Chapitre 15 Les niveaux de protéine C-réactive sont inversement corrélés avec le taux de production des lipoprotéines riches en triglycérides contenant l'apolipoprotéine B-48 chez des hommes résistants à l'insuline

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C-reactive protein levels are inversely correlated with the apolipoprotein B-48-containing triglyceride-rich lipoprotein production rate in insulin resistant men

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Résumé

L'état pro-inflammatoire et l'hypertriglycéridémie postprandiale sont deux éléments associés à une augmentation du risque de MCV. Il a récemment été suggéré que l'augmentation du taux de production des LRT intestinales en état postprandial observé chez les patients avec RI serait causée, du moins en partie, par une altération de la sensibilité à l'insuline de l'intestin, elle-même causée par l'inflammation.

Objectif : L'objectif de cette étude était d'évaluer les associations entre la RI, les concentrations plasmatiques de CRP et la cinétique des LRT contenant l'apo B-48 dans un large échantillon d'hommes sensibles et résistants à l'insuline.

Méthodes : La cinétique des LRT contenant l'apo B-48 a été mesuré chez 151 hommes à l'aide d'une infusion de leucine deutérée. Les sujets RI (n=91) présentaient des niveaux de TG à jeun ≥ 1,5 mmol/L et un index HOMA-IR ≥ 2,5 ou un diabète de type 2. Les sujets insulino-sensibles (n=24) présentaient un index HOMA-IR < 2,5 et des niveaux de TG à jeun < 1,5 mmol/L.

Résultats : Comparativement aux sujets insulino-sensibles, les sujets RI présentaient un taux de production de LRT contenant l'apo B-48 (+202% ; P<0,0001) et des concentrations de CRP supérieures (+51% ; P=0,01). Chez les sujets RI, le taux de production de LRT contenant l'apo B-48 et les concentrations de CRP étaient inversement associés (r=-0,32 ; P=0,002). En effet, les sujets RI avec des concentrations de CRP supérieures à la médiane (2,20 mg/L) présentaient un taux de production de LRT contenant l'apo B-48 inférieur comparativement aux sujets RI avec des concentrations de CRP inférieures à la médiane (Δ=-24% ; P<0,05).

Conclusions : Ces résultats confirment que la RI est associée à une sécrétion accrue de LRT contenant l'apo B-48. Ces résultats suggèrent aussi qu'un état pro-inflammatoire important est associé à une diminution de la sécrétion de ces mêmes lipoprotéines chez des sujets RI.

Title page

C-reactive protein levels are inversely correlated with the apolipoprotein B-48-containing triglyceride-rich lipoprotein production rate in insulin resistant men

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TRL apoB-48 kinetics and inflammation in insulin resistance

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Abstract

The pro-inflammatory state and elevated plasma levels of post-prandial triglycerides (TG) are associated with increased cardiovascular disease risk. Recent studies suggested that the increase in the production rate of post-prandial lipoproteins observed in patients with insulin resistance (IR) may be caused, at least in part, by the dysregulation of intestinal insulin sensitivity triggered by inflammation.

Objective: The objective of the present study was to evaluate the association between IR, plasma C-reactive protein (CRP) levels and the kinetics of TG-rich lipoprotein (TRL) containing apolipoprotein (apo) B-48 in a large sample of insulin sensitive (IS) and IR men.

Methods: The *in vivo* kinetics of TRL apoB-48 were measured in 151 men following a primed-constant infusion of L-[5,5,5-D_3]leucine. IR subjects (n=91) were characterized by fasting TG levels ≥ 1.5 mmol/L and an index of homeostasis model assessment of IR (HOMA-IR) ≥ 2.5 or type 2 diabetes, while IS subjects (n=24) were characterized by an HOMA-IR index < 2.5 and TG levels < 1.5 mmol/L.

Results: IR subjects had higher TRL apoB-48 production rate (+202%; $P<0.0001$) and CRP levels (+51%; $P=0.01$) than IS subjects. TRL apoB-48 production rate and CRP levels were inversely correlated in IR subjects ($r=-0.32$; $P=0.002$). IR subjects with CRP levels above the median (2.20 mg/L) had lower TRL apoB-48 production rate than IR subjects with CRP levels below the median ($\Delta=-24\%$; $P<0.05$).

Conclusion: Our results confirm that IR is associated with increased TRL apoB-48 secretion and suggest that a higher inflammatory status is associated with decreased TRL apoB-48 secretion among IR subjects.

Keywords: Inflammation, CRP, apolipoprotein B-48, insulin resistance

Introduction

Insulin resistance (IR) is a plurimetabolic condition associated with a dysregulation of glucose and insulin metabolism, an atherogenic dyslipidemia, a pro-inflammatory state, and an increased risk of cardiovascular disease (CVD).[1,2] Elevated triglyceride-rich lipoprotein (TRL) levels are an important feature of IR, which also contributes to the increase in CVD risk.[1] In vivo kinetic studies in humans suggested that the overaccumulation of TRLs observed in patients with IR or type 2 diabetes (T2D) is attributable to increased production rates (PR) of both intestinally derived apolipoprotein (apo) B-48-containing lipoproteins and TRL apoB-100 of hepatic origin and to decreased catabolism of these subfractions.[3,4] The elevation of intestinally derived apoB-48-containing TRL levels is of significant interest because substantial evidence indicates that chylomicron remnants are associated with the development of atherosclerotic lesions[5] and CVD risk.[6]

Inflammation is an important contributor to the development of IR and CVD.[7] C-reactive protein (CRP), a pro-inflammatory acute phase reactant secreted from the liver, is a major surrogate marker of inflammation and its association with IR and CVD risk has been well documented.[8,9] Several studies have suggested that intestinal inflammation plays a key role in the development of both intestinal and systemic IR.[10] In animal models in which intestinal inflammation was induced, a reduction in insulin sensitivity and an elevation of TRL apoB-48 secretion at the intestinal level have been shown.[10,11] In humans, associations between increased TRL apoB-48 secretion, intestinal inflammation and impaired insulin signaling have been reported.[12-14] Although it is well documented that fasting triglyceride (TG) levels, insulinemia and circulating free fatty acids have significant impact on intestinal TRL metabolism, the role of inflammation as a modulator of the apoB-48-containing TRL production is not fully understood.[14-17] Limited data are available on the association between pro-inflammatory molecules, such as CRP, and TRL apoB-48 metabolism in humans.[18]

The general objective of the present study was to gain further insight into intestinal TRL apoB-48 metabolism by examining the correlation between TRL apoB-48 kinetics, IR and CRP levels. We first aimed to evaluate the association between IR and TRL apoB-48 kinetics in a large sample of IS and IR men. We also aimed to evaluate the independent associations between CRP levels and TRL apoB-48 production and fractional catabolic rates (FCR) and to document these associations in IS and IR subjects separately. Based on recent literature, we hypothesized that IR and CRP levels are positively associated with TRL apoB-48 PR and inversely associated with TRL apoB-48 FCR, independently of other correlates of TRL apoB-48 kinetics.

Methods

Study Subjects

Data from 151 male subjects who participated in lipoprotein kinetic studies were analyzed.[4,19-24] None of the participants had symptomatic CVD, monogenic hyperlipidemia, an acute inflammatory state, evidenced by the presence of fasting CRP levels > 10 mg/dL,[25] type 1 diabetes, insulin therapy, acute hepatic or renal disease, cancer history, uncontrolled arterial hypertension, and recent history of drug or alcohol abuse. Subjects with T2D (n=31), as defined by the American Diabetes Association,[26] were receiving stable dose of metformin for at least 3 months prior to the kinetic study. All subjects were withdrawn from lipid-lowering medication for at least 6 weeks prior to the kinetic study.

Subjects with fasting TG levels ≥ 1.5 mmol/L (132 mg/dL) and an index of homeostasis model assessment of IR (HOMA-IR) ≥ 2.5 or T2D were categorized as dyslipidemic and IR.[4,27] Subjects with TG levels < 1.5 mmol/L and HOMA-IR < 2.5 were categorized as non-dyslipidemic and IS. TG levels of 1.5 mmol/L refer to the 50th percentile for Canadian men aged 35-44 years.[28]

Biochemical Measurements

Blood samples were collected after a 12-hour fast from an antecubital vein prior to the beginning of the kinetic study in tubes containing disodium EDTA and benzamidine (0.03%).[29] Blood lipids were measured using enzymatic methods and ultracentrifugation, as previously described.[30] Glucose levels were measured using colorimetry, and insulin levels were examined using electrochemiluminescence (Roche Diagnostics, Indianapolis, IN, USA). Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure CRP levels (Biocheck Inc., Foster City, CA, USA).

Experimental Protocol for In Vivo Stable Isotope Kinetics

To determine the kinetics of TRL apoB-48, subjects underwent a primed-constant infusion of L-[5,5,5-D_3]leucine while they were maintained in a constant fed state. Starting at 0700, the subjects received 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.[31] Three snack types were used during the experimental protocol: 1) low-fat (22.4% of total caloric intake from fat), 2) moderate-fat (35.1%) or 3) high-fat (41.1%) (**Supplemental table 1**). At 1000, with 2 intravenous lines in place, one for the infusate and one for blood sampling, 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

The quantification and the isolation of apoB-48 were performed using two different highly correlated methods.[24,32,33] In 110 subjects, the apoB concentration in TRL was determined after performing a noncompetitive ELISA using immunopurified polyclonal antibodies (Alerchek Inc., Springvale, ME, USA). ApoB-100 and apoB-48 were subsequently separated using SDS-PAGE according to standardized electrophoresis procedures.[34] Densitometry was used to measure the relative proportions of apoB-100 and apoB-48.[35] Three different time points were scanned to calculate the ratios and to estimate the average concentration of apoB-100 and apoB-48 using the total apoB concentration. In 41 subjects, apoB-48 concentration in TRL was determined using a noncompetitive ELISA (Shibayagi Co. Ltd., Gunma, Japan[36]) with immuno-purified polyclonal antibodies. Three different time points during the infusion protocol were used to estimate the average concentration of apoB-48 and to confirm steady states.

Isotopic Enrichment Determinations

The isotopic enrichment of leucine in apoB was determined using gas chromatography-mass spectrometry (GC-MS) in 110 subjects and using liquid-chromatography with multiple reaction monitoring (LC-MRM) in 41 subjects. These two procedures have been previously described.[24,33] The two methods have been compared and are highly correlated (R^2=0.98, P<0.0001).[33]

Kinetic Analysis

The TRL apoB-48 FCR (pools/d) was derived using a previously described multi-compartmental model.[22,37] The apoB-48 PR was determined using the formula: PR (mg/kg/d) = [FCR ● apoB-48 concentration (mg/dL) ● plasma volume (L)]/body weight (kg).[38] 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.

Sample size estimate

Sample size calculation was conducted using the difference in TRL apoB-48 PR between IS and IR subjects as primary outcome. Previous studies from our group[20,22] showed that the within-patient standard deviation (SD) for TRL apoB-48 PR represents approximately 50% of the mean value. Based on these data, power analysis indicated that a sample size of 34 subjects would allow us to detect a difference of 50% in TRL apoB-48 PR between IS and IR subjects with a power of 80% at a two sided 5% significance level.

We also conducted sample size calculation on the expected association between TRL apoB-48 FCR and CRP levels as secondary outcome. The recent study by Thongtang et al.[18] reported a significant association between CRP pool size and TRL apoB-48 FCR (Pearson correlation coefficient=-0.90). No data were available on the association between TRL apoB-48 PR and CRP levels. Based on

these data, our power analysis indicated that 29 subjects would be required to detect a true change in plasma TRL apoB-48 FCR of 0.9 units per unit change in CRP levels with a power of 80% at a two-sided 0.05 significance level. This is based on the conservative assumption that the standard deviation of CRP levels is 30% and the standard deviation of the TRL apoB-48 FCR is 50%.[18]

Statistical Analyses

Statistical analyses were conducted using JMP Pro software (version 12.2.0, SAS Institute, Cary, NC, USA). Non-normally distributed variables were transformed using the Box-Cox transformation. Associations were assessed using Spearman's correlation test. Multiple linear regression models were used to evaluate independent associations between various factors and TRL apoB-48 kinetics. Differences between IS and IR subjects were assessed using the least-square means Student's t-test. For these specific analyses, subjects with TG levels ≥ 1.5 mmol/L and HOMA-IR <2.5 or TG levels <1.5 mmol/L and HOMA-IR index ≥ 2.5 (n=36) were excluded. In sensitivity analyses, the method used to quantify apoB-48 and to determine the isotopic enrichment, the nature of the original protocol (nutritional, pharmaceutical, cross-sectional) were added in multiple linear regression models as covariables. Statistical significance was considered at $P<0.05$.

Results

Characteristics of the subjects

Anthropometric and fasting biochemical characteristics of the 151 men included in the study are presented in **Table 1**. Mean age and BMI were 42.5 ± 12.4 y and 30.5 ± 4.3 kg/m^2, respectively. Subjects as a group had high insulin levels (118 ± 65 pmol/L) and high HOMA-IR index (4.6 ± 3.7). Mean TG levels were also elevated (2.27 ± 1.30 mmol/L), and HDL-C levels were low (1.02 ± 0.22 mmol/L). The mean CRP levels were moderately elevated (2.84 ± 2.32 mg/L).

Factors associated with TRL apoB-48 kinetics

Fig. 1 presents the association between various anthropometric and metabolic factors and TRL apoB-48 kinetics among the 151 subjects. TRL apoB-48 PR was significantly and positively associated with fasting TG levels (r=0.45; $P<0.0001$), HOMA-IR (r=0.29; $P=0.0009$) and BMI (r=0.26; $P=0.008$). CRP levels (r=0.02; $P=0.8$) and age (r=0.01; $P=0.9$) were not associated with TRL apoB-48 PR. TRL apoB-48 FCR was significantly and negatively correlated with fasting TG levels (r=-0.36; $P<0.0001$) and age (r=-0.23; $P=0.005$), while no correlations were observed with HOMA-IR (r=-0.10; $P=0.2$), CRP levels (r=-0.03; $P=0.8$) and BMI (r=0.09; $P=0.3$). **Fig. 2** presents the differences in TRL apoB-48 kinetics according to the dietary fat content of the snacks provided during the kinetic study. TRL apoB-48 PR and TRL apoB-48 FCR were both significantly higher in subjects fed with the moderate-fat and the high-fat snacks compared with the subjects who were fed the low-fat snacks. There was no significant difference in TRL apoB-48 kinetics between moderate- and high-fat snacks.

Multiple linear regression analyses indicated that the fasting TG levels and the fat content of the kinetic snacks were the most important predictors of the TRL apoB-48 PR, representing 32.1% of the variance (**Table 2**). BMI and HOMA-IR were also independently associated with TRL apoB-48 PR variance (2.7% and 1.8%, respectively). CRP levels showed no independent association with TRL apoB-48 PR variance (0.9%; *P*=0.2). The fasting TG levels and the fat content of the kinetic snacks were the only factors significantly associated with TRL apoB-48 FCR, representing 18.4% of the variance.

Comparisons Between IS and IR Subjects

Table 3 presents the differences between IS (n=24) and IR (n=91) subjects. As expected, IR subjects had significantly higher BMI, HOMA-IR and TG levels than IS subjects. Moreover, CRP levels were significantly higher in the IR group (Δ=+51%; *P*=0.01). **Fig. 3** shows that TRL apoB-48 pool size (PS) and PR were both higher in IR subjects than in IS subjects (Δ=+320% (*P*<0.0001) and Δ=+202% (*P*<0.0001), respectively). TRL apoB-48 FCR was lower in IR subjects than in IS subjects (Δ=-11%; *P*=0.06). The differences in TRL apoB-48 kinetics were independent of the dietary fat content provided during the kinetic studies, age, BMI and therapy with metformin.

A significant interaction was measured in a regression model between subject groups (IS vs. IR) and CRP levels for TRL apoB-48 PR (*P*=0.02). This interaction was independent of the fat content of the kinetic snacks, age, BMI and therapy with metformin. TRL apoB-48 PR was inversely correlated with CRP levels in IR subjects (r=-0.32; *P*=0.002) while no significant correlations were observed in IS subjects (**Fig. 4**). In IR subjects, CRP levels represented 4.4% (*P*=0.006) of TRL apoB-48 PR variance, and this association was independent of the fasting TG levels, HOMA-IR, BMI, age, therapy with metformin and fat content of the kinetic snacks (**Table 4**). When the method used to quantify apoB-48 and to determine the isotopic enrichment or the nature of the original protocol was included in the model, CRP levels remained significantly associated with TRL apoB-48 PR in IR subjects (data not shown). IR and IS subjects were subsequently stratified according to CRP levels. As presented in **Fig. 5**, TRL apoB-48 PR was 24.1% higher (*P*<0.05) in IR subjects with CRP levels below the median (2.20 mg/L) compared with IR subjects with CRP levels higher than the median. TRL apoB-48 PR was lower in IS subjects than in IR subjects while the difference in TRL apoB-48 PR between IS subjects with low and high CRP levels did not reach statistical significance (*P*=0.5). Again, differences noted in **Fig. 5** were independent of BMI, age, fat content of the kinetic snacks and therapy with metformin. Among IR subjects only, no interaction was measured between CRP levels and therapy with metformin for TRL apoB-48 PR. In addition, no interaction was measured between study groups (IS vs. IR) and CRP levels for TRL apoB-48 FCR or PS.

Discussion

In the present study, the relationships between TRL apoB-48 metabolism and CRP levels were investigated in a large sample of 151 males with various degrees of IR. TRL apoB-48 kinetics were measured using the constant infusion of a stable isotope during a protocol where subjects were constantly fed and maintained in a steady state for 15 h. Fasting TG levels and dietary fat content were the most significant predictors of TRL apoB-48 PR and TRL apoB-48 FCR. Although CRP levels were not associated with TRL apoB-48 PR or TRL apoB-48 FCR in the whole cohort, IR subjects had higher CRP levels than IS subjects, and an inverse correlation was observed between TRL apoB-48 PR and CRP levels in the sub-group of subjects with IR. More specifically, IR subjects with high CRP levels had lower TRL apoB-48 PR than IR subjects with low CRP levels. This study confirms that IR and inflammation are associated with the secretion of apoB-48-containing TRL and suggests that inflammation could decrease TRL apoB-48 secretion in IR subjects.

Many factors are known to stimulate TRL apoB-48 secretion, including elevated fasting TG levels,[17] hyperinsulinemia[15] and high levels of circulating free fatty acids.[16] Dietary fat is another major factor contributing to TRL apoB-48 secretion. It is well documented that increasing amounts of dietary fat enhance TRL apoB-48 secretion, although the impact of the different fatty acids is not fully understood.[39] Therefore, the present study confirms and extends these previous findings on TRL apoB-48 secretion in humans. The results of the present study showed that fasting TG levels were the main predictor of TRL apoB-48 PR, independently of IR, dietary fat content and BMI. In addition, dietary fat content was a major contributor of TRL apoB-48 PR representing 11.8% of its variance. This Study confirms that IR is associated with an increased production of apoB-48 containing lipoproteins resulting in the overaccumulation of circulating apoB-48-containing lipoproteins.[3,4,15] Moreover, TRL apoB-48 FCR was only slightly decreased in IR subjects, which is consistent with previous kinetic studies.[4,15]

Inflammation is recognized as a key etiological factor in IR, but its effect on intestinal lipoprotein metabolism is not fully understood. The study by Thongtang et al.[18] is the only one so far that evaluated the association between CRP and TRL apoB-48 metabolism using an *in vivo* kinetic model. This study was conducted in 8 subjects with combined hyperlipidemia, and no association was reported between CRP PR or PS and TRL apoB-48 PR.[18] Nonetheless, several lines of evidence support the physiological construct where intestinal inflammation increases apoB-48 secretion through decreasing enterocytes insulin sensitivity.[14,40] *In vivo* and *ex vivo* studies conducted in hamsters showed that the low-dose infusion of tumor necrosis factor (TNF)-α, a pro-inflammatory cytokine, inhibited intestinal insulin receptor-β and increased the phosphorylation of many kinases involved in intestinal insulin signaling, namely the extracellular signal-regulated kinases 1 and 2, the p38 mitogen-activated protein kinases and the c-JUN N-terminal kinase.[40] The infusion of TNF-α in hamsters induced intestinal insulin resistance and increased apoB-48 production as well as increased

fasting and post-prandial TRL apoB-48 levels.[11,40] The adverse effects of TNF-α on intestinal insulin sensitivity and apoB-48 secretion in hamster enterocytes were reversed through anti-inflammatory polyphenols.[41] In humans, various studies reported positive associations between systemic and intestinal inflammation, apoB-48 secretion and IR.[12,14] Monteiro-Sepulveda et al.[12] recently showed increased jejunal T cells and decreased intestinal insulin sensitivity in obese humans relative to lean subjects. Veilleux et al.[14] reported alterations in insulin signaling, as well as increased oxidative stress, inflammation and apoB-48 production in duodenal specimens of IR subjects compared with IS subjects. In summary, these studies in animals and humans strongly suggested that the impact of intestinal IR on chylomicron secretion is mediated at least in part through inflammation. Therefore, the results of the present study are, to some extent, consistent with previous studies that reported, relative to IS subjects, higher CRP levels and increased TRL apoB-48 PR in IR subjects. The non-significant +24% increase in TRL apoB-48 PR among IS subjects with CRP levels above the median, compared with IS subjects with CRP levels below the median is also concordant with previous studies. However, the absence of significant statistical difference is likely to be related to the limited number of IS subjects.

According to the studies mentioned above, IR subjects with CRP levels above the median were expected to also have higher TRL apoB-48 PR. However, TRL apoB-48 PR was -24% lower in subjects with high levels of CRP compared with IR subjects with CRP levels below the median. The inverse association between TRL apoB-48 PR and CRP levels in IR subjects observed in the present study has not previously been documented *in vivo*. Nonetheless, similar observations have been previously reported in cultured human intestinal CaCo-2 cells.[42-45] Mehran et al.[42] showed a ~30% reduction of TG secretion and apoB-48 synthesis in CaCo-2 cells following incubation with TNF-α. Murthy et al.[43-45] reported reductions from 40% to 60% in apoB secretion from CaCo-2 cells following incubation with TNF-α, IL-1β or IL-6. It has been suggested that cytokines cause this impairment in lipid metabolism through the activation of the epidermal-growth factor receptor signaling pathway.[45] Although the insulin sensitivity of CaCo-2 cells was not assessed in these studies, previous studies have provided evidence that, when exposed to pro-inflammatory cytokines, intestinal epithelial cells divert normal metabolic activity to cell growth and restitution.[45] Interestingly, these effects on CaCo-2 cells were measured following incubation at a higher, non-cytotoxic, concentration of pro-inflammatory cytokines (100-500 ng/mL[42-45]) than in the study on hamster enterocytes mentioned above (10-50 ng/mL[11,40]). More recently, Hoffmanova et al.[46] showed that subjects with T2D exhibited elevated circulating concentrations of markers of both intestinal epithelial apoptosis and enterocyte damage. The present study is consistent with these findings and suggests that the impact of inflammation on TRL apoB-48 secretion could be modulated through the insulin sensitivity of the enterocytes. Indeed, one might speculate that inflammation enhances TRL apoB-48 secretion through the induction of IR in enterocytes. However, it is likely that higher levels of inflammation could decrease TRL apoB-48 secretion through the induction of cell growth and restitution in IR enterocytes.

This hypothesis needs further investigation. Moreover, it remains unlikely that increased TRL apoB-48 PR reduces CRP levels. In fact, it is well documented that higher TRL apoB-48 PR increases hepatic TG uptake leading to elevated CRP secretion.[18] Moreover, high chylomicrons secretion has been associated with high post-prandial endotoxemia as pro-inflammatory intestinal lipopolysaccharides are absorbed and carried in the circulation through binding to dietary lipids.[47]

This study encompasses several strengths and limitations. CRP is a well-recognized circulating pro-inflammatory marker reflecting the systemic inflammatory status. Even when CRP correlates with intestinal inflammation,[12,48] the lack of a specific intestinal inflammatory marker limits the interpretation of the present results. In addition, these results are based on a single assessment of inflammation and TRL apoB-48 kinetics, which may reflect intra-individual variability.[49] Nonetheless, this limitation is compensated through the relatively large sample size. Kinetic studies are usually conducted on a limited number of participants (n= 5 to 30) because it requires extensive laboratory work. The large and unique cohort of participants in the present study with a wide range of IS and IR facilitated the detection of associations unlikely observed in a standard kinetic study. Moreover, this male cohort facilitated the examination of intestinal TRL metabolism while limiting the confounding effect of the menstrual cycle and female hormones on lipid metabolism.[50]

Conclusions

In conclusion, our results confirm that IR is associated with increased TRL apoB-48 secretion and suggest that a higher inflammatory status is associated with decreased TRL apoB-48 secretion in IR subjects. In sum, the present study suggests that increasing insulin sensitivity, independently of the inflammatory status, could be an effective strategy to reduce the oversecretion of TRL apoB-48 associated with IR. Further studies are needed to investigate the impact of pro-inflammatory molecules on intestinal IR and TRL apoB-48 metabolism in humans.

Author Contributions

All the authors have read and approved the final manuscript. PC and BL designed the research; AJT, ML, JM, MEL and JCH conducted the research; JPDC, PC, BL, and AJT analyzed the data; JPDC, PC, AJT, and BL drafted the manuscript; and PC had primary responsibility for the final content.

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Disclosure statement

BL leads the Chair on Nutrition at Laval University. All authors declare that they have no relevant conflicts of interest.

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Tables

Table 15-1 Anthropometric and fasting biochemical characteristics of the subjects (n=151)

	Mean ± SD	**Range (min-max)**
Age (y)	42.5 ± 12.4	21.0-66.1
Weight (kg)	94.2 ± 15.4	66.4-140.1
BMI (kg/m^2)	30.5 ± 4.3	22.0-42.5
Insulin (ρmol/L)	118 ± 65	18-403
Glucose (mmol/L)	5.8 ± 1.5	3.9-12.2
HOMA-IR	4.6 ± 3.7	0.46-29.7
Total-C (mmol/L)	5.19 ± 0.86	3.15-8.62
TG (mmol/L)	2.27 ± 1.30	0.42-8.08
LDL-C (mmol/L)	3.24 ± 0.80	1.60-5.67
HDL-C (mmol/L)	1.02 ± 0.22	0.60-1.69
Total-C/HDL-C ratio	5.30 ± 1.36	2.28-11.6
CRP (mg/L)	2.84 ± 2.32	0.03-9.68

BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; C: cholesterol; TG: triglyceride; CRP: C-reactive protein.

Table 15-2 Multiple linear regression analysis showing the independent contribution of various factors on the TRL apoB-48 kinetics (n=151)

Dependent variables	Independent variables	Partial (R^2 x 100)	*P*
TRL apoB-48 PR	Fasting TG	20.3	<0.0001
	Fat content of the kinetic snacks	11.8	<0.0001
	BMI	2.7	0.02
	HOMA-IR	1.8	0.05
	CRP	0.9	0.2
	Age	0.1	0.6
	Metformin	0.0	0.8
	Total	**37.7**	**<0.0001**
TRL apoB-48 FCR	Fasting TG	9.4	<0.0001
	Fat content of the kinetic snacks	9.0	0.0001
	HOMA-IR	1.8	0.06
	BMI	1.6	0.07
	Metformin	1.3	0.1
	Age	0.2	0.5
	CRP	0.0	0.9
	Total	**23.3**	**<0.0001**

TRL apoB-48 PR, TRL apoB-48 FCR, fasting TG, HOMA-IR and CRP level distributions were normalized using the Box-Cox transformation. TRL: Triglyceride-rich lipoproteins; apo: apolipoprotein; PR: production rate; TG: triglyceride; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; CRP: C-reactive protein; FCR: fractional catabolic rate.

Table 15-3 Anthropometric and fasting biochemical characteristics of IS (n=24) and IR (n=91) subjects

	IS	**IR**	**Δ (%)**	***P***
Age (y)	37.8 ± 12.0	44.9 ± 12.7	+19	0.02
Weight (kg)	83.8 ± 14.5	97.0 ± 14.8	+16	0.0002
BMI (kg/m^2)	27.3 ± 3.8	31.4 ± 4.0	+15	<0.0001
Insulin (ρmol/L)	56 ± 16	141 ± 68	+152	<0.0001
Glucose (mmol/L)	4.89 ± 0.52	6.32 ± 1.67	+29	<0.0001
HOMA-IR	1.64 ± 0.57	5.86 ± 4.19	+257	<0.0001
TG (mmol/L)	1.04 ± 0.30	2.90 ± 1.28	+179	<0.0001
CRP (mg/L)	2.00 ± 1.94	3.01 ± 2.25	+51	0.01

Insulin, glucose, HOMA-IR, fasting TG and CRP level distributions were normalized using the Box-Cox transformation. IS: insulin sensitive subjects (TG < 1.5 mmol/L and HOMA-IR < 2.5); IR: insulin resistant subjects (TG ≥ 1.5 mmol/L and HOMA-IR ≥ 2.5); BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; TG: triglycerides; CRP: C-reactive protein.

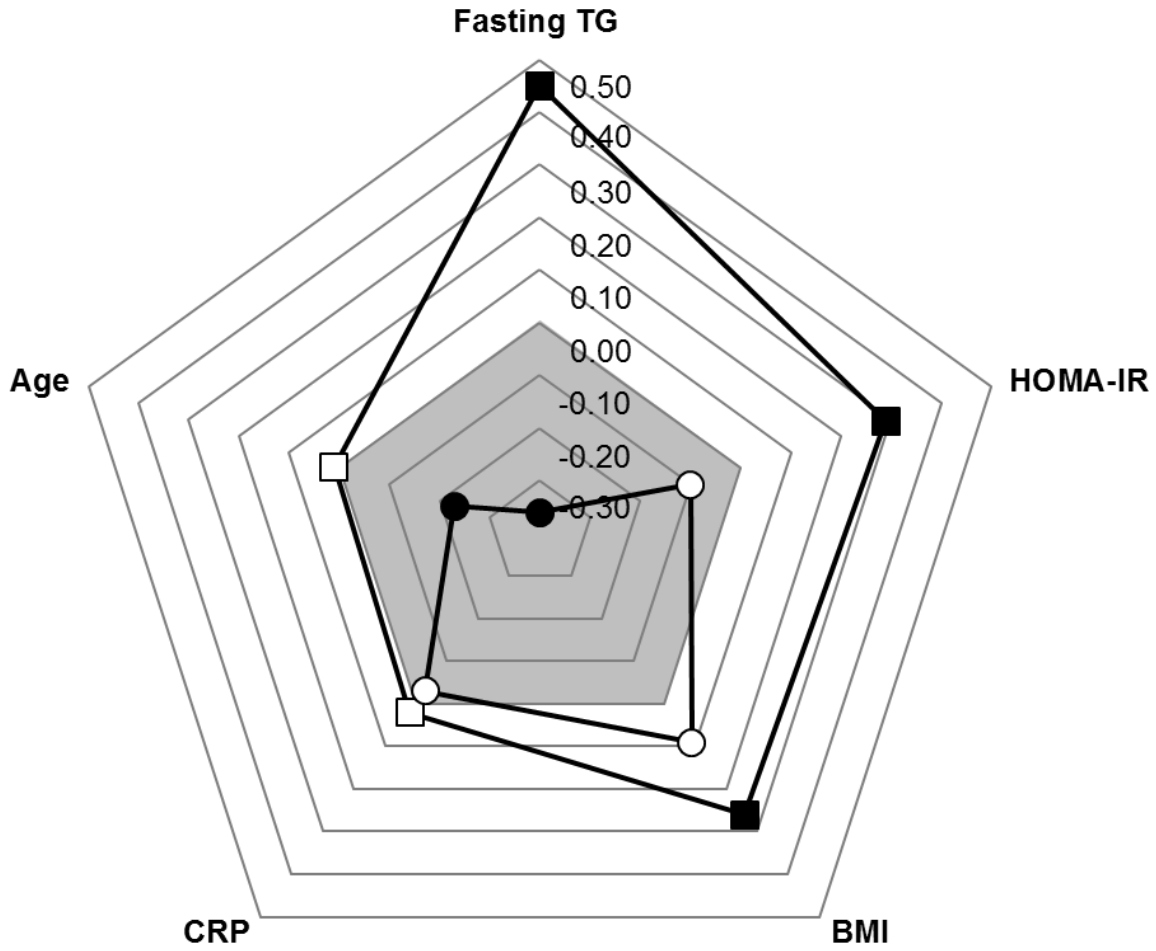
Table 15-4 Multiple linear regression analysis showing the independent contribution of various factors on the TRL apoB-48 production rate in IR subjects (n=91)

Independent variables	**Partial (R^2 x 100)**	***P***
Fasting TG	5.2	0.003
CRP	4.4	0.006
HOMA-IR	3.3	0.02
BMI	2.5	0.03
Age	2.1	0.05
Metformin	1.3	0.1
Fat content of the kinetic snacks	0.6	0.6
Total	**19.4**	**0.001**

TRL apoB-48 PR, fasting TG, HOMA-IR and CRP level distributions were normalized using the Box-Cox transformation. TRL: triglyceride-rich lipoproteins; apo: apolipoprotein; IR: insulin resistant subjects (TG ≥ 1.5 mmol/L and HOMA-IR ≥ 2.5); TG: triglycerides; CRP: C-reactive protein; HOMA-IR: homeostasis model assessment of insulin resistance; BMI: body mass index.

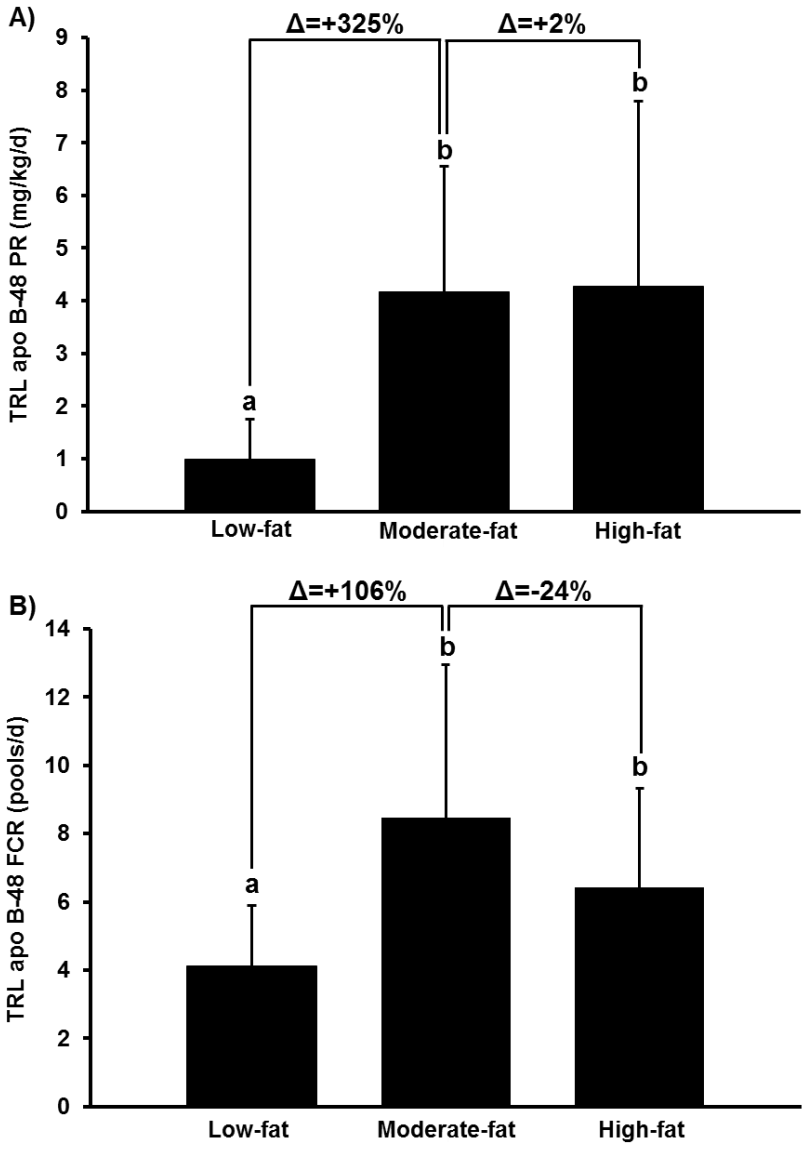
Figures

Figure 15-1 Radar plot presenting the associations among various metabolic factors and the TRL apoB-48 production rate (PR) and the TRL apoB-48 fractional catabolic rate (FCR)



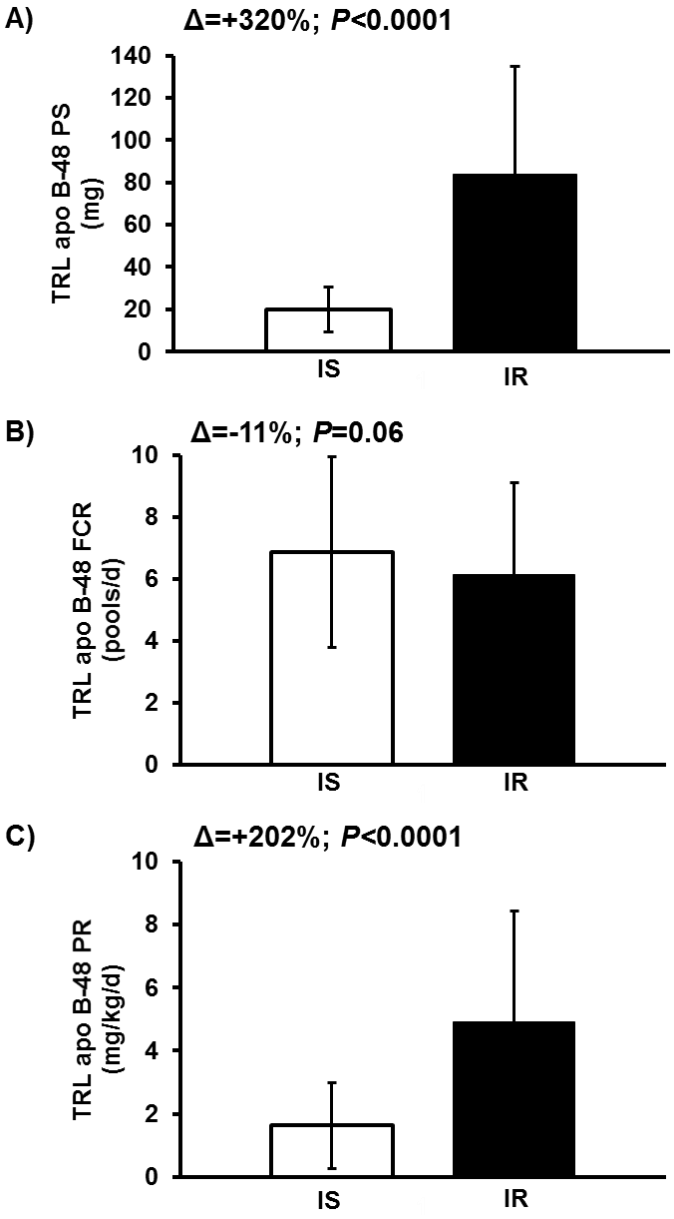
Radar lines represent Spearman's correlation coefficient. Squares represent TRL apoB-48 PR and circles represent TRL apoB-48 FCR. Black-filled marks identify significant association (P<0.05), and white-filled marks represent non-significant association. TRL: triglyceride-rich lipoproteins; apo: apolipoprotein; TG: triglyceride; HOMA-IR: homeostasis model assessment of insulin resistance; BMI: body mass index; CRP: C-reactive protein.

Figure 15-2 The TRL apoB-48 production rate (PR) and the TRL apoB-48 fractional catabolic rate (FCR) in subjects fed with low-fat snacks, moderate-fat snacks and high-fat snacks



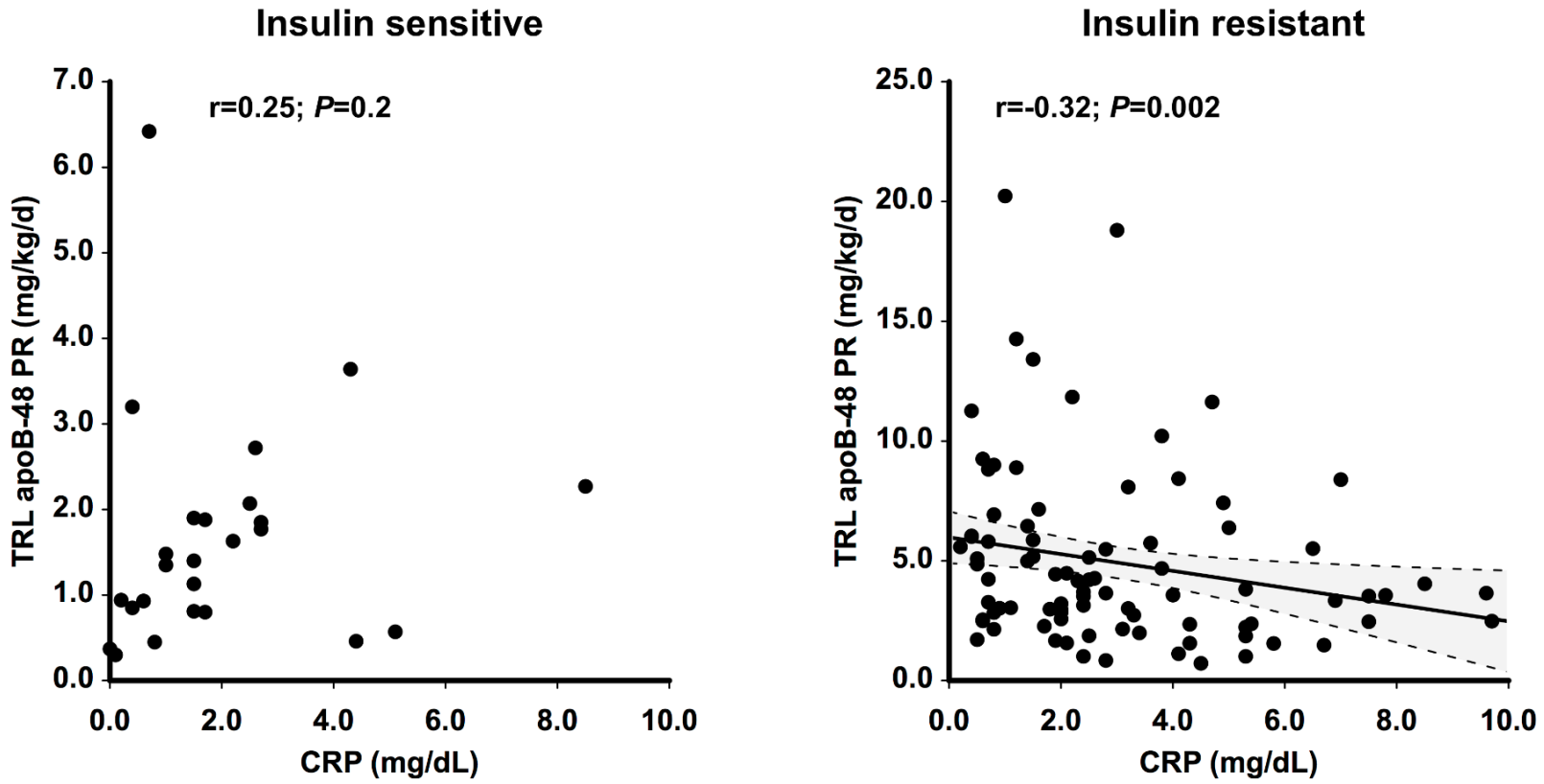
The TRL apoB-48 production rate (PR) and the TRL apoB-48 fractional catabolic rate (FCR) in subjects fed with low-fat snacks (22.4% of caloric intake, n=11), moderate-fat snacks (35.1% of caloric intake, n=27) and high-fat snacks (41.1% of caloric intake, n=113). The values are presented as the means ± SD. Body mass index, HOMA-IR and fasting triglyceride levels were added in the model as covariables. Columns with different letters are significantly different (P<0.05). TRL apoB-48 PR, TRL apoB-48 FCR, HOMA-IR and fasting TG level distributions were normalized using the Box-Cox transformation. TRL: triglyceride-rich lipoproteins; apo: apolipoprotein.

Figure 15-3 TRL apoB-48 kinetics in IS subjects and IR subjects



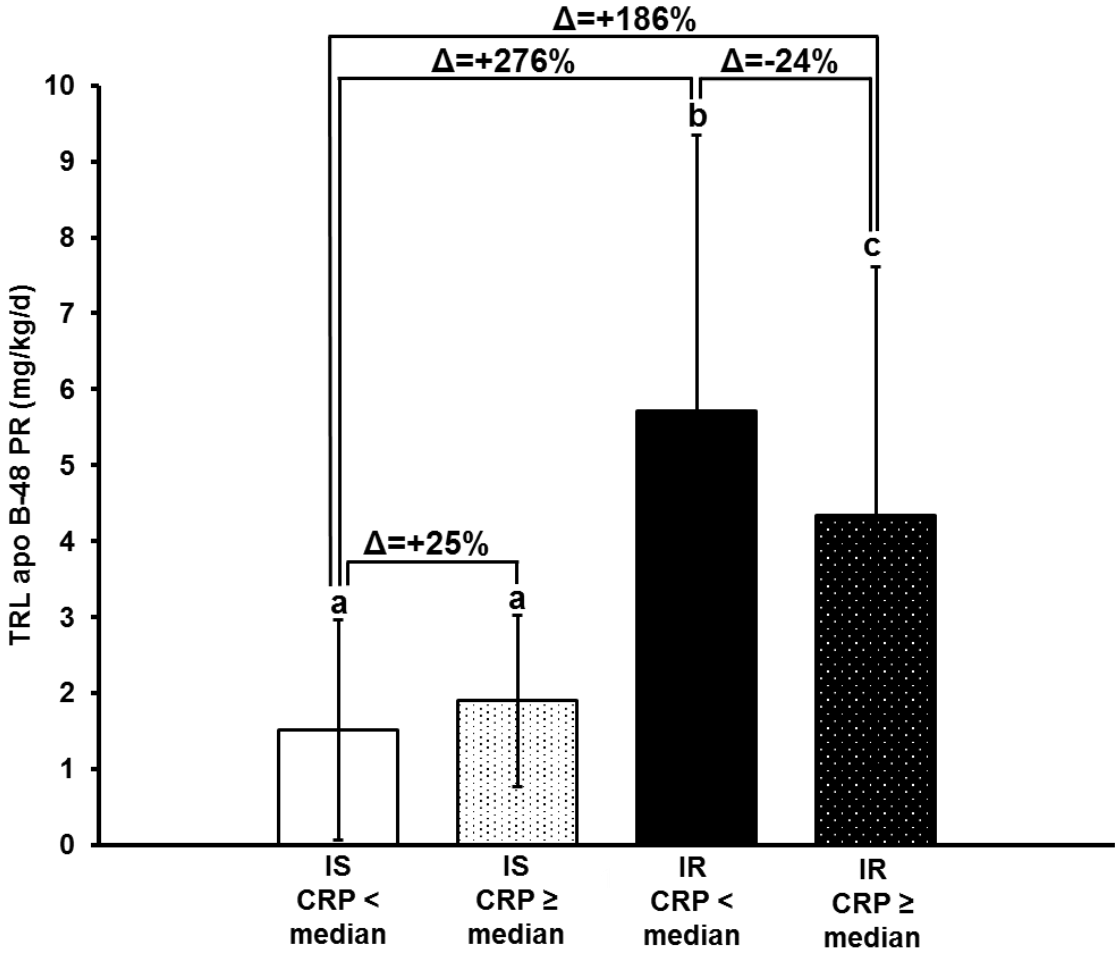
TRL apoB-48 kinetics in IS subjects (HOMA-IR<2.5 and TG < 1.5 mmol/L, n=24) and IR subjects (HOMA-IR ≥ 2.5 and TG ≥ 1.5 mmol/L or type 2 diabetes, n=91). A) TRL apoB-48 pool size (PS); B) TRL apoB-48 fractional catabolic rate (FCR); C) TRL apoB-48 production rate (PR). The values are presented as the means ± SD. Fat content of the kinetic snacks, age, BMI and therapy with metformin were added as a covariables in the models. TRL apoB-48 PS, TRL apoB-48 PR, TRL apoB-48 FCR distributions were normalized using the Box-Cox transformation. TRL: triglyceride-rich lipoproteins; apo: apolipoprotein; IS: insulin sensitive; IR: insulin resistant**.**

Figure 15-4 Correlation between TRL apoB-48 production rate (PR) and fasting C-reactive protein (CRP) levels in IS and IR subjects



Correlation (Spearman's coefficient) between TRL apoB-48 production rate (PR) and fasting C-reactive protein (CRP) levels in IS (HOMA-IR < 2.5 and TG < 1.5 mmol/L, n=24) and IR subjects (HOMA-IR ≥ 2.5 and TG ≥ 1.5 mmol/L or type 2 diabetes, n=91). The dotted lines indicate the 95% confidence intervals for the regression line. Prior to the assessment of correlations, a significant interaction was measured in a regression model between subject groups (IS vs. IR) and CRP levels for TRL apoB-48 PR (*P*=0.02). TRL: triglyceride-rich lipoproteins; apo: apolipoprotein; IS: insulin sensitive; IR: insulin resistant.

Figure 15-5 The TRL apoB-48 production rate (PR) in IS and IR subjects according to fasting C-reactive protein (CRP) levels



White columns represent IS subjects. Black columns represent IR subjects. Dotted columns represent subjects with CRP levels ≥2.20 mg/L (median). Values are presented as the means ± SD. Columns with different letters are significantly different (P<0.05). Differences (Δ) are expressed as % vs. IS subjects with CRP levels < median or vs. IR subjects with CRP levels < median. Fat content of the kinetic snacks, age, BMI and therapy with metformin were added as a covariables in the models. TRL apoB-48 PR distribution was normalized using the Box-Cox transformation. TRL: triglyceride-rich lipoproteins; apo: apolipoprotein; IS: insulin sensitive; IR: insulin resistant.

Supplemental material

Supplemental table 15-1 Nutritional composition of the snacks during the kinetic study

	Low-fat	Moderate-fat	High-fat
Total fat, %	22.4	35.1	41.1 ± 0.2
MUFA, %	11.0	14.9	24.2 ± 1.7
PUFA, %	5.6	4.7	8.5 ± 1.6
SFA, %	3.6	13.5	6.0 ± 0.0
Carbohydrates, %	62.7	49.0	44.0 ± 0.2
Proteins, %	16.1	15.9	15.4 ± 0.1

Subjects were fed 30 identical snacks during the kinetic study. MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.