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► **To cite this version:**

A. Jovanovic, E. Leverton, B. Solanky, B. Ravikumar, J. E. M. Snaar, et al.. The second meal phenomenon is associated with enhanced muscle glycogen storage in humans. *Clinical Science*, 2009, 117 (3), pp.119-127. 10.1042/CS20080542 . hal-00496946

HAL Id: hal-00496946

<https://hal.science/hal-00496946>

Submitted on 2 Jul 2010

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The second meal phenomenon is associated with enhanced muscle glycogen storage in humans

A. Jovanovic¹, E. Leverton², B. Solanky², B. Ravikumar¹, J. E. M. Snaar², P.G. Morris², R. Taylor¹

¹Diabetes Research Group & Newcastle Magnetic Resonance Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne and ²Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, Nottingham NG7 2RD, United Kingdom

Running title: Second meal phenomenon and muscle glycogen storage in humans

Corresponding author:

Professor Roy Taylor, Newcastle Magnetic Resonance Centre, Newcastle University, Campus for Ageing and Health, Westgate Road, Newcastle upon Tyne, NE4 5PL, UK.
Telephone: + 44 191 248 1172; *Fax:* +44 191 2240 115156 3692; *Email:* Roy.Taylor@ncl.ac.uk

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Abstract

Background: The rise in blood glucose after lunch is less if breakfast has been eaten. The metabolic basis of this second meal phenomenon remains uncertain. We hypothesized that storage of ingested glucose as glycogen could be responsible during the post meal suppression of plasma FFA.

Objective: To determine the metabolic basis of the second meal phenomenon.

Design: Healthy subjects were studied on two separate days, with breakfast and without breakfast in random order. We studied metabolic changes after a standardized test lunch labeled with 3 g of ^{13}C - labeled (99%) glucose. Changes in postprandial muscle glycogen storage were measured using ^{13}C magnetic resonance spectroscopy.

Results: The rise in plasma glucose after lunch was significantly less if breakfast had been taken (0.9 ± 0.3 vs. 3.2 ± 0.3 mmol/l, with and without breakfast respectively; $p < 0.001$) despite comparable insulin responses. Pre-lunch FFA were suppressed after breakfast (0.13 ± 0.03 vs. 0.51 ± 0.04 mmol/l) and levels correlated positively with the maximum glucose rise after lunch ($r = 0.62$; $p = 0.001$). The increase in muscle glycogen signal was greater 5 hours after lunch on the breakfast day (103.2 ± 20.7 vs. 47.6 ± 12.4 Units; $p < 0.007$) and correlated negatively with plasma FFA concentrations before lunch ($r = -0.48$; $p < 0.05$).

Conclusion: The second meal effect is associated with priming of muscle glycogen synthesis consequent upon sustained suppression of plasma FFA concentrations.

Key Words: second meal, glucose, free fatty acids, glycogen, magnetic resonance spectroscopy, Type 2 diabetes.

Abbreviations: FFA, free fatty acids

Introduction

The extent of post-prandial rise in plasma glucose depends not only upon the quantity and nature of food ingested but also upon the metabolic state immediately prior to eating. In a previous study we made the incidental observation that the rise in blood glucose was considerably less after the second of two similar meals(1). On searching the literature it became clear that this had first been described almost a century ago as the second meal phenomenon or Staub-Trautgott effect(2; 3). Despite renewed interest in the phenomenon several decades ago(4-7) the mechanism underlying this effect remains unknown.

Limiting the rise in plasma glucose after meals is important, as the extent of the postprandial rise in blood glucose is a risk factor for cardiovascular disease independent of fasting plasma glucose (8-11). The relationship applies within the normal range of glucose values as well as in impaired glucose tolerance and diabetes. Considerable effort is being directed towards development of therapeutic agents which specifically target post-prandial hyperglycaemia (12-14). However, there is a potential health advantage to the whole population of being able to understand the second meal phenomenon as a physiological means of minimising the rise in blood glucose concentration after eating.

The present study was undertaken to establish whether the second meal effect was a consequence of enhanced rates of glycogen synthesis in muscle secondary to post-first meal suppression of plasma FFA. The development of ^{13}C magnetic resonance spectroscopy has allowed direct, non-invasive quantification of glycogen storage.

Subjects and Methods

Subjects

Healthy subjects on no medication were recruited. Ethical permission was obtained from the Newcastle and North Tyneside Local Research Ethics Committee. Informed consent was obtained from each subject before commencement of studies. 10 subjects were recruited, mean age 46.7 ± 3.9 years, weight 76.3 ± 3.1 kg, and BMI 26.1 ± 1.1 kg/m²

Study Design

Subjects were studied on 2 separate days in random order. On one day subjects consumed a standard breakfast followed by the standard lunch including ^{13}C -labeled glucose 4 hours later. On the other day breakfast was omitted. Subjects abstained from alcohol or vigorous exercise for 3 days before each study and followed their habitual diet. They fasted from 18:00 on the evening before the study, although water was permitted.

Protocol

On the morning of the study, subjects were transported to the Magnetic Resonance Centre by taxi. A venous cannula was placed in the dorsum of one hand. This hand was kept in a heated box at 55°C to allow sampling of arterialized blood, and during periods in the scanner the hand was kept at the same temperature using a heat retaining kaolin pack. The subject was positioned in the scanner and fasting muscle glycogen was measured. The baseline blood samples were then taken and the standard breakfast was provided. On the breakfast day, time 0 was set as the commencement of the standard breakfast. On the day without breakfast the subjects fasted until lunchtime but otherwise the protocol was the same. Repeat glycogen measurements were taken at +2, and +4

hours. At +4h, the subjects were given the standard lunch which incorporated 3 g of [U-¹³C] glucose. Muscle glycogen was subsequently measured at +6, and +9h. Subjects were asked to eat the test meals at their normal rate. Both breakfast and lunch meals were consumed within 15 minutes in all cases. Subjects remained in a sitting position (except for the standardized supine periods in the magnet) to avoid postural differences in rate of gastric emptying between test days. Arterialized blood samples were collected hourly. Breath samples for ¹³CO₂ were obtained before the labeled lunch and at 1-h intervals thereafter.

Meal composition

The standard breakfast consisted of 50g muesli, 100g milk, 2 slices of toast (56g), 20g marmalade, 20g margarine and 200 ml orange juice (106g carbohydrate, 18g fat, 15 g protein, 646 kcal).

The standard lunch (103g carbohydrate, 30g fat, 44g protein, 858 kcal) comprised a cheese sandwich, orange juice 200ml, yogurt 170g and jelly. A 100 ml jelly contained 3g [U-¹³C] glucose (atom 99%; Cambridge Isotopes, Andover, MA) was divided in 4 cubes and eaten at equally timed intervals during meal to allow even dispersion throughout gastric contents. Subjects were asked to chew each cube thoroughly before swallowing.

Breath ¹³C enrichments

Breath samples for ¹³C enrichments were collected at intervals. The subject was asked to blow through a straw into a glass tube. This was immediately capped with a rubber top at the end of a full exhalation. ¹³C enrichments of breath samples were determined by continuous flow isotope ratio mass spectrometry (ABCA, PDZ Europa Ltd. Crewe Cheshire, UK; CV analysis 0.07%, CV collection 0.3%). All results of ¹³C enrichment of expired air are expressed as atom percent excess (APE).

¹³C glucose enrichments

¹³C glucose plasma enrichments were determined by elemental-analyzer combustion continuous flow isotope ratio mass spectrometry using an automated nitrogen carbon analysis unit for solids and liquids (ANCA-SL) linked to a 20/20 mass spectrometer (PDZ Europa, Crewe, UK). The reference material used in the analysis was IA-R005 with an accepted delta ¹³C value of -26.1%.

Blood Glucose, Plasma Insulin, and Metabolites

Blood glucose was measured with a HemoCue photometer analyzer (HemoCue, Ängelholm, Sweden). Plasma FFA was measured on a Roche Cobas centrifugal analyzer by a commercially available enzymatic colorimetric kit (Wako Chemicals, Neuss, Germany). Serum insulin and C-peptide were both measured using ELISA kits (Dako; Ely, Cambridge, UK). Plasma glucagon concentration was measured by radioimmunoassay (Linco; Linco Research, St Charles, MO). Metabolites (glycerol, lactate, pyruvate, beta-hydroxybutyrate and alanine) were measured using a COBAS biocentrifugal analyzer (Roche Diagnostics, Welwyn Garden City, UK). Plasma triglycerides were measured on a Roche Cobas centrifugal analyzer, using a colorimetric assay (ABX Diagnostics, Montpellier, France). Plasma catecholamines were measured using enzyme immunoassay (Labor Diagnostica Nord GmbH&KG, Nordhorn, Germany).

¹³C MRS and Quantitation of Spectra

MR data were acquired on a 3 Tesla whole body MR scanner. Muscle glycogen measurements were taken from the quadriceps muscle using a surface coil comprising a 7cm diameter carbon coil for transmission and reception and quadrature proton coils for ¹H decoupling. A small vial, containing ¹³C formate and fixed at the centre of the ¹³C coil, was used as a reference for determining glycogen concentrations. The subject was placed in a comfortable supine position and the coil was placed directly over the mid-

anterior aspect of the thigh and held firmly in place with a Velcro strap. The area was marked to allow the coil to be repositioned accurately.

Manual shimming was performed on the water resonance peak, and the broadband decoupling frequency was centred on the glycogen resonance. The coils were tuned and matched before each measurement using a network analyser (HP model 8751A, 5-500KHz). The ^{13}C spectra were acquired using a pulse acquire sequence with proton decoupling. The excitation pulse was a 100 μs hard pulse at a peak power of 390 W with CYCLOPS phase cycling. Broadband decoupling was achieved using the WALTZ-8 sequence during signal acquisition with a peak power of $50 \pm 2\text{W}$. A repetition interval of 360 ms was used and 3000 acquisitions were averaged for each spectrum giving a temporal resolution of 18 min. RF power was monitored throughout the acquisition period to ensure that it did not exceed the maximum value allowed according to the specific absorption rate limits recommended by the National Radiological Protection Board.

The spectra were processed using the Matlab version of MRUI. The integral of the glycogen peak was expressed as a fraction of the integral of the formate peak arising from the vial containing ^{13}C -formate at the centre of the ^{13}C coil. Quantification was achieved by comparison with a phantom containing 184 mmol/l oyster glycogen and 150 mmol/l KCl. . The concentration of muscle glycogen, $[\text{Glyc}]_{\text{muscle}}$ was calculated using the formula:

$$1) [\text{Glyc}]_{\text{muscle}} = \frac{R_{\text{muscle}} \times [\text{Glyc}]_{\text{phantom}}}{R_{\text{phantom}}}$$

where R_{phantom} and R_{muscle} are the ratios of the integrals of glycogen to formate peaks in the phantom and muscle, respectively, and $[\text{Glyc}]_{\text{phantom}}$ is the concentration of glycogen in the phantom (184mmol/l).

Before lunch the ^{13}C -glycosyl units were at natural abundance and the rise in absolute concentrations can be determined as glycogen in mmol/l of muscle). After the lunch containing ^{13}C -glucose, the percentage enrichment of the glycosyl units in muscle glycogen is no longer known, and glycogen concentration is reported as total signal intensity in U/l.

The coefficient of variation for ^{13}C MRS muscle glycogen measurement has previously been shown to be $4.3 \pm 2.1\%$ compared with $9.3 \pm 5.9\%$ by biopsy measurement (15).

Statistical Analysis

Data are presented as mean and standard error of the mean. All statistical calculations were performed using MINITAB software (Release 15; Minitab, State College, PA). Comparisons were carried out using paired Student's *t*-test (two tailed), and relationships were tested using the linear correlation analysis. Statistical significance was accepted at $P < 0.05$. A prior power calculation identified that to detect a 40% change in muscle glycogen concentration with 95% power 8 subjects had to be studied.

Results

Glucose

On the breakfast day blood glucose rose from 4.5 ± 0.2 mmol/l to peak at 7.8 ± 0.3 mmol/l at 1 hour after breakfast returning to 5.0 ± 0.2 mmol/l before lunch (Fig 1a). After lunch, the peak postprandial glucose was lower than after breakfast (6.1 ± 0.3 mmol/l, $p < 0.001$) and the increment was considerably less (1.1 ± 0.3 vs. 3.3 ± 0.3 mmol/l, $p < 0.001$). On the day without breakfast, blood glucose fell from 4.7 ± 0.1 to 4.4 ± 0.2 mmol/l, during the prolonged fast before lunch. The glucose level at 1 hour after the lunch was significantly greater compared to that on the breakfast day (7.6 ± 0.3 vs. 5.9 ± 0.2 mmol/l, $p < 0.004$). The difference remained significant when the glucose increments were compared (3.2 ± 0.3 vs. 0.9 ± 0.3 mmol/l, $p < 0.001$, Fig. 1b).

Serum insulin and C-peptide

On the breakfast day, serum insulin rose from 4.5 ± 0.4 mU/l to peak level of 63.5 ± 10.4 mU/l at 1 hour after breakfast and fell to 18.0 ± 4.2 mU/l at lunch-time. The post lunch insulin peak was 64.5 ± 9.3 mU/l 30 minutes later with a slow return to the baseline at the end of the study. On the day without breakfast insulin levels declined slowly until lunch (5.5 ± 0.8 mU/l at 0h; 3.4 ± 0.5 mU/l at 4h, $p = 0.002$). Serum insulin peaked at 68.1 ± 10.7 mU/l, 1 hour after lunch ($p = 0.72$ compared with the breakfast day at 30 minutes after lunch). Subsequently, insulin levels remain higher on the day without breakfast (25.4 ± 6.1 vs. 16.3 ± 4.0 ; $p < 0.05$) (Fig. 2a).

The change in C-peptide levels mimicked the change in insulin levels. On the breakfast day C-peptide levels rose to maximum 3.3 ± 0.3 nmol/l at 1 hour after breakfast returning to 2.0 ± 0.3 nmol/l before lunch. C-peptide rose back to 3.3 ± 0.4 nmol/l at 1 h after lunch and slowly declined from then on. On the day without breakfast C-peptide concentrations peaked at 3.0 ± 0.3 nmol/l at 1h and were still elevated by the end of the day (Fig. 2b).

Glucagon and catecholamines

Mean fasting glucagon levels were similar on the two test days (52.7 ± 4.5 pg/ml and 57.6 ± 6.2 pg/ml, respectively). Glucagon levels were stable until lunch on both days and gradually increased towards the end of the study days (Fig. 2C).

Immediately before lunch plasma noradrenaline levels were similar on 2 study days (1.51 ± 0.18 , 1.47 ± 0.19 nmol/l) with no further change 30 minutes after lunch. Pre-lunch mean plasma adrenalin concentration was slightly higher on the day without breakfast (prolonged fast) (0.37 ± 0.04 vs. 0.27 ± 0.01 nmol/l, $p < 0.05$). Post-lunch, plasma adrenaline decreased to 0.33 ± 0.03 nmol/l ($p = 0.07$) on the day without breakfast, remaining constant on the breakfast day (0.27 ± 0.01 and 0.28 ± 0.01 nmol/l).

Plasma FFA, triglycerides and metabolites

Plasma FFA were suppressed rapidly on the breakfast day, reaching a nadir at +2h (basal 0.48 ± 0.04 ; 2h 0.08 ± 0.01 mmol/l) and remaining suppressed until the end of the study day. On the day without breakfast plasma FFA remained elevated until 1 h after lunch falling to a nadir at +6 h (0.09 ± 0.01 mmol/l) with no further change until the end (Fig. 3).

Plasma FFA concentrations pre-lunch-time correlated positively with the maximum glucose rise after lunch ($r = 0.70$; $p < 0.001$) (Fig. 4). Glycerol and beta-hydroxybutyrate levels are shown in Figure 3. Both suppressed rapidly after the first meal of the day. Plasma lactate, pyruvate and alanine showed the expected rise in concentrations after meals as the splanchnic tissues switches from lactate uptake to release. Most of the lactate originates from the liver while the gut contributes the majority of circulating alanine, based on the previous studies in the conscious dog.

Fasting triglyceride levels were comparable on the 2 test days (1.27 ± 0.19 and 1.28 ± 0.20 mmol/l). On the breakfast day, triglyceride levels rose steadily during the course of the study day, reaching levels significantly greater from baseline and peaking at +6 h (2.09 ± 0.26 mmol/l, $p < 0.001$). On the day without breakfast, triglyceride levels started to rise from 1.08 ± 0.15 mmol/l at 1 hour after lunch to its peak 1.66 ± 0.27 mmol/l at the end of the study day.

Muscle glycogen

Mean fasting glycogen concentrations were similar on two days (59.6 ± 8.2 vs. 62.8 ± 12.8 mmol/l, $p = 0.72$). On the breakfast day, the mean glycogen concentration rose to 77.4 ± 5.6 mmol/l at +2 hours. Before lunch the ^{13}C -glycosyl units are at natural abundance, therefore the rise in absolute concentrations can be determined (glycogen mmol/l). After the lunch containing ^{13}C -glucose, the percentage enrichment of the glycosyl units in muscle glycogen is not directly measurable and total signal intensity U/l is reported. After ingestion of the labeled lunch there was a rapid increase in the glycogen signal intensity at 2 hours after the meal (123.4 ± 23.5 U/l) peaking at 171.2 ± 28.2 U/l at the end of the study day.

On the day without breakfast, mean fasting glycogen concentration remained stable until lunch (62.8 ± 12.8 mmol/l at 0h, 58.5 ± 12.7 mmol/l at +4h). Following lunch there was a slower rise in the muscle glycogen signal intensity to 97.5 ± 15.5 U/l at 2 h and 104.7 ± 17.9 U/l at the end of the study day. The increment over baseline was significantly greater on the breakfast day at the end of the study day (103.2 ± 20.7 vs. 47.6 ± 12.4 U/l; $p < 0.007$; Fig. 5a). There was an inverse correlation between this increase in muscle glycogen signal and plasma FFA concentrations before lunch ($r = -0.48$; $p < 0.05$; Fig. 5b).

Breath ^{13}C enrichments

The appearance of ^{13}C in expired breath was similar on days with and without breakfast. ^{13}C APE steadily increased to a maximum at 4 hours after lunch of 0.81 ± 0.06 vs 0.76 ± 0.06 ; $p = 0.36$ on the two study days (Fig. 5c).

Discussion

This study has demonstrated for the first time that the second meal phenomenon is associated with increased glycogen synthesis in skeletal muscle in humans. The rate of incorporation of dietary glucose into muscle glycogen after lunch is approximately 50% greater within 2 hours and approximately doubled within 5 hours when breakfast had been taken compared with no-breakfast days. The meal induced insulin response was similar on the two days. The plasma FFA concentration before lunch correlated positively with the post-lunch rise in blood glucose, and negatively with the increase in the glycogen signal after lunch. Our data suggest that insulin secretion after breakfast suppresses plasma FFA, facilitating carbohydrate economy after lunch and permitting greater storage of glycogen in muscle. An additional direct effect of recent exposure of muscle to insulin cannot be excluded (16). The conclusions of the study may be of practical benefit to athletes wishing to maximize performance and to people with type 2 diabetes, a condition in which muscle glycogen synthesis is impaired. Furthermore, the response of counter-regulatory hormones glucagon, adrenalin and noradrenalin following the second meal were similar.

The present study was triggered by our previous incidental observation of a 70 % lesser rise in blood glucose after lunch than after a similar meal at breakfast time (1). However, this could have been at least partially an effect of the diurnal cortisol profile. In the present study we examined the rise in blood glucose only at lunchtime thus avoiding this complicating factor. As lunch when given after a breakfast, rather than after no breakfast, was associated with a 73% reduction in blood glucose increment, the major influence is established as the second meal phenomenon rather than a time of day effect. Although this was first observed almost a century ago (2; 3) the underlying mechanism has not been elucidated. Re-discovery of the phenomenon decades later (4; 5) was followed by a series of investigations upon anaesthetized animals using intravenous glucose administration (6; 17). These studies showed no effect of a prior dose of glucose on glycogen storage in liver or muscle, nor on glucose oxidation, but the effects observed are likely to have been affected by increasing duration of anaesthetic. By investigating the second meal phenomenon in humans, direct observation of effect upon muscle glucose storage as glycogen and meal derived glucose oxidation was possible.

The second meal is given after the body has already taken in energy and it may be considered that the greater total carbohydrate intake would lead to increased glycogen stores later in the day. However, the study design of using a ^{13}C labelled lunch allows storage of glycogen derived from carbohydrate in the lunch to be assessed as the detected increment in muscle glucose. The breakfast carbohydrate itself is not likely to contribute to the increase glycogen storage in the period after lunch, as storage of the first meal carbohydrate as glycogen is complete within the first 5 hours (19). Hence, there would be negligible contribution of breakfast carbohydrate to the observed increase in ^{13}C -glycogen concentration 2 and 5 hours after lunch as glycogen derived from breakfast would not only be at natural abundance but also would be very small in absolute amount. The loss of possibility of interpreting signal change as representing total muscle glycogen is a necessary and small price to pay for the definitive tracing of lunch derived glucose.

Non-invasive magnetic resonance spectroscopy has been shown to measure muscle glycogen concentration accurately and precisely in comparison to biopsy (15). Using this technique we have previously demonstrated that, storage of glucose as muscle glycogen accounts for 30% of ingested carbohydrate at peak, five hours after the test meal in healthy subjects (18). Thereafter the glycogen concentration in muscle was

observed to fall. Total glucose oxidation has been observed to account for approximately 50% of ingested carbohydrate over the same period (19). Modulation of either or both of these processes is capable of changing the plasma glucose rise after eating. When two successive meals are taken, muscle glycogen concentration rises in a stepwise fashion after each meal (1). Muscle glycogen is in a state of continuous synthesis and degradation, in the fasting state (20) and during gentle exercise (21). However, in the insulin stimulated state after a meal, muscle glycogen breakdown is minimised and the glucose stored as glycogen will reflect the ^{13}C -glucose APE in plasma (22). As only the lunch was isotopically enriched, the post-lunch marked rise in glycogen concentration reflects uptake and storage as glycogen of ingested carbohydrate. The present data demonstrate a clear effect of a meal taken 4 hours previously upon uptake of meal derived glucose and storage as muscle glycogen. The question arises as to the underlying mechanism for modulation of glycogen synthesis, and the inverse relationship of plasma FFA concentration with postprandial rates of glycogen storage suggests this as a potential mediator of the second meal phenomenon.

Following intravenous administration of glucose under insulin-stimulated conditions, muscle glycogen synthesis is the major pathway of glucose storage (23). Increase in plasma FFA for over 3 hours induces insulin resistance in humans by inhibition of muscle glucose transport resulting in the reduction in the rate of muscle glycogen synthesis (24). This study elucidates the mechanism of the glucose – FFA interaction first described by Randle (25). Conversely, prolonged pharmacological suppression of plasma FFA with the nicotinic acid analogue acipimox improves insulin action in type 2 diabetic patients by increasing nonoxidative glucose disposal with skeletal muscle biopsies showing increase in glycogen concentration (26-28). In patients with HIV lipodystrophy, overnight suppression of FFA increased glucose uptake and muscle glycogen synthase activity during euglycemic-hyperinsulinemic clamp (29). One study of oral glucose administration has examined the effect of FFA suppression and this observed improved glucose tolerance and muscle glucose uptake (30). We have previously demonstrated that suppression of FFA with acipimox in normal subjects brought about a postprandial plasma glucose rise following a mixed meal which was 25% lower than after placebo (31). This was not due to any component of more rapid suppression of hepatic glucose output and was mainly a direct effect upon glucose storage as glycogen. The possibility of some direct effect of prior insulinisation of muscle has to be recognised (16) and there may be synergy between any such effect and suppression of plasma FFA. However, the magnitude of the effect of acipimox alone in bringing about a 25% improvement in peak meal plasma glucose (31) suggests that the FFA effect is likely to be the predominant mechanism in facilitating postprandial muscle glycogen storage.

In the 1980's there was considerable interest in the effect of low-glycaemic index breakfasts in improving the post-lunch blood glucose profile (32; 33). This effect on the total area under the glycaemic response curve to lunch was subsequently shown to relate directly to the concentration of plasma FFA at the time of lunch (34). If the quantity of carbohydrate consumed at breakfast was insufficient to suppress plasma FFA then no effect of glycaemic index of breakfast upon post-lunch glycaemia was observed (35). To preserve the second meal phenomenon throughout the day it would appear necessary that meals or snacks are taken frequently enough to maintain suppression of plasma FFA and hence perpetuate the post-prandial state.

Application of such dietary manipulation could have considerable implications in people with diabetes. However, one study has suggested that the second meal

phenomenon does not occur in diabetes (36). This study used intravenous glucose loads in diabetic subjects with very poor insulin secretory response, and although not measured it is probable that FFA suppression was not achieved. We have recently demonstrated that the second meal phenomenon is potently expressed in people with type 2 diabetes when day to day conditions are reproduced using mixed meals (37).

These observations raise the possibility that mimicking the second meal phenomenon could be utilised to limit the relatively large rise in blood glucose seen after the first meal of the day in people with type impaired glucose tolerance or 2 diabetes. This could be achieved by taking a low carbohydrate, high protein snack to bring about insulin secretion and FFA suppression pre-breakfast or by pharmacological reduction of pre-breakfast plasma FFA. The minimal size of first meal to achieve adequate suppression of plasma FFA is now being investigated.

In summary, the second meal phenomenon is associated with enhanced muscle glycogen storage and appears to be determined by suppression of plasma FFA. These findings have implications for controlling excessive swings in postprandial glycaemia by therapeutic application of the second meal effect.

Acknowledgment

We are grateful to the volunteers for their commitment during the study. We thank Ms Heather Gilbert and Ms Annette Lane for technical assistance.

This study was supported by a grant from the Wellcome Trust UK (WellcomeTrust Grant No. GR073561).

Figures

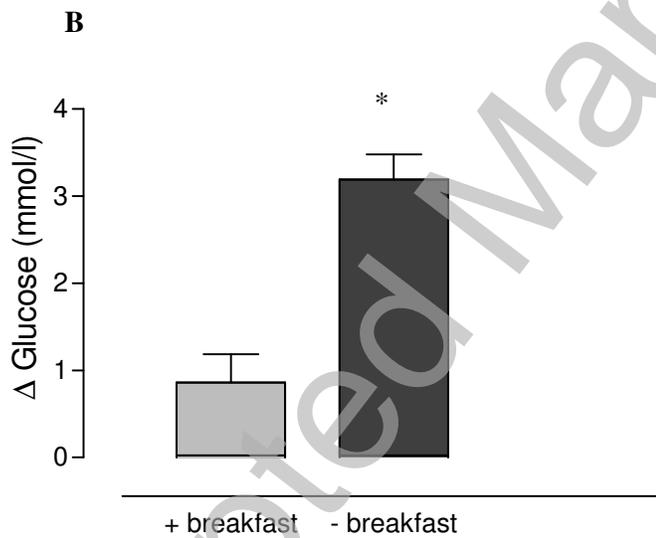
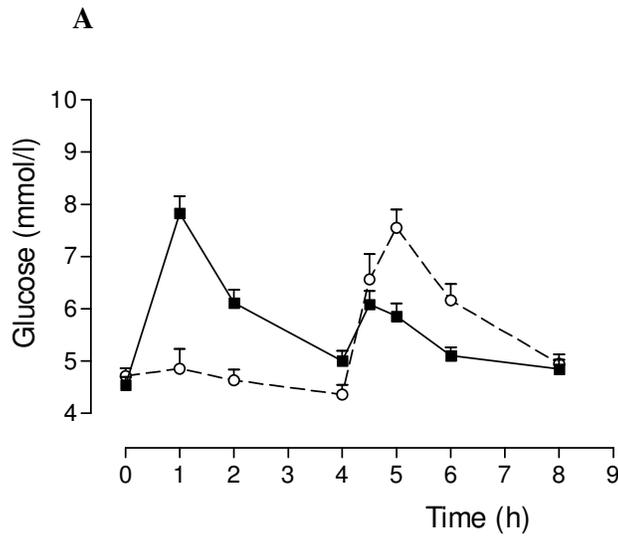


Figure 1. A: Changes in blood glucose concentrations on days with (—■—) and without breakfast (---○---); B: Increment in blood glucose 1 h after lunch, * p<0.001.

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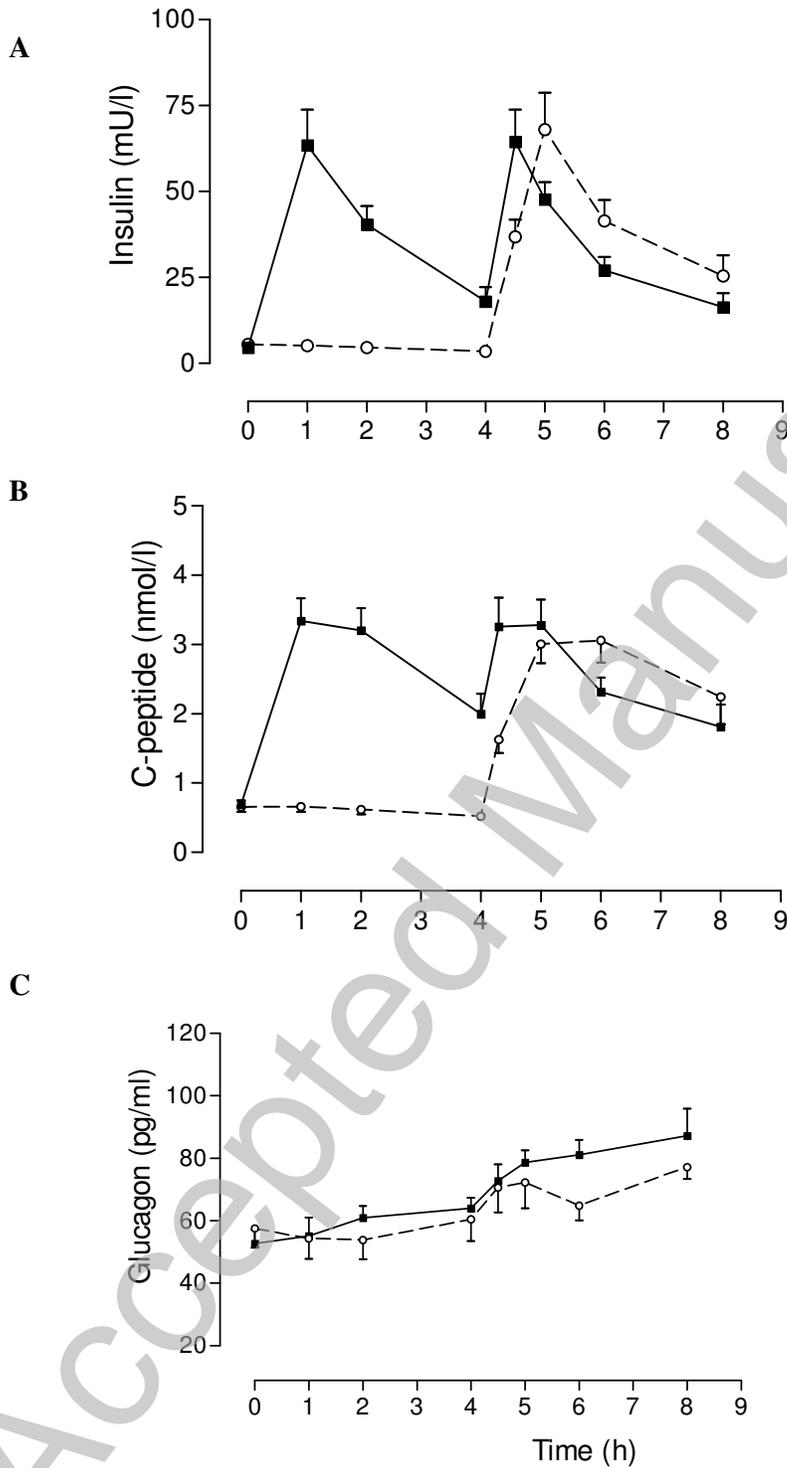


Figure 2. Changes in serum insulin, C-peptide, and glucagon during the study period on breakfast days (—■—) and days without breakfast (—○—). Data are shown as means ± SE.

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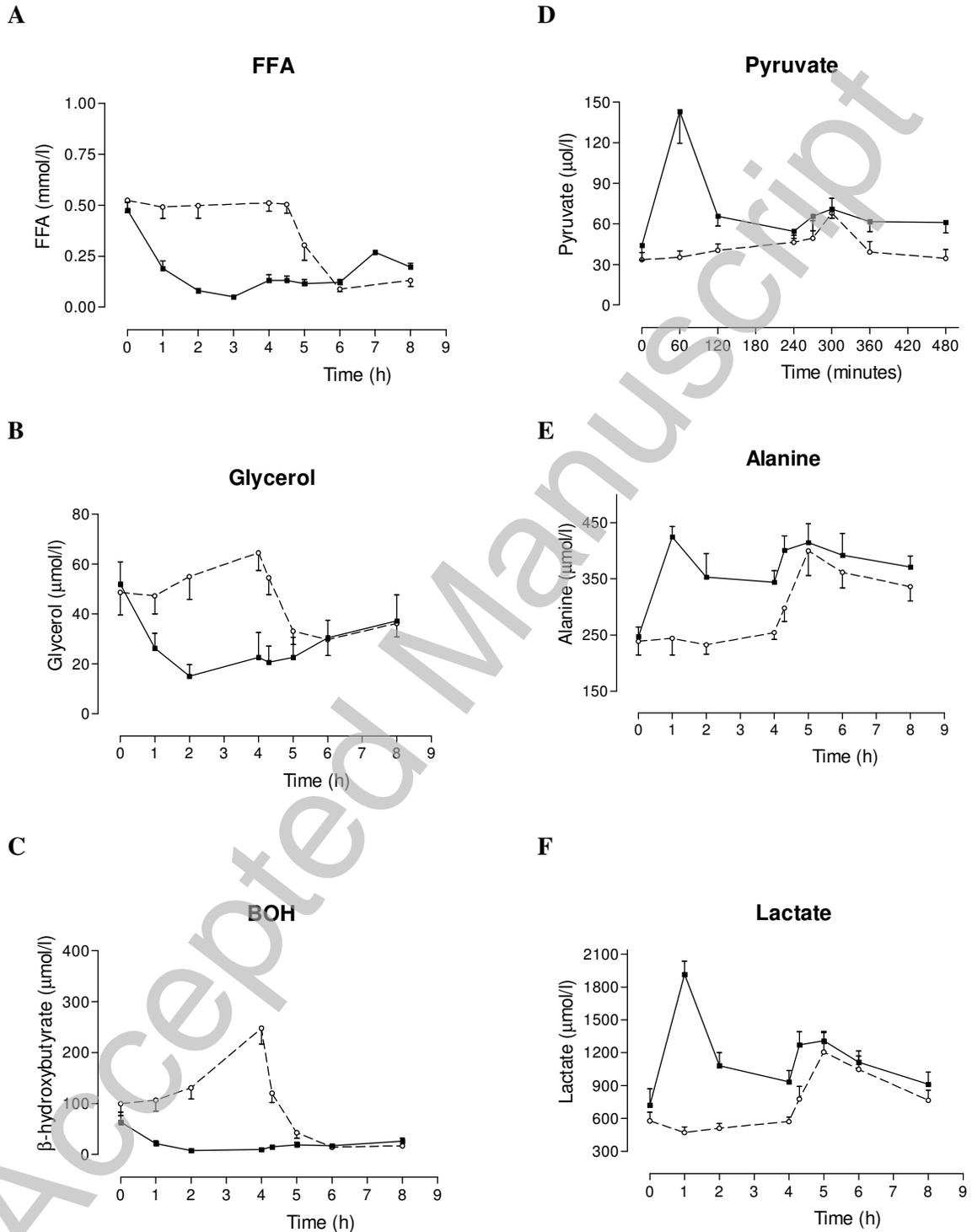


Figure 3. Changes in plasma free fatty acids (FFA), glycerol, beta-hydroxybutyrate (BOH), pyruvate, alanine and lactate during the study period on breakfast days (—■—) and days without breakfast (---○---). Data are shown as means ± SE.

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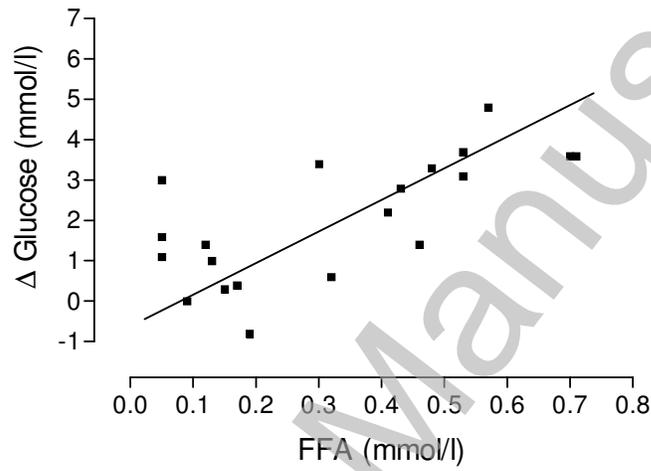
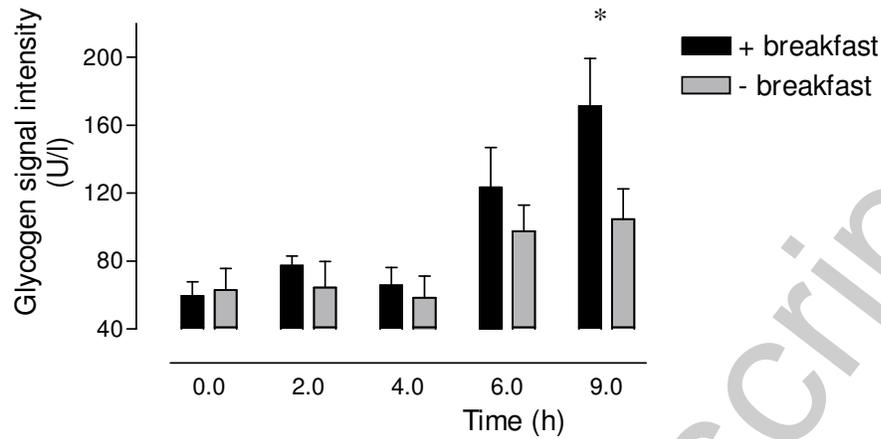


Figure 4. Relationship between pre-lunch FFA and the maximal increase in blood glucose concentration after lunch; ($r=0.70$; $p<0.001$).

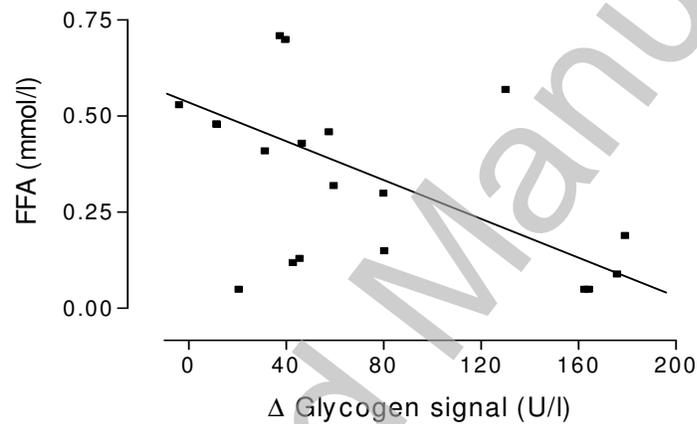
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B



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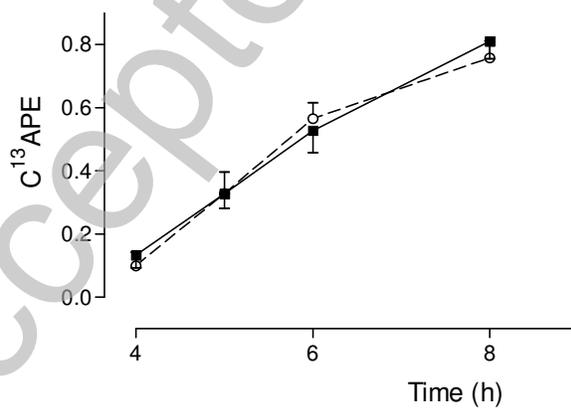


Figure 5. A: Change in muscle glycogen signal on 2 days, * $p < 0.05$; B: Relationship between increment in glycogen signal and pre-lunch plasma FFA, $r = -0.48$; $p < 0.05$; C: Change in breath ^{13}C APE on breakfast days (—■—) and on days without breakfast (---○---). Isotopic data are shown as atoms percent excess (APE). Data are shown as means \pm SE.

References:

1. Carey PE, Halliday J, Snaar JE, Morris PG, Taylor R. 2003. Direct assessment of muscle glycogen storage after mixed meals in normal and type 2 diabetic subjects. *American journal of physiology* 284(4):E688-694.
2. Staub H. 1921. Untersuchungen über den Zuckerstoffwechsel des Menschen. *Z Clin Med* 91:44-48.
3. Traugott K. 1922. Über das Verhalten des Blutzucker Spiegels bei Wiederholter und verschiedener Art enteraler Zuckerzufuhr und dessen Bedeutung für die Leberfunktion *Klin Wochenschr* 1:892-894.
4. Szabo AJ, Maier JJ, Szabo O, Camerini-Davalos RA. 1969. Improved glucose disappearance following repeated glucose administration. Serum insulin growth hormone and free fatty acid levels during the Staub-Traugott effect. *Diabetes* 18(4):232-237.
5. Metz R, Friedenberg R. 1970. Effects of repetitive glucose loads on plasma concentrations of glucose, insulin and free fatty acids: paradoxical insulin responses in subjects with mild glucose intolerance. *The Journal of clinical endocrinology and metabolism* 30(5):602-608.
6. Buchanan B, Abaira C, Hodges L. 1984. Potentiation of ¹⁴C-glucose oxidation by priming glucose loads: effect of starvation. *Metabolism: clinical and experimental* 33(5):411-414.
7. Abaira C, Lawrence AM. 1977. Improved tolerance to successive glucose loads in acromegaly. *Metabolism: clinical and experimental* 26(3):287-293.
8. Haffner SM. 1998. The importance of hyperglycemia in the nonfasting state to the development of cardiovascular disease. *Endocrine reviews* 19(5):583-592.
9. Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, Ziegler HJ, Lindner J. 1996. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. *Diabetologia* 39(12):1577-1583.
10. 1999. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. *Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe. Lancet* 354(9179):617-621.
11. Cavalot F, Petrelli A, Traversa M, Bonomo K, Fiora E, Conti M, Anfossi G, Costa G, Trovati M. 2006. Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. *The Journal of clinical endocrinology and metabolism* 91(3):813-819.
12. Heise T, Nosek L, Spitzer H, Heinemann L, Niemoller E, Frick AD, Becker RH. 2007. Insulin glulisine: a faster onset of action compared with insulin lispro. *Diabetes, obesity & metabolism* 9(5):746-753.
13. Singhal P, Caumo A, Cobelli C, Taylor R. 2005. Effect of repaglinide and gliclazide on postprandial control of endogenous glucose production. *Metabolism: clinical and experimental* 54(1):79-84.
14. Ahren B. 2007. Dipeptidyl peptidase-4 inhibitors: clinical data and clinical implications. *Diabetes care* 30(6):1344-1350.
15. Taylor R, Price TB, Rothman DL, Shulman RG, Shulman GI. 1992. Validation of ¹³C NMR measurement of human skeletal muscle glycogen by direct biochemical assay of needle biopsy samples. *Magn Reson Med* 27(1):13-20.

16. Geiger PC, Han DH, Wright DC, Holloszy JO. 2006. How muscle insulin sensitivity is regulated: testing of a hypothesis. *American journal of physiology* 291(6):E1258-1263.
17. Abaira C, Buchanan B, Hodges L. 1987. Modification of glycogen deposition by priming glucose loads: the second-meal phenomenon. *The American journal of clinical nutrition* 45(5):952-957.
18. Taylor R, Price TB, Katz LD, Shulman RG, Shulman GI. 1993. Direct measurement of change in muscle glycogen concentration after a mixed meal in normal subjects. *The American journal of physiology* 265(2 Pt 1):E224-229.
19. Singhal P, Caumo A, Carey PE, Cobelli C, Taylor R. 2002. Regulation of endogenous glucose production after a mixed meal in type 2 diabetes. *American journal of physiology* 283(2):E275-283.
20. Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI, Shulman RG. 1994. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *Journal of applied physiology: respiratory, environmental and exercise physiology* 76(1):104-111.
21. Price TB, Taylor R, Mason GF, Rothman DL, Shulman GI, Shulman RG. 1994. Turnover of human muscle glycogen with low-intensity exercise. *Medicine and science in sports and exercise* 26(8):983-991.
22. Jue T, Rothman DL, Shulman GI, Tavitian BA, DeFronzo RA, Shulman RG. 1989. Direct observation of glycogen synthesis in human muscle with ¹³C NMR. *Proceedings of the National Academy of Sciences of the United States of America* 86(12):4489-4491.
23. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. 1990. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by ¹³C nuclear magnetic resonance spectroscopy. *The New England journal of medicine* 322(4):223-228.
24. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI. 1999. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103(2):253-259.
25. Randle PJ, Garland PB, Hales CN, Newsholme EA. 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1:785-789.
26. Vaag A, Skott P, Damsbo P, Gall MA, Richter EA, Beck-Nielsen H. 1991. Effect of the antilipolytic nicotinic acid analogue acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 88(4):1282-1290.
27. Johnson AB, Argyraki M, Thow JC, Cooper BG, Fulcher G, Taylor R. 1992. Effect of increased free fatty acid supply on glucose metabolism and skeletal muscle glycogen synthase activity in normal man. *Clin Sci (Lond)* 82(2):219-226.
28. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI. 1996. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97(12):2859-2865.
29. Lindgaard B, Frosig C, Petersen AM, Plomgaard P, Ditlevsen S, Mittendorfer B, Van Hall G, Wojtaszewski JF, Pedersen BK. 2007. Inhibition of lipolysis stimulates peripheral glucose uptake but has no effect on endogenous glucose production in HIV lipodystrophy. *Diabetes* 56(8):2070-2077.
30. Piatti PM, Monti LD, Davis SN, Conti M, Brown MD, Pozza G, Alberti KG. 1996. Effects of an acute decrease in non-esterified fatty acid levels on muscle

- glucose utilization and forearm indirect calorimetry in lean NIDDM patients. *Diabetologia* 39(1):103-112.
31. Carey PE, Gerrard J, Cline GW, Dalla Man C, English PT, Firbank MJ, Cobelli C, Taylor R. 2005. Acute inhibition of lipolysis does not affect postprandial suppression of endogenous glucose production. *American journal of physiology* 289(6):E941-947.
 32. Jenkins DJ, Wolever TM, Taylor RH, Griffiths C, Krzeminska K, Lawrie JA, Bennett CM, Goff DV, Sarson DL, Bloom SR. 1982. Slow release dietary carbohydrate improves second meal tolerance. *The American journal of clinical nutrition* 35(6):1339-1346.
 33. Jenkins DJ, Wolever TM, Nineham R, Sarson DL, Bloom SR, Ahern J, Alberti KG, Hockaday TD. 1980. Improved glucose tolerance four hours after taking guar with glucose. *Diabetologia* 19(1):21-24.
 34. Wolever TM, Bentum-Williams A, Jenkins DJ. 1995. Physiological modulation of plasma free fatty acid concentrations by diet. Metabolic implications in nondiabetic subjects. *Diabetes care* 18(7):962-970.
 35. Clark CA, Gardiner J, McBurney MI, Anderson S, Weatherspoon LJ, Henry DN, Hord NG. 2006. Effects of breakfast meal composition on second meal metabolic responses in adults with type 2 diabetes mellitus. *Eur J Clin Nutr*.
 36. Clark CA, Gardiner J, McBurney MI, Anderson S, Weatherspoon LJ, Henry DN, Hord NG. 2006. Effects of breakfast meal composition on second meal metabolic responses in adults with Type 2 diabetes mellitus. *Eur J Clin Nutr* 60(9):1122-1129.
 37. Jovanovic A, Gerrard J, Taylor R. 2007. The second meal effect in type 2 diabetes: A physiologic means of limiting postprandial hyperglycaemia. *Diabetes* 56 (Sup 1):A 1599.