

# Effects of a National Cholesterol Education Program Step II Diet on apolipoprotein A-IV metabolism within triacylglycerol-rich lipoproteins and plasma<sup>1-3</sup>

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## ABSTRACT

**Background:** Apolipoprotein (apo) A-IV is a major component of triacylglycerol-rich lipoprotein (TRL) apolipoproteins.

**Objective:** We investigated the effects of dietary saturated fat and cholesterol restriction on the metabolism of TRL and plasma apo A-IV.

**Design:** We assessed TRL and plasma apo A-IV kinetics in 16 and 4 subjects, respectively, consuming an average US (baseline) diet for 6 wk and a National Cholesterol Education Program Step II diet for 24 wk, respectively. At the end of each diet period, all subjects received a primed, constant infusion of deuterated leucine for 15 h with hourly feeding. Ratios of stable-isotope tracer to tracee were measured by using gas chromatography–mass spectrometry, and kinetic data were modeled by using SAAM II.

**Results:** Mean apo A-IV concentrations during the isotope infusion period were  $6.9 \pm 2.6$  mg/L in TRL and  $2.2 \pm 3.2$  mg/L in plasma with the baseline diet; these values were 37.7% ( $P < 0.001$ ) and 19.4% ( $P < 0.01$ ) lower with the Step II diet. Similar changes were observed in the fasting state between the 2 diets. The mean apo A-IV secretion rate decreased significantly from baseline by 59.6% in TRLs and by 40.2% in plasma. Significant correlations were observed between TRL apo A-IV concentrations and the secretion rate ( $r = 0.94$ ,  $P < 0.001$ ) and between TRL apo A-IV pool size and TRL-cholesterol concentrations ( $r = 0.48$ ,  $P < 0.01$ ).

**Conclusions:** Our data indicate that the National Cholesterol Education Program Step II diet significantly decreases TRL and plasma apo A-IV concentrations compared with the average US diet and that this decrease is due to a decreased secretion rate. *Am J Clin Nutr* 2001;74:308–14.

**KEY WORDS** Apolipoprotein A-IV, kinetics, National Cholesterol Education Program Step II diet, NCEP, metabolism, stable-isotope tracer, triacylglycerol-rich lipoproteins

## INTRODUCTION

It is well documented that National Cholesterol Education Program (NCEP) Step II diets can significantly lower blood total cholesterol and LDL-cholesterol concentrations compared with those diets commonly consumed in the United States (1–6). An elevated LDL-cholesterol concentration is a primary risk factor for cardiovascular disease (7–9). There is increasing evidence that alterations in triacylglycerol-rich lipoprotein (TRL) metab-

olism are important in the pathogenesis of atherosclerosis and its clinical consequences (10, 11). The role of apolipoprotein (apo) A-IV, a protein component of TRLs, in TRL metabolism is currently under investigation. After food consumption, human apo A-IV synthesis substantially increases in the intestine and is secreted into the circulation through the lymph (12–14). Apo A-IV is associated with TRLs and plays a role in lipid absorption, transport, and TRL cellular internalization via many different mechanisms (15, 16). Ordovas et al (17) reported that a patient with familial apo A-I, C-III, and A-IV deficiencies had evidence of fat malabsorption. This finding was not seen in patients with apo A-I and C-III deficiencies, suggesting that apo A-IV plays a critical role in fat absorption. It has not been established how diets that are restricted in saturated fat and cholesterol content influence apo A-IV metabolism, especially in the TRL and plasma fractions. To further understand the effects of NCEP Step II diets on apo A-IV metabolism, TRL and plasma apo A-IV kinetics were studied in 16 and 4 healthy subjects, respectively, with the use of a stable-isotope-tracer method.

## SUBJECTS AND METHODS

### Subjects

Sixteen subjects (11 men and 5 women) aged >40 y participated in this study. The women were postmenopausal. The subjects did not take any medication known to affect lipid metabolism and had no thyroid disease, diabetes mellitus, kidney disease, or liver disease. All subjects were nonsmokers and consumed no alcohol during the study. The study protocol was approved by the Tufts University School of Medicine and the New England Medical Center Human Investigation Review Committee.

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**TABLE 1**

Compositions of the baseline and the National Cholesterol Education Program (NCEP) Step II diets

	Baseline diet	Step II diet
Carbohydrate (% of energy)	49.4 ± 2.2	58.2 ± 1.8
Protein (% of energy)	15 ± 1.2	16.3 ± 0.7
Fat (% of energy)	35.4 ± 2.3	25.5 ± 1.8
Saturated	14.1 ± 2.2	4.0 ± 0.4
Monounsaturated	14.5 ± 1.0	10.8 ± 0.9
Polyunsaturated	6.9 ± 1.2	10.5 ± 0.2
Cholesterol (mg/kJ)	0.904 ± 0.15	0.284 ± 0.10

<sup>1</sup> $\bar{x} \pm SD$ .

### Experimental protocol

The compositions of the diets are shown in **Table 1**. All subjects consumed a typical US diet (18), which consisted of 15% of energy as protein, 49.4% as carbohydrate, 35.4% as fat (14.1% saturated, 14.5% monounsaturated, and 6.9% polyunsaturated with <1% n-3 fatty acids), and 0.904 mg cholesterol/kJ (146 mg cholesterol/1000 kcal) as the baseline diet for 6 wk. Furthermore, all subjects consumed an NCEP Step II diet consisting of 16.3% of energy as protein, 58.2% as carbohydrate, 25.5% as fat (4.0% saturated, 10.8% monounsaturated, and 10.5% polyunsaturated with <1% n-3 fatty acids), and 0.284 mg cholesterol/kJ (46 mg cholesterol/1000 kcal) for 24 wk (1, 19). All meals were prepared by the Metabolic Research Unit Kitchen of the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University. The chemical content of the diet was determined by food analysis at Hazelton Laboratories (Madison, WI). Energy intake was adjusted to maintain body weight. Subjects were allowed to eat and drink only what was given to them by the center, except for water and non-energy-containing beverages. Subjects were encouraged to maintain their usual level of physical activity.

For the baseline diet, blood samples (30 mL) were drawn into tubes containing 0.1% EDTA at weeks 4, 5, and 6 for the measurement of lipid, lipoprotein, and apolipoprotein concentrations. During the last week (week 6), primed, constant stable-isotope-tracer infusions were administered for 15-h to define the metabolic variables of apo A-IV, as previously described (20–22). A bolus (10  $\mu\text{mol/kg}$ ) of stable-isotope tracer, deuterated leucine ( $[^2\text{H}_3]$ leucine, 10  $\mu\text{mol/kg}$ ), was injected and followed by a 15-h constant infusion of  $[^2\text{H}_3]$ leucine (10  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). Subjects were fed hourly. The amount of food given at each hour was 5% of the daily energy intake. The composition of the portioned meal approximated that of the diet that was provided before the infusion period. The primed, constant infusion was initiated 5 h after the feeding began. Blood samples (20 mL) were drawn just before the infusion (0 h) and at hours 1, 2, 3, 4, 6, 8, 10, 12, and 15 during the infusion. For the NCEP Step II diet, blood samples (30 mL) were drawn to measure lipid concentrations at the end of each 4-wk period during the 24 wk. During the last week (week 24), the primed, constant stable-isotope-tracer infusions were repeated by using the same protocol as that which was conducted at the end of the baseline diet.

### Lipid and apolipoprotein A-IV measurement

Blood samples were centrifuged at  $1000 \times g$  and 4°C for 30 min to obtain plasma samples. Plasma samples were then subjected to single-spin ultracentrifugation at  $109000 \times g$  and

4°C for 18 h and at a density of 1006 g/L to separate the TRLs (23). Sample aliquots were stored at  $-70^\circ\text{C}$ . Plasma total triacylglycerol and cholesterol concentrations were measured by use of a standardized automated enzymatic technique (24). Apo A-IV concentrations in plasma and TRL fractions were measured by using a commercially available kit (HYDRAGEL Apo A-IV; Sebia, Issy-les-Moulineaux, France). The assay was based on an immunoprecipitation rocket technique. The CV of the apo A-IV assay was <10%.

### Apolipoprotein A-IV isolation and tracer-to-tracee ratio measurement

TRL apo A-IV was isolated by use of a 4–22.5% gradient SDS polyacrylamide gel electrophoresis (PAGE). Low-molecular-weight markers, a Western immunoblotting assay, and 2-dimensional electrophoresis were used to identify the gel band corresponding to apo A-IV. Whole plasma A-IV was isolated by immunoprecipitation. Briefly, 25  $\mu\text{L}$  plasma was reacted with 100  $\mu\text{L}$  apo A-IV antiserum at 37°C for 1 h. The precipitated protein pellets were dissolved in a 2-mercaptoethanol-containing buffer via incubation at 37°C for 1.5 h. Apo A-IV was then isolated by SDS PAGE. Apo A-IV bands from the SDS PAGE gels were hydrolyzed in 12N HCl. Sample hydrolysates were then dried under nitrogen. A mixture of 250  $\mu\text{L}$  *n*-propanol and acetyl chloride (5:1) was added to each sample tube and allowed to react to form leucine *N*-acetyl *n*-propyl ester. Samples were dried again under nitrogen and further dried with a Speed-Vac (Savant Instruments Inc, Farmingdale, NY). After the sample was dried, 1 drop of heptafluorobutyric anhydride was added to each sample tube. Sample tubes were incubated at 60°C for 1 h to synthesize the final derivative (leucine *N*-heptafluorobutyl *n*-propyl ester). After complete evaporation of extra heptafluorobutyric anhydride, the leucine derivatives were dissolved in ethyl acetate, transferred to glass sample vials, and stored at 4°C for subsequent isotopic ratio measurement. The isotopic ratios were measured by using gas chromatography–mass spectrometry (GC/MS 5890 Series-II/5988A; Hewlett Packard, Palo Alto, CA) in the methane negative chemical ionization mode. Tracer-to-tracee ratios were calculated by using the following equation (25, 26):

$$[E_t/(E_{\text{infusate}} - E_t)] \times 100\% \quad (1)$$

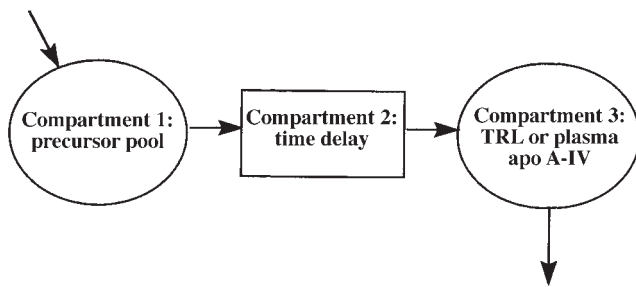
where  $E_t$  is the tracer enrichment of a sample at time point  $t$  (in h) and is equivalent to the isotopic ratio/(1 + isotopic ratio)  $\times$  100% and  $E_{\text{infusate}}$  is the tracer abundance of infusate.

### Kinetic modeling

The kinetics of apo A-IV in the TRL fraction are shown as a multicompartamental model in **Figure 1**. The model consists of a precursor compartment (compartment 1), which is the plasma amino acid forcing function. Compartment 2 is an intracellular delay compartment accounting for the synthesis of apo A-IV and the assembly of lipoproteins. Compartment 3 accounts for the kinetics of the TRL-containing apo A-IV. The fractional catabolic rate (FCR) of TRL apo A-IV corresponds to the rate of irreversible loss from compartment 3 ( $k_{0,3}$ ). The SAAM II program (SAAM Institute Inc, Seattle) was used to find the best fit of the model to the observed tracer data with a weighted-least-squares.

### Statistical analysis

Data were analyzed with the SYSTAT 7.0 (1997; SPSS Inc, Chicago) software program and are presented as means  $\pm$  SDs.



**FIGURE 1.** Kinetic model of triacylglycerol-rich lipoprotein (TRL) apolipoprotein (apo) A-IV. The tracer, [ $^3\text{H}$ ]leucine, was administered into compartment 1 by a primed, constant infusion.

Paired *t* tests were performed. Spearman correlation coefficients were determined and linear regression analysis was performed. *P* values <0.05 were considered to be significant.

## RESULTS

The subjects had a mean age of  $57 \pm 12$  y, a mean weight of  $77.6 \pm 12.9$  kg, and a mean body mass index ( $\text{kg}/\text{m}^2$ ) of  $26.3 \pm 3.1$ . The plasma lipid and lipoprotein concentrations of the 16 subjects who consumed the baseline and Step II diets are summarized in **Table 2**. The lipid concentrations during the baseline diet represent the mean of the values at weeks 4, 5, and 6 and those during the Step II diet represent the mean of values at the end of months 4, 5, and 6. The mean total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations were significantly lower in the subjects consuming the Step II diet were significantly than in the subjects consuming the baseline diet. The mean total triacylglycerol and TRL-cholesterol concentrations tended to be lower with the Step II diet than with the baseline diet, but not significantly so. The TRL and plasma apo A-IV concentrations with the baseline and Step II diets in fasting or infusion (fed) status are shown in **Table 3**. The fasting data represent the mean concentrations of the 3 time points at weeks 4, 5, and 6 for the baseline diet and at the end of months 4, 5, and 6 for the Step II diet. The fed data represent mean apo A-IV concentrations at hours 3, 6, 12, and 15 during infusion. During the fed state, TRL and plasma apo A-IV concentrations were 38% and 19% lower, respectively, with the baseline diet. In the fasting state, TRL and plasma apo A-IV concentrations were 28% and 17% lower, respectively, with the Step II diet than with the baseline

**TABLE 2**

Lipid concentrations of participants who consumed the baseline and the National Cholesterol Education Program (NCEP) Step II diets<sup>1</sup>

	Baseline diet	Step II diet
	<i>mmol/L</i>	
Triacylglycerol	$2.08 \pm 0.87$	$1.85 \pm 0.70$
Triacylglycerol-rich lipoprotein cholesterol	$0.70 \pm 0.39$	$0.57 \pm 0.36$
Total cholesterol	$5.70 \pm 1.27$	$4.43 \pm 0.91^2$
LDL cholesterol	$4.07 \pm 1.17$	$2.93 \pm 0.67^2$
HDL cholesterol	$1.01 \pm 0.23$	$0.85 \pm 0.17^3$

<sup>1</sup> $\bar{x} \pm \text{SD}$ ; *n* = 16.

<sup>2,3</sup>Significantly different from baseline diet (paired *t* test): <sup>2</sup>*P* < 0.01, <sup>3</sup>*P* < 0.05.

**TABLE 3**

Effects of the baseline and the National Cholesterol Education Program (NCEP) Step II diets on triacylglycerol-rich lipoprotein (TRL) and plasma apolipoprotein (apo) A-IV concentrations<sup>1</sup>

	Baseline diet	Step II diet
	<i>mg/L</i>	
TRL apo A-IV		
Fed state	$6.9 \pm 2.6$	$4.3 \pm 2.0^2$
Fasting state	$5.3 \pm 1.9^3$	$3.8 \pm 1.0^4$
Plasma apo A-IV		
Fed state	$217.7 \pm 31.6$	$175.5 \pm 26.0^2$
Fasting state	$192.3 \pm 41.5^5$	$159.2 \pm 29.0^{2,6}$

<sup>1</sup> $\bar{x} \pm \text{SD}$ ; *n* = 16.

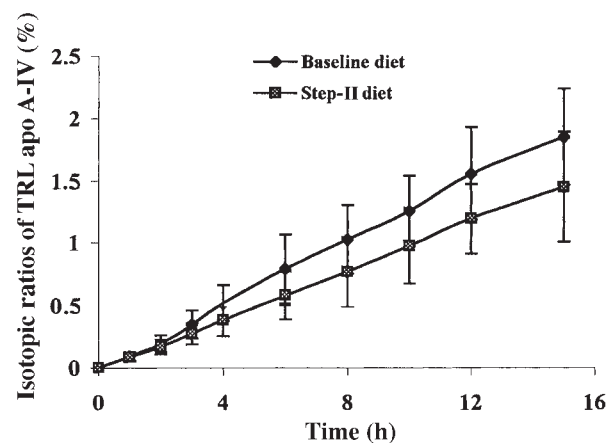
<sup>2,4</sup>Significantly different from baseline diet: <sup>2</sup>*P* < 0.001, <sup>4</sup>*P* < 0.01.

<sup>3,5,6</sup>Significantly different from fed state: <sup>3</sup>*P* < 0.05, <sup>5</sup>*P* < 0.001, <sup>6</sup>*P* < 0.01.

diet concentrations. TRL and plasma apo A-IV concentrations were significantly higher in the fed state than in the fasting state, except for TRL apo A-IV concentrations with the Step II diet.

TRL apo A-IV tracer-to-tracee ratio data during the 15-h tracer infusion are shown in **Figure 2** for subjects who consumed the 2 diets. The tracer-to-tracee ratio curves for apo A-IV did not plateau within the 15-h experimental period; therefore, the measured TRL apo B-48 plateaus of tracer-to-tracee ratios of each subject were used as estimates of apo A-IV tracer-to-tracee ratio plateaus because both proteins are derived from the intestine. The mean TRL apo A-IV tracer-to-tracee ratios were  $1.85 \pm 0.38\%$  and  $1.45 \pm 0.44\%$  at the at the last time point (15 h) for the baseline and Step II diets, respectively. Plasma apo A-IV kinetics were conducted in a subset of 4 subjects. Plasma apo A-IV had similar tracer-to-tracee ratio trends and relatively lower isotopic ratios than did TRL apo A-IV. The kinetic variables of TRL and plasma apo A-IV for the 2 diets are shown in **Tables 4** and **5**, respectively. All of the variables were significantly different between the 2 diets, except for FCR in Table 5.

The variability in the reduction of the secretion rate and the FCR between the Step II diet and baseline diets is shown in **Figure 3**. TRL apo A-IV concentrations were significantly correlated with the secretion rate in subjects who consumed the baseline or



**FIGURE 2.** Tracer-tracee ratios for triacylglycerol-rich lipoprotein (TRL) apolipoprotein (apo) A-IV during the 15-h tracer infusion in subjects who consumed baseline and Step II diets.

**TABLE 4**Triacylglycerol-rich lipoprotein apolipoprotein A-IV metabolic variables of the baseline and the National Cholesterol Education Program (NCEP) Step II diets<sup>1</sup>

	Baseline diet	Step II diet	Percentage change
			%
Fractional catabolic rate (pools/d)	0.56 ± 0.13 <sup>2</sup>	0.40 ± 0.13 <sup>3</sup>	-28.6
Secretion rate (mg · kg <sup>-1</sup> · d <sup>-1</sup> )	0.188 ± 0.084	0.076 ± 0.036 <sup>4</sup>	59.6
Residence time (d)	1.86 ± 0.44	2.75 ± 0.88 <sup>3</sup>	47.6
Pool size (mg)	23.49 ± 7.36	14.88 ± 6.44 <sup>4</sup>	-36.7

<sup>1</sup>n = 16.<sup>2</sup> $\bar{x} \pm$  SD.<sup>3,4</sup>Significantly different from baseline diet (paired *t* test): <sup>3</sup>*P* < 0.01, <sup>4</sup>*P* < 0.001.

Step II diet (**Figure 4**). However, no significant correlation was observed between TRL apo A-IV concentrations and the FCR. TRL apo A-IV pool sizes were significantly correlated with TRL-cholesterol concentrations (**Figure 5**).

## DISCUSSION

The NCEP Step II diet is the cornerstone of dietary therapy before the implementation of drug treatment for elevated LDL-cholesterol concentrations. It is useful to fully understand how this diet changes plasma lipid profiles and lowers the risk factors for cardiovascular disease. The present study investigated the effects of the NCEP Step II diet on TRL metabolism and plasma apo A-IV. After its synthesis in the intestine and entry into circulation, most apo A-IV leaves the TRL because of the exchange with apo C-II and apo E in HDL (15, 16). Apo C-II activates lipoprotein lipase, which enables lipoprotein lipase-mediated TRL hydrolysis, whereas apo E binds to specific receptors (mainly in the liver and muscle) to facilitate TRL clearance. Thus far, kinetic studies on human apo A-IV are limited and there is no reported data of how diet influences TRL apo A-IV metabolism.

In the present study, TRL and plasma apo A-IV concentrations were significantly lower with the Step II diet than with the baseline diet. Similar changes occurred in the fasting state between the 2 diets. These results are consistent with previous studies that showed that food intake stimulates apo A-IV synthesis (12, 27–29) and that diets high in fat can more effectively induce apo A-IV synthesis and secretion (12, 13, 30, 31). Stimulation of apo A-IV synthesis in response to graded doses of dietary fat was shown in animal studies (32–36). The secretion rate was 59.6% lower in subjects consuming the Step II diet than in those consuming the baseline diet (*P* < 0.001); therefore, the decrease in TRL apo A-IV concentrations in those consuming the Step II diet was primarily due to a decrease in the secretion rate. The signi-

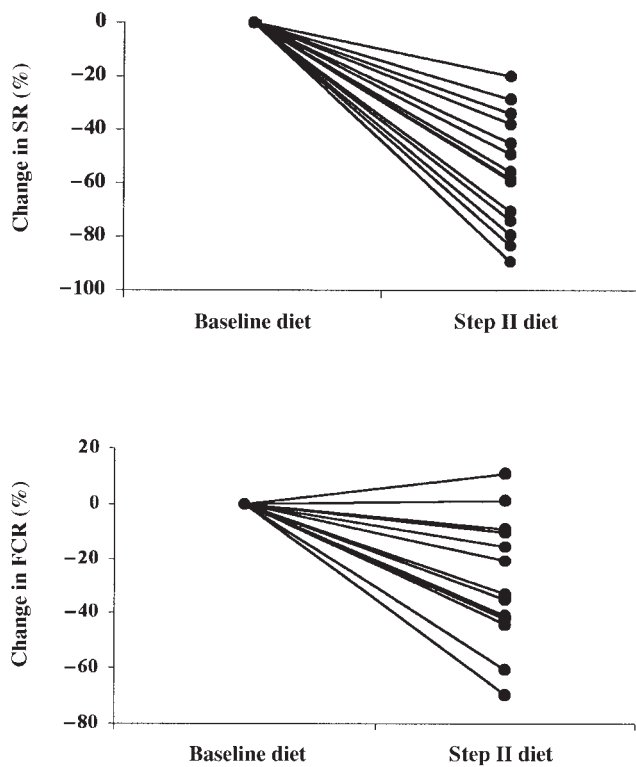
ficant correlations between TRL apo A-IV concentrations and the secretion rate (Figure 4) also indicate that these concentrations are determined by the secretion rate. Although the FCR was significantly lower with the Step II diet than with the baseline diet (*P* < 0.01), it could not compensate for the intensely reduced synthesis. However, the lower FCR suggests that the Step II diet also prolongs the residence time (RT) of TRL apo A-IV and the mechanism responsible for the decrease is not clear. The mechanism may relate to component and size changes of lipoprotein particles induced by the NCEP Step II diet. The changes may prolong turnover rates of these particles and consequently cause a reduction in the FCR of associated apolipoproteins. One could also speculate whether the NCEP Step II diet down-regulates cell membrane receptors that are related to apo A-IV-associated particle cell internalization. The down-regulation would lead to a low apo A-IV FCR. Plasma apo A-IV kinetic variables exhibited changes similar to those of TRL apo A-IV kinetics.

The observed RT of TRL apo A-IV were 1.86 ± 0.44 and 2.75 ± 0.88 d with the baseline and Step II diets, respectively. As we know, TRL triacylglycerol is catabolized rapidly, whereas apo B-48 has an RT of ≈ 5 h (21). The present study showed that TRL apo A-IV has a longer RT than does apo B-48, indicating that TRL apo A-IV must leave TRL particles and circulate as free apo A-IV or transfer to other lipoproteins, namely HDL. HDL apo A-IV then transfers back to newly secreted TRLs, most likely in the lymph. We previously observed a similar situation for TRL apo A-I, which has a RT of ≈ 4 d, which it is comparable with that of HDL apo A-I (37). This dynamic transport causes TRL apo A-IV to stay at a low enrichment level and concentration. The similarity of TRL apo A-IV RT (1.86 d) observed in the present study with the RT of HDL apo A-IV (1.61 and 1.43 d) reported by Ghiselli et al (29) and Malmendier et al (38), respectively, indicates that some TRL apo A-IV may be derived from HDL apo A-IV by reverse transport. This recirculation of apo A-IV between the 2 lipoprotein fractions may accompany

**TABLE 5**Plasma apolipoprotein A-IV metabolic variables of the baseline and the National Cholesterol Education Program (NCEP) diets<sup>1</sup>

	Baseline diet	Step II diet	Percentage change
			%
Fractional catabolic rate (pools/d)	0.385 ± 0.092 <sup>2</sup>	0.342 ± 0.064	-11.2
Secretion rate (mg · kg <sup>-1</sup> · d <sup>-1</sup> )	1.47 ± 0.49	0.88 ± 0.18 <sup>3</sup>	-40.2
Residence time (d)	2.72 ± 0.66	3.01 ± 0.69 <sup>3</sup>	10.6
Pool size (mg)	710.5 ± 103	529.1 ± 68 <sup>4</sup>	-25.5

<sup>1</sup>n = 4.<sup>2</sup> $\bar{x} \pm$  SD.<sup>3,4</sup>Significantly different from baseline diet (paired *t* test): <sup>3</sup>*P* < 0.05, <sup>4</sup>*P* < 0.01.

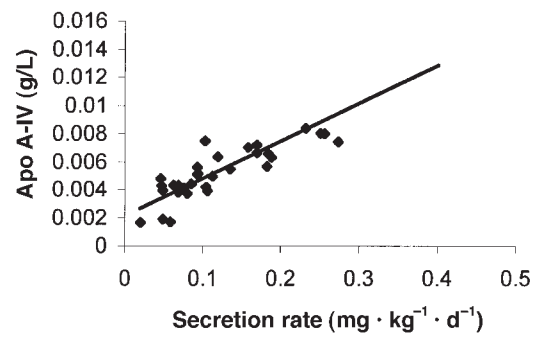


**FIGURE 3.** Changes in triacylglycerol-rich lipoprotein (TRL) apolipoprotein (apo) A-IV secretion rate (SR) and fractional catabolic rate (FCR) between the time of consumption of the baseline and Step II diets.

cholesterol ester transport and lipolysis. Apo A-IV in intestinal lymph would be expected to have a high tracer enrichment and a short RT. For these reasons, the true secretion rate of TRL apo A-IV could not be obtained. The secretion rate, in this case, is an apparent secretion rate, ie, it actually represents a transport rate of TRL apo A-IV, which is the same as its absolute catabolic rate.

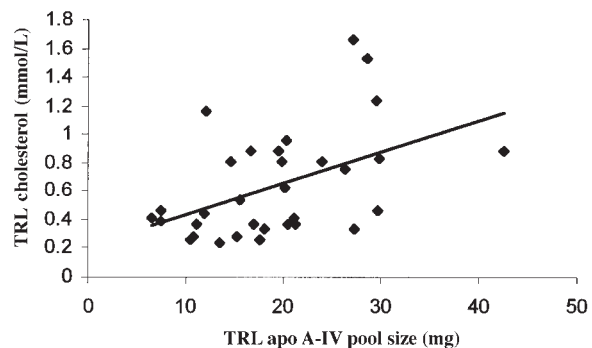
In the present study, the apo A-IV tracer-to-tracee ratio enrichment curves did not plateau within the 15 h of primed, constant tracer infusion. We used the tracer-to-tracee ratio plateaus of apo B-48 to estimate that of apo A-IV because only the intestine secretes significant amounts of apo B-48. Therefore, the tracer enrichment plateaus of apo B-48 should reflect that of enterocyte transfer RNA (tRNA) leucine (21). Thus, these values were used to generate the parameters of the forcing function used in the apo A-IV kinetic modeling. Considering that the intestine has multiple sources of amino acid supply and uses absorbed amino acids to synthesize protein preferentially (39–41), the plasma leucine tracer-to-tracee ratio plateaus, or the  $\alpha$ -ketoisocaproic acid tracer-to-tracee ratio plateaus, may be less precise for estimating enterocyte [ $^2\text{H}_3$ ]leucine tRNA enrichment in the fed state.

The significant correlation between TRL apo A-IV concentrations and its secretion rate and lack of correlation with the FCR indicate that TRL apo A-IV concentrations are determined by the secretion rate. The TRL apo A-IV pool size correlates with the TRL-cholesterol concentration. This relation may imply that apo A-IV is involved in cholesterol transport. On the other hand, a high TRL-cholesterol concentration could be created by a large TRL pool and this large TRL pool may result in an increase in TRL-associated apo A-IV.



**FIGURE 4.** Correlation between triacylglycerol-rich lipoprotein (TRL) apolipoprotein (apo) A-IV concentrations and the secretion rate (SR).  $r = 0.94$ ,  $P < 0.001$ .

Previous human apo A-IV metabolic data are summarized in **Table 6**. The RT of plasma apo A-IV in the present study was longer than that previously reported. The difference may have been due to the fact that our studies were conducted in a constant fed state with use of a stable-isotope method, whereas previously reported studies used radioactive tracer methods in the fasting state. Using a radioactive tracer, Ghiselli et al (29) reported a mean apo A-IV RT in plasma after 0.64 d, which was almost 3-fold shorter than the 1.61 d observed for HDL apo A-IV. Moreover, these investigators examined the lipoprotein-free fraction and found that the RT for apo A-IV was 0.55 d. The data from this study suggest that apo A-IV within a lipoprotein particle is catabolized at a substantially slower rate than either whole plasma or the lipoprotein-free fraction. We conducted this kinetic study with small, hourly feedings to maintain a steady state, especially with regard to TRL particles. It is known that in the fed state there is a substantial increase in TRL particles, which can be >3-fold higher than in the fasting state. Such a state may possibly account for the differences that we observed for plasma apo A-IV kinetics in comparison with those of other investigators. We previously observed a 30% prolongation of the FCR for LDL apo B-100 and a decreased input of apo B in LDL in the fed state when compared with the fasting state (20). Therefore, the fed state can cause significant alterations in apolipoprotein kinetics compared with the fasting state. In 1994 Verges et al (43) examined plasma apo A-IV metabolism with stable-isotope and radioactive tracers in 2 healthy subjects and 2 hypertriglyceridemic patients and



**FIGURE 5.** Correlation between triacylglycerol-rich lipoprotein (TRL) apolipoprotein (apo) A-IV pool size and TRL-cholesterol (TRL-C) concentrations.  $r = 0.48$ ,  $P < 0.01$ .

**TABLE 6**  
Summary of human metabolic studies of apolipoprotein (apo) A-IV<sup>1</sup>


Reference	Tracer	Isoforms	Fraction	Residence time	Secretion rate
				<i>d</i>	<i>mg · kg<sup>-1</sup> · d<sup>-1</sup></i>
Ghiselli et al, 1986 (29)	Radioiodine [3]	—	Plasma	0.64	8.69
	Radioiodine [2]	—	LFF	0.55	7.67
	Radioiodine [2]	—	HDL	1.61	0.86
Malmendier et al, 1991 (38)	Radioiodine [5]	—	HDL	1.43	2.71
Rader et al, 1993 (42)	Radioiodine [15]	A-IV-1	Plasma	0.41	16.3
	Radioiodine [13]	A-IV-2	Plasma	0.45	16.3
Verges et al, 1994 (43)	Radioiodine [2]	—	Plasma	0.88	10.4
	Stable isotope [2] <sup>2</sup>	—	Plasma	0.80	10.4
	Radioiodine [2]	—	Plasma	0.44	10.8
	Stable isotope [2]	—	Plasma	0.42	10.1
Sun et al (present study) <sup>3</sup>	Stable isotope [16]	—	TRL	1.86–2.75	0.08–0.19
	Stable isotope [4]	—	Plasma	2.72–3.01	0.88–1.47

<sup>1</sup>*n* in brackets. A-IV-1, A-IV isoform 1; A-IV-2, A-IV isoform 2; LFF, lipoprotein-free fraction; RT, residence time; TRL, triacylglycerol-rich lipoprotein.

<sup>2</sup>Subjects were hypertriglyceridemic.

<sup>3</sup>Data from 2 diets.

reported metabolic variables that were similar with both methods. In these studies, the reported precursor pool [<sup>2</sup>H<sub>3</sub>]leucine enrichment and tracer-to-tracee ratio plateaus of apo A-IV were 5.07% and 2.65%, which are lower than those reported in other studies with stable isotopes (41, 44–49).

In conclusion, our data indicate that 1) the NECP Step II diet significantly lowers TRL and plasma apo A-IV synthesis; 2) the RTs of TRL apo A-IV with both diets are much longer those of TRL apo B-48, suggesting that apo A-IV recirculates between TRLs and other lipoproteins (ie, HDL) and that apo A-IV is not the primary catabolic pool for apo A-IV; 3) the primary determinant of apo A-IV concentrations is its secretion rate; and 4) TRL apo A-IV concentrations are significantly correlated with TRL-cholesterol concentrations. 

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