

De novo lipogenesis during controlled overfeeding with sucrose or glucose in lean and obese women¹⁻³

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ABSTRACT

Background: The results of previous studies suggest that de novo lipogenesis may play an important role in the etiology of obesity, particularly during overconsumption of different carbohydrates.

Objective: We hypothesized that de novo lipogenesis would increase during overfeeding, would vary depending on the type of carbohydrate consumed, and would be greater in obese than in lean women.

Design: De novo lipogenesis was measured during 96 h of overfeeding by 50% with either sucrose or glucose and during an energy balance treatment (control) in 8 lean and 5 obese women. De novo lipogenesis was determined by measuring the amount of deuterium incorporation into plasma triacylglycerols. Fat and carbohydrate balance were measured simultaneously by continuous whole-body calorimetry.

Results: De novo lipogenesis did not differ significantly between lean and obese subjects, except with the control treatment, for which de novo lipogenesis was greater in the obese subjects. De novo lipogenesis was 2- to 3-fold higher after overfeeding by 50% than after the control treatment in all subjects. The type of carbohydrate overfeeding (sucrose or glucose) had no significant effect on de novo lipogenesis in either subject group. Estimated amounts of absolute VLDL production ranged from a minimum of 2 g/d (control) to a maximum of 10 g/d after overfeeding. This compares with a mean fat balance of ≈ 275 g after 96 h of overfeeding. Individual subjects showed characteristic amounts of de novo lipogenesis, suggesting constitutive (possibly genetic) differences.

Conclusion: De novo lipogenesis increases after overfeeding with glucose and sucrose to the same extent in lean and obese women but does not contribute greatly to total fat balance. *Am J Clin Nutr* 2001;74:737-46.

KEY WORDS De novo lipogenesis, deuterium, overfeeding, carbohydrate, glucose, sucrose, lean women, obese women, calorimetry

INTRODUCTION

Although excess dietary fat can be stored extensively in adipose tissue, the storage of excess dietary carbohydrate is more limited and the metabolism of dietary carbohydrate more complex (1). Only a small, fairly stable reserve of 200-500 g carbohydrate can be stored as glycogen in the human body (1, 2). The fate of dietary carbohydrate not stored as glycogen is to either be oxidized in response to immediate energy demands or be con-

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verted to fat by hepatic de novo lipogenesis (2, 3). Some evidence also suggests that de novo lipogenesis in adipose tissue may have a role in the disposal of dietary carbohydrate (4). De novo lipogenesis from carbohydrate is energetically expensive (5) and evidence to date suggests it does not contribute significantly to increased fat balance in persons consuming a typical high-fat Western diet (6). However, there are concerns that because highly refined carbohydrates constitute an increasing proportion of the diet, de novo lipogenesis may play a more significant role in the general increase of fat stores at a population level (6, 7).

In humans, de novo lipogenesis was shown to respond to several energy supply scenarios (3, 8). For example, de novo lipogenesis increases linearly with increased energy intakes of 25% and 50% above energy balance and decreases with underfeeding at the same levels of energy deficiency in lean subjects (8). Even in the absence of overfeeding, de novo lipogenesis changes in response to alterations in the macronutrient composition of the diet, specifically by increasing in response to an increase in the proportion of carbohydrate in the diet (8, 9). Additionally, some preliminary evidence suggests that not only the quantity but also the type of carbohydrate in the diet affects the rate of de novo lipogenesis. Fructose, in particular, has been identified as a monosaccharide with an especially potent effect on de novo lipogenesis (10, 11). Most previous studies have examined the effect of diet composition or energy levels on de novo lipogenesis in lean subjects; however, there is some evidence that de novo lipogenesis is greater in hyperinsulinemic obese subjects (11, 12). An experimental feature shared by many of these previous studies was that the response time to the dietary manipulation was short, generally ≤ 6 h duration (10, 11). It is feasible that over a

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TABLE 1

Protocol of $^2\text{H}_2\text{O}$ dosing, blood sampling, and mealtimes carried out for each treatment while the subjects resided continuously in a whole-body indirect calorimeter

Day	Time or volume
Day 3	
Time of $^2\text{H}_2\text{O}$ dose	0800, ¹ 1210, 1600, 2010, and 2400
Time of blood sample	0730, ² 1200, 1500, and 2000
Blood volume (mL)	25, 5, 25, and 5
Mealtimes	0930, 1100, 1400, 1630, and 1900
Day 4	
Time of $^2\text{H}_2\text{O}$ dose	0410, 0800, 1200, 1600, 2010, and 2400
Time of blood sample	0400, 0930, 1500, and 2000
Blood volume (mL)	5, 25, 5, and 25
Mealtimes	0830, 1130, 1400, 1630, and 1900
Day 5	
Time of $^2\text{H}_2\text{O}$ dose	0410
Time of blood sample	0400 and 0800
Blood volume (mL)	5 and 25
Mealtimes	—

¹Priming dose of $^2\text{H}_2\text{O}$; all other doses were additional doses given to maintain a plateau of enrichment.

²Background blood sample; all subsequent samples contained $^2\text{H}_2\text{O}$.

longer time period, during which a sustained energy imbalance or alteration in macronutrient composition is achieved, de novo lipogenesis might prove to be a more significant process.

The design of this study permitted us to measure fractional rates of hepatic de novo lipogenesis by using deuterated water ($^2\text{H}_2\text{O}$) incorporated into total plasma triacylglycerol (5, 13), while simultaneously measuring macronutrient intake, oxidation, and balance in a whole-body calorimeter during 4 d. The level of overfeeding used (50% of the energy intake provided by the control diet) was carefully chosen to ensure that the excess carbohydrate could be disposed of as 1) glycogen, 2) fat stored via hepatic de novo lipogenesis, 3) fat stored through sparing of fat oxidation, or 4) any combination of these. We addressed 3 main questions within the context of this study. 1) How does the rate of de novo lipogenesis respond to controlled overfeeding during 4 d, especially when most of the overfeeding is supplied as carbohydrate? 2) Will different sources of dietary carbohydrate (sucrose and glucose) elicit the same amount of de novo lipogenesis? 3) Is there an underlying metabolic difference in de novo lipogenesis between lean and obese (nonhyperinsulinemic) subjects that can identify individuals with a predisposition to the development of obesity?

SUBJECTS AND METHODS

Study design

Thirteen volunteers were recruited to take part in a dietary intervention and isotope tracer study at the MRC-Dunn Clinical Nutrition Centre. The volunteers were 8 lean and 5 obese non-smoking, nonvegetarian women who, apart from those who were overweight, were otherwise healthy. The body weight (in kg) and height (in m) of each subject were recorded before and after each calorimetry period. Percentage body fat (%BF) was measured after each treatment period by use of dual-energy X-ray absorptiometry (DXA; QDR1000; Hologic Inc, Waltham, MA). The physical characteristics ($\bar{x} \pm \text{SD}$) of the 2 subject groups were as follows. Body weight, body mass index (BMI; in kg/m^2), and

%BF were significantly greater ($P < 0.01$, Student's *t* test) in the obese subjects (body weight, 81.0 ± 4.5 kg; BMI, 31 ± 4.0 ; %BF, $44.8 \pm 4.6\%$) than in the lean subjects (body weight, 65.6 ± 6.0 kg; BMI, 25 ± 1.0 ; %BF, $35.1 \pm 5.6\%$). There was no significant difference in age (lean, 53.1 ± 0.3 y; obese, 52.4 ± 4.8 y) or height (lean, 1.63 ± 0.05 m; obese, 1.63 ± 0.07 m) between the 2 subject groups. Three of the lean subjects and 2 of the obese subjects were premenopausal. In these subjects, all measurements were made in the same phase of their menstrual cycles, as determined by taking each subject's case history. All of the remaining subjects were postmenopausal. All subjects underwent a medical examination before taking part in the study, and plasma triacylglycerol and cholesterol concentrations were screened to ensure that they fell within the normal range for sex and age. The study was approved by the MRC-Dunn Nutrition Centre Ethical Committee, and written, informed consent was obtained from each subject.

Protocol overview

Each subject was required to attend the research center on 3 occasions and to spend a total of 4.5 d (108 h) continuously in a whole-body indirect calorimeter. Subjects entered the calorimeter at 2000 the evening before the study began (day -1), when the calorimeters began to operate. However, to allow for adjustment effects, the first 12 h of calorimetry data were excluded from the study. That is, calorimetry data were used only during the period from 0800 on day 1 to 0800 on day 5, a total of 96 h. The subjects were allowed to acclimate to the experimental dietary regimen in the calorimeters for the first 48 h of each treatment period (days 1 and 2) before isotope dosing and blood sampling for de novo lipogenesis measurements began (days 3–5). The subjects left the calorimeter after the final blood sample was taken at 0800 on day 5 (Table 1).

While in the calorimeters, the subjects followed an identical protocol of sleep, rest, meals, and exercise. A detailed description of the calorimeters is given elsewhere (14); briefly, the calorimeters were comfortably furnished with a desk, armchair, bed, television, videocassette recorder, radio and compact disk player, wash basin, and portable toilet. The door of the calorimeters contained a 2-way hatch with a removable cover and an air-tight rubber sleeve to permit blood sampling. While in the calorimeter, all subjects adhered to an identical fixed activity regimen of light exercise on a cycle ergometer. One subject performed the same work but used stepping instead of cycling as her exercise routine. On each occasion the subjects consumed 1 of 3 diets, which were allocated in random order.

On the morning of day 3, an indwelling cannula was inserted into an arm vein. During the following 2 d, a total of 10 blood samples were drawn (Table 1). Different volumes were required for the blood samples because on certain occasions additional biochemical assays (triacylglycerol, glucose, leptin, and insulin) were carried out on the samples. The cannula was kept patent with a fresh solution of heparin-containing saline. After the first blood sample was drawn (background sample), the subjects began taking a regimen of regular doses of $^2\text{H}_2\text{O}$. Each dose was calculated on a weight-specific basis for each subject and was ≈ 100 mL, 98‰. The $^2\text{H}_2\text{O}$ doses were kept in sterile bottles at $5 \pm 2^\circ\text{C}$. The $^2\text{H}_2\text{O}$ dosing was designed to provide the subjects with an initial priming dose, followed by additional doses that ensured that $^2\text{H}_2\text{O}$ concentrations were maintained at an elevated plateau for the duration of the dietary treatment period. The priming dose provided 20 mmol/kg body wt and the additional doses provided 0.25 mmol/kg body wt 6 times/d (Table 1). During this latter period, blood samples were drawn regularly over



the next 48 h to obtain plasma samples to determine the incorporation of deuterium into triacylglycerol and thus measure de novo lipogenesis.

Dietary treatments

The treatments comprised 2 overfeeding treatments and a control treatment in which energy intake was calculated as basal metabolic rate (measured for each subject on a previous occasion in the calorimeter) multiplied by a fixed constant of 1.3, to maintain energy expenditure while in the calorimeter, and fed to the nearest 0.5 MJ/d. The control diet provided 48% of energy as carbohydrate, 40% of energy as fat, and 12% of energy as protein. The 2 overfeeding diets provided 50% more energy than did the control diet. There was no protein in the overfeeding portion of the diet and the proportion of carbohydrate to fat was kept the same as in the control diet (the amount of carbohydrate was 1.2 times that of fat). Thus, the 50% extra energy of the overfeeding diets consisted of 27.3% carbohydrate and 22.7% fat. The overall macronutrient composition of the overfeeding diets was 50% of energy as carbohydrate, 42% of energy as fat, and 8% of energy as protein. For example, a subject with a daily energy intake of 8.0 MJ was fed 240 g carbohydrate, 86 g fat, and 56 g protein daily. An additional 4.0 MJ energy was fed during an overfeeding treatment for the same subject. This 4.0 MJ comprised an extra 135.0 g sugar and 49.7 g fat daily, giving a total macronutrient intake of 375 g carbohydrate, 136 g fat, and 56 g protein daily. The overfeeding diets were designed to meet clinical concerns about chronic metabolic disturbances that might occur if >30% of daily energy intake is fed as a simple sugar (15).

The carbohydrate component of the 50% extra energy of the overfeeding diets consisted of either glucose (OFglu) or sucrose (OFsuc) administered as a lemon drink sweetened with the appropriate sugar. No complex carbohydrates were included in the carbohydrate overfeeding portion of the diet. The fat component of the overfeeding portion was simply incorporated into each meal as mixed fats such as oils or butter. All experimental diets were prepared in the metabolic kitchen at The MRC-Dunn Clinical Nutrition Centre. A washout period of ≥ 1 mo was allowed between each dietary treatment. During each treatment, subjects were fed 5 meals/d: breakfast, morning snack, lunch, evening snack, and dinner (Table 1). The meals were spaced out throughout the day as evenly as possible to minimize postprandial changes in plasma lipids and to attain a plateau. Aside from the number of meals given, the diets were designed to be as normal as possible with regard to appearance and palatability and were isoenergetic for a given energy intake. No alcohol was included in the diet.

Analysis of plasma samples for incorporation of deuterium into triacylglycerol

Blood samples were centrifuged immediately at $1500 \times g$ and 4°C for 10 min, and the plasma obtained was frozen at $-20 \pm 2^\circ\text{C}$. Lipids were extracted from four 0.5-mL aliquots of plasma per sample by mixing the sample with 5 mL chloroform:methanol (2:1). An additional 1.5 mL water was added, the samples were mixed again, and the phases were separated by centrifugation ($1500 \times g$, 4°C , 10 min). The aqueous layer was extracted again with 5 mL chloroform. The combined organic layers were evaporated to dryness under nitrogen and then dissolved in 1 mL hexane. Lipid classes were separated by elution on Isolute SI silica columns (Jones Chromatography, Hengoed, United Kingdom) and the triacylglycerol fraction was checked for integrity by thin-

layer chromatography. This was accomplished by spotting a 10- μL plasma sample onto a thin-layer chromatography silica plate (Camlab, Cambridge, United Kingdom) and running it in petroleum ether:diethyl ether:acetic acid (80:20:1). Triolein, cholesterol, cholesteryl oleate, and oleic acid standards (Sigma-Aldrich, Dorset, United Kingdom) were run on the same plate, and the samples were visualized in iodine vapor.

The remainder of the triacylglycerol fraction was transferred to a 15-cm heat-resistant glass combustion tube and evaporated to dryness under nitrogen. Then 500 mg fine copper wire (Elemental Microanalysis Ltd, Okehampton, United Kingdom) and 1 cm silver wire (precleaned in ammonium hydroxide) were added. The tube was evacuated on a vacuum manifold and sealed. Samples were combusted at 500°C in a muffle furnace for 2 h. After cooling, the tubes were cracked in a vacuum line and the combustion water was trapped at -78°C with use of a methylated spirit-dry ice bath. The water trap was then heated and the combustion water was vacuum distilled into a heat-resistant glass tube at -78°C containing 150 mg zinc shot (Biogeochemical Laboratories, Indiana University, Bloomington, IN). The tubes were then sealed under a vacuum before being heated for 45 min at 500°C to reduce the water to hydrogen gas. Hydrogen gas samples were prepared in triplicate from the triacylglycerol water samples and a laboratory standard calibrated to the international standard V-SMOV (International Atomic Energy Agency, Vienna), and their isotope ratios measured relative to tank hydrogen on a SIRA-10 dual-inlet gas isotope ratio mass spectrometer (Micromass, Manchester, United Kingdom). Enrichments relative to the reference water standards were then calculated and these values converted to fractional abundance.

Duplicate aliquots of urine samples (0.4 mL) and the laboratory reference were analyzed for deuterium enrichment at each time point after equilibration for 3 d with ≈ 4 mg 5% Pt on an alumina catalyst, 325 mesh (Sigma Aldrich), in septum-capped glass vials (Labco, High Wycombe, United Kingdom). Equilibrated hydrogen from the samples and the references were then introduced to the mass spectrometer through a Multi Prep system interfaced to a SIRA-10 gas isotope ratio mass spectrometer (Micromass).

Total triacylglycerol concentrations in plasma were measured on a Cobas Fara centrifugal analyzer (Roche Products Ltd, Welwyn Garden City, United Kingdom) after enzymatic hydrolysis with a triacylglycerol assay kit (Randox Laboratories Ltd, Crumlin, United Kingdom). Plasma glucose concentrations were also measured by Cobas with the appropriate assay kit. Insulin concentrations were analyzed with a commercial immunoassay kit. Leptin concentrations were measured by radioimmunoassay with a commercially available kit (Linco Research, St Charles, MO) with a detection limit of 0.5 mg/L. Leptin concentrations were measured in the final blood sample of each dietary treatment period (0800 on day 5). Each sample was assayed in duplicate on 2 separate occasions and the in-house intra- and interassay CVs were 3% and 5%, respectively.

Isotope kinetics

A development of the 2-compartment system previously used by Leitch and Jones (5) was used to model de novo lipogenesis. The modified system is illustrated in **Appendix A**. With use of the original model, and with a primed infusion of label into the body pool, it would be expected that the measured incorporation into plasma lipid should rise exponentially to a plateau and that the rate constant of the exponential portion of the curve should



TABLE 2

Rates of de novo lipogenesis after 96 h of dietary treatment in lean and obese women¹

Subject group	Dietary treatment		
	Control	OFglu	OFsuc
	<i>% hepatic VLDL production</i>		
Lean (<i>n</i> = 8)	8.70 ± 1.33 ^{a,2}	23.91 ± 5.04 ^b	26.56 ± 5.03 ^b
Obese (<i>n</i> = 5)	14.63 ± 1.73 ^a	26.68 ± 2.97 ^{a,b}	32.58 ± 3.91 ^b

¹ $\bar{x} \pm \text{SEM}$. Means within rows with different superscript letters are significantly different, $P \leq 0.05$ (general linear model procedure, Scheffe post hoc). There was also a significant interaction between diet and subject type, $P \leq 0.01$. OFglu, overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose; OFsuc, overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose.

²Significantly different from obese subjects, $P \leq 0.05$ (ANOVA).

reflect the rate of removal of VLDL from the plasma. Plasma VLDL has a half-life of ≈ 3 h (16), but in all cases in the present study the observed rate of appearance of label in plasma indicated kinetics much slower than this. For this reason, we interposed an extra compartment between the body water pool and the plasma. It is assumed that the rate-limiting step is the transfer of VLDL from the site of lipogenesis to the plasma, and this is the parameter reflected in the experimental curve.

The primed constant infusion method, if perfectly executed, puts a certain level of label into a pool and then maintains it at that value by an infusion at a rate exactly balancing the losses from that pool. If the method is imperfectly applied, ie, if the infusion rate does not quite match the losses, then the quantity of tracer in all the pools will be a function of time. For the pool into which the dose is put it is an easy matter to show that if the time of observation is short compared with the time constant for the losses, then the observed quantity of tracer will be a linear function of time. This linear function can then be used as an input function for tracer into the second pool and so on, leading to a final expression for the excess abundance of tracer in the plasma VLDL pool (F_3) as

$$F_3(t) \frac{Q_3'(t)}{Q_3(t)} = (\text{FSR}) \left\{ \left(\frac{\alpha}{Q_1} \right) t + \left[\left(\frac{q_1(0)}{Q_1} \right) - \left(\frac{\alpha}{Q_1} \right) \frac{1}{k_{32}} \right] [1 - e^{-k_{32}t}] \right\} \quad (1)$$

where $\left(\frac{\alpha}{Q_1} \right)$ and $\left(\frac{q_1(0)}{Q_1} \right)$ are the slope and intercept of the plot of the excess fractional abundance of isotope in the body water pool, respectively; k_{32} is a rate constant; and FSR is the fractional synthetic rate.

In equation 1, the factor $\left(\frac{Q_3'(t)}{Q_3(t)} \right)$ is inserted to allow for the presence of plasma triacylglycerol other than VLDL. The time course of total plasma triacylglycerol was measured during the period of sampling for the de novo lipogenesis measurements. A diurnal variation with minima corresponding to overnight fasting was observed in all cases, and the fasting VLDL concentrations, $Q_3(t)$, were interpolated over the full 48-h period by fitting a parabola between the 3 points of lowest plasma triacylglycerol concentrations. The values used for $Q_3'(t)$ were the discrete total triacylglycerol measurements made for each of the samples used in the isotopic determination.

In accordance with a previous study (5), the fractional abundance of the triacylglycerol is calculated by scaling the equivalent

values for the hydrogen generated therefrom by assuming that the typical synthesized triacylglycerol is tripalmitin with 98 hydrogen atoms, only 69 of which are available for incorporation (5, 17). Furthermore, isotope effects give a deuterium:hydrogen incorporation ratio of 0.702. The product of these 3 considerations gives a scaling factor of $98/69/0.702 = 2.02$.

After application of this factor, the experimental data were compared with the model by calculating the sum of the squared residuals for all data points. This sum was minimized by varying the model parameters k_{32} and FSR by using the built-in nonlinear optimization function in EXCEL (version 5.0; Microsoft Corporation, Redmond, WA).

Statistics

Fractional rates of de novo lipogenesis (%) were analyzed for the effect of dietary treatment and subject type (lean or obese) and for within-subject variability by the general linear model (GLM) procedure (DATA DESK 4.1; Data Description Inc, Ithaca, NY). The main statistical model therefore described de novo lipogenesis as a function of subject type (lean or obese), dietary treatment, and individual subject (also with subject nested within subject type). Where there were significant main effects and significant interactions between the main effects in the model, Scheffe's post hoc analysis was used to separate means. The same GLM procedure was used to test for the effects of diet, subject type, and their interaction on macronutrient and energy intakes, expenditure, and balance.

The effect of subject type, diet, and their interaction on plasma concentrations of glucose, insulin, leptin, and triacylglycerol were also analyzed by using the GLM procedure (Minitab 12; Minitab Inc, State College, PA). In addition, the relations between de novo lipogenesis and macronutrient intake, oxidation and balance, and body composition were analyzed by linear regression (Minitab 12). The relation between de novo lipogenesis and each of these biochemical variables was analyzed by linear regression with the biochemical variable, diet, and subject all included as predictor variables (Minitab 12). Because each subject was represented 3 times in these regression analyses (control and both overfeeding treatments), subject was also included as a predictor variable in the regression model.

RESULTS

Effect of dietary treatment on de novo lipogenesis

The rate of de novo lipogenesis was significantly affected by dietary treatment ($P \leq 0.001$), subject type ($P \leq 0.001$), and individual subject ($P \leq 0.001$). Because dietary treatment had a significant effect on de novo lipogenesis, Scheffe's post hoc analysis was used to separate the means (Table 2). The main reason for a significant effect of treatment on de novo lipogenesis was the response to overfeeding in both groups. In the lean subjects, de novo lipogenesis was significantly higher by a factor of >2.5 after overfeeding with either sucrose or glucose than after the control diet. In the obese subjects, overfeeding with sucrose resulted in a significant doubling of de novo lipogenesis. Although de novo lipogenesis was higher in response to the OFglu treatment than in response to the control diet in the obese subjects, this difference was not significant. There was no significant difference in the rate of de novo lipogenesis measured in response to the different sources of dietary carbohydrate in either group.

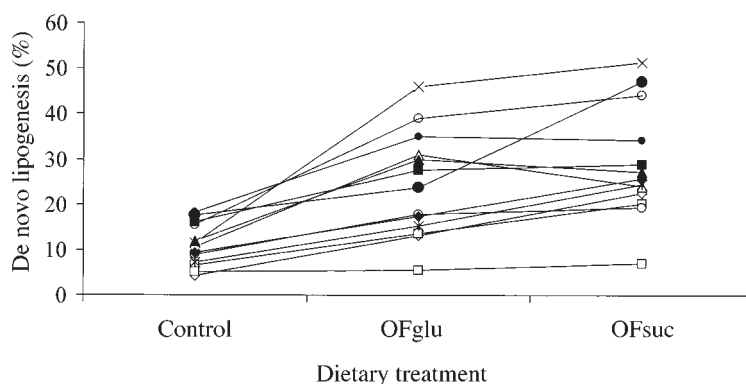


FIGURE 1. Individual de novo lipogenesis measured after 96 h of control or overfeeding. OFglu, overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose; OFsuc, overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose. Obese subjects ($n = 5$) are denoted by solid symbols and lean subjects ($n = 8$) by open symbols. De novo lipogenesis values for each subject for the 3 dietary treatments are linked by solid lines.

The effect of subject type on de novo lipogenesis was largely due to the difference in de novo lipogenesis seen in response to the control treatment. After the control treatment, de novo lipogenesis was $14.63 \pm 1.73\%$ in the obese and $8.70 \pm 1.33\%$ in the lean subjects ($\bar{x} \pm \text{SEM}$; Scheffe's post hoc analysis), a difference of 40% (Table 2). Although de novo lipogenesis in the obese subjects was also higher than that in the lean subjects with the OFglu or OFsuc treatments, by 19% and 11% respectively, these differences were not significant. There was a significant interaction between diet and subject type: de novo lipogenesis after overfeeding treatments in the obese subjects was significantly greater than de novo lipogenesis after the control treatment in the lean subjects. By contrast, however, de novo lipogenesis after overfeeding in the lean subjects was not significantly greater than de novo lipogenesis after the control treatment in the obese subjects.

Within-subject variability in fractional rates of de novo lipogenesis

In all subjects, de novo lipogenesis was higher after overfeeding than after the control treatment (Table 2) and there was a high level of within-subject consistency in this response. That is, with few exceptions, subjects with a high amount of de novo lipogenesis measured in response to the control diet also tended to have a high amount of de novo lipogenesis in response to the overfeeding treatments (Figure 1). In the same way, those subjects with a low amount of de novo lipogenesis after the control treatment had a low amount of de novo lipogenesis after both overfeeding treatments. There was a high level of correlation between the control and both the OFglu ($r = 0.676$, $P \leq 0.01$) and OFsuc ($r = 0.704$, $P \leq 0.01$) treatments for all subjects (Figure 1). In addition, there was a significant correlation within subjects between de novo lipogenesis measured in response to overfeeding with glucose and that measured in response to overfeeding with sucrose ($r = 0.762$, $P \leq 0.01$).

The rate of de novo lipogenesis increased significantly with increasing concentrations of plasma triacylglycerol ($P \leq 0.001$), although there was no significant effect of dietary treatment on triacylglycerol concentrations (Table 3). Another indication of the within-subject consistency of de novo lipogenesis is to include variables such as triacylglycerol concentration (which is positively correlated with de novo lipogenesis, $r = 0.503$) and

energy intake, expenditure, and balance, which might contribute to the significant subject effect, in the main model. When triacylglycerol concentration and energy expenditure were included in the analysis, neither was significantly related to de novo lipogenesis, but dietary treatment and subject were highly related to de novo lipogenesis ($P \leq 0.01$). The addition of energy intake or energy balance to the model resulted in only subject being significantly related to de novo lipogenesis ($P \leq 0.001$).

Concentrations of serum insulin, plasma glucose, and plasma leptin were measured after 96 h of each dietary treatment (Table 3). There was no significant linear relation between the rate of de novo lipogenesis and any of these biochemical indexes. None of these indexes entered as significant in the main model used to examine within-subject variability, although subject, subject type, and dietary treatment were significantly related to de novo lipogenesis in each case ($P \leq 0.001$).

TABLE 3

Plasma concentrations of triacylglycerol, insulin, glucose, and leptin after 96 h of dietary treatment in lean ($n = 8$) and obese ($n = 5$) women¹

	Dietary treatment		
	Control	OFglu	OFsuc
Triacylglycerol (mmol/L)			
Lean	1.71 ± 1.04	1.93 ± 1.08	1.62 ± 0.74
Obese	2.52 ± 1.04	3.34 ± 2.08	3.09 ± 1.44
Insulin (mmol/L)			
Lean	27.3 ± 12.6	60.7 ± 91.2	36.1 ± 17.1
Obese	41.4 ± 7.8	39.0 ± 23.4	58.4 ± 24.4
Glucose (mmol/L)			
Lean	5.91 ± 0.88	5.60 ± 0.34	6.37 ± 1.37
Obese	5.73 ± 0.55	6.71 ± 2.27	5.81 ± 0.57
Leptin (μg/L)			
Lean	21.0 ± 11.9	21.5 ± 16.4	22.4 ± 12.1
Obese	46.2 ± 23.2	53.2 ± 17.6	51.2 ± 17.9

¹ $\bar{x} \pm \text{SD}$. Data represent the mean concentrations recorded at 5 time points per treatment for each group of subjects. There was no significant effect of dietary treatment, subject type, or their interaction on any biochemical variable. OFglu, overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose; OFsuc, overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose.

TABLE 4

Fat and carbohydrate balance after 96 h of dietary treatment in lean ($n = 8$) and obese ($n = 5$) women¹

	Dietary treatment		
	Control	OFglu	OFsuc
Fat balance (g)			
Lean	-22 ± 75	248 ± 89	275 ± 58
Obese	-34 ± 80	303 ± 57	308 ± 50
All subjects	-27 ± 74 ^a	269 ± 80 ^b	288 ± 56 ^b
Carbohydrate balance (g)			
Lean	-43 ± 104	148 ± 83	107 ± 66
Obese	13 ± 107	89 ± 126	104 ± 128
All subjects	21 ± 104 ^a	125 ± 101 ^b	106 ± 89 ^b

¹ $\bar{x} \pm \text{SEM}$. Means within rows with different superscript letters are significantly different, $P \leq 0.05$ (general linear model procedure, Scheffe post hoc). There was no significant difference in fat or carbohydrate balance between lean and obese subjects in response to any dietary treatment (no significant interaction). OFglu, overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose; OFsuc, overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose.

Macronutrient and energy balances

Macronutrient and energy balances were measured over the 96 h of each dietary treatment (Table 4). These data were reported elsewhere in detail (14). In brief, there was no significant difference in macronutrient balance between subject groups. Dietary treatment had a significant effect on both fat ($P \leq 0.001$) and carbohydrate ($P \leq 0.01$) balance. These effects were entirely due to the difference in macronutrient balance between the control and the overfeeding treatments. There was no significant difference in either carbohydrate or fat balance between the OFglu and OFsuc treatments. Protein balance was not significantly affected by either dietary treatment or subject group.

Energy intake was significantly affected by dietary treatment and subject type, but there was no significant interaction between these 2 variables (Table 5). Energy expenditure was significantly affected by subject type, being higher in the obese than in the lean subjects for all dietary treatments, but was not significantly affected by dietary treatment. After 96 h of dietary treatment, energy balance was significantly affected by dietary treatment, although this was entirely due to the difference between the control diet and the 2 overfeeding diets. There was no significant difference in energy balance between the OFglu and the OFsuc treatments in either the lean or obese subjects.

We examined the relation between the amount of de novo lipogenesis and macronutrient intakes, oxidation, and balances during each dietary treatment by using linear regression analysis for all subjects pooled together. We also included the variable of subject in the regression model to allow for the fact that we measured de novo lipogenesis in each subject on 3 occasions, under 3 different dietary conditions. There was a significant positive relation between dietary fat intake and the rate of de novo lipogenesis in all subjects ($P \leq 0.001$), which explained 43% of the variation in de novo lipogenesis. There was no significant relation between de novo lipogenesis and fat oxidation in the pooled subjects, but there was a significant negative relation between de novo lipogenesis and fat oxidation in the obese subjects alone ($P \leq 0.01$). Both fat intake ($r = 0.655$) and fat balance ($r = 0.562$) were significantly positively correlated with de novo lipogenesis ($P \leq 0.001$). However, there was a significant negative correlation ($r = -0.337$, $P \leq 0.05$)

between fat oxidation and de novo lipogenesis when the data for the subjects were pooled. The significant positive relation between de novo lipogenesis and fat balance accounted for 34% of the variation in de novo lipogenesis (Figure 2).

As might be expected, there was a highly significant positive relation between carbohydrate intake and the amount of de novo lipogenesis measured in the pooled subjects ($r^2 = 43\%$, $P \leq 0.001$). The more carbohydrate supplied in the diet, the greater the response of de novo lipogenesis, regardless of subject type. In contrast with the relation between de novo lipogenesis and fat oxidation, the amount of de novo lipogenesis increased significantly with increasing carbohydrate oxidation ($P \leq 0.001$), with 41% of the variation in de novo lipogenesis due to variation in carbohydrate oxidation. De novo lipogenesis was significantly positively related to carbohydrate balance in the pooled analysis (Figure 2). There was also a significant ($P \leq 0.01$) positive correlation between de novo lipogenesis and carbohydrate intake ($r = 0.654$), oxidation ($r = 0.540$), and balance ($r = 0.481$). Those subjects fed the greatest amounts of dietary carbohydrate had the highest amount of carbohydrate oxidation, were in the highest positive carbohydrate imbalance, and had the highest amount of de novo lipogenesis. These same subjects also had the lowest amount of fat oxidation, indicating that carbohydrate oxidation was sparing fat oxidation and thus increasing fat balance in these subjects.

Body composition

We tested the relation between de novo lipogenesis and 3 gross indexes of body composition: body weight, BMI, and %BF. We used the pooled subject group to optimize the full spectrum of variation in body composition within the subject group. Although there was a significant positive relation between de novo lipoge-

TABLE 5

Cumulative energy intake, expenditure, and balance after 96 h of dietary treatment in lean ($n = 8$) and obese ($n = 5$) women¹

	Dietary treatment		
	Control	OFglu	OFsuc
Energy intake (kJ) ²			
Lean	30855 ± 1458	46165 ± 2251	46065 ± 2255
Obese	33280 ± 1788	49772 ± 2556	49772 ± 2556
Energy expenditure (kJ) ³			
Lean	32351 ± 2735	33762 ± 2616	33335 ± 2751
Obese	34616 ± 1609	36081 ± 1354	35670 ± 1478
Energy balance (kJ) ⁴			
Lean	-1496 ± 2635	12402 ± 2899	12839 ± 3110
Obese	-1343 ± 1453	13691 ± 1271	14101 ± 1887

¹ $\bar{x} \pm \text{SD}$. OFglu, overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose; OFsuc, overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose.

²Significantly affected by both dietary treatment and subject type ($P < 0.001$) but no significant interactive term [general linear model (GLM) procedure]. The Scheffe post hoc test showed that the control group was significantly different from both overfeeding groups, which were not significantly different from each other.

³Significantly affected by subject type ($P < 0.001$) but not by dietary treatment; no significant interactive term (GLM procedure).

⁴Significantly affected by dietary treatment ($P < 0.001$) but not by subject-type; no significant interactive term (GLM procedure). The Scheffe post hoc test showed that the control group was significantly different from both overfeeding groups, which were not significantly different from each other.

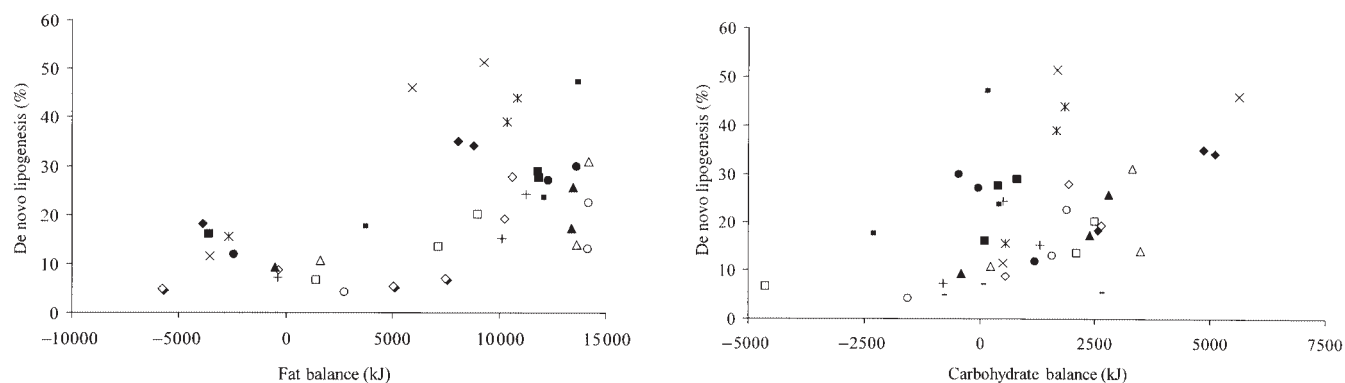


FIGURE 2. The relation between de novo lipogenesis and fat and carbohydrate balance in lean (○; $n = 8$) and obese (●; $n = 5$) subjects. Each subject is also individually represented by a unique symbol for each of the 3 dietary treatments: control, OFglu (overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose), and OFsuc (overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose). There was a significant positive relation between de novo lipogenesis and both fat ($r = 0.562$, $P \leq 0.001$) and carbohydrate ($r = 0.481$, $P \leq 0.001$) balance (linear regression).

nesis and body weight ($P \leq 0.05$), we found no significant relation between de novo lipogenesis and either BMI or %BF in the pooled sample. One measure of body composition with regard to fat content that was significantly related to de novo lipogenesis was fat balance, as discussed above. This relation remained significant when total energy intake was included in the analysis. Fat balance, as determined by gaseous exchange, is a precise means of measuring subtle changes in fat stores in response to dietary manipulations, and we previously showed that the precision estimates for fat storage are ≈ 200 kJ or 5 g (14). Thus, a precise means of detecting small, rapid changes in fat balance was related to de novo lipogenesis, whereas gross measurements of body composition were not sensitive enough to detect any relation with relatively short-term changes in de novo lipogenesis.

Estimated absolute fat production from dietary carbohydrate

There are several ways to estimate rates of actual fat production by using the values for de novo lipogenesis generated in the present study combined with estimates of VLDL production rates (Table 6). One can make the assumption either that there is a constant VLDL pool of ≈ 30 g/d [method 1; (8)] or that the daily VLDL production rate is 300 mg/kg body wt [method 2; (13)]. The amount of dietary carbohydrate provided in the control diet ranged from 230 ± 12 to 250 ± 13 g/d, depending on subject type. The estimated amount of fat produced by de novo lipogenesis in response to this diet ranged from a minimum of 0.7 to a maximum of 4.5 g/d. The amount of dietary carbohydrate supplied to the subjects during the overfeeding treatments varied from 360 ± 17 to 389 ± 21 g/d, depending on whether the subjects were lean or obese. Estimated absolute fat production in response to the overfeeding was similar regardless of which method was used and averaged 3.7 ± 0.5 g/d when VLDL production was estimated by method 1 and 6.2 ± 1.2 g/d when estimated by method 2.

DISCUSSION

De novo lipogenesis is the primary route by which dietary carbohydrate is converted into fat in the liver. In reality, however, it has been suggested that hepatic de novo lipogenesis is a rela-

tively unimportant metabolic pathway in humans consuming a standard Western diet but that it may become more significant under conditions of excess dietary intake, especially of carbohydrate (6, 7). In the present study, we showed that de novo lipogenesis was significantly higher by 2–3 times after overfeeding with predominantly carbohydrate than after the control treatment, to a maximum amount of 36% (Table 2). Previously (8), de novo lipogenesis of $\approx 10\%$ and 20% were recorded in lean humans ($n = 6$) in response to 25% and 50% overfeeding, respectively, with complex carbohydrate, which represented a 6–10-fold increase over the control treatment in that study. The same study also showed that de novo lipogenesis decreased to unmeasurably low amounts in response to underfeeding of predominately carbohydrate by 25% and 50%. In addition, overfeeding by 50% with fat resulted in de novo lipogenesis amounts that were not significantly different from those with a control euenergetic treatment (8). Thus, an excess amount of carbohydrate stimulates de novo

TABLE 6

Daily carbohydrate intake and estimated amounts of absolute fat production from rates of de novo lipogenesis (Table 2) after 96 h of dietary treatment in lean ($n = 8$) and obese ($n = 5$) women¹

	Dietary treatment		
	Control	OFglu	OFsuc
Carbohydrate intake (g/d) ²			
Lean	231 ± 12	360 ± 17	360 ± 17
Obese	250 ± 13	389 ± 21	389 ± 21
VLDL (g/d) ³			
Lean	1.17 ± 0.51	3.23 ± 1.93	3.59 ± 1.92
Obese	1.98 ± 0.52	3.60 ± 0.90	4.40 ± 1.18
VLDL (g/d) ⁴			
Lean	1.79 ± 0.84	4.97 ± 3.23	5.51 ± 3.29
Obese	3.55 ± 0.99	6.49 ± 1.77	7.84 ± 1.92

¹ $\bar{x} \pm$ SEM. OFglu, overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose; OFsuc, overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose.

²Actual intake.

³Estimated VLDL production rate of 30 g/d (8).

⁴Estimated VLDL production rate of 300 mg · kg body wt⁻¹ · d⁻¹ (13).

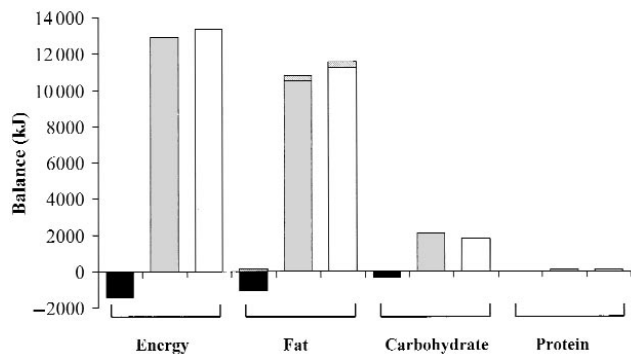


FIGURE 3. Energy, fat, carbohydrate, and protein balance after 96 h of control (■), OFglu (□; overfeeding in which the carbohydrate component of the 50% extra energy consisted of glucose), and OFsuc (▨; overfeeding in which the carbohydrate component of the 50% extra energy consisted of sucrose) dietary treatments. Data are pooled for both lean and obese subjects ($n = 13$). The hatched areas of the fat balance bars represent the contribution of de novo lipogenesis to overall fat balance.

lipogenesis significantly more than does an isoenergetic quantity of fat. Studies that failed to measure any response of de novo lipogenesis to overfeeding, even with the use of amounts of carbohydrate that exceed normal physiologic amounts, tended to have used indirect calorimetry. Thus, these negative findings may be a reflection of methodologic constraints as opposed to the absence of any de novo lipogenesis (2, 3, 18).

When measured directly (5, 19, 20), de novo lipogenesis is strongly influenced by the macronutrient composition of the diet, even in the absence of excess energy, and can increase dramatically under certain dietary conditions. De novo lipogenesis increased 5-fold in lean subjects fed a low-fat, high-carbohydrate diet (70% carbohydrate, 10% fat) compared with a typical Western diet containing 45% carbohydrate and 40% fat (20).

There is also some evidence that the type of dietary carbohydrates consumed affects de novo lipogenesis differently. Fructose, in particular, has been identified as having a more powerful effect on de novo lipogenesis than glucose in both laboratory rats (21) and humans (10, 11), albeit in the short-term (6 h). This has been attributed to fructose's rapid utilization by the liver, which avoids the rate-limiting 6- or 1-phosphofruktokinase step in glycolysis that limits glucose metabolism (22). Schwarz et al (10, 12) showed that short-term feeding of fructose increased de novo lipogenesis in humans by a factor of between 3 and 10 compared with an isoenergetic load of glucose, which failed to significantly increase de novo lipogenesis at all. These authors postulated that fructose and sucrose would have the same response in de novo lipogenesis, because fructose is one of the monosaccharides of the disaccharide sucrose, but that glucose and starch would have the same nonstimulatory effect. In contrast, the results of the present study show that overfeeding with mainly glucose or sucrose for 96 h increased de novo lipogenesis to the same extent as did the control condition, which suggests that in the longer term the fructose component of sucrose ceases to have a strong lipogenic effect. Indeed, preliminary data from our laboratory also show that de novo lipogenesis in subjects (2 lean and 1 obese women) overfed mainly with fructose averaged 23%, which is not significantly different from de novo lipogenesis measured in response to glucose or sucrose (Table 2). In addition, we showed

that there is no difference in overall fat balance, as measured by continuous indirect calorimetry for 96 h, in subjects overfed predominantly with either sucrose, fructose, glucose, or fat (14).


One of the aims of the present study was to evaluate the difference in de novo lipogenesis between lean and obese women under both control and overfeeding conditions. We found that de novo lipogenesis was significantly higher in the obese subjects than in the lean ones when subjects were fed to energy balance. However, this difference disappeared during conditions of overfeeding (Table 2). The same trend of obese subjects having higher rates of de novo lipogenesis than do their lean counterparts while in energy balance (12, 18) but of no difference between subjects during conditions of overfeeding by as much as 50% was previously reported (8). We found a high degree of within-subject consistency in the measurements of de novo lipogenesis from the present study (Figure 1). That is, subjects with a high amount of de novo lipogenesis with the control treatment had a high amount of de novo lipogenesis with the overfeeding treatments, and the opposite was also true. These data provide some evidence that some subjects may have an intrinsically higher, perhaps genetic, potential for de novo lipogenesis under certain dietary circumstances. De novo lipogenesis was significantly positively related to body weight, but there was no relation between de novo lipogenesis and 2 gross indexes of body composition, BMI and %BF, measured in the present study. However, previously, a significant negative correlation between fat-free mass and de novo lipogenesis was reported in patients with a wasting disease (23).

A more sensitive measure of change in body composition than either BMI or %BF is macronutrient balance (fat and carbohydrate) as measured by gaseous exchange with the use of indirect calorimetry. We measured precise differences in fat balance of between -13.6 and 187 g/d in our subjects, depending on dietary treatment, with a precision of ≈ 5 g fat (200 kJ) over 4 d and found a significant positive relation between fat balance and de novo lipogenesis. Previously, Hellerstein (6) also suggested that measurements of de novo lipogenesis would be an accurate tool for assessing macronutrient intake because de novo lipogenesis in their study was highly correlated with recent carbohydrate intake. In the present study, there was a significant positive correlation between de novo lipogenesis and carbohydrate intake, oxidation, and balance, although the relation between de novo lipogenesis and carbohydrate balance was weak ($r^2 = 21\%$).

Both whole-body fat and carbohydrate balance increased significantly after overfeeding in the present study but did not differ significantly between overfeeding treatments. Additionally, there was no significant difference in either macronutrient balance between lean and obese subjects (14). During overfeeding, subjects were given an average of 1398 g carbohydrate and oxidized ≈ 1280 g, which resulted in a mean carbohydrate balance of ≈ 115 g (Table 4). Concomitant measures of hepatic de novo lipogenesis showed that the mean de novo lipogenesis was 27% (averaged for both overfeeding treatments and for pooled subject data). The estimated amount of absolute fat production via de novo lipogenesis that this represents is only ≈ 4 g; however, this may well be an underestimation because these estimates are dependent on an index that was not measured, namely, VLDL-triacylglycerol production. Nonetheless, even when VLDL-triacylglycerol production was measured from kinetic modeling, absolute fat production is similarly low (8). Dietary fat intake during this period was 512 g, of which ≈ 233 g was oxidized, leaving all subjects with an average positive fat bal-

ance of 278 g (Table 4), of which the de novo lipogenesis contribution was clearly a tiny proportion.

The energy, fat, carbohydrate, and protein balances of the pooled subject set are summarized in **Figure 3**. As is clearly shown, the de novo lipogenesis proportion (hatched portion of bar chart) of the overall fat balance was very small. This agrees with previous studies in which hepatic de novo lipogenesis was found not to be a significant contributor to overall fat balance, with estimated fat production ranging from 1–2 g/d in lean men in energy balance (13, 18) to <5 g/d during overfeeding with carbohydrate in lean subjects of both sexes (24) or 7 g/d in exsmokers consuming an ad libitum diet (25). Adipose tissue de novo lipogenesis in lean and obese subjects was shown to be as low as hepatic de novo lipogenesis (2–5 g/d) in free-living conditions in which subjects consumed their usual (in terms of energy and macronutrient composition) diet (4). Regardless of which dietary manipulations have been considered to date—excess energy or excess carbohydrate in lean or obese subjects or in men or women—the amount of de novo lipogenesis measured still represents a minor fraction of the disposal of dietary carbohydrate.

The design of the present study allowed us to address 3 main questions about the process of de novo lipogenesis in humans. Fractional de novo lipogenesis more than doubled in response to overfeeding by 50% in energy terms in lean and obese women but did not differ significantly when the source of the excess dietary carbohydrate was either glucose or sucrose. Although de novo lipogenesis was greater in the obese subjects than in the lean ones while the subjects were in energy balance, there was no significant difference between subject groups after overfeeding. However, de novo lipogenesis was highly positively correlated with both body weight and with precise measurements of whole-body fat balance. Quantitatively, however, absolute amounts of fat synthesized from carbohydrate did not represent a significant contribution to overall fat stores after 96 h of excess carbohydrate intake. 

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REFERENCES

- Flatt JP. Use and storage of carbohydrate and fat. *Am J Clin Nutr* 1995;61(suppl):952S–9S.
- Acheson KJ, Flatt JP, Jequier E. Glycogen synthesis versus lipogenesis after a 500 gram carbohydrate meal in man. *Metabolism* 1982;31: 1234–40.
- Acheson KJ, Schultz Y, Bessard T, Anantharaman K, Flatt JP, Jequier E. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. *Am J Clin Nutr* 1988;48:240–7.
- Guo ZK, Cella LK, Baum C, Ravussin E, Schoeller DA. De novo lipogenesis in adipose tissue of lean and obese women: application of deuterated water and isotope ratio mass spectrometry. *Int J Obes* 2000; 24:932–7.
- Leitch CA, Jones PJH. Measurement of triglyceride synthesis in humans using deuterium oxide and isotope ratio mass spectrometry. *Biol Mass Spectrom* 1991;20:392–6.
- Hellerstein MK. Synthesis of fat in response to alterations in diet: insights from new stable isotope methodologies. *Lipids* 1996;31: S117–25.
- Parks EJ, Hellerstein MK. Carbohydrate-induced hypertriglycerolemia: historical perspective and review of biological mechanisms. *Am J Clin Nutr* 2000;71:412–33.
- Schwarz JM, Neese RA, Turner SM, Dare D, Hellerstein MK. Short-term alterations in carbohydrate energy intake in humans—striking effects on hepatic glucose production, de novo lipogenesis, lipolysis and whole-body fuel selection. *J Clin Invest* 1995;96: 2735–43.
- Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J, Hirsch J. Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. *J Clin Invest* 1996;97: 2081–91.
- Schwarz JM, Neese RA, Basinger A, Hellerstein MK. Effect of oral fructose on lipolysis, fat oxidation, fractional and absolute de novo lipogenesis (DNL) using mass isotope distribution analysis (MIDA). *FASEB J* 1993;7:867 (abstr).
- Schwarz JM, Neese RA, Turner SM, Nguyen C, Hellerstein MK. Effect of fructose ingestion on glucose production (GP) and de novo lipogenesis (DNL) in normal and hyperinsulinemic obese humans. *Diabetes* 1994;43:52 (abstr).
- Schwarz JM, Neese RA, Schackleton C, Hellerstein MK. De novo lipogenesis during fasting and oral fructose in lean and obese hyperinsulinemic subjects. *Diabetes* 1993;42(suppl):A39 (abstr).
- Leitch CA, Jones PJH. Measurement of human lipogenesis using deuterium incorporation. *J Lipid Res* 1993;34:157–63.
- McDevitt RM, Poppitt SD, Murgatroyd PR, Prentice AM. Macronutrient disposal during controlled overfeeding with glucose, fructose, sucrose, or fat in lean and obese women. *Am J Clin Nutr* 2000;72: 369–77.
- Committee on Medical Aspects of Food Policy. Department of Health report on health and social subjects 37. Dietary sugars and human disease. London: Her Majesty's Stationery Office, 1989.
- Beltz WF, Kesaniemi YA, Howard BY, Grundy SM. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma very low density lipoproteins, intermediate density lipoproteins, and low density lipoproteins. *J Clin Invest* 1985;76: 575–85.
- Jungas RL. Fatty acid synthesis in adipose tissue incubated in tritiated water. *Biochemistry* 1968;7:3708–17.
- Acheson KJ, Schultz Y, Bessard K, Ravussin E, Jequier E, Flatt JP. Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. *Am J Physiol* 1984;246:E62–70.
- Hellerstein MK, Christiansen M, Keampfer S, et al. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J Clin Invest* 1991;87:1841–52.
- Hudgins LC, Seidman CE, Diakun J, Hirsch J. Human fatty acid synthesis is reduced after the substitution of dietary starch for sugar. *Am J Clin Nutr* 1998;67:631–9.
- Park O-J, Cesar D, Faix D, Wu K, Schackleton HL. Mechanisms of fructose-induced hypertriglyceridaemia in the rat; activation of hepatic pyruvate dehydrogenase through inhibition of pyruvate dehydrogenase kinase. *Biochem J* 1992;282:753–7.
- Mayes PA. Intermediary metabolism of fructose. *Am J Clin Nutr* 1993;58(suppl):754S–65S.
- Hoh R, Pelfini A, Neese RA, et al. De novo lipogenesis predicts short-term body-composition response by bioelectrical impedance analysis to oral nutritional supplements in HIV-associated wasting. *Am J Clin Nutr* 1998;68:154–63.
- Schwarz JM, Neese RA, Dare D, Turner ST, Hellerstein MK. Effect of carbohydrate overfeeding and underfeeding on de novo lipogenesis and insulin levels. *Proc Nutr Soc* 1995;54:47A (abstr).
- Neese RA, Benowitz NL, Hoh R, et al. Metabolic interactions between surplus dietary energy-intake and cigarette-smoking or its cessation. *Am J Physiol* 1994;30:E1023–34.

APPENDIX A

The differential equations describing the flow of labeled material through the compartments of the model illustrated in Figure A1 are

$$\left. \begin{aligned} \frac{dq_1}{dt} &= f_{10} - (k_{01} + k_{21})q_1 \\ \frac{dq_2}{dt} &= k_{21}q_1 - k_{32}q_2 \\ \frac{dq_3}{dt} &= k_{32}q_2 - k_{03}q_3 \end{aligned} \right\} \quad (A1)$$

where q_i is the amount of tracer in the i th compartment, f_{10} is the constant rate of infusion of tracer into compartment 1, and k_{ji} is the fractional rate constant of transfer of label from compartment i to compartment j .

These equations can be solved in turn. For the first, direct integration gives

$$[f_{10} - (k_{01} + k_{21})q_a(t)] = [f_{10} - (k_{01} + k_{21})q_a(0)]e^{-(k_{01} + k_{21})t} \quad (A2)$$

Because water turnover is slow, the exponential can be approximated

$$e^{-(k_{01} + k_{21})t} \approx 1 - (k_{01} + k_{21})t$$

and thus

$$q_1(t) \approx q_1(0) + [f_{10} - (k_{01} + k_{21})q_1(0)]t \quad (A3)$$

which is the linear function found when the primed infusion technique is used but the infusion rate is imperfectly matched to the priming dose.

For the second compartment, we can substitute the approximation for q_1 and solve by using an integrating factor $e^{k_{32}t}$. Proceeding in this way and introducing the boundary condition $q_2(0) = 0$ gives

$$q_2(t) = \alpha \frac{k_{21}}{k_{32}} t + \frac{k_{21}}{k_{32}} \left[q_1(0) - \frac{\alpha}{k_{32}} \right] [1 - e^{-k_{32}t}] \quad (A4)$$

where $a = [f_{10} - (k_{01} + k_{21})q_1(0)]$. The final equation is solved in the same way.

Equation A5 is used to substitute for q_2 ; making use of the integrating factor $e^{k_{03}t}$ and the boundary condition $q_3(0) = 0$ gives

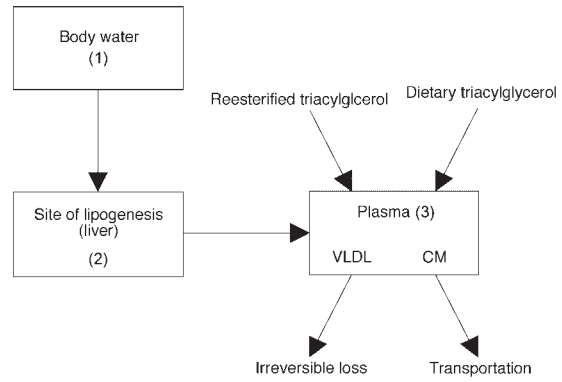


FIGURE A1. Compartmental model of lipogenesis. CM, chylomicron.

$$q_3(t) = \frac{k_{21}}{k_{03}} \left\{ \alpha + \left[q_1(0) - \alpha \left(\frac{1}{k_{03}} + \frac{1}{k_{32}} \right) \right] [1 - e^{-k_{03}t}] + \frac{k_{03}(k_{32}q_1(0) - \alpha)}{k_{32}(k_{32} - k_{03})} [e^{-k_{03}t} - e^{-k_{32}t}] \right\}$$

In the particular instance where $k_{03} \gg k_{32}$ this reduces to

$$q_3(t) \approx \frac{k_{21}}{k_{03}} \left\{ \alpha + \left[q_1(0) - \frac{\alpha}{k_{32}} \right] [1 - e^{-k_{32}t}] \right\} \quad (A5)$$

The trace kinetics give (using the conventional nomenclature of Q_i to denote amount of tracer) an expression for the rate of de novo lipogenesis as $k_{21}Q_1$, and a rate of total VLDL appearance, which is equal to the rate of VLDL irreversible loss as $k_{03}Q_3$. From this, the fractional synthetic rate (FSR) is given by

$$(\text{FSR}) = \frac{k_{21}Q_1}{k_{03}Q_3} \quad (A6)$$

and, therefore, the excess fractional abundance of tracer in the plasma VLDL pool is given by

$$\frac{q_3}{Q_3} = (\text{FSR}) \left\{ \left(\frac{\alpha}{Q_1} \right) t + \left[\left(\frac{q_1(0)}{Q_1} \right) - \left(\frac{\alpha}{Q_1} \right) \frac{1}{k_{32}} \right] [1 - e^{-k_{32}t}] \right\} \quad (A7)$$

where $\left(\frac{\alpha}{Q_1} \right)$ and $\left(\frac{q_1(0)}{Q_1} \right)$ are the slope and intercept of the plot of the excess fractional abundance of isotope in the plasma pool.

