Chapitre 14 Les concentrations plasmatiques d'insuline et de glucose sont associées différemment à l'expression intestinale des gènes clés impliqués dans le métabolisme des chylomicrons

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L'article présenté dans ce chapitre s'intitule :

Differential associations between plasma concentrations of insulin and glucose and intestinal expression of key genes involved in chylomicron metabolism

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Résumé

Contexte et objectif : Certains mécanismes causant la surproduction de LRT contenant l'apo B-48 chez des humains RI demeurent incompris. L'objectif de cette étude était d'évaluer l'association entre les concentrations plasmatiques d'insuline et de glucose et l'expression intestinale des gènes clés impliqués dans l'assemblage et la sécrétion des chylomicrons dans un grand échantillon d'hommes non diabétiques présentant des niveaux différents de RI.

Méthodes : Des biopsies duodénales ont été collectées par gastroduodénoscopie chez 127 hommes sans maladie intestinale. L'expression génique intestinale était mesurée par *PCR* quantitatif à partir des échantillons duodénaux. Les concentrations plasmatiques d'insuline et de glucose ont été mesurées à jeun. La cinétique postprandiale des LRT contenant l'apo B-48 a été mesurée en utilisant une infusion de leucine deutérée pendant 12 heures dans un sous-groupe de 75 sujets maintenus en état postprandial constant.

Résultats : Les concentrations plasmatiques d'insuline étaient négativement associées à l'expression intestinale des gènes *ACS1* (β type=-0,20; *P*=0,007), *DGAT1* (β type=-0,18; *P*=0,001), *DGAT2* (β type=-0,20; *P*=0,02) et *MTP* (β type=-0,27; *P*=0,0005), alors que les concentrations de glucose était positivement associées à l'expression du gène *MTP* (β type=0,15; *P*=0,04), indépendamment de l'âge, de l'indice de masse corporelle, du tour de taille, de l'alimentation et de l'expression duodénale du gène *SREBP1c*. Les concentrations d'insuline, mais pas de glucose, étaient positivement corrélées avec le taux de production postprandial de LRT contenant l'apo B-48 (r=0,24; *P*=0,04) et la quantité plasmatique de ces mêmes lipoprotéines (r=0,27; *P*=0,03).

Conclusions : Les concentrations plasmatiques d'insuline et de glucose sont différemment associées à l'expression intestinale des gènes clés impliqués dans le métabolisme des chylomicrons. Ces résultats suggèrent que les altérations dans le métabolisme des LRT intestinales chez des sujets RI pourraient être régulées par les concentrations plasmatiques d'insuline et de glucose et donc modifiées avec le développement de l'insuffisance insulinémique.

Title page

Differential associations between plasma concentrations of insulin and glucose and intestinal expression of key genes involved in chylomicron metabolism

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Short title

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PC, JPDC and BL designed the research; AJT, VL and JPDC conducted the research; JPDC, AJT, BL and PC analyzed the data; JPDC, AJT, BL and PC wrote the paper; and PC had primary responsibility for the final content.

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Abbreviations:

ACS1: acetyl-CoA synthetase 1;

apo: apolipoprotein;

BMI: body mass index;

DGAT: diacylglycerol O-acyltransferase:

FABP2: fatty acid-binding protein 2;

FATP4: fatty acid transport protein 4;

HOMA-IR: homeostasis model of insulin resistance;

IR: insulin resistance;

MGAT2: mannosyl (α-1,3-)-glycoprotein β-1,2-N-acetylglucosaminyltransferase;

MTP: microsomal triglyceride transfer protein;

PCR: polymerase chain reaction;

SAR1B: SAR1 homolog B; SREBP: sterol regulatory element-binding protein;

TG: triglyceride;

TRL: triglyceride-rich lipoproteins.

Abstract

The mechanisms underlying the oversecretion of apolipoprotein (apo) B-48-containing triglyceride-rich lipoproteins (TRL) in insulin-resistance (IR) states in humans remain to be fully understood. The objective of this study was to evaluate the association between the plasma levels of insulin and glucose and the intestinal expression of key genes involved in chylomicron metabolism in a large sample of nondiabetic men displaying various degrees of IR. Duodenal biopsies were obtained by gastroduodenoscopy in 127 men free of intestinal disease. Gene expression was measured using quantitative PCR in duodenal samples. Plasma insulin and glucose concentrations were measured in the fasting state. Postprandial TRL apoB-48 kinetics were measured using a primed-constant infusion of L-[5,5,5-D_3]leucine for 12 hours in a subgroup of 75 subjects maintained in a constant fed state. Plasma insulin levels were negatively associated with intestinal expression of *ACS1* (standard β=-0.20; *P*=0.007), *DGAT1* (β=-0.18; *P*=0.001), *DGAT2* (β=-0.20; *P*=0.02) and *MTP* (β=-0.27; *P*=0.0005), while glucose levels were positively associated with *MTP* expression (β=0.15; *P*=0.04), independent of age, BMI, waist circumference, dietary intake and duodenal expression of *SREBP1c.* Insulin levels, but not glucose concentrations, were positively correlated with postprandial TRL apoB-48 production rate (r=0.24; *P*=0.04) and pool size (r=0.27; *P*=0.03). In conclusion, plasma insulin and glucose levels are differentially associated with the expression of key genes involved in chylomicron metabolism. These results suggest that alterations in intestinal lipoprotein metabolism associated with IR may be regulated by plasma levels of both insulin and glucose concurrently and are therefore likely modified by the onset of insulin insufficiency.

Keywords: insulin resistance, intestine, gene expression, chylomicron.

Introduction

Insulin resistance (IR) is a chronic metabolic disorder responsible for an important proportion of the cardiovascular disease (CVD) public health burden.[1, 2] Postprandial hypertriglyceridemia is a key clinical feature of this condition that originates, in part, from an overproduction of intestinally derived apolipoprotein (apo) B-48-containing triglyceride (TG)-rich lipoproteins (TRLs).[3] Given the association between postprandial lipemia and CVD risk as well as the atherogenicity of cholesterol-rich chylomicron remnants, the oversecretion of chylomicrons associated with IR is of particular concern.[4-8]

In the postprandial state, dietary fat lipolytic products are absorbed by enterocytes and transported to the endoplasmic reticulum by fatty acid transport protein 4 (FATP4) and fatty acid-binding protein 2 (FABP2).[9] Fatty acids are then esterified and converted to TGs by mannosyl (α-1,3-)-glycoprotein β-1,2-N-acetylglucosaminyltransferase (MGAT2) and diacylglycerol O-acyltransferase (DGAT) 1 and 2.[9] Pre-chylomicrons are subsequently formed by the interaction between apoB-48, microsomal triglyceride transfer protein (MTP) and newly synthesized TGs.[9] The nascent lipoprotein is transported to the Golgi apparatus by SAR1 gene homolog B (SAR1B) for lipidation and is finally released in circulation by exocytosis.[9]

Previous studies have shown that plasma insulin inhibits chylomicron secretion,[10] while it was recently demonstrated that plasma glucose stimulates chylomicron secretion.[11] Conflicting observations regarding the intestinal expression of key genes involved in chylomicron assembly and secretion were, however, reported in humans with IR exhibiting an overproduction of intestinal lipoproteins. Phillips et al. observed that duodenal expression of *MTP* was upregulated in type 2 diabetic patients compared with non-diabetic patients, supporting the notion that the suppressive effect of insulin on chylomicron synthesis is blunted and intestinal *de novo* lipogenesis is enhanced by IR.[12] On the other hand, our group observed a downregulation of intestinal expression of key genes involved in lipoprotein metabolism, including *MTP*, in nondiabetic IR subjects. The absence of a direct correlation between gene expression in the fasting state and postprandial chylomicron secretion was explained by the important repressive effect of hyperinsulinemia on genes involved in chylomicron secretion and an increased flux of free fatty acids to the enterocytes.[13-15] These observations are based on data from studies with a limited number of subjects, and the effects of IR on the intestinal expression of genes involved in chylomicron metabolism need to be confirmed in studies with much larger sample sizes.

The general objective of this study was to evaluate the association between fundamental features of IR and intestinal expression of key genes involved in chylomicron metabolism. More specifically, we aimed to evaluate in a large sample of nondiabetic men displaying various degrees of IR the association between the plasma levels of insulin and glucose and the intestinal expression of sterol

regulatory element-binding protein 1c *(SREBP1c)*, *FATP4*, *FABP2*, *DGATs*, acetyl-CoA synthetase 1 (*ACS1)*, *ApoB*, *MTP* and *SAR1B*. We hypothesized that fasting intestinal expression of genes involved in chylomicron metabolism is inversely associated with insulin concentrations[16, 17] and positively associated with plasma glucose levels.[11]

Methods

All authors had access to the study data and reviewed and approved the final manuscript. The present study is a cross-sectional analysis of data from 127 male subjects who participated in intestinal gene expression studies in our laboratory.[14, 18-21] These studies were approved by the Laval University Ethical Review Committee, and written consent was obtained from all subjects.

Study subjects

The participants who composed the present sample were healthy and exhibited various degrees of IR. None of the subjects had diabetes, acute gastrointestinal, hepatic or renal disease, symptomatic CVD, monogenic hyperlipidemia, acute inflammatory state (evidenced by fasting C-reactive protein levels >10 mg/dL),[22] history of cancer, uncontrolled arterial hypertension, or recent history of drug or alcohol abuse. Prior to data collection, all subjects were free from lipid-lowering medication for at least 6 weeks and had a stable weight for more than 3 months.

Intestinal biopsies and extraction and quantification of total RNA

In all subjects, duodenal biopsies were collected from the second portion of the duodenum during gastroduodenoscopy after a 12 h fast. The procedure was conducted in the fasting state and limited to the second portion of the duodenum to avoid complications (e.g. pulmonary aspiration, perforation). Samples (3 x 3 mm) were collected using single-use biopsy forceps, immediately flash-frozen in liquid nitrogen and stored at -80°C before RNA extraction. Intestinal tissue samples were homogenized in 1 mL of Qiazol (Qiagen, Hilden, Germany). RNA was extracted using an RNeasy kit (Qiagen, Hilden, Germany). To eliminate any contaminating DNA, biopsies were treated with an RNase-free DNase set. Total RNA extraction and quantitative real-time polymerase chain reaction (PCR) were performed using standard procedures as previously described.[20] Primer sequences and gene descriptions are available in **Table 1**. The expression of the housekeeping gene glucose-6-phosphate-dehydrogenase (G6PD) was used as a reference. The intestinal expression of ACS1 and SAR1B was quantified in n=95 and n=84 subjects, respectively, whereas the expression of SREBP1c, FATP4, FABP2, ACS1, DGAT1, DGAT2, MTP, ApoB and G6PD was quantified in all subjects (n=127). Gene expression measurements were performed by the CHU de Québec-Université Laval Research Center Gene Expression Platform (Quebec, Canada).

The validity of the intestinal gene expression model used in the present study was demonstrated in previous studies by the inter-relationship of the expression of coregulated genes.[14, 18-21, 23] The validity

of the intestinal gene expression model is also supported by positive associations between the expression of the gene and the mass of the protein in biopsy samples as previously reported.[14, 23] Finally, the fact that the changes observed in intestinal gene expression following pharmaceutical,[18, 23] or nutritional[19, 21] interventions reflect the expected homeostatic mechanisms also validates this model.

Biochemical Measurements

A blood sample was obtained from all subjects after a delay ranging from 0 to 48 hours from the gastroduodenoscopy. Blood samples were collected after a 12-hour fast from an antecubital vein in tubes containing disodium EDTA and benzamidine (0.03 %).[24] Blood lipids were measured using enzymatic methods and ultracentrifugation as previously described.[25] Glucose levels were measured using colorimetry, and insulin levels were dosed using electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA).

Dietary assessment

In 89 subjects, assessment of dietary intake was conducted using a validated food frequency questionnaire, which assessed food intake from the four weeks preceding the gastroduodenoscopy.[26, 27] In the other 38 subjects, the diet on the days preceding clinical measurements was fully controlled, and the diet composition was measured using Nutrition Data System for Research software (University of Minnesota, MN, USA).

In Vivo Stable Isotope Kinetics

An *in vivo* stable isotope study to assess postprandial TRL apoB-48 intravascular kinetics was conducted within 24 to 48 h of the gastroduodenoscopy in a subgroup of 75 subjects. Subjects underwent a primed-constant infusion of L-[5,5,5-D_3]leucine. Starting at 0700, subjects were maintained in a constant fed state by eating 30 small, identical snacks every half hour for 15 hours, each containing 1/30th of their estimated daily food intake based on the Harris-Benedict equation. Two snack types were used during the experimental protocol. One provided 35.1 % of total caloric intake from fat, and the other provided 41.1 % of total caloric intake from fat. At 1000, L-[5,5,5-D_3]leucine (10 µmol/kg body weight) was injected as a bolus intravenously and then by continuous infusion (10 µmol/kg body weight/h) over a 12-h period. Blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 11, and 12 h.

Quantification and Isolation of ApoB-48

In 46 subjects, TRL apoB-100 and TRL apoB-48 were separated using SDS-PAGE according to standardized electrophoresis procedures, and densitometry was used to measure the relative proportion of apoB-48. [28, 29] Three different time points were scanned to estimate the average concentration of apoB-48 and to confirm steady states. In the other 29 subjects, the apoB-48

concentration in TRL was determined using a noncompetitive ELISA kit (Shibayagi Co. Ltd., Gunma, Japan). The assay was calibrated according to manufacturer's instructions. The within assay variation was 3.5% and the between assay variation was 2.8-8.6%. Three different time points during the infusion protocol were also used to estimate the average concentration of apoB-48 and to confirm steady states. The two methods are highly correlated.[20, 30, 31]

Isotopic Enrichment Determinations

The isotopic enrichment of leucine in apoB-48 was determined using gas chromatography-mass spectrometry in 46 subjects and using liquid-chromatography with multiple reaction monitoring in 29 subjects. These two procedures have been previously described and are highly correlated.[20, 31]

Kinetic Analysis

The TRL apoB-48 fractional catabolic rate (FCR; pools/d) was derived using a multicompartmental model.[32] We assumed a constant enrichment of the precursor pool and used the TRL apoB-48 plateau tracer/tracee ratio data as the forcing function to drive the appearance of tracer into apoB-48 [32]. Assuming that each subject remains in steady state with respect to apoB-48 metabolism during the study as previously shown [32], the FCR is equivalent to the fractional synthetic rate. The apoB-48 production rate (PR) was determined using the formula: PR (mg/kg/d) = [FCR ● apoB-48 concentration (mg/dL) ● plasma volume (L)]/body weight (kg)]. The plasma volume was estimated at 4.5 % of body weight. The SAAM II program (SAAM Institute, Seattle, WA, USA) was used to fit the model to the observed tracer data.

Minimal detectable association

Calculation of the minimal detectable association was conducted on the expected association between plasma insulin concentrations and duodenal expression of *MTP* as the primary outcome. Our calculation indicated that a sample size of 127 subjects would allow us to detect a correlation coefficient of 0.251 or greater with a power of 0.8 at a two-sided 0.05 significance level, with the conservative assumption that the standard deviation of insulin concentrations and *MTP* intestinal expression is 50 % of the mean. This calculation is consistent with a previous study by Gutierrez-Repiso et al.[33], who found an inverse association between the fasting insulin concentrations and jejunal expression of *MTP* (standard β=-0.746) in 45 morbidly obese IR subjects.

Statistical Analyses

Non-normally distributed variables were transformed prior to Pearson's correlation analyses. Pearson's correlations were systematically adjusted for intestinal mRNA level of the *G6PD* housekeeping gene. The adaptive Holm-Bonferroni method was used to adjust Pearson's correlation *P* values for multiple testing.[34] This sequential stepdown approach is a rigorous and recognized technique to control for a family-wise error rate [34]. The method ensures that the probability of ≥1 false

discovery under the null hypothesis is fixed at the α level while maintaining high statistical power. *P* values are adjusted and evaluated against α<0.05.

Differences among subjects with plasma levels of insulin above and below the median were analyzed using mixed models. Only covariates with significant effects were maintained in the models as fixed effects. The same approach was used to compare subjects with plasma levels of glucose above and below the median.

Backward stepwise linear regression models were used to identify independent correlates of intestinal gene expression. The models were systematically adjusted for the duodenal expression of *G6PD* to limit the heterogeneity between samples. Statistical significance was considered *P*<0.05. Data were analyzed using SAS Studio University Edition v3.5 (SAS Institute Inc., Cary, NC, USA).

Results

Anthropometric and fasting biochemical characteristics of the 127 male subjects included in the study are presented in **Table 2**. Participants' mean age (±SD) was 37.8 ± 10.9 years (range: 21.0 to 65.0 years). As a group, subjects exhibited features of IR with abdominal obesity (mean waist circumference: 107.0 ± 12.6 cm), hyperinsulinemia (mean insulin levels: 116 ± 45 ρmol/L) and normoglycemia (5.4 ± 0.5 mmol/L).

Figure 1 presents Pearson's correlations between fasting plasma concentrations of insulin and glucose and the intestinal expression of key genes involved in chylomicron metabolism. Correlations were adjusted for age, BMI, waist circumference, glucose levels (for insulin), insulin levels (for glucose), and dietary intake of energy, fat and carbohydrates. Significant inverse associations were observed between plasma insulin levels and intestinal expression of *ACS1* (r=-0.22; *P*=0.02), *DGAT1* (r=-0.24; *P*=0.009), *DGAT2* (r=-0.19; *P*=0.04) and *MTP* (r=-0.26; *P*=0.004). Insulin levels also tended to be inversely associated with expression of *FATP4* (r=-0.17; *P*=0.06), *FABP2* (r=-0.15; *P*=0.1) and *ApoB* (r=-0.17; *P*=0.06). No correlation was observed between plasma glucose concentrations and intestinal expression of key genes involved in chylomicron assembly and secretion. The HOMA-IR index was inversely associated with expression of *DGAT1* (r=-0.20; *P*=0.03) and *MTP* (r=-0.18; *P*=0.05) and tended to be associated with expression of *ACS1* (r=-0.18; *P*=0.07) and *DGAT2* (r=-0.16; *P*=0.07) (data not shown).

To assess the magnitude of the associations between plasma concentrations of insulin or glucose and intestinal expression of key genes involved in chylomicron assembly and transport, subjects were first separated according to the median value of plasma insulin (113 ρmol/L). The mean insulin levels were 151 ± 33 ρmol/L and 80 ± 21 ρmol/L among subjects with high and low insulin levels, respectively. Both groups had identical mean plasma glucose concentrations (5.4 ± 0.5 mmol/L). As presented in **Figure 2**, downregulation of intestinal expression of *ACS1* (Δ=-20.6 ± 8.9 %; *P*=0.03),

DGAT1 (Δ=-18.7 ± 6.1 %; *P*=0.005), *DGAT2* (Δ=-27.5 ± 10.1 %; *P*=0.008), and *MTP* (Δ=-18.9 ± 6.4 %; *P*=0.007) was observed among subjects with plasma insulin levels above the median compared with subjects with insulin levels below the median. These differences were independent of age, BMI, waist circumference, glucose levels, dietary intake of energy, fat and carbohydrate.

Subjects were subsequently separated according to plasma glucose median values (5.3 mmol/L). Mean glucose levels were 5.7 ± 0.4 mmol/L and 5.0 ± 0.2 mmol/L in subjects with high and low glucose levels, respectively, while plasma insulin concentrations were matched among the two groups (116 ± 45 ρmol/L and 116 ± 46 ρmol/L). Subjects with plasma glucose levels above the median exhibited upregulation of expression of *MTP* (Δ=+13.3 ± 7.7 %; *P*=0.03) compared with that in subjects with plasma glucose below the median (**Figure 3**). These differences were independent of age, BMI, waist circumference, insulin levels, dietary intake of energy, fat and carbohydrate.

Backward stepwise multiple linear regression analyses were conducted to identify independent predictors of the expression of key genes involved in chylomicron metabolism (**Table 3**). Models initially included, as independent variables, plasma levels of insulin and glucose, age, BMI, waist circumference, energy intake, dietary fat and carbohydrate intake and expression of the nuclear transcription factor *SREBP1c* (except for the model that used *SREBP1c* as the dependent variable). Plasma insulin concentrations were independently and inversely associated with intestinal expression of *ACS1*, *DGAT1*, *DGAT2* and *MTP*. Plasma glucose concentrations were independently and positively associated with expression of *MTP*.

Finally, plasma insulin levels were positively correlated with TRL apoB-48 PR (r=0.24; *P*=0.04) and PS (r=0.27; *P*=0.03) but not with TRL apoB-48 FCR (r=-0.01; *P*=0.9). Plasma glucose levels were associated with TRL apoB-48 FCR (r=-0.24; *P*=0.049) but not with TRL apoB-48 PR (r=-0.15; *P*=0.2) or PS (r=0.05; *P*=0.7). As presented in **Figure 3**, subjects with insulin above the median exhibited significantly higher TRL apoB-48 PR (Δ=+35.0 ± 19.6 %; *P*=0.04) compared with subjects with low insulin levels. Conversely, subjects with high glucose levels had a similar TRL apoB-48 PR but a lower TRL apoB-48 FCR (Δ=-20.7 ± 10.8 %; *P*=0.02) and a higher TRL apoB-48 PS (Δ=+34.8 ± 15.6 %; *P*=0.03) compared with subjects with low glucose levels. These results were independent of age, BMI, waist circumference, levels of glucose (for insulin) or insulin (for glucose), energy intake, dietary fat and carbohydrate intake and fat content of the kinetic snacks. Further adjustment for the method used to quantify apoB-48 and to determine the isotopic enrichment of leucine in apoB-48 had no significant effect on these associations.

Using backward stepwise regression models, no independent association was observed between the plasma concentrations of insulin and glucose and TRL apoB-48 PR and FCR.

Discussion

In the present study, the association between the plasma concentrations of insulin and glucose and fasting intestinal expression of key genes involved in chylomicron assembly and secretion was assessed in a large sample of nondiabetic men displaying various degrees of IR. Intestinal gene expression was quantified from duodenal biopsies obtained from 127 subjects. This study demonstrates that the fasting plasma insulin and glucose concentrations are differentially associated with the expression of key genes involved in chylomicron assembly and secretion. More specifically, this study provides strong evidence that hyperinsulinemia in the fasting state is associated with a downregulation of intestinal expression of *ACS1*, *DGAT1*, *DGAT2* and *MTP*, whereas the fasting glucose levels are positively associated with intestinal expression of *MTP*. Fasting plasma insulin and glucose levels are also differentially associated with the postprandial intravascular kinetics of TRL apoB-48. Higher insulin levels were associated with increased postprandial secretion of TRL apoB-48, while higher glucose levels were negatively associated with TRL apoB-48 clearance. These data suggest that alterations in intestinal lipoprotein metabolism associated with IR may be regulated by both plasma levels of insulin and glucose concurrently.

Characterization of the expression of major genes involved in chylomicron metabolism is a key issue to understanding the mechanisms underlying the oversecretion of atherogenic TRL apoB-48 particles in the IR state. However, access to intestinal specimens of humans free of gastrointestinal disease is highly challenging because of the invasive aspect of gastroduodenoscopy. Thus, in recent years, most studies were conducted in animal models of IR. Experiments conducted in fructose-fed Syrian golden hamsters,[35] obese IR JCR:LA-cp rats,[36] and sand rats[37, 38] reported upregulation of intestinal expression of *MTP*, concomitant with an increased secretion of chylomicrons in comparison with insulin-sensitive animals. In these studies, chylomicron oversecretion associated with IR was caused by increased intracellular fatty acid transport capacity, *de novo* lipogenesis and the biogenesis and lipidation capacities of apoB-48.[39] Inconsistent data from studies in animal models and those in humans are not unexpected because these discrepancies may be related to the differences between species. For example, IR is usually induced within a short period of time in animal models,[40] whereas exposure to the deleterious effects of IR and hyperinsulinemia in humans is chronic.[41]

Other groups have collected intestinal biopsies from morbidly obese patients undergoing bariatric surgery.[33, 39] Veilleux et al. observed upregulation of jejunal expression of *MTP*, intestinal-FABP and acetyl-CoA-carboxylase as well as downregulation of *SREBP2*, ATP-binding cassette 1 and proprotein convertase subtilisin/kexin type 9 along with IR.[39] Gutierrez-Repiso et al. observed that fasting insulin levels were inversely associated with jejunal expression of *MTP* and positively associated with the expression of *SREBP1c.*[33] Although IR was associated with alterations in intestinal expression of genes involved in lipoprotein metabolism in morbidly obese subjects, no consistent pattern relating these alterations to chylomicron overproduction was observed.

In the present study, we took advantage of our large sample of nondiabetic subjects who volunteered to undergo gastroduodenoscopy and kinetic study in recent years to provide new insights into the respective associations between plasma insulin and glucose and chylomicron metabolism in IR. Indeed, our study provides strong evidence that IR, as demonstrated by hyperinsulinemia,[17] is inversely associated with intestinal expression of key genes involved in chylomicron metabolism in the fasting state. In the IR state, the acute action of insulin on plasma glucose and FFA is reduced, partly because of the saturation of the adipose tissue-storage capacity.[42] To compensate, insulin secretion is increased and chronic hyperinsulinemia is observed.[17] Insulin also represses the synthesis of hepatic and intestinal lipoproteins by reducing *MTP* expression in a dose- and time-dependent manner.[43, 44] Therefore, the inverse association observed between plasma insulin levels and intestinal expression of *MTP* most likely results from the important inhibitory effect of hyperinsulinemia.[14] Furthermore, compensatory hyperinsulinemia also promotes the ectopic accumulation of plasma FFAs in various tissues, including the intestine.[45-47] Given that intra-enterocyte lipid content modulates the expression of genes involved in lipid transport and synthesis,[18, 23] the inverse association observed between IR and intestinal expression of *FATP4*, *ACS1*, *DGAT1* and *DGAT2* is consistent with recent observations relating obesity to increased intra-enterocyte lipid concentrations.[15]

The positive association between plasma glucose concentrations and intestinal expression of *MTP* from the present report supports the concept that intestinal expression of *MTP* and *de novo* lipogenesis are increased in subjects at advanced stages of IR, such as type 2 diabetes.[12] Recent data suggest that regulating the intestinal expression of the genes involved in chylomicron metabolism is dependent on insulin sufficiency and bridges a gap between two opposed paradigms.[12, 14] In the early stage of IR, when hyperinsulinemia and normoglycemia are observed concomitantly, intestinal expression of genes involved in chylomicron metabolism is decreased. At the advanced stage of IR, when compensatory hyperinsulinemia is accompanied by impaired fasting glucose, intestinal expression of the same genes tends to increase. Finally, as previously observed by Phillips et al. in type 2 diabetic subjects, insulin insufficiency and hyperglycemia are associated with a marked increase in the expression of these genes.[12] In sum, alterations in intestinal lipoprotein metabolism associated with IR are highly associated with concurrent levels of plasma insulin and glucose. The differential association between insulin and glucose and the intestinal expression of genes involved in chylomicron assembly and secretion suggests that the expression of these genes is most likely to be modified by the progression of IR to insulin insufficiency, frank hyperglycemia and type 2 diabetes.

The current study confirmed that hyperinsulinemia and impaired fasting glucose are associated with elevation of intestinally derived lipoprotein concentrations in the postprandial state. Hyperinsulinemia was associated with increased TRL apoB-48 secretion, and impaired glucose tolerance was associated with reduced TRL apoB-48 clearance. The elevation in intestinal lipoprotein secretion

associated with hyperinsulinemia is consistent with the lack of an acute inhibitory effect of insulin to repress postprandial lipoprotein secretion after enteral stimulation,[48] the increased plasma FFA flux to the intestine[46] and the increased lipid availability within enterocytes.[14] It is also believed that enteral stimulation is a more potent regulator of *MTP* expression and chylomicron secretion than insulinemia, but extensive investigations during the postprandial state remain warranted to confirm this assumption. By contrast, reduced TRL apoB-48 clearance associated with slightly high glucose levels may indirectly reflect the reduced stimulatory effect of insulin on lipoprotein lipase.[49] Moreover, these differential associations between insulin and glucose concentrations and TRL apoB-48 kinetics may explain that reduced TRL apoB-48 clearance was not consistently observed in subjects with IR in previous studies.[14, 50]

In the current study, the combination of gene expression and *in vivo* kinetic data allowed the acquisition of a broad perspective of the alterations in chylomicron metabolism associated with IR. This unique and large sample composed of men displaying various degrees of IR allowed the detection of smaller effect sizes. Nonetheless, the lack of type 2 diabetic subjects and the small number of insulin-sensitive subjects limited the extrapolation of the present results to higher levels of insulin sensitivity or IR. The lack of duodenal specimens collected in the postprandial state also limited the interpretation of the inverse association between fasting intestinal gene expression and apoB-48 secretion. Finally, the study was conducted among men only, and further investigations in women are needed to assess these associations.

In conclusion, the present data indicate that plasma insulin and glucose levels are differentially associated with the expression of key genes involved in chylomicron assembly and secretion. The fasting insulin levels were inversely associated with the expression of key genes involved in chylomicron metabolism, whereas the plasma glucose levels tended to be positively associated with the expression of these genes. These data suggest that alterations in intestinal lipoprotein metabolism associated with IR may be regulated by concurrent plasma levels of insulin and glucose and are therefore likely modified by the progression of IR to insulin insufficiency, frank hyperglycemia and type 2 diabetes.

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Tables

Table 14-1 Sequence primers and gene descriptions

Gene Symbol	Description	GenBank	Size (bp)	Primer Sequence 5'→3' S/AS
FABP2	Homo sapiens fatty acid binding protein 2, intestinal (FABP2)	NM_000134	137	TCAGGCTGGAATGTAGTGGAGAGA/CAAAACAAAAATTAGCTGGGCACTG
FATP4	Homo sapiens solute carrier family 27 (fatty acid transporter), member 4 (SLC27A4)	NM_005094	139	TGGCTGCCCTGGTGTACTATG/TTCCGAATCACCACCGTCATG
DGAT1	Homo sapiens diacylglycerol O-acyltransferase 1 (DGAT1)	NM_012079	135	TGCAGGATTCTTTATTCAGCTCT/CCACCAGGATGCCATACTTGAT
DGAT2	Homo sapiens diacylglycerol O-acyltransferase 2 (DGAT2), 2 transcripts	NM_032564	215	CCGATGGGTCCAGAAGAAGTT/TCACCAGGGCCTCCATGTACA
ACS1	Homo sapiens acyl-CoA synthetase long-chain family member 1 (ACSL1), 5 transcripts	NM_001995	262	GGCAACCCCAAAGGAGCAATG/TTGGAACCACGGGGAAGACAGT
ApoB	Homo sapiens apolipoprotein B (APOB)	NM_000384	274	CTGCGCAACGAGATCAAGACA/CATGCTGGGAATCGACTTGTGA
MTP	Homo sapiens microsomal triglyceride transfer protein (MTTP), 2 transcripts	NM_000253	210	CAGGGTGGTCTAGCTATTGATATTTC/TGGGTACTGAGAAAACTGCACTGT
SAR1B	Homo sapiens SAR1 homolog B (S. cerevisiae)	NM_016103	203	TGGCATTGTATTTCTGGTGGATTGTG/TCCCCTTTCCTGTTGTCTGACCATATA
SREBP1c	Homo sapiens sterol regulatory element binding transcription factor 1 (SREBF1), 2 transcripts	NM_004176	283	TGCGGAGAAGCTGCCTATCAACC/TTTGTGGACAGCAGTGCGCAGAC
G6PD	Homo sapiens glucose-6-phosphate dehydrogenase (G6PD), nuclear gene encoding mitochondrial protein	NM_000402	121	GATGTCCCCTGTCCCACCAACTCTG/GCAGGGCATTGAGGTTGGGAG

Table 14-2 Characteristics of the subjects (n=127)

	Mean ± SD	**Range (min-max)**
Age (y)	37.8 ± 10.9	21.0 - 65.0
Weight (kg)	97.3 ± 15.4	66.0 - 151.0
BMI (kg/m^2)	31.3 ± 4.4	20.4 - 43.9
Waist circumference (cm)	107.0 ± 12.6	71.5 - 141.3
Insulin (ρmol/L)	116 ± 45	30 - 277
Glucose (mmol/L)	5.4 ± 0.5	4.4 - 6.7
HOMA-IR	3.98 ± 1.61	1.02 - 10.02
Total-C (mmol/L)	5.18 ± 0.92	3.29 - 7.72
TG (mmol/L)	1.92 ± 1.00	0.43 - 5.62
LDL-C (mmol/L)	3.32 ± 0.85	1.74 - 5.76
HDL-C (mmol/L)	1.08 ± 0.24	0.54 - 1.77
Total-C/HDL-C ratio	5.02 ± 1.25	2.44 - 8.78
Energy intake (kcal)	2932 ± 728	1207 - 5642
Fat intake (% energy)	35.0 ± 4.4	22.2 - 48.1
SFA intake (% energy)	12.4 ± 2.4	6.7 - 22.0
CHO intake (% energy)	47.1 ± 5.7	32.5 - 62.3

BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; C: cholesterol; TG: triglyceride; SFA: saturated fatty acid; CHO: carbohydrate.

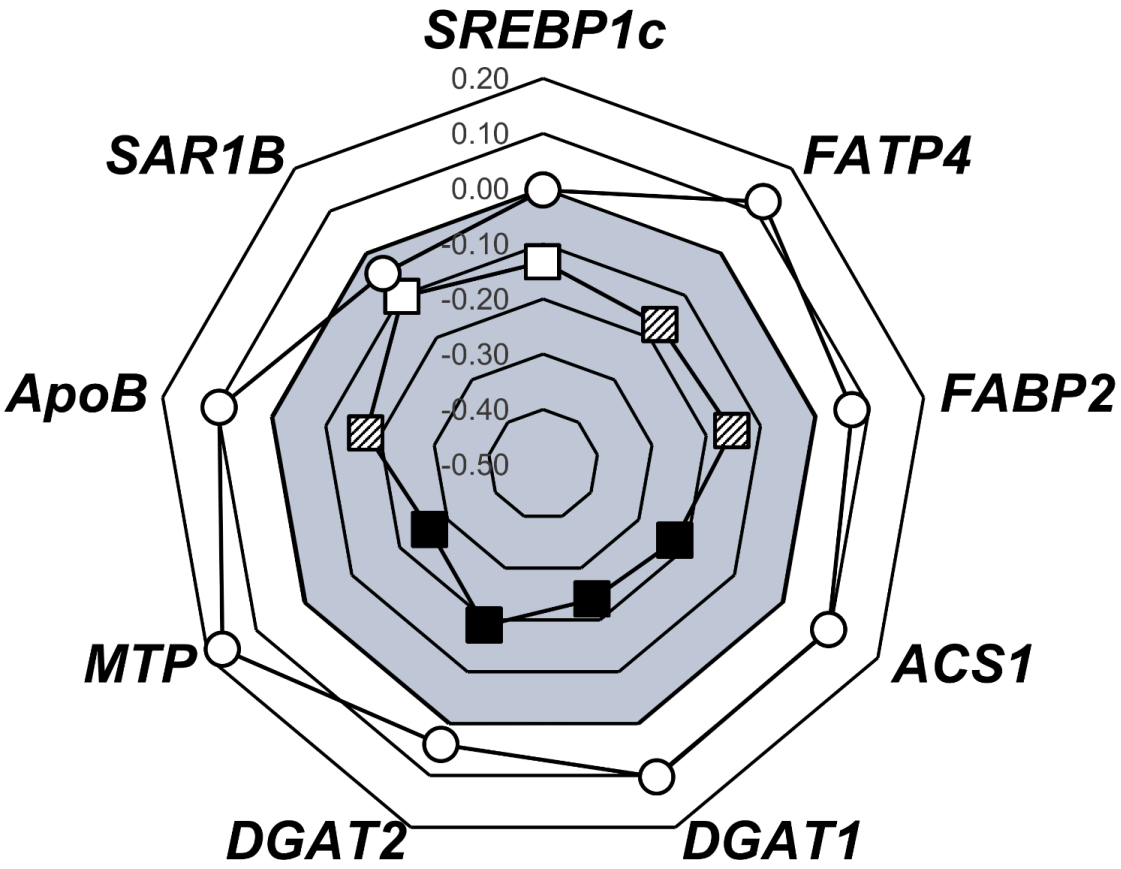
Table 14-3 Stepwise multiple regression analyses of predictors of intestinal expression of key genes involved in chylomicron assembly and secretion

Genes	Independent variable	Standard β	Partial R^2 (%)	*P*
SREBP1c	Carbohydrate intake	0.18	2.6	0.03
FATP4	*SREBP1c* expression	0.48	33.5	<0.0001
	Waist circumference	-0.17	5.4	0.001
FABP2	No independent correlate	---	---	---
ACS1	Insulin concentrations	-0.20	4.5	0.007
DGAT1	*SREBP1c* expression	0.24	8.5	<0.0001
	Insulin concentrations	-0.18	5.6	0.001
	Age	-0.13	2.9	0.02
DGAT2	*SREBP1c* expression	0.29	7.9	0.002
	Insulin concentrations	-0.20	4.3	0.02
MTP	Insulin concentrations	-0.27	10.8	0.0005
	SREBP1c expression	0.21	5.4	0.01
	Age	-0.19	5.5	0.01
	Glucose concentrations	0.15	3.6	0.04
ApoB	*SREBP1c* expression	0.69	45.2	<0.0001
SAR1B	*SREBP1c* expression	0.37	19.4	<0.0001

Models initially included, as independent variables, insulin and glucose levels, age, body mass index, waist circumference, dietary intake of energy, fat (% of total energy) and carbohydrate (% of total energy) and *SREBP1c* expression (except for the model with *SREBP1c* as the dependent variable). The expression of the housekeeping gene *G6PD* was systematically included and maintained as a covariate in all models.

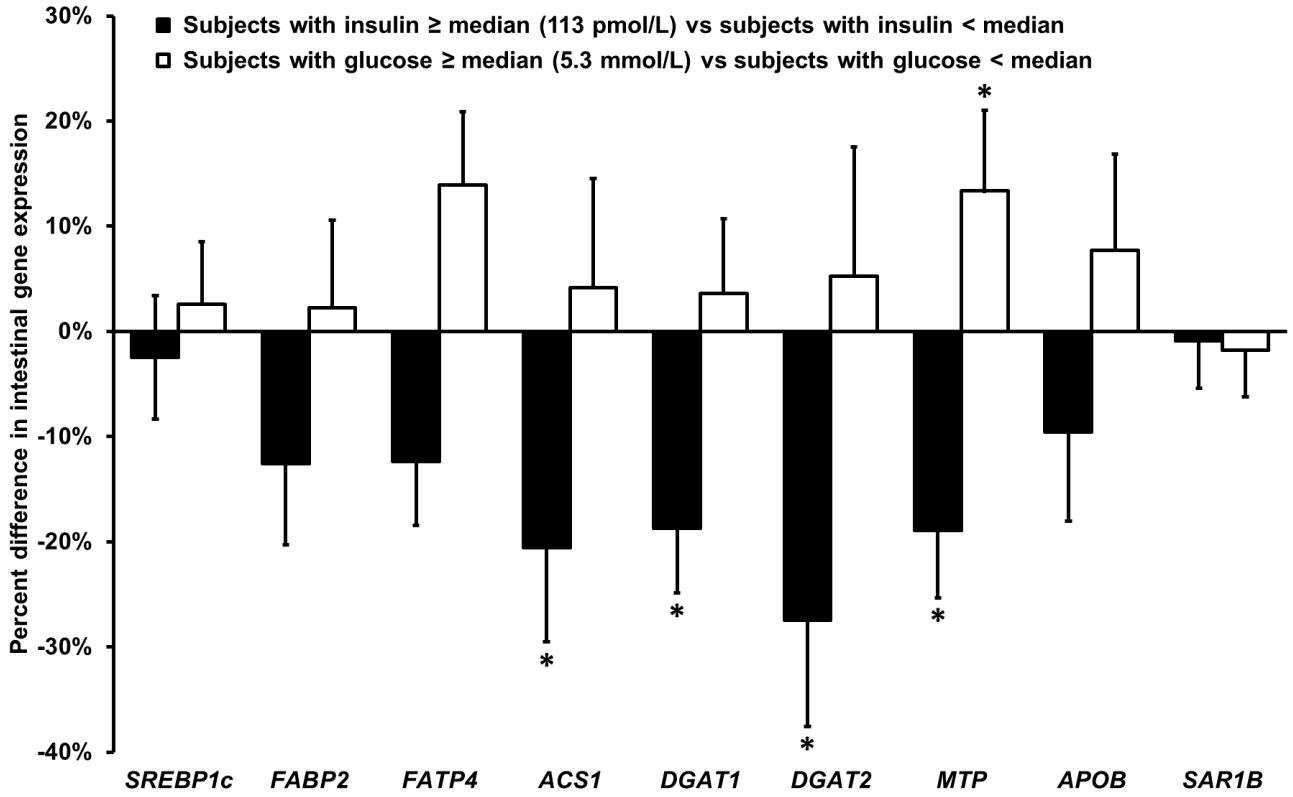
Figures

Figure 14-1 Associations among plasma concentrations of insulin and glucose and the intestinal expression of key genes involved in chylomicron metabolism



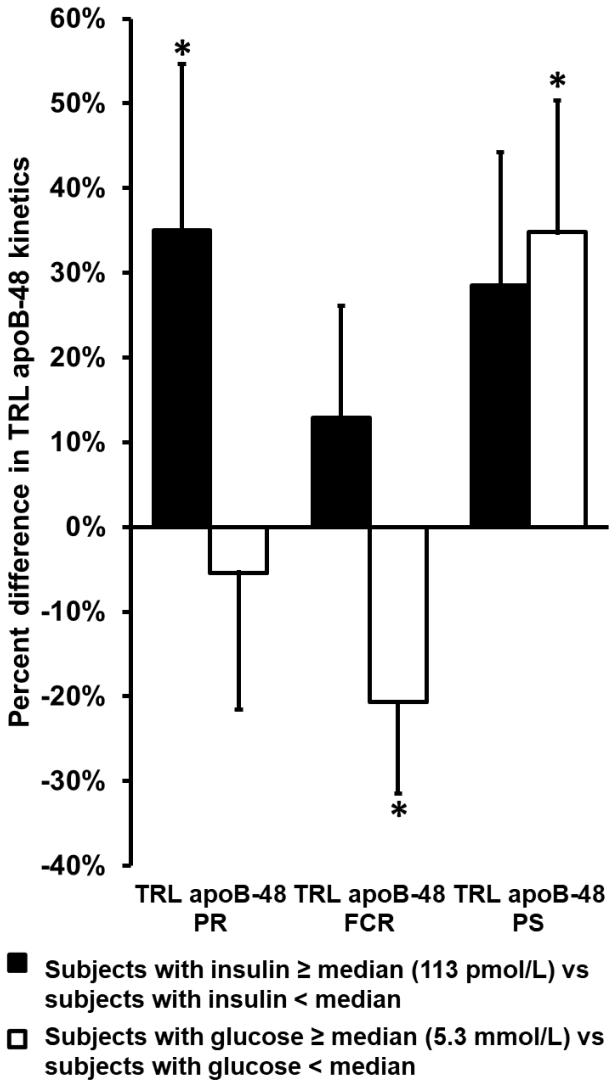
Radar plot presenting the associations among plasma concentrations of insulin (■) and glucose (●) and the intestinal expression of key genes involved in chylomicron assembly and secretion, after adjustment for intestinal mRNA level of the housekeeping gene *G6PD*, age, body mass index, waist circumference, glucose levels (for insulin), insulin levels (for glucose), and dietary intake of energy, fat and carbohydrate. The adaptive Holm-Bonferroni method was used to adjust *P* values for multiple comparisons. Radar lines represent Pearson's correlation coefficient. Black-filled symbols identify a significant correlation ($P<0.05$), lined symbols identify a statistical trend ($0.05\leq P\leq 0.1$) and white-filled symbols identify a non-significant association ($P>0.1$).

Figure 14-2 Percent difference in intestinal gene expression according to fasting plasma concentrations of insulin or glucose



Black bars: differences in gene expression when subjects were separated according to the median insulin concentration (113 pmol/L). White bars: differences in gene expression when subjects were separated according to the median glucose concentration (5.3 mmol/L). Data are presented as the percent difference in gene expression between subjects with insulin/glucose ≥ median vs subjects with insulin/glucose < median. *P* values were calculated using mixed models after adjusting for significant covariates among age, body mass index, waist circumference, glucose (for insulin analysis), insulin (for glucose analysis), energy intake, and dietary fat and carbohydrate intake. All models were adjusted for intestinal mRNA level of the housekeeping gene *G6PD*. *: $P<0.05$.

Figure 14-3 Percent difference in postprandial TRL apoB-48 intravascular kinetics according to fasting plasma concentrations of insulin or glucose



Black bars: differences in gene expression when subjects were separated according to the median insulin concentration (113 pmol/L). White bars: differences in gene expression when subjects were separated according to the median glucose concentration (5.3 mmol/L). Data are presented as the percent difference in TRL kinetics between subjects with insulin/glucose ≥ median vs subjects with insulin/glucose < median. *P* values were calculated using mixed models with adjustment for significant covariates among age, BMI, waist circumference, glucose (for insulin analysis), insulin (for glucose analysis), energy intake, dietary fat and carbohydrate intake and fat content of the kinetic snacks. TRL: triglyceride-rich lipoprotein; apo: apolipoprotein; PR: production rate; FCR: fractional catabolic rate; PS: pool size. *: $P<0.05$.