P < 0.05) to approach baseline levels just before lunch (0 min vs. 265 min, P > 0.05). Plasma insulin also increased during the breakfast (0 min vs. 30 min, P < 0.05) and decreased thereafter (30 min vs. 90 min, P < 0.05; 0 min vs. 265 min, P < 0.05), but was still higher than baseline at 265 min (P < 0.05). Plasma insulin tended to have a second albeit smaller peak in particular in the HF group at 130 min after the onset of breakfast, but the increase between 90 and 130 min did not reach statistical significance. Finally, plasma lactate concentration also increased in response to the breakfast (0 min vs. 50 min, P < 0.05) and later decreased (50 vs. 265 min) to about baseline levels (0 min vs. 265 min, P > 0.05). The group comparison of the integrated glucose, insulin, and lactate responses to the meal, as reflected by the areas under the curve, revealed that the insulin response to the meal was bigger in the HF than in the LF group (t [19] = 2.16, P < 0.05).

For glucose and lactate the areas under the curves did not significantly differ between the HF and LF groups. The baseline insulin concentration ($r[28] = 0.37$, $P < 0.05$) and the insulin area under the curve ($r[28] = 0.49$, $P < 0.01$) correlated positively, and the baseline lactate level negatively ($r[28] = -0.54$, $P < 0.01$) with the fat level of the usual diet, whereas no such correlation was observed for the baseline glucose level ($r[28] = 0.26$, $P = 0.19$) or for the glucose area under the curve ($r[28] = 0.18$, $P = 0.36$). There was also no correlation between the plasma glucose level right before lunch and hunger ratings ($r[28] = 0.03$, $P = 0.87$).

Plasma triglycerides (TG), free fatty acids (FFA), and β-hydroxy-butyrate (BHB)

The plasma TG, FFA, and BHB levels (Figure 4) also changed over time (TG: $F(6,114) = 43.44$, $P < 0.0001$; FFA: $F(6,114) = 11.36$, $P < 0.0001$; BHB: $F(6,114) = 21.61$, $P < 0.0001$), but again there were no group differences (TG: $F(1,19) = 1.08$, $P = 0.31$; FFA: $F(1,19) = 2.39$, $P = 0.14$; BHB: $F(1,19) = 0.00$; $P = 0.98$) and no group x time interactions (TG: $F(6,114) = 0.38$, $P = 0.89$; FFA: $F(6,114) = 1.41$, $P = 0.22$; BHB: $F(6,114) = 0.29$, $P = 0.94$) for any of the three. In response to the breakfast, plasma TG continuously increased, and this increase reached significance at 70 min (0 and 30 min vs. 70 min, $P < 0.05$). Plasma FFA initially decreased during breakfast (0 min vs. 30 min, $P < 0.05$), than increased above baseline levels (0 and 30 min vs. 90 min, $P < 0.05$), and reached again about baseline levels before lunch (90 min vs. 265 min, $P < 0.05$). Plasma BHB continuously decreased between breakfast onset and 130 min (0 vs. 130 min, $P < 0.05$) and remained at about the same level until lunch. The areas under the curves for plasma TG, FFA and BHB did not differ between groups. There was no significant correlation between plasma BHB or TG and percentage of fat in the usual diet or RQ. There was also no correlation between the plasma FFA level and the percentage of fat in the usual diet. However, plasma FFA level was negatively correlated with the nearest RQ value at baseline ($r[28] = -0.49$, $P < 0.01$), 30 min ($r[28] = -0.38$, $P < 0.05$), and 265 min ($r[28] = -0.45$, $P < 0.05$). In addition, plasma FFA and BHB levels correlated positively at baseline ($r[28] = 0.76$, $P < 0.001$), 70 minutes ($r[28] = 0.60$, $P < 0.01$), 90 minutes ($r[28] = 0.43$, $P < 0.04$), and 130 minutes ($r[28] = 0.56$, $P < 0.01$) after baseline, and plasma TG and plasma FFA correlated at 265 minutes ($r[28] = 0.64$, $P < 0.01$).

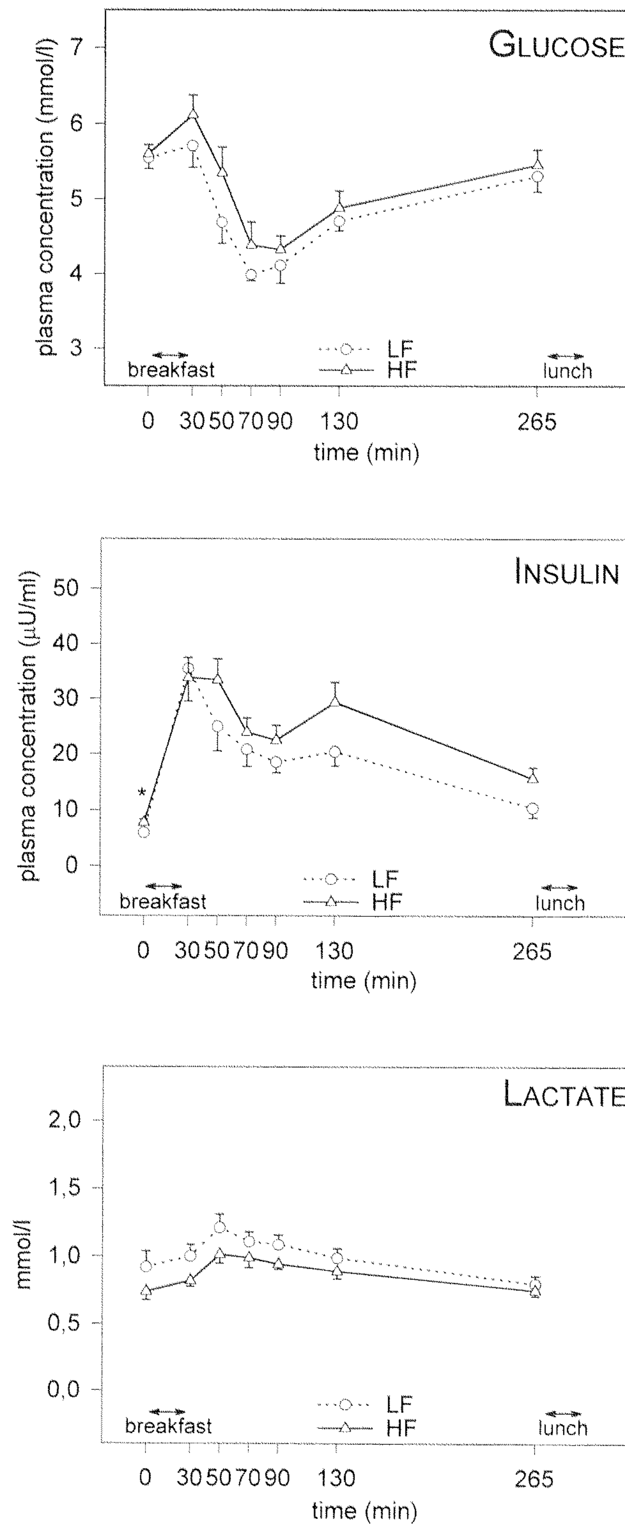


Figure 3: *Effect of the high fat breakfast on plasma glucose (top), plasma insulin (middle) and plasma lactate (bottom) concentrations in the LF and HF groups. Values are means ± SEM of 10 (LF) and 11 (HF) subjects, respectively.*

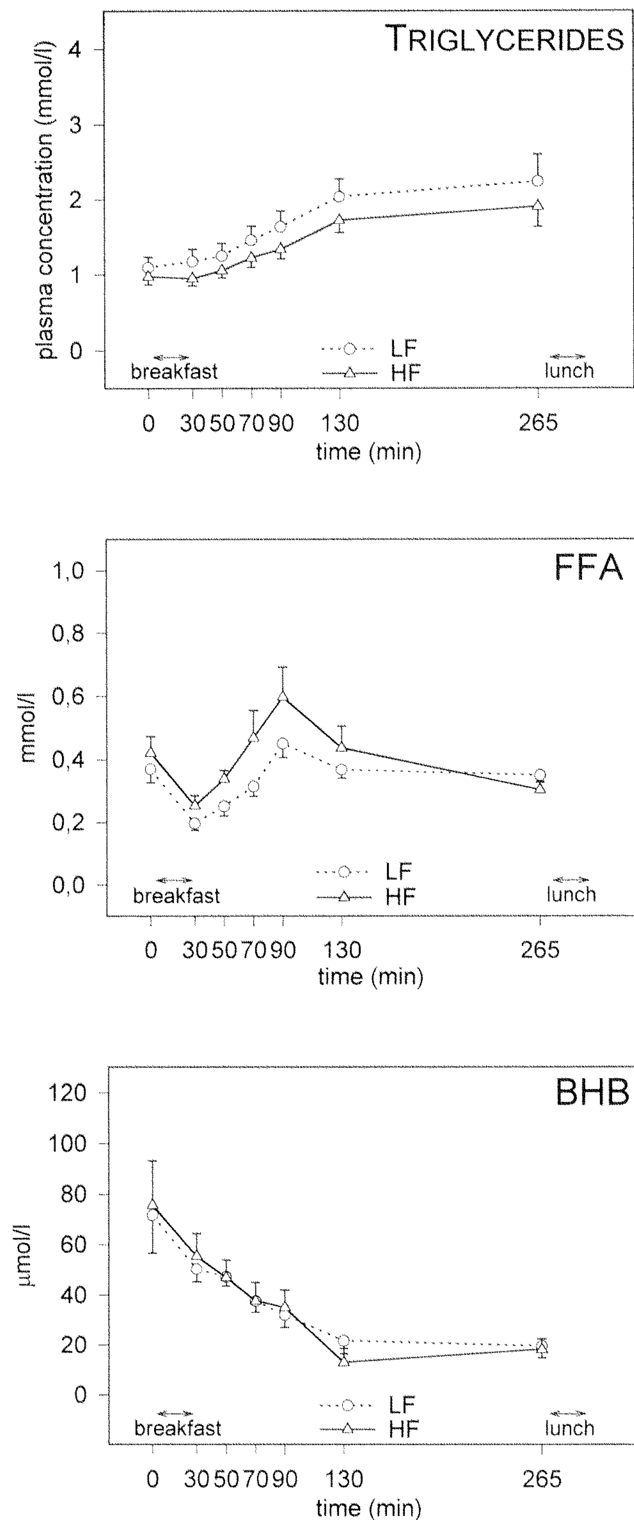


Figure 4: *Effect of high fat breakfast on plasma triglycerides (TG)(top), plasma free fatty acids (FFA) (middle) and plasma β-hydroxy-butyrate (BHB) (bottom) concentrations in the LF and HF groups. Values are means ± SEM of 10 (LF) and 11 (HF) subjects, respectively.*

Lunch intake and microstructure of eating

Lunch size and duration did not differ between the LF and HF groups (size: t [19] = 0.66, P > 0.05, duration: t [19] = 0.58, P > 0.05, Figure 5). Lunch size did also not correlate with the percentage of fat in the usual diet (r [28] = 0.06, P = 0.76). Yet, lunch size correlated positively with lunch duration (r [28] = 0.71, P < 0.001) and daily energy intake, as assessed in phase I of the study (r [28] = 0.53, P < 0.01). The quadratic equation was the best fit for the cumulative intake curves (r > 0.98) (Figure 6). Neither the slope coefficient "b" nor the deceleration coefficient "c" differed significantly between the LF and HF groups. Neither the slope b (r [28] = -0.24, P = 0.23), nor the deceleration c (r [28] = 0.33, P = 0.10) correlated with the percentage of fat intake in the usual diet. Lunch size was also not correlated with the slope b (r [28] = 0.33, P = 0.09) or the deceleration c (r [28] = -0.03, P = 0.87) of lunch intake. The slope change (< 10%) was generally not big enough to characterize the subjects as decelerated eaters.

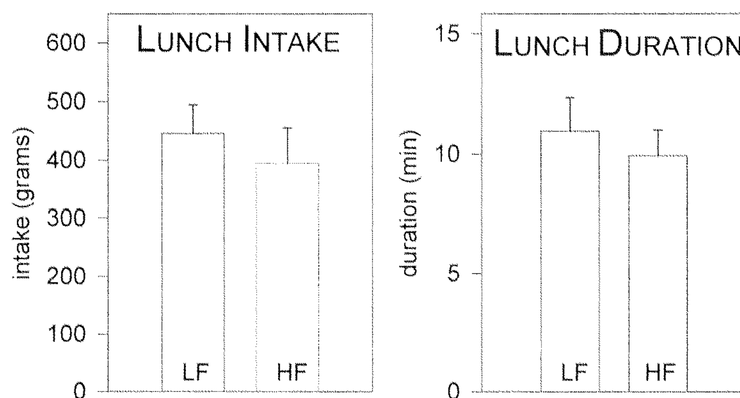


Figure 5: *Size and duration of lunch for the LF and HF groups after the high fat breakfast. Bars represent means ± SEM of 10 (LF) and 11 (HF) subjects, respectively.*

Questionnaire ratings

As expected, questionnaire ratings changed significantly with time (hunger: F(4,55) = 28.84, P < 0.0001; satiety: F(4,55) = 53.92, P < 0.0001) but there were no group differences (Hunger: F(1,19) = 0.03, P > 0.05; satiety: F(1,19) = 1.68, P > 0.05) (Table 3) and no group x time interactions. Hunger ratings decreased and satiety ratings increased significantly during breakfast and lunch. Likewise, hunger ratings increased and satiety ratings decreased between breakfast and lunch. The percentage of fat in the usual diet did not correlate with the hunger (r [28] = 0.04, P > 0.84) or satiety (r [28] = 0.02, P > 0.91) ratings before lunch intake. Hunger and satiety ratings before lunch correlated with lunch size (hunger ratings: r [28] = 0.57, P > 0.01, satiety ratings: r [28] = -0.55, P < 0.01).

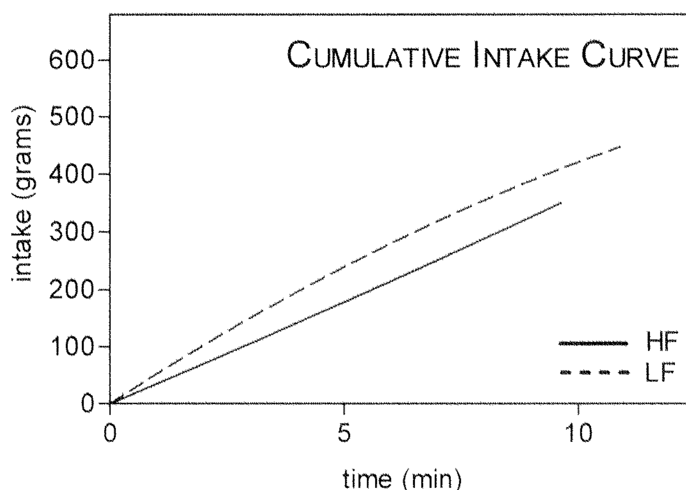


Figure 6: *Fitted cumulative intake curves at lunch plotted with the mean coefficients of the LF and HF groups. See text for further details. As the UEM data from one HF subject are missing, the endpoint of the HF curve does not exactly correspond to the values given in Figure 5.*

DISCUSSION

The results demonstrate that the fat level in the habitual diet does not acutely affect fatty acid oxidation in response to a fat-rich breakfast, and does also not affect the satiating potency of that meal in lean, male subjects. At least within the range of percent fat intakes observed (21-44% of the total energy intake) and in the subjects of the present study, there was no acute increase in postprandial fatty acid oxidation with increasing levels of fat in the habitual diet. There were also no differences in physical activity between the HF and LF groups, which might bias the metabolic measurements.

Surprisingly, no significant correlation between the usual fat consumption and the baseline RQ or fat metabolite levels could be detected. However basal RQ tended to be lower in the HF compared to the LF group, suggesting that a high habitual fat intake favors baseline fatty acid oxidation. This should be expected based on previous observations of a close relationship between RQ and fat intake (e.g. Rumpler *et al.* 1991; Verboeket-van de Venne *et al.* 1994) and probably reflects an enzymatic adaptation of fatty acid oxidation to the level of dietary fat (Bremer *et al.* 1984). The impact of the habitual fat intake on metabolism may be more pronounced in the postabsorptive than in the postprandial state and may depend on body fat, which did not vary significantly in the subjects of the present study. This may explain the lack of a significant effect of habitual fat intake on most metabolic parameters. In addition, a stronger influence of habitual fat intake on baseline RQ may have been obscured by the comparatively high baseline RQ levels in our study. Apparently the body's carbohydrate stores were not depleted by the overnight fast. The transient increase in RQ during the meal in the LF and HF groups is in line with previous findings (Surina *et al.* 1993; Flatt *et al.* 1985) and indicates that, independent of the habitual fat intake, carbohydrates are preferentially oxidized even after a high fat meal.

TABLE 3: QUESTIONNAIRE RATINGS CONCERNING HUNGER AND SATIETY SENSATIONS BEFORE AND AFTER MEALS

BREAKFAST		Questions	LF	HF
Before breakfast		How strong is your desire to eat?	62.7 ± 7.4	63.5 ± 8.1
		How full do you feel?	20.0 ± 7.2	20.1 ± 5.1
	A	How hungry are you right now?	62.7 ± 7.4	63.0 ± 7.3
After breakfast		How did the meal taste?	62.8 ± 8.2	57.0 ± 7.2
		How full do you feel after this meal?	91.5 ± 2.5	93.5 ± 2.2
	B	How hungry are you after this meal?	10.7 ± 5.9	6.1 ± 1.8
	Δ^1	Prandial decrease in hunger (= B - A)	-52.6 ± 8.8	-56.9 ± 7.1
		How rich in fat did you consider this meal?	65.4 ± 6.1	61.5 ± 4.4
		How thirsty are you after this meal?	21.1 ± 5.9	17.5 ± 3.4
		How long do you think does the meal last?	63.3 ± 8.9	61.2 ± 10.7
LUNCH				
Before lunch		How strong is your desire to eat?	55.9 ± 9.7	52.3 ± 8.0
		How full do you feel?	32.9 ± 6.7	52.6 ± 8.4
	C	To what extent are you hungry right now?	60.1 ± 9.1	50.0 ± 7.4
	Δ^2	Postprandial increase in hunger (= C - B)	60.1 ± 8.3	43.9 ± 6.2
After lunch		How did the meal taste?	65.5 ± 4.4	59.3 ± 7.0
		How full do you feel after this meal?	84.4 ± 3.0	85.7 ± 2.3
		How hungry are you after this meal?	10.5 ± 3.1	21.7 ± 7.9
		How thirsty are you after this meal?	26.1 ± 7.7	20.0 ± 3.1
		How long do you think does the meal last?	69.9 ± 6.3	74.5 ± 7.7

Values are means ± SEM; 0 = weakest and 100 = strongest sensation of the feeling (in mm of visual analogue scale). LF = low fat group (n = 10), HF = high fat group (n = 11)

In both groups, plasma FFA decreased during the breakfast and increased to above baseline levels somewhat later. Plasma levels of TG also increased in response to the high fat breakfast in both groups of the present study, as well as in a previous study of ours (Surina *et al.* 1993). The initial prandial decrease in plasma FFA is presumably due to an inhibition of lipolysis, mainly mediated by the concomitant increase in plasma insulin. The postprandial rise in plasma free fatty acids is in line with previous reports (Surina *et al.* 1993; Griffiths *et al.* 1994) and can be attributed to lipoprotein lipase-mediated hydrolysis of dietary TG (see Nilsson-Ehle *et al.* 1980). The pronounced postprandial increase in plasma FFA coincided with the reversal of the prandial increase in the RQ value, indicating that at least some of the fatty acids were oxidized. At the same time, plasma BHB continued to decrease, suggesting that the resumption of whole body fatty acid oxidation after the fat-rich breakfast was not accompanied by an increase in hepatic fatty acid oxidation and ketogenesis. This may be due to the

postprandial inhibition of hepatic fatty acid oxidation by insulin and glucose (Sidossis *et al.* 1996).

Most of the postprandial metabolic changes observed in particular in the LF group were strikingly similar to the changes reported by Surina *et al.* (Surina *et al.* 1993). However, the transient increase in plasma BHB level in response to the high fat breakfast described in this previous study (Surina *et al.* 1993) was not replicated. Closer examination of the data revealed that the baseline BHB level in the present study was much higher than in the previous study (Surina *et al.* 1993). Therefore, the high baseline BHB level may have obscured a subtle effect of the high fat breakfast on plasma BHB. The high baseline BHB level is by itself surprising considering the comparatively high baseline RQ values. Taken together, these data suggest that in the morning after an overnight fast, hepatic fatty acid oxidation and ketogenesis are more and/or earlier activated than whole body fatty acid oxidation. Low insulin and glucose levels together with high glucagon levels before breakfast may favor ketogenesis, unlike after breakfast, when plasma BHB is much lower although plasma FFA are even higher than at baseline.

Fasting plasma glucose was similar in both groups and glucose did scarcely increase in response to the high fat breakfast. This is again compatible to previous findings of an insignificant prandial and postprandial increase in plasma glucose in response to a high fat breakfast under similar conditions (Surina *et al.* 1993). In both studies there was also a pronounced reactive hypoglycemia between 50 and 130 min after the onset of the high fat breakfast. The reactive hypoglycemia followed the meal-induced peak in plasma insulin levels, and its time course fits previous reports by other authors (e.g. Raben *et al.* 1994a). Interestingly, the plasma insulin response to the high fat breakfast tended to be biphasic, with the second peak at about 130 min appearing somewhat more pronounced in the HF group. In various other studies with different types of test meals a similar though generally smaller second peak in plasma insulin has been observed (Surina *et al.* 1993; Raben *et al.* 1994a; Flatt 1995). Since these studies did not differentiate subjects according to habitual fat intake, the second postprandial rise in insulin may have been obscured. The increase in plasma lactate together with high plasma insulin levels indicates that the postprandial hypoglycemia reflects enhanced glucose utilization. This is possible despite the beginning decline in the RQ value around that time because energy expenditure, which was not evaluated in the present study, can be expected to increase in response to a meal (e.g. Flatt *et al.* 1985). The lactate/insulin ratio in the HF group was significantly ($t[19] = 2.75$, $P < 0.05$) lower than in the LF group, indicating that less lactate was produced in response to insulin with high fat intake. Such a diminished lactate appearance after a glucose and insulin challenge is in fact considered to be an early sign of insulin resistance (Lovejoy *et al.* 1992). Interestingly, plasma TG were somewhat lower and plasma FFA were somewhat higher in the HF group. Both changes suggest a higher LPL activity in the HF group, which may be explained by a higher insulin level (Schrezenmeir 1996). Together, the metabolite data are therefore consistent with a diminished insulin sensitivity of carbohydrate metabolism and an unaltered insulin sensitivity of lipid metabolism. Such a dissociation of insulin sensitivity is characteristic for the so-called metabolic syndrome (Schrezenmeir 1996). As body weight, BMI, and body fat content did not differ between the LF and HF groups, the results suggest that early stages of the metabolic syndrome can develop with long-term high fat consumption prior to any overt signs of adiposity. Although this assumption needs further and more direct proof, it is

in line with previous reports of the development of insulin resistance and reduced glucose tolerance in rats consuming high fat diets (Kraegen *et al.* 1991; Nagy *et al.* 1990; Storlien *et al.* 1986; Storlien *et al.* 1987). It is noteworthy, however, that in some of these animal studies diets with extreme fat or carbohydrate content were used, whereas the changes in the present study could be observed with subjects adapted to moderate levels of dietary fat (around 40% of the energy). It is generally believed that chronic increases in circulating fatty acids decrease glucose utilization and ultimately cause insulin resistance (Randle *et al.* 1963). It has also been shown that lipid oxidation acutely affects subsequent glucose utilization in obese patients (Golay *et al.* 1995). However, the exact mechanisms of the inhibiting effect of fatty acids on glucose utilization are still not completely elucidated (e.g. Sidossis *et al.* 1996; Magnan *et al.* 1996) and, to our knowledge, the onset of insulin resistance in lean human subjects eating a moderate fat diet has not been identified yet.

The size and duration of the target lunch as well as hunger and satiety ratings between breakfast and lunch were not correlated to the level of fat in the habitual diet and did not differ between the LF and HF groups, indicating that under the present test conditions the usual fat consumption had no influence on the satiating effect of a fat-rich breakfast and on the amount eaten during the target lunch. There was also no difference between the LF and HF groups in the rate of eating and in the deceleration of the cumulative intake curve during the target lunch, demonstrating that the microstructure of eating in the target meal was also not affected. In general, the lack of a strong deceleration of the food intake curves towards the end of the meal is not unusual for young male subjects (Westerterp-Plantenga *et al.* 1990; Kaplan 1980).

Hepatic fatty acid oxidation has been proposed to contribute to the maintenance of postabsorptive satiety at least in rats (Scharrer *et al.* 1986; Langhans *et al.* 1987; Langhans *et al.* 1992). According to the LF and HF groups' similar plasma BHB level between breakfast and lunch, habitual fat intake did not affect postprandial hepatic fatty acid oxidation. Thus, the present findings do not allow drawing conclusions about the satiating potency of postprandial hepatic fatty acid oxidation in humans. Clearly, further studies are necessary to test whether experimentally induced bigger changes in hepatic fatty acid oxidation do affect eating in humans when carbohydrate supply is limited.

In conclusion, the results indicate that the habitual level of fat intake (between 21 and 44%) fails to acutely affect whole body or hepatic fatty acid oxidation in response to a high fat breakfast in lean, male subjects, and does not affect the satiating effect of this meal and the size and microstructure of the subsequent lunch. However, even at this level, continuous high fat intake appears to have subtle effects on postprandial metabolism consistent with early signs of a beginning insulin resistance, despite unchanged body weight or BMI. This is very interesting, but the presently available data do not allow us to judge the potential clinical relevance of this observation.

LACTATE AS SATIETY SIGNAL IN RATS

BASED ON:

Silberbauer C.J., Surina-Baumgartner D.M., Arnold M., Langhans W.: Prandial lactate infusion inhibits spontaneous feeding in rats.
American Journal of Physiology – Regulatory, Integrative and Comparative Physiology (2000) 278: R646-R653

INTRODUCTION

Plasma lactate increases after ingestion or intragastric administration of carbohydrates. In response to intragastric glucose loads, portal vein plasma lactate concentration increased in unrestrained, chronically catheterized rats (Niewoehner *et al.* 1994) and dogs (Shoemaker *et al.* 1963). The increase was generally greater in the portal vein than in the periphery (Kimura *et al.* 1988; Niewoehner *et al.* 1984). An increase in portal vein, systemic, and hepatic lactate concentration also accompanied feeding after mild food deprivation in rats (Langhans 1991), and in man, systemic plasma lactate increases in response to carbohydrate containing meals (Felig *et al.* 1975; Silberbauer *et al.* 1996; Surina *et al.* 1993). The increase in circulating lactate and, to a lesser extent, pyruvate reflects the low phosphorylating capacity of hepatocytes for glucose (Foster 1984). Consequently, much of the ingested glucose enters hepatic glycogen by an indirect route that requires glucose transformation into three-carbon units by extrahepatic tissues (Newgard *et al.* 1983).

Lactate reduces food intake after parenteral administration in monkeys (Baile *et al.* 1970) and rats (Langhans *et al.* 1985a; Langhans *et al.* 1985b; Racotta *et al.* 1977). The meal-induced increase in plasma lactate may therefore contribute to the control of feeding. To further explore this possibility, we investigated the effects of remotely controlled, meal contingent hepatic portal infusions of lactate on spontaneous feeding in undisturbed rats. In previous studies, the suppression of food intake in response to lactate was blocked after hepatic branch vagotomy (Langhans *et al.* 1985b; Nagase *et al.* 1996). This suggests that lactate inhibits feeding by acting in the liver; however, it is not proof for this assumption because the hepatic branch of the vagus also carries afferent fibers of extrahepatic origin (Berthoud *et al.* 1992). Therefore, as an alternative approach to examine whether or not lactate's effect on food intake originates in the liver, we compared feeding after infusing lactate directly into the liver through the hepatic portal vein or into the inferior vena cava just proximal to the junction of the hepatic veins. To judge the physiological relevance of the observed effects, we measured plasma lactate and glucose concentrations in the vena cava after hepatic portal vein lactate infusion.

METHODS

Animals

Male Sprague-Dawley rats (body weight: 280 – 300 g at study onset; Institute for Laboratory Animals, University of Zurich) were individually housed in a temperature-controlled (21 ± 2°C) colony room and kept on a reversed 12:12-h light-dark cycle (dark onset: 1300). The rats had ad-libitum access to water and ground rat chow (NAFAG 890, Gossau, Switzerland) with a metabolizable energy content of about 12 kJ/g. Rats were adapted to housing conditions for at least 8 days before surgery.

Surgery

Catheters were assembled as previously described and were sterilized in alcohol before use. The protruding dorsal end of a silicon catheter [Silastic (Dow Corning, Midland, MI, USA); ID 0.508 mm, OD 0.940 mm, length 27 cm] was slipped onto a headpiece (a modified cannula with a screw top), reinforced with a 2.5 cm piece of larger silicon catheter (ID 1.016 mm, OD 2.159 mm), and led through a folded polypropylene surgical mesh (Bard Marlex Mesh, Davol Inc.) to improve adhesion to skin and fascia. The rats were anesthetized by intraperitoneal injection (1.25 ml/kg) of a mixture of ketamine (80 mg/ml; Ketasol-100), xylazine (4.66 mg/ml; Rompun, Bayer, Leverkusen, Germany), and acepromazine (0.05 mg/kg; Sedaline, Chassot, Berne, Switzerland). The topless headpiece was led subcutaneously from a 2 cm intrascapular incision to a puncture wound 1cm rostral to the incision, and the threaded end was exteriorized. The top was screwed on to secure the headpiece in place. The catheter was led subcutaneously from the neck to the 5 cm incision in the midline of the abdomen. The distal end of the catheter was implanted into the ileocolic vein and led rostrally to the hepatic portal vein. The catheter was fixed to the vein with sutures and Histoacryl glue (B. Braun, Melsungen, Germany). Skin and muscle were closed with resorbable sutures. A cap was put on the headpiece to close the catheter. Chloramphenicol (500 mg = 5 ml of chloromycetin; Parke-Davis, Grub, Switzerland) was injected subcutaneously for infection prophylaxis, and novaminsulfon (50 mg = 0.1 ml of Vetalgin; Veterinaria, Zurich, Switzerland) was injected intramuscularly for analgesia. For further details of the surgical procedure refer to the work by Surina-Baumgartner *et al.* (1995).

In one group of rats, a second catheter was placed in the vena cava within the same surgery, to investigate whether hepatic portal vein and vena cava lactate infusion affect eating differently. The surgical procedure was the same as for the hepatic portal vein catheterization. The inferior vena cava was exposed and penetrated with one cannula rostral to the renal veins using Kaufman's method (Kaufman 1980). The distal tip of the cannula was advanced 3 - 4 cm, so that it lay near the juncture of the hepatic vein with the inferior vena cava. The cannula was anchored to the abdominal muscles with non-resorbable sutures. The dorsal end of the vena cava catheter was exteriorized with a headpiece 1 cm rostral to the headpiece of the hepatic portal vein catheter. After a 3-day recovery period, the catheters were flushed every second day with 0.3 ml 0.9% saline to ensure patency.

Test procedure

Rats were placed into individual open-topped Plexiglas infusion cages (37 x 21 x 41 cm) with stainless steel grated floors and hinged doors. Rats had ad-libitum access to water, and ground chow was available ad-libitum in food cups mounted on electronic balances (Mettler PM 3000). The rats could reach the food cup over a short bridge 5 cm from the cage floor. The balances were interfaced with a computer (Olivetti M 300) in an adjacent room, and a custom-designed program (VZM, Krügel, Munich, Germany) recorded the weights of the food cups every 30 s. In addition, a video surveillance camera (VSS 3440; Phillips) connected to a 13-in monitor in the adjacent room allowed for continuous observation of the rats.

After at least 5 days of adaptation to the cages, the rats were adapted to the experimental procedure for 3 days. The procedure entailed connecting the catheter to a syringe pump (model A99; Razel, Stamford, CT) via two segments of Tygon tubing (0.76 mm ID, 2.29 mm OD) separated by a swivel joint suspended ~45 cm above the cage floor, which allowed the rat to move freely. The lower tubing segment was sheathed with a stainless steel spring. The syringe, both tubing segments, and the dead space of the indwelling cannula were filled with saline. In the rats with both hepatic portal vein and vena cava catheters, the two catheters were attached to the syringe pump alternately on consecutive days. The food cups were filled with fresh food and the experimenter left the room. The whole procedure required ~30 min and was completed 2.5 hours before dark onset.

The infusion pumps were remotely controlled from the adjacent room. The infusions started 2 min into the first spontaneous nocturnal meal and lasted for 5 min. If a meal was under way when the lights went out, the infusion was done during the next meal. The criterion for meal onset was a ≥ 0.3g decrease in the food cup weight and visual verification of concomitant feeding activity on the monitor. After all rats had consumed the first and the second spontaneous meal, ~3 h after dark onset, the catheters were detached and flushed with 0.3 ml bacteriostatic saline, and the headset was capped. A similar procedure (Le *et al.* 1991) had no detectable effects on spontaneous nocturnal feeding patterns.

Experiments

The effects of hepatic portal vein infusions of lactate on feeding were tested in a total of 32 rats (body wt on experimental days: 324-463 g), using individual crossover tests. A group of 12 rats was used to investigate the effect of 0.5 mmol lactate; these 12 plus an additional 10 rats were used in the test of 1.0' mmol lactate; and 10 of the original 12 rats plus an additional 10 rats were used in the test of 1.5 mmol lactate. On the day before each crossover test, rats received a 0.9% saline (mock) infusion. On the next 2 days, lactate (sodium L-lactate, pH 7.4) or saline (sodium chloride, pH 7.4) was infused in the hepatic portal vein, with the order randomized between rats. Infusion rates of 0.1 or 0.15 ml/min were combined with infusate concentrations of 1 or 2 M to yield doses of 0.5 mmol/rat (0.1 ml/min x 1 M x 5 min), 1.0 mmol/rat (0.1 ml/min x 2 M x 5 min), and 1.5 mmol/rat (0.15 ml/min x 2 M x 5 min). After each crossover test, catheter patency was tested by infusing 0.1 ml of the anesthesia mixture and flushing with 0.3 ml saline. Data from rats that were not anesthetized within 1 min (n = 5) were

excluded from analysis, yielding 12, 19, and 18 rats for the 0.5, 1.0, and 1.5 mmol doses, respectively.

Two counterbalanced within subjects tests, each on four consecutive days and preceded by a single mock infusion, were performed in the 12 rats with catheters in both the hepatic portal vein and the vena cava. One or 1.5 mmol were infused in either the hepatic portal vein or the vena cava, and feeding was recorded. The single mock infusion before each 4-day trial was supposed to be sufficient to lessen possible carryover effects because each rat received hepatic portal vein and vena cava infusions on alternate days and because vena cava infusions should not promote the learning effects observed after hepatic portal vein glucose infusion by Tordoff and Friedman *et al.* 1988. The patency of the hepatic portal vein catheter was tested as described above. The vena cava catheter was presumed to be patent if blood could be aspirated.

Blood sampling

The effects of hepatic portal vein lactate infusions on systemic plasma lactate and glucose levels were investigated in 13 rats (body weight 435 - 581 g) with only a hepatic portal vein catheter. Rats received infusions of 1.5 mmol lactate (n = 7) or saline (n = 6) beginning 2 min into the first spontaneous nocturnal meal as described above. One min after infusion end (i.e. 8 min after meal onset) the rats were anesthetized with ether, and a laparotomy was performed; blood was drawn from the vena cava in 2-mil sodium fluoride tubes (6.1 mg NaF added) and was centrifuged immediately (1500 g, 15 min, 4°C). The plasma was stored at -20°C for later analysis of lactate and glucose concentrations by standard colorimetric and enzymatic methods adapted for the Cobas Mira auto-analyzer (Hoffman LaRoche, Switzerland) (Langhans 1991; Silberbauer *et al.* 1996).

Data collection and analysis

Meals were defined as weight changes ≥ 0.3 g, lasting ≥ 1 min, and separated by ≥ 15 min from other weight changes, as described previously (Langhans, Scharrer 1987). Meal duration was the time from the first to the last weight change in a single meal, and the intermeal interval (IMI) was the time from the last weight change in a meal to the first change in the next meal. Meals defined and recorded this way accounted for 94% of total daily food intake. Meal size divided by meal duration described the mean feeding rate within meals, and the IMI after a meal (min) divided by the size of that meal (g) described the satiety ratio. In the experiments that tested the effects of hepatic portal vein lactate infusions on meal patterns, only 8 of the 36 rats contributed data for all three lactate doses. Therefore, the data were analyzed in two ways: first, by paired t-tests of each of the three crossover tests, and, second, by a repeated-measures ANOVA of the data from the eight rats that completed all three crossover tests. The comparisons of the effects of hepatic portal and inferior vena cava lactate infusions were also analyzed with repeated-measures ANOVA for each dose separately and across the two doses for the eight rats that completed both trials. Bonferroni's modified t-test was used for post-hoc comparisons. Student's t-test was used to detect treatment differences in systemic plasma glucose and lactate concentrations. P-values ≤ 0.05 were considered significant.

RESULTS

Feeding responses to hepatic portal vein infusions

Separate analysis of the three crossover tests (n = 12, 19, and 18) indicated that meal-contingent hepatic portal vein infusions of 1.0 and 1.5 mmol lactate reduced the size of the first spontaneous nocturnal meal by 28% (1.0 mmol: t [18] = 2.45, $P < 0.05$) and 67% (1.5 mmol: t [17] = 4.19, $P < 0.001$), respectively (Fig. 1, top). Infusion of 0.5 mmol lactate did not affect meal size. Only infusion of 1.5 mmol lactate/meal decreased meal duration (Table 1). None of the lactate doses affected the average feeding rate within the meal (Table 1) or the subsequent IMI (Fig. 1, middle). As a result of the reduced meal size and unaffected postmeal IMI, hepatic portal infusion of 1.5 mmol lactate increased (t [17] = 2.62, $P < 0.05$) the satiety ratio of the meal (Fig. 1, bottom). The size of the subsequent (second) nocturnal meal (range: 0.9 – 7.1 g) increased from 2.4 ± 0.3 (mean ± SEM) to 3.6 ± 0.4 g (t [18] = 2.45, $P < 0.05$) after hepatic portal infusion of 1.0 mmol, but was not affected by infusion of 1.5 mmol lactate. None of these meal parameters differed between control infusions and mock (0.9% saline) infusions on the days before a lactate test, and none of the lactate doses affected 2h cumulative food intake (data not shown). Overall analysis of these meal parameters in the eight rats that contributed data to all three crossover tests also indicated that 1.5 mmol lactate significantly decreased meal size [$F(5,35) = 3.26$, modified t = 2.71, $P < 0.05$] and meal duration [$F(5,35) = 3.11$, modified t = 2.64, $P < 0.05$] and increased the satiety ratio [$F(5,35) = 2.58$, modified t = 3.08, $P < 0.05$; Table 2]

Comparison between hepatic portal vein and vena cava infusions

Meal contingent hepatic portal and vena cava infusion of 1.0 and 1.5 mmol lactate reduced the size of the first nocturnal meal similarly (Figs. 2 and 3). Independent of the infusion route, only the higher dose of lactate also decreased meal duration (repeated measures ANOVA: $F(3,21) = 3.43$, $P < 0.05$). None of the lactate infusions affected the average feeding rate within meals (data not shown). The subsequent IMI tended to decline after vena cava infusion of both lactate doses (Figs. 2 and 3), but the differences did not reach statistical significance (1.0 mmol: $F(3,27) = 2.59$, $P = 0.07$; 1.5 mmol: $F(3,21) = 2.84$, $P = 0.06$). As a result of the reduced meal size and unaltered IMI, hepatic portal vein lactate infusion of 1.5 mmol increased the satiety ratio of the meal by 175 ± 32% (mean ± SEM; $F(3,21) = 4.45$, modified t = 3.31, $P < 0.01$) (Fig. 3). This increase was much higher (t [7] = 4.08, $p < 0.01$) than the insignificant ($F(3,27) = 1.37$, $P = 0.27$) 43 ± 21% satiety ratio increase after vena cava infusion. After infusion of 1.0 mmol lactate through either route, the size of the second nocturnal meal increased from 2.6 ± 0.3 g (mean ± SEM) in both control groups to 3.7 ± 0.4 g (lactate/hepatic portal vein) and 3.5 ± 0.3 g (lactate/vena cava; $F(3,27) = 2.90$, $P = 0.053$). Again, overall analysis of these meal parameters in the eight rats that completed both trials revealed essentially the same effects, i.e., a significant reduction of meal size by both lactate doses independent of the infusion route ($F(7,49) = 5.92$, $P < 0.001$), and a significant increase of the satiety ratio by 1.5 mmol lactate infused in the hepatic portal vein ($F(7,49) = 3.30$, modified t = 3.36, $P < 0.01$).

TABLE 1: EFFECT OF MEAL CONTINGENT HEPATIC PORTAL VEIN LACTATE INFUSION ON MEAL DURATION AND MEAN FEEDING RATE WITHIN MEALS

Dose	Meal duration (min)		Mean feeding rate (g/min)	
	Saline	Lactate	Saline	Lactate
0.5 mmol	13 ± 3	11 ± 2	0.33 ± 0.04	0.34 ± 0.04
1.0 mmol	14 ± 3	11 ± 3	0.36 ± 0.09	0.51 ± 0.12
1.5 mmol	13 ± 3	4 ± 1*	0.36 ± 0.07	0.36 ± 0.04

Values are means ± SEM of 12, 19, and 18 rats for 0.5, 1.0 and 1.5 mmol doses, respectively.
** Significant decrease in meal duration by lactate (paired t test, $t[17] = 2.97$, $p < 0.01$)*

TABLE 2: EFFECT OF MEAL CONTINGENT HEPATIC PORTAL VEIN LACTATE INFUSION ON MEAL PARAMETERS IN 8 RATS THAT CONTRIBUTED DATA TO ALL THREE CROSSOVER TESTS

Dose	Meal size (g)		Meal duration (min)		Satiety Ratio (min/g)	
	Saline	Lactate	Saline	Lactate	Saline	Lactate
0.5 mmol	2.6 ± 0.5	2.5 ± 0.5	10.1 ± 2.9	11.1 ± 2.7	74 ± 18	84 ± 14
1.0 mmol	3.2 ± 0.5	2.5 ± 0.6	12.5 ± 2.8	13.5 ± 2.2	52 ± 18	60 ± 11
1.5 mmol	2.5 ± 0.4	0.8 ± 0.2*	10.8 ± 1.6	2.5 ± 0.5	46 ± 5	118 ± 22

*All values are means ± SEM of 8 rats. * Significant differences between lactate and saline values $P < 0.05$ in Bonferroni t-test after significant repeated-measures ANOVA)*

Plasma glucose and lactate in response to hepatic portal vein infusion

One min after the infusion of 1.5 mmol lactate into the hepatic portal vein, the vena cava plasma lactate concentration was significantly higher than after 1.5 mmol saline infusion ($t[11] = 3.49$, $P < 0.05$) (Fig. 4). Lactate infusion did not affect the plasma glucose concentration (Fig. 4).

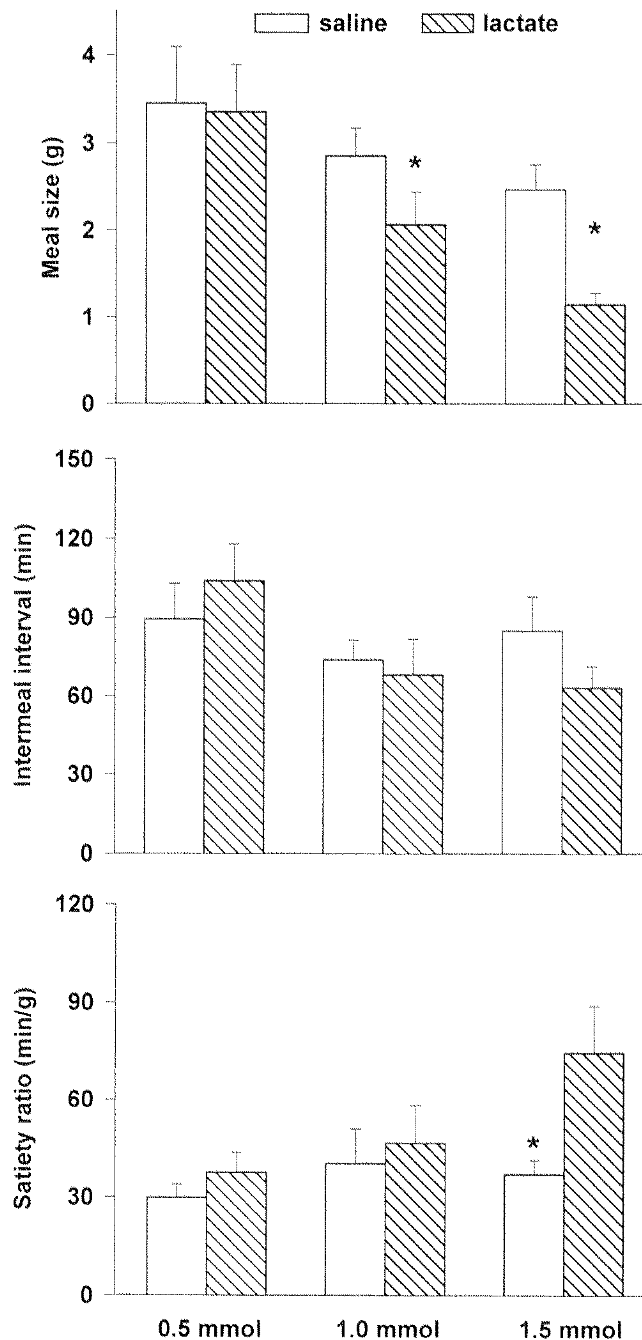


Figure 1: *Effects of prandial hepatic portal infusion of lactate on the size of the first spontaneous nocturnal meal (top), the duration of the subsequent IMI (middle), and the satiety ratio (subsequent IMI [min] / meal size [g]) (bottom) in ad-libitum fed rats. Data are means ± SEM of 12 (0.5 mmol lactate/meal), 19 (1.0 mmol lactate), and 18 (1.5 mmol lactate) rats used in the individual crossover tests. * P < 0.05 (paired t-test for each dose).*

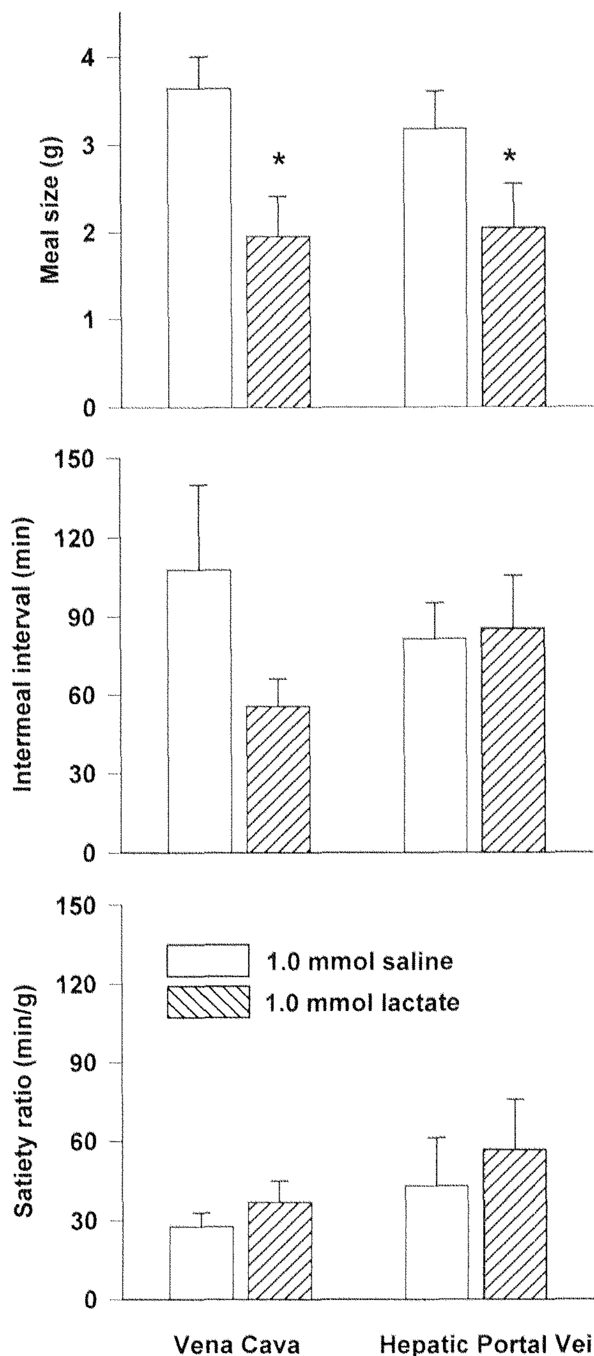


Figure 2: *Effects of prandial hepatic portal or vena cava infusion of 1.0 mmol lactate/meal on the size of the first spontaneous nocturnal meal (top), the duration of the subsequent IMI (middle), and the satiety ratio (subsequent IMI [min] / meal size [g]) (bottom) in ad-libitum fed rats. Data are means ± SEM of 10 rats used in a within subject design. * $P < 0.05$ (Bonferroni t-tests after significant repeated-measures ANOVA: $F(3,27) = 7.25$, modified t for hepatic portal vein infusion = 2.59, modified t for vena cava infusion = 3.83).*

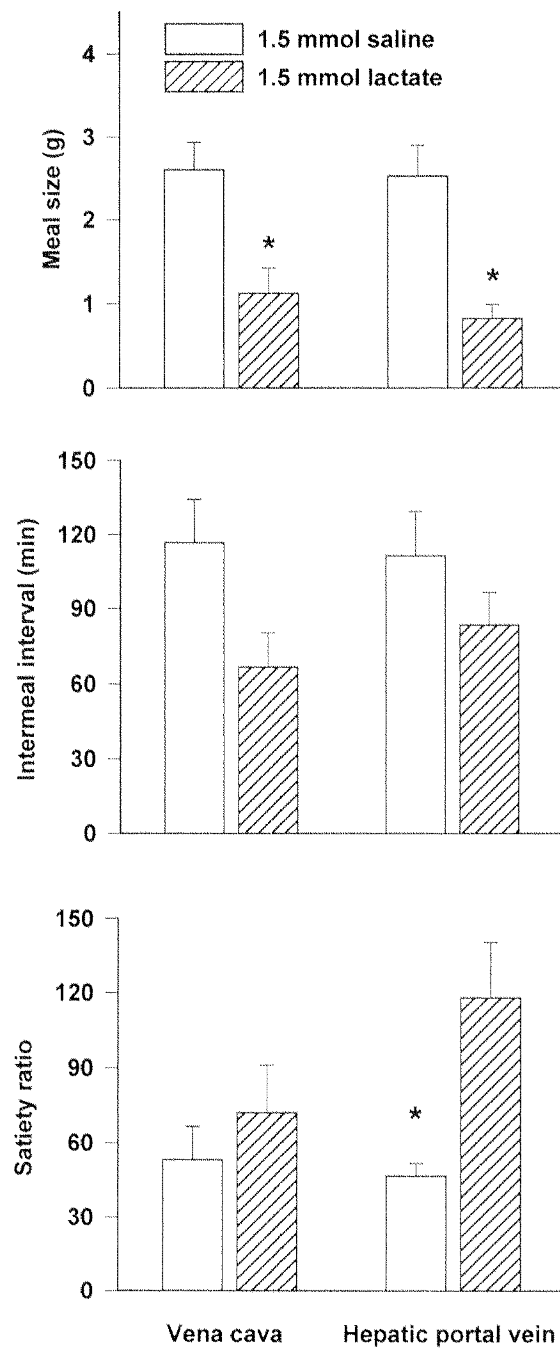


Figure 3: *Effects of prandial hepatic portal or vena cava infusion of 1.5 mmol lactate/meal on the size of the first spontaneous nocturnal meal (top), the duration of the subsequent IMI (middle), and the satiety ratio (subsequent IMI [min] / meal size [g]) (bottom) in ad-libitum fed rats. Data are means ± SEM of 8 rats used in a within subject design. * $P < 0.05$ (Bonferroni t-tests after significant repeated-measures ANOVA: $F(3,21) = 8.21$, modified t for hepatic portal vein infusion = 3.72, modified t for vena cava infusion = 3.23).*

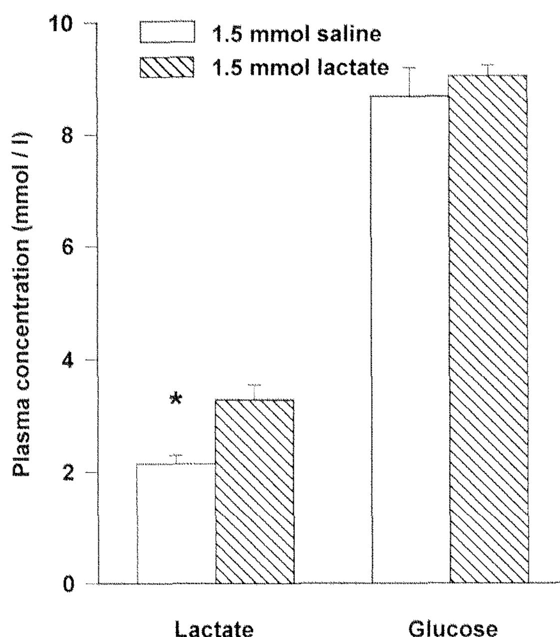


Figure 4: *Effects of prandial hepatic portal infusion of 1.5 mmol lactate on systemic plasma levels of lactate and glucose. N = 7 and 6 for lactate and control, respectively. * P < 0.05 (Student's t-test).*

DISCUSSION

The results of this study demonstrate that hepatic portal vein and vena cava infusion of lactate (1.0 and 1.5 mmol) in the rat during the first spontaneous meal of the dark phase can prematurely end the meal. To our knowledge, this is the first evidence for such acute effects of an intravenously administered metabolite on meal termination (satiation). In addition, hepatic portal vein infusion of 1.5 mmol lactate also prolonged postprandial satiety. Because circulating lactate normally increases in response to carbohydrate-containing meals, the findings are consistent with the idea that endogenous lactate plays a role in the physiological control of meal taking.

Lactate has long been known to inhibit feeding after parenteral administration in animals (Baile *et al.* 1970; Langhans *et al.* 1985a; Langhans *et al.* 1985b; Racotta *et al.* 1977), but previous studies did not record meal patterns or address possible acute effects of lactate on feeding. Baile *et al.* (1970) observed a reduction of cumulative food intake in monkeys within the first 30 min after a 30 min intravenous lactate infusion. Extending those findings, the present study revealed an immediate effect of intravenous lactate infusions on the size of the ongoing meal. This effect was more than caloric compensation for the administered lactate because the energy equivalent of the meal size suppression (~20 kJ after 1.5 mmol lactate) exceeded the gross energy of the administered lactate (~2 kJ) by a factor of 10. Moreover, feeding stopped after just 2 min of the 1.5 mmol lactate infusion, when only ~0.6 mmol lactate had been

administered. Considering the weaker effect on meal size and the lower infusion rate of the 1 mmol lactate dose (1 mmol: 0.1 ml/min vs. 1.5 mmol: 0.15 ml/min), the rate of lactate delivery into the hepatic portal vein (i.e. portal vein lactate appearance) appears to be more important for meal termination than the administered lactate dose.

The smallest dose of intravenous lactate that reduced meal size under the present test conditions (1.0 mmol/meal) did not significantly affect the subsequent IMI or the 2-h cumulative food intake. Yet, the size of the second nocturnal meal increased, which may reflect a compensation for the decreased size of the first meal. Such an increase in the size of the second meal did not occur after hepatic portal infusion of 1.5 mmol lactate/meal, perhaps because the residual inhibitory effect of the higher lactate dose prevented a compensatory increase in the size of the second meal. As a result of the decrease in meal size and the unaffected IMI after the 1.5 mmol infusion, the satiety ratio of the first nocturnal meal (duration of the subsequent IMI [min]/amount eaten during the meal [g]) increased, indicating that hepatic portal infusion of 1.5 mmol lactate also enhanced the postingestive satiating effect of food, i.e. prolonged postprandial satiety.

Glucose has often been shown to reduce food intake after hepatic portal vein infusion. Russek (1970) first demonstrated such a hypophagic effect of glucose in 22-h fasted dogs. He postulated the existence of hepatic glucosensors involved in food intake control (Russek *et al.* 1963). Glucosensors have been shown to exist in the portal-hepatic area (Niijima 1969), and now it is widely accepted that they play a role in the control of food intake (see Langhans 1996). In various past studies (Tordoff *et al.* 1986; Tordoff *et al.* 1988; Tordoff *et al.* 1989) hepatic portal vein glucose administration reduced cumulative food intake when glucose doses between 3 and 12 mmol were infused over 2 h. In contrast, tests of a possible acute effect of glucose on intake are scarce. One recent study addressed the time course of the glucose effect on intake by infusing similar doses of glucose over various times before and during an intraoral glucose solution feeding test (Baird *et al.* 1997). The primary finding in this study was a delayed effect of glucose infusion on oral ingestion. In all studies mentioned, the food intake suppression by hepatic portal vein glucose infusion did not depend on the amount of glucose infused or the infusion rate. Also, glucose did not affect feeding after jugular vein infusion. Therefore, the effect of lactate on feeding reported here differed from the effect of glucose described above, as the effect of lactate on meal size appeared to be immediate, not restricted to the hepatic portal route of infusion, and dependent on the rate of lactate delivery. Also, preliminary studies of ours indicate that glucose doses of 0.5 – 1.5 mmol, delivered at equivalent infusion rates under similar conditions, do not consistently affect meal size and postprandial satiety. Together these findings suggest that hepatic portal glucose and lactate infusions affect food intake through at least partially separate mechanisms. However, a direct comparison between the effects of various lactate and glucose doses is difficult because the two metabolites have different plasma levels and turnover rates.

In previous studies, the hypophagic effect of lactate after subcutaneous (Langhans *et al.* 1985b) and intraperitoneal (Nagase *et al.* 1996) injection depended critically on an intact hepatic branch of the vagus. Moreover, the critical lesion in these experiments appeared to be afferent and not efferent because subcutaneously injected lactate still reduced food intake after peripheral atropinization (Langhans *et al.* 1985b). The hepatic branch of the vagus is not the only afferent connection between liver and brain, and does not only carry fibers originating in the liver (Berthoud *et al.* 1992). Therefore,

differential feeding effects after hepatic portal and jugular vein or vena cava infusions provide more compelling evidence for a hepatic origin than data from hepatic branch vagotomy studies (see Friedman 1997). In the present study, lactate's similar effect on meal size after the hepatic portal and the vena cava infusion does not support the hypothesis that lactate acts in the liver to limit meal size. On the other hand, the data also raise a critical question concerning the possibility that lactate reduces food intake by acting at an extrahepatic site. Because the liver presumably removes lactate from the portal vein (see also below), more lactate should reach an extrahepatic site of action and thus reduce meal size more after vena cava than after hepatic portal vein infusion. However, this did not occur, and this discrepancy requires further investigation. Perhaps the amount of lactate reaching the liver after vena cava infusion was sufficient to inhibit feeding, or lactate can reduce meal size through both a hepatic and an extrahepatic mechanism. Although lactate reduced meal size through both routes of infusion similarly, the 1.5 mmol dose increased the satiety ratio of the meal and, hence, prolonged postprandial satiety more after hepatic portal vein than after vena cava infusion, for which the increase was not significant. This suggests that the effect of lactate on postprandial satiety is due to a hepatic action. Thus, lactate may terminate a meal and enhance postprandial satiety by acting at different sites and perhaps through different mechanisms. Interestingly, results of hepatic portal vein glucose infusion indicate that a glucose-related hepatic metabolic signal also controls postprandial satiety rather than meal termination (Baird *et al.* 1997).

Lactate (1.5 mmol) infused into the hepatic portal vein did not increase the systemic plasma lactate concentration above the level usually seen after a carbohydrate-containing meal in the rat (Langhans 1991; Surina *et al.* 1993) or in humans (Felig *et al.* 1975; Silberbauer *et al.* 1996). This corresponds with previous findings of a moderate increase in circulating lactate after intravenous L-lactate infusion in monkeys (Baile *et al.* 1970). Yet, in the present study the infusion-induced increase in portal vein lactate concentration and appearance rate were probably higher than normal. Wet liver weight in the rat is ~3.5% of body weight (Niewoehner *et al.* 1994), and total liver blood flow is around 2 ml/min x g liver (Richardson *et al.* 1981), with ~75% of that provided through the portal vein (Greenway *et al.* 1971). Thus, after a meal, when splanchnic blood flow usually increases (Hernandez *et al.* 1986), hepatic portal vein blood flow in a 350 g rat is presumably ~20 ml/min (2ml x 12.25 g liver x 0.75 = 18.4 ml + x ml, due to meal-induced increase). Consequently, the 0.3 mmol lactate/min administered in this study should yield portal vein blood concentrations of ~15 mmol/l lactate (1000ml : 20ml x 0.3mmol/l). This estimate corresponds very well with the systemic plasma lactate concentration measured at the end of a 5 min, 1.5 mmol infusion of lactate in the jugular vein of rats without concomitant access to food; in this situation, plasma lactate rose from a baseline of 0.9 ± 0.1 (mean ± SEM, n = 6) to 16.5 ± 0.7 mmol/l at infusion end and was still at 5.2 ± 0.6 mmol/l 5 min thereafter. Therefore, the comparatively low systemic lactate level found 1 min after the end of hepatic portal vein infusion was presumably, at least in part, due to hepatic lactate removal from the portal blood. In any case, in response to a meal, the rate of portal vein lactate appearance and the maximum lactate levels in the portal vein and in the vena cava are lower (Kimura *et al.* 1988; Langhans 1991; Niijima 1983) than after lactate infusion. Because the meal-induced increase in circulating lactate is certainly not the only metabolic feedback signal in the control of food intake, supraphysiological increases in lactate appearance and/or level are presumably necessary to affect meal termination and/or postprandial satiety.

Lactate did not affect the average feeding rate within the meal, and no signs of illness in response to the 0.5 to 1.5 mmol/meal lactate infusions were noticed by frequent observation. The rats merely stopped feeding earlier. The observed reduction in meal size by lactate was unlikely to be an osmotic effect because the control infusion consisted of equiosmotic saline in all experiments. Higher doses of lactate ($\geq$ 2 mmol/rat) reduced meals size even more; yet, the data are not reported here because these lactate doses and the corresponding saline control infusions occasionally triggered some unusual behavior such as intensive grooming. Again, however, no signs of illness were observed. All in all, it appears unlikely that the effect of lactate on feeding is due to malaise or some other nonspecific effect of the infusion. To absolutely exclude this possibility, however, it should be addressed directly in further studies.

Control meal size appeared to be smaller in the trials in which the higher doses were administered. Because the dose was increased with each test, it is possible that learning somehow influenced meal sizes (Tordoff *et al.* 1986). In addition, a high osmotic load with the high dose might decrease meal size. In any case, however, the phenomenon did not significantly influence the feeding-suppressive effect of lactate, and also control meal size did not differ significantly across doses in any of the trials (hepatic portal vein infusion: $F (5,35) = 3.26$, modified $t = 0.10$, $P > 0.05$; comparison between hepatic portal vein and vena cava infusion: $F (7,49) = 5.92$, modified $t = 1.17$ and 2.18 for hepatic portal vein and vena cava infusion, respectively, $P > 0.05$). The results do not allow determination of whether lactate acted as a signal or whether it inhibited feeding through its metabolic actions. Yet, the failure of lactate infusion to increase the systemic blood glucose level corresponds with previous observations in monkeys (Baile *et al.* 1970) and indicates that the reduction of food intake by lactate was not based on an increase in circulating glucose subsequent to conversion of the infused lactate to glucose. This is consistent with previous findings suggesting that oxidation of lactate beyond pyruvate, rather than stimulation of gluconeogenesis, contributes to the inhibition of feeding (Langhans *et al.* 1985a). Yet, a stimulation of liver glycogen synthesis by lactate may also play a role, at least for the enhancement of postprandial satiety. In the perfused rat liver, addition of lactate to the perfusion medium increased hepatic insulin clearance (Pagano *et al.* 1996) and greatly enhanced, and in fact determined, the rate of glycogen synthesis from glucose (Zhang *et al.* 1994). Interestingly, an increase in liver glycogen was the only metabolic measure related to changes in food intake after hepatic portal vein glucose infusions in another study (Tordoff *et al.* 1989). It is presently unknown, however, how changes in liver glycogen could affect feeding. Other possible mechanisms for the effect of lactate on food intake include a hyperpolarization of hepatocyte membranes, which has also been shown to occur in response to lactate addition to the perfusate in the perfused rat liver preparation (Dambach *et al.* 1974; Rossi *et al.* 1995), and a decrease in the discharge rate of hepatic branch vagal afferents. The latter effect could be subsequent to or independent of an effect on hepatocyte membranes. A decrease in hepatic vagal afferent activity has been shown in response to hepatic portal infusion of pyruvate (Niijima 1983), which forms easily from lactate.

Lactate is used by astrocytes and neurons as a source of energy, and it can replace glucose as a fuel for the brain under certain conditions (e.g. see Wiesinger *et al.* 1997). As lactate is rapidly taken up by neurons through a saturable transport system (Dringe *et al.* 1993), a central mechanism may contribute to the acute meal size effect of intravenously infused lactate. This interesting possibility merits further investigations.

To the best of our knowledge, the effect of intracerebroventricular lactate infusion on food intake has not yet been investigated, and intracerebroventricular administrations of glucose and other metabolites that inhibited eating were usually sustained for several hours (Davis *et al.* 1982).

In conclusion, intravenous infusion of lactate acutely reduced spontaneous nocturnal meal size in the rat. When infused into the hepatic portal vein, lactate also prolonged postprandial satiety. These results are consistent with a physiological role of endogenous lactate in the control of meal taking and suggest that the liver is crucial for the effect of lactate on postprandial satiety. The exact mechanisms of these inhibitory effects of lactate on feeding and the site where lactate acts to terminate meals remain to be identified.

Perspectives

Lactate is generated in response to meals and is released by several tissues (muscle, intestine, erythrocytes) including adipose tissue, where its production is modulated by nutritional state and adiposity (e.g. see DiGirolamo *et al.* 1992). Moreover, lactate is increasingly recognized as an important brain fuel (e.g. Wiesinger *et al.* 1997), and it potently inhibits feeding after parenteral administration. This feeding suppressive effect appears to be strong or even stronger than that of glucose, the leading candidate for a metabolite feedback signal in food intake control. These features and the profound modulation of circulating lactate by short-term events (such as meals or acute physical activity), as well as by long-term phenotypic characteristics (adiposity), make lactate a very interesting intermediary metabolite with potential signaling functions in the control of food intake and energy balance.

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GENERAL DISCUSSION

The control of eating behavior of humans in their natural environment is very complex, and eating is affected by many nutritional, physiological, psychological, sociological and cultural factors (e.g. de Castro 1996). In the work presented in this thesis, I intended to address the physiological role of some of the so-called pre- and postabsorptive satiety signals. The main emphasis in this context was on short-term prandial or postprandial mechanisms, which may affect the size and duration of a meal and/or the duration of the subsequent intermeal interval.

Traditionally, preabsorptive satiety signals have mainly been linked to satiation, i.e. they supposedly contribute to the termination of an ongoing meal and therefore influence meal size and duration. On the other hand, postabsorptive satiety signals are believed to mainly determine the duration of the intermeal interval. Moreover, the microstructure of eating is proposed to be a useful parameter to characterize eating behavior (Westerterp-Plantenga *et al.* 1990b, Westerterp-Plantenga *et al.* 1992) because it may reflect the prandial evolution of satiation, expressed as the deceleration characteristics of the cumulative intake curve (CIC). Some data suggest that the deceleration of the rate of intake during a meal is absent in obese people (Meyer & Pudel, 1972). Although the subjects participating in our studies were normal-weight, the shape of the cumulative intake curve during the target lunch in Study I did not show the deceleration characteristics described by Westerterp *et al.* (1990). This is, however, not necessarily unusual for normal weight, young males (Westerterp *et al.* 1990). Perhaps the within and between subject variability of the CIC must be specifically investigated under more controlled conditions in order for the deceleration of the CIC to be used as a crucial parameter in similar experimental settings. More importantly, the type of breakfast served in Study I did not influence the shape of the CIC, i.e. there was neither a statistically significant difference in the rate nor in the deceleration coefficients as determined by a non-linear regression analysis on the microstructure data. Thus, at least under the present experimental conditions, high and low fiber ingestion did not appear to influence the rate of intake during a meal and, hence, the development of preabsorptive satiety.

According to several findings dietary fiber has an inhibitory effect on gastric emptying and may thus lead to early meal termination (Eastwood 1992). In demonstrating that a low fiber breakfast with a high content of readily available carbohydrates leads to greater increases in plasma glucose, insulin, and lactate concentrations, the findings of Study I are in line with previous data suggesting that fiber slows down the rate of nutrient absorption in the intestine (e.g. Eastwood 1992). This presumably results in a delayed appearance of nutrients in the blood plasma, which in turn may influence metabolic satiety signals (Burley *et al.* 1993). Therefore, the presence of fiber in the intestinal tract may enhance preabsorptive and postabsorptive signals, thus contributing to satiation as well as satiety. In Study I, the pronounced postprandial glucose, insulin and lactate responses to the breakfast after the low fiber meal resulted in a reactive hypoglycemia just before the target lunch. The absence of this reactive hypoglycemia in response to the other breakfasts may have been due to the preabsorptive fiber action. Yet, as the carbohydrate and fiber content of the breakfasts varied - which was unavoidable since the meals were intended to reflect the "real" breakfast situation - the

postprandial glycemic response cannot be solely attributed to the differences in fiber content.

One theory holds that the initiation of a meal is provoked by the depletion of energy yielding nutrients, concurrent with a low fuel utilization, which weakens the satiety signals in the postabsorptive state. This appears to be particularly relevant for glucose. The blood glucose level has often been shown to decrease prior to spontaneous meals in rats, and this decrease appears to be causally related to meal onset. Recently, the existence of a similar premeal decline in blood glucose, which may be causally related to meal onset, was convincingly demonstrated also for humans by Margriet Westerterp and her group (Melanson *et al.* 1999a; Melanson *et al.* 1999b). In our study, the observed correlation between the plasma glucose concentration just before the target lunch and the duration of the self-determined intermeal interval prior to it is the only sign of a possible causal relationship between blood glucose and meal initiation. Apart from that, the reactive hypoglycemia in the low fiber group of our first study did not increase hunger before the target lunch (as determined by questionnaire ratings), nor did it increase meal size or induce a change in the microstructure of the meal. The reasons for these seemingly discrepant findings are not clear. Perhaps the signal derived from the reduced availability of glucose shortly before lunch after the low fiber breakfast was just not strong enough to translate into a statistically significant perceptional or behavioral difference in the current setting, or it was counteracted by other signals. All in all, the results of Study I suggested that energy content of a meal is the major determinant of subsequent food intake in man and that fiber content within a realistic range and nutrient composition have but a modulating effect on subsequent energy intake.

A special experimental setup was chosen in Study II, in order to investigate whether a high fat meal has different short-term effects on food intake and metabolism dependent on habitual dietary fat consumption. This question of course relates to the presumed obesity promoting effects of high dietary fat intake. Previous investigators found that baseline fatty acid oxidation in humans increased with increases in the dietary fat level (e.g. Rumpler *et al.* 1991; Verboeket-van de Venne *et al.* 1994). In Study II, a habitual high fat consumption appeared to favor baseline fatty acid oxidation because the basal RQ tended to be lower in the group usually consuming a high fat diet. This may reflect an enzymatic adaptation of fatty acid oxidation to the level of dietary fat (Bremer *et al.* 1984). There was, however, no acute increase in postprandial fatty acid oxidation following a high fat meal with increasing levels of habitual dietary fat in our subjects. Independent of the fat content of the habitual diet ($\geq$40% or $\leq$35%), prandial and postprandial changes in plasma FFA and BHB concentrations indicated that there was a prandial inhibition of lipolysis, which was possibly mediated by the concomitant increase in plasma insulin. Whereas the data also suggested that at least some of the fatty acids were oxidized postprandially, the continuing postprandial decrease in plasma BHB concentration suggested that resumption of whole body fatty acid oxidation was not accompanied by an increase in hepatic fatty acid oxidation and ketogenesis. High plasma levels of glucose and insulin might have had an inhibitory effect on fatty acid oxidation as proposed by Sidossis *et al.* (1996). All in all, the metabolic changes after the high fat breakfast did not differentially affect the size, duration and microstructure of the target lunch in the high and low fat groups, nor were there any measurable effects on subjective hunger and satiety ratings. Therefore, habitual fat intake does not appear to affect the satiating effect of such a high fat meal nor does it measurably influence

subsequent meal intake. The lack of differences in postprandial hepatic fatty acid oxidation between habitual high and low fat consumers did not allow assessing the satiety effect of hepatic fatty acid oxidation in humans. While this thesis was in preparation, however, another study from our laboratory revealed that an inhibition of hepatic fatty acid oxidation leads to a stimulation of food intake also in humans (Kahler *et al.* 1999).

In Study II, the overall changes in carbohydrate (glucose, lactate) and fat (FFA, BHB) metabolites in association with the observed changes in the plasma insulin level suggest that a habitual high fat consumption may bear early signs of insulin resistance even without or before a measurable increase in body weight. Consequently, the consumption of a high fat diet may contribute to the development of the metabolic syndrome, which at some later point in time may increase the predisposition for adiposity.

The observed prandial and postprandial changes in the plasma level of lactate in Studies I and II as well as reports of a marked food intake suppressive effect of parenteral lactate administrations in animals prompted us to study the prandial and postprandial effects of lactate on food intake more closely. To do so, we performed a series of experiments in rats. The prandial and postprandial increase in plasma lactate in response to carbohydrate ingestion reflects the low phosphorylating capacity of hepatocytes for glucose (Foster 1984). Glucose is therefore transformed into three-carbon units by extrahepatic tissues (Newgard *et al.* 1983). The findings in Study III were highly interesting since they showed for the first time an acute effect of a metabolite on an ongoing meal. At a dose of 1.0 mmol, lactate infusion reduced the size of the current meal. With the 1.5 mmol infusions an increase in the satiety ratio of the first meal was observed, i.e. at that dose, lactate did not only affect satiation, but also postprandial satiety, i.e. the satiating effect of a meal. This latter effect was only present after hepatic portal vein, but not after vena cava infusion, suggesting that the liver is somehow involved. Further studies are necessary, however, to critically examine the exact contribution of hepatic and extra-hepatic sites to the feeding inhibitory effects of lactate. All in all, the findings support the idea that the prandial and postprandial increase in circulating lactate contributes to satiation and satiety, and therefore plays a role in the physiological control of meal taking, at least in rats. Although the data cannot be directly transferred to man, they point towards the possibility that metabolites, such as lactate, may acutely affect eating and also suggest that the liver contributes to this effect.

Cognitive factors and believes about the foods eaten may play a major role for the control of eating behavior in man, as opposed to other species. The questionnaire ratings given by the subjects in Studies I and II yielded at least some subjective data and may help to explain some of the findings. Thus, the questionnaire ratings in Study I may partly reflect such cognitive factors and their effects (or lack of effects) on a subject's actual eating behavior. It was striking that the ratings did not reveal group differences for any of the questions, except that participating subjects seemed to unanimously assume that the high fiber breakfast would keep them more satiated for a longer period of time than any of the other breakfast types. This perception, however, did not influence food intake during the target lunch, and no increased postmeal satiety, preprandial hunger rating or decrease in target lunch size compared to the other groups were evident with the high fiber breakfast.

PERSPECTIVES

The present studies yielded some data, which support the theory of a metabolic control of food intake, but not all results were consistent with findings reported in the literature. This may be due to procedural differences. With the experimental designs chosen, however, new and interesting findings were obtained that do deserve closer attention and should stimulate further, more specific experiments. In general, the human studies should be extended by investigating a larger study population and by applying more sophisticated instrumentation such as a respiratory chamber. In my opinion, the inclusion and exclusion criteria at screening should be even more specific in order to obtain an even more homogenous subject sample. Moreover, the results of Study II, although mainly negative in the light of the question addressed, still suggest to me that the dietary habits of subjects participating in investigations of food intake regulation need to be better considered in future setups of experiments because, in addition to physical activity level, dietary habits may affect results in a subtle way. Finally, the advantages of the rat model for addressing some specific questions are apparent: the homogeneity of the studied population, the conditions which can be kept constant around the clock, and the possibility to easily limit external as well as internal influences facilitate the interpretation of the obtained results. Of course the rat model has also disadvantages, in particular the question of applicability of the data to human beings. As long as the regulation of food intake behavior is still unsatisfactorily known, a combination of human as well as a limited number of targeted animal studies presumably provide the best basis for understanding the physiologic control mechanisms involved.

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ACKNOWLEDGEMENTS

I am deeply grateful to Prof. Dr. Wolfgang Langhans for his support, trust and guidance in completing this work. His extraordinary knowledge, ideas, and professionalism motivated me throughout the past years when I was working under his supervision. He distinguishes himself by allowing independent work and providing an open door to any problem that crossed my path. He gave me the chance to learn from him and the opportunity to extend my skills needed for scientific research. His devotion to keeping the mind on the essential matters in science is a principle that I will carry with me on my future path.

I am sincerely thankful for the opportunity and trust Prof. Dr. Wolfgang Langhans has given me to give his lecture "Eating Behavior of Man" for the postgraduate course in Human Nutrition in the summer semester of 1996. Due to him only providing me with the basic structure of the lecture I was able to apply and extend my knowledge on human nutrition and to add to my teaching experience.

I also would like to deeply thank Prof. Dr. Caspar Wenk for his support and his valuable input and trust in finalizing my thesis.

I want to extend my most special thanks to Dr. Denise Surina-Baumgartner for her constant support and her cooperation in the experiments. She kept me on track more than she will ever know.

I want to thank Anthony Moses for the lab analyses, and Myrtha Arnold for helping with blood sampling and lab analyses that were invaluable for the completion of this work.

Special thanks go to Dr. Peter Driscoll, Dr. Rinaldo Rossi, Andrea Kahler, Marty Porter, Dr. Petra Frey-Rindova, Andrea Krampf, Rachel Marchal, and Martina Gantar for their active support in the experiments. My thanks also go to all the subjects participating in the experiments. I also want to thank the members of the institute for being there when I needed them, especially Dr. Markus Senn for his technical assistance.

I want to express my deepest appreciation to Dr. Margriet Westerterp-Plantenga for our instructive discussions and constructive support in the evaluation of the experiments using the universal eating monitor. I am grateful to Dr. Harry R. Kissileff, Dr. Karl. M. Pirke, and Dr. Ibrahim Elmadfa for letting me visit their institutes and giving me an insight into their research.

A special thanks goes to Patricia Kunstenaar for helping me find my way.

From the bottom of my heart I want to thank my family and friends for being there for me and for their constant support and understanding.

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