Synthesis of Fat in Response to Alterations in Diet: Insights from New Stable Isotope Methodologies

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ABSTRACT: Synthesis of fatty acids, or *de novo* lipogenesis (DNL), is an intensively researched metabolic pathway whose functional significance and metabolic role have nevertheless remained uncertain. Methodologic problems that limited previous investigations of DNL *in vivo* and recent methodologic advances that address these problems are discussed here. In particular, deuterated water incorporation and mass isotopomer distribution analysis techniques are described. Recent experimental results in humans based on these techniques are reviewed, emphasizing dietary and hormonal factors that modulate DNL and quantitative significance of DNL under various conditions, including carbohydrate overfeeding. The somewhat surprising finding that DNL appears not to be a quantitatively major pathway even under conditions of surplus carbohydrate energy intake, at least in normal adults on typical Western diets, is discussed in depth. Nutritional and metabolic implications of these results are also noted, and some speculations on possible functional roles of DNL in normal physiology and disease states are presented in this context. In summary, methodologic advances have added to our understanding of DNL and its regulation, but many questions concerning quantitation and function remain unanswered.

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The enzymatic machinery for endogenous synthesis of fatty acids (FA) from acetate subunits is present in microbes, plants, and animals. Many biochemists have devoted their careers to studying genes and proteins involved in this pathway, also termed *de novo* lipogenesis (DNL). Nevertheless, a central functional question has remained unanswered: How much DNL actually occurs in organisms that can also ingest exogenous fats? A widely held concept is that DNL serves as a disposal route for surplus nonfat calories, especially carbohydrates (CHO) and ethanol, into the storage pool of body fat. This model of DNL as an overflow valve within the cellular or organismal energy economy has been difficult to evaluate because reliable techniques for measuring true rates and metabolic sources of DNL in living organisms until recently had not been available. Over the past three or four years, however, two methods have been developed that allow direct and accurate measurement of DNL in humans: mass isotopomer distribution analysis (MIDA) and deuterated water incorporation. A somewhat surprising experimental result has emerged, based on these methods—namely, that DNL is not a quantitatively important pathway under most circumstances in humans. These findings make the functions of DNL less certain, but potentially more interesting.

Here I review recent advances related to measurement and physiologic roles of DNL. First, I discuss the central methodologic problem that had to be resolved for measurement of DNL with isotopes (labeling of the intracellular true precursor pool) and how MIDA and ^2H_2O incorporation resolved this problem. Then I describe recent investigations using MIDA to measure DNL in humans under a variety of conditions, and I conclude with a discussion of the possible functions of DNL.

Overview of DNL as a pathway. Several features of DNL must be noted when considering the synthesis of FA as an integrated pathway rather than as a series of component enzymes. The first point is the thermogenic cost. Conversion of CHO to FA prior to oxidation dissipates an estimated 28% of the energy available to the organism if the carbohydrates were oxidized directly (1). This high thermogenic cost has variously been considered a reason for the organism to avoid DNL (energy wasteful) or a useful mechanism of thermogenesis (e.g., in brown fat, or to prevent weight gain during CHO overfeeding). The second point is that true DNL implies synthesis from 2-carbon units and should be distinguished from elongation of preformed FA. Though both DNL and elongation involve addition of activated acetate units in the form of malonyl-CoA, the former process produces nonessential FA, by definition, while the latter interacts with essential FA as well. The third aspect to consider is the current enzymologic model of the regulation of DNL. The malonyl-CoA/acetyl-CoA carboxylase (ACC) regulation model of DNL (2–7) is one of the most powerful and unifying of contemporary meta-

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Abbreviations: ACC, acetyl-CoA carboxylase; AIDS, acquired immune deficiency syndrome; CHO, carbohydrates; CS, cigarette smoking; DNL, *de novo* lipogenesis; FA, fatty acids; HGP, hepatic glucose production; MIDA; mass isotopomer distribution analysis; NIDDM, noninsulin-dependent diabetes mellitus; RQ, respiratory quotient; TG, triacylglycerols; VLDL, very low density lipoprotein.

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bolic hypotheses. The model is that malonyl-CoA concentrations integrate FA oxidation and synthesis (2,3) and prevent concurrent activity of these opposing pathways. Furthermore, malonyl-CoA concentrations are in turn sensitive to CHO-derived metabolites in the cell (oxaloacetate, pyruvate, citrate) and to the activity of the hormonally controlled enzyme ACC (4–7). In this biochemical context, the fourth point is the presumed function of DNL in the cellular and organismal energy economy. Based on its obvious function in plants and single-cell organisms as a route for storage as lipids of excess non-lipid-derived energy, the same function for DNL has been presumed for omnivorous mammals such as humans. Enzymology and molecular genetics of regulation have supported this view in that factors relating to CHO availability (substrates and hormones) regulate activity and synthesis of the enzymes of DNL (8–10). As we see later in the paper, however, quantitative investigations have not supported this as the usual physiologic function of DNL, at least in adults with typical Western diets. Finally, tissue sites of DNL should be noted. Species differences exist regarding relative importance of liver vs. adipose tissue as the major site of DNL. In rodents, adipose DNL may be at least as important as hepatic DNL (11), whereas for humans the general consensus is that liver is the predominant site (12,13). This formulation may not always be correct for humans, however. In addition, other specialized tissues may also perform DNL under certain circumstances, e.g., lactating mammary gland (14).

Measurement of DNL: historical problems. It may seem peculiar that some of the questions just mentioned remain unanswered. Why don't we know more definitively the quantitative importance of DNL, or the tissue site of DNL in humans, or the relation between molecular genetic and physiologic events? The main reason for persistent uncertainty in this field is methodologic: Reliable techniques for measuring true rates and metabolic sources of DNL in living organisms, including humans, had not been available until recently. It is therefore worth discussing in some detail methods used for measuring DNL.

Indirect calorimetry. Indirect calorimetry, or measurement of gas exchange, has been used for many years as an index of DNL (1,15). When nonprotein respiratory quotient (RQ) is >1.0, net DNL is believed to be occurring in the organism or tissue being studied, since the RQ of DNL is well above 1.0, whereas the RQ of CHO, protein, and FA oxidation are 1.0, ~0.83, and 0.70, respectively (1,16). The problems are that this is a nonmechanistic "black-box" measurement based on many assumptions (16) and, more importantly, that it only can measure net DNL rather than the true unidirectional synthesis of FA. If fat is being both oxidized and synthesized from CHO in the organism concurrently, the net RQ becomes 1.0, and indirect calorimetry reports only that CHO oxidation is occurring. Thus, indirect calorimetry cannot exclude DNL/FA oxidation substrate cycles, which may be important in metabolic regulation (17), and indirect calorimetry cannot quantify true flux rates through DNL. Nevertheless, indirect calorimetric measurements have generally indicated that *net* DNL is unusual in humans, even when fed surplus CHO energy in a single meal or for several days (18–22). Unfortunately, these results could not definitively establish rates of DNL.

Isotopic methods. Dynamic biochemical processes, such as DNL, are most appropriately measured by use of isotopes. Administration of labeled precursors allows newly synthesized molecules to be identified over an experimentally defined time period and permits the relationships among regulatory factors, specific metabolic precursors, and chemical fluxes to be quantified. Measurement of any metabolic process that involves polymerization biosynthesis (combination of repeating monomeric subunits into a polymer) has a fundamental requirement, however (23,24). It is necessary not only to measure how much of the labeled substrate is converted to the polymer of interest, but also to measure the degree of labeling of the true intracellular precursor (the monomeric pool from which the polymer is synthesized). For DNL, the biosynthetic precursor is cytosolic acetyl-CoA (Fig. 1A). How to gain access to the labeling (specific activity or enrichment) of the lipogenic acetyl-CoA pool has presented a long-standing problem for measurement of DNL. The acetyl-CoA pool is particularly complex, in that there are numerous potential metabolic sources of acetyl-CoA whose input varies in a regulated manner according to metabolic conditions. Moreover, cellular acetyl-CoA pools used for different metabolic pathways are compartmentalized and inhomogeneous. For example, acetyl-CoA for hepatic ketogenesis exhibits different labeling than acetyl-CoA for tricarboxylic acid cycle, lipogenesis, and cholesterogenesis in liver (25). It is therefore not possible to infer how much of a labeled substrate has actually entered the lipogenic acetyl-CoA pool relative to unlabeled substrates, or to use surrogate measures of acetyl-CoA (e.g., ketone bodies) to represent the true precursor pool for DNL (26).

The older literature attempting to measure lipid synthesis from ^{14}C-labeled acetate in fact represents an excellent example of the general methodologic principle concerning the importance of measuring precursor pool-specific activity. Several early investigators concluded that insulin plus glucose inhibits cholesterogenesis by hepatocytes, based on lower incorporation of ^{14}C-acetate into cholesterol. It is now clear that these conclusions were entirely wrong because dilution of acetyl-CoA by unlabeled glucose (from activation of glycolysis and pyruvate dehydrogenase activity by insulin) was not accounted for. Cholesterogenesis was in fact stimulated, although total label incorporation was reduced, as would have been apparent if the precursor specific activity had been measured.

An experimental solution to the problem of the lipogenic precursor pool: labeled water incorporation. Awareness of the precursor pool problem 30 years ago led to the development of the $^{3}H_2O$ incorporation technique for measuring DNL and cholesterogenesis (27). This approach solves the problem of the precursor pool by bypassing it. Instead of labeling lipids with a traditional labeled metabolite, a labeled cofactor (H_2O that exchanges with NADPH) is used. Because water mixes freely across almost all biological membranes,

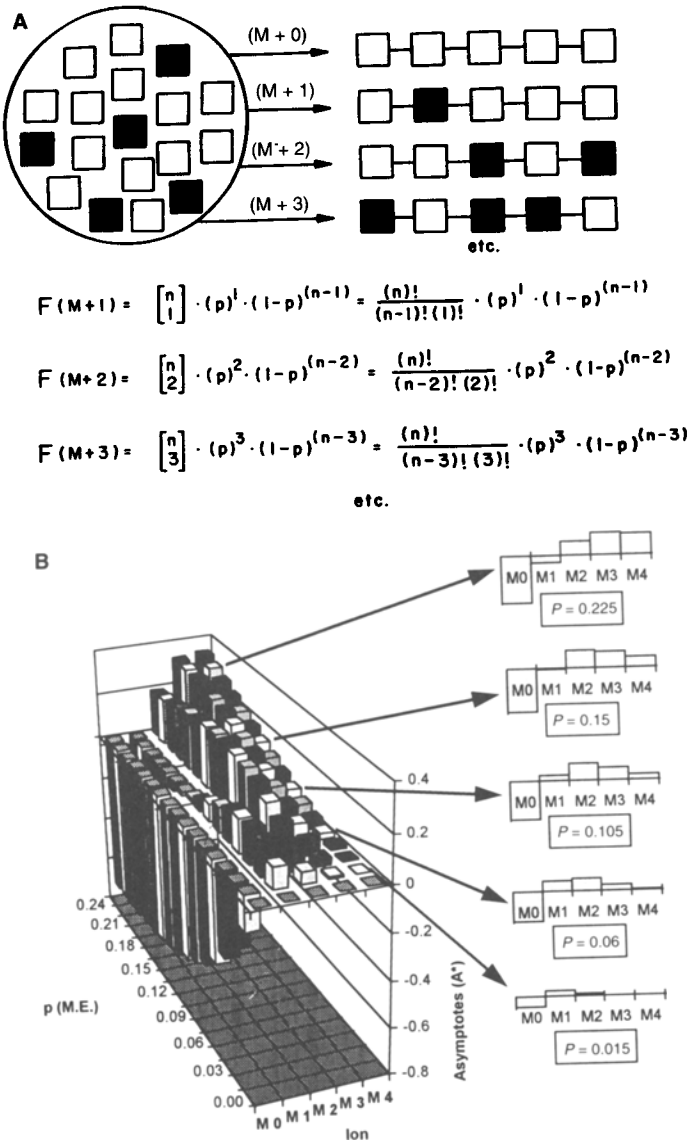

FIG. 1. A) Model of the relationship between isotope enrichment of the monomeric subunits in a precursor pool and the abundance of mass isotopomer species in its polymeric biosynthetic product. Frequency (f) of each isotopomeric species containing *x* labeled subunits [■] out of a total of *n* subunits is calculated from the probability (*p*) that each precursor subunit is labeled, according to the binomial-expansion (shown) (35). B) Three-dimensional histogram showing the effect of varying acetyl-CoA precursor pool enrichment (*p*) on the pattern of mass isotopomer enrichments in a polymer composed repeating acetate submits, in this example, cholesterol. The excess fractional abundances of M_0- to M_4-cholesterol isotopomers from [2-^{13}C]-acetate label are shown. Each cross-sectional slice shown represents the isotopomer pattern characteristic of a particular value of *p*. By analysis of the isotopomer pattern, it is therefore possible to infer the value of *P* (34).

isotopic compartmentalization is considered unlikely, and the specific activity of any body water pool can be used to represent the specific activity of water at the site of synthesis. More recently, ^2H_2O incorporation was pioneered by Jones *et al.* (28–31) for measuring lipid synthesis in humans. Although long considered the gold standard for lipid synthesis, labeled water-incorporation methods are based on a number of assumptions and nonmeasured parameters that may compromise interpretability of results, particularly in human subjects.

Hydrogen is in fact incorporated into lipids both from NADPH and H_2O itself. It is therefore necessary to choose a hydrogen:carbon (H:C) ratio for a newly synthesized lipid molecule that reflects the proportion of H atoms in NADPH that are in equilibrium with cellular water. Unfortunately, the proportion of NADPH hydrogen atoms that derive from water is variable, according to the metabolic sources of NADPH in the cell at a particular time—pentose phosphate pathway, malic enzyme, and so forth. (31). Administration of ethanol, for example, changes the H:C ratio (32). Techniques for estimating the H:C ratio experimentally have also been problematic. Early techniques used the ^{14}C-specific activity of cellular ketone bodies to represent true ^{14}C-acetyl–CoA specific activity (33) and to calculate H:C incorporation ratios. It is now clear that this approach is flawed, since cellular acetyl-CoA pools are highly compartmentalized and differentially labeled (25) and since ketone bodies are synthesized in mitochondria; thus these do not even reflect the cytosolic acetyl-CoA pool in theory. Despite these concerns, a constant standard value for the H:C ratio based on these early *in vitro* studies has typically been used for studies in whole animals and humans (28–30). A recent study using a more rigorous technique for directly measuring the H:C ratio in cholesterol (34), for example found that the ratio was quite variable, ranging from 20 to 30, in contrast to the constant value of 22 typically assumed by researchers. A significant overestimation of cholesterol synthesis would occur if a value of 22 is used for H:C in cholesterol when the true number is 27 or 30. The same considerations apply for DNL as for cholesterol synthesis.

Nevertheless, ^2H_2O incorporation has provided one technique for measuring DNL in humans that makes an attempt to define the true precursor isotope enrichment. Using this approach, Jones *et al.* (28–31) have investigated effects of diet composition and temporal patterns on FA synthesis.

Another experimental solution to the precursor pool problem: mass isotopomer distribution analysis (MIDA). A more recent solution has been developed for measuring polymerization biosynthesis and the true precursor pool, based on the principle of combinatorial probabilities (34–37). Mass isotopomer distribution analysis (MIDA) uses nonradioactive (stable) isotopes with quantitative mass spectrometry. The basic principle of MIDA is that the isotope distribution or labeling pattern of a polymer synthesized from an isotopically perturbed monomeric precursor pool conforms to the binomial (or multinomial) expansion (Fig. 1). The proportion of unlabeled, single-labeled, double-labeled (and so forth) molecular species of a polymer is a function of the probability that each precursor subunit was isotopically labeled (*p*) and the fraction of newly synthesized polymers present (dilution of newly synthesized polymers by preexisting natural abundance molecules). Calculations are somewhat more complex

than analyzing a simple binomial distribution, because carbon in nature contains ^{13}C at an abundance of approximately 1.09% (34); one must therefore calculate the difference between natural and isotopically perturbed distributions (34,36). It can be shown mathematically (34,36), however, that the internal relationship between mass isotopomers (e.g., the ratio between single-labeled and double-labeled molecules) is uniquely determined by p, the true precursor enrichment, independent of dilution of the polymer by unlabeled molecules (Fig. 1B). One can therefore calculate p from the isotope pattern in the polymer. Alternatively, a matrix correction for natural abundance contribution to the labeling pattern (40) or nonlinear best-fit regression equations (41) can be used to calculate p. Once p is known, it is a simple matter to calculate fractional synthesis (f), according to the standard precursor/product relationship (34,36). MIDA and analogous combinatorial probability methods have been used for measuring endogenous synthesis of FA (35–38,42,44), cholesterol (38,45), gluconeogenesis (39), glyconeogenesis (39,46), protein synthesis (47,48) and bile acid synthesis (49) [Kuipers, F., Bandsma, R., Neese, R., and Hellerstein, M.K. (1995) unpublished observation].

MIDA provides a number of advantageous theoretical and practical features for measuring DNL. First, there can be no disputation as to whether the correct acetyl-CoA pool was isolated for the particular lipid product of interest, since calculation is based on the lipid molecule itself. Acetate units in a molecule itself obviously reflect the acetyl-CoA pool from which the molecule was synthesized. Second, metabolic contribution to the lipogenic acetyl-CoA pool from various substrates (glucose, acetate, ethanol, and so forth) can be measured (38,44). Also, precursor pools for synthesis of different lipids can be compared, to determine, for example, whether cholesterol and FA derive from the same precursor pool (38) and thus might compete for substrate, whether *de novo* and chain-elongated FA share a common acetyl-CoA pool (35), or whether the metabolic source of different lipids varies according to physiologic conditions [Kuipers, F., Bandsma, R., Neese, R., and Hellerstein, M.K. (1995) unpublished observation]. Higher-order polymers can also be studied by MIDA; triglycerides can in principle be analyzed as polymers of palmitoyl-CoA, for example, of glycogen as a polymer or glucose subunits. Finally, from the analytic perspective, MIDA requires only a single biochemical isolation and measurement step, performed on the polymer of interest itself. The need for isolating putative biochemical precursors (cellular acetyl-CoA, triosephosphate, and so forth) is obviated. For secreted polymers, MIDA is therefore completely noninvasive (no tissue sample is required); for retained polymers, it is still simpler and less laborious than biochemical isolation of a precursor molecule.

In summary, there now exist two techniques for measuring DNL in humans: MIDA and deuterated water incorporation. This represents significant progress, since only a few years ago no rigorous methods were available. Both techniques use stable isotopes with mass spectrometry, thereby avoiding exposure to radioactivity. MIDA possesses certain advantages over water-incorporation techniques, particularly the ability to characterize metabolic behavior of the intracellular precursor pool (sources, fates, turnover, compartments, and so forth) and freedom from reliance on unmeasured correction factors (the H:C ratio assumed for water-incorporation calculations).

DNL in humans on standard Western diets. Based on these methodologic advances, hepatic DNL has been measured in humans under a variety of experimental conditions. We (35) first measured hepatic DNL in normal men (nonobese, nondiabetic, not overfed, on their standard *ad libitum* diets). The fraction of circulating very low density lipoprotein (VLDL)-palmitate and stearate derived from DNL after a 16-h infusion of [1-^{13}C]-acetate was 1–2% fasted and <5% after 8 h of feeding, whether with a food meal, intravenous glucose (at 7–10 mg/kg/min), or an oral liquid formula (at 7–10 mg CHO/kg/min). Total hepatic DNL was therefore <1 g palmitate per day, a trivial contribution in comparison to typical dietary fat intake (100 g/day or more). Jones and Schoeller (28) have presented a similar estimate of DNL (<2 g/day) using ^2H_2O incorporation. These results support previous conclusions from indirect calorimetry (18–22) and comparison of adipose tissue to diet FA composition (49) that DNL is not an active pathway under usual conditions on a modern (high-fat) diet.

We then measured DNL in young women and obese men (38). Although a significant menstrual cycle variation was observed in women, with three-fold higher values of fractional DNL in the follicular phase compared to the luteal phase, the calculated fat burden imposed by DNL per year was small (a couple of pounds) in comparison to the dietary fat burden (about 100 pounds a year). It is therefore unlikely that DNL explains body-composition differences between men and women. DNL was also slightly elevated in obese, hyperinsulinemic men (38), but again could not account for body-composition differences.

Effects of dietary CHO manipulations. Are there any dietary circumstances where DNL is substantially increased? Schwarz *et al.* (50,51) have measured the effects of oral fructose on DNL. When given at equicaloric doses as glucose (7 mg/kg/lean tissue/min for 6 h), fructose caused an almost twenty-fold increase in fractional DNL above fasting values, whereas glucose had almost no effect. Close to 30% of circulating triacylglycerol (TG)-palmitate came from DNL after this rather moderate dose of fructose. Even so, only a small proportion of the fructose load was converted to fat (less than 5%). Hudgins *et al.* (52,53) (Rockefeller University) have studied DNL in normal subjects placed on very-low-fat/high-CHO eucaloric liquid formula diets (10% fat, 70% CHO) for 25 days under metabolic ward conditions. Fractional DNL measured by MIDA increased to about 20% (fasted) and 30% (fed). A nonisotopic approach (dilution of VLDL-TG 18:2 relative to dietary and adipose FA 18:2, which were matched in the study) confirmed a substantial contribution from DNL to FA in VLDL-TG. Absolute hepatic DNL calculated by MIDA was nevertheless rather modest (e.g., less than 5 or 10 g fat synthesized per day) on these eucaloric, very low fat diets.

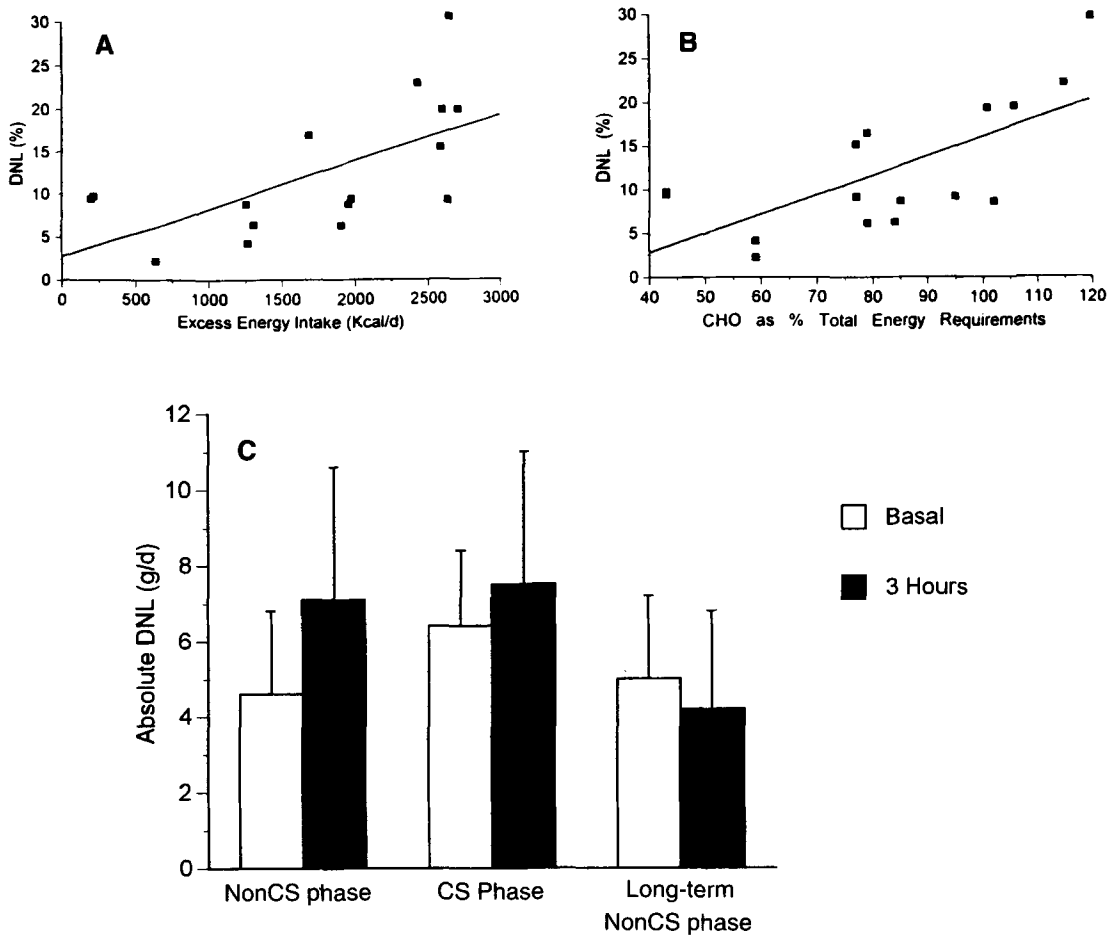

FIG. 2. Effect of *ad libitum* overfeeding on *de novo* lipogenesis (DNL) in humans, and interaction with cessation of cigarette smoking (CS): A) relationship between excess energy intake (kcal/day above estimated daily requirements) and fractional DNL ($r^2 = 0.39$, $P < 0.01$); B) relationship between percent of daily energy requirements provided by carbohydrates (CHO) and fractional DNL ($r^2 = 0.47$, $P < 0.01$); C) absolute DNL during CS and non-CS phases. Basal refers to fasting value. 3 hours refers to value after 3 h of CS of nonsmoking.

Effects of overfeeding. It can legitimately be argued that these conditions do not represent a true test of DNL if the pathway functions as a disposal route for *surplus* CHO energy. It might be necessary to overfeed CHO—that is, provide surplus energy in the form of CHO to the whole body—to induce DNL. It should be noted that previous indirect calorimetric data have generally failed to show much net DNL in overfed humans, at least on a mixed diet, unless ingested at enormous excess (20–22). Our first data in this area came out of a study in which we were asking whether the weight gain after cessation of cigarette smoking (CS) could be due in part to increased DNL. To address this question, Hellerstein *et al.* (43,55) studied smokers under metabolic ward conditions during a week of CS, then a week of no CS, and repeated measurement following four more weeks of abstention from CS. We evaluated two dietary conditions—one in which a constant metabolic diet was given (43) and one in which *ad libitum* intake was allowed (54). DNL was not increased by cessation of CS per se. Several subjects on *ad libitum* diets overate to a remarkable extent, however (54). Some subjects consumed 5,500–6,000 kcal/day, with 3,000–4,000 kcal from CHO. Fractional DNL was substantially increased in the *ad libitum* diet group, and a strong positive correlation was observed between calculated surplus energy intake or CHO energy intake and fractional DNL (Figs. 2A and 2B), implying that a dose–response relationship exists. When absolute DNL was calculated, however, <10 g/day of FA (palmitate and stearate) was synthesized even in subjects with the highest DNL rates (Fig. 2C). Synthesis of new fat was therefore quantitatively minor compared to ingestion of dietary fat (>100 g/day) in these individuals (54).

We have since followed up on these observations by administering defined diets containing up to 50% excess or deficit of energy as CHO or fat for 5-day periods (55). Fractional DNL was found to be a remarkably sensitive index of recent CHO energy intake—increasing more than ten-fold on

surplus CHO and becoming unmeasurably low on CHO-deficient diets, while exhibiting a linear dose–response relationship to CHO intake. DNL did not increase above baseline values on 50% excess *fat* calories. CHO intake over the preceding 5 days could in fact be diagnosed with near 100% sensitivity and specificity by DNL, which therefore represented an objective biomarker of recent CHO energy intake. Nevertheless, absolute hepatic DNL accounted for <5 g of fat synthesized, even on 50% surplus-CHO diets (55).

Several obvious questions are raised by these findings. First, where do excess CHO calories go, if not into DNL? Second, are surplus CHO calories therefore "free"—i.e., do they not contribute to body-fat stores? And third, what may be the function of DNL, if storage of surplus CHO energy as fat is not a quantitatively important process? The answers to the first two questions are clear from our substrate oxidation measurements (43,55) and those of previous investigators (20–22). Addition of CHO in excess to a mixed diet results in preferential oxidation of dietary CHO and sparing of dietary fat from being oxidized. Thus, surplus CHO may not itself be converted to fat, but it nevertheless will result in increased body-fat stores by preventing dietary fat from being oxidized. The answer to the second question is therefore no, CHO calories are not free. Nonprotein RQ rises to close to 1.0 on surplus CHO intake even after an overnight fast (22,55), indicating nearly complete (>90%) suppression of fat oxidation.

The distinction between excess CHO being converted to fat vs. sparing oxidation of fat is not just a technicality, however, despite the shared consequence of either process on body-fat stores. Attempts to understand or prevent adverse effects of overfeeding depend on establishing the precise metabolic fate and consequences of surplus energy intake. For example, some health-food entrepreneurs have proposed use of an inhibitor of DNL, (-)hydroxycitrate, for treatment of obesity. If this is intended to reduce storage of fat by reducing its synthesis from CHO, our results make it clear that the strategy will not be quantitatively effective. Also, we observed that short-term changes in CHO intake and energy balance exerted striking effects on fasting hepatic glucose production (HGP) in normal humans (55) and patients with non-insulin-dependent diabetes mellitus (NIDDM) (56). Increased HGP accounted for most of the increased CHO oxidation that occurred in the fasted state in subjects who had been overfed CHO for 5 days. Increased fasting HGP therefore serves a metabolic function in the context of a system where surplus CHO energy ingested during the day is not immediately converted to fat for later oxidation at night (i.e., a cyclic system with RQ >1.0 and <1.0 during the day and night, respectively), but instead is stored as liver glycogen, which provides fuel for oxidation at night (a noncyclic system with a steady RQ close to 1.0 during both day and night). Since increased HGP is a major contributing factor to insulin resistance and NIDDM (57), the inability of humans to use the DNL pathway as a disposal route for excess CHO in mixed diets may be an important factor in the etiology of NIDDM.

Studies of overfeeding on mixed diets cannot establish what would happen to DNL if a true surplus of CHO energy were present in the absence of dietary fat—i.e., if a very-low-fat surplus-CHO diet were administered for long enough to saturate the maximal whole body glycogen storage capacity. This experiment has yet to be done under controlled conditions, as will be explained later.

What are the functions of DNL? If DNL does not generally act as a sink for disposal of overflow CHO energy, what then are its functions? There are several possible answers to this question.

(1) DNL has no physiologic functions, but is a vestigial pathway in humans. This seems unlikely a priori, in view of the complex machinery for DNL and the regulation that clearly occurs *in vivo* as well as *in vitro*.

(2) DNL is important *in utero* for embryonic development, but becomes unimportant postnatally when exogenous FA can be ingested and absorbed (i.e., it is a vestigial pathway developmentally). This is an interesting possibility that has some support. The apparently limited ability of FA to cross the placenta combined with enormous lipid demands of the developing fetus (especially for myelination in the central nervous system and deposition of subcutaneous fat during the third trimester of pregnancy) suggest that DNL may be very active *in utero*. One model is that after delivery, ingestion of a high-fat diet (in breast milk) suppresses DNL. This has not been established in humans, however.

Two recent observations provide some indirect evidence on this hypothesis. The first is from a mouse knock-out model (58). Knockout of the gene for apolipoprotein B results in lethality *in utero*. It may be speculated that inability to transport lipid synthesized in the fetal liver to the central nervous system is responsible. The second observation is clinical (Chwals, W., personal communication). Premature infants who are stressed can exhibit remarkably high RQ values (1.10–1.20) when treated with intravenous nutrition, consistent with massive *net* DNL. Perhaps this reflects persistence of the prenatal pattern, in the face of relative energy excess. Both these speculations must be tested experimentally, however.

(3) DNL is important on very-low-fat diets but is unimportant on the unnaturally high-fat modern diet (i.e., is a vestigial pathway culturally). As noted above, DNL is at least qualitatively increased on eucaloric very-low-fat diets in normal adults (52,53). It will be of interest to establish whether large short-term adaptations in DNL do occur in response to day-to-day variations in energy balance in subjects on a very-low-fat diet. If so, DNL may function in the energy balance economy but only if modulation of dietary fat oxidation is not available as a first metabolic option.

(4) DNL is in fact important for storage of surplus CHO energy, but the proper experiment has yet to be carried out. Effects of true CHO energy surplus (CHO in excess of total energy expenditure, for long enough to saturate glycogen stores and in the presence of a low-fat diet) on DNL have not

yet been determined. The simple expedient of replacing fat with CHO in the fuel mixture or expanding whole body glycogen stores would no longer be an option for disposal of surplus CHO energy in this setting. Another possibility to consider is that until now the wrong tissue has been evaluated. Perhaps adipose tissue can become a significant site of DNL under certain circumstances, in which case adipose biopsies for measurement of DNL would be required. Some indirect evidence has been presented that, at least during intravenous feeding of sick patients, adipose DNL may become active [Wolfe, R.R. (1994) personal communication].

(5) DNL serves regulatory roles, perhaps as a signal of CHO availability in tissues. Malonyl-CoA concentrations and perhaps DNL influence fuel selection (FA oxidation vs. reesterification) in liver and muscle (2–4) and secretion of insulin in the pancreatic ß-cell (59). The sensitive qualitative response of DNL to CHO energy balance (55) may therefore influence fat balances not through the input of new FA but by controlling FA oxidation.

(6) DNL is necessary for synthesis of special pools of lipids. The metabolic source of the myristate used for acylation of proteins (60), for example, is not known. Is there a dietary myristate requirement, or is this FA synthesized *de novo*? This will be interesting to establish and is amenable to experimental study using the techniques previously described.

(7) DNL becomes important in specialized tissues or under special conditions. The lactating mammary gland appears to have a very active DNL pathway, at least in animal models (14). The metabolic source of lipids in breast milk of humans has not yet been established, however. Brown fat also may have extremely active DNL, which may be one mechanism of adaptive thermogenesis in this organ (Danforth, E., personal communication). Ob/Ob mice exhibit up to fifty-fold increased DNL (61), although contribution to their increased body-fat stores is not clear.

Does DNL have a role in disease? There are several disease conditions to which DNL might contribute. Some speculations are as follows: (i) hypertriglyceridemia (especially CHO-induced) (64–66), (ii) wasting syndromes [e.g., acquired immune deficiency syndrome (AIDS)] and partitioning of nutrients (42,69–73), (iii) alcoholic liver disease (fatty liver) (68), (iv) membrane FA composition (e.g., insulin resistance) (75). Hypertriglyceridemia, especially in response to high-CHO diets (62–64) may be influenced by DNL either directly (through addition of FA to VLDL-TG) or indirectly (through inhibition of tissue FA oxidation and stimulation of hepatic reesterification). Alcoholic liver disease is preceded by fatty infiltration of the liver, which could be at least in part, a consequence of ethanol-stimulated DNL or secondary inhibition of tissue FA oxidation (65,66). Body-composition abnormalities characteristic of patients with wasting in AIDS— preservation of body fat relative to lean body mass (67) and repletion of body fat rather than lean body mass during refeeding (68–70)—may be influenced by DNL. We reported (42) that DNL is paradoxically elevated in patients with weight loss due to AIDS. Moreover, the presence of increased DNL predicted poor lean-tissue response to nutrient supplementation (71), consistent with either a causal role for DNL in altered nutrient partitioning or a role for DNL as a marker of underlying cytokine abnormalities (72). Finally, altered membrane FA composition has been associated with a number of clinical conditions, including insulin resistance (73). If DNL alters qualitative FA composition in susceptible individuals, it could contribute to disease without being an important pathway quantitatively in the whole body.

In conclusion, before a process can be discussed, it must be amenable to measurement. This is now possible for DNL by two methods: MIDA and deuterated water incorporation. Based on these direct isotopic measurement techniques, a somewhat surprising model has emerged of how the human body handles excess CHO energy. DNL does not seem to be a highly traveled pathway, at least in normal adults on typical Western diets. A more detailed understanding of the quantitative contribution from DNL and testing of other potential functions of DNL are required. These remaining areas of uncertainty should now be accessible to experimental analysis, however, based on the recent methodologic advances.

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