SHORT-TERM ADMINISTRATION OF ISOTRETINOIN ELEVATES PLASMA TRIGLYCERIDE CONCENTRATIONS WITHOUT AFFECTING INSULIN SENSITIVITY IN HEALTHY HUMANS

THÈSE

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Une courte administration d'acide 13-cis rétinoïque chez l'homme en bonne santé augmente les taux plasmatiques de triglycérides sans influencer la sensibilité à l'insuline

Les mécanismes responsables de la résistance à l'insuline associée à l'hypertriglycéridémie chez l'homme sont mal connus. Il a été proposé que l'hypertriglycéridémie n'engendrait une résistance à l'insuline que lorsqu'elle est associée à une augmentation du transfert de lipides dans le muscle. Selon cette hypothèse, une hypertriglycéridémie secondaire à la diminution de l'élimination de particules riches en triglycérides ne devrait pas engendrer de résistance à l'insuline.

Afin de vérifier cette hypothèse, nous avons étudié la sensibilité à l'insuline (au niveau du corps entier et du tissu adipeux) chez 15 sujets volontaires masculins avant et après 5 jours d'un traitement par l'acide 13-cis rétinoïque (1 mg/kg/j), un dérivé de la vitamine A qui diminue l'élimination des particules riches en triglycérides. Au cours d'un clamp hyperinsulinémique euglycémique à 3 paliers, nous avons mesuré le métabolisme global du glucose dépendant de l'insuline (6,6 $^2$H$_2$ glucose), l'oxydation du glucose (calorimétrie indirecte), la lipolyse ($^3$H$_5$ glycérol) et la lipolyse du tissu adipeux sous-cutané (microdialyse). L'acide 13-cis rétinoïque a augmenté le taux plasmatique de triglycérides de $0.97 \pm 0.15$ à $1.30 \pm 0.22$ mmol/l ($p < 0.02$) mais n'a pas eu d'effet sur le métabolisme global du glucose et la lipolyse.

Ces observations sont compatibles avec une diminution de l'élimination des particules riches en triglycérides induite par l'acide 13-cis rétinoïque. L'inhibition de la production endogène du glucose et la diminution du glycérol sous-cutané induites par l'insuline n'ont pas été affectées par l'administration d'acide 13-cis rétinoïque.

Nous concluons que la diminution de l'élimination des particules riches en triglycérides induite par 5 jours d'acide 13-cis rétinoïque n'a pas d'influence sur les mécanismes antilipolytiques ou sur le métabolisme du glucose dépendant de l'insuline. Ces résultats soutiennent le concept que la résistance à l'insuline associée à l'hypertriglycéridémie se développe principalement quand la production de triglycérides est augmentée.
Short-Term Administration of Isotretinoin Elevates Plasma Triglyceride Concentrations Without Affecting Insulin Sensitivity in Healthy Humans

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The mechanism underlying hypertriglyceridemia-associated insulin resistance in humans remains unclear. Several observations made in animals suggest that an increase in very-low-density lipoprotein (VLDL) assembly and secretion by the liver may lead to an increase in free fatty acid (FFA) delivery to the extrahepatic tissues, which may induce insulin resistance. High-fructose diet increases VLDL production, raises plasma triglycerides concentration, and induces insulin resistance in rodents. Furthermore, recent studies have shown that overexpression of muscle and/or liver lipoprotein lipase in rodents causes tissue-specific insulin resistance. In addition, we recently reported that the inhibitory effects of lipids on whole body glucose utilization were increased in athletes during exercise and hyperinsulinemia, suggesting that endurance training increases muscle lipid uptake during contraction while reducing glucose transport and oxidation.

In contrast, hypertriglyceridemia secondary to reduced utilization of triglyceride-rich lipoproteins appears to be associated with unchanged or increased whole body insulin sensitivity. Overexpression of apolipoprotein (apo)C-III, which inhibits lipoprotein lipase, reduces the clearance of triglyceride-rich particles and does not change or increases insulin action in mice. More recently, it was observed that hypertriglyceridemia can be induced by overexpressing human apoC-I in mice, presumably through a decrease in the hepatic clearance of VLDL. These animals were shown to have increased whole body insulin sensitivity. Altogether these observations suggest that increased delivery of VLDL-triglycerides or uptake of VLDL-associated lipids by peripheral tissues may be an important factor in the regulation of tissue insulin sensitivity. Hyperinsulinemia secondary to insulin resistance may subsequently further elevate plasma triglyceride concentrations by increasing FFA reesterification and VLDL secretion, thus generating a vicious cycle.

Isotretinoin, a vitamin A derivative used in the treatment of acne, elevates plasma triglyceride levels in approximately 1 of 5 subjects. A reduction in the clearance of VLDL particles has been identified as the most likely mechanism underlying this adverse effect. It appears secondary to an increase in the content of apoC-III in VLDL, which interferes with lipoprotein lipase-mediated intravascular lipolysis. Alternatively, an increase in VLDL production was postulated and may result from a stimulation of the re-esterification of FFA in the liver or from a stimulation of hepatic de novo lipogenesis. Here, we performed a detailed assessment of the effects of a short-term administration of isotretinoin on lipid metabolism and insulin sensitivity in healthy humans. Our results corroborate that isotretinoin elevates plasma triglyceride concentrations through a reduction of triglyceride clearance. Moreover, they indicate that isotretinoin does not impair insulin sensitivity, a finding consistent with the hypothesis that hypertriglyceridemia primarily induces insulin resistance when associated with increased lipid delivery to insulin-sensitive tissues.

MATERIALS AND METHODS

Subjects
A total of 15 male healthy volunteers were enrolled in this study. Each volunteer had previously been treated with isotretinoin on average 5 years earlier (range, 3 to 10). Seven of them had increased their plasma triglyceride concentration by more than 1 mmol/L during this
treatment and the remaining 8 had had unchallenged levels. All volunteers were in good physical condition, had no personal history of diabetes, alcoholism, renal, or hepatic insufficiency, and had normal liver function tests and plasma triglyceride concentrations. Their characteristics are shown in Table 1. Mean age was 28.3 ± 1.7 years (range, 20 to 40), body mass index (BMI) 24.5 ± 0.9 kg/m² (range, 20 to 24), percentage body fat (determined using skinfold thickness measurements) 22.8 ± 1.3% (range, 15% to 30%), fat-free mass 68.1 ± 1.8 kg (range, 46 to 74), and waist-to-hip ratio 0.8 ± 0.6 (range, 0.6 to 0.9). The Ethical Committee of the Lausanne University Medical School approved the experimental protocol, and every subject provided an informed written consent.

### General Procedure

Experiments began in the morning after an overnight fast. Volunteers were requested not to consume caffeine or alcohol containing drinks at least 12 hours before the study; furthermore, they were asked not to get involved in any strenuous physical activity during the 2 days preceding the study. Each volunteer took part in the same protocol twice, once before and once after 5-day isotretinoin treatment. Volunteers had received isotretinoin as a treatment for acne, are included in this study and were recruited over the same time period. The 5-day isotretinoin treatment significantly increased fasting plasma triglyceride values (Table 1). The changes in plasma triglyceride levels observed 3 to 10 years earlier, when the subjects had received isotretinoin as a treatment for acne, are also shown for comparison. There was no correlation between the changes in plasma triglyceride concentrations observed in this study and those observed 3 to 10 years previously.

### Lipid Metabolism

A 5-day administration of isotretinoin increased total plasma triglyceride and VLDL-triglyceride on average by 33% (P < 0.01 and 0.03, respectively) (Fig. 1). The ratio of palmitic to linoleic acid in VLDL-triglyceride was the same before (2.1 ± 0.4) and after (2.2 ± 0.55) isotretinoin administration. Total cholesterol (4.3 ± 0.3 vs. 4.5 ± 0.3 mmol/L) and high-density lipoprotein (HDL)-cholesterol (1.0 ± 0.9 vs. 0.1 ± 0.0 mmol/L) levels were unchanged. Fasting plasma FFA were identical after isotretinoin and in control experiments and were suppressed to the same extent at each step of euglycemic insulin infusions (Fig 2). Similarly, fasting glycerol turnover and its suppression by insulin were not affected by isotretinoin administration (Table 3). Adipose interstitial glycerol concentrations were identical in the basal state irrespective of stimulation by epinephrine. Furthermore, suppression of adipose interstitial glycerol by insulin, both without and with epinephrine, was identical before and after isotretinoin (Fig 3). Lipid oxidation was progressively suppressed by graded doses of insulin, but this effect was not affected by isotretinoin (Table 3).
Glycemic Metabolism
Glucose production, utilization, and oxidation in basal and insulin-stimulated conditions are shown in Table 4. Before isotretinoin administration, all of these parameters of glucose metabolism were stimulated in a dose-dependent fashion by insulin. However, there was no difference between the test performed at baseline and after a 5-day isotretinoin administration. Endogenous glucose production was inhibited at each step of hyperinsulinaemia. Here again, isotretinoin administration did not alter the suppressive effect of insulin.

Relationship Between Changes in Fasting Plasma Triglyceride and Insulin Sensitivity
Figure 4 shows a plot of changes in insulin sensitivity (evaluated as GR3 during infusion of 1.2 and 2.4 pmol insulin/min; y changes in fasting plasma triglyceride concentrations). There was no correlation between these 2 variables.

DISCUSSION
A 5-day administration of isotretinoin at a dose of 1 mg/kg/d produced an average of 33% increase in fasting triglyceride concentration, and an average of 37% increase in basal VLDL-triglyceride concentration in these healthy male volunteers. Hypermiglyceridemia can develop as the result of either an increase in hepatic triglyceride synthesis and secretion, or a decreased clearance of triglycerides from the circulation. Several reports in the literature indicate that isotretinoin reduces the clearance of VLDL-triglyceride in animals. Various mechanisms have been shown to be possibly involved in this process. In humans, it has been reported that isotretinoin increases the level of VLDL apoCII and hence inhibits lipoprotein lipase. In rats, isotretinoin has also been observed to decrease lipoprotein lipase activity at a post-transcriptional level.

In contrast, the effects of isotretinoin on hepatic triglyceride synthesis have not been documented in humans. Stimulation of isotretinoin of triglyceride secretion would imply either an increased hepatic fatty acid re-esterification or a stimulation of hepatic de novo lipogenesis. Our results indicate that neither of these processes was acutely stimulated after isotretinoin. First, isotretinoin did not increase basal plasma FFA concentration or whole body glycerol turnover, nor basal subcutaneous adipose tissue glyceral concentration. In order to assess adipose tissue lipolysis more sensitively, we also measured epinephrine-stimulated lipolysis. This remained possible that isotretinoin altered hepatic lipolysis specifically, but that this effect was not detected by whole body calorimetry. Second, an increased hepatic de novo lipogenesis would result in an increased ratio of palmitic acid to linoleic acid, an essential fatty acid, in VLDL-triglyceride. Isotretinoin, however, did not alter the ratio of palmitate to linoleate. In view of these considerations, our data support the hypothesis that isotretinoin inhibited VLDL-triglyceride clearance. It remains possible that a stimulation of VLDL-triglyceride secretion occurred as a result of changes in intrathoracic triglyceride sorting, in the absence of any changes in the net lipoprotein and fatty acid re-esterification. Further studies, with a detailed evaluation of VLDL-triglyceride kinetics, will be required to unambiguously evaluate the mechanisms responsible for isotretinoin-induced hypertriglyceridemia.

Approximately 11 of 12 patients treated with isotretinoin developed a significant hypertriglyceridemia. We therefore selected 2 subgroups of patients previously treated with isotretinoin; at this first occasion, patients of one group had marked hypertriglyceridemia whereas the other had not shown changes in plasma triglyceride concentration (data shown in Table 1). We had expected that the same pattern of response to isotretinoin would be repeated in the present study and would allow us to clearly sort out the effects of isotretinoin per se or of those related to hypertriglyceridemia. Contrary to our expectation, there was no correlation between the increase in plasma triglyceride after the first exposure and those observed in this study (data shown in Table 1). The reason for this remains unclear, but it is likely that some environmental factors changed over the 3- to 10-year period that separated the 2 exposures and accounted for this discordant response. Further studies will be required to identify such factors. The increase in plasma triglyceride concentration was also much smaller in the present study than after the first exposure, possibly due to the shorter time of exposure to isotretinoin. It therefore remains possible that a larger increase in plasma triglyceride concentration may have been required to reduce insulin sensitivity. The present study allowed us to sensitively assess the effect of isotretinoin on whole body and regional insulin sensitivity. It included hyperinsulinemic clamp studies at 3 levels of insulinosis. During the first step, low insulin concentrations were attained and provided insight into the effects of insulin in inhibiting adipose tissue lipolysis. The second and third steps involved higher insulin concentrations extending graded effects to inhibit hepatic glucose production and stimulate skeletal muscle glucose utilization. Similarly, suppression of endogenous glucose production or of adipose tissue lipolysis (indi-
human lipoprotein lipase in mouse skeletal muscle is associated with

insults to the liver and inflammation in insulin resistance. Proc Natl Acad Sci USA 98:7322-7327, 2001

isotretinoin-induced hypertriglyceridemia, therefore, differs from familial combined hyperlipidemia or hypertriglyceridemia secondary to high-fructose diets, which are both associated with insulin resistance. This novel observation allows more in-depth focus on the mechanisms linking hypertriglyceridemia and insulin resistance in these latter conditions. An increased secretion of triglyceride-rich particles may lead to both hypertriglyceridemia and insulin resistance, the latter by increasing the amount of fat delivered to insulin-sensitive tissue. Alternatively, insulin resistance in extrahepatic tissue may be the primary event, and secondarily may lead to hypertriglyceridemia by increasing adipose tissue lipolysis and hepatic fatty acid re-esterification.

In conclusion, the present study emphasizes recent observations that elevated triglyceride concentrations may occur without insulin resistance. These observations are consistent with the hypothesis that an increased hepatic triglyceride secretion, together with an increased delivery of triglyceride to skeletal muscle or other organs and tissues involved in metabolic control, are required to induce insulin resistance.

REFERENCES

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Isotretinoin-induced hypertriglyceridemia is associated with insulin resistance. Proc Natl Acad Sci USA 98:7322-7327, 2001