UNIVERSITÉ DU QUÉBEC À MONTRÉAL

INFLUENCE DU PROFIL LIPIDIQUE MATERNEL SUR L'EXPRESSION DE RÉCEPTEURS À LIPOPROTÉINE DE FAIBLE DENSITÉ

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ABCA-1	Transporteur avec domaine liant l'ATP de type A1
ACAT	Acyltransférase acyl-CoA:cholestérol
Apo	Apolipoprotéine
ARN	Acide ribonucléique
ARNm	ARN messager
BCA	Acide bicinchoninique
BMI	Indice de masse corporelle
CE	Ester de cholesterol
CEH	Hydrolase d'ester de cholestérol
CEPT	Protéine de transfert des esters de cholestérol
CHUM	Centre hospitalier de l'Université de Montréal
CHUQ	Centre hospitalier de l'Université de Québec
FC	Cholestérol libre
FFA	Acide gras libre
GAPDH	Glyséraldéhyde-3-phosphate déhydrogenase
GDM	Diabète gestationnel
GDP	Gain de poids
HC	Cholestérol maternel plasmatique inférieur à la médiane
hCG	Hormone gonadotrophine chorionique humaine
HDL	Lipoprotéine de haute densité

HMGR	HMG-CoA réductase
hPL	Hormone placentaire lactogène humaine
IDL	Lipoprotéine de densité intermédiaire
IL-1β	Interleukine-1 bêta
IMC	Indice de masse corporelle
Insig	Protéine du gène activé par l'insuline
LC	Cholestérol maternel plasmatique inférieur à la médiane
LDL	Lipoprotéine de faible densité
LDL-R	Récepteur de lipoprotéine de faible densité
LOX-1	Récepteur au LDL oxydées de type lectine 1
NF-Y	Facteur nucléaire Y
nSREBP	SREBP mature, nucléaire
PPAR	Prolifératéur de peroxysome alpha
pSREBP	SREBP immature, précurseure
RE	Réticulum endoplasmique
S1P	Site 1 protéase
S2P	Site 2 protéase
SCAP	Protéine activatrice du clivage par les stérols
SD	Déviation standart
SEM	Erreur standard des moyennes
Sp1	Protéine de spécificité 1
SR-BI	Récepteur scavenger de classe B-I

SRE	Élément réponse aux stérols
SREBP	Protéines liant l'élément de réponse aux stérols
TC	Cholestérol total
TG	Triglycéride
TNF-α	Facteur de nécrose tumorale-alpha
VLDL	Lipoprotéine de très faible densité

RÉSUMÉ

L'obésité, avec les conséquences physiologiques qu'elle engendre, est sans doute une des pathologies entraînant le plus grand nombre de décès en Amérique du Nord. On note un accroissement de l'obésité chez les enfants qui semble pouvoir être corrélée au profil lipidique de la mère durant la grossesse. Cependant, les échanges lipidiques placentaires demeurent un volet de la science encore peu étudié. Dans le plasma, le cholestérol est véhiculé via les lipoprotéines, principalement par un certain type, soit les lipoprotéines à faible densité (LDL). Ces LDL natives sont reconnues par le récepteur aux LDL (LDLr) présent à la surface des syncytiotrophoblastes. Chez l'humain, le troisième trimestre de la grossesse associé à une hypercholestérolémie prononcée pouvant avoir comme conséquence d'engendrer des LDL oxydées. Ces particules sont considérées comme étant plus arthérogéniques, puisque leur affinité avec le LDLr est réduite. Ces LDL oxydées sont les ligands d'autres récepteurs soit le «lectinlike oxidized low density lipoprotein receptor-1» (LOX-1) et le «scavenger receptor type BI» (SR-BI). Par contre, la régulation de l'expression de ces différents récepteurs au niveau du placenta, en cas d'hypercholestérolémie, reste en grande partie inexplorée. Le but de cet étude est de caractériser la régulation de l'expression des différents récepteurs de LDL dans le placenta en fonction du profil lipidique maternel, soit de la concentration de cholestérol plasmatique, de l'indice de masse corporel pré-grossesse (IMC) et du gain de poids (GDP) en cours de grossesse. Les femmes sélectionnées sont dans un premier temps classées selon leur concentration plasmatique (inférieur à 7mM ou supérieur à 8mM), puis rétrospectivement classées selon leur IMC (normal, inférieur ou supérieur à la normale établie), ainsi qu'en fonction de leur GDP durant le grossesse (normal, inférieur ou supérieur à la normale). De plus, l'expression de ces différents récepteurs sera aussi quantifiée chez un groupe de femmes présentant un diabète gestationnel. Enfin, la localisation de ces récepteurs au niveau du syncytiotrophoblaste, soit au niveau de la membrane à bordure en brosse (BBM) ou de la membrane basale plasmique (BPM), sera déterminée. L'expression protéique des différents récepteurs dans le placenta est quantifiée par immunobuvardage de type Western et par immunohistochimie. Les résultats démontrent une diminution de l'expression du LDLr, chez les femmes ayant une concentration de cholestérol >8mM, qui semble être corrélée négativement à l'IMC et au GDP. Inversement, une augmentation de l'expression de LOX-1 est observée chez les femmes ayant une concentration de cholestérol <7mM et semble être corrélée positivement avec l'IMC, mais négativement avec le GDP. L'expression de SR-BI ne semble pas être modifiée. Ainsi, l'environnement placentaire est influencé par les IMC et GDP hors norme, ce qui, à long terme, pourrait être associé à une augmentation des problèmes cardiovasculaires. (Subventionné par IRSC)

Mots clés : Placenta, LDL, LDLoxydées, LDLr, LOX-1, SR-BI, diabète gestationnel

INTRODUCTION

L'obésité, aux États-Unis, a été identifiée comme étant devenue une épidémie, par le Centre du Contrôle et de la Prévention des maladies [1]. En effet, le taux d'enfants et d'adolescents ayant un poids au-dessus de la normale a triplé depuis les années 1980 se situant maintenant à environ 15% [1]. L'obésité est susceptible d'entraîner diverses pathologies, telles que l'athérosclérose, le diabète, l'hypertension, ainsi que des hypercholestérolémies, entraînant des maladies cardiovasculaires.

L'obésité complique entre 18% et 38% des grossesses [2]. Ainsi, des études récentes ont démontré une association étroite entre l'obésité et les complications prénatales, incluant la prééclampsie, le diabète gestationnel, la macrosomie ainsi que les accouchements par césarienne [3]. Il a été établi qu'un indice de masse corporelle (IMC) normal se situant entre 20 et 26 Kg/m^2 et un gain de poids idéal durant la grossesse (variant de 11 à 18 Kg) chez la mère diminuent les risques de complications périnatales [4]. Évidemment, des femmes ayant un poids anormal avant la grossesse, soit un IMC supérieur à 26 Kg/m^2 ou inférieur à 20 Kg/m^2, voient leurs risques de développer des complications durant la grossesse et à l'accouchement s'accroître [5].

Le placenta humain, un organe essentiel à la grossesse, est d'une très grande complexité. Fonctionnel après seulement quelques semaine suivant la conception, il assure à lui seul la responsabilité du transport des nutriments, des minéraux, des gaz et des déchets, donc, par le fait même, la survie du fœtus. À cet effet, l'échange du cholestérol entre la mère et son fœtus via les lipoprotéines demeure encore obscur. Le cholestérol est une molécule précurseur d'hormones importante et est le principal constituant des membranes cellulaires [6]. Le projet présent vise donc à confirmer par quels récepteurs, le cholestérol (via des lipoprotéines à faible densité, natives ou oxydées) est intégré dans les cellules trophoblastiques, et si l'expression de ces récepteurs est influencée par le profil lipidique de la mère au cours de la grossesse.

1. CHAPITRE I

ÉTAT DES CONNAISSANCES

1.1 LE PLACENTA HUMAIN

1.1.1 Caractéristiques générales du placenta

Le placenta humain est un organe fascinant par la complexité de son développement et la diversité de ces fonctions, toutes indispensables au déroulement adéquat de la grossesse et à la croissance fœtale. Cet organe temporaire consiste en une partie fœtale (le chorion) et une partie maternelle (la décidua). Le développement du fœtus est assuré par le transfert de nutriments du sang maternel et ce, via le placenta, un organe spécialisé dans l'échange de nutriments et dans l'excrétion de déchets produits par le fœtus [7]. Le placenta humain est de type hémochorial, c'est-à-dire que le chorion (la membrane entourant le fœtus) est en contact direct avec le sang maternel [8]. Il est composé du cordon ombilical, des membranes amniotiques et chorioniques, du parenchyme villeux et de la décidua [9].

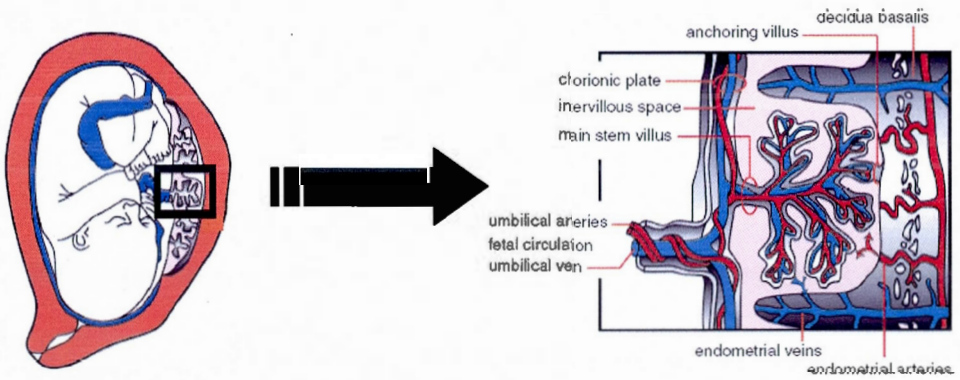

Figure 1.1 : Représentation schématique de la structure placentaire. Tiré de Fuchs et Elliger, 2004.

Le placenta étant indispensable à la survie du fœtus, il n'est pas surprenant qu'il croisse à un rythme qui excède la croissance du fœtus dans le premier trimestre de la grossesse [10]. Le cordon ombilical, assurant la liaison entre le placenta et le fœtus, est constitué d'une veine et de deux artères ombilicales. La veine ombilicale est chargée d'acheminer les nutriments au fœtus alors que l'artère ombilicale voie à l'excrétion des déchets, soit à l'inverse de la circulation normale [11].

1.1.2 Le développement

Suivant la fécondation, il y a transformation du zygote en morula, débutant ainsi le stade embryonnaire. Dès la deuxième semaine de gestation, il y a formation du blastocyte. Le détachement de la membrane pellucide de l'ovocyte permet à l'œuf de s'implanter dans la muqueuse utérine par son pôle embryonnaire. La blastocyte possède la capacité de se différencier en deux structures bien distinctes soit l'embryoblaste et le trophoblaste. C'est donc à partir de ce stade que se produit l'implantation; une étape caractérisée par un envahissement graduel de la paroi utérine par les cellules trophoblastiques. Via les enzymes protéolytiques, sécrétées par les trophoblastes, l'embryoblaste est maintenu par l'apport continuel en nutriments provenant de la digestion graduelle de la paroi utérine. Les nutriments libérés par cette digestion sont véhiculés dans la cavité du blastocyte où ils serviront au développement de l'embryoblaste [11]. Conjointement à l'implantation, l'instauration de mécanismes hormonaux, visant au maintien de la grossesse, se met en marche [12]. Via la gonadotrophine chorionique, l'action du corps jaune peut-être maintenue jusqu'au deuxième mois et assurer une division cellulaire continue ainsi que la poursuite de la grossesse. Avec la maturation, et de façon graduelle, le placenta devient autonome dans le contrôle hormonal régissant la grossesse [13].

Suivant l'implantation, le trophoblaste primaire se différencie en deux couches de cellules distinctes. La couche cellulaire interne se nomme cytotrophoblaste et la couche cellulaire externe se nomme syncytiotrophoblaste [14]. Dans la zone

d'implantation du blastocyste, le cytotrophoblaste extravilleux prolifératif, prolifère puis migre vers l'endomètre maternel et devient le cytotrophoblaste extravilleux (ou invasif). Durant sa différentiation, il remplace les cellules endothéliales des artères de l'endomètre maternel, devant les cellules géantes bi-ou trinucléées au niveau de la décidua. Enfin, les cytotrophoblastes villeux créent par fusion cellulaire le syncytiotrophoblaste. Le syncytiotrophoblaste qui résulte de la fusion des cytotrophoblastes recouvre les villosités choriales, établissant la principale interface mère-foetus. Dans le placenta humain mature, les cellules cytotrophoblastiques forment une unité relativement dispersée; leur nombre diminue graduellement au profit des cellules syncytiotrophoblastiques jusqu'à la fin de grossesse [14].

1.1.3 Le syncytiotrophoblaste humain

Le placenta est un syncytium composé de plusieurs types de cellules. La barrière placentaire se forme à partir d'une couche de trophoblastes, laquelle contrôle le passage des molécules de la circulation maternelle à la circulation fœtale [12]. La villosité choriale, l'unité structurale et fonctionnelle du placenta humain, apparaît dans sa construction définitive vers la troisième semaine suivant la fécondation [9]. Ces villosités sont grandement vascularisées et présentent des sinusoïdes afin de ralentir le sang maternel, ce qui facilite les échanges mère-fœtus. Au début de la grossesse, la couche de cytotrophoblastes se différencie en un syncytiotrophoblaste, celui-ci, très invasif, permet l'ancrage puis l'enfouissement complet du blastocyste dans la muqueuse utérine pour finalement s'y implanter [12].

1.1.4 Les membranes syncytiotrophoblastiques

Le syncytiotrophoblaste est une structure polynucléée caractérisée par la présence d'une membrane à bordure en brosse (BBM) se situant sur la face maternelle, ainsi que d'une

membrane plasmique basale faisant face à la circulation fœtale (BPM) [15]. De ce fait, le syncytiotrophoblaste est l'unique barrière sélective entre le sang maternel et fœtal, bien que certaines substances traversent le placenta via l'espace extracellulaire [16]. Chacune de ces 2 membranes possède son propre système structural et fonctionnel. Les BPM possèdent une activité adénylate cyclase hormono-dépendante [17, 18], des récepteurs β-adrénergique [19], une activité Na^+/K^+- ATPase [20], ainsi que deux types de transport de Ca^{2+}, dont l'un est dépendant de l'ATP [17, 18]. Quant aux BBM, celles-ci possèdent une activité phosphatase alcaline [17, 18], un seul système de transport de Ca^{2+} indépendant de l'ATP [21], et une série de récepteurs hormonaux [17, 18, 20, 22]. Ainsi, baignant dans le sang maternel, le syncytiotrophoblaste est capable d'assurer les fonctions d'échanges entre la mère et le fœtus, et ce, par divers mécanismes tels la diffusion simple, le transport facilité actif et via différents récepteurs [12]. En plus de ces fonctions d'échanges, le syncytiotrophoblaste a une fonction endocrine; il permet la sécrétion d'hormones stéroïdiennes (progestérone et œstrogène) et des hormones polypeptidiques, chorionique-gonadotrophine humaine (hCG), lactogène placentaire humaine (hPL), l'hormone de croissance placentaire (PGH)), ces hormones étant déterminantes dans le maintien et le bon développement de la grossesse [9]. Enfin, comme mentionné antérieurement, le syncytiotrophoblaste, qui est hautement polarisé, possède sur sa membrane apicale de nombreuses villosités, celles-ci étant grandement vascularisées et constituées de sinusoïdes qui ralentissent le sang maternel pour faciliter les échanges. Ces microvillosités sont le site de nombreux récepteurs, en particulier le récepteur aux lipoprotéines de faible densité (LDL), indispensable à l'apport du cholestérol maternel au placenta [12]. Bien que le transfert placentaire des lipides soit limité, ceux-ci jouent un rôle de premier plan au niveau du développement fœtal; le cholestérol étant essentiel à la croissance du fœtus. Par ailleurs, le mécanisme de transport des lipides au niveau placentaire demeure encore non résolu.

1.2 LIPIDES ET GROSSESSE

Durant la grossesse, le fœtus croit de manière exponentielle et requiert une grande quantité de cholestérol pour supporter la formation de membranes cellulaires, la synthèse d'hormones et enfin pour assurer le développement du système nerveux. En effet, le métabolisme maternel doit satisfaire aux demandes du fœtus en développement ainsi qu'aux demandes énergétiques de la mère. Le cholestérol sert, entre autre, à la production de la progestérone et la 17β-estradiol, deux hormones essentielles au bon déroulement de la grossesse [23]. Le début de la grossesse est considéré comme la phase anabolique, caractérisée par une augmentation de la production de triglycérides et une augmentation de la sortie des triglycérides (TG) de la circulation, ceci résultant en une augmentation du dépôt de gras dans les tissus maternels [24]. La fin de la grossesse est considérée comme la phase catabolique où il y a une augmentation de la libération d'acides gras libres par les adipocytes, causée par le fait que la mère soit insulino-résistante et par la stimulation d'hormones sensibles à la lipase, telle la lipoprotéine lipase via les hormones placentaires [25]. Il existe donc deux sources potentielles de cholestérol pour le fœtus. La source première étant le cholestérol endogène, synthétisé par le fœtus lui-même, et la seconde est exogène et provient des lipoprotéines maternelles qui sont transportées et internalisées par le placenta [26]. L'apport de cholestérol maternel est très important au début de la grossesse et devient minimal à la fin de la grossesse puisque les tissus fœtaux ont une grande capacité à synthétiser le cholestérol [6].

Ainsi, le fœtus et le placenta requièrent une grande quantité de divers composés lipidiques, notamment les acides gras à longues chaînes, pour la synthèse de composés plus complexes tels les glycérolipides, sphingolipides, glucolipides et esters de cholestérol [27]. Ces lipides complexes sont essentiels à la formation de composantes structurales et de réserves énergétiques [28, 29]. Les acides gras polyinsaturés sont aussi les précurseurs des prostaglandines et de la thromboxane, impliquées dans la modulation

de nombreux mécanismes cellulaires comme la prolifération cellulaire, les réponses immunes et l'activité des canaux ioniques [27, 30]. Tout au long de la vie, les acides gras et leurs dérivés peuvent induire des modifications fonctionnelles de divers mécanismes cellulaires au niveau d'une variété de tissus (poumons, foie, intestin, cœur, rein, muscles squelettiques, placenta). Parmi ces mécanismes, notons l'activation des canaux K^+, la stimulation du transport du glucose, la perturbation du flux calcique, la modulation de l'activation de la protéine kinase C, les modifications structurales et fonctionnelles des membranes, inhibition de la Na^+/K^+ ATPase, réduction de la capacité de liaison de l'angiotensine [27, 31] etc.

1.2.1 Les lipides

Les lipides forment un groupe très hétérogène, dont les structures peuvent varier énormément. Ils ont été principalement réunis en raison de leur insolubilité dans l'eau et de leur solubilité dans les solvants organiques [32]. Les lipides ont aussi été définis comme des molécules comportant une chaîne aliphatique (formé de groupements CH_2), seuls quelques acides gras à courte chaîne font exception à cette règle, tel l'acide butyrique [33]. Les acides gras sont rarement retrouvés à l'état libre dans la nature, et se trouvent essentiellement sous forme estérifiée comme constituants majeurs des différents autres lipides [32]. En règle générale, les acides gras, pouvant être saturés, insaturés et parfois hydrolysés ou ramifiés, sont de type monocarboxylique à chaîne linéaire non ramifiée, comprenant un nombre pair d'atomes de carbone, soit entre 4 et 36 [33].

1.2.2 Les triglycérides (TG)

Les graisses et les huiles retrouvées dans les plantes et les animaux sont essentiellement des TG (figure 1.2). Ces substances non polaires, insolubles dans l'eau sont des triesters d'acides gras et de glycérol :

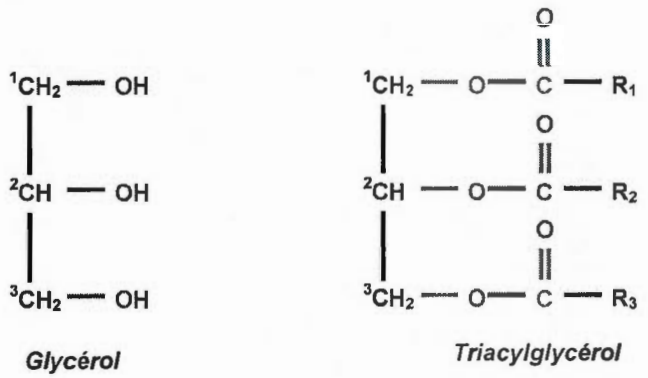

Figure 1.2. Structure chimique des TG. Tiré de **Voet et Voet, 1998.**

Les TG jouent un rôle de réserves énergétiques chez les animaux et ils constituent la catégorie des lipides la plus abondante, même s'ils ne sont pas des constituants des membranes biologiques [32]. En effet, ils représentent 10% du poids total d'un animal sain, et peuvent être plus élevés en cas d'obésité [33]. Les TG diffèrent selon la nature et la position de leurs trois résidus d'acides gras. Les TG simples ne contiennent qu'un seul type d'acide gras, tandis que les TG mixtes les plus courants contiennent deux ou trois résidus d'acide gras différents et sont nommés en fonction de leur position sur le résidu glycérol [32]. Chaque glycérol est estérifié avec trois acides gras, habituellement à longues chaînes tel le palmitate, le stéarate, l'oléate et le linoléate [34]. Durant la grossesse, une augmentation des TG de trois à quatre fois est observée [35]. Par contre, chez l'humain, il semble que ceux-ci ne puissent traverser directement le placenta; ils doivent être préalablement hydrolysés par une lipase. Ainsi, l'élévation de la lipoprotéine lipase durant les derniers jours de la grossesse, serait un indicatif du rôle primordial joué par les TG [36].

1.2.3 Le cholestérol

Le cholestérol (figure 1.3) est un constituant majeur des membranes plasmiques animales et se trouve en moindre quantité dans les membranes des organites cellulaires. Son groupe OH lui confère un léger caractère amphiphile, tandis que sa structure cyclique fusionnée lui confère une rigidité supérieure à celle des autres lipides membranaires [32].

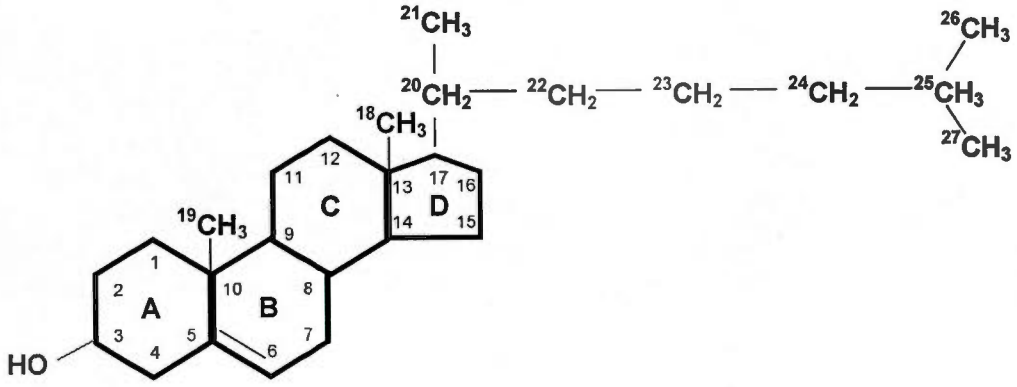

Figure 1.3. Structure chimique du cholestérol. Tiré de Voet et Voet, 1998.

1.3 LES LIPOPROTÉINES

Le cholestérol, de même que les TG et les phospholipides, sont transportés dans le plasma par un complexe nommé «lipoprotéine» (figure 1.2) [32]. Étant donné la nature hydrophobe des lipides, on ne les retrouve pas sous forme libre dans le plasma sanguin, ils sont liés par des interactions non covalentes à des protéines amphipathiques appelés apolipoprotéines afin de former des particules nommées lipoprotéines. Les lipoprotéines sont caractérisées par un cœur insoluble d'ester de cholestérol (EC) et de TG entouré par une coquille de phospholipides amphipathiques et spécialisés, les apolipoprotéines [37]. La disposition des constituants de la lipoprotéine est déterminée par la polarité de ses composants. Une molécule polaire se situera à la périphérie (soit en contact avec le sang), tandis qu'une molécule hydrophobe se retrouve au centre (soit dans la cœur de la lipoprotéine) [38]. La fonction principale des lipoprotéines est de transporter un bon nombre de nutriments hydrophobes, principalement des lipides, à travers la circulation systémique.

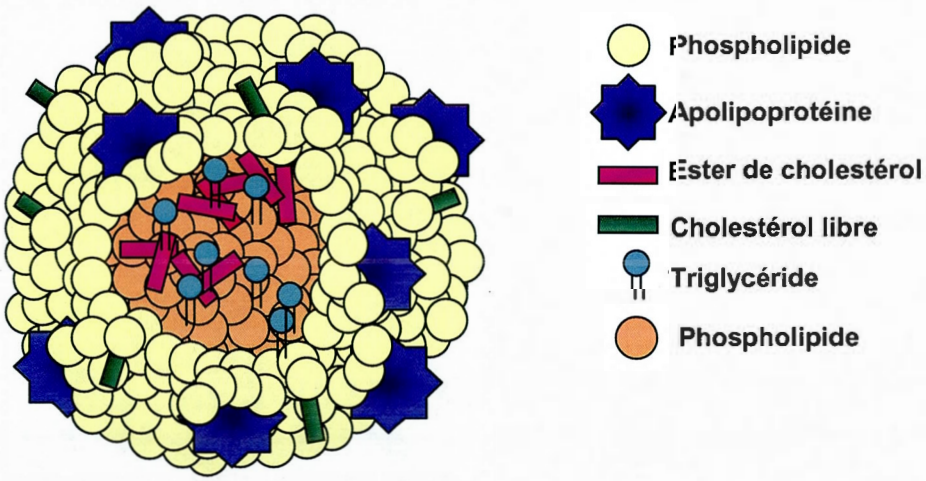

Figure 1.4 : Structure de la lipoprotéine. La lipoprotéine est composée d'un cœur contenant des esters de cholestérols ainsi que des triglycérides Cette particule sphérique est formée majoritairement de phospholipides, d'apolipoprotéines et de cholestérol libre.

1.3.1 Les classes de lipoprotéines

Les lipoprotéines sont classées selon leurs densités, en ordre croissant, soit les chylomicrons, les VLDL (very low density lipoprotein), les IDLs (intermediary density lipoprotein), les LDL (low density lipoprotein) et les HDL, (high density lipoprotein) (Tableau I). Ces différentes densités sont liées à la quantité relative de lipides comparativement aux protéines de la lipoprotéine. Les apolipoprotéines (apo), différant pour chacun des types de lipoprotéines, ont un rôle important dans le transport et le métabolisme des lipides. Elles ont un domaine structural reconnu par les différents récepteurs [32].

Tableau I. Propriétés physiques et chimiques des différentes classes de lipoprotéines plasmatiques humaines

CLASSE	DENSITÉ (g/ml)	APO	FONCTION	ORIGINE
Chylomycron	0,93	A, B100, C, E	Transport de TG	Intestin
VLDL	0,95-1,006	B100, C, E	Transport de TG	Foie (85%) Intestin (15%)
IDL	1,006-1,019	B100, C, E	Transport de TG	Catabolisme des VLDL
LDL	1,019-1,063	B100	Transport du cholestérol	Catabolisme des IDL
HDL	1,063-1,210	A, C, E, D	Transport du cholestérol	Intestin Foie Catabolisme des VLDL et chylomycrons

1.3.2 Les chylomycrons

Ayant la plus faible densité, les chylomycrons, composé majoritairement de TG, forment la première classe de lipoprotéines. Ce sont les lipoprotéines responsables du transport des lipides alimentaires du tractus intestinal jusqu'à la circulation sanguine. Ils sont synthétisés par l'intestin dans les cellules mucosales du duodénum et du jéjunum pendant l'absorption des lipides [39]. Leurs fonctions principales se situent au niveau du transport d'acides gras sous forme de TG aux sites cellulaires de captation et d'utilisation [39].

1.3.3 Les VLDL et les LDL

Les VLDL contiennent environ 10% de protéines, 65% de TG et 25% de cholestérol [40]. Ils jouent un rôle dans le transport des TG des cellules hépatiques vers les autres cellules. De ce fait, un régime alimentaire riche en lipides favorise la production de VLDL et la formation de plaques athéromateuses associées aux maladies cardiovasculaires. Les lipoprotéines majeures chez l'humain sont les LDL et les HDL. Les LDL contiennent 25% de protéines, 20% de TG et 55% de cholestérol [40]. Les LDL proviennent du catabolisme des VLDL. La cascade VLDL/LDL commence avec l'assemblage des lipoprotéines contenant l'apo-B100 dans le réticulum endoplasmique des hépatocytes, suivi par une maturation dans l'appareil de Golgi et enfin de leur sécrétion [41]. Une fois dans la circulation, les TG des VLDL sont hydrolysés par la lipoprotéine lipase, un processus menant à la formation de lipoprotéine de densité intermédiaire (IDL), ceux-ci étant des résidus transitoires du catabolisme des VLDL. Certaines IDL produites peuvent être catabolisées directement par le foie tandis que celles séjournant dans le plasma sont transformées par la lipase hépatique (LH) et la lipoprotéine lipase (LPL) en LDL [42].

1.3.4 Les HDL

Enfin, la dernière classe de lipoprotéines regroupe les lipoprotéines de haute densité (HDL). Ces dernières contiennent 50% de protéines, 37% de TG et 13% de cholestérol [43]. Elles sont synthétisées au niveau du foie et de l'intestin ou sont des descendantes directes des produits du catabolisme des chylomycrons et des VLDL. Les HDL assurent une fonction inverse des LDL; elles participent au transport inverse du cholestérol des tissus extrahépatiques aux cellules du foie pour la réutilisation ou l'excrétion biliaire [32]. Les HDL sont formées suivant l'action de trois enzymes principales : la lécithine : cholestérol transférase (LCAT), la protéine de transfert de phosphatidylcholine (PCTP) ainsi que la protéine de transfert d'ester cholestérol (CETP) [32]. La cascade des HDL débute lorsqu'une HDL naissante, provenant d'une molécule d'apo-AI pauvre ou dépourvue de lipides, sécrétée par le foie ou dissociée d'une HDL mature, s'associe à du cholestérol libre ainsi que des phospholipides provenant des tissus périphériques et de la lipolyse des lipoprotéines riches en TG [32]. Ensuite la LCAT se charge, sur plusieurs cycles, de convertir le cholestérol libre en esters de cholestérol, pour finalement produire une HDL mature et sphérique. Grâce à l'action de la CEPT, qui échange des TG provenant des VLDL, IDL et LDL contre des esters de cholestérol, les HDL matures perdent leurs esters de cholestérol [32].

1.4 MÉTABOLISME GÉNÉRAL DU CHOLESTÉROL

Les LDL sont les principaux transporteurs de cholestérol estérifié et non estérifié aux tissus périphériques. Produits à partir des VLDL, ce type de lipoprotéines est associé aux apoB-100. Le rôle principal des LDL est de distribuer le cholestérol aux tissus extra-hépatiques pour l'utilisation de nombreux processus, incluant la production d'hormones stéroïdiennes et la formation de membranes cellulaires [26]. Le maintien de la concentration intracellulaire de cholestérol est attribuable à plusieurs éléments régulateurs : (a) l'activité de l'acyl-CoA : cholestérol *O*- acyltransférase (ACAT) (b) l'activité de la 3-hydroxy-3-méthylglutaryl-CoA réductase (HMG-CoAr) ; (c) l'activité de la 7∝-hydroxylase (7-OH Chol) et (d) à l'expression des récepteurs à LDL à la surface de la cellule [37].

1.4.1 ACAT

L'ACAT, limitante dans le métabolisme du cholestérol, permet aux cellules de stocker le cholestérol, en catalysant son estérification [44]. En effet l'ACAT, une enzyme microsomale catalyse la formation intracellulaire d'ester de cholestérol à partir du cholestérol et de l'oléyl-CoA [45]. Dans les cellules riches en cholestérol, l'estérification est augmentée, régulant à la hausse l'activité de l'ACAT, sans aucune modification de celle-ci au niveau de l'ARNm [46].

1.4.2 HMG-CoA réductase

L'enzyme HMG-CoA réductase, une glycoprotéine située au niveau du réticulum endoplasmique des cellules du foie et du placenta [47], catalyse la réduction de l'HMG-CoA et de l'acétate, en présence de deux molécules de NADPH, afin de générer de l'acide mévalonique, le mévalonate étant une molécule précurseur du cholestérol [46, 48]. Ainsi, quand les quantités de mévalonate ou de cholestérol dans les LDL chutent, la quantité de HMG-CoA réductase exprimé est fortement augmentée (jusqu'à 20 fois), cela grâce à une augmentation de la synthèse de l'ARNm de l'enzyme accompagnée d'une diminution de sa dégradation [32, 49].

1.4.3 7α-hydroxylase

La 7∝-hydroxylase catalyse la dégradation du cholestérol dans les cellules, via la formation d'acides biliaires. Cette voie de dégradation par la 7∝-hydroxylase entraîne la transformation du cholestérol en 7∝-OH cholestérol [50]. Cette enzyme est localisée dans le cytoplasme des hépatocytes et son activité est dépendante du NADPH [46]. Enfin, cette enzyme est principalement régulée par le taux de sels biliaires arrivant dans le foie [46].

1.5 LES LDL NATIVES

1.5.1 Le récepteur à LDL native

Les LDL se lient à des récepteurs spécifiques localisés sur la même membrane plasmique des cellules libres. Le récepteur aux LDL (LDLr) est un récepteur à lipoprotéine prototype exprimé dans tous les tissus utilisant les lipoprotéines [37]. Le LDLr a été découvert par Brown et Goldstein, en 1973, durant leurs recherches sur les bases moléculaires découlant de l'hypercholestérolémie familiale [51, 52]. Leurs travaux ont démontré que des fibroblastes d'individus normaux lient les LDL avec une grande affinité et suppriment la synthèse de cholestérol endogène lorsqu'ils sont en culture, en présence de LDL, contrairement aux fibroblastes provenant d'individus souffrants d'hypercholestérolémie familiale qui ne suppriment pas la synthèse de cholestérol endogène et qui ne peuvent incorporer les LDL-cholestérol du milieu de culture. Ce n'est qu'environ 10 ans plus tard que le gène encodant le LDLr fut cloné et séquencé [53, 54]. Il est estimé que 70% des LDLr se retrouvent au niveau des cellules hépatiques, mais, toutes les cellules ayant besoin de cholestérol sont susceptibles d'exprimer la protéine du LDLr à leur surface [55]. En effet, l'association du LDL avec le LDLr a été démontrée sur des fractions de membranes non-purifiées de placenta humain à terme [56], ainsi qu'au niveau des membranes en bordure en brosse (BBM) de placenta humain, et ce, dès la sixième semaine de grossesse [57], ainsi que dans du tissus à terme [58].

Le LDLr fait partie de la superfamille des récepteurs à LDL comprenant aussi le VLDLr (récepteur à VLDL), la LRP (protéine apparenté au LDLr) et la mégaline. Le LDLr présente une capacité de liaison relativement étendue au niveau des lipoprotéines, via les apolipoprotéines; il peut reconnaître l'apoB-100 et l'apoE [59]. Le LDLr possèdent 2 principales fonctions; il fournit le cholestérol aux cellules, et il retire les lipoprotéines riches en cholestérol de la circulation afin de prévenir leur accumulation. Ainsi, une déficience en LDLr, connue sous le nom d'hypercholestérolémie familiale, peut engendrer des taux de cholestérol dans le sang, de trois à cinq fois plus élevés que la

normale [32]. Il s'ensuit alors des dépôts de cholestérol dans leur peau et dans leur tendon sous la forme de nodules jaunes, appelés xanthomes. Les hétérozygotes développent des symptômes de maladies coronariennes après 30 ans. Par contre, les homozygotes, beaucoup plus sévèrement atteints décèdent d'infarctus du myocarde dès l'âge de 5 ans, provoqué par la formation rapide d'athéromes [32].

1.5.2 La structure protéique du LDLr

Le LDLr mature est une glycoprotéine transmembranaire de 839 acides aminés possédant un seul domaine transmembranaire. Lors de sa migration vers la surface de la cellule, la protéine du LDLr subit une glycosylation dans l'appareil de Golgi qui donne lieu à la protéine mature. Le LDLr est constitué de 5 domaines distincts ayant des fonctions individuelles (figure 1.3) :

1) Une région extracellulaire N-terminal formé de 292 acides aminés ayant une répétition de 7 séquences d'environ 40 acides aminés chacune. Ces séquences contiennent 6 cystéines impliquées dans la formation de ponts disulfures afin de maintenir la stabilité de la protéine. De charge négative, ce domaine est responsable de la liaison du récepteur à ces ligands (apoB-100 et apoE) chargés positivement [60].
2) Une seconde région extracellulaire, cette fois formée de 400 acides aminés contenant des répétitions (riches en cystéine) de type précurseur du facteur de croissance épithéliale (EGF) [53] qui sont séparées par des régions contenant les motifs YWTD (pauvres en cystéine) responsables de la dissociation des ligands dans les endosomes en présence d'un faible pH [61] ;

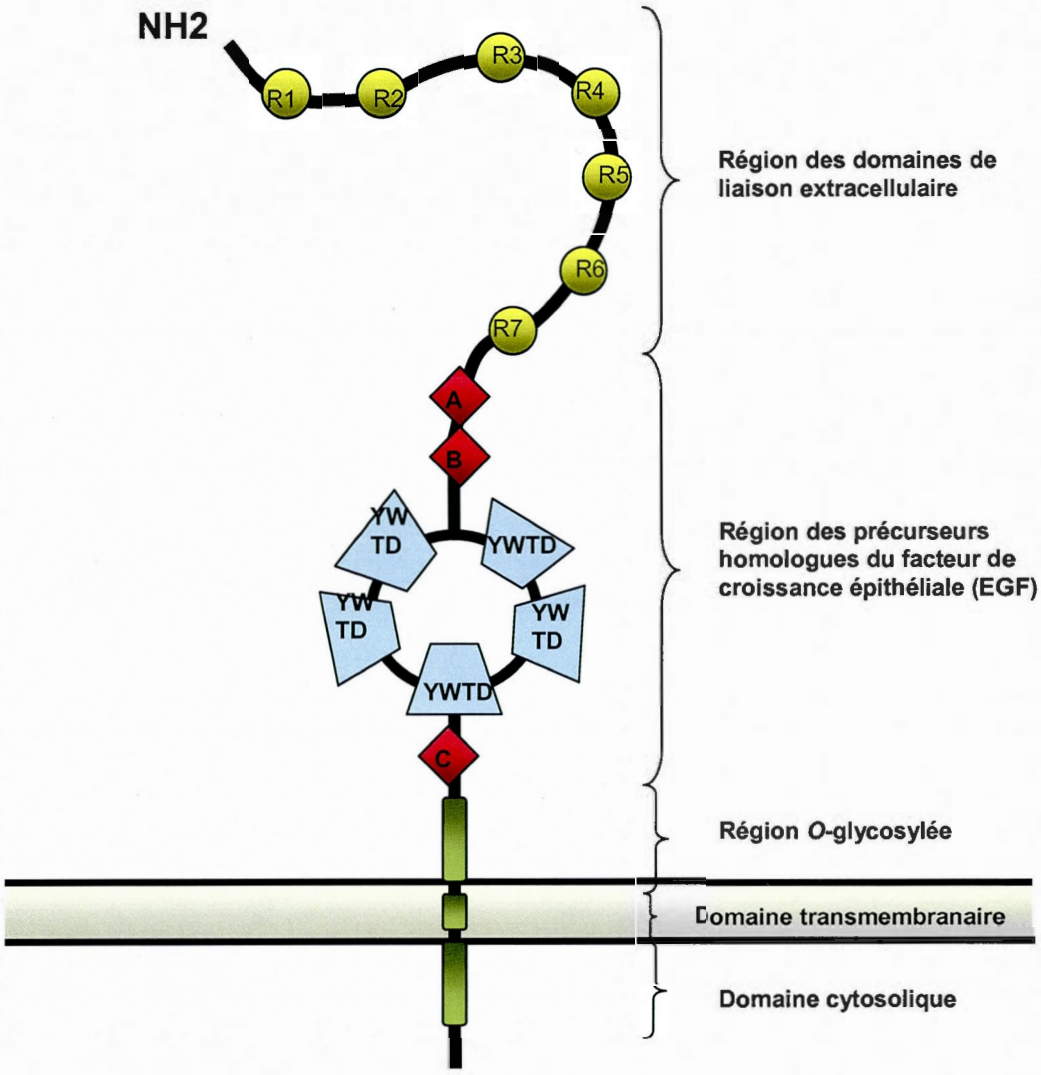

Figure 1.5 : Représentation schématique de la structure protéique du LDLr

3) Une région glycolysée par des sucres *O*-liés servant d'espace entre le domaine fonctionnel du LDLr et la surface de la cellule [60]. Cette région, composée de 58 acides aminés (riche en sérine et thréonine) est située juste au dessus de la membrane cytoplasmique. Ces chaînes de carbohydrates permettraient de tenir les récepteurs éloignés de la surface membranaire [54] ;
4) Un domaine transmembranaire servant à l'ancrage à la membrane. Cette région de 22 à 25 acides aminés possède aussi un rôle probable dans la transduction de signaux [60];
5) Une queue cytoplasmique de 50 acides aminés contenant entre autres des éléments séquentiels de type motif NPXY (asparagine (N)-proline (P)-acide aminés X- tyrosine (Y) [37], ces motifs permettent de diriger le LDLr dans les puits tapissé [62].

Le LDLr est synthétisé en un précurseur ayant un poids moléculaire de 120 kDa, les sucres N et *O*-liés sont ajoutés au précurseur du LDLr dans le réticulum endoplasmique ainsi que dans l'appareil de Golgi, ce qui résulte en une augmentation du poids apparent du LDLr à environ 160kDa [63]. Cette glycolysation fournit une tige rigide permettant au domaine intracellulaire de s'étendre dans l'espace extracellulaire [64]. Enfin, cette glycolysation protège le récepteur de la dénaturation durant le recyclage [64].

1.5.3 Mécanisme de la captation du cholestérol par endocytose

Le LDLr lie les lipoprotéines riches en cholestérol, contenant l'apoB-100 et/ou apoE et entraîne leur internalisatios endocytique. Le domaine du récepteur liant les LDL a des interactions électrostatiques avec les résidus chargés positivement de l'arginine et la lysine de l'apo-B100 présente sur la LDL. Le LDLr migre sur une région de la membrane plasmique spécialisée dans l'endocytose; les puits tapissés de clathrine [32]. La clathrine est une protéine couvrant le côté cytoplasmique de la membrane. Une fois la LDL liée au LDLr, les protéines de clathrine entraînent l'endocytose. Lorsque la vésicule est dans la cellule, les protéines de clathrine se dissocient spontanément de la vésicule endosomale [32]. Puisque le pH de la vésicule diminue pour former le lysosome, la LDL se dissocie alors de son récepteur lequel est recyclé à la surface de la cellule. La lipoprotéine est ensuite dégradée en acides aminés et les esters de cholestérol sont hydrolysés en cholestérol libre et en acide gras, libérant les lipides dans le cytosol [32]. Puisqu'un excès de cholestérol dans la cellule pourrait nuire à l'intégrité structurelle de ses membranes, divers moyens sont mis en branle afin d'éviter une accumulation excessive de cholestérol. Ainsi, ce nouvel arrivage de cholestérol libre réduit temporairement le nombre de LDLr à la surface de la cellule afin de diminuer l'incorporation de LDL [65] et diminue l'activité de HMG-CoA réductase, une enzyme clé de la synthèse du cholestérol endogène [66]. Enfin, le surplus de cholestérol libre dans la cellule peut être entreposé de façon sécuritaire sous forme estérifiée grâce ACAT [67]. En fait, la captation des LDL par le LDLr est indispensable à deux niveaux dans l'homéostasie des lipides : il distribue les lipides essentiels requis pour maintenir les fonctions cellulaires en plus de réguler les concentrations de lipoprotéines riches en cholestérol dans la circulation [32].

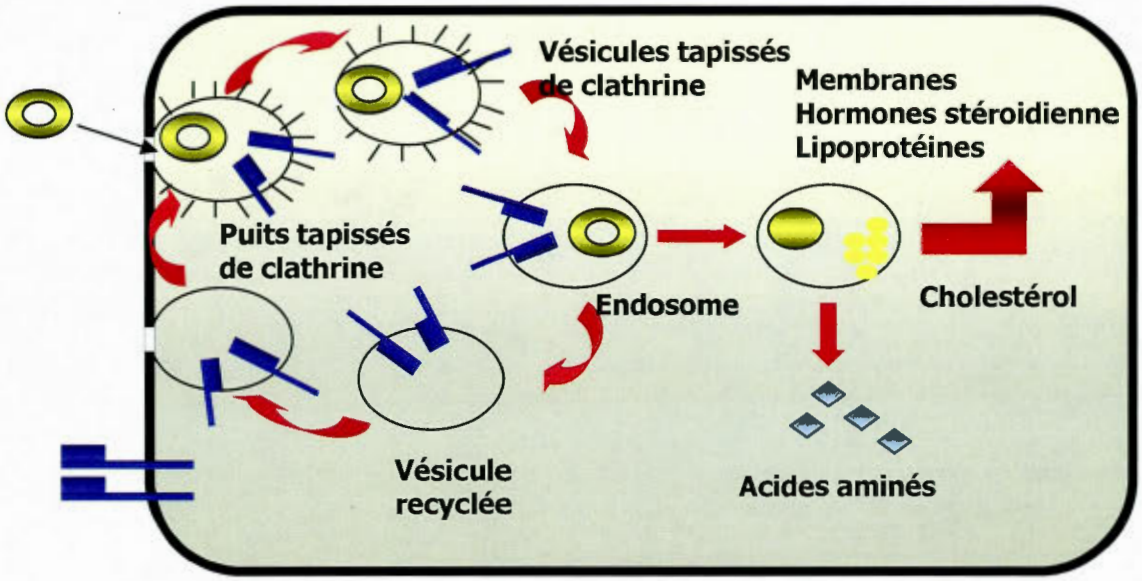

Figure 1.6 : Mécanisme de captation du cholestérol par endocytose.

1.6 Les LDL oxydées

Le métabolisme lipidique, altéré durant la grossesse, est caractérisé par un niveau de cholestérol normal ou faible durant les deux premiers trimestres, suivi par une hypercholestérolémie durant le dernier trimestre [68]. La phase anabolique du début de la grossesse produit des changements métaboliques qui contribuent à augmenter la lipogénèse et le stockage des graisses en vue de la phase catabolique de fin de grossesse au cours de laquelle le fœtus croit rapidement [24]. La résistance à l'insuline durant la grossesse augmente la lipolyse au niveau des tissus adipeux, menant à une hausse du flux d'acide gras vers le foie, ceci entraînant à son tour la synthèse de VLDL [24]. Aussi, la résistance à l'insuline réduit l'activité de la lipoprotéine lipase, une enzyme insulino-dépendante responsable de la clairance des VLDL du plasma [69]. Ainsi, les VLDL restent plus longtemps dans le plasma et mènent ultimement à l'accumulation de LDL. Enfin, une augmentation de LDL est directement associée avec le développement de l'athérosclérose. Le stress oxydatif, fortement lié à l'athérosclérose, peut être définit comme étant un déséquilibre entre les radicaux libres dommageables (par exemple les lipides oxydés) et les antioxydants protecteurs. Ainsi, les modifications oxydatives au niveau des LDL sont une étape importante dans le développement de l'athérosclérose. Le processus d'oxydation des LDL est un phénomène qui altère aussi bien la composition de la lipoprotéine en cholestérol, en acides gras et en apolipoprotéines. Étant donné leurs doubles liaisons, les lipides les plus vulnérables à l'oxydation, par les radicaux libres, sont les acides gras polyinsaturés. Ensuite, ces modifications chimiques vont se propager aux autres lipides selon une réaction qui s'attaque aux acides gras dans un ordre aléatoire, entraînant la dégradation et la libération de fragments lipidiques. Il y aura alors formation de peroxydes lipidiques et du produit de leur dégradation, les aldéhydes, dont l'accumulation peut-être directement cytotoxique. Ainsi formé, les aldéhydes peuvent se lier à l'apo-B100 des LDL (partie protéique) et donc modifier son activité physiologique [70]. Lorsqu'il y a transformation de l'apoB-100 et que chargée négativement, celle-ci n'est plus reconnue par le LDLr, mais bien par des récepteurs macrophages

«scavengers» retrouvés, entre autre au niveau de la membrane plasmique des macrophages. Ces mêmes récepteurs entraînent les LDL modifiées dans un processus dit arthérogène, puisque cette voie ne subit aucun rétrocontrôle métabolique : il n'y a donc aucune diminution de la synthèse intracellulaire de cholestérol, ni de limitation de la synthèse intracellulaire de cholestérol à la surface des cellules. Ceci étant, il se produit une absorption excessive de cholestérol dans les macrophages, menant à la formation de cellules spumeuses «foam cells», une étape clé dans le développement de l'athérosclérose [71].

Il existe une grande variété de particules correspondant aux LDL oxydées, caractérisées par des différences fonctionnelles; (a) les LDL légèrement oxydées (LDL-Lox) qui elles entraînent la sécrétion de facteurs inflammatoires et (b) les LDL fortement oxydées (LDL-Fox) [72, 73], celles-ci étant cytotoxiques et conduisent à la formation de cellules spumeuses. Enfin, le degré d'oxydation des LDL semble avoir un effet sur l'affinité des LDL avec leurs récepteurs [74]. Une fois oxydés, les LDL sont responsables d'effets variés; elles peuvent, entre autres, induire des changements fonctionnels au niveau des cellules endothéliales, en stimulant l'expression des molécules d'adhésion [75] et induire le développement de lésions athérosclérotiques. Une étude effectuée en 2002 confirme que, durant la grossesse, il se produit des changements marquants au niveau du cholestérol, des TG et des sous-fractions de LDL (petites et denses) sériques, lesquels étant normalement associés à une augmentation du risque de maladie coronarienne [24].

1.6.1 Récepteur aux LDL oxydées : LOX-1

Les LDL oxydées n'étant pas reconnues par le LDLr, celles-ci se fixent à d'autres récepteurs aussi exprimés au niveau du placenta, soit le Leptinlike Oxidized Low Density Lipoprotein Receptor-1 (LOX-1). Cloné en 1997, sur des cellules endothéliales aortiques bovine, par Sawamura et ses collaborateurs, LOX-1 est un récepteur à LDLox relativement récent [76]. Les chercheurs ont par la suite démontré la capacité de LOX-1 à lier, internaliser et dégrader les LDLox au niveau des cellules endothéliales [76]. La présence d'ARN messager de LOX-1 a été démontrée dans des organes hautement vascularisés, tels que le placenta, les poumons et les reins [77]. D'ailleurs, une étude récente, faite au niveau de cellules de lignée trophoblastique (JAR), indique que LOX-1 y est exprimé et que son expression protéique semble varier entre 40 et 50% en fonction de l'apport en LDL oxydées [78].

1.6.2 La structure protéique de LOX-1

LOX-1 est une protéine membranaire de type II d'environ 47 kDa. De part sa structure, il appartient à la famille des lectines de type C, lesquelles lient les hydrates de carbones via un mécanisme Ca^{2+}-dépendant et possèdent 4 domaines distincts : une courte région cytoplasmique N-terminal, un domaine transmembranaire, un domaine «neck» connecteur et un domaine «lectin-like» à l'extrémité C-terminale [79].

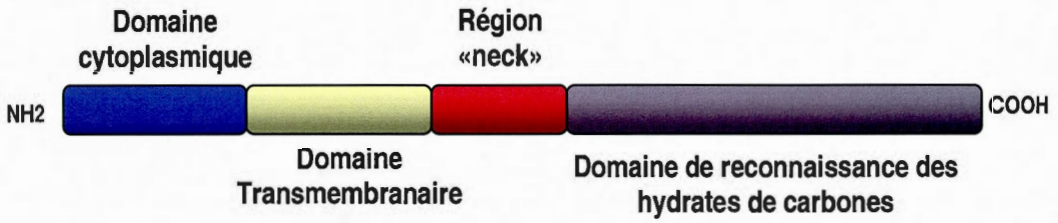

Figure 1.7 : Représentation schématique des domaines composant LOX-1. Adapté de Mehta et al. (2006).

Ce récepteur, composé de 273 acides aminés, est initialement synthétisé en une protéine précurseur de 40 kDa, nommée pré-LOX-1. Pré-LOX-1 est éventuellement glycolysée en une protéine mature et transportée à la surface de la cellule [80]. Afin de comprendre comment LOX-1 reconnaît les LDLox, il est impératif de caractériser les structures fonctionnelles de LOX-1. Malgré les recherches intensives de nombreux chercheurs dans la dernière décennie, les fonctions de ces différents domaines demeurent encore obscures. En 2005, Chen et ses collaborateurs, ont confirmé que la fonction du domaine «lectin-like» de LOX-1 est impliquée dans la liaison des LDLox [81]. Cette même équipe a trouvé que la localisation à la surface cellulaire est dépendante d'un motif chargé positivement présent dans la région cytosolique juxta-membranaire de LOX-1 [81]. Enfin, leurs travaux les plus récents ont démontré que le domaine «neck» n'a pas de rôle spécifique au niveau de la liaison du ligand [81, 82]. Par ailleurs, une autre étude récente effectuée par Ohki et ses collaborateurs, a démontré, suivant une analyse de la structure cristalline du domaine extracellulaire de LOX-1, qu'une courte région située dans le domaine «neck» formait un homodimère lequel était lié par un pont disulfure interchaîne indispensable pour l'activité de liaison de LOX-1 [83].

1.6.3 Les différents ligands de LOX-1

Il existe quatre groupes de substances ayant été identifiées comme étant des ligands potentiels de LOX-1 : (1) les lipoprotéines modifiées (LDLox, LDL acétylées, HDL hypo-chloridées) [84, 85]; (2) les agents chimiques polyanioniques [86]; (3) les phospholipides anioniques [85, 87] et (4) des ligands cellulaires (cellules apoptotiques et âgées, plaquettes activées et bactéries) [87-89]. Ainsi, le potentiel de liaison de ces ligands à LOX-1 explique la versatilité de ces fonctions physiologiques.

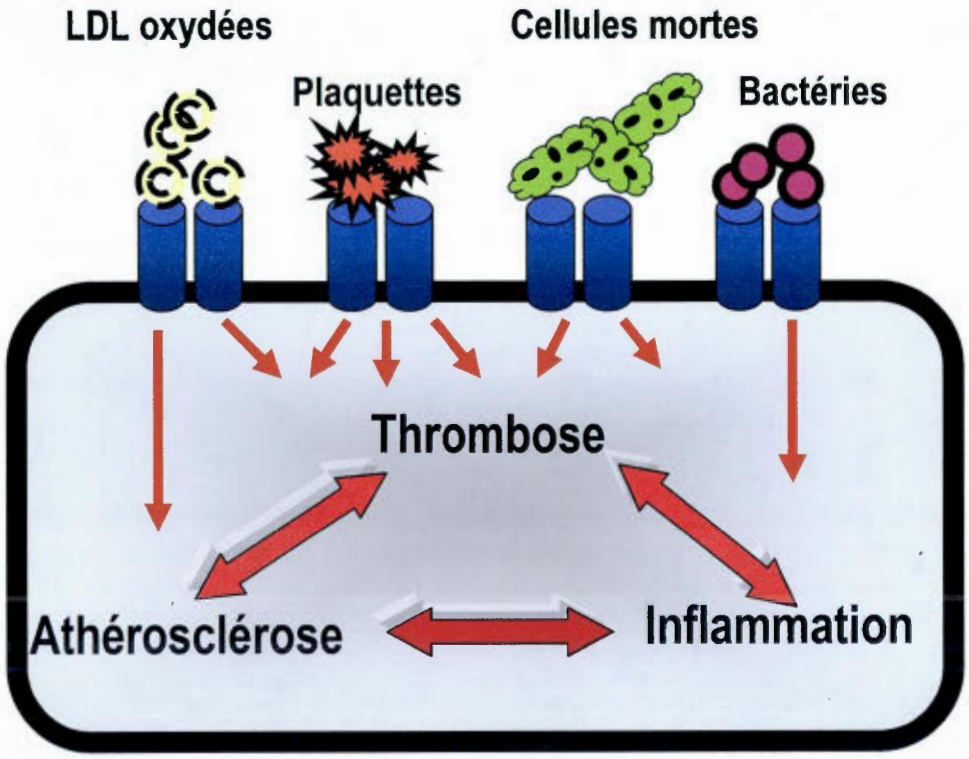

Figure 1.8 : Schéma des différentes interactions de LOX-1 avec ces différents ligands, résultant en une variété d'états physiologiques. Tiré de Chen et al., 2002.

1.6.1.3 Relation de LOX-1 avec l'athérosclérose

La contribution de LOX-1 au niveau de l'athérosclérose est supportée par plusieurs évidences : (I) LOX-1 démontre une forte activité pour la liaison, l'internalisation et la dégradation protéolytique des LDLox [76]; (II) les LDLox activent LOX-1 et induisent des dysfonctions endothéliales [90, 91]; (III) outres les LDLox, d'autres médiateurs de l'athérosclérose tel l'angiotensine II et certaines des cytokines régulent à la hausse LOX-1; (IV) LOX-1 est dynamiquement régulé à la hausse par des conditions proathérogéniques comme le diabète, l'hypertension et une dyslipidémie [92-96] et finalement (V) LOX-1 est présent dans les cellules dérivées d'athéromes et il est retrouvé en grande quantité *in vivo*, dans les lésions athérosclérotiques chez l'humain et les animaux [92, 97]

1.7 Récepteur à LDL oxydés : SR-BI

Un second récepteur pouvant lier les LDL modifiées, soit oxydées et acétylées, mais principalement les HDL, est le récepteur Scavenger de type 1 (SR-BI) [98]. Le récepteur humain est appelé CLA-1 (CD36 and LIMPII-analogous-1) [99]. CLA-1 fait donc référence au SR-BI humain, puisqu'il présente une séquence similaire à 81% avec celle du hamster [100]. En plus de leurs séquences très similaires, ces 2 récepteurs possèdent une distribution tissulaire identique et ils sont en mesure de lier les mêmes ligands [102, 103]. Ainsi, dans le présent document le terme «SR-BI» inclut aussi l'homologue humain «CLA-1», afin de faciliter la compréhension du texte.

1.7.1 La structure protéique de SR-BI

Le SR-BI est une glycoprotéine membranaire, membre de la famille des CD36, de 509 acides aminés avec un poids moléculaire apparent de 82 kDa [100]. Le SR-BI est constitué d'une large boucle extracellulaire ancrée dans la membrane à chaque extrémité par un domaine transmembranaire (figure 1.7). La partie intracellulaire amino-terminale est courte, comparativement à la partie intracellulaire carboxy-terminale. La boucle extracellulaire contient de nombreuses cystéines, des sites de glycosylation (pouvant expliquer différence entre le poids prédit de SR-BI à partir de la séquence d'acide aminés, soit de 57 kDa, et le poids réel obtenu lors d'immunobuvardage, de 82 kDa. [104]), ainsi que des sites d'acétylation par des acides gras [104]. Dans la partie intracellulaire caboxy-terminale, il existe une séquence de ciblage peroxysomale PTS1 (peroxysomal targeting sequence) [101]. Le PTS1 est reconnu par un récepteur spécifique dirigeant les protéines contenant cette séquence dans les peroxysomes (un des sites de la β-oxydation des acides gras et du cholestérol) [105]. Enfin, le domaine intracellulaire de SR-BI contient des sites potentiels de phosphorylation pour différentes kinases susceptibles de contrôler l'activité de SR-BI [101].

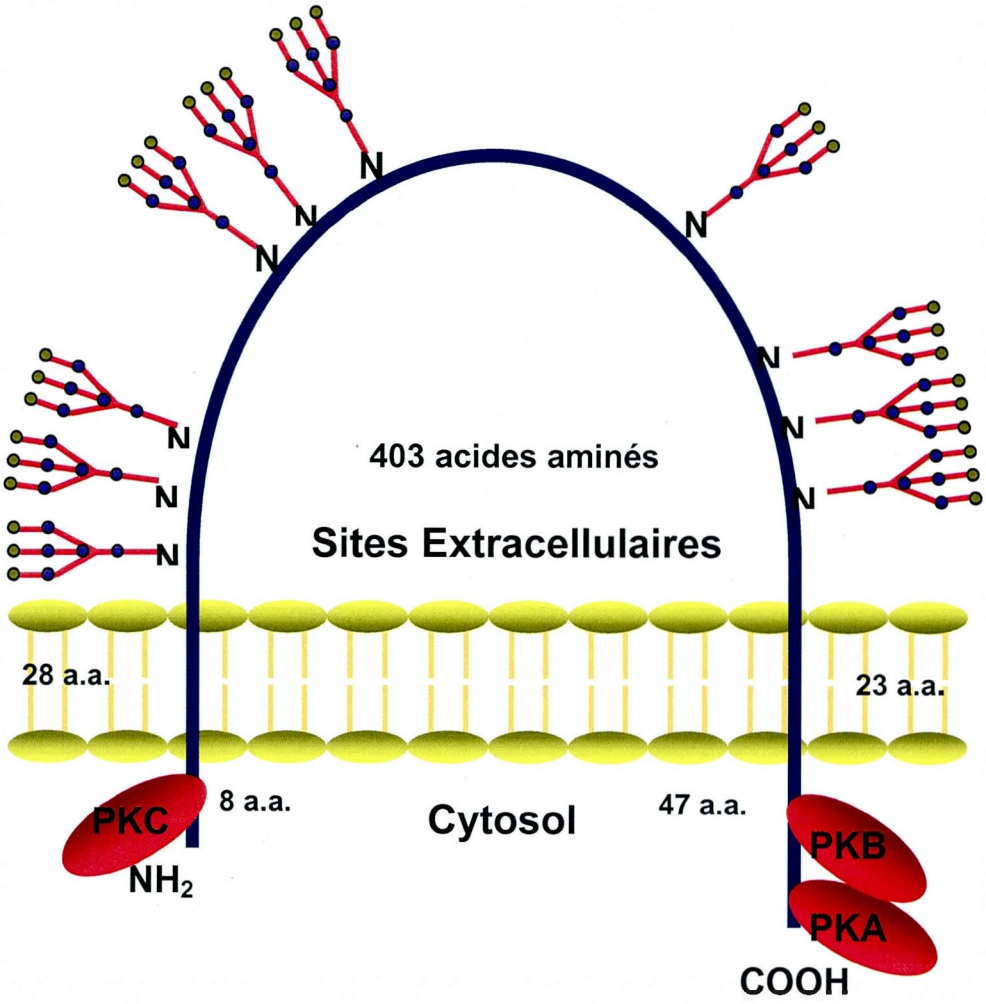

Figure 1.9 : Représentation schématique du récepteur SR-BI. Adapté de Rhainds et Brissette (2004).

1.7.2 Les différents ligands de SR-BI

SR-BI est un récepteur possédant de nombreux ligands. Il a d'abord été caractérisé comme étant un récepteur capable de lier les LDL modifiées, soit oxydées ou acétylées [106]. Par la suite, des études effectuées par ces mêmes chercheurs ont démontré qu'il reconnaît également des LDL [106], VLDL [107] et des HDL [108]. Même si SR-BI est en mesure de lier les LDL natives avec une grande affinité, ce sont les HDL qui sont considérées comme les ligands physiologiques du SR-BI. Des études de compétition ont démontré la possibilité que le site de liaison des LDL ne soit pas le même que celui des HDL, car les LDL ne peuvent empêcher la liaison des HDL; par contre, les HDL sont en mesure de bloquer les LDL [108]. Enfin, ce récepteur a également la capacité de lier les phospholipides anioniques tels la phosphatidylsérine, le phosphatidylinositol, l'acide phosphatidique ainsi que l'albumine maléylée [106, 109]. Ces derniers se trouvant principalement dans les feuillets internes de la membrane plasmique et étant libérés lors de l'apoptose, il est fort probable que SR-BI ait un rôle dans la liaison des cellules mortes ou endommagées [108].

1.7.3 Le mécanisme de captation sélective du cholestérol

Contrairement au mécanisme endocytique des récepteurs aux LDL, SR-B1 délivre les esters de cholestérol des lipoprotéines au foie par un processus de captation sélective. Lors de la captation sélective SR-B1 (figure 1.8), lie la lipoprotéine et ensuite, le cœur composé d'esters de cholestérol est livré à la membrane plasmique et ce, sans l'incorporation concomitante et la dégradation de la particule entière par la cellule [110]. Longtemps attribué seulement aux HDL, il est maintenant connu que ce phénomène de captation sélective peut être attribué aux IDL [111] ainsi qu'aux LDL [112].

Ainsi, SR-BI facilite l'incorporation sélective d'EC provenant des lipoprotéines par un processus suivant deux étapes : la première impliquant une liaison de la lipoprotéine au domaine extracellulaire de SR-BI, la seconde est le transfert sélectif de lipides à la membrane plasmique. Par ailleurs, le mécanisme exact permettant la prise sélective d'esters de cholestérol à partir des lipoprotéines demeure encore nébuleux. Via SR-BI, l'accolement de la lipoprotéine à la membrane plasmique semble essentiel; par contre cela ne semble pas suffisant pour produire un transport maximal de cholestérol entre la lipoprotéine et la cellule. Durant les années 1990, l'hypothèse la plus répandue proposait la formation d'un canal hydrophobe permettant le transfert des EC [113]. Cependant, une étude effectuée plus récemment suggère que la lipoprotéine soit internalisée suivant la liaison à SR-BI et qu'une partie du cholestérol de la lipoprotéine subissent une transcytose sélective pour ensuite être transportée vers la membrane apicale [114]. Quant à elle, la lipoprotéine subit une rétro-endocytose vers la membrane basolatérale pour être retournée dans la circulation [114].

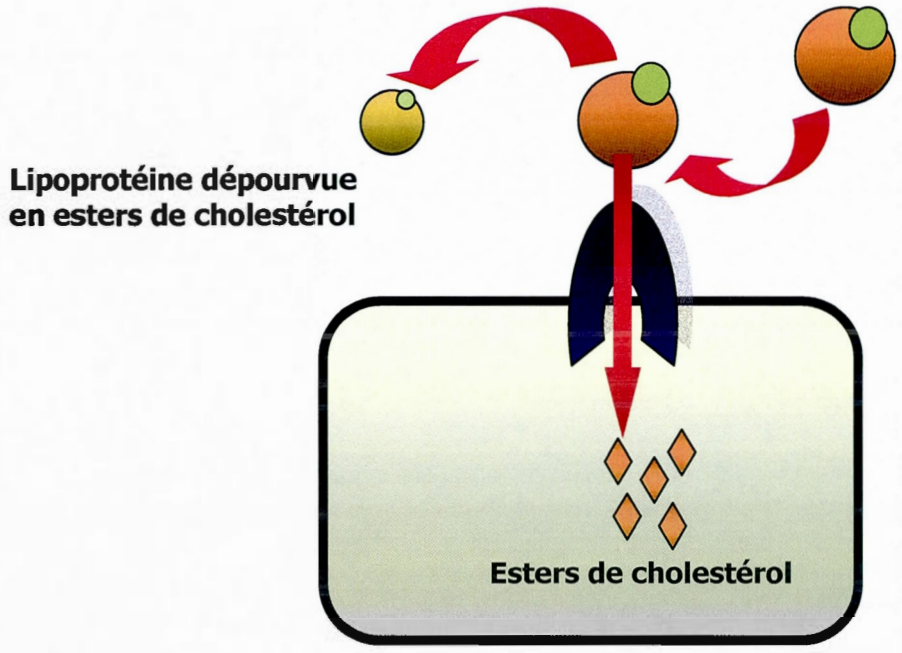

Figure 1.10: Mécanisme de captation du cholestérol par la captation sélective.

La présence de SR-B1 a été démontrée dans les syncytiotrophoblastes, au niveau des BBM (membrane de bordure en brosse) et des BPM (membrane basale plasmique) [99, 115]. Bien que la signification physiologique de cette présence demeure nébuleuse, il est possible que les HDL maternels puissent fournir le cholestérol ainsi que d'autre lipides, via SR-B1, pour les besoins croissants du fœtus en développement [116]. Enfin, une étude effectué en 2002 suggère que SR-BI serait régulé à la hausse en présence de LDL modifiées [117].

1.8 Buts du projet

Le transport des lipides par les lipoprotéines est d'une importance capitale dans le métabolisme lipidique. Via les différents récepteurs, les lipoprotéines maternelles sont transférées au fœtus. Afin de mieux comprendre les échanges lipidiques materno-fœtal, il est important d'étudier l'expression des récepteurs à lipoprotéines dans le placenta humain. De plus, en dépit de l'abondante littérature sur la nutrition au cours de la grossesse, l'influence des lipides maternels sur la croissance du fœtus demeure obscur. Actuellement, près du tiers de la population féminine présente un niveau d'adiposité préjudiciable à son état de santé. Un excès de poids pré-grossesse, associé à un gain excessif de poids en cours de grossesse peuvent augmenter les risques de macrosomie fœtale associée à des incidences de diabète et des risques arthérogéniques au cours de la vie adulte. L'obésité maternelle est aussi associée à la mort fœtale, des risques de malformations fœtales, etc. Ce projet vise donc à apporte plus d'information à ce sujet, puisque les niveaux d'expression de différents récepteurs à lipoprotéines seront corrélés à l'IMC maternelle pré-grossesse, le gain de poids ainsi qu'au niveau du cholestérol total maternel. Enfin, une connaissance plus approfondie du niveau de l'expression placentaire des récepteurs à lipoprotéine pourrait permettre, en outre, de déterminer si le placenta est apte à réguler l'apport du cholestérol maternel vers le fœtus, afin de protéger celui-ci contre les dommages pouvant être liés à l'hypercholestérolémie.

1.8. 1. Hypothèses de travail

Ainsi, les hypothèses de ce projet seront les suivantes :

Le niveau de cholestérol total maternel affecte l'expression protéique placentaire des différents récepteurs à lipoprotéines (LDLr, SR-BI et LOX-1). Des niveaux élevés de cholestérol total, ainsi qu'un IMC au dessus de la norme vont favoriser une diminution de l'expression protéique du LDLr, tandis que ces mêmes élévations entraîneront une augmentation de l'expression protéique de SR-BI et de LOX-1. Cette régulation à la hausse de LOX-1 pourrait favoriser le développement de l'athérosclérose au courant de la vie adulte.

Enfin, le diabète gestationnel affecte les niveaux d'expression de ces mêmes récepteurs : les femmes atteintes de cette pathologie devraient avoir un niveau d'expression placentaire de LDLr, SR-BI et LOX-1 plus élevé que les femmes non atteintes.

2. CHAPITRE II

ARTICLE 1

2.1 Contributions

Titre de l'article
MODULATION OF THE LDLR AND THE SR-BI EXPRESSION IN HUMAN PLACENTA IN RESPONSE TO CHANGE IN THE MATERNAL LIPID PROFILE.

Auteure principale: Maude Ethier Chiasson

Collaborateurs : A. Duchesne, J.-C. Forest, Y. Giguère, C. Mounier

Directrice de recherche : J. Lafond

L'article suivant a été rédigé dans sa totalité par moi-même, Maude Ethier Chiasson. La réalisation des expériences ainsi que les tests statistiques en découlant ont aussi été réalisés par moi-même. L'échantillonnage de la population, le suivi des patientes et les accouchements ont été effectués à l'unité d'obstétrique et de gynécologie de l'hôpital St-Luc, sous la direction du Dr André Masse et ses collaborateurs. Les dosages sanguins ont été effectués à l'hôpital St-François d'Assise, dans les laboratoires des Dr Yves Giguères et Jean-Claude Forest.

L'article suivant est présenté sous la forme exigée par l'université concernant la mise en forme, cependant, la version soumise au journal *Biology of Reproduction* a été modifiée selon les exigences du journal.

2.2 MODULATION OF THE LDLR AND THE SR-BI EXPRESSION IN HUMAN PLACENTA IN RESPONSE TO CHANGE IN THE MATERNAL LIPID PROFILE.

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ABSTRACT

Maternal hyperlipidemia is a characteristic feature during pregnancy, it has been reported that modification of the maternal lipid profile can induce disturbance during pregnancy. The low density lipoprotein receptor (LDLr) is responsible for transporting cholesterol-containing lipoprotein particles from the circulation into the cells. Also presents in placenta, the scavenger receptor type B1 (SR-B1) is known for its role in cholesterol metabolism. In the present study, we evaluated the impact of hypercholesterolemia on the protein expression level of theses receptors in human term placenta. Women were divided in 2 groups according to their level of total plasma cholesterol: the low cholesterol group, <7mM (LC), and the high cholesterol group >8mM (HC). We demonstrate here that, in placenta, an increase in the level of maternal total circulating cholesterol leads to a significant decrease in the placental level of the LDLr protein expression, while the level of the SR-BI expression remains unchanged. A similar change, in the expression of the LDLr, is observed in association with the maternal pre-pregnancy body mass index and weight gain. Our study also reveals that changes in the maternal plasma cholesterol concentration do not modify the fetal lipid profile, suggesting a compensatory mechanism realized by the placenta. Finally, we studied the specific localization of both receptors in syncytiotrophoblast membranes. We observe that the SR-BI is mainly expressed in brush border membrane, while the LDLr is mainly expressed in the basal plasma membrane of syncytiotrophoblasts. Taken together, our data suggest that the LDLr

plays a key role in regulating the level of cholesterol delivered to the baby from the placenta.

INTRODUCTION

Maternal hyperlipidemia is one of the most consistent and striking changes to take place in lipid metabolism during late pregnancy. Normal human pregnancy is characterized by a progressive increase in the low density lipoprotein (LDL) and the very low density lipoprotein (VLDL) concentrations in the maternal circulation, as reflected by the increase of serum triglycerides (TG) and cholesterol [118]. This modification of lipid metabolism, principally observed in the second half of the gestation, may be capital for fetal growth and development [99]. Cholesterol is an essential component for adequate development of the fetus, being used by the placenta for the synthesis of steroid hormones, while fatty acids are oxidized or esterified and used for cellular membrane synthesis [119]. The placenta is also the central support organ for the developing fetus, serving as the site of maternal-fetal exchange of ions and lipids [120]. Human placenta is formed by trophoblast cell differentiation which is characterized by the formation of a specific multinuclear structure, named the syncytiotrophoblast (ST). The ST possesses two structurally and functionally different membranes necessary for adequate transplacental exchange: a brush border membrane (BBM) facing the maternal circulation, and a basal plasma membrane (BPM) facing the fetal circulation [17, 121]. Because placental *de novo* cholesterol synthesis is not sufficient to support steroid hormonal production, a major part of the hormones produced by placenta are derived from maternal plasma cholesterol [122]. For this, maternal lipoproteins are taken up by the ST where they

are hydrolyzed and their products used for energy purpose and steroid hormone synthesis [123]. ST cells express many lipoproteins receptors, including the low density lipoprotein receptor (LDLr) [40], the acetylated LDLr [124], the LDLr related protein (LRP) [125], the VLDL receptor [126], the scavenger receptors, such as the scavenger receptor class B type I (SR-BI) [69, 99, 103] and the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) [77].

Among them, the LDLr is of primary importance in the binding and the internalization of the plasma-derived LDL-cholesterol and in regulating the plasma LDL concentration. The LDLr was first discovered by Brown and Goldstein in their search for the molecular basis underlying familial hypercholesterolemia. They found that the LDLr is less expressed in hypercholesterolemic subjects compared to normal [51, 52]. In addition, they observed that the LDLr activity, in fibroblasts, is highly dependent of the intracellular cholesterol concentration [127]. In various tissues, this mechanism maintains a constant level of plasma cholesterol by controlling the rates of both cholesterol synthesis and uptake from LDL [40, 55, 127].

The mature LDLr is a modular, single-pass transmembrane glycoprotein of 839 amino-acid [60]. It is first synthesized by ribosomes in the cytosol and folded as a 120 kDa precursor in the endoplasmic reticulum [128]. It is then transported to the Golgi apparatus, when it undergoes extensive O-linked glycosylation leading into the mature 160 kDa form found at the cell surface, and where it can binds lipoproteins

[64, 129]. The physiologic ligands of the LDLr are the LDL, which represents 65 to 70% of circulating plasma cholesterol in human. The LDL contain apolipoprotein B-100 (apoB-100) as primary protein component [60]. The LDLr also exhibits a high-affinity binding for lipoproteins that contain apolipoprotein E (apoE), such as different forms of VLDL and intermediate density lipoprotein (IDL) [51, 130]. Internalization of the lipoprotein by the LDLr occurs via the clathrin-coated pits system, which ultimately delivered the lipoprotein to the late endosomes releasing the bound lipoprotein particles [60]. Subsequently, the receptors return to the cell surface in a process called receptor recycling. By contrast, the released lipoprotein particles proceed to lysosomes, where the cholesterol esters are hydrolyzed to free cholesterol [40]. A typical LDLr molecule is recycled at least 100 times in an acid environment before being degraded [131].

In addition to the LDLr, the LDL particles binds to other receptor such as the SR-BI, also express in ST cells [99]. The SR-BI, a 82 kDa glycoprotein, is a receptor that principally mediates the selective uptake of lipoproteins associated with cholesteryl esters. In this process, lipids are transported into the cells with a process that does not involve uptake and lysosomal degradation of the lipoprotein particle [132]. The SR-BI is expressed at high levels in many tissues and cell types that are most active in selective uptake such as: the liver, the adrenal glands, the testis and the ovaries [108, 133-135]. The SR-BI receptor is also highly expressed in the human placenta cells,

allowing the growing fetus to obtain a considerable portion of cholesterol from maternal lipoproteins [136].

The contribution of maternal cholesterol to the fetus under normal as well as pathological circumstances is poorly understood. Few studies suggest that maternal contribution can vary with the maternal metabolic environment during pregnancy [137, 138]. Therefore, the aim of the present study is to 1) analyse the impact of changes in maternal and fetal circulating lipids on the LDLr and the SR-BI protein expression in human term placenta and 2) to evaluate if any correlation is observed with body mass index (BMI) and weight gain during pregnancy. We showed in our study that elevation of maternal total cholesterol plasma level decreased the expression of the LDLr protein in human term placenta, without affecting the level of the SR-BI protein expression. We also observed that these changes in maternal lipid profiles do not affect the lipid profile of the fetus which is correlated with a decreased of the LDLr expression in the BBM of the ST. In conclusion, our study suggests an important role of the LDLr in maintaining the fetal cholesterol concentration during pregnancy-related disorders/pathologies in which the lipid profile may be disturbed.

MATERIALS AND METHODS

The women participating in the study were recruited at their first prenatal visit, before their tenth week of pregnancy, at the Clinique Fidès of Montreal and at the Service of Perinatalogy, of the Centre Hospitalier de l'Université de Montréal (CHUM), Pavilion St-Luc, Canada, from 2002 to 2006. The study was approved by the ethical committee of St-Luc Hospital and Université de Montréal (Montréal, Qc, Canada). After signing a consent form, each woman filled out an interview-administrated questionnaire, which contained general sociodemographic data, medical history, drinking and smoking habit. The total study population consisted of 900 pregnant women. To conduct this study, 74 women were selected, based on her health status. Women were classified in two groups according to the plasma total cholesterol concentration at term. To establish the group, we established the median of the maternal plasma total cholesterol at term (6,42 mM). Women with cholesterol concentration lower than 7mM referred to the low cholesterol group (LC) (n=44) while women with cholesterol concentration higher than 8mM referred to the high cholesterol (HC) group (n=30). Subsequently they were reclassified into three groups according their pre-pregnancy body mass index (BMI). The BMI, representing a measure of adiposity, was calculated as pre-pregnancy kg/m2. The established normal values for BMI, according to the Health Canada (2002), were between 20-26 kg/m^2, while a low BMI is <20 kg/m^2, and high BMI is >26 kg/m^2. Finally, women were divided according to their weight gain during pregnancy. Maternal weight gain

represents the placental and the baby weight, amniotic liquid volume, maternal breast weight and adipose tissue. The normal values established for weight gain during pregnancy ranged from 11 to 18kg. All of these women were non-smokers and did not receive any medication known to interfere with lipid metabolism. Finally, a post natal follow-up was made to assess newborns health status as well as collecting data relative to the weight and height of the babies.

Blood and tissue samples

Blood samples were collected at each trimester and at delivery, from the mother and the cord blood. Blood samples were collected in 10 ml gel Vacutainer tube (BD, Oakville, ON, Canada), centrifuged 15minutesat 3500 x g, and plasma samples were kept at -20°C until analysis. The placentas, from vaginal delivery, were obtained from the collaborating hospital (Centre Hospitalier de l'Université de Montréal, Pavilion St-Luc, (QC, Canada)), and were immediately immersed in Dulbecco's modified Eagle Medium (DMEM) (Sigma, Oakville, ON, Canada) containing a mixture of antibiotic (penicillin, streptomycin and neomycin, Invitrogen, Burlington, ON, Canada) and $NaHCO_3$. After the removal of the amnion, the chorion and the decidual layer, the placental tissue, was cut in $5cm^2$sections and immediately frozen to liquid nitrogen and then kept at -80°C until use.

Lipids determination

The plasma levels of total cholesterol (TC), LDL, high density lipoprotein (HDL), VLDL and TG were measured using the Unicel 36 DX600 Synchron Clinical System (Beckman-Coulter, Mississauga, ON, Canada), at the Clinical Biochemistry Service of Saint-François d'Assise Hospital at Québec (Qc, Canada).

Proteins extraction

Placental frozen tissue samples were washed 3-4 times with cold NaCl 0,9% solution containing anti-protease (1 µM leupeptin, 1,46 µM pepstatin and 2 µg/ml aprotinin) to remove blood from tissue. 1g of tissue was homogenized (125 mM Tris-HCl, pH 8,0, 2Mm $CaCl_2$, 1.4% (v/v) Triton X-100, 1 µM leupeptin, 1,46µM pepstatin, 2 µg/ml aprotinin and 1 mM phenylmethylsulphonylfluoride (PMSF)) using a Polytron Tissue homogeniser PT 3000 (Brinkmann, NY, USA) in 1ml ice-cold hypertonic buffer. The homogenate was than kept on ice for 30 min and centrifuged at 10 000 X g for 25 minutes at 4°C. The supernatant resulting was stored at -80°C until used. Protein concentration content was determined by spectrophotometric quantification using the bicinchoninic acid (BCA) reagent (Pierce, Brockville, ON, Canada) using bovine serum albumin (BSA) as standard.

Purification of syncytiotrophoblast brush border (BBM) and basal plasma membranes (BPM) from human term placenta

Membranes were purified from placental tissues collected from fresh human placenta as described . The tissue obtained after removal of the amnion, the chorion and the decidual layer were minced and stirred for 45 minutes in 10 mM Tris-HEPES (pH 7.,4) containing 270 mM mannitol, 0.1mM PMSF, 1 mg/ml benzamidin and 10mM leupeptin. The membranes were then purified using a technique currently used in our laboratory [17] with some minor modifications [121].

Western blot analyses of the LDLr and the SR-BI

Total placental proteins (150 µg for total placental protein and syncytiotrophoblast membranes) were solubilized in a sample buffer (4% SDS, 30mM dithiotreitol, 10% β-mercaptoethanol, 0,25 M sucrose, 0,01 M EDTA-Na_2 and 0,075% bromophenol blue), and heated at 95°C for 5 min. The proteins were resolved in a 8% SDS-PAGE and electroblotted to PDVF membrane (Millipore, Cambridge, ON, Canada) at 20 Volts for 40 min. Membranes were then blocked overnight at 4°C in TBS-T (20 mM Tris Base, pH 7,6, 137 mM NaCl, 0.1% Tween-20) containing 5% skimmed milk. Thereafter, membranes were washed 3 x TBS-T and incubated for 90 minutes at room temperature, with either a human low density lipoprotein receptor (LDLr) rabbit monoclonal antibody (Fitzgerald, Concord, MA, USA) (1/500 in TBS-T), or a human scavenger receptor type BI (SR-BI) rabbit monoclonal antibody (Novus Biological, Littleton, CO, USA) (1/8000 in TBS-T/ 5% skimmed milk) or a human

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mouse monoclonal antibody (Chemicon International, Temecula, California, USA) (1/5000 in TBS-T/5% BSA). Blots were washed 3X with TBS-T, and probed with anti-mouse and anti-rabbit IgG horseradish peroxidase-conjugated secondary antibodies (for LDLR (1/3500) and SR-BI (1/12 000) respectively), (1/3500 TBS-T 5% BSA) and with an GAPDH antimouse-IgG (1/2500) (TBS-T 5% skimmed milk) (Chemicon International, Temecula, California, USA) for 90 minutes at room temperature. Blots were then washed 3X with TBS-T and the detection was performed using the BM Chemiluminescence system (Roche, Laval, Qc, Canada) and visualized by autoradiography (Hyperfilm ECL, GE Healthcare, Baie d'Urfée, Qc, Canada). Each data were normalized with a control sample which one serve to express each data as percentage of control. For semiquantitative analyses of the bands, the film was digitized and intensity of the band is doing by the Quantity One Software (Bio-Rad Laboratories, Mississauga, ON, Canada).

Immunohistochemistry

An immunohistochemistry study was performed to analyze the cellular localization of the LDLr. After removal of the amnion, the chorion and the decidual layer, the placental tissue was cut in sections of 5 mm^3, embedded with cold (4°C) Tissue-tek OCT (Electron Microscopy Sciences, Hatfield, PA, USA) and immediately frozen in -80°C isopentane (Sigma-Aldrich, St-Louis, MO, USA). Sections were then stored at

-80°C until further processing. For immunohistochemical staining, 8µm thick frozen sections (Cryostat, Leica, Microsystems CM1505S, Wtzlar, Germany) were put on SuperFrost Plus glass slides (Fisher Scientific, Pittsburgh, PA, USA) and air dried overnight. Sections were then fixed with freshly prepared cold 4% paraformaldehyde and 0,2% picric acid in PBS for 20 minutes at 4°C. To complete fixation, cryosections were air dried at room temperature. Tissues sections were rinsed with PBS 1X for 3 minutes and then, sections were incubated with permeabilisation solution (PBS 1X and 1% Triton X-100) for 30 minutes at room temperature. Sections were blocked in 0,5 % donkey serum and 0,1% Tween-20 for 1 hour at room temperature, after quenching endogenous peroxidase activity by exposing slides to «Peroxidase Block» (Kit LSAB+ System-HRP, DAKO, Denmark) for 5 minutes. The sections were then incubated with primary antibody diluted in blocking solution overnight at 4°C. The primary antibody used was rabbit monoclonal anti-human low density lipoprotein receptor (LDLr) rabbit monoclonal antibody (Fitzgerald, Concord, MA, USA, rabbit monoclonal anti-SR-BI (Novus Biological, Littleton, CO, USA) polyclonal and anti-hCG (Chemicon, Temecula, CA). Subsquently, slides were incubated with endogenous avidin and biotin blocking solution (Biomeda, Foster City, CA) for 15 minutes at room temperature. Tissue sections were incubated with "Link" universal secondary antibody solution (DAKO) for 1 hour at room temperature and then with "Streptavidin-peroxydase" for 30 minutes. They were stained with «Substrate-chromogen solution» (DAKO), and counterstained with

Mayer's hematoxylin (Fluka, Buchs, Switzerland). Sections were viewed and photographed under interference-contrast Leitz Diaplan microscope (Leica, Microsystems CM1505S, Wtzlar, Germany) equipped with a Nikon CoolPix 990 camera. Cells having intense brownish staining were considered immunopositive. To ascertain protein specific antibody binding, controls were performed omitting primary antibodies.

Statistical analyses

Data were expressed as the mean ± SEM, and analysed with the unpaired Student *t*-test at $p < 0,05$ level of significance, to evaluated difference between groups. For the relationship between two variables of the same population, results are expressed as Spearman correlation and the curve represent Pearson linear correlation. All statistical analyses were performed using the Prism software (version 4.0.2; GraphPad Software, 2004).

RESULTS

Population characteristics

Characteristics of mothers and newborns are presented in Table I. A total of 74 women gave birth and their blood samples were collected at term. The participants were split in two groups according to their plasma cholesterol concentration: LC women (n=44) where cholesterol concentration is below 7mM and HC women (n=30) with a cholesterol concentration higher than 8mM (this does not represent a pathologic state). In both group, the age of women was ±31 years old and their gestational age was about 39 weeks. No significant difference was observed in the BMI and the weight gain between the 2 groups, as well as on babies' weight and height at birth. It is of interest to note that a higher non pathologic cholesterol level has also no effect on the weight of placenta.

Maternal plasma lipids at delivery and in venous cord blood

In LC and HC groups the concentrations of TC, HDL-cholesterol, LDL-cholesterol, TG, ApoA-1 and ApoB-100 were measured at delivery in both maternal and in the venous cord blood (Table II). At delivery, the maternal plasma concentrations of TC, TG and LDL-cholesterol were significantly increased into the HC group compared to LC group, corresponding to an increased level of ApoB-100, while the levels of HDL-cholesterol and ApoA-1 remain unchanged. No significant differences were observed in the cord blood samples between both groups regarding all studied

parameters. Taking together, our data suggest that hypercholesterolemia at term modify the circulating lipid profile in the mother without affecting the lipid profile in newborn.

Protein expression of the LDLr and the SR-BI

As hypercholesterolemia appears to affect circulating lipid profile only on the mother side, we hypothesize that cholesterol transport in placenta could be affected. Therefore, the expression of the LDLr, the major transporter of LDL particles, was evaluated by Western blot analysis in placenta. 150 µg of total proteins extracted from LC and HC placentas, were deposited on SDS-PAGE and blotted with a specific human LDLr antibody, as described under the "material and methods" section. As showed in figure 1, the level of the LDLr expressed in placenta from HC mothers is decreased by more than 60% (1,081±0,088 *vs* 0,3793±0,04223) compared to the level measured in the LC group. These data suggest that the placenta is able to compensate for an increase of the maternal plasma cholesterol concentration by decreasing the level of the LDLr expression. At the opposite, the SR-BI protein levels are not affected by the charge of circulating cholesterol as shown in figure 2, where no change has been measured between the LC and HC groups.

We then evaluated the impact of pre-pregnancy BMI on the modulation of the LDLr expression in term placenta. Each LC and HC groups were divided into 3 subgroups:

BMI below 20 kg/m^2 (low BMI), between 20-26 kg/m^2 (normal BMI) and above 26 kg/m^2 (high BMI). Of interest, our analysis reveals that the level of the LDLr protein expression in placenta is modulated by the pre-pregnancy BMI of the mother. In LC group (Fig. 3A (white bars)), a lower maternal pre-pregnancy BMI leads to a significant increase in the expression of the LDLr compared to normal BMI (0,5299±0,07002 *vs* 0,3831±0,03810, **p*=0,0438), while no such significant observation is made in the HC group (black bars) (0,5299±0,07002 *vs* 0,3831±0,03810). However, for HC women, a dramatic decrease of the receptor expression in the highest BMI group is observed compared to the lowest BMI group (0,5299±0,07002 *vs* 0,06766±0,03897, $^{\S\S\S}p$=0,0007), since there no difference for LC women (1,431±0,2341 *vs* 0,9022±0,1693). These results suggest that even if the BMI is not modified by changes in the cholesterol concentration (Table I), the BMI has probably an impact on maternal lipid metabolism during pregnancy. In fact, the level of the LDLr expression in placenta appears to be increased when the BMI is decreased even in absence of plasma hypercholesterolemia suggesting another of regulation of the LDLr expression.

Also, we evaluate the impact of weight gain during pregnancy (< 11 kg (low), 11-18 kg (normal) and >18 kg (high)) on the LDLr expression in the placenta of LC and HC groups. As shown in figure 3B, in LC group (white bars), a low weight gain leads to a significant increase of the LDLr expression compared to a normal and a high weight gain (1,708±0,2061 *vs* 1,000±0,1193, ***p*=0.0033 and 1,708±0,2061 *vs*

0,9811±0,9811, *p=0.0115 respectively). However, in the HC group, the weight gain during pregnancy does not influence the expression of the LDLr in the placenta. In conclusion, our data suggest that at least in the LC group, the increase in weight gain during pregnancy down-regulated the expression of the LDLr in the placenta. Taking together, our results reveal that an increase in maternal weight acquired either before or during pregnancy, appears to be compensated by the placenta, resulting in a decrease in the level of the LDLr. This suggests that the LDLr is probably the principal cholesterol receptor in placenta and its expression may be modulated by the level of maternal circulating cholesterol. Therefore, the placenta may possibly play an important role in cholesterol compensation for the fetus.

Protein expression of the LDLr in ST membranes

Our data presented above suggest that the LDLr is the major cholesterol receptor in human placenta, while the SR-BI seems to be less important. We then evaluated if their cellular localizations can be correlated with their modulation in response to changes in maternal plasma concentration. We analyzed the expression of both receptors by Western blot analysis in BPM (contact with fetal blood) and BBM (contact with maternal blood) of ST cells isolated from fresh placenta. Our analysis reveals that the LDLr is mainly located in the BPM (Fig. 4A), while the SR-B1 expression is equivalent in both fraction (Fig. 4B). As shown in figure 5, immunohistochemical analysis of the LDLr and the SR-BI expression in the human placenta also demonstrates that the LDLr (Fig. 5A) is principally localized at the

BBM of the ST, while the SR-BI (Fig. 5B) appears to be localized on both maternal (BBM) and fetal side (BPM). To ascertain that cells were ST we used human chorionic gonadotropin (HGC) as a positive control (Fig. 5C). These results further emphasize a key role for the LDLr in placenta modulating the level of cholesterol available for the fetus.

Correlations between the LDLr and the SR-BI proteins expressions and circulating plasma lipids at delivery

The correlation between the LDLr protein expression and many of the maternal plasma circulating lipids was evaluated by Spearman correlation and Pearson linear correlation curves. Negative correlation were observed between the LDLr protein expression and total maternal plasma cholesterol (r= -0,4864) level, plasma LDL level (r=-0,5304) and plasma apoB-100 (r=-4340) at term, (Fig. 6A-B and D respectively). No correlation was demonstrated between the LDLr protein expression and maternal plasma HDL level (r=-0,02610) at term (figure 6C). As expected, no correlation was found between the SR-BI protein expression and all the examines factors, maternal total cholesterol (r=0,01736), LDL-cholesterol (r=-0,04968) HDL-cholesterol (r=0,02548), and plasma apoB-100 (r=0,05475) levels.

DISCUSSION

In the present study we have analyzed the lipid profile in LC and HC pregnant women, since abnormal high plasma lipid level even in fetal life has been associated with increasing risk of coronary heart diseases [139, 140]. It has been reported that maternal plasma lipid status is modified during pregnancy [6, 141]. In our study, we observed that in HC pregnant women, total plasma cholesterol, LDL cholesterol, TG and apoB-100 concentrations are increased compared to the levels measured in LC women. No significant difference was observed for HDL cholesterol and apo-A1 levels between the two groups. In addition, we showed that modulation of maternal cholesterol concentration does not affect the fetal lipid profile measured in the venous cord blood, suggesting a modulation of the fetal metabolism or the presence of a compensatory mechanism in the placenta.

In order to verify if the placenta is able to modulate the amount of cholesterol distributed to the baby, we analyzed the expression of cholesterol transporters in HC and LC placentas. Placenta is a crucial organ for cholesterol transfer from the mother to the fetus. Cholesterol is taken up from maternal plasma LDL by the LDLr and by SR-BI [142] and next transported into cells by the ATP binding cassette transporter 1 (ABCA-1) [143]. We demonstrated in this paper that increase of the concentration of plasma cholesterol, and especially changes of the level of circulating LDL-cholesterol, modifies the expression of the LDLr protein in placenta, while the level

of the SR-B1 protein is not affected. This suggests that in placental cells as in hepatocytes, the expression of the LDLr protein is under the control of cholesterol concentration [55].

The mechanism of cholesterol action on the regulation of the LDLr expression is complex. It appears to be mediated by different transcription factors (TFs) such as sterol regulatory element-binding proteins (SREBPs), the Sp1 YY1 and the NF-Y/CBF [144]. Both SREBP-1 and 2 are crucial molecules in the regulation of cholesterol metabolism [145-147] however, SREBP-2 has been preferentially implicated in the control of the LDLr gene expression [148]. It was showed that when cellular cholesterol concentrations decreased, SREBP-2 is released from the Golgi [149]. The mature SREBP-2 is subsequently translocated to the nuclei [150] where it can bind to the LDLr promoter increasing gene transcription [149]. However, we did not detect any difference in the level of mature SREBP-2 expression in HC *versus* LC placentas (*unpublished data*). This may suggest that in placenta, cholesterol does not regulate the LDLr gene transcription *via* SREBP-2 but probably *via* other TFs such as Sp1 YY1 and NF-Y/CBF.

Postmenopausal women, with a higher BMI have increased plasma estrogen levels, compared to women with low BMI, a consequence of a greater peripheral conversion of androgens to estrogens by the adipose tissue [151-153]. Previous studies reported that expression of the LDLr is stimulated by estrogen in human cultured ST and in

placental minces [154, 155]. However, in the present study, we showed that in the LC group, an increase in the placental LDLr protein expression is only observed in women with a low pre-pregnancy BMI. In addition, our analyses revealed that in HC women, elevation of the pre-pregnancy BMI is associated with a significant decrease of the placental LDLr protein expression. This suggests that during pregnancy, the effect of estrogen on the LDLr expression must be impaired. It was also demonstrated that insulin activates the gene expression of the LDLr [156, 157]. This may explain while in HC women, augmentation of the BMI increases the LDLr expression as hyperinsulinemia is often observed when obesity is associated with hypercholesterolemia [158, 159].

The expression of the placental LDLr is not affected by the weight gain in the HC group, while in the LC group its expression is significantly increased in women with a low weight gain. This increase may be attributed to a mechanism in which the LC women are able to compensate for a lower level of circulating cholesterol especially when it is associated with a low weight gain. It has been previously reported that a high weight gain during pregnancy is associated with an increase in the concentration of the serum lipoproteins correlating with fetal macrosomia [160]. However, in our study, no fetal macrosomia has been observed in the HC group despite a significant increase in the level of plasma LDL. This may be explained by the fact that in the HC group, the level of the placental LDLr expression is not affected by the weight gain. Taking together, our data demonstrate that weight gain during pregnancy may

affect the expression of the LDLr but only when it is associated with a low circulating concentration of cholesterol.

Our data also revealed that the LDLr is preferentially expressed in BPM (facing the fetus) of ST cells. An association of LDL with the LDLr has been previously observed on microvillous membrane prepared from 6^{th} week of gestation and mid term placentas [57] and from term tissues [58]. The level of the LDLr mRNA expression is however significantly decreased in placenta along pregnancy [161]. In the present study, we also showed that the expression of the LDLr protein is detected in BBM fractions but in a greater amount in BPM. This striking observation cannot be attributed to a contamination of our BPM fractions since their purities were controlled by the measure, currently used in our laboratories, of the alkaline phosphatase activity, a BBM marker, and the Na^+, K^+, ATPase activity, a BPM marker (*data not shown*). In addition, Western blot analyses were confirmed by immunohistochemical studies. The difference between our observation and the previous data reporting the presence of LDLr only in BBM [58] is still difficult to understand. However, the presence of a higher level of the LDLr protein on BPM correlates with the fact that in both HC and LC groups, the fetus is hypocholesterolemic compared to the mother (*see Table II*). The high level of LDLr expression on the fetus side may controls the level of cholesterol in the fetal blood

which is necessary for optimal fetal development [162].The significant decrease of the LDLr expression observed in the total placenta extracts of HC women could be therefore attributed to a decrease of the receptor expression principally on the mother side (BBM). This may reflect a compensatory mechanism develop by the placenta in order to maintain a physiological cholesterol level in the tissue necessary for adequate placental exchanges [163]. In agreement with this hypothesis, the concentration of placental cholesterol is similar in both LC and HC group (*data not shown*).

Cholesterol may be transfer from the placenta to the fetal circulation by either aqueous gradient diffusion or from newly synthesized lipoproteins in placenta *via* the SR-B1 receptor [164]. SR-BI selectively takes up cholesteryl esters primarily from HDL, which are then hydrolyzed by neutral or acidic cholesterol esterases [108, 165]. A previous study suggests that SR-BI plays a major role during different stages of fetal development as in human lacking functional LDLr, fetal development and cholesterol supply to the fetus are normal compared to controls [166]. In addition, a decrease in SR-BI expression and in the selective uptake of cholesterol ester is observed in cultured term cytotrophoblast cells compared with first trimester cytotrophoblast cells suggesting a modulation of SR-BI function during development [166]. In our study, we showed that expression of placental SR-BI is not modified by changes in maternal plasma cholesterol level. In addition, no correlation was found between the placental expression level of SR-BI and the maternal plasma HDL-cholesterol and LDL-cholesterol concentrations. Taking together, our study suggests

that at term, SR-BI does not play a critical role in controlling the plasma cholesterol concentration in both placenta and fetus.

Taken together, our study shows the importance of the modulation of placental LDLr expression in the control of cholesterol concentrations in both term placenta and fetus. More precisely, we demonstrated that expression of the LDLr protein level in term placenta is inversely correlates with maternal total plasma cholesterol, plasma LDL-cholesterol, and plasma apoB-100 concentrations probably explaining the absence of hypercholesterolemia in the fetus. In addition, we showed that increase in maternal pre-pregnancy BMI is associated with a decrease in the LDLr expression in both HC and LC women, while a low maternal weight gain during pregnancy increased the LDLr expression only in LC women. Finally, we demonstrated that the LDLr is more expressed in BPM than in BBM probably reflecting a compensatory mechanism performed by the placenta in order to maintain an adequate cholesterol concentration in both term placenta and fetus. In conclusion, our study clearly demonstrates that during pregnancy, the LDLr in human term placenta plays a crucial role in the control cholesterol homeostasis in both placenta and fetus and this in response to changes in maternal lipid profile.

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Table I: Population characteristics

Maternal plasmatic cholesterol concentration	LC (n=44) < 7 mM	HC (n=30) > 8 mM
Mother age (year)	30.9 ± 5.3	31.4 ± 4.2
Gestational age (week)	38.9 ±1.5	39.2 ± 1.6
Mother BMI (kg/m^2)	23.5 ± 5.0	22.41 ± 4.36
Mother weight gain (kg)	14.98 ± 6.09	18.62 ± 6.81
Newborn birth weight (g)	3282 ± 648	3404 ± 462
Newborn height (cm)	51.4 ± 2	52.0 ± 5.0
Placenta weight (g)	590.2 ± 154.1	608.4 ± 142.7

Results are expressed as mean ± SD where compared to LC group

Table II: Maternal plasma lipids at delivery and in cord blood

	LC group (n=44)		**HC group (n=30)**	
	Maternal plasma	Venous cord blood	Maternal plasma	Venous cord blood
Total cholesterol (mM))	5.74 ± 1.15	1.88 ± 0.93	8.30 ± 1.03 *	1.67 ± 0.51
HDL-cholesterol (mM)	1.70 ± 0.46	0.75 ± 0.31	1.72 ± 0.60	0.72 ± 0.31
LDL-cholesterol (mM)	2.79 ± 0.83	0.83 ± 0.54	4.95 ± 0.94 *	0.67 ± 0.25
Triglycerides (mM)	2.73 ± 1.11	0.63 ± 0.51	3.65 ± 1.23 *	0.60 ± 0.26
Apo-AI (g/L)	2.05 ± 0.41	0.91 ± 0.36	2.21 ± 0.46	0.83 ± 0.22
ApoB-100 (g/L)	1.14 ± 0.26	0.30 ± 0.23	1.64 ± 0.25 *	0.25 ± 0.10

Results are expressed as mean ± SD, where * $p < 0.05$ compared to the LC group

FIGURE LEGEND

Figure 1. ***Western blot analysis of the LDLr in placenta at term***. The level of the LDLr expression was evaluated by Western blot analysis using 150 µg of total protein isolated from placenta of LC and HC groups. (A)- The autoradiography shows a representative Western Blot performed on 4 different placentas. (B)- LDLr proteins expressed in placenta of LC (white bars) and HC groups (black bars). Results are expressed as the level of the LDLr measured in tissue standardized by the level of GAPDH measured in the same sample and the same membrane after the stripping of the first antibody. Results are the mean ± SEM (n=44 for LC and n=30 for HC) *** $p < 0{,}0001$ comparing HC *vs* LC.

Figure 2. ***Western blot analysis of the SR-BI in placenta at term***. The level of the SR-BI expression was evaluated by Western blot analysis using 150 µg of total protein isolated from placenta of LC and HC groups. (A)- The autoradiography shows a representative Western Blot performed on 4 different placentas. (B)- SR-BI proteins expressed in placenta of LC (white bars) and HC groups (black bars). Results are expressed as the level of the SR-BI measured in tissue standardized by the level of GAPDH measured in the same sample and the same membrane after the stripping of the first antibody. Results are the mean ± SEM (n=44 for LC and n=30 for HC).

Figure 3. ***Expression of the LDLr in human placenta at term according to the maternal pre-pregnancy BMI.*** The level of the LDLr expression was evaluated by Western blot analysis using 150µg of total protein isolated from placenta of LC (white bars) and HC (black bars) group. Each group were then divided in 3 sub-groups (A)- according to pre-pregnancy BMI of the mother and (B)- according the weight gain. Results are expressed as the level of the LDLr measured in tissue standardized by the level of GAPDH measured in the same sample. Results are expressed as the mean ± SEM. (n=44 for LC group and n=30 for HC). (A) * $p < 0{,}05$ comparing LC women with low BMI *vs* women with normal BMI, and §§§ $p < 0{,}0001$ comparing HC women with high BMI *vs* women with low and normal BMI, (B) ** $p < 0{,}001$ comparing LC women with a low weight gain and those with a normal weight gain (11-18kg) * $p < 0{,}05$ comparing LC women with a low weight gain and those with a high weight gain. .

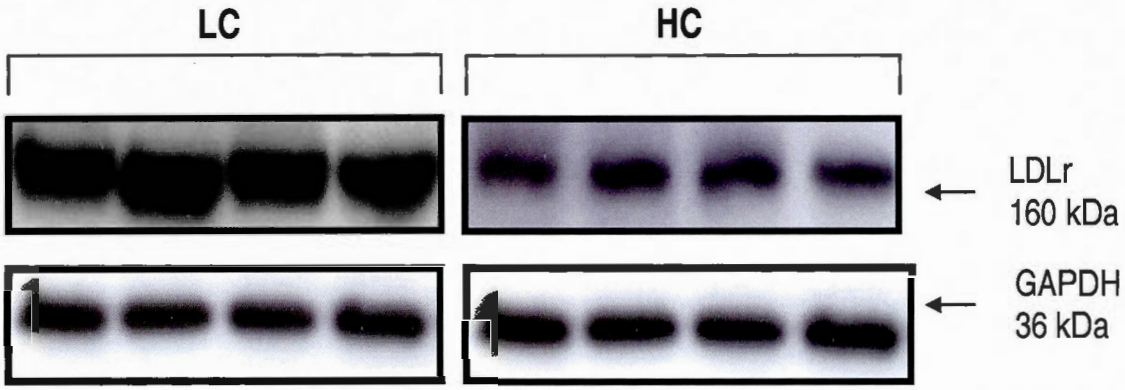
Figure 4. ***Expression of the LDLr and the SR-BI in BPM and BBM prepared from human placenta at term.*** The level of (A) LDLr and (B) the SR-BI expression were evaluated by Western blot analysis using 150µg of total protein isolated from BPM and BBM prepared from human placenta at term as described under the "material and methods" section. The autoradiography shows a representative Western blot performed on 3 different preparations.

Figure 5. ***Immunohistochemical expression of the LDLr and the SR-B1 in ST, in both maternal (BBM) and fetal side (BPM) of term placenta.*** Positive staining is in brown color and nuclear counterstaining is blue. (A) Expression of the LDLr in ST. (B) Expression of SR-B1 in ST. (C) The positive control (HCG) is present in ST, (D) negative control (NC): ST cells without antibody.

Figure 6. ***Relationship of the LDLr (left panels) and the SR-BI (right panels) protein expression and different maternal plasma lipids concentration at delivery*** for (A) total cholesterol, (B) LDL-cholesterol, (C) HDL-cholesterol and (D) plasma apoB100. Results are expressed as Spearman correlation and the curve represent Pearson linear correlation.

Figure 1

A


LC
HC
LDLr
160 kDa
GAPDH
36 kDa

B

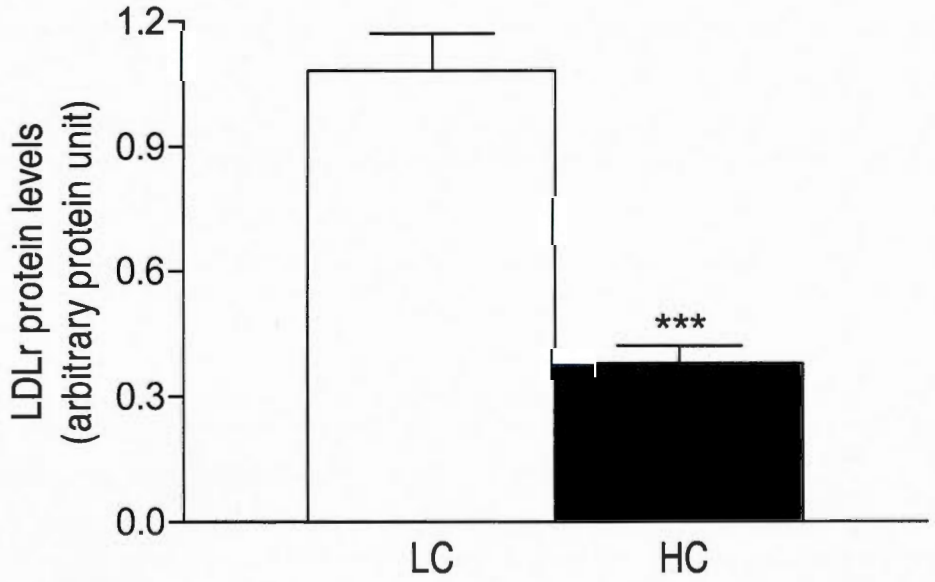
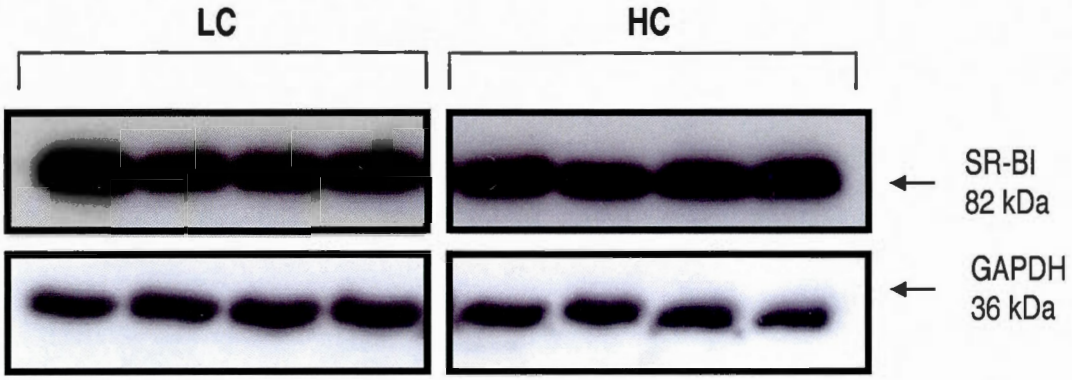

LDLr protein levels
(arbitrary protein unit)
1.2
0.9
0.6
0.3
0.0
***
LC
HC

Figure 2


LC
HC
SR-BI
82 kDa
GAPDH
36 kDa

B

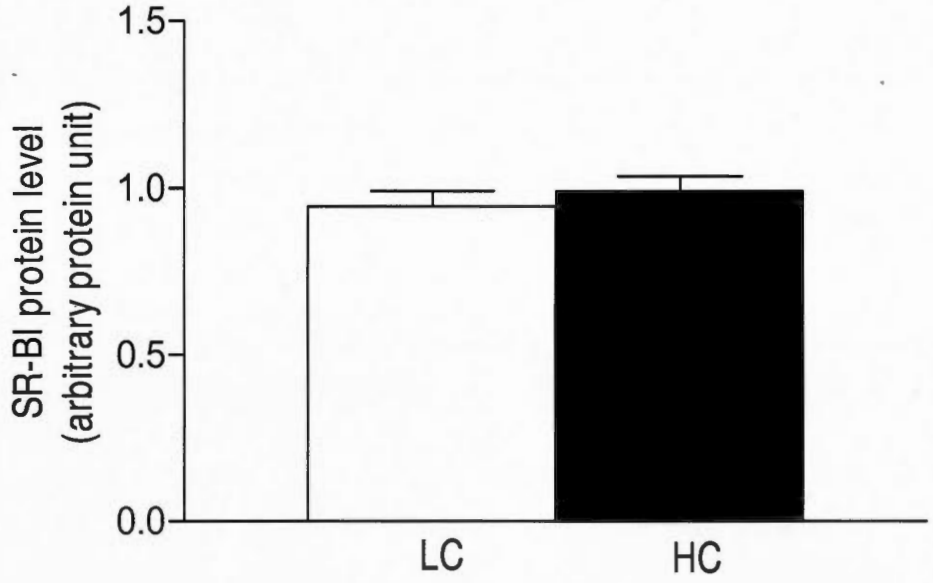
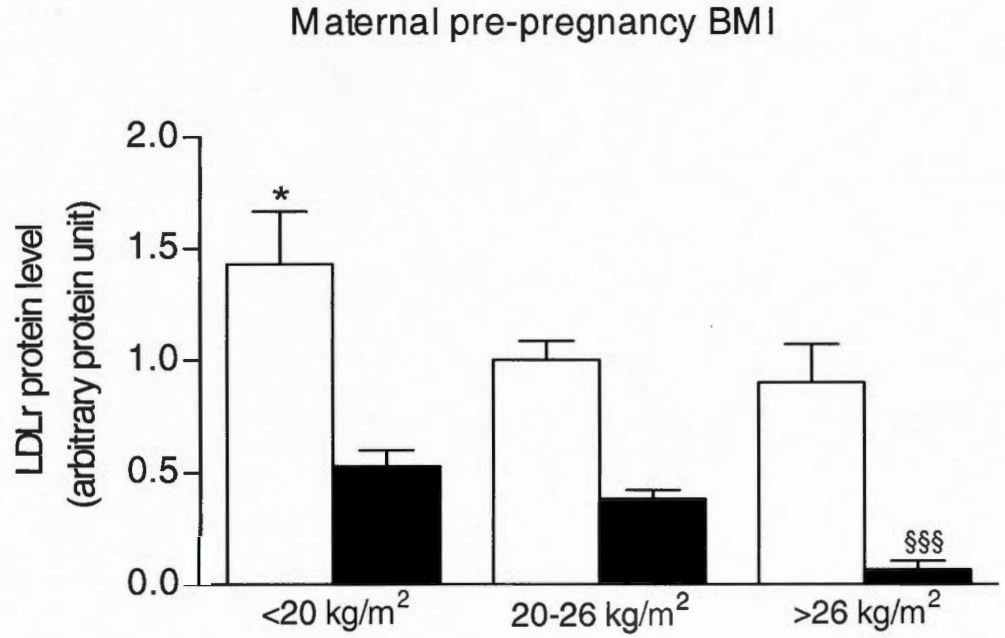

SR-BI protein level
(arbitrary protein unit)
1.5
1.0
0.5
0.0
LC
HC

Figure 3

A


Maternal pre-pregnancy BMI
LDLr protein level (arbitrary protein unit)
2.0
1.5
1.0
0.5
0.0
*
§§§
<20 kg/m2
20-26 kg/m2
>26 kg/m2

B

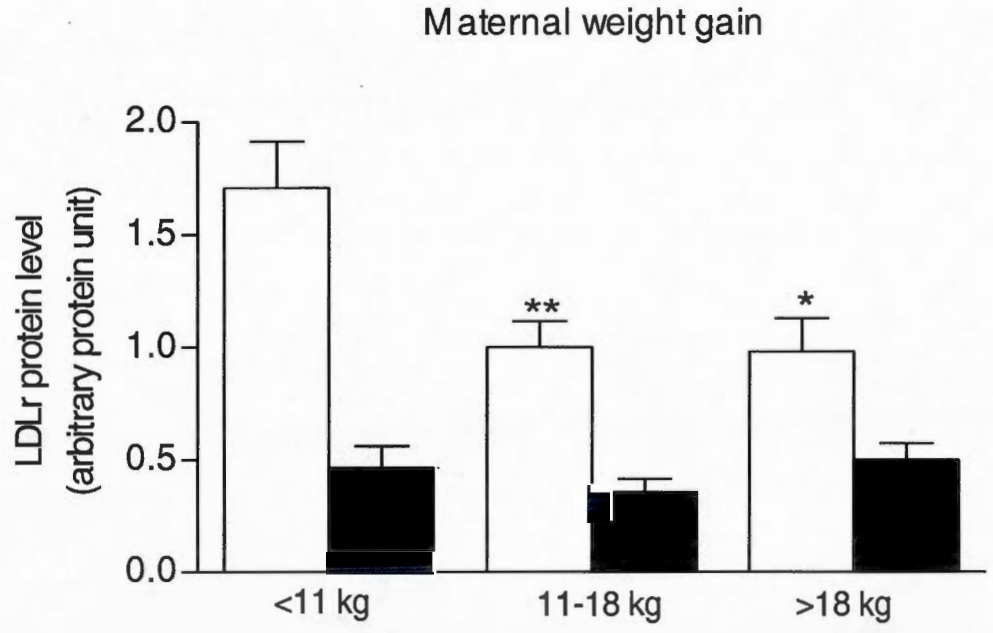
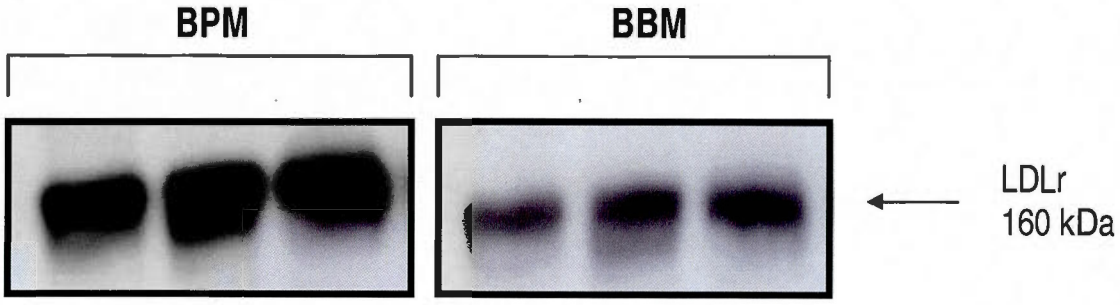

Maternal weight gain
LDLr protein level (arbitrary protein unit)
2.0
1.5
1.0
0.5
0.0
**
*
<11 kg
11-18 kg
>18 kg

Figure 4

A



B

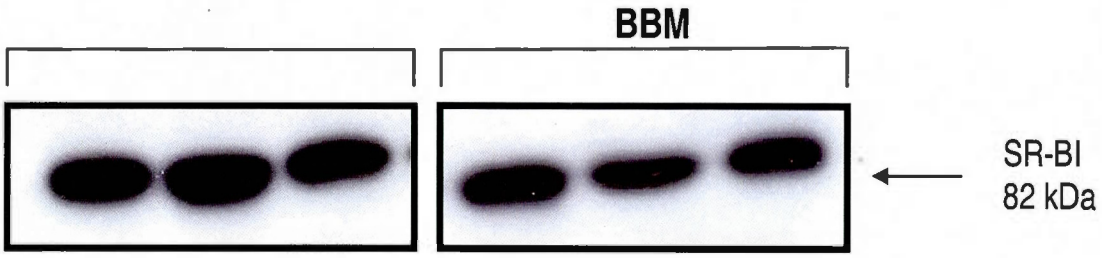


Figure 5

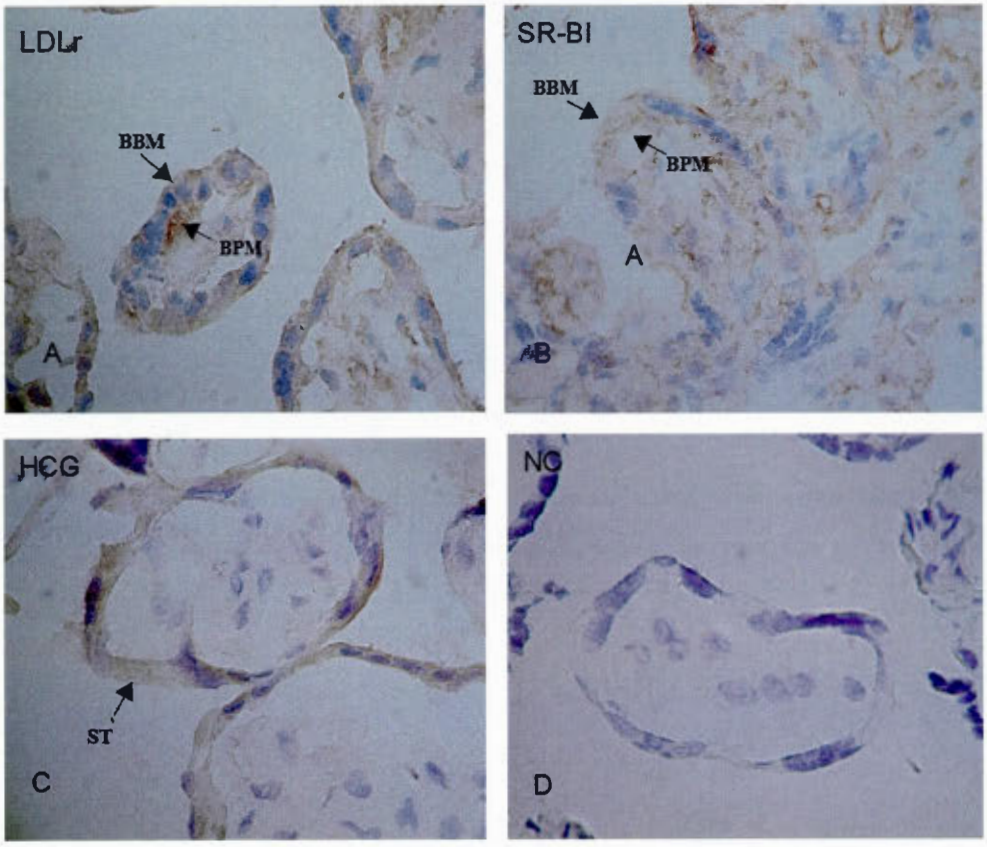
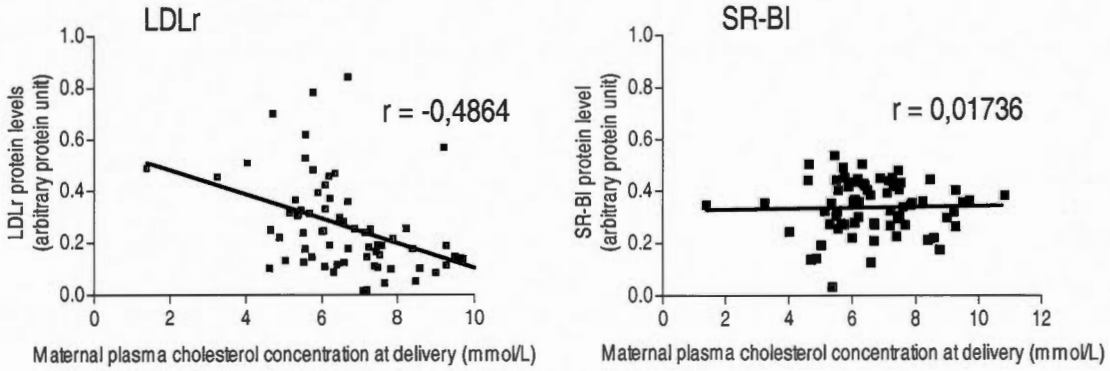
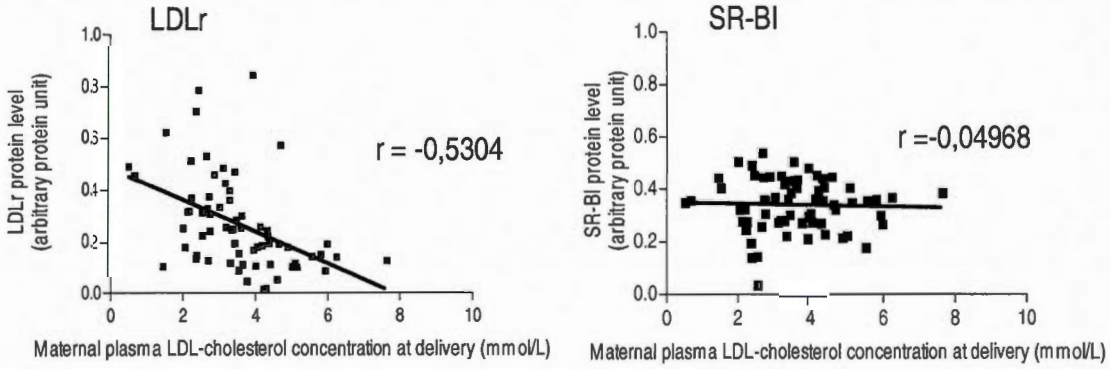


Figure 6

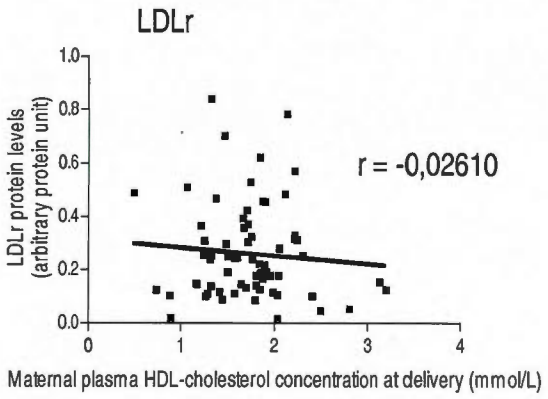
A- Total cholesterol concentration

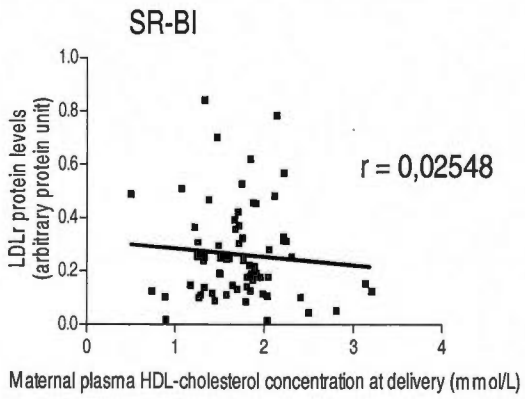


B- LDL-cholesterol concentration

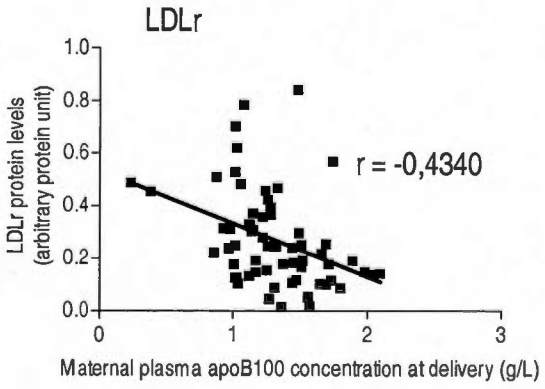


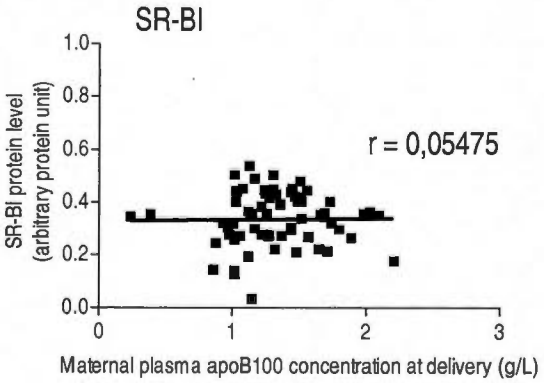
C- HDL-cholesterol concentration





D- ApoB100 concentration





3. CHAPITRE III

ARTICLE 2

3.1 Contributions

3.2 Titre de l'article :

MODULATION OF PLACENTAL PROTEIN EXPRESSION OF LECTIN-LIKE OXIDIZED LOW DENSITY LIPOPROTEIN RECEPTOR-1 (LOX-1): IMPLICATION IN PREGNANCY-RELATED DISORDERS OR PATHOLOGIES

Auteure principale: Maude Ethier Chiasson

Collaborateurs :J.-C. Forest, Y. Giguère, C. Marseille-Tremblay

Directrice de recherche : J. Lafond

L'article suivant a été rédigé dans sa totalité par moi-même, Maude Ethier Chiasson. La réalisation des expériences ainsi que les tests statistiques en découlant ont aussi été réalisés par moi-même, exception faite quant aux dosages des cytokines inflammatoires effectué par Charles Marseille Tremblay. L'échantillonnage de la population, le suivi des patientes et les accouchements ont été effectués à l'unité d'obstétrique et de gynécologie de l'hôpital St-Luc, sous la direction du Dr André Masse et ses collaborateurs. Les dosages sanguins ont été effectués à l'hôpital St-François d'Assise, dans les laboratoires des Dr Yves Giguères et Jean-Claude Forest.

L'article suivant est présenté sous la forme exigée par l'université concernant la mise en forme, cependant, la version soumise au journal *Placenta* a été modifiée selon les exigences du journal.

3.2 MODULATION OF PLACENTAL PROTEIN EXPRESSION OF LECTIN-LIKE OXIDIZED LOW DENSITY LIPOPROTEIN RECEPTOR-1 (LOX-1): IMPLICATION IN PREGNANCY-RELATED DISORDERS OR PATHOLOGIES

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Key words: Placenta, LOX-1, LDLox, cholesterol, lipid profile, BMI, BPM, BBM

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ABSTRACT

The lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a newly described receptor for oxidatively modified low-density lipoprotein. The human pregnancy is associated with hyperlipidemia and oxidative stress. It has been reported that modification in maternal lipid profile can induce disturbance during pregnancy. In this study we have evaluated by Western-blot analysis the expression protein level of LOX-1 in human term placenta of women having plasma cholesterol level lower to 7mM or higher to 8mM and women of gestational diabetes mellitus. The present study demonstrates that the maternal lipid profile is associated with placental protein expression of LOX-1. A significant increased of the protein expression of LOX-1 was observed in placenta of women with elevated plasmatic total cholesterol level (>8mM). In addition, the placental protein expression of LOX-1 is increased in mother having the highest pre-pregnancy body mass index (BMI) and low (<7mM) plasmatic total cholesterol level at term. Interestingly, the placental protein expression of LOX-1 is increased in presence of gestational diabetes mellitus compared to normal pregnancy. In conclusion, placental LOX-1 protein expression is associated with maternal lipid profile and pre-pregnancy BMI, as well as in case of gestational diabetes mellitus.

INTRODUCTION

The placenta is the central support organ for the developing fetus, serving as the site of maternal-fetal exchange of ions and lipids [120]. Human trophoblast differentiation is characterized by the formation of a specific multinuclear structure, named the syncytiotrophoblast. The syncytiotrophoblast cell possesses two structurally and functionally different plasma membranes: a brush border membrane (BBM) facing the maternal circulation and a basal plasma membrane (BPM) facing the fetal circulation [17, 121]. During pregnancy, cholesterol is an essential component for placental and fetal development, used by the placenta for the synthesis of steroid hormones [119]. Physiological adaptations of maternal lipoprotein metabolism occur throughout pregnancy, leading to increasing lipoprotein concentrations from 2nd trimester to term. Maternal hypercholesterolemia is a characteristic feature during pregnancy and corresponds to an accumulation of triglycerides (TG) in very low density lipoprotein (VLDL), in low density lipoprotein (LDL) and in high-density lipoprotein (HDL) [6, 35]. Although the exact mechanism of cholesterol transport across placenta remains unclear, many studies suggests the implication of HDL as well as LDL, both providing cholesterol for placental hormone synthesis [167]. Early in pregnancy, maternal anabolic phase produces metabolic changes to support lipogenesis and fat storage in preparation for the catabolic phase of late pregnancy, in which there is rapid fetal growth [168]. The maternal insulin resistance observed during this period increases lipolysis in adipose tissue, leading to an enhanced flux of fatty acids to the liver. This promotes the synthesis of VLDL resulting in an increased

of TG concentrations. Moreover, insulin resistance reduces the activity of lipoprotein lipase, the enzyme responsible, for VLDL clearance from plasma. Then, VLDL remains in the plasma for longer period and ultimately leads to the accumulation of LDL. Thus this increase in LDL is directly associated with the development of atherosclerosis [169].

Human placental cells express many lipoprotein receptors that can bind many lipoproteins such as the low density lipoprotein receptor (LDLr) [56], the LDL receptor related protein (LRP) [125], the very low density lipoprotein receptor (VLDL receptor) [126], the scavenger receptors [69, 99, 103] and the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) [77]. This latter one was recently cloned by Sawamura and al. [76, 85], and it can bind, internalize and degrade the oxidized LDL (ox-LDL). LOX-1 is a type II membrane glycoprotein belonging to the C-type lectin family with a short N-terminal cytoplasmic tail and a long C-terminal extracellular domain [80]. It has a molecular weight of 47 kDa and is initially synthesized as a 40 kDa precursor protein (pre-LOX-1), that has a minimal N-linked high mannose type carbohydrate, which is further glycosylate subsequently into the mature protein and transported to the cell surface [170]. LOX-1 binds ox-LDL, delipidated and solubilized ox-LDL, indicating that oxidatively modified apolipoprotein B-100 (apo-B) or some oxidized phospholipids firmly attached to the apo B might be ligands of LOX-1 [85]. In addition, this receptor is upregulated by

pro-atherogenic conditions, such as dyslipidemia, hypertension and diabetes [92, 93, 96, 171].

During human pregnancy, the maternal lipoproteins profiles is greatly modified [6]. Fundamentally, pregnancy is associated with an oxidative stress state arising from the increasing metabolic activity of placental mitochondria and the decreasing scavenging by antioxidants [172]. Production of lipids peroxidation product has been detected in human placenta; LDL are one of the targets for lipid peroxidation which become in their oxidized form [173]. This process represent a key step in the development of atherosclerosis, since several studies showed that LDL become atherogenic when they are converted to ox-LDL [70, 174]. Because they have reduced affinity for the native LDL receptor (LDLr), smaller and denser particles are considered to be more atherogenic. Thus, they are longer retained in the circulation, and become more inclined to oxidation, maybe because they contain less intrinsic antioxidants [175, 176]. Ox-LDL becomes ligands for scavenger receptors, including LOX-1.

Affecting 3 to 7% of women, gestational diabetes is characterized by endothelial dysfunction and reactive nitrogen and oxygen species contribute to the progression of diabetes [177, 178]. In diabetes, ROS (reactive oxygen species) including superoxide are thought to be produced as a result of prolonged periods of exposure to hyperglycaemia, which is known to cause non-enzymatic glycation of plasma

proteins [179]. In addition, gestational diabetes mellitus is generally associated with disturbances in lipoprotein metabolism with a tendency toward hypercholesterolemia [180]. During pregnancy, women with gestational diabetes mellitus have higher serum TG concentrations, but lower HDL-cholesterol concentrations than normal pregnant women [181].

This study was designed to observe the modifications of the metabolism of circulating lipids on the protein expression of LOX-1 in human term placenta and to correlate the modification of LOX-1 with BMI and some inflammatory cytokines. The aimed was to evaluate the expression and localisation of LOX-1 in placentas of normal, hypercholesterolemic and gestational diabetes mellitus women, using Western blot analysis and immunohistochemistry. Thus, this study could lead to a better understanding of the regulation of important receptor involved in oxidized cholesterol metabolism and pregnancy-related disorders/pathologies in which the lipid profile may be disturbed.

MATERIALS AND METHODS

Study population

The women participating in the study were recruited at their first prenatal visit, before their tenth week of pregnancy, at the Clinique Fidès of Montreal and at the Service of Perinatalogy, of the Centre Hospitalier de l'Université de Montréal (CHUM), Pavilion St-Luc, Canada, from 2002 to 2006. The study was approved by the ethical committee of St-Luc Hospital and Université de Montréal (Montréal, Qc, Canada). After signing a consent form, each woman filled out an interview-administrated questionnaire, which contained general sociodemographic data, medical history, drinking and smoking habit. The total study population consisted of 900 pregnant women. To conduct this study, 79 women were selected. Of these women, 72 have a normal pregnancy, while 7 developed gestational diabetes mellitus (GDM). The ones from normal pregnancy were classified in two groups according to the plasmatic total cholesterol concentration at term. To establish the group, we based ourselves on the median of the maternal plasmatic total cholesterol at term (6,42 mM). Women with cholesterol concentration lower than 7mM referred to the low cholesterol group (LC) (n=43) while women with cholesterol concentration higher than 8mM referred to the high cholesterol (HC) group. Subsequently they were reclassified into three groups according their pre-pregnancy body mass index (BMI). The BMI, representing a measure of adiposity, was calculated as pre-pregnancy kg/m2. The established normal values for BMI, according to the March of Dimes recommendations, were between

20-26 kg/m^2. For women who developed gestational diabetes they were matched with 12 controls women giving birth about 37 to 40 weeks, having normal lipid profile, normal values for BMI and weight gain. All of these women were non-smokers and did not receive any medication known to interfere with lipid metabolism, except for women with gestational diabetes who received insulin. Finally, a post natal follow up was made to assess newborns health status as well as collecting data relative to the weight and height of the babies.

Blood samples

Blood samples were collected at delivery, from the mother and the cord blood. The blood samples were collected in 10 ml gel Vacutainer tube (BD, Oakville, Canada), centrifuged 15 min at 3500 x g, less than 1h after delivery and plasma samples were kept at -20°C until analysis. The placentas, from vaginal delivery, were obtained from the collaborating hospital and immediately immersed in Dulbecco's modified Eagle Medium (DMEM) (Sigma, Oakville, ON, Canada) containing a mixture of antibiotic (penicillin, streptomycin and neomycin, Invitrogen) and $NaHCO_3$. After the removal of the amnion, the chorion and the decidual layer, the placental tissue, was cut in sections of 5cm^2, and immediately frozen in liquid nitrogen and kept at -80°C until use.

Lipid assays

The plasma levels of total cholesterol and LDL, HDL, VLDL, TG were individually measured using the Unicel 36 DX600 Synchron Clinical System (Beckman-Coulter, Mississauga, Canada), at the Clinical Biochemistry Service of Hôpital Saint-François d'Assise, Québec (Qc, Canada).

Total proteins extraction

The placental frozen tissue samples were washed 3-4 times with cold NaCl 0,9% solution containing anti-protease (1 μM leupeptin, 1,46 μM pepstatin and 2 μg/ml aprotinin) to remove blood from tissue. The tissue (1g) was homogenised using a Polytron Tissue homogeniser PT 3000 (Brinkmann, Canada) in 1ml ice-cold hypertonic buffer (125 mM Tris-HCl, pH 8,0, 2Mm $CaCl_2$, 1.4% (v/v) Triton X-100, 1 μM leupeptin, 1,46μM pepstatin, 2 μg/ml aprotinin and 1 mM phenylmethylsulphonylfluoride (PMSF)). The homogenate was kept on ice for 30 min, centrifuged at 10 000 X g for 25 min at 4°C, the supernatant was collected and stored at -80°C until used. The protein concentration content was determined by spectrophotometric quantification using the bicinchoninic acid (BCA) reagent (Pierce, Brockville, ON, Canada) with bovine serum albumine (BSA) as standard.

Purification of syncytiotrophoblast brush border (BBM) and basal plasma membranes (BPM)

Briefly, membranes were purified from placental tissues collected from fresh human placenta obtained from full-term normal vaginal delivery, Centre Hospitalier de

l'Université de Montréal, Pavilion St-Luc, Canada. After the removal of the amnion, the chorion and the decidual layer, the tissues were minced and stirred for 45 min in 10 mM Tris-HEPES (pH 7.,4) containing 270 mM mannitol, 0.1mM PMSF, 1 mg/ml benzamidin and 10mM leupeptin. The membranes were purified, as described by our laboratory [17] with some modifications [121].

Western blot analyses of LOX-1

Total placental proteins (30 μg for total placental protein and 150μg for syncytiotrophoblastics membranes) were solubilized in a sample buffer (4% SDS, 30mM dithiotreitol, 10% β-mercaptoethanol, 0,25 M sucrose, 0,01 M EDTA-Na_2 and 0,075% bromophenol blue), and heated at 95°C for 5 min. The proteins were resolved in a 8% SDS-PAGE and electroblotted to PDVF membrane (Millipore, Cambridge, ON, Canada) at 20 Volts for 40 min. Membranes were blocked overnight at 4°C in TBS-T (20 mM Tris Base, pH 7,6, 137 mM NaCl, 0.1% Tween-20) containing 5% skimmed milk. Thereafter, membranes were washed 3X TBS-T and anti-human LOX-1 goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA) (1/1000 in TBS-T/5% BSA, 1% skimmed milk), or human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mouse monoclonal antibody (Chemicon International, Temecula, California, USA) (1/5000 in TBS-T/5% BSA,) was added for 90 min at room temperature. Blots were washed 3X with TBS-T, and probed with horseradish peroxidase-conjugated secondary antibodies, for LOX-1,

anti-goat IgG (1/6500 TBS-T 5% BSA and 1% skimmed milk) and for GAPDH antimouse-IgG (1/3000 TBS-T 5% skimmed milk) (Chemicon International, Temecula, California, USA) for 90 min at room temperature. Blots were then washed 3X with TBS-T and the detection was performed using the BM Chemiluminescence system (Roche, Laval, Qc, Canada) and visualized by autoradiography (Hyperfilm ECL, GE Healthcare, Baie d'Urfée, Qc, Canada). The PDVF membranes were stripped with a stripping solution (HCl 12 M (pH 2,0) glycine 25 mM, SDS 1%) for 10 min at room temperature. Thereafter, membranes were rinsed 3X for 5 min with a washing solution (1 M Na_2HPO_4, 1 M NaH_2PO_4 (pH 7.2) 9 g/L NaCl) and blocked for 1h. Thereafter, the membrane is ready to be reprobed with GAPDH. Subsequently, the membrane is ready to be reprobed with GAPDH for loading standardization. We normalized each data with a control sample which one serve to express each data as percentage of control. For semiquantitative analyses of the bands, the film was digitized and was analyzed by the Quantity One Software (Bio-Rad Laboratories, Mississauga, ON, Canada).

Immunohistochemistry

An immunohistochemistry study was performed to investigate the localisation of LOX-1 protein. We used appropriate placental tissues from fresh placentas. After the removal of the amnion, the chorion and the decidual layer, the placental tissue was cut in sections of 5x5x5 mm, embedded with cold (4°C) Tissue-tek OCT (Electron

Microscopy Sciences, Hatfield, PA) and immediately frozen in isopentane at -80°C (Sigma-Aldrich, St-Louis, MO). Sections were stored at -80°C until further processing. For immunohistochemical staining, frozen sections (8µm thick) were mounted onto SuperFrost Plus glass slides (Fisher Scientific, Pittsburgh, PA) and air dried overnight. Section were then fixed with freshly prepared cold 4% paraformaldehyde and 0,2% picric acid in PBS for 20 min at 4°C. To complete fixation, cryosections were air dried at room temperature. Tissues sections were rinsed with PBS for 3 min and then, sections were incubated with permeabilisation solution (1% Triton X-100 in PBS) for 30 min at room temperature. Sections were blocked in 0,5 % donkey serum and 0,1% Tween-20 for 60 min at room temperature, after quenching endogenous peroxidase activity by exposing slides to «Peroxidase Block» (Kit LSAB+ System-HRP, DAKO, Denmark) for 5 min. The sections were then incubated with primary antibodies diluted in blocking solution (Biomeda, Foster City, CA) overnight at 4°C. The primary antibodies used were goat polyclonal anti-LOX-1 antiserum (1:100, Santa Cruz Biotechnology, CA), rabbit polyclonal anti-hCG (1:500, Chemicon) and mouse anti-CD34 (1:1000, NeoMarker). To ascertain protein specific antibody binding, controls were performed omitting primary antibodies. Subsequently, they were incubated with endogenous avidin and bitotin blocking solution for 15 min at room temperature respectively. Tissue sections were incubated with "Link" universal secondary antibody solution (DAKO) for 60 min at room temperature and then with "Streptavidin-peroxydase" for 30 min. They were

stained with «Substrate-chromogen solution» (DAKO), and counterstained with Mayer's hematoxylin (Fluka, Buchs, Switzerland). Sections were viewed and photographed under interference-contrast Leitz Diaplan microscope equipped with a Nikon CoolPix 990 camera. Cells having intense brownish staining were considered immunopositive.

Cytokines quantification

Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) concentrations were measured in the total protein fraction extracted from placental tissue, using the Human IL-1 (IL-1b) ELISA Ready-SET-Go! and Human TNFa (Tumor Necrosis Factor alpha, TNF-alpha, TNF-a) ELISA Ready-SETGo! Kits (eBioscience, San Diego, USA) according to the manufacturer's instructions.

Statistical analyses

Data were expressed as the mean ± SEM, and analysed with unpaired Student *t*-test at $p < 0,05$ level of significance, to evaluated difference between groups. For the relationship between two variables of the same population, results are expressed as Spearman correlation and the curve represent Pearson linear correlation. All statistical analyses were performed using the Prism software (version 4.0.2; GraphPad Software, 2004).

RESULTS

Subject characteristics

Basic characteristics of mothers and newborns are given in Table I. A total of 72 women gave birth and their blood samples were collected at term. The participants were split in two groups: LC women (n=43) and HC women (n=29). The age or the women was ±32 years old and their gestational age was about 39 weeks and there was no difference in the BMI of both groups. No differences were observed concerning the weight gain. No differences were observed for weight and height at birth for babies of both groups. In addition, no differences were observed for placental weight.

Plasma lipids at birth and in cord blood

Table II shows total cholesterol, HDL-cholesterol, LDL-cholesterol, TG, Apo-AI and Apo-B concentrations at delivery and in cord blood for LC and HC groups. Total cholesterol, LDL-cholesterol and Apo-B100 concentrations were different between both groups at delivery, but no differences were observed for HDL. The HC women had significantly higher level of TG at delivery than LC women (p= 0,0029). The concentrations of apo-AI showed no differences between both groups. Finally, no differences were observed in cord blood sample for both groups regarding all studied parameters.

Protein expression of LOX-1

The expression of LOX-1 was evaluated by Western blot using total protein isolated from placental tissue. Figure 1A shows representative Western blot of the expression of LOX-1 and GAPDH from placental tissue extracts. Following normalization with the GAPDH protein level, our results demonstrates that the expression of LOX-1 is increased (68%) in HC group compared to LC one (Fig. 1B). Figure 2 shows the expression of LOX-1 after its normalization with GAPDH, in relation with different (A) pre-pregnancy BMI (<20 kg/m^2, 20-26 kg/m^2 and >26 kg/m^2). For the LC group (<7 mM), this figure shows the increase of the protein expression of LOX-1 in placenta of the women with the highest BMI as compared to those with the lowest BMI (p=0,0072). While, for the HC women, no difference were observed between all different BMI groups. In addition, our results show an increase of the protein expression of LOX-1 between the LC and HC groups for the 2 lowest BMI groups (p=0,0002 and p<0,0001, respectively).

Level of inflammatory cytokines in term placenta

To evaluate the role of inflammatory markers on LOX-1 placental protein expression, the quantification of IL-1β and TNF-α was done. These cytokines are highly expressed in placenta, principally IL-1β. Figure 1C shows the IL-1β and TNF-α concentrations in human term placenta in relation of maternal plasmatic total cholesterol level at term. Our results demonstrated that theses cytokines were not

influenced by maternal plasmatic total cholesterol level. Also, as shown in figure 2B, maternal pre-pregnancy BMI do not affect the level of these cytokines. However, in HC women placental IL-1β was influenced by weight gain; our result show an increases between women gained >11 kg and women with normal weigh gain (*139,2±11,54 versus 235,2±38.03*) (data not shown). No difference was showed for TNF-α related to weigh gain (data not shown).

Protein expression of LOX-1 in syncytiotrophoblast membranes

The expression of LOX-1 was evaluated by Western blot in syncytiotrophoblast BPM and BBM, isolated form fresh placenta. Figure 3 shows representative Western blot of the expression of LOX-1 in syncytiotrophoblast plasma membranes (BPM and BBM), syncytiotrophoblast cells and total protein from placental tissue. Our results demonstrated that LOX-1 protein expression was detected in both placental membranes, in syncytiotrophoblastic cells and in total protein extract from placenta and correspond to a molecular weight of 47 kDa

Immunohistochemical study of LOX-1

As show in figure 4 immunohistochemical study of LOX-1 expression in human placenta demonstrated that it was localized in both maternal (BBM) and fetal (BPM) side of syncytiotrophoblast cell and in fetal capillaries. LOX-1 stained strongly immunopositive principally in BBM.

Correlations between LOX-1 protein expression and circulating plasma lipids

The correlation between LOX-1 protein expression and many maternal plasma circulating lipids was evaluated by Spearman correlation and Pearson linear correlation curve. Positive correlations were observed between LOX-1 protein expression and maternal plasmatic total cholesterol level (r= 0,4248, *p*=0,0010), LDL plasma level (r =0,4102, *p*=0,0019) and apo-B100 level (r = 0.3885, *p*=0,0018) at term, as shown in figure 5A-B-D (left blots), respectively. In contrast, no correlation was marked between the LOX-1 protein expression and maternal plasma HDL level (r=0.1292, *p*=0,4619) at term (Fig. 5C). In addition, no correlation was found between LOX-1 protein expression and cord blood, total plasma cholesterol (r=-0,0773, *p*=0,5725), LDL-cholesterol (r=-0,1680, *p*=0,3737), HDL-cholesterol (r= -0,03027, *p*=0,7236) and apo-B100 (r= 0,07784, *p*=0,8223) levels (Fig. 5 A,B,C and D, right blots).

Population characteristics for gestational diabetes mellitus women

Basic characteristics for mothers and newborns are given in Table III. A total of 19 women gave birth and their blood samples were collected at term. The participants

were split in two groups: Control women (n=12) and GDM women (n=7). The age of the women was ± 31 years old, and their gestational age was about 39 weeks. Compared with controls women, the GDM women had significant highest BMI (22,2 ± 2,1 versus 28,3 ± 6,6; p= 0,0082). No differences were observed concerning the maternal weight gain. No differences were observed for the weight and height at birth for babies of both groups. In addition, no differences were observed for the placental weight.

Plasma lipids during pregnancy and at birth of gestational diabetes mellitus

Table IV presents total cholesterol, HDL-cholesterol, LDL-cholesterol, TG, apo-AI and apo-B100 concentrations at delivery and in cord blood for control and GDM women. No differences were observed for total cholesterol, LDL-cholesterol, apo-AI and apo-B100 at delivery. GMD subjects had significantly lower concentration of HDL-cholesterol at delivery than control women (p=0,0415). No differences were observed in cord blood samples for both groups for all studied parameters.

Protein expression of LOX-1 in gestational diabetes mellitus

The expression of LOX-1 was evaluated by Western blot using total protein isolated from placental tissue. Figure 6A shows representative Western blot of the expression of LOX-1 and GAPDH from placental tissue (n=3 for each group). Following normalization with the GAPDH protein level, our results (Fig. 6B) show that

expression of LOX-1 in GDM women is significantly increased by 63% compared to the control ones ($p = 0,0078$).

Inflammatory cytokines level in term placenta of women with GDM

Figure 6C shows concentration of IL-1β and TNF-α in term placenta of GDM women and control women (CTL). Our results clearly demonstrated that theses cytokines were modified in GDM group, while both placental IL-1β and TNF-α were increased (0,0139 and 0,0437 respectively).

DISCUSSION

Our study shows that maternal lipid profile is associated with LOX-1 protein expression in human term placenta. Placental LOX-1 protein expression is increased in case of maternal hypercholesterolemia and is affected by pre-pregnancy BMI. In addition, in the present study we show for the first time the expression of LOX-1 in maternal (BBM) and fetal side (BPM) of normal human placentas.

Abnormally high plasma lipids concentration is thought to be an important pathogenic factor, principally in atherogenesis. In apo-E knockout mice, a model for spontaneous hypercholesterolemia, the stimulated LOX-1 expression is reduced by feeding mice with an HMG-CoA reductase inhibitor [182], which decreases LDL-cholesterol level and reduces the formation and uptake of ox-LDL [183]. Our data are in accordance with these observations since we showed a positive correlation between plasma total cholesterol, LDL-cholesterol and apo-B100 levels with placental protein expression of LOX-1. The oxidative modification hypothesis, proposed by Goldstein et al. [184], focuses on the concept that LDL in its native form is not atherogenic [185]. However, LDL chemically modified is readily internalized by macrophages through the scavenger receptor pathways [184]. The only mechanism whereby cells use LDL as substrate for the scavenger receptor pathways is, *in vitro*, via an oxidation of LDL which results in a modification of apo-B100 [186]. LDL-cholesterol are subject to oxidation and modification of the lysine residues on apo-B100 leading to an increase of the net negative charge on the lipoprotein particles [187]. A study

realized with JAR cells (a choriocarcinoma cell line) indicates that LOX-1 mediates 40-50% of ox-LDL uptake [78]. However, lipids peroxides are important because their uncontrolled production may result in oxidative stress with significant damage to cell integrity [173]. The importance of oxidative stress in pregnant women is beginning to be studied to an increasing extend, because of the current little of knowledge. In contrast, in non-pregnant women, there is a physiological change in plasma lipids and an increased oxidative turnover that is controlled by countervailing antioxidative mechanisms in healthy pregnant women [24]. Considering the high demand by the foetus, there is an increase production of ROS by the placenta during pregnancy, on the other hand, placenta is a source of an antioxidative enzyme and hormone system to control placental lipid peroxidation in healthy pregnancy [188]. Thus, the excessive lipid peroxidation due to an increased of pro-oxidants or caused by increased susceptibility of LDL to oxidation, is associated with pregnancy disorder like preeclampsia and IUGR [189]. Many studies reported higher levels of placental lipid peroxides in preeclampsia than in normal pregnancies [190-192], suggesting that placental tissues could be the source of elevated levels of serum lipid peroxides. Thus the high level of LOX-1 placental protein expression can be explain by enhancement of oxidative stress in HC women and thus this condition could be responsible of some pregnancy disorders, like preeclampsia.

In this study, we demonstrated that pre-pregnancy BMI significantly increased placental LOX-1 protein expression in LC women. We firstly hypothesized that this

increase could be attributes to the inflammatory status observed in obesity [193]. It is well known that adipose tissue is a highly active endocrine organ secreting a number of hormones that alter the circulation of metabolites, cytokines and growth factor [194, 195]. Thus, obese women are more likely than normal-weight women to enter pregnancy in a subclinical inflammatory state since increases in body fat are associated with elevated cytokine levels and inflammation [196]. Surprisingly, our data showed no difference for both IL-1β and TNF-α in relation to maternal BMI. However, this effect could be attributed to the fact that maternal adiposity could also produced a hypoxic state if glycosylated haemoglobin levels are increased and affinity for oxygen is reduced, decreasing oxygen transfer to the uterus and impairing placentation [197]. Thus, many molecules related to oxidative stress can induce the expression of LOX-1, such as isoprostanes which are prostaglandin isomers formed by free radical peroxidation of arachidonic acid presents in phospholipids [198]. Among these isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ a well known marker of oxidative stress [198], has the potential to increased the uptake of oxidized LDL and the expression of LOX-1 in JAR cells [199]. In addition, they showed that 8-iso-prostaglandin $F_{2\alpha}$, induced the activation of NF-κB, which is a redox sensitive transcription factor [199]. Some of our data showed that maternal weight gain had no effect on placental LOX-1 protein expression. In contrast, our results showed and increased in IL-1β content for HC women with normal weight gain (11-18kg) compared to HC women with lowest weight gain (<11kg). The precise relationship

between this cytokines and maternal weight gain remains unclear and further investigations will be necessary.

The present study demonstrates the localisation of LOX-1 in both maternal (BBM) and fetal (BPM) side of syncytiotrophoblast. The liaison of ox-LDL with LOX-1 induces superoxide generation, inhibits NO production, enhances endothelial adhesiveness for leucocytes and induces chemokine expression [91, 200]. Thus, the presence of LOX-1 in both side of syncytiotrophoblast may promote apoptosis [201]. A recent study show that the functional role of trophoblasts in placental dysfunction results from the localization and the upregulation of LOX-1 in the preeclamptic placenta, which possibly promote apoptotic activity [201]. Since LOX-1 is a receptor for ox-LDL, a highly atherogenic lipoprotein, physiological functions of placental cell may be altered. Thus maternal hypercholesterolemia, could promote fetal atherosclerosis development via LOX-1. Finally, further investigations will be required to understand the precise role of this two binding sites.

An important finding of our study is that GDM increased placental LOX-1 protein expression. This result is in accordance with studies that demonstrated the increase of the expression of LOX-1 in diabetes in different tissues. It has been reported that LOX-1 expression is increased by glucose both in macrophage and endothelial cells [202, 203]. Another study using diabetic rats found that LOX-1 expression is increased in aortas of diabetic rats, compared with non-diabetic rats [204].

Effectively, diabetes is characterized by the presence of an important oxidative stress, endothelial dysfunction and increased expression of endothelial molecules in the inflammatory cells [205]. Thus, this effect could be attributed to the maternal inflammatory status; a recent study show that an increase of cytokines concentration was observed in maternal blood with GDM [206].This finding is in agree with our results where we demonstrated an significant increased of two central mediators of inflammatory reaction, IL-1β and TNF-α, in placenta of women with GDM. Finally, we show that this increase is more pronounced for placental IL-1β, while this cytokine being associated with the risk of developing type 2 diabetes [207]. In fact, several studies shows that pro-inflammatory cytokines regulates the expression of LOX-1 [94, 208, 209]. A study using smooth muscles cells showed that strong codistribution of the pro-inflammatory cytokines IL-1α and IL-1β, and TNF-α with LOX-1 expression in advance atherosclerosis lesions [208]. Moreover, pregnancy induces inflammatory changes characterized by activation of peripheral blood leucocytes [210], and the production of pro-antiflammatory cytokines such as IL-6 and TNF-α. In human, placental tissue expresses different pro- and anti-inflammatory cytokine [211]. TNF-α and its receptors have been shown to be significantly produced by human placenta; TNF-α gene is expressed in decidua and trophoblast [212]. It has been shown that TNF-α does not cross the placenta [213, 214], suggesting that the TNF-α is synthesized by the placenta and/or the fetus itself [212].

Thus, the increase of the expression of LOX-1 observed in women with GDM could be regulated by theses cytokines.

Of particular mention, GDM women had higher BMI that control ones. It is known that hyperlipidemia is exaggerated in obese pregnant women [215]. This fact could be attributed to the inability of insulin to suppress whole-body lipolysis leads to a marked increase in plasma free fatty acids (FFA) in obese women compared to control women [216]. Obese women also had higher serum TG levels packaged into VLDL, as well as LDL and HDL [6, 215]. Abundance of lipoprotein-triacylglycerols is associated with accelerated transfer of TG toward HDL by cholesterol ester transfer protein (CETP) [119]. Thus, reduced hepatic lipase activity accounts for the formation of larger HDL particles, rich in TG and less dense, typically occurring during advanced stages of gestation [217]. Concomitantly, LDL particles become richer in TG, while reducing their size and increased their density [218-220]. Ultimately, these changes in lipoprotein profile may favor endothelial damage in pregnancy and activation of atherogenesis [221]. The marked increase in serum lipids in obese pregnant women are attributed to the enhanced of insulin-resistant condition and to an increase in plasma estrogens [6]. In adipose tissues of obese women with GDM, Catalano and co-workers (2002) found that insulin receptor substrate-1 protein levels were 43% lower than in controls [222]; this fact will account, at least in part, for the increase of insulin resistance seen in obese women.

Finally, circulating levels of peroxisome proliferators-activated receptor-γ (PPARγ) mRNA and protein are reduced in obese women with GDM compared that healthy women [222]. Moreover, Hofnagel and co-workers (2006) found that activator of PPARγ inhibit upregulation of LOX-1 by IL-1β [208]. Moreover, babies delivered under GDM were normal, resulting in the maternal intake of insulin during the third trimester of pregnancy [223]. To conclude, all of these fact could are in accordance with our data, where we found that placental protein expression of LOX-1 is increased in women with GDM.

In conclusion, our results corroborate the importance of maternal lipid profile during pregnancy. Thus, maternal hypercholesterolemia, as well as GDM, could promote fetal atherosclerosis development. The modulation of LOX-1 protein expression by disturbance or pathologies during pregnancy can ultimately influence fetal growth and development.

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Table I: Population characteristics

	LC group (< 7 mM) n=43	HC group (> 8 mM) n=29
Mother age (year)	31.2 ± 5.3	31.2 ± 4.2
Gestational age (week)	39.0 ±1.5	39.3 ± 1.5
Mother BMI (kg/m2)	23.5 ± 5.0	22.41 ± 4.36
Mother weight gain (kg)	15.01 ± 6.09	18.83 ± 6.81
Newborn birth weight (g)	3291 ± 648	3420 ± 462
Newborn height (cm)	51.5 ± 2	52.0 ± 5.0
Placenta weight (g)	592.4 ± 154.1	609.8 ± 142.7

LC= Low cholesterol women group and HD High cholesterol women group. Results are expressed as mean ± SD

Table II: Plasma lipids at delivery and in cord blood

	LC group (<7 mM) n=43		HC group (>8 mM) n=29	
	At delivery	Cord blood	At delivery	Cord blood
Total cholesterol (mM))	5.75 ± 1.6	1.88 ± 0.94	8.21 ± 1.00 *	1.68 ± 0.51
HDL-cholesterol (mM)	1.70 ± 0.46	0.75 ± 0.31	1.76 ± 0.57	0.73 ± 0.31
LDL-cholesterol (mM)	2.80 ± 0.84	0.84 ± 0.54	4.84 ± 0.94 *	0.67 ± 0.28
TG (mM)	2.71 ± 1.12	0.64 ± 0.52	3.59 ± 1.21 *	0.60 ± 0.27
Apo-AI (g/L)	2.08 ± 0.38	0.90 ± 0.36	2.21 ± 0.46	0.83 ± 0.23
ApoB-100 (g/L)	1.14 ± 0.26	0.30 ± 0.23	1.64 ± 0.25 *	0.26 ± 0.10

LC= Low cholesterol women group and HC High cholesterol women group. Results are expressed as mean ± SD, where * $p < 0.05$ compared to the maternal plasma cholesterol concentration < 7 mM group

Table III: Population characteristics of gestational diabetes mellitus (GDM) women

	Control group n=12	**GDM group (>8 mM) n=7**
Mother age (year)	31.0± 7.3	30.0 ± 5.3
Gestational age (week)	38.8 ±1.4	39.0 ± 0,8
Mother BMI (kg/m^2)	22.2 ± 2.1	28.3 ± 6.6 **
Mother weight gain (kg)	14,7 ± 2,6	11.54 ± 5.5
Newborn birth weight (g)	3355 ± 393	3362 ± 356
Newborn height (cm)	51.2 ± 2.1	50.8 ± 1,6
Placenta weight (g)	547.9± 131.6	555.0 ± 175.0

Results are expressed as mean ± SD where * $p < 0.05$ compared to the control group

Table IV: Plasma lipids during pregnancy and at birth of gestational diabetes mellitus (GDM) women

	Control group n=12		**GDM group (>8 mM) n=7**	
	At delivery	Cord blood	At delivery	Cord blood
Total cholesterol (mM))	6.06 ± 0.51	1.82 ± 0.48	5.24 ± 1.87	1.48 ± 0,16
HDL-cholesterol (mM)	1.80 ± 0.32	0.76 ± 0.22	1.40 ± 0.47 *	0.83 ± 0.49
LDL-cholesterol (mM)	3.04 ± 0.45	0.79 ± 0.35	2.60 ± 1.07	1.10 ± 1.24
TG (mM)	2.66 ± 0,72	0.60 ± 0.25	2.71 ± 1.09	0.57± 0.33
Apo-AI (g/L)	2.20 ± 0.31	0.89 ± 0.17	1.89 ± 0.48	1.15 ± 0.83
ApoB-100 (g/L)	1.16 ± 0.11	0.27 ± 0.15	1.07 ± 0.42	0.43 ± 0.54

Results are expressed as mean ± SD, where * $p < 0.05$ compared to the control group

Figure 1. Representative protein expression of LOX-1 and inflammatory cytokines concentration from total proteins extracted from term placenta related to maternal plasmatic total cholesterol concentration. (A). Western blot analysis was performed using LOX-1 antibody on total protein (150μg) isolated from placental tissue. Thereafter, the blot was stripped and reprobed with an anti-GAPDH antibody. (B) Densitometric analysis of LOX-1 protein level after normalization with GAPDH protein level. LC (n=43) and HC (n=29). (C) Inflammatory cytokines, IL-1β (right) and TNF-α (left), content in term placenta. LC (n=31) and HC (n=21). Results are expressed as mean ± SEM, where *** $= p < 0.001$

Figure 2. Representative protein expression of LOX-1 and inflammatory cytokines concentration from total proteins in total proteins extracted from term placenta related to maternal pre-pregnancy BMI. (A) Densitometric analysis of LOX-1 protein level after normalization with GAPDH protein level. Western blot analysis was performed using LOX-1 or GAPDH antibody in total proteins (150μg) extracted from placental tissue. (B) Inflammatory cytokines, IL-1β (up) and TNF-α (down), content in term placenta. Results are expressed as mean ± SEM where ++ = $P < 0.01$ and *** = $p < 0.001$, n= ?????

Figure 3. Representative protein expression of LOX-1 in human purified, BBM BPM, syncytiotrophoblast (STB)(n=3) and total protein extract from placental tissue (PLA). (n=3) Proteins (150μg) were migrated on a 8 % SDS-PAGE under reducing conditions. Blots were done with LOX-1 antibody.

Figure 4. Immunohistochemical expression of (A) LOX-1 in syncytiotrophoblast (ST), in both maternal (BBM) and fetal side (BPM) of term placenta. Positive staining is in brown color and nuclear counterstaining is blue. (B) The positive control (HCG) is present in syncytiotrophoblast, (C) the endothelial marker, CD34, is present on fetal capillaries.

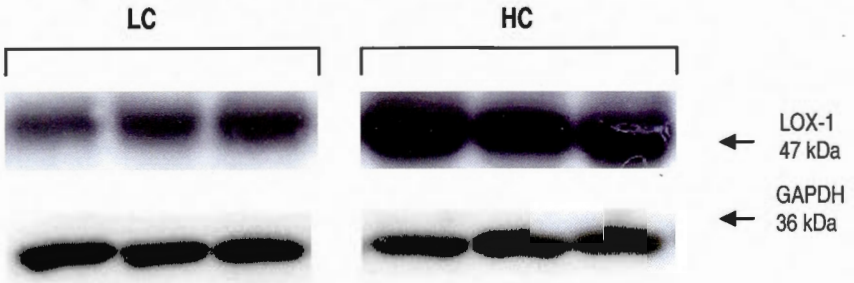
Figure 5. Relationship of LOX-1 protein expression and maternal plasmatic lipids concentration at delivery and in cord blood for (A) total-cholesterol (n= 83), (B) LDL-cholesterol (n= 68), (C) HDL-cholesterol (n= 70), (D) ApoB100 (n= 79). Results are expressed as Spearman correlation and the curve represent Pearson linear correlation.

Figure 6. Representative protein expression of LOX-1 in total proteins and, inflammatory cytokines content, extracted from term human placenta of CTLand GDM women. (A) Western blot analysis was performed using LOX-1 on total protein (150μg) isolated of placental tissue. Thereafter, the blot was stripped and reprobed with an anti-GAPDH antibody. (B) Densitometric analysis of LOX-1 protein level after normalization with GAPDH protein level, CTL (n=12) and GDM (n=7). (C)

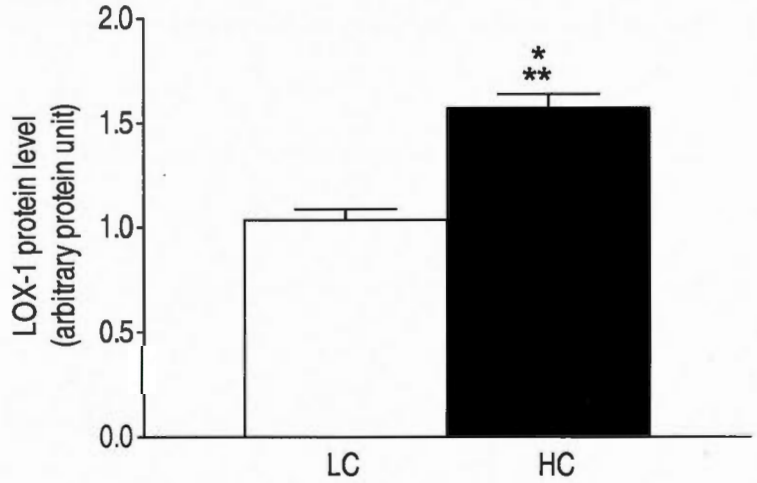
Inflammatory cytokines, IL-1β (right) and TNF-α (left), content in term placenta. CTL (n=10) and GDM (n=6) Results expressed mean ± SEM where ** $=p < 0.01$ and * $=p < 0.05$

Figure 1

A- Representation Western-Blot



B- Placental protein level



C- Cytokines content in term placenta

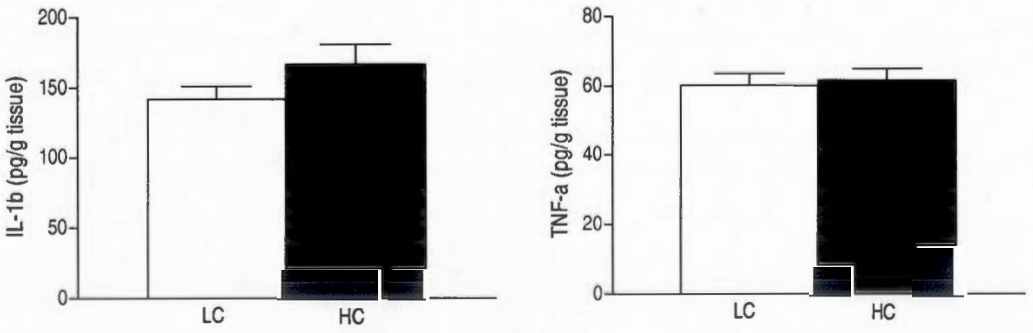
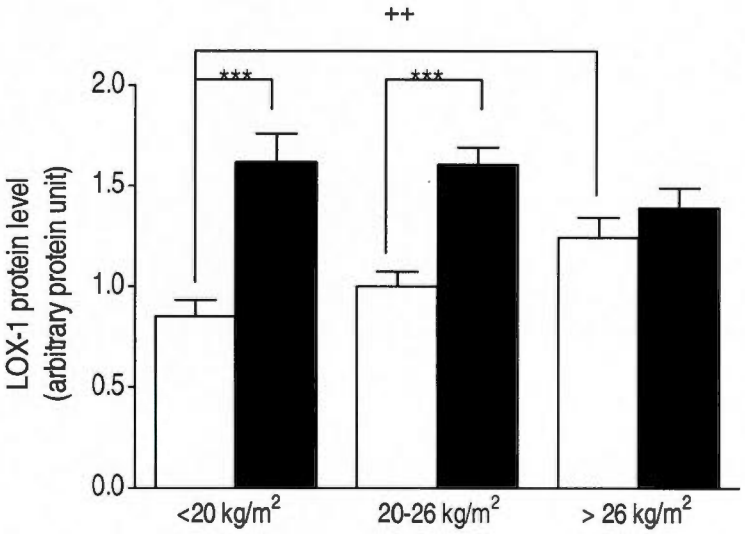


Figure 2

A- Placental protein level



B- Cytokines content in term placenta

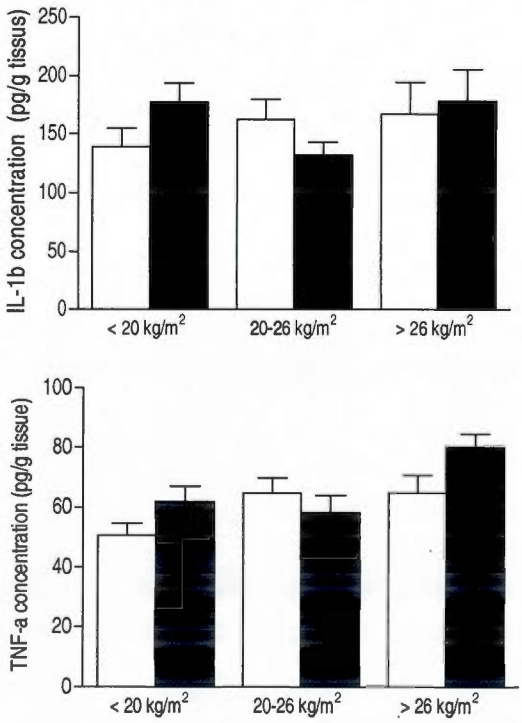


Figure 3

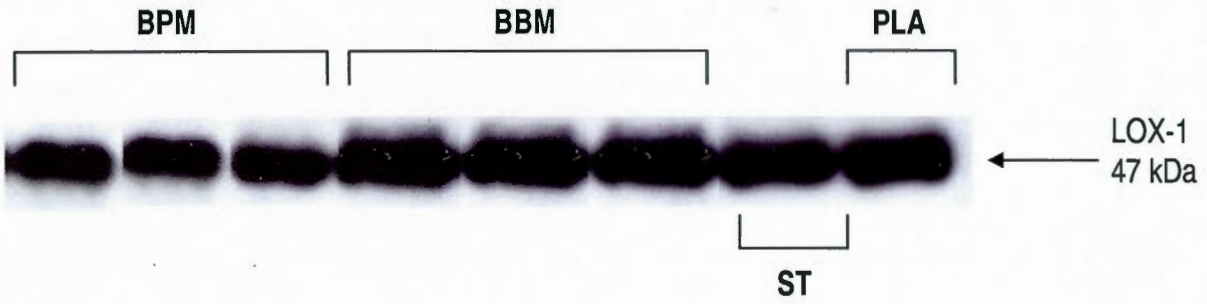


Figure 4

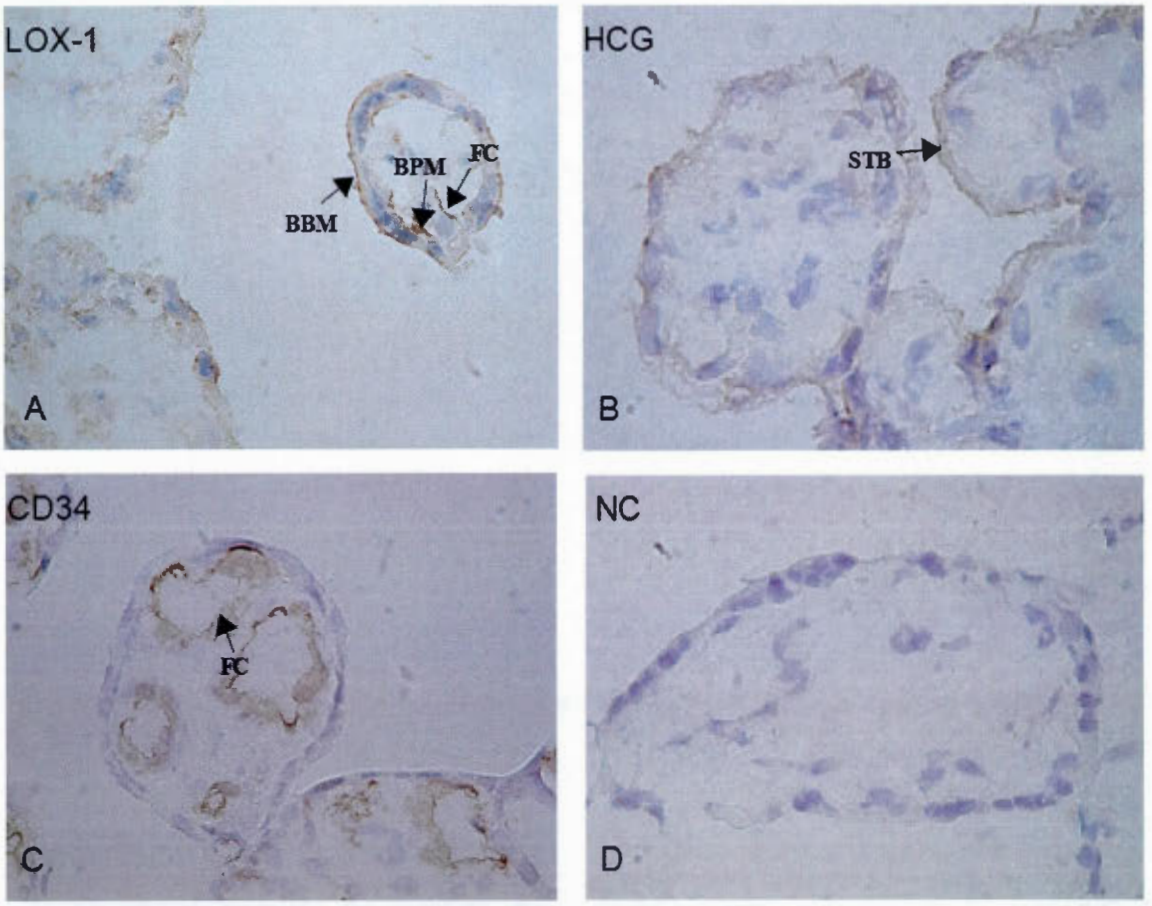
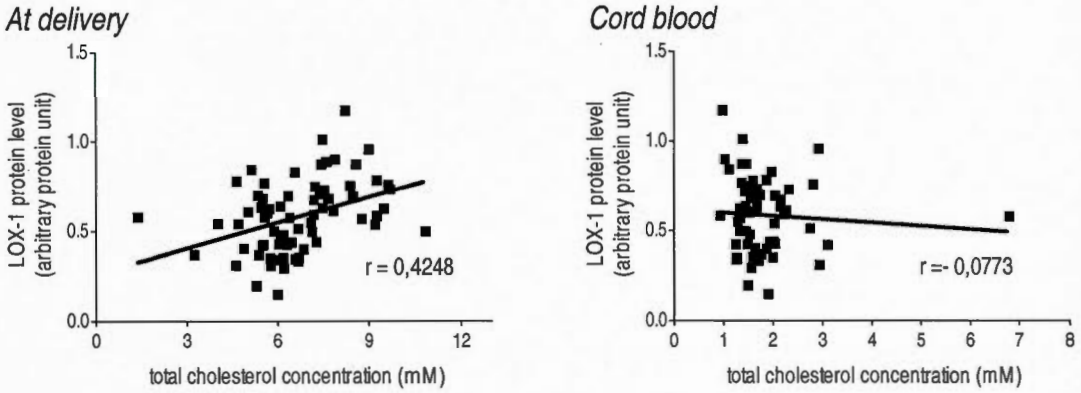
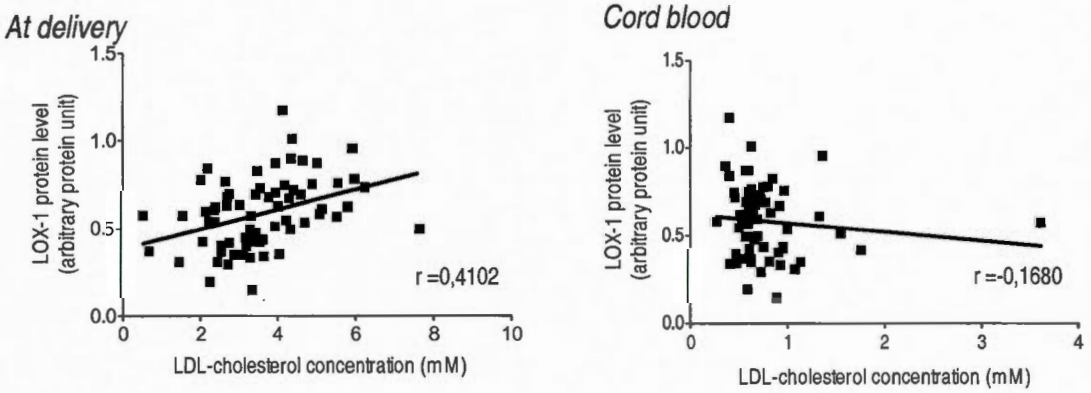


Figure 5

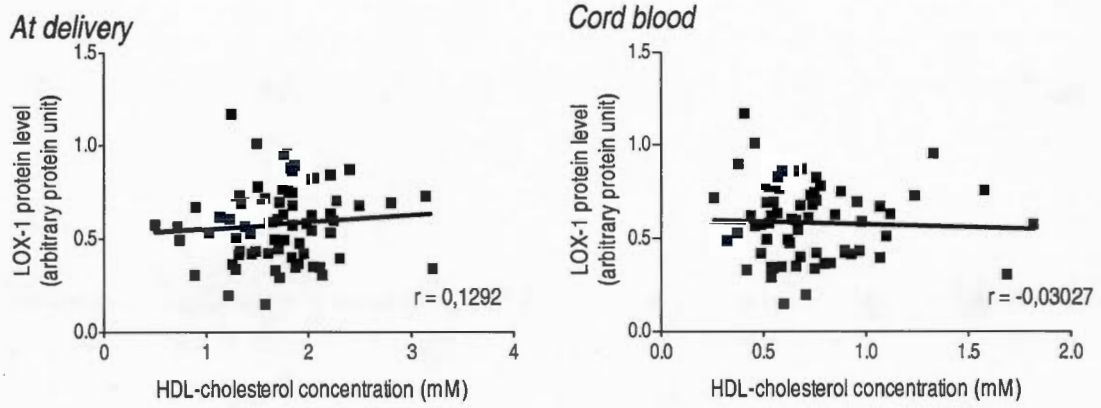
A- Total Cholesterol



B- LDL-cholesterol

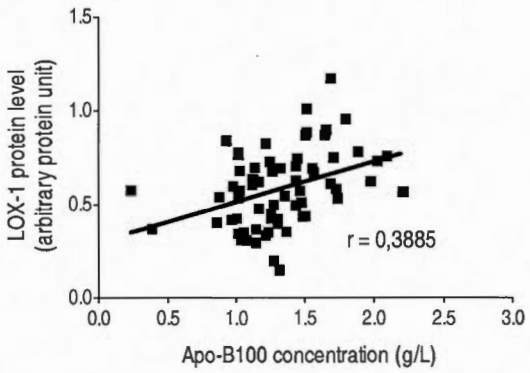


C- HDL-cholesterol



D- ApoB100

At delivery



Cord blood

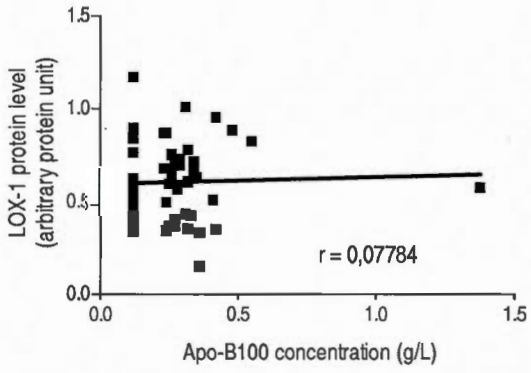
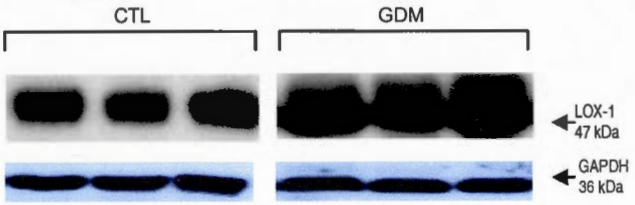
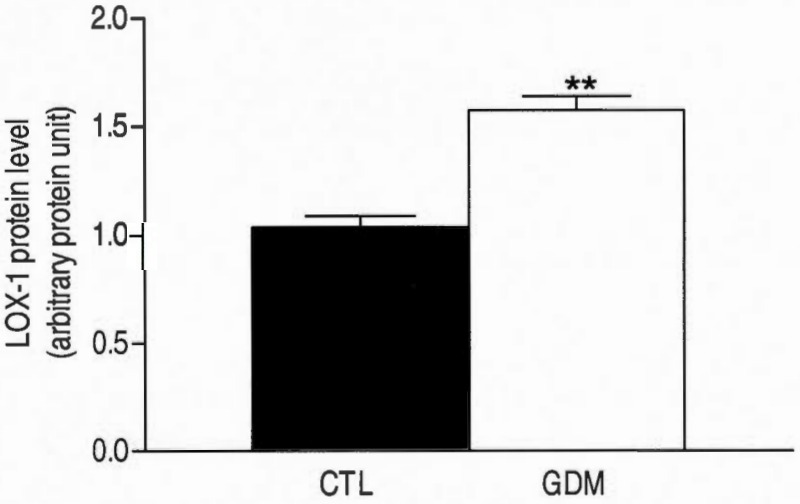


Figure 6

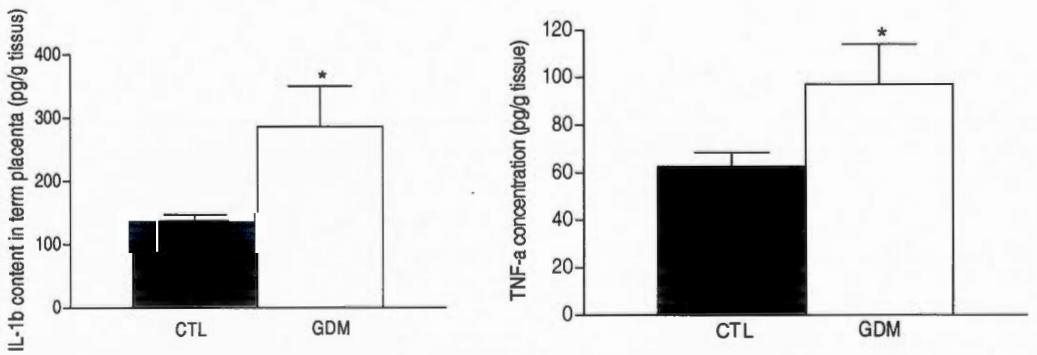
A-Representative Western-Blot



B-LOX-1 protein level



C- Cytokines content in term placenta



4. CHAPITRE IV

CONCLUSION ET DISCUSSION

Le placenta est un organe fascinant qui se développe tout au long de la grossesse afin de subvenir aux besoins croissants du fœtus. En dépit de l'abondante littérature sur la nutrition en cours de grossesse, l'influence des lipides maternels ainsi que leurs mécanismes de transfert par le placenta, n'ont peu ou pas été étudiés. Ainsi, lors de la grossesse, le placenta est apte à transporter jusqu'à 50% des lipides et 100% des acides gras essentiels consommés dans la diète journalière de la mère [28, 224, 225]. À la lueur de ces informations, il est donc aisé de constater que l'environnement lipidique maternel pourrait altérer le profil lipidique du nouveau né. À notre connaissance, notre laboratoire est la première équipe à étudier, chez l'humain, l'influence d'une modification du profil lipidique maternel sur le développement néonatal, tout en s'interrogeant sur le rôle du placenta et des ses composantes lipidiques dans le développement néonatal.

Dans le cadre du présent projet, le but était de déterminer si l'IMCpré-grossesse, le gain de poids ainsi que la concentration de cholestérol plasmatique chez la mère pouvaient influencer l'expression protéique placentaire de différents récepteurs à lipoprotéine de faible densité natives et/ou oxydées, soit LDLr, SR-BI et LOX-1. De tous les travaux effectués à ce jour, aucun ne s'est attardé à l'étude des échanges placentaires lipidiques lors de modulation de l'expression génétique de certains composants placentaires en cours de grossesse en fonction des profils lipidiques maternels, dont le développement fœtal est certainement tributaire. La présence de plusieurs récepteurs à lipoprotéines dans le placenta, supporte l'habileté du placenta à prendre le cholestérol des lipoprotéines maternelles, même si la contribution de ce cholestérol au pool du cholestérol plasmatique foetal est très faible [226]. Ainsi, d'autres études, elles aussi effectuées sur le placenta humain, ont démontré que lors des premiers stades de la gestation, il existe une

contribution substantielle du cholestérol maternel au pool du cholestérol fœtal. Par ailleurs, à terme, la concentration de HDL, de LDL et de cholestérol total est plus grande dans la veine ombilicale qu'au niveau de l'artère [227].

Les premiers travaux ont été effectués sur des placentas à terme provenant de femmes, sélectionnées et ayant remplit un formulaire de consentement, n'ayant aucune pathologie connue, non-fumeuses et ne prenant pas de médicaments pouvant altérer le métabolisme des lipides. Au niveau de l'expression placentaire protéique de LDLr, nos résultats, ont démontré une diminution de l'expression en fonction de la concentration plasmatique de cholestérol de la mère. Aussi, chez les femmes LC (ayant un niveau inférieur de cholestérol plasmatique (<7 mM)), on note une différence significative, soit une augmentation, de l'expression placentaire du LDLr, seulement chez les femmes ayant un IMC inférieur à la normale (< 20 kg/m^2) et chez les femmes ayant un gain de poids inférieur à la normale (<11 kg). Par ailleurs, chez les femmes HC (ayant un niveau supérieur de cholestérol plasmatique (>8 mM)), l'expression du LDLr diminue en fonction de l'indice de masse corporelle, tandis que, chez ces femmes, le gain de poids ne semble avoir aucun impact sur l'expression du LDLr. Enfin, il a été possible d'établir des corrélations avec différents dosages de composants lipidiques plasmatiques à l'accouchement, soit des corrélations inverses avec le cholestérol total, les LDL et l'apo-B100. Ainsi, ces résultats suggèrent que dans les cellules placentaires, la régulation du LDLr serait régie par un système de rétro-contrôle, dépendant de la concentration de cholestérol, comme dans les hépatocytes et les autres cellules.

Des expérimentations analogues ont été effectuées afin de déterminer l'influence de ces mêmes facteurs au niveau de l'expression de LOX-1. Pour ce récepteur, les résultats ont démontré une augmentation de son expression en fonction de la concentration de cholestérol plasmatique de la mère. De même, chez les femmes LC, il y a une augmentation entre les femmes ayant un indice de masse corporelle inférieur à la normale (< 20 kg/m^2) et supérieur à la normale (>26 kg/m^2). Par contre, le gain de poids

maternel ne semble pas influencer l'expression de LOX-1. Les corrélations effectuées entre l'expression placentaire de LOX-1 et différents paramètres lipidiques plasmatiques révèlent une corrélation positive avec le cholestérol total, les LDL ainsi que l'apo-B100. Ainsi, ces différences d'expression du LOX-1 pourraient être en grande partie attribuable au stress oxydatif et à l'inflammation très prononcés durant la grossesse.

Enfin, en ce qui concerne SR-BI, les résultats ont été tout autres. En effet, aucune différence n'a été notée entre les femmes contrôles (<7 mM) et HC (>8 mM). Cette absence de différence pourrait être attribuable au fait que le récepteur SR-BI ait une multitude de ligands, pouvant donc être régulé par des composantes non étudiées dans le présent projet. Par contre, lorsqu'on s'attarde à l'indice de masse corporelle, il est possible de constater une augmentation de l'expression placentaire de SR-BI chez les femmes HC comparé au groupe de femmes LC, lorsqu'elles ont un indice de masse corporelle inférieur à la normale (<20 kg/m^2), tandis que le phénomène inverse se produit chez les femmes ayant un indice de masse corporelle supérieur à la normale (>26 kg/m^2). Concernant l'influence du gain de poids, la seule différence se situe au niveau des femmes HC, alors qu'il y a une augmentation de l'expression de SR-BI entre les femmes ayant un gain de poids normal (11-18 kg) et les femmes ayant un gain de poids supérieur à la normale (>18 kg).

Dans un deuxième temps, les travaux ont été effectués sur des placentas, à terme, provenant de femmes présentant un diabète gestationnel. Ces résultats ont démontré une augmentation de l'expression protéique placentaire, des trois récepteurs étudiés, chez les femmes présentant un diabète gestationnel. Enfin, dans l'optique de déterminer où se trouvaient précisément les récepteurs susmentionnés, soit au niveau des BBM ou des BPM, il a été impératif de bien séparer ces deux membranes. Leur composition protéique étant différente, une séparation inadéquate aurait faussé les résultats et par le fait même, une mauvaise compréhension des mécanismes régissant ces échanges placentaires en découlerait automatiquement. Ainsi, nos résultats dévoilent, pour la première fois, que le

LDLr est exprimé principalement au niveau des BPM, tandis que LOX-1 est exprimé sur les BPM, mais principalement sur les BBM. Ces résultats pour le LDLr confirment donc que le transfert de LDL natives, au troisième trimestre est plus faible. De plus, le haut niveau d'expression du LDLr du côté fœtal peut être le reflet d'un contrôle du cholestérol dans le sang fœtal, lequel est indispensable au développement fœtal. La diminution significative de l'expression du LDLr observé dans les extraits de placenta total chez les femmes HC, peut être attribué à une diminution de l'expression du récepteur, principalement du côté fœtal. Enfin, ces résultats semblent démontrer un mécanisme compensatoire, développé par le placenta, afin de maintenir un niveau de cholestérol physiologique indispensable pour des échanges placentaires adéquats. Pour SR-B1, nos résultats confirment d'autres études démontrant que celui-ci est principalement exprimé au niveau des BBM.

En conclusion, nos travaux ont démontré que l'IMC pré-grossesse, le gain de poids et la concentration plasmatique de cholestérol peuvent modifier l'expression de récepteurs impliqués dans le métabolisme du cholestérol. Par ailleurs, ces résultats n'étaient pas associés à des cas de macrosomie foetale. Néanmoins, une multitude d'incertitudes restent encore à élucider en ce qui concerne les mécanismes menant à des malformations néonatales. Évidemment, les rôles et les fonctions des récepteurs, étudiés dans le présent projet, au niveau du placenta humain restent encore obscurs. Il n'en demeure pas moins un champ de recherche fort intéressant sur lequel des études ultérieures auront l'opportunité de se baser. Ainsi, énormément de travail reste à faire afin de mieux comprendre les mécanismes régissant les échanges lipidiques par lesquels des anomalies peuvent entraîner des malformations fœtales ou des pathologies se développant plus tard au courant de la vie. Ainsi, afin de mieux comprendre les échanges materno-fœtal, plusieurs points doivent être éclaircis. L'étude de l'expression placentaire des transporteurs comme ABCA-1 et la MTP (microsomale transfert protein) est capitale afin de comprendre la voie qu'empruntent les lipides lorsqu'ils sont incorporés dans le placenta. Enfin, l'étude de l'activité des enzymes clés du métabolisme du cholestérol,

soit ACAT, 7-α-hydroxylase et la HMG-CoA réductase, doivent être étudiées afin d'évaluer l'influence du cholestérol maternel au niveau de la biosynthèse et du catabolisme du cholestérol dans la placenta. Enfin, nous espérons que nos résultats auront apporté une contribution, aussi infime soit-elle, dans le mystérieux domaine qu'est la biologie de la reproduction.

5. CHAPITRE V

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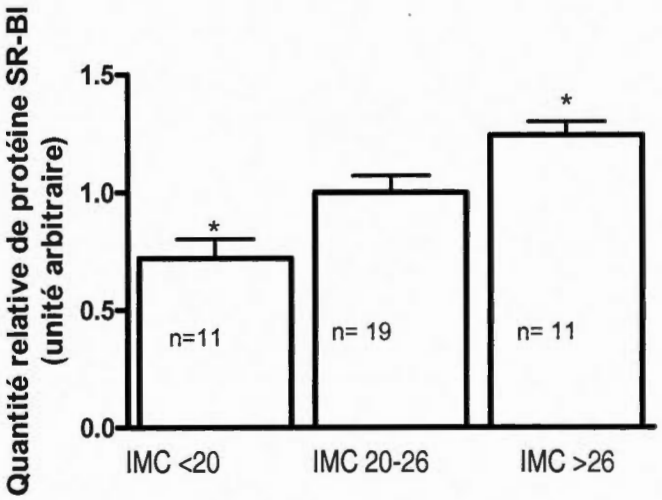
6. CHAPITRE VI : ANNEXES

6.1 Annexe I

A. Femmes LC (<7 mM)



B- Femmes HC (>8 mM)

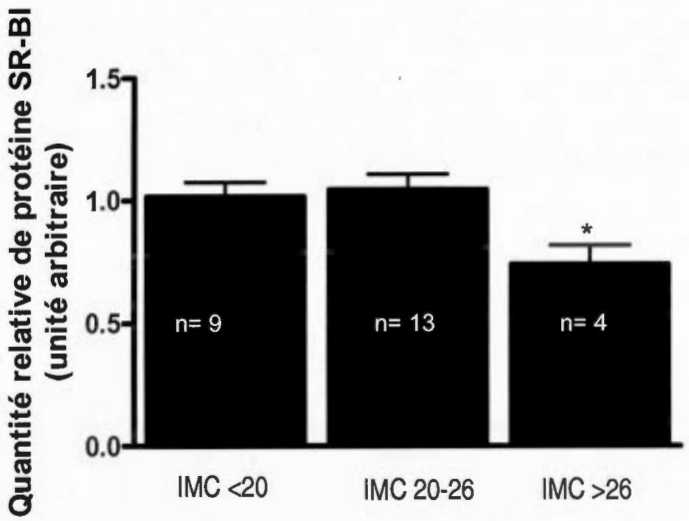
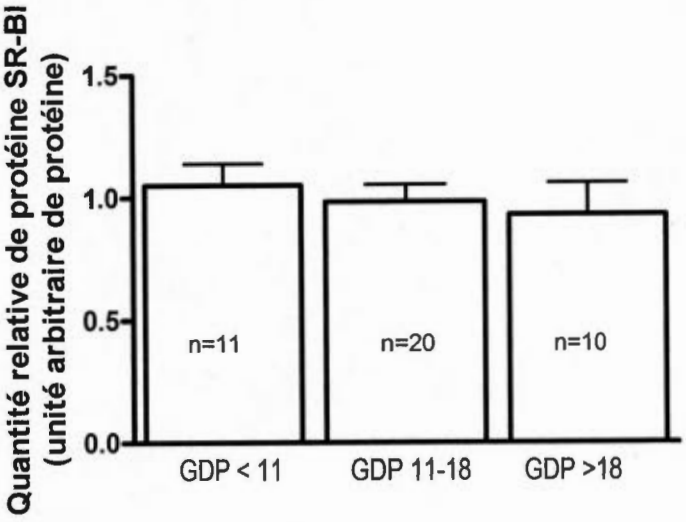


Figure I : Expression protéique représentative du LDLr provenant d'extrait de placenta humain à terme, en relation avec l'IMC (A) femmes LC (< 7mM), (B) femmes HC. Les résultats représentent la moyenne ± SEM, * p<0,05 comparé aux femmes ayant un IMC normal (20-26 kg/m^2)

6.2 Annexe II

A-Femmes LC (<7 mM)



B- Femmes HC (>8 mM)

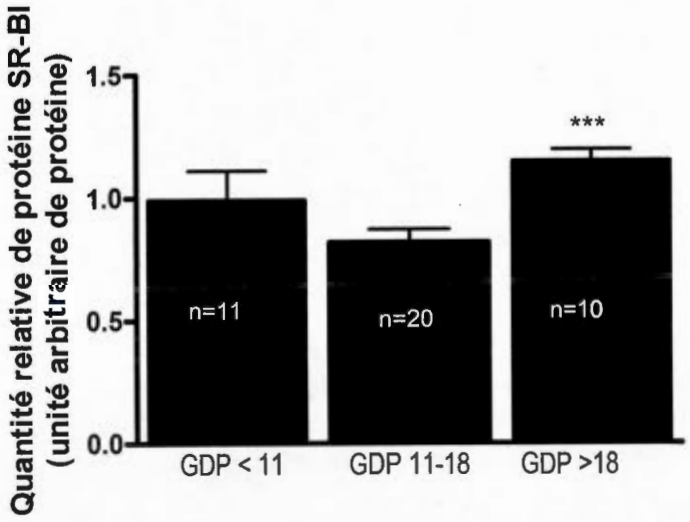


Figure II : Expression protéique représentative du LDLr provenant d'extrait de placenta humain à terme, en relation avec le gain de poids (GDP), (A) femmes LC (< 7mM), (B) femmes HC. Les résultats représentent la moyenne ± SEM, *** p<0,0001 comparé aux femmes ayant un GPD normal (11-18 kg)

6.3 Annexe III

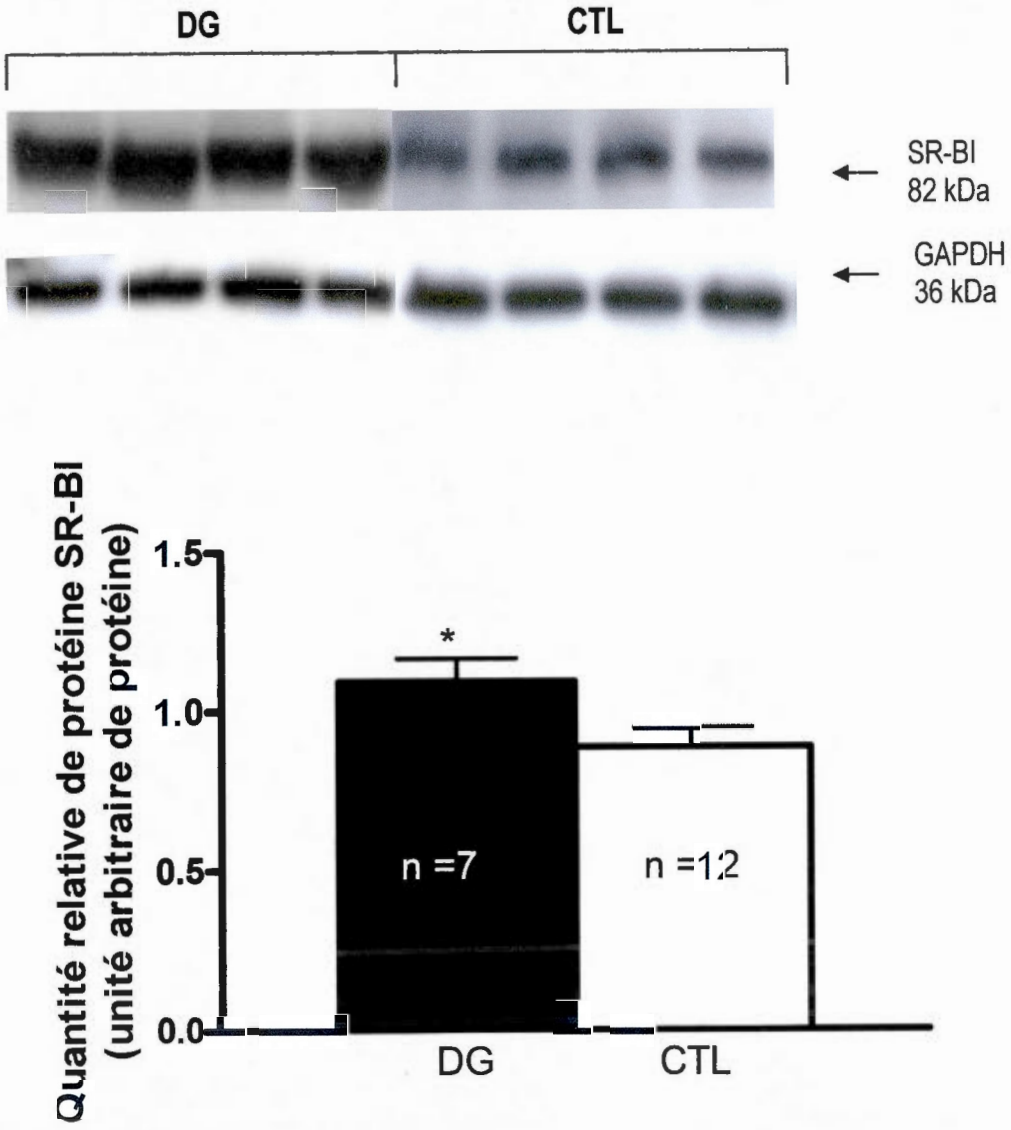


Figure III : Expression protéique représentative du SR-BI provenant d'extrait de placenta humain à terme, en relation avec le diabète gestationnel. Les résultats représentent la moyenne ± SEM, * $p<0,05$ comparé aux femmes CTL

6.4 *Annexe IV*

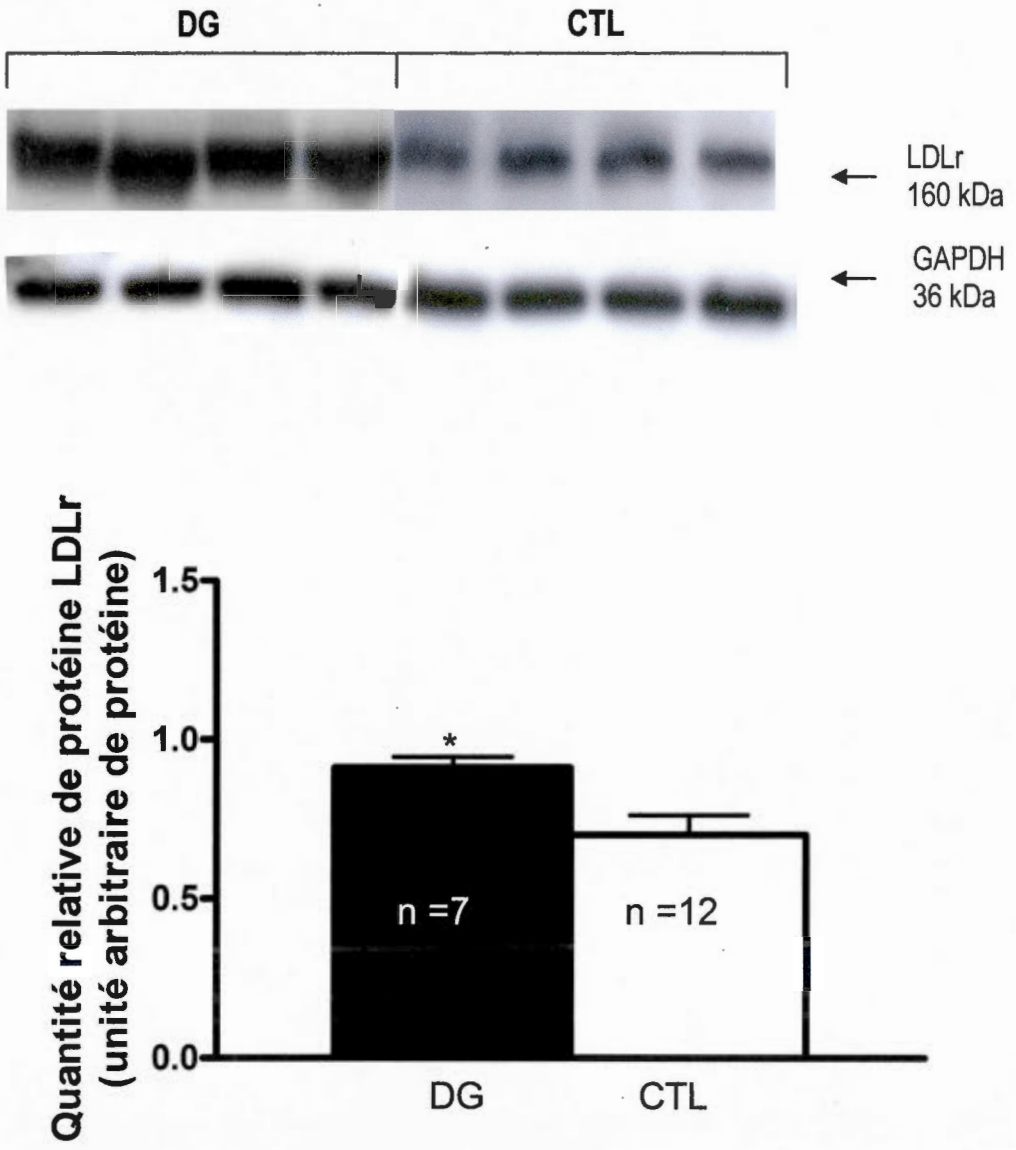


Figure IV : Expression protéique représentative du LDLr provenant d'extrait de placenta humain à terme, en relation avec le diabète gestationnel. Les résultats représentent la moyenne ± SEM, * $p<0,05$ comparé aux femmes CTL